# THE DESIGN, OPTIMIZATION AND TESTING OF Y CHROMOSOME SHORT TANDEM REPEAT MEGAPLEXES

by

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ABSTRACT

A multiplex polymerase chain reaction (PCR) assay capable of the simultaneous amplifying 20 Y chromosome short tandem repeat (STR) markers has been developed and tested to aid human testing and population studies. These markers include all of the Y-STR markers that make up the "extended haplotype" used in Europe (DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and YCAII a/b) plus the additional polymorphic Y-STR markers (DYS437, DYS438, DYS439, DYS447, DYS448, DYS388, DYS460, and GATA H4). The Y-STR 20plex is the first to include a simultaneous amplification of all the markers within the European "minimal" and "extended haplotype." A subset of the Y-STR 20plex primers, the Y-STR 9plex was also developed and tested. The Y-STR 9plex contains only the markers within the European minimal haplotype. Lastly, a Y-STR 11plex was designed and tested. The markers within the Y-STR 11plex are DYS385 a/b, DYS447, DYS448, DYS450, DYS456, DYS458 and DYS 464 a/b/c/d.

Validation experiments were performed in order to assess the reliability of the haplotypes generated by these newly designed Y-STR multiplexes. The validation experiments included concordance, precision, specificity and sensitivity studies. Additionally, a total of 647 male samples from three different U.S. populations were analyzed for all the loci included in the Y-STR 20plex and Y-STR 11plex. Allelic frequencies for all of the Y-STR markers were tabulated as well as haplotype diversity data for various combinations of markers.

Y-STR multiplexes were tested against samples from forensic cases that have already been closed. Analysis of these samples demonstrate that the Y-STR multiplexes developed here can handle real casework samples. Finally, the prototype Y Standard Reference Material (SRM) 2395 was characterized. SRMs are often used to validate a laboratory's measurement capability. SRM 2395 is a Y-STR profiling standard that consists of five components, four male (A-D) and one female (E). The characterization included the DNA sequencing of 18 different Y-STR loci for each component (including all of the markers in the European minimal haplotype), presenting a suggested nomenclature for allele designations, and running these components against commercially available Y-STR kits and the Y-STR multiplexes designed in this study.

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# CHAPTER 1

## INTRODUCTION

The determination of the structure of deoxyribonucleic acid (DNA) by James Watson and Francis Crick in 1953 is often said to mark the birth of modern molecular biology.[1] Watson and Crick's representation of the double helical structure DNA held together by hydrogen bonds helped to define the two purposes of DNA. First, DNA specifies the sequences of amino acids in a polypeptide chain, and second, it provides a template for its own replication.

Nucleic acids are linear polymers of nucleotides whose phosphates bridge the 3' and 5' positions of successive sugar residues. Nucleotides are made up of three parts: a nucleobase, a sugar, and a phosphate. DNA, a nucleic acid, consists of two polynucleotide strands containing nucleotides.[2] These strands wind about a common axis with a right handed twist to form a double helical structure. The two strands are antiparallel and wrap around each other such that they cannot be separated without unwinding the helix. Each strand of the helix consists of phosphodiester groups joining deoxyribofuranoses with 3' to 5' linkages. The bases occupy the core of the helix while its sugar-phosphate chains are coiled about its periphery thereby minimizing the repulsions between charged groups. Each base of the nucleotide units is hydrogen bonded to a base of a nucleotide on the opposite strand to form a base pair. These

hydrogen-bonding interactions, termed complementary base pairing, result in the specific association of the two chains of the double helix. According to Watson and Crick the double helical structure can accommodate only two types of base pairing. Each Adenine (A) residue must pair with a Thymine (T) residue and vice versa, and each Guanine (G) residue must pair with a Cytosine (C) residue and vice versa. It is the sequence of the bases contained within DNA that determine an individual's genetic make-up.

The initial sequencing and analysis of the human genome has been recently completed and it is estimated to contain approximately 3.1 billion base pairs.[3,4] This large amount of genetic material is found within the nucleus of human cells and is divided into chromosomes. Chromosomes are dense packets of DNA and proteins called histones. A normal human genome consists of 23 pairs of chromosomes per cell. Chromosomes 1-22 are not involved in sex determination and are termed autosomes while chromosomes X and Y are the sex chromosomes. Females are designated XX because they contain two copies of the X chromosome while males are designated XY and contain only one copy of the X chromosome. Chromosomes in all somatic cells contain two sets of each chromosome, and are termed diploid. Conversely, gametes, the sex determining chromosomes have only a single set of chromosomes and are designated as haploid.

The DNA within chromosomes is composed of both 'coding' and noncoding regions. Coding regions contain genes, which serve as the blueprint for self-replication and protein synthesis. Genes can vary in size from a few thousand to tens of thousand base pairs. It is estimated that there are 30,000-40,000 protein-coding genes contained within

the human genome.[3,4] A copy of each gene resides at the same locus on each chromosome pair. Alternate possibilities for a gene or genetic locus are termed alleles. Approximately 99.7% of human DNA is the same between individuals. The remaining 0.3%(~ one million nucleotides) is different from individual to individual.[5] These differences provide the opportunity for using DNA sequence information for such endeavors as human identification.

## DNA Polymorphisms and Short Tandem Repeats (STRs)

The location of a gene or a DNA marker on a chromosome is termed a locus. One type of variation that exists at a particular locus at the DNA level is termed a length polymorphism. Variable number tandem repeats (VNTR), "minisatellite" and short tandem repeat (STR), "microsatellite" markers are length polymorphisms, and can vary between individuals based on the number of nucleotide repetitions within a given allele. VNTRs consist of sets of tandemly repeated base pair sequences that can vary in length from 9 – 70 base pairs.[6] An example of a VNTR is the forensic DNA marker D1S80. The D1S80 marker is a minisatellite with a 16bp repeat unit and contains alleles in the range of 16-41 repeats.[7]

STRs were first reported in the late 1980s and are repeated base pair sequences that vary in length from 2 – 6 base pairs.[8] A schematic of a STR is given in Figure 1-1. STRs are categorized by the length of the repeat unit. For example, tetranucleotide repeats have four nucleotides repeated next to one another over and over again. An example of a STR commonly used in the forensic community is D5S818. D5S818 is a tetranucleotide AGAT repeat with an allele range of 7-16.[5] Thousands of these

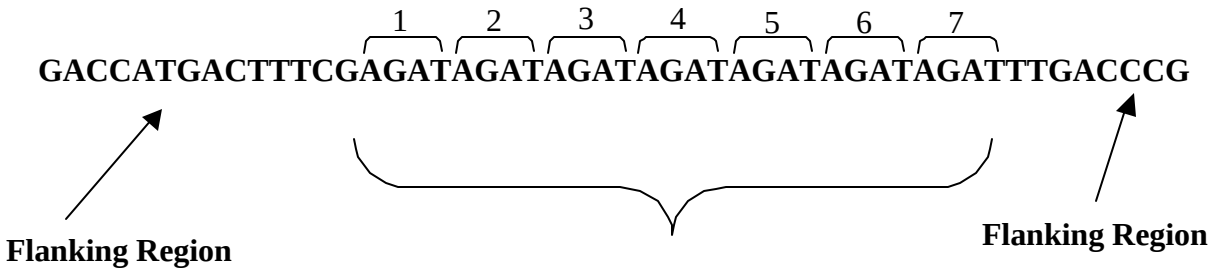



Figure 1-1
Short Tandem Repeat (STR) Marker
Illustration showing the AGAT repeat unit for the D5S818 STR marker. The number of AGAT repeat units varies between individuals.

polymorphic microsatellites have been identified in human DNA. It is estimated that STR makers in the human genome occur every 10,000 nucleotides.[9] STRs found within chromosomes 1-22 are termed autosomal DNA markers. These markers are recombined with each mitotic event because half of the genetic information comes from the mother and half comes from the father. An individual is homozygous if the alleles at a specific location are identical and heterozygous if the alleles are different.[10]

STRs are broken up into individual categories based on the repeat motif. Simple repeats such as D5S818 contains a repeat of identical length and sequence. Compound repeats consist of two or more adjacent simple repeats with different repeat motifs. Complex repeats may contain several repeat blocks of variable unit length as well as intervening sequences. The last STR category termed complex hypervariable repeats are those with a number of non-consensus alleles that differ in both sequence and size.[5]

There are instances where an allele for a STR locus contains an incomplete number of repeats. Microvariants is the term given to alleles that contain incomplete repeat units. An example of a microvariant is allele 9.3 at the TH01 locus. The TH01 9.3 allele contains nine tetranucleotide repeats and one incomplete repeat of three nucleotides because the seventh repeat is missing a single adenine out of the normal AATG repeat unit.[11]

Over the past ten years the utility and validity of STR typing for forensic applications such as human identity testing have been substantiated.[12-14] Multiple markers from individuals are examined in order to determine a person's DNA profile. The more markers examined improves the chances of obtaining a unique STR profile. The individual genotype frequencies from each marker can be multiplied together in order to determine the DNA profile frequency for a particular set of STR markers. For example the four polymorphic STRs D16S539, D13S317, D7S820, and D5S818 provide a matching probability of 1 in $1.8 \times 10^4$ for a Caucasian population.[5]

## Polymerase Chain Reaction (PCR) and Multiplex PCR

Most STR analysis works best with approximately 1 ng of template DNA. There are around 333 copies of each locus in 1 ng of human genomic DNA.[5] In order to increase the number of DNA molecules to a level suitable for STR analysis, the DNA must be amplified. Although molecular cloning techniques are indispensable to modern biochemical research, the polymerase chain reaction (PCR) is often a faster and more convenient method for amplifying DNA.[15-16]

In PCR, DNA is separated into single strands and incubated with DNA polymerase, deoxyribonucleotide triphospates (dNTPs), and two oligonucleotide primers whose sequence flank the DNA segment of interest (Figure 1-2). The primers direct the DNA polymerase to synthesize complementary strands of the target DNA. Multiple cycles of this process, each doubling the amount of target DNA, exponentially amplify the DNA starting with as little as a single gene copy. In each cycle, the two strands of the duplex DNA are separated by heating, the primers are annealed to their complementary segments on the DNA, and the DNA polymerase directs the synthesis of the complementary strands. The use of a heat-stabile DNA polymerase, such as *Taq* polymerase isolated from *Thermus aquaticus*, eliminates the need to add fresh enzyme after each round of heating.[15] Twenty cycles of PCR increase the amount of the target sequence around a million fold with high specificity.

Two of the most important components of a successful PCR reaction are primer design and primer quality. The amount of PCR product, or amplicon, is directly affected by the annealing characteristics of the primers. If PCR is to work well, the forward and reverse primers must be specific for the target region, have similar annealing temperatures, and not interact with one another. Additionally, the sequence to which the primers will bind must be fairly conserved. If the region where the primer is to bind is not conserved, primer annealing may not occur. This could result in a lack of amplification of the desired locus, termed null allele.[17]

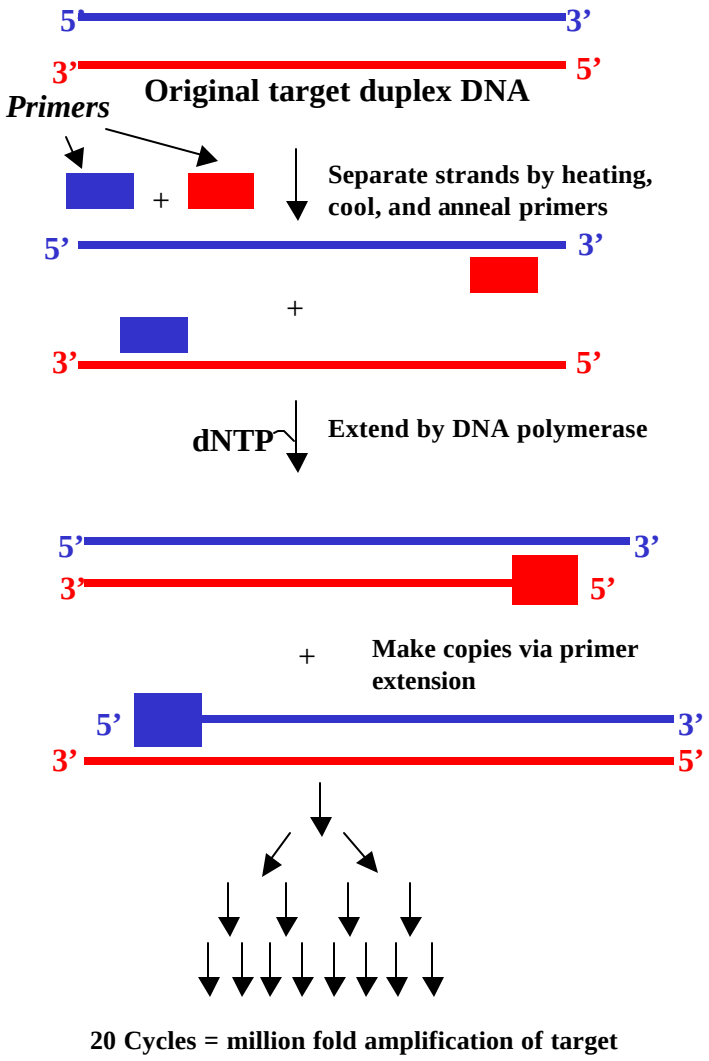



Figure 1-2
Polymerase Chain Reaction (PCR)
In each cycle of PCR, the template DNA strands are separated by heating and cooling to allow the primers to anneal to complementary sequences on each strand. DNA polymerase then extends the primers. The number of DNA copies doubles with every cycle after the second cycle. Twenty cycles can result in the million-fold amplification of the target DNA.

Multiplex polymerase chain reaction (PCR) is defined as the simultaneous amplification of multiple regions of DNA templates by adding more than one primer pair to the amplification reaction mixture. Figure 1-3 is an illustration showing the multiplex PCR process. In this example, three separate loci are simultaneously amplified using

three different primer sets. The resulting amplification products are different sizes and can be differentiated using an appropriate analysis technique.

Multiplex PCR primer design and optimization is a greater challenge than designing singleplex PCR primer pairs because multiple primer annealing events need to occur under the same annealing conditions without interfering with one another. Extensive optimization is normally required to obtain good balance between amplicons of the various loci being amplified.[18-19] Previous efforts on multiplex PCR design and optimization have focused on chimeric primers and on varying experimental conditions such as $MgCl_2$ concentration, reaction buffer concentrations, annealing temperature, and polymerase concentration.[18,20]

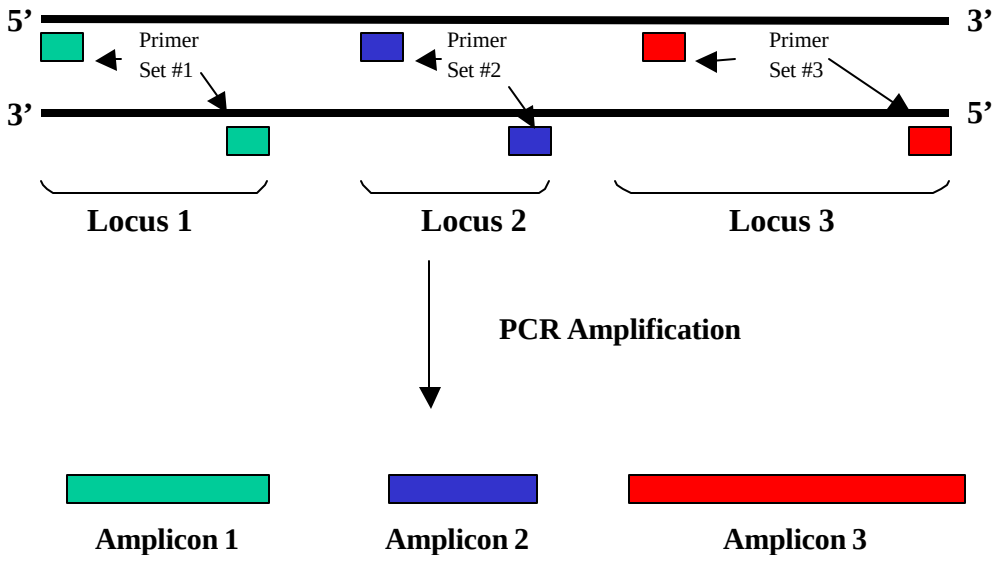



Figure 1-3
Mutliplex PCR
Simultaneous amplification of target DNA involving more than one primer set. Each primer set is designed to amplify a different locus. Different colored boxes represent the different primer sets. The resulting amplicons are the PCR amplification products for that particular primer set.

For a multiplex PCR reaction to work properly, primer pairs need to be compatible. Since multiplex assays are run under the same PCR conditions, the primers used need to have fairly similar characteristics such as melting temperature ($T_m$) and should not exhibit significant interactions with each other, themselves, or unwanted regions of the template. Excessive regions of complementarity between primers must be avoided to prevent the formation of primer-dimers that may cause the primers to bind to one another instead of the template DNA. Through stringent initial primer selection, the time-consuming and often costly process of optimization can be reduced. The primary experimental conditions used to optimize the multiplex PCR system in this study are the primer pair concentrations; other variables such as thermal cycling number, reaction components, annealing temperatures, etc, are fixed.

High quality primers are essential to successful multiplex amplification reactions. Both high performance liquid chromatography (HPLC) and time-of-flight mass spectrometry (TOF-MS) have been used as techniques of quality control assessment of the PCR primers used in multiplex STR amplification reactions.[21] For example, it has been shown that TOF-MS provides a rapid and accurate check of primer quality prior to using the oligonucleotides in a PCR reaction.[21,22] From examining a TOF-MS spectra of a particular primer, the molecular weight determined using the TOF-MS can be compared to the calculated value determined from the mass of the dye (if present) and the sum of the masses of the nucleotides expected. Primers with either incorrect sequences or oligonucleotide failures can compete for binding sites on the DNA template and can negatively impact the presence and/or length of the subsequent amplicon.

Since first being described in 1988,[23] PCR multiplexing has been applied in many areas of DNA testing including the analysis of deletions,[24] mutations,[25] autosomal STRs[10-13] and Y chromosome STRs.[26-28] Furthermore, the wide availability of genetic information due to the publishing of the sequence of the human genome[3,4] makes the demand for multiplex PCR even greater.

## Y Chromosome STRs and Y-STR Multiplex Assays

The Y chromosome is becoming a useful tool for tracing human evolution through male lineages[29] as well as application is to a variety of forensic situations[30] including those involving evidence from sexual assault cases containing a mixture of male and female DNA.[27-28, 31-32] In 1998 the report titled "Jefferson fathered slave's last child" used Y chromosome DNA markers to trace the Jefferson family line by linking the modern-day descendants of Thomas Jefferson and Eston Hemmings.[33] More recently, Y-STR markers were used to study the Y-chromosomal lineages of the likely male-line descendants of Genghis Khan.[34]

Up until a few years ago the use of Y chromosome for forensic purposes was restricted by a lack of polymorphic markers.[5] These markers have differing degrees of polymorphism and ability to differentiate between two unrelated male DNA samples. Table 1-1 lists some of the Y chromosome STR markers available in the literature, including the ones studied in this work. All of the nomenclature listed for each Y-chromsome STR marker follows the recommendations provided by the International

Table 1-1
Common Y-STR markers
Information includes the GenBank® Accession number for each locus, respective repeat motif, and number of repeats within the GenBank sequence (termed reference allele). Some sequences were made reverse and complement (R&C) in order to maintain consistency with previously used forward and reverse primer designations. Repeats motifs for each locus defined using ISFG recommendations as a guide.[35]

| Marker Name | Repeat Motif | GenBank Accession | Reference Allele |
|---|---|---|---|
| DYS19 | TAGA | AC017019 (r&c) | 15 |
| DYS385 a/b | GAAA | AC022486 (r&c) | 11 |
| DYS389 I<br>DYS389 II | (TCTG) (TCTA)<br>(TCTG) (TCTA) | AC004617 (r&c) | 12<br>29 |
| DYS390 | (TCTA) (TCTG) | AC011289 | 24 |
| DYS391 | TCTA | AC011302 | 11 |
| DYS392 | TAT | AC011745 (r&c) | 13 |
| DYS393 | AGAT | AC006152 | 12 |
| YCAII | CA | AC015978 | 23 |
| DYS388 | ATT | AC004810 | 12 |
| DYS426 | GTT | AC007034 | 12 |
| DYS434 | TAAT (CTAT) | AC002992 | 10 |
| DYS435 | TGGA | AC002992 | 9 |
| DYS436 | GTT | AC005820 | 12 |
| DYS437 | TCTA | AC002992 | 16 |
| DYS438 | TTTTC | AC002531 | 10 |
| DYS439 | AGAT | AC002992 | 13 |
| DYS441 | CCTT | AC004474 | 14 |
| DYS442 | TATC | AC004810 | 12 |
| DYS446 | TCTCT | AC006152 | 14 |
| DYS447 | TAA**W**A compound | AC005820 | 23 |
| DYS448 | AGAGAT | AC025227 | 23 |
| DYS449 | TTTC | AC051663 | 29 |
| DYS450 | TTTTA | AC051663 | 9 |
| DYS456 | AGAT | AC010106 | 15 |
| DYS458 | GAAA | AC010902 | 16 |
| DYS459 a/b | TAAA | AC010682 | 9 |
| DYS460 (A7.1) | ATAG | AC009235 (r&c) | 10 |
| DYS461 (A7.2) | (TAGA) CAGA | AC009235 (r&c) | 12 |
| DYS462 | TATG | AC007244 | 11 |
| DYS464 a/b/c/d | CCTT | AC006338 | 15 |
| Y-GATA-H4 | TAGA | AC011751 (r&c) | 12 |
| Y-GATA-C4 | T**S**TA compound | G42673 | 21 |
| Y-GATA-A10 | TAGA | AC011751 | 13 |

Society of Forensic Genetics (ISFG).[35] The ISFG is an international organization consisting of members from 49 countries who provide guidelines concerning the application of DNA polymorphisms to the area of human identification. Whereas most Y-linked STR systems exhibit a single polymorphic fragment when amplified by PCR, a number of Y-STRs originate from regions that are duplicated on the Y-chromosome, and can exhibit two PCR amplicons of variable size. Examples of Y-STR loci whose primer pairs amplify two PCR products are 389I/II, YCAII, and DYS385 and DYS459.[36-39] Recently, a new Y-chromosome STR marker has been discovered that is quadruplicated on the Y chromosome, DYS464.[39] DYS464 is a simple tetranucleotide consisting of the repeat sequence CCTT. Due to its quadruplication on the Y-chromosome it can provide up to four polymorphic peaks for a particular primer set.

Microvariants have also been described for Y-STR markers. As was the case for autosomal alleles such as TH01 alleles, alleles with incomplete repeats have been found for DYS385.[40] Microvariants can be the result of deletions in the regions flanking the repeat motif. For the DYS385 locus, the intermediate alleles originally designated 17-1 and 18-1 with the consensus structures of $(GAAA)_{17}$ and $(GAAA)_{18}$, respectively were found to lack a T in the same $(T)_7$ stretch located within the 3' flanking region of each allele.[40] If ISFG guidelines are followed, the 17-1 and 18-1 DYS385 alleles are designated as 16.3 and 17.3 respectively.

Included on the list in Table 1-1 are a core set of Y STR markers that are currently being used by the European Y chromosome typing community. They have established a "minimal haplotype" and an "extended haplotype" for inclusion of common loci into a

central DNA database (see http://www.ystr.org). The minimal haplotype consists of results for the following Y STR markers: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385.[41] The extended haplotype includes all of the markers from the minimal haplotype plus the highly polymorphic dinucleotide repeat YCAII.[41]

Since the Y chromosome is passed down from father to son without any recombination, there is a prevalence of patrilineages in a population. This can result in an overrepresentation of some haplotypes.[30] Also, individual Y STR markers cannot be combined using the product rule because of its non recombing nature, as is the case with autosomal STRs.[5] The combination of the markers included in the minimal haplotype can distinguish approximately 78-97% of male individuals in various local European populations.[30] If the YCAII a/b Y-STR marker is included the discriminatory capacity reached as high 99% in these same European populations. The Y chromosome haplotype must include as many polymorphic loci as possible to raise the exclusion chance to nearly 100% leaving only male relatives undiscriminated.[30]

In order to examine as many Y markers simultaneously as possible, Y-STR multiplex PCR assays should be used. Within the last five years, a number of Y chromosome STRs have been combined into Y STR multiplex assays.[26-28,31] Unfortunately these multiplexes involve the simultaneous amplification of six or less loci. The loci used in these multiplexes included some or all of the following STR markers DYS19, DYS389 I and II, DYS390, and DYS391, and DYS393. Recently, Y-STR assays have become available to purchase commercially. The first two commercially available Y STR multiplex kits

are the Y-Plex$^{TM}$ 6, and Y-Plex$^{TM}$ 5 (ReliaGene Technologies, Inc., New Orleans, LA). The Y-Plex$^{TM}$ 6 kit includes the markers DYS19, DYS390, DYS391, DYS393, DYS389II, and DYS385. The Y-Plex$^{TM}$ 5 kit includes the markers DYS389I/II, DYS392, DYS438, and DYS439. Taken together, one can obtain the minimal haplotype using these kits. The minimal haplotype, amplifiable in two or three multiplexes has been accepted for court use in Europe.[42] Up until this point there has not been a Y STR multiplex available that includes the simultaneous amplification of all the markers that make up the "extended haplotype". Additionally, the majority of the Y-STR multiplexes presented in the literature do not include many of the recently discovered Y-STR markers (Table 1-1) such as DYS464, DYS447, and DYS448.

## Standard Reference Materials (SRMs)

The US National Institute of Standards and Technology is responsible for developing national and international standard reference materials (SRMs). SRMs are used to validate a particular assay used in a laboratory, calibrate instrumentation, troubleshoot protocols, and check laboratory proficiency. For example, the SRM 2391a, PCR-based DNA profiling standard was released in December 1999. SRM 2391a is designed to provide quality assurance to laboratories that perform DNA profiling using PCR methods and STRs.[43] The Federal Bureau of Investigation's (FBIs) DNA Advisory Board standard 9.5 states: "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocols against an appropriate NIST standard reference material or standard traceable to a NIST standard".[5] If Y STR testing

is to be performed in a forensic laboratory, a suitable standard reference material is needed.

## Capillary Electrophoresis

Since first being introduced in the mid 1980's Capillary Electrophoresis has become a widely used analytical technique by the scientific community. The method of CE was first reviewed in 1990 by Kuhr in *Analytical Chemistry*.[44] CE has been extensively used to separate DNA fragments containing STRs with highly reproducible results.[45-48] Over the past 5 years, the commercial availability of CE instruments has grown. Two such instruments are the ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 3100 Genetic Analyzer 16 Capillary System (Applied Biosystems). These instruments provide a semi-automated and accurate means of analyzing STR amplicons.[13-14]

The heart of a CE instrument such as the ABI PRISM® 310 Genetic Analyzer is the high voltage power supply providing up to 15,000 volts. Connected to it are two electrodes, the ends of which are immersed in a conductive buffer at the inlet and outlet reservoirs, along with both ends of a fused silica capillary (Figure 1-4). The buffers used in CE can also be used in gel electrophoresis. The fused silica capillary, which serves as the separation tube in CE, is of very narrow internal diameter, typically 20 to 100 μm. The high surface area to volume ratio of the capillaries allows for the application of very high electric field strengths without the generation of excessive Joule heat. A window is "burned" towards the outlet end of the capillary for the purpose of on-column detection, generally by UV or laser-induced fluorescence as in the case of the ABI PRISM® 310 Genetic Analyzer.

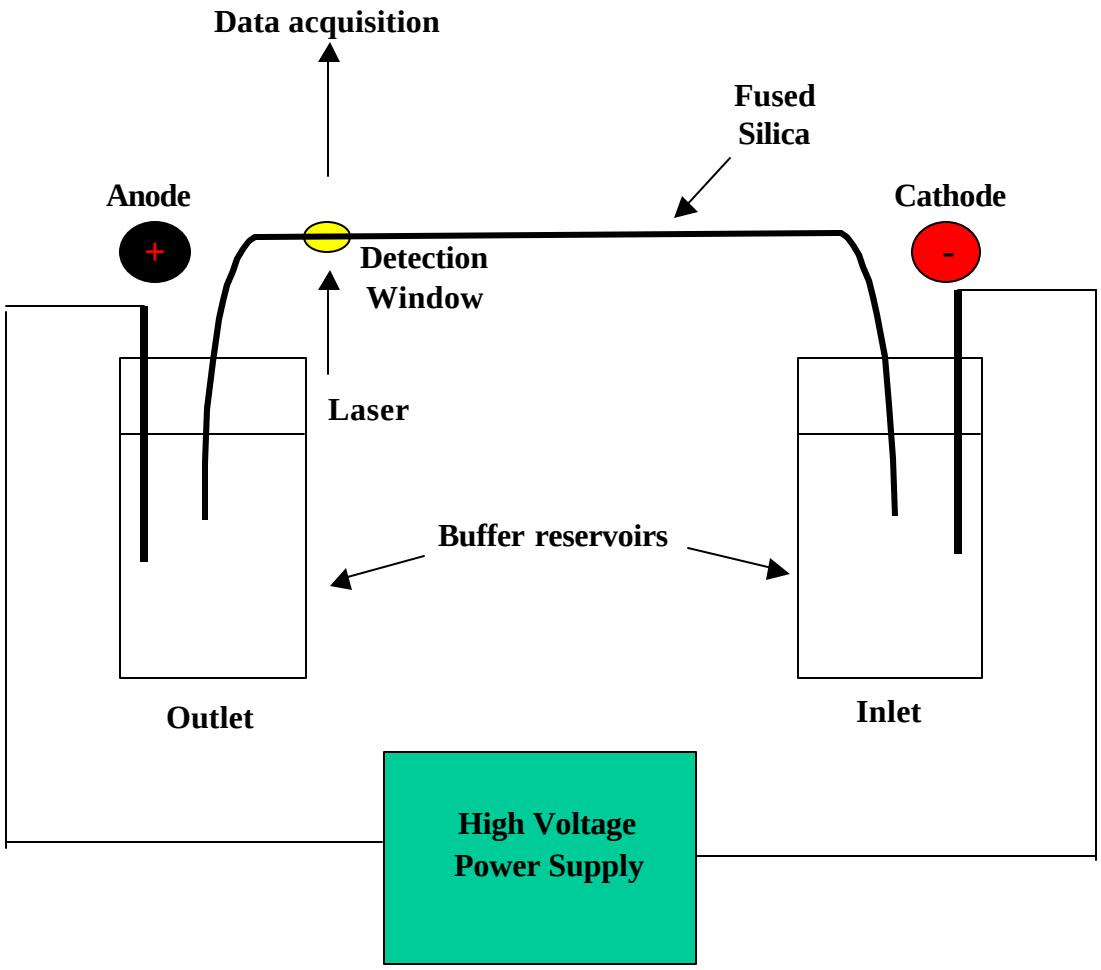



Figure 1-4
Capillary Electrophoresis (CE) Schematic Used for DNA Analysis
CE consists of fused silica capillary, two electrodes, and a conductive buffer. Samples are injected onto the capillary by applying a voltage to each sample. A high voltage is applied across the capillary after the electrokinetic injection in order to separate the DNA molecules.

Analysis begins by filling the capillary with the viscous polymer solution. CE separates molecules based on differences in their electrophoretic mobility in a conductive buffer. Electrophoretic mobility is proportional to the charge of the molecule divided by its frictional coefficient.[49] This is approximately equal to the charge to mass ratio of the molecule. Under the influence of an electric field the negatively charged DNA molecules will migrate away the cathode and move towards the anode. Since DNA molecules

posses a constant charge to mass ratio (one phosphate group for every nucleotide), a sieving material is required to resolve DNA fragments that are different in size. Separation of DNA is accomplished using gels or polymer solutions that retard larger DNA molecules as they pass through the sieving material.[5] Instead of an agarose or polyacrylamide gel used in slab gels, the DNA molecules pass through a viscous polymer solution that is pumped into the capillary prior to the addition of the sample. Instead of pores as in the case of gel electrophoresis, the DNA molecules are retarded by the polymer chains contained within the polymer solution.[5]

The sample is injected at the inlet by pressure or electrokinetically as is the case for the ABI PRISM® 310 Genetic Analyzer. Electrokinetic injection is a process that is influenced by the ionic strength of the sample, the amount of template DNA in the sample, and the geometry of the electrode and capillary tip within the sample.[50] The process of electrokinetic injection involves the transfer of charged ions in an electrical field onto the capillary separation matrix. Because only ions transfer in this process, no liquid volume loss occurs from the sample. Thus, samples can be reloaded if a run is aborted or lost. Following injection, both ends of the fused silica capillary are immersed in the inlet and outlet buffer reservoirs. A separation voltage (15,000 volts – typical setting for separations using ABI PRISM® 310) is then applied and data collection is initiated.

## Fluorescence Detection

The majority of CE is performed using UV absorbance detection often at low wavelengths such as 260 nm to maximize sensitivity.[49] Fluorescence and Laser Induced

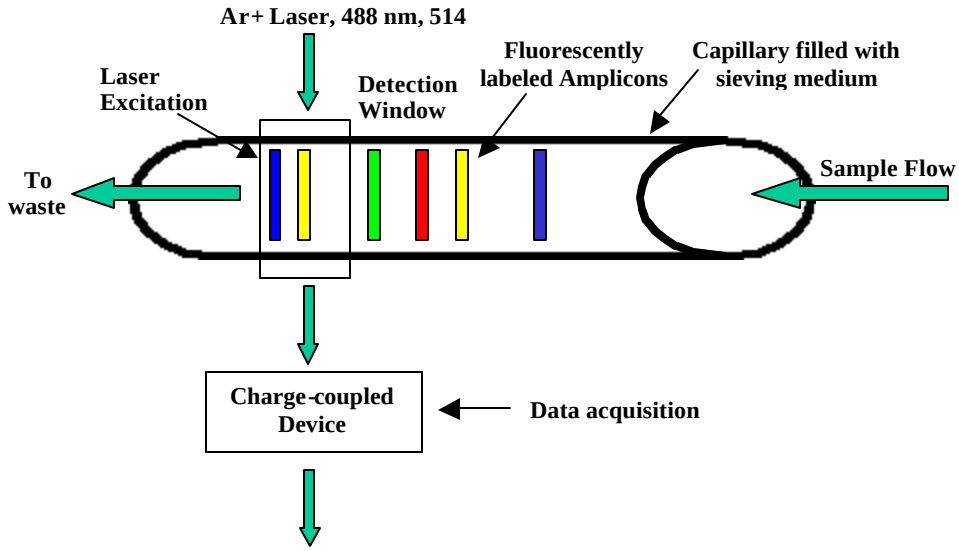
Fluorescence (LIF) are further detection options which can offer more sensitive and selective detection compared to UV absorbance.[49] The sensitivity (1000x greater than UV detection) and specificity of LIF make it an ideal strategy for DNA detection.[51-52]

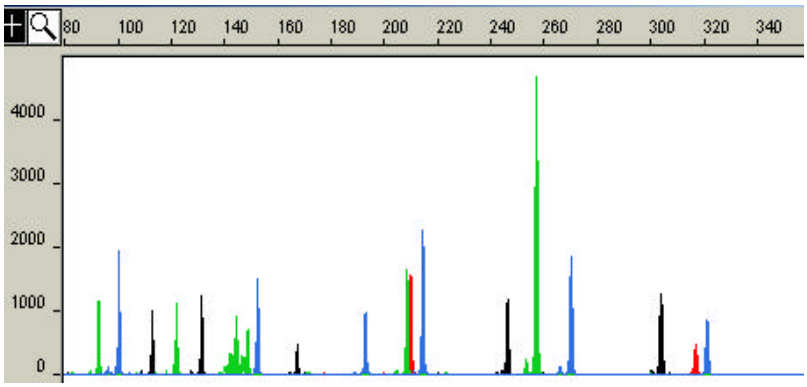
Since DNA has little native fluorescence, analysis using LIF detectors requires that the amplicons be fluorescently labeled. In the application to DNA typing with STR markers, the fluorescent dye is attached to the 5' end of a PCR primer that is incorporated into the amplified target region of DNA. Table 1-2 lists some of the fluorescent dyes commonly used to label PCR products analyzed on the ABI PRISM® 310 and ABI PRISM® 3100. Each of these dyes has its own unique excitation and emission maximum. Filters are designed to capture the emission photons for each fluorescent dye. Each dye set used must be matched to the excitation source and the instrument optics involved in their detection.

Table 1-2
Fluorescent dye labels used in STR kits. Information includes chemical name, excitation maximum (nm) and emission maximum (nm). Adapted from Table 10-2 shown by Butler.[5]

| DYE | Chemical name | Excitation maximum (nm) | Emission maximum (nm) |
|---|---|---|---|
| 6-FAM | 6-Carboxy fluorescein | 494 | 522 |
| JOE | 6-Carboxy-2',7'-dimethoxy-4',5'-dichlorofluorescein | 528 | 554 |
| NED | Proprietary to Applied Biosystems | 553 | 575 |
| ROX | 6-Carboxy-X-rhodamine | 587 | 607 |
| Fluorescein | Fluorescein | 490 | 520 |
| TAMRA | 4,7,2',7'-Tetramethyl-6-carboxyrhodamine | 560 | 583 |
| VIC | Proprietary to Applied Biosystems | 533 | 554 |
| PET | Proprietary to Applied Biosystems | 558 | 595 |
| LIZ | Proprietary to Applied Biosystems | 638 | 655 |

The ABI PRISM® 310 and 3100 instruments perform detection of the DNA sample automatically. The time span from sample injection to sample detection is measured by a laser placed near the end of the capillary. The laser (Ar+ 488 nm, 514nm – for both ABI PRISM® 310 and 3100) excites the fluorescent dye in the visible spectrum and illuminates the DNA fragments as they pass by a window that has been burned into the capillary at a fixed position (Fig. 1-5). The smaller molecules pass by the window first followed by the larger molecules. Amplicons of the same size are distinguished by using different fluorescent dye labels. The emitted photons are captured by a photosensitive device. For the detection of low- intensity light, a photoelectric cell with a built in amplifier (photomultiplier tube, PMT) or a charged-coupled device (CCD) camera as is the case for the ABI PRISM® 310 and ABI PRISM® 3100 is commonly used. In both cases, the photons of light hitting the detector are converted to an electrical signal. The resulting data is graphically displayed (termed electropherogram) as colored peaks noted by height in relative fluorescent units (RFUs) versus time scan number.




**CE Electropherogram (Size bp vs. rfu)**
**Obtained using GeneScan® software**

Figure 1-5
Illustration of CE Using Fluorescence Detection.
Negatively charged fluorescently labeled DNA passes through capillary and by detection window. Samples are separated on the basis of size and type of fluorescent label. Fluorescently labeled PCR products are induced to fluoresce by Ar+ laser. Emitted photons are captured by Charged Couple Device (CCD). The resulting data is converted to an electrical signal and can be displaced as a CE electropherogram using GeneScan® software (Applied Biosystems, Foster City, CA)

# CHAPTER 2

## STATEMENT OF PURPOSE

The purpose of this study is to present a systematic approach to the design, optimization and testing of multiplexes capable of simultaneously amplifying numerous Y-STR markers. The Y-STR markers chosen should be highly polymorphic and in use by the forensic community. The first Y-STR multiplex constructed, the Y STR 20plex, was capable of the simultaneous amplification of 20 polymorphic Y chromosome-specific STR markers.[53] This 20plex includes all of the markers in the European extended haplotype and also contains the trinucleotide loci DYS388[30] and DYS426[54], the tetranucleotide loci DYS437[55,56], DYS439[55,56], GATA A7.1 (DYS460)[57,58] and H4[57,59], the pentanucleotide loci DYS438[55,56], and DYS447[39], and the hexanucleotide marker DYS448[39]. The second multiplex is a subset of the Y-STR 20plex called the Y-STR 9-plex. The Y-STR 9plex included all of the markers currently within the European "minimal haplotype". Lastly, a Y-STR 11plex was constructed and tested. It included the Y-STR markers DYS447[39], DYS448[39], DYS450[39], DYS456[39], DYS458[39], DYS385[38] and the DYS464.[39]

After the successful design, optimization and testing of the Y-STR multiplexes, an assessment of the reliability of the analytical platform, including the ABI PRISM® 3100 Genetic Analyzer was undertaken. This evaluation hopes to demonstrate that the

analytical platform described herein can provide accurate and reliable analysis of Y-STR markers. Ideally, Y-STR multiplexes should only contain the most highly polymorphic markers in order to increase their respective discriminatory capacities. With the exception of the markers in the extended haplotype, little information existed as to the discriminatory capacity of additional Y-STR markers in the same set of DNA samples. Various combinations of markers were evaluated in order to ascertain which had the greatest power of discrimination.

Any multiplex PCR assay, if it is to be performed in a laboratory receiving federal funding must test their procedures against a suitable reference material.[5] Until now no such standard exists for Y-STR markers. In order to meet this need, a prototype Y Standard Reference Material® (SRM) 2395 was characterized. This characterization is to include the DNA sequencing of numerous Y-STR loci for five male components, and the presentation of standard nomenclature for each marker according to ISFG guidelines.[35]

Finally, the Y-STR multiplexes have been evaluated as to their efficacy in analyzing actual forensic casework samples. The forensic casework samples were analyzed at the United States Criminal Investigation Laboratory (USACIL) in Forest Park, Georgia.

# CHAPTER 2

# MATERIAL AND METHODS[1]

## DNA Samples

A variety of DNA samples were used to study all of the Y STR multiplexes presented here. First were those coming from the Y Chromosome Consortium (YCC) panel.[60] The YCC panel consists of 74 male samples and 2 female samples composed of individuals from diverse world populations. These samples were originally established as cell lines in the Laboratory of Human Genetics at the New York Blood Center (see http://ycc.biosci.arizona.edu). The YCC samples were provided for this work from Dr. Michael Hammer's laboratory at the University of Arizona as extracted DNA that had been previously quantified.

Second, the samples used for the population studies and Prototype Y SRM 2395 components were obtained from anonymous human blood samples purchased from two commercial blood banks (Millennium Biotech, Inc., Ft. Lauderdale, FL and Interstate Blood Bank, Memphis, TN). These samples were approved for use through an institutional review board at the National Institute of Standards and Technology (NIST) and were extracted using a modified salt-out procedure method.[61]

[1] Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or the U.S. Depart ment of Defense nor does it imply any of the materials, instruments or equipment identified are necessarily the best available for this purpose.

Finally, the DNA samples analyzed in the forensic casework samples were obtained from the United States Army Criminal Investigation Laboratory (USACIL) in Forest Park Georgia. These samples were extracted using conventional organic extraction methods by USACIL personnel.[62]

## Development of Reference Sequences

Sequences for each locus were obtained from GenBank® (www.ncbi.nlm.nih.gov) using the standard nucleotide BLAST (Basic Local Alignment Search Tool).[63] Previously published Y STR primers were chosen as the query sequences for the respective Y STR markers. BLAST search results listed the accession numbers that contained sequences producing significant alignments to the query sequences. The accession numbers are listed in order of their alignment score. The alignment score is defined as the degree of similarity between the query sequence primer and the sequence being compared.[64] The sequences with the higher alignment scores are listed first. The higher the score, the greater extent the nucleotide sequences are related.

The reference sequence and accompanying GenBank® accession number for each sequence that contains a Y STR marker is defined as the one that returns the maximum alignment score for the query sequence. If a query sequence primer returned multiple GenBank® accession numbers with a maximum score, the most recent entry and/or sequence with the larger flanking region to the repeat was used for further evaluation including sequence alignments. Sequence alignments of the STR repeat and 200-300 base pair (bp) flanking regions for the various GenBank® sequence entries were performed to aid detection of polymorphic nucleotides and regions where X chromosome

homology was known to exist (see multiplex PCR design). Sequence alignments were performed for all of the queries that returned multiple GenBank® accession numbers with a maximum alignment score. The alignments were conducted using the Baylor College of Medicine (BCM) search launcher available at http://searchlauncher.bcm.tmc.edu/multi-align.html.

From these sequence alignments, a consensus reference sequence was produced that originated from the GenBank® accession number listed for each Y STR Marker in Table 1-1 in the introduction. The number of repeats contained within each reference sequence was then determined using ISFG recommendations as a guide.[35]

## Primer Design

Primer 3 version 0.2 over the World Wide Web www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi was used to design the individual primer sets used in each of the newly designed Y-STR multiplexes.[65] The Primer3 software allows the user to manipulate certain parameters during the design process. Some of these parameters include, minimum and maximum primer length, minimum and maximum melting temperatures, and optimal product size. For the multiplex assay design described here, default parameters were used. Primers for each locus were designed using a fixed size for each PCR product. Primer sequences used for each multiplex are given in Tables 3-1 and 3-2. The Y-STR 9plex is composed of only those primers from the 20plex set needed to amplify the minimal haplotype loci.

Table 3-1
Primer sequences for PCR amplification of loci in the Y STR 20plex[53] and 9plex. The Y-STR 9plex is a subset of the Y-STR 20plex and contains primer sets for DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392 and DYS393. Predicted primer melting temperatures ($T_m$) were calculated using a total primer concentration = 0.05 μM and $[Na^+]$ = 50 mM.[65]

| Locus | | Primer Sequences (5'-to-3') | Primer Conc (μM) | Primer3 $T_m$ (°C) |
|---|---|---|---|---|
| DYS19 | F | **NED**-ACTACTGAGTTTCTGTTATAGTGTTTTT | 1.8 | 55.0 |
| | R | GTCAATCTCTGCACCTGGAAAT | 1.8 | 60.5 |
| DYS385 | F | **VIC**-AGCATGGGTGACAGAGCTA | 0.6 | 56.9 |
| a/b | R | GCCAATTACATAGTCCTCCTTTC | 0.6 | 54.7 |
| DYS388 | F | **NED**-GAATTCATGTGAGTTAGCCGTTTAGC | 1.8 | 63.4 |
| | R | GAGGCGGAGCTTTTAGTGAG | 1.8 | 59.2 |
| DYS389 | F | **6FAM**-CCAACTCTCATCTGTATTATCTATG | 1.3 | 54.2 |
| I/II | R | GTTATCCCTGAGTAGTAGAAGAATG | 1.3 | 59.0 |
| DYS390 | F | **VIC**-TATATTTTACACATTTTTGGGCC | 0.2 | 57.2 |
| | R | GTGACAGTAAAATGAAAACATTGC | 0.2 | 57.0 |
| DYS391 | F | **6FAM**-TTCAATCATACACCCATATCTGTC | 0.2 | 57.9 |
| | R | GATAGAGGGATAGGTAGGCAGGC | 0.2 | 60.0 |
| DYS392 | F | **NED**-TAGAGGCAGTCATCGCAGTG | 1.8 | 60.2 |
| | R | GACCTACCAATCCCATTCCTT | 1.8 | 57.3 |
| DYS393 | F | **VIC**-GTGGTCTTCTACTTGTGTCAATAC | 0.4 | 54.7 |
| | R | GAACTCAAGTCCAAAAAATGAGG | 0.4 | 57.7 |
| DYS426 | F | **VIC**-CTCAAAGTATGAAAGCATGACCA | 0.2 | 59.3 |
| | R | GGTGACAAGACGAGACTTTGTG | 0.2 | 59.8 |
| DYS437 | F | **6FAM**-GACTATGGGCGTGAGTGCAT | 0.2 | 61.1 |
| | R | GAGACCCTGTCATTCACAGATGA | 0.2 | 59.6 |
| DYS438 | F | **6FAM**-CCAAAATTAGTGGGGAATAGTTG | 0.2 | 58.9 |
| | R | GATCACCCAGGGTCTGGAGTT | 0.2 | 62.6 |
| DYS439 | F | **6FAM**-TCGAGTTGTTATGGTTTTAGGTCT | 0.2 | 58.3 |
| | R | GTGGCTTGGAATTCTTTTACCC | 0.2 | 60.3 |
| DYS447 | F | **PET**-GGTCACAGCATGGCTTGGTT | 0.7 | 63.4 |
| | R | GGGCTTGCTTTGCGTTATCTCT | 0.7 | 64.0 |
| DYS448 | F | **PET**-TGGGAGAGGCAAGGATCCAA | 1.1 | 65.2 |
| | R | GTCATATTTCTGGCCGGTCTGG | 1.1 | 64.3 |
| DYS460 | F | **NED**-GAGGAATCTGACACCTCTGACA | 0.7 | 59.3 |
| (A7.1) | R | GTCCATATCATCTATCCTCTGCCTA | 0.7 | 59.1 |
| H4 | F | **NED**-ATGCTGAGGAGAATTTCCAA | 0.4 | 57.3 |
| | R | GCTATTCATCCATCTAATCTATCCATT | 0.4 | 56.2 |
| YCAII | F | **VIC**-TGTCAAAATTTAACCCACAATCA | 0.9 | 59.3 |
| a/b | R | GCAGTCTTTCACCATAAGGTTAGC | 0.9 | 57.2 |

Table 3-2
Primer sequences for PCR amplification of loci in the Y STR 11plex. Predicted primer melting temperatures ($T_m$) were calculated using a total primer concentration = 0.05 μM and $[Na^+]$ = 50 mM.[65]

| Locus | | Primer Sequences (5'-to-3') | Primer Conc (μM) | Primer3 $T_m$ ($^oC$) |
|---|---|---|---|---|
| DYS385 a/b | F | **VIC**-TAGACACCATGCCAAACAACA | 0.4 | 60.0 |
| | R | GGCTGCTGACCAGATTTCTTT | 0.4 | 60.8 |
| DYS447 | F | **FAM**-GGTCACAGCATGGCTTGGTT | 0.4 | 63.4 |
| | R | GGGCTTGCTTTGCGTTATCTCT | 0.4 | 64.0 |
| DYS448 | F | **FAM**-GGAGAGGCAAGGATCCAAATA | 0.4 | 60.4 |
| | R | GTTGATTCCCTGTGTTGGAGAC | 0.4 | 60.0 |
| DYS450 | F | **NED**-TGCAGCTGTTTGTAGATCTGGT | 0.4 | 60.0 |
| | R | GCCTTTCCAATTTCAATTTCTG | 0.4 | 60.0 |
| DYS456 | F | **NED**-GGACCTTGTGATAATGTAAGATAG | 0.4 | 54.0 |
| | R | GTAGAGGGACAGAACTAATGGAA | 0.4 | 55.7 |
| DYS458 | F | **NED**-GCAACAGGAATGAAACTCCAAT | 0.4 | 60.4 |
| | R | GTTCTGGCATTACAAGCATGAG | 0.4 | 59.8 |
| DYS464 a/b/c/d | F | **VIC**-CTTTGGGCTATGCCTCAGTTT | 0.4 | 60.6 |
| | R | GCCATACCTGGGTAACAGAGAGAC | 0.4 | 59.0 |

## Primer Modification

All of the reverse primers which lacked a guanosine residue at the 5'end of their original sequence were modified by adding a guanosine to the 5' end to promote non-template addition.[66,67] Non-template addition is most often adenosine and is often referred to as adenylation or the "plus A" form of the amplicon. The resulting amplicon is one base pair longer than the actual target sequence and also can result in a split peak containing –A and +A sequences. With the addition of a non-template base plus the G 5'-tail, the labeled, fully adenylated PCR product is two base pairs longer than an amplicon predicted on a reference sequence from the primer position alone. Without the

addition of the G 5' tail, the fully adenylated PCR product is one base pair longer than an amplicon predicted on a reference sequence from the primer position alone.

To maintain consistency the forward primers are labeled with a fluorescent dye to generate a labeled PCR product for detection purposes. For the work with the Y-STR 20 plex, Y-STR 9plex and Y-STR 11plex on the ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 3100 Genetic Analyzer 16 capillary array system, 6-FAM (blue), VIC (green), NED (yellow), and PET (red) were the fluorescent dyes covalently linked to the 5' end of the forward primers and the fluorescent dye LIZ (orange) for the labeling of the internal size standard. If 4-dye chemistry is desired, the red dye ROX can be used as the internal standard with 6FAM (blue), VIC (green), and NED (yellow) being used to label the PCR amplicons.

The following fluorescent dyes were used for the Y-STR loci in both the Y-STR 20plex and Y-STR 9plex: 6FAM (DYS391, DYS389I/II, DYS437, DYS438, DYS439), VIC (DYS426, DYS393, YCAII a/b, DYS390, DYS385 a/b), NED (A7.1, H4, DYS388, DYS19, DYS392) and PET (DYS447, DYS448).

The following fluorescent dyes were used for the Y-STR loci in the Y-STR11plex: 6FAM (DYS447, DYS448), VIC (DYS385, DYS464) and NED (DYS450, DYS456, DYS458).

## Primer Quality Control

Primers were purchased from MWG Biotech (High Point, NC), Operon Technologies (Alameda, CA), or Applied Biosystems (Foster City, CA) and upon receipt were quality

control tested prior to further use to confirm proper synthesis using methods previously described.[21-22]

## PCR Amplification Conditions

### Singleplex PCR

Singleplex PCR amplifications were performed in reaction volumes of 20.0 µL with 2 units of AmpliTaq Gold® DNA polymerase (Applied Biosystems), 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphates (dNTPs: dATP, dCTP, dGTP, dTTP), 5% glycerol by volume, 4.4 µL of the respective primer set (See Table 3-1 and 3-2 for concentrations), and 2 µL DNA template. DNA template varied between 50 pg and 10 ng depending on the study. Thermal cycling was performed using a GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e., ramp speeds of $1^0$C/s):

95 $^o$C for 10 minutes

28 cycles:
- 94 $^o$C for 1 minute
- 55 $^o$C for 1 minute
- 72 $^o$C for 1 minute

60 $^o$C for 45 minutes
25 $^o$C hold

### Y-STR 20plex, Y-STR 9plex, Y-STR 11plex

The PCR amplifications for each Y-STR multiplex were performed in reaction volumes of 20.0 µL with 2 units of AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.75 mM $MgCl_2$,

300 µM deoxynucleotide triphosphates (dNTPs: dATP, dCTP, dGTP, dTTP), 5% glycerol by volume, 3.2 mg/ml of Bovine Serum Albumin, 4.4 µL of the Y-STR primer mix (See Tables 3-1 and 3-2 for concentrations), and 2 µL DNA template. The concentrations of the Y-STR 9plex primer sets were the same as the Y-STR 20plex, except the primer sets for the additional loci in the Y-STR 20plex have been removed. The primer concentrations stated in Tables 3-1 and 3-2 were calculated based on their concentration in the 20.0 µL PCR reaction volume. The male DNA template amount in the PCR varied between 50 pg and 10 ng depending on the study. Thermal cycling was performed using the same conditions as in singleplex PCR given above. Please note for some of the population samples PCR reaction vessels were scaled down to 5.0 or 10.0 µL. In the case of 5.0 µL PCR, 1.0 µL of DNA template was used (approximately 1 ng) and the number of amplification cycles was cut to 26 instead of the standard 28. In the case of 10.0 µL PCR, 2.0 µL of DNA template was used (approximately 2 ng) and the number of amplification cycles was cut to 27 instead of 28.

For the forensic casework samples, all PCR amplifications were performed in reaction volumes of 25.0 µl with 2.5 units of AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA). All of the concentrations of the other reagents were unchanged.

## Y-PLEX™ 6 Kit

The Y-PLEX™ 6 kit is a commercially available Y STR multiplex kit manufactured by ReliaGene Technologies (New Orleans, LA). This kit was used to amplify DYS19,

DYS385, DYS389II, DYS390, DYS391, and DYS393. PCR amplification were performed according to the manufacturer instructions outlined in the Y-PLEX™ 6 instruction manual version 1.0, except the reaction volumes were reduced by 50%.

## Detection and Analysis of PCR Products in Y-STR Analysis

The separation and detection of Y-STR 20plex products was accomplished with the ABI PRISM® 3100 Genetic Analyzer 16-capillary array system (Applied Biosystems) following manufacturer's protocols using the G5 matrix filter set to determine the five dyes, 6FAM™(blue), VIC™ (green), NED™ (yellow), PET™ (red), and LIZ™ (orange), available from Applied Biosystems. Prior to sample analysis, a spectral matrix was established using matrix standard set DS-33 (Applied Biosystems, P/N 4323016). Samples were prepared with 18.6 µL Hi-Di™ formamide (Applied Biosystems, P/N 4311320), 0.4 µL GS500 LIZ size standard (P/N 4322682), and with 1.0 µL of PCR product. Samples were injected 16 at a time for 10 s at 3,000 volts and separated at 15,000 volts for 44 minutes with a run temperature of 60°C. Separations were performed using the GeneScan POP-6 module, 3100 POP™-6 sieving polymer matrix (Applied Biosystems P/N 4316357), 1X Genetic Analyzer Buffer with EDTA (P/N 402824), and a 36 cm x 50 µm capillary array (P/N 4315931). Following data collection, samples were analyzed with GeneScan® 3.7 (Applied Biosystems), and allele designations were made in Genotyper® 3.7 (Applied Biosystmes) based on a modified genotyping macro from one used in the AmpFISTR® Identifiler™ kit (Applied Biosystems). Allele calls were made

based on sizing bin windows of up to +/- 1.50 bp rather than by comparison to allelic ladders.

The separation and detection of the Y-PLEX™ 6 kit generated PCR products were accomplished with an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) using filter set A. The matrix was established with matrix standards for the four dyes FAM (blue), JOE (green), TAMRA (yellow), and ROX (red) (Applied Biosystems, P/N 401546). Each sample was prepared by adding 1.0 µL PCR product to 20 µL of deionized formamide containing 0.75 µL GS500 ROX size standard. Samples were injected for 5 seconds at 15,000 volts and separated at 15,000 volts for 26 minutes with a run temperature of 60°C using Genetic Analyzer POP™-4 (P/N 402838), 1X Genetic Analyzer Buffer with EDTA, and a 47 cm x 50 µm capillary (J & W Scientific, Folsom, CA). Following data collection, samples were analyzed with GeneScan® 3.1 for Macintosh (Applied Biosystems) and allele designations were determined by comparison to allelic ladders using Genotyper® 2.5 (Applied Biosystems) and the Y-PLEX™ 6 310 *v*3.0 Genotyping template provided by ReliaGene with their kit.

## DNA Sequencing

For PCR products used in DNA sequencing of Y-STR loci in the Prototype Y SRM 2395 components, the following modifications were made to the singleplex PCR protocol for Y-STR analysis. First, the amount of template amplified was kept constant at approximately 7.0 ng. Second, the number of amplification cycles was set to 35 not 28 in order to increase the amount of product. Finally, the forward primer of each respective

primer set used for amplification was not labeled with a fluorescent dye as was the case in regular Y-STR typing. The sequences of the primers used for the PCR and subsequent DNA sequencing reactions are given in Table 3-3. In many cases, these primers are outside the ones used in the multiplex assays to enable inspection of the primer binding sites in the 20plex and 11plex multiplexes.

For DNA sequence analysis of the loci duplicated on the Y-chromosome (389I/II, and 385 a/b) gel electrophoretic separations of the amplified PCR products were performed by Margaret Kline at NIST in order to separate the respective allelic fragments. Separations for 385 were accomplished with polyacrylamide gel (10% total acrylamide with 3% bis acrylamide crosslink). The 389I and 389II fragments were separated using an agarose gel (3% Nu-Sieve-1% SeaKemGTG). All gel fragments were visualized with SYBR® Green II DNA stain (Molecular Probes, Eugene, OR). The fragments were cut from the gel, and placed in 100 µL TE buffer and incubated overnight at 37° C. A 5.0 µL aliquot of each washing containing the isolated alleles was amplified for another 28 cycles using the same cycling parameters described earlier.

Following PCR amplification, products were purified using ExoSap-IT™ (USB, Cleveland, OH, P/N 78201) according to manufacturer's protocol. The ExoSap-IT™ degrades nucleotides and any DNA primers remaining after PCR. The protocol involves adding 2.0 µL of Exo-Sap IT™ per 5.0 µL of PCR volume into each tube. The samples are then placed into a GeneAmp 9700 and incubated for 15 minutes at 37° C and then for another 15 minutes at 80° C.

Table 3-3
Primer sequences for PCR amplification of loci used for DNA sequencing. All primer concentrations were 1.0 μM. Primer sequences differing from those used in the multiplex assays are in bold.

| Locus | | Primer Sequences (5' to 3') |
|---|---|---|
| DYS19 | F | ACTACTGAGTTTCTGTTATAGTGTTTTT |
| | R | GTCAATCTCTGCACCTGGAAAT |
| DYS385 | F | **GCTGAGGCAGGGTAATTGTT** |
| a/b | R | **TAAGGGCTGCTGACCAGATT** |
| DYS388 | F | GAATTCATGTGAGTTAGCCGTTTAGC |
| | R | GAGGCGGAGCTTTTAGTGAG |
| DYS389 | F | CCAACTCTCATCTGTATTATCTATG |
| I/II | R | TTATCCCTGAGTAGTAGAAGAATG |
| DYS390 | F | **TGGGATGGGAAATGATGTTT** |
| | R | **ACAATGCAAATGATGAAAGAAA** |
| DYS391 | F | **GCTGAGGTCAAGTAACCCTGTC** |
| | R | **TTTGTGGTGGGTCTGTCTTG** |
| DYS392 | F | **CCCACTCTTGGCATCTAGTGA** |
| | R | **GGAAGCTAGCAAGACTTCAGAAA** |
| DYS393 | F | GTGGTCTTCTACTTGTGTCAATAC |
| | R | **CTAAATAAAGTCATATCAGCTGC** |
| DYS426 | F | **TGGCCATCTCCTTTATTTGG** |
| | R | **GTGAGCCGAGAGAGAGATCG** |
| DYS435 | F | GGGTTGTCCAGAGAAACAGC |
| | R | CCCCCTCCTCTCGTCTATC |
| DYS436 | F | **GTTTCACAGCGTTCATTGGA** |
| | R | **GCTACTCCCAGCCAAAACTG** |
| DYS437 | F | GACTATGGGCGTGAGTGCAT |
| | R | AGACCCTGTCATTCACAGATGA |
| DYS438 | F | CCAAAATTAGTGGGGAATAGTTG |
| | R | ATCACCCAGGGTCTGGAGTT |
| DYS439 | F | TCGAGTTGTTATGGTTTTAGGTCT |
| | R | TGGCTTGGAATTCTTTTACCC |
| DYS447 | F | GGTCACAGCATGGCTTGGTT |
| | R | GGGCTTGCTTTGCGTTATCTCT |
| DYS448 | F | TGGGAGAGGCAAGGATCCAA |
| | R | TCATATTTCTGGCCGGTCTGG |
| DYS460 | F | **CCACAGTAAAAGGGCTCCAC** |
| (A7.1) | R | **TGATGCTGTGTCACTATATTTCTGTT** |
| H4 | F | **TGGACAGAGTGGGTTCTGAAG** |
| | R | **TGGTCAAAACACCATTTCCTC** |
| DYS461 | F | CCACAGTAAAAGGGCTCCAC |
| | R | TGATGCTGTGTCACTATATTTCTGTT |

The DNA sequencing reactions were prepared using the BigDye™ Terminator *v* 3.0 sequencing kit (P/N 4390242) from Applied Biosystems. The BigDye™ dye structures contain a fluorescein donor linked to one of four dichlororhodamine acceptor dyes. The BigDye™ Terminators are designated according to the dideoxynucleotide to which they are attached. The BigDye™ Terminators and their corresponding dRhodamine acceptor dyes are given in Table 3-4. DNA sequencing reactions were performed in reaction volumes of 20.0 µL with 8.0 µL of Terminator Mix, 3.2 µL of either the forward or reverse primers (1 µM) for the loci of interest, 8.8 µL of $H_2O$ and 1.0 µL of purified PCR product (approximately 5-10 ng). Each set of samples prepared for DNA sequencing was run in conjunction with a pGEM™ control sample. Running of the pGEM™ control ensures that both the instrument and DNA sequencing kits are in working order. The pGEM™ controls were performed in reaction volumes of 20.0 µL with 8.0 µL of Terminator Mix, 4.0 µL of the control primer, 7.0 µL of $H_2O$ and 1.0 µL of the pGEM™ control. DNA sequencing reactions were also performed using half-reactions. In half reactions the amount of Terminator mix is cut in half to 4.0 µL and that volume is replaced with 4.0 µL of 2.5X DNA sequencing buffer (Edge Biosystems, Gaithersburg, MD; P/N 78901). Cycle sequencing was performed as follows:

25 cycles of

96 °C for 10 seconds
50 °C for 5 seconds
60 °C for 4 minutes

4 °C hold

Table 3-4
BigDye™ Terminator Dye/Base Relationships
A= Adenine, C= Cytosine, G = Guanine, and T = Thymine

| Terminator | BigDye™ Terminator |
|---|---|
| A | dRG |
| C | dROX |
| G | dR110 |
| T | dTAMRA |

Extension products resulting from the DNA sequencing reactions were then prepared for electrophoresis. First, an aliquot of 15.0 µL of DNA sequencing product was filtered using Performa DTR Gel filtration columns (P/N 42453) obtained from Edge Biosystems, according to manufacturer's protocol. This is done to remove any unicorporated dye terminators left in the reaction mixture. Excess dye terminators in sequencing reactions obscure data in the early part of the sequencing and can interfere with the base calling. Second, the microcentrifuge tubes containing the PCR products were placed into an ISSLLO Speed Vac System and vacuum dried for 30 minutes using the medium setting. Once dry, the resulting pellets in the bottom of the microcentrifuge tubes were reconstituted with 10.0 µl of $H_2O$.

Detection of the sequencing products was accomplished with the ABI PRISM® 3100 Genetic Analyzer 16-capillary system following manufacturer's protocols using the E matrix filter to detect four dyes. Prior to sample analysis, a spectral matrix was established according to manufacturer's protocol using the BigDye™ Terminator v3.0 Matrix Standard (Applied Biosystems, P/N 4390436). Samples were prepared with 10.0 µL Hi-Di™ formamide (Applied Biosystems, P/N 4311320) and with 1.0 µL of DNA

sequencing product. Samples were then injected onto the 16-capillary system using the RapidSeq6_POP6Default Module and the DT3100POP6{BDv3}v1.mob mobility file.

Separations were performed using the 3100 POP-6™ sieving polymer (Applied Biosystems, P/N 4316357), 1X Genetic Analyzer Buffer with EDTA (P/N 402824), and 36 cm array (P/N 4315931). Following data collection samples were analyzed with Sequencing Analysis® 3.7 (Applied Biosystems) and top and bottom strands were aligned and edited using Sequencher® 4.1 for Macintosh (Gene Codes Corporation, Ann Arbor, MI).

## Statistical Analysis of Population Data

Allele frequencies for each locus were calculated by counting the number of individual alleles and dividing by the total number of alleles in the respective population. DYS385 and YCAII are multicopy Y-STRs and represent variations at two loci simultaneously. These regions cannot be differentiated using the any of the primers and methods described herein and thus are analyzed as a phenotype. For example, if a particular male DNA sample has two alleles with 11 and 14 repeats for the DYS385 locus, it is not known which region contains the respective length variation. Meaning, the sample could be designated as an 11-14 or a 14-11. Thus, the sample is analyzed as a phenotype and is given the allele designation of 11-14.

DYS464 primers target a quadruplicated region of the Y-chromosome. Up to four polymorphic peaks can be generated per PCR amplification. Allele designations for DYS464 were based on the presence or absence of peaks of a different base pair size.[39]

Additionally, because of this quadruplication the allele calls for DYS464 in a given male sample are designated as a phenotype for the same reasons as indicated above.

STR diversity, or the probability that two alleles, chosen at random, are different, was calculated using the formula $D = (n/n-1)(1 - Ó p_i^2)$, where n is the sample size and $p_i$ is the allelic frequency.[58] Haplotype diversity was computed in the same way with the same equation using haplotype frequencies instead of allele frequencies. The probability of obtaining an identical haplotype in a pair of random unrelated males can be estimated as 1-D. Discriminatory capacity was determined by dividing the number of different haplotypes seen in a given population by the total number of samples in that population.[39]

# CHAPTER 4

# RESULTS AND DISCUSSION

## Multiplex PCR Primer Design, Optimization and Testing

Due to the complexity involved in the design of multiplex PCR primer sets, it is helpful to view the strategy for primer selection in a systematic fashion. Figure 4-1 illustrates the process of multiplex PCR primer design, optimization and testing used in this study.[22] It starts with the selection of the loci to be examined and ends with the empirical test of the primer mix. Figure 4-1 is divided into two parts. Part A focuses on the multiplex PCR primer mixture design while part B describes the process of multiplex PCR primer mixture testing and optimization. Part A is the most time consuming and when done successfully limits the amount of actual primer testing and optimization. A successfully designed and optimized multiplex should result in the amplification of all desired loci and have similar yields (balance) between respective amplicons. The resulting multiplex PCR reaction should be free of non-specific amplicons, and amplicons that utilize the same fluorescent dye (if used) must be distinguishable from one another. That is they must be resolvable regardless of the size of the alleles present.

The multiplex design and testing process outlined in Figure 4-1 originated from the results obtained during construction of the Y-STR 20plex. Later, the flow-chart shown in Figure 4-1 was followed for the design and testing of the Y-STR 11plex.

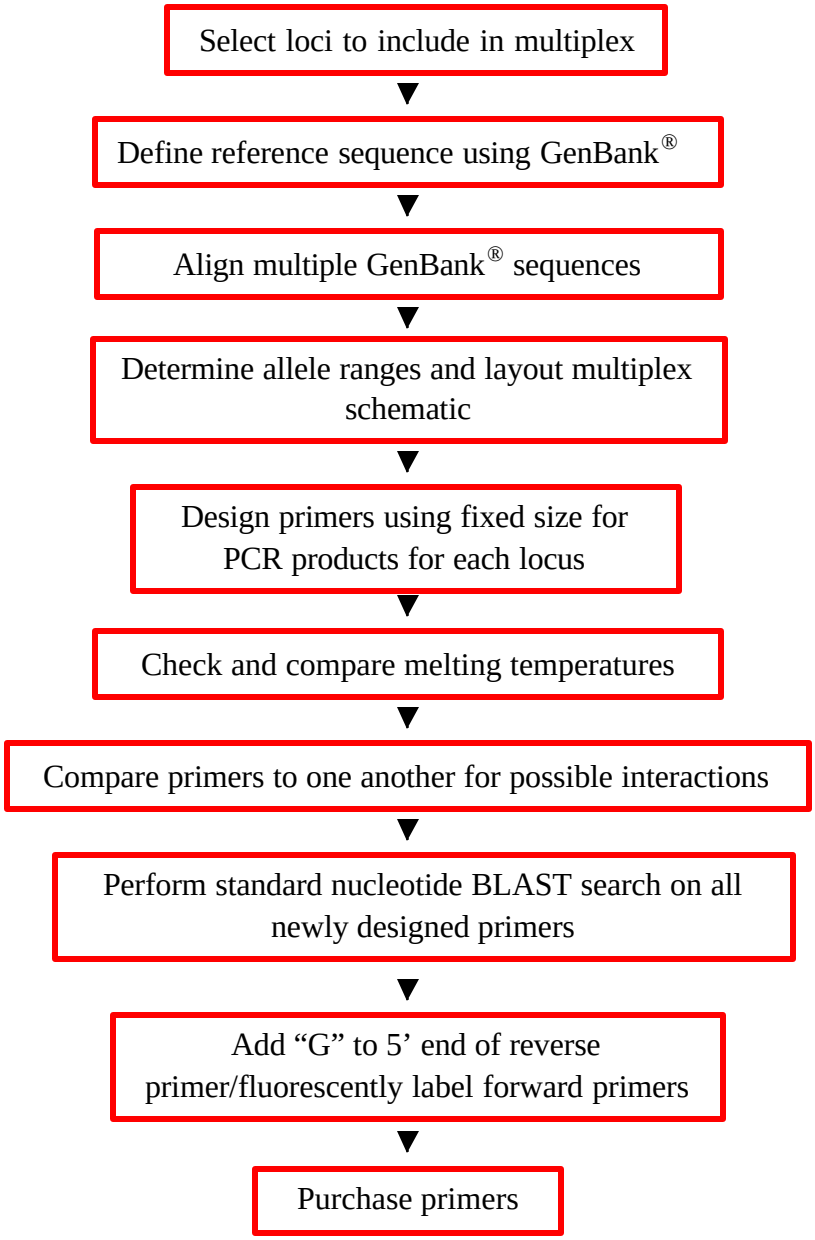



Figure 4-1A
Part A of the multiplex design, optimization and testing process.
Part A is a flow chart outlining steps in the multiplex design part of the entire process. Part A consists of loci selection, primer design, and primer purchase. Adapted from Schoske et al.[22]

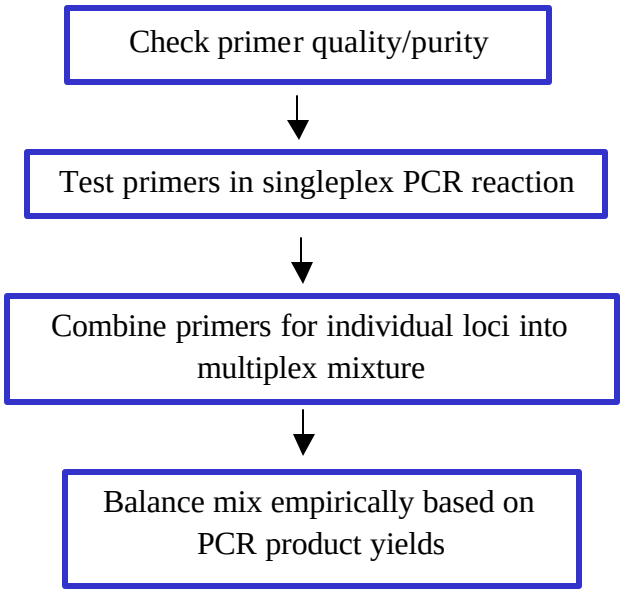



Figure 4-1B
Part B of the multiplex design, optimization and testing process.
Part B is a flow chart outlining steps in the multiplex optimization and testing part of the entire process. Part B starts with an analysis of primer quality and ends with balancing primer concentrations based on PCR product yields. Adapted from Schoske et al.[22]

## Y-STR 20plex Multiplex Design

### Selection of loci to be included in the Y-STR 20plex

Upon undertaking the project of Y-STR multiplex PCR assay development and testing in 2000, there were approximately 23 markers being used in Y-chromosome studies.[5] The first Y marker discovered was DYS19 by Roewer et al.[68] in 1992. Then in 1997 Kayser et al.[30] released information on the discovery of seven new Y-STR markers. In 1998, the highly polymorphic tetranucleotide DYS385 Y-STR marker was introduced.[38] When this project first started DYS385 was the most polymorphic Y-STR currently available (See population studies for diversity value). The number of available Y-STRs grew in 1999-2000 when White et al.[57] and Ayub et al.[55] introduced information

on 13 new Y-STR markers. These markers included DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, Y-GATA-A4, Y-GATA A7.1 (DYS460), Y-GATA-A7.2 (DYS461), Y-GATA-A8, Y-GATA-A10, Y-GATA-C4, and Y-GATA-H4. These markers range in degree of polymorphism and their ability to differentiate between two male specimens.

An initial review of Y-STR multiplexing in the literature revealed that the main focus of Y-STR multiplexing efforts at the time was to include markers that were eventually chosen to be included in the European minimal haplotype.[26-30,31] However, none of these multiplexes included primer sets for the simultaneous amplification of the entire minimal haplotype. In 1997, Kayser et al.[30] published a study which required three separate PCR reactions in order to obtain the minimal haplotype. The first two were multiplex PCR assays were termed Triplex I and Quadruplex I. Triplex I contained primer sets for DYS391, DYS392, and DYS393, while Quadruplex I contained primer sets for DYS19, DYS390, and DYS 389I/II. The PCR product for the final member of the minimal haplotype, DYS385 was performed in a singleplex fashion. Later, Redd et al.[26] and Prinz et al.[27] presented Y-STR multiplex assays that included some of the minimal haplotype markers, however the number of Y-STR markers included in each of these multiplex was four. Then in 1999, Gusmao et al.[28] published information of a Y-STR multiplex assay which included primer sets for markers DYS390, DYS19, DYS389I/II, and DYS393. At the time the Gusmao et al.[28] pentaplex as it was termed was the largest Y-STR multiplex available.

While the European's had settled on the previous described "minimal haplotype" for use by its forensic science community, little was known about the Ayub et al.[55] and White et al.[57] Y-STR markers. A Y-STR 10plex was designed; tested and constructed that incorporated some of the markers from these two references.[69] This Y-STR 10plex included DYS435, DYS436, DYS437, DYS438, DYS439, GATA-A7.1 (DYS460), GATA H4, DYS19, DYS391 and DYS392. The Y-STR haplotypes of a small sample set was reviewed and the data showed that DYS435 and DYS436 were not varying much.[69] Thus, these two markers were not included in future Y-STR multiplexes. All of the other markers in the Y-STR 10plex did exhibit alleles of varying size in the small population set.

It was decided that any Y-STR multiplex that was to be designed and tested should include at a minimum the European "minimal haplotype" and the most polymorphic markers from the Y-STR 10plex. This decision was based on three facts. One, as stated above, none of the current Y-STR multiplexes in the literature were capable of the simultaneous amplification of the minimal haplotype loci. Two, the minimal haplotype markers were being used by the European forensic community. Finally, one way to increase the discriminatory capacity of a Y-STR haplotype is to increase the number of Y-STR markers analyzed. So markers from White et al.[57] and Ayub et al.[55] were to be included into the Y-STR multiplexes.

The first Y-STR multiplex constructed contained primer sets that targeted 14 regions of the Y chromosome. The markers were DYS19, DYS389I, DYS389II, DYS385 a/b, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, and H4.

The next two markers originally slated to be added to the Y-STR multiplex were DYS388[30] and G09411 (DYS462)[58]. DYS388 a trinucleotide repeat was added because when it was tested against the same sample set used by Ruitberg et al.[69] on DYS435 and DYS436, it showed a couple of different alleles while DYS435 and DYS436 did not vary at all. Dr. Peter de Knijff of Leiden University in the Netherlands provided the sequence information for the Y-STR marker G09411, later termed DYS462[58], to NIST. At the time, no information on G09411 existed in the literature. Population data on this marker did not become available until 2002.[58] G09411 was not included in the Y-STR multiplex because the first primers designed for G09411 resulted in the formation of a number of PCR amplicons. One of amplicons was for the G09411 but the others were non-specific amplicons whose sizes did not vary in the male samples tested. G09411 was then pulled from consideration in the multiplex and was not investigated any further.

The next set of Y-STR loci targeted for inclusion into the Y-STR multiplex was DYS426, DYS441 and DYS442. At the time there wasn't any population data in the literature on DYS426. It was added because its previously published primer set[54] would generate PCR amplicon sizes that could be easily incorporated into the Y-STR multiplex (see allele and size range determination for the Y-STR 20plex). DYS441 and DYS442, discovered by Ida et al.[70] in 2001were selected because they were more polymorphic than DYS435 and DYS436. For example, Ida et al.[70] published STR diversity values for DYS441 and DYS442 that were 0.72 and 0.51 respectively, however DYS435 and DYS436 published STR diversity values were only 0.070 and 0.064 respectively.[55] Primers were designed and ordered for DYS426, DYS441 and DYS442. When tested in

singleplex, the primer sets for DYS441 and DYS442 failed to yield PCR amplicons. They were not evaluated any further and removed from consideration for inclusion into the Y-STR multiplex.

Up until this point the Y-STR multiplex included primer sets for the following markers: DYS19, DYS388, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS426, DYS437, DYS438, DYS439, DYS460, and H4. The Y-STR marker, YCAII a/b, a dinucleotide repeat that is duplicated on the Y-chromosome was then considered for inclusion into the multiplex. This brought the number of markers in the multiplex to 18. YCAII a/b was added because it was highly polymorphic and being widely studied by the European forensic science community.[30]

Finally, through a collaboration with Mike Hammer's Group at the University of Arizona established by Dr. John Butler, access was gained to some of their newly discovered Y STR Loci.[39] As a result DYS447 and DYS448 were added to the Y STR Megaplex. The Y-STR 20plex described here consisted of 17 primer pairs, three of which (DYS389I/II, YCAII a/b, and DYS385 a/b) target duplicate regions of the Y chromosome and can provide two polymorphic peaks for each marker. This brought the total number of possible simultaneous amplifications to 20. In summary the Y-STR 20plex includes primer sets for the amplification of the following Y-STR markers: DYS19, DYS388, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS447, DYS448, DYS460, GATA H4 and YCAII a/b.

Allele and Size Range Determination for Loci in the Y-STR 20plex

The allele ranges of the Y-STR 20plex loci listed in Table 4-1 were defined through a combination of extensive literature searches, testing of the YCC panel by Butler et al.[53] and the population data presented in this work (see population studies). The results of the literature searches for each Y-STR 20plex loci are given in the Appendix (A-1). The data provided in A-1 is a summary of the alleles and/or phenotypes (i.e. in case of DYS385) discovered for a number of different populations. The accompanying size ranges in (Table 4-1) for the alleles were determined using the GenBank® accession sequence as the standard reference point for each locus. For example, the GenBank® accession number BAC Clone AC002992 contains within it the reference sequence for DYS438. The DYS438 reference sequence contains a simple pentanucleotide repeat motif consisting of 10 TTTTC repeats. Template DNA containing 10 TTTTC repeats amplified using the primers reported by Ayub and coworkers should yield an amplicon 222 bp in length.[55] The allele range from the literature for DYS438 is 6 to 14 repeats. If these previously published primers were used, amplicons in the size range of 202-242 bp would be expected. In the absence of literature information on the allele range (i.e. DYS426, DYS447, and DYS448) of a particular STR locus, the size range was based entirely on the running of the YCC panel and running the population samples in this work.

A visual schematic for the Y-STR 20plex was prepared using the size ranges in Table 4-1 and is shown in Figure 4-2. In these schematics loci were laid out in terms of possible PCR product size and dye color. In order to get as many Y chromosome

Table 4-1
Allele and estimated size ranges for Y STR 20plex Markers.
The original allele ranges were published by Butler et al.[53] New allele ranges based on literature searches, and/or population studies (see population studies) performed since Butler et al.[53] publication. Size ranges given below are based on new allele ranges. The nomenclature for DYS439 and DYS448 have been changed since the original publication of the Y-STR20plex.[53] The values in parenthesis are the allele calls if the nomenclature provided in this work is used (see section on Prototype Y-SRM 2395).

| STR Locus | Original Allele Range[53] | New Allele Range | Size Range Using Primers in Table 3-1 | Size Range using primers in the literature | Reference to locus |
|---|---|---|---|---|---|
| DYS19 | **10-19** | **10-19** | **233-269 bp** | 176-212 bp | 36 |
| DYS385 | **7-23** | **7-28** | **242-326 bp** | 241-325 bp | 38 |
| DYS388 | **10-18** | **9-18** | **148-175 bp** | 129-146 bp | 30 |
| DYS389I | **9-17** | **9-17** | **143-175 bp** | 142-174 bp | 30 |
| DYS389II | **26-34** | **24-34** | **255-295 bp** | 254-294 bp | 30 |
| DYS390 | **17-28** | **17-28** | **189-233 bp** | 188-232 bp | 30 |
| DYS391 | **7-14** | **6-14** | **89-121 bp** | 268-300 bp | 30 |
| DYS392 | **6-16** | **6-17** | **290-323 bp** | 234-267 bp | 30 |
| DYS393 | **9-16** | **9-17** | **109-137 bp** | 108-136 bp | 30 |
| DYS426 | **10-12** | **9-13** | **89-101 bp** | 89-101 bp | 54 |
| DYS437 | **14-17** | **13-18** | **182-202 bp** | 181-201 bp | 55,56 |
| DYS438 | **6-13** | **6-14** | **300-340 bp** | 202-242 bp | 55,56 |
| DYS439* | **16(9)-21(14)** | **8-15** | **206-234 bp** | 233-261 bp | 55,56 |
| DYS447 | **22-29** | **19-33** | **192-261 bp** | 192-261 bp | 39 |
| DYS448* | **20(21)-26(27)** | **19-27** | **293-341 bp** | 283-331 bp | 39 |
| A7.1 (DYS460) | **7-12** | **7-13** | **101-125 bp** | 162-186 bp | 57 |
| H4 | **8-13** | **8-14** | **122-146 bp** | 353-377 bp | 57 |
| YCAII | **11-24** | **11-25** | **135-165 bp** | 135-167 bp | 37 |

markers into the Y-STR multiplex, the markers were placed close together with each dye color. This strategy required a prior knowledge of previous known alleles in order to minimize the chance for overlap between PCR products amplified with the same fluorescent dye.

The allele and size ranges on the schematic provide for visual representation of the loci and highlight areas of possible size overlap. If an overlap in the possible alleles from

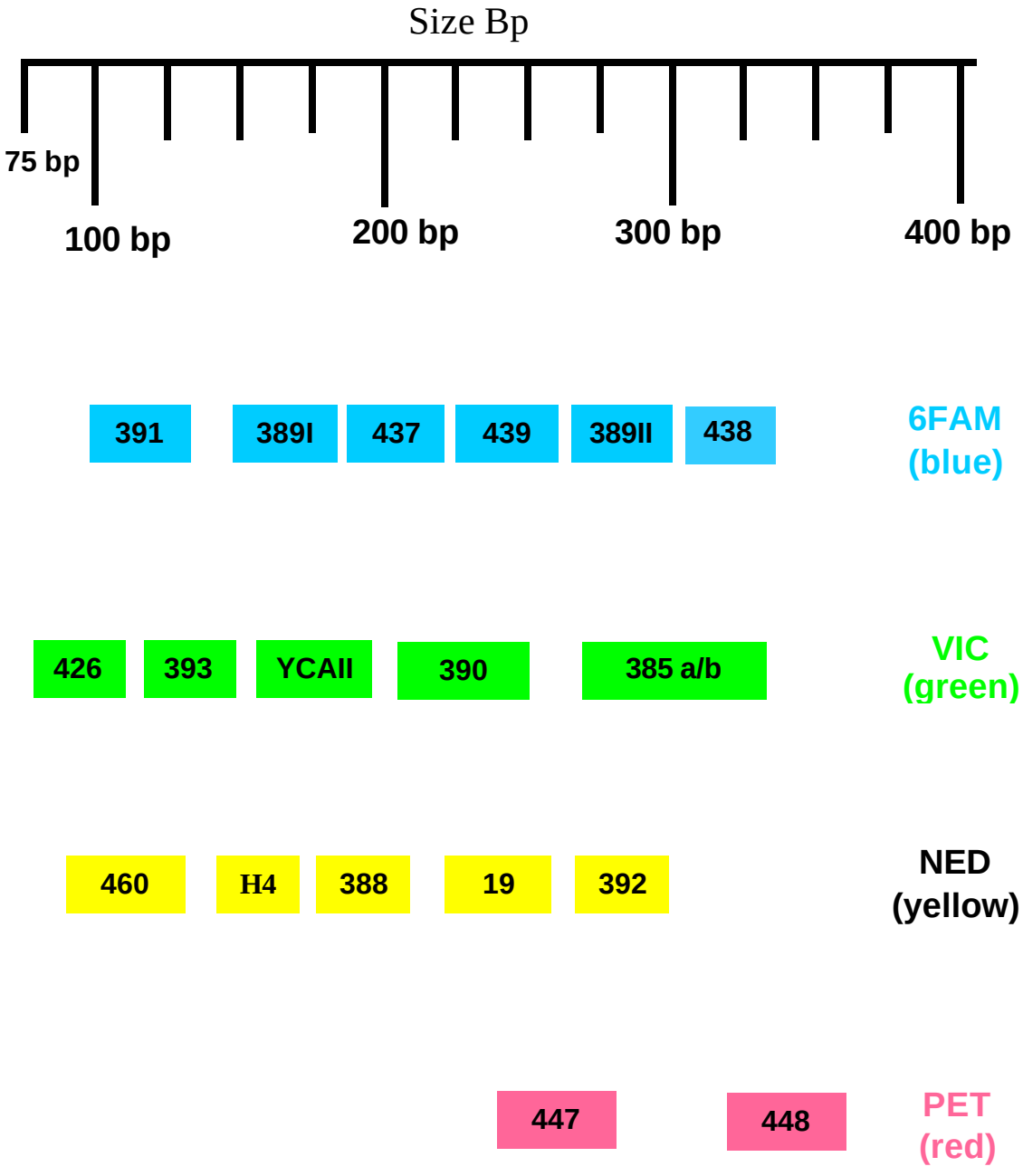



Figure 4-2
Schematic for the Y-STR 20ple x
Schematic of PCR product sizes produced with the known allele size ranges for the loci in the Y-STR 20plex. Markers names have been abbreviated (e.g. DYS391) is listed as 391.

two PCR products adjacent in size exists in the same dye color, then it may become difficult to assign the observed allele to its correct locus. For this reason, it would be beneficial if all of the amplicons within a single fluorescent dye color used in the Y-STR 20plex had size ranges at least 10 bp apart. It was thought that a 10 bp gap could accommodate the discovery of new alleles if they should arise. The 10 bp gap would allow for the discovery of up to two tetranucleotide repeats (8 base pair total) and three trinucleotide repeats (nine base pair total) before the size ranges would overlap.

Between the first iteration of the Y-STR 20plex[111], the one published by Butler et al.[53] and the one presented in this work new alleles were discovered that could impact proper allele designation. The discovery of new alleles has lead to the overlap of some of the allele size ranges within color. For example, in earlier versions of the Y-STR 20plex schematic[111] there was a nine base pair difference between the established size range for DYS460 and GATA H4.[111] The original size range, obtained from White et al.[57] for H4 was determined to be 10-13 TAGA repeats. However, a new GATA H4 allele, one with 8 TAGA repeats was discovered which expanded the expected size range by 8 base pairs.[53] Furthermore, the discovery of an allele 13 for DYS460 by Bosch et al.[58] and Uchihi et al.[97] has resulted in the overlap of these two size ranges. The 101-125 and 122-142 bp size ranges are for DYS460 and H4 respectively.

Besides overlapping, the base pair size between a number of the loci has been reduced. For example, in Butler et al.[53] the number of base pairs separating the DYS437 and DYS439 loci was 12 bp (Table 4-1). The discovery of an allele 18 (202 bp) for DYS437 and an allele 8 (206 bp) for DYS439 has reduced the size difference to 4 base

pairs. Thus, the discovery of an allele 19 or 7 for DYS437 and DYS439 respectively will result in an overlap of the size ranges.

These overlaps can be accommodated in a couple of ways. First, new primer sets could be designed to try and adjust the size ranges to eliminate the overlap. Second, the primer sets for an affected locus (i.e. YCAII and DYS460) could be moved into PET™. During the initial stages of the construction of Y-STR multiplexes, many laboratories did not possesses instrumentation with the capability of analyzing 5 fluorescent dyes. The initial Y-STR multiplex designs did not include primer sets labeled in PET™. The advent of GeneScan® software capable of analyzing 5 dyes allowed DYS447 and DYS448 to be added to the multiplex and provided added flexibility to the design process.

Primer Design Issues for Y-STR 20plex Primers

Previous published primers set were used for DYS385[38], DYS390[30], DYS426[54], DYS393[30], DYS437[55], and DYS447.[39] The design of the Y-STR 20plex was focused around a primer set proven to successfully amplify the DYS385 a/b locus. This was due to the fact that DYS385 a/b was the most polymorphic Y-STR currently available at the time the Y-STR 20plex was being designed.

Primers were redesigned for most of the remaining loci for four reasons. First, PCR product size ranges had to be adjusted from previous studies in order to optimize their size in each dye color. For example, the original designed primer sets for DYS438 and DYS439 could not be labeled with the same dye color if the original primers from the literature were used (Table 4-1). The size ranges for DYS438 and DYS439 are 202-242

and 237-257 bp respectively when previous primers were used to perform PCR. Thus, an allele in the same dye color registering 237 bp could be identified as either a DYS438 or DYS439 allele. PCR product sizes were adjusted by moving the primer positions in the flanking regions surrounding the Y STR repeat.

Second, primers need to have similar annealing characteristics in order to generate a balanced yield from all PCR products during a simultaneous amplification. Most Y-STR loci primer sets from the literature were initially designed to amplify the marker in a singleplex fashion or limited multiplexes and therefore could not be selected to work together. The previously used YCAII a/b primers afford an example of this phenomenon.[37] These primers, 5'-TATCGATGTAATGTTATATTA-3 and 5'-TAT ATTAAATAGAAGTAGTGA-3 have a predicted melting temperature ($T_m$) of 43.9 and 39.2 $^oC$ respectively. The newly designed primers, listed in Table 3-1 produce amplicons of identical sizes to the original primer set, have a $T_m$ of 59.3 and 57.2 $^oC$. The higher $T_m$ improved their amplification efficiency particularly in a multiplex PCR environment.

Third, the latest information about chromosome homology or polymorphic nucleotides in the primer binding regions was applied to avoid regions that would generate non-specific amplicons (i.e. homology to X or other regions of the genome) or impact PCR amplification. This information was gathered through the use of sequence alignments. Sequence alignments proved useful by identifying both multiple potential priming sites and/or polymorphisms that may be present within the proposed primer binding site. If multiple potential primer binding sites exist, the primers ability to amplify the target of interest can be affected resulting in the amplification of a region of

template other than the allele of interest. The presence of polymorphisms within the primer binding regions could result in allele dropout (a null allele).[17] In the case of a null allele, a DNA template exists for a particular locus but fails to amplify due to primer hybridization difficulties in the presence of this polymorphism.[5] A summary of sequence alignments that were performed for loci within the Y-STR 20plex is presented in Table 4-2. The table contains the GenBank accession numbers for each locus, any homologs and results of the sequence alignments. The sequence alignment results for DYS391, DYS19 and DYS460 will be detailed in order to further illustrate the importance of avoiding primer binding sites that have homologs or that may contain polymorphisms.

Three examples of chromosome homology were noted in the construction of the Y-STR 20plex, two involved X chromosome homology and the other involved Y chromosome homology. The X homolog problem for DYS393 was first discovered by Dupuy et al.[112] In order to capitalize on these sequence differences, Dupuy and coworkers[112] described alternate primers that targeted the sequences differences between the X homolog and the DYS393 locus.

The DYS391 provided another good example of X homologs and the difficulty they present to the development of Y-STR multiplex assays.[53] Figure 4-3 illustrates the individual sequences for X and Y homologs of the DYS391 locus along with sequence alignment of these two regions. The newly designed primers targeted sequence differences in order to maximize primer binding potential to only the Y homolog in order to make the amplification Y chromosome specific. Previously published primers targeted only a four sequence difference in their improved reverse primer for DYS391.[113] The

Table 4-2
Summary of Sequence Alignment of Top Strands for loci in the Y-STR 20plex
Sequence alignments are of top strands. The reference sequence listed first is compared to the other GenBank® accessions. Positions of alignment differences are given in relation to the repeat motif. Polymorphisms will be designated Upstream (US) of the 5' end of the repeat or Downstream (DS) of the 3' end of the repeat.

| Locus | Sequences aligned | Seq. differences Relative to Repeat | |
|---|---|---|---|
| 19 | AC017019 (R&C) v. AF140632 | G/- 51 bp DS | |
| | AC017019 v. AC006335 (Y-Homolog) | T/G 2 bp US | T/C 3 bp DS |
| | | A/T 15 bp US | C/T 52 bp DS |
| | | A/- 17 bp US | C/T 59 bp DS |
| | | A/C 34 bp US | G/A 61 bp DS |
| | | A/- 47 bp US | A/G 90 bp DS |
| | | T/A 50 bp US | A/T 117 bp DS |
| | | T/C 54 bp US | T/C 119 bp DS |
| | | A/T 58 bp US | T/C 120 bp DS |
| | | T/A 62 bp US | |
| 385 | AC0022486 (R&C) v. AC007379 | No differences | |
| 388 | AC004810 v. AF140633 | -/T 43 bp DS | |
| 389I/II | AC004617 (R&C) v. AF140634 | G/A pos 4 of 389I repeat motif | |
| 392 | AC011745 (R&C) v. AF140638 | No differences | |
| 393 | AC006152 v. AF272856 | No differences | |
| | AC006152 v. AF133512 (X-Homolog) | G/T 2bp DS of 3' end of Forward Primer | |
| | | CC/TT 3' end of Reverse Primer | |
| 460 | AC009235 (R&C) v. G42675 | -/T 84 bp US | |
| 391 | AC011302 v. AF140637 | No differences | |
| | AC011302 v. AF055717 (X-Homolog) | T/C 9 bp DS | G/A 52 bp DS |
| | | G/A 12 bp DS | C/A 67 bp DS |
| | | A/G 38 bp DS | A/G 90 bp DS |
| | | C/T 42 bp DS | A/- 96 bp DS |
| | | G/A 45 bp DS | A/G 153 bp DS |
| | | X-homolog missing 29 nucleotides compared to DYS391 | |
| H4 | AC011751 (R&C) v. G42676 | No differences | |
| YCAII | AC015978 v. AC007241 (R&C) | No differences | |

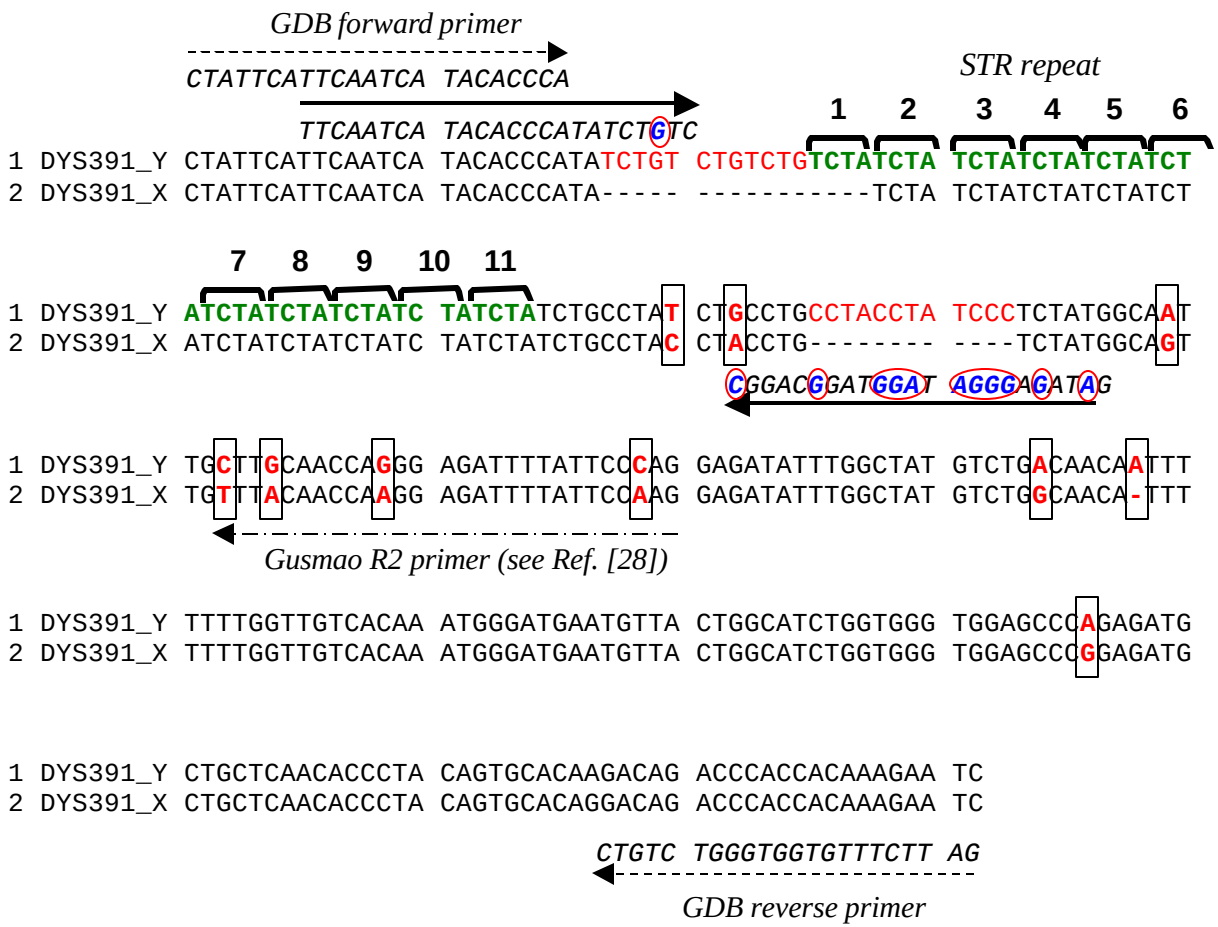



Figure 4-3
Alignment of top strands from DYS391 X and Y homologous sequences
The Y sequence comes from GenBank accession AC011302 while the X sequence is from AF055717. The X sequence is missing 29 nucleotides (indicated by dashes) compared to the Y sequence for DYS391. Sequence differences between the X and Y homologs are boxed. The primers indicated by the dotted arrows are the commonly used ones that are listed in the Genome Database (GDB). Taken from Butler et al.[53]

primers presented in this work target one sequence difference for the forward primer and eleven sequence differences in the reverse primer to improve Y allele specific amplification of DYS391.[53]

The design of primers sets for DYS19 illustrated how the design process was influenced by both the annealing characteristics of the previously published primer and the discovery of a Y-homolog. As it was for YCAII, the predicted melting temperatures of the original primers were not adequate for the set multiplex conditions. The previously used DYS19 primer set, 5'-CTACTGAGTTTC TGTTATAGT-3' and 5'-ATGGCCATGTAG TGAGGACA-3, have a predicted melting temperature of 43.0 and 58.9 $^{o}$C respectively. The new primers for DYS19, 5'-AGGTATGAGATCAAATTGA CTGTG-3' and 5'-TGAGGACAAGGAGTCCATCTG-3' have a predicted melting temperature of 57.3 and 60.3 $^{o}$C respectively. As stated in the multiplex design flow-chart (Figure 4-1) these new primers were reblasted into GenBank in order to identify possible regions of homology. No regions of homology except that of the reference sequence AC017019 were identified at that time. These primers were subsequently ordered in FAM, quality controlled (as in material and methods) and incorporated into the multiplex. These primers were not tested in a singleplex fashion. At the time it was felt that since the primers were only homologous to the marker of interest (according to GenBank) that singleplex testing was not needed.

Upon running the Y-STR 20plex with the newly designed DYS19 primers originally labeled in FAM generated a duplicate peak within the size range for FAM labeled DYS437 amplicons.[111] The resulting amplicon was not polymorphic in over 100

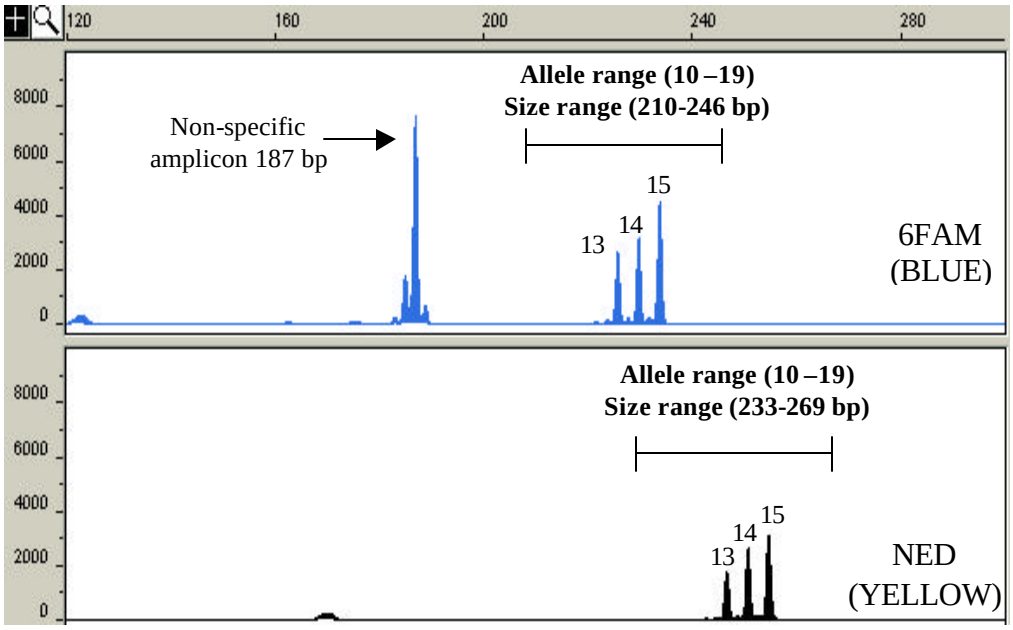



Figure 4-4
GeneScan® Result Obtained for Three Male Samples Using Different DYS19 Primer Sets The top panel is an overlay of three different male samples each with different DYS19 alleles that have been labeled with 6FAM (Blue). A non-specific peak was identified at 187.00 bp for each sample. The bottom panel is an overlay of the same three male samples as in the top panel but a redesigned primer set was used in order to make the primer set more DYS19 specific. The alleles in the bottom panel are labeled with NED (yellow). The nonspecific peak is no longer evident. Each DYS19 amplicon is labeled with the number of repeats present. The expected size ranges for each primer set are also given.

male samples tested and measured 187 bp. The size of this amplicon fell within the size range of alleles for DYS437 and may have had a negative effect on allele calling for DYS437 (see Table 4-1). This non-specific amplicon for DYS19 is shown in Figure 4-4. Figure 4-4 is an overlay of GeneScan result for three male samples that each have different alleles for the DYS19 locus. The top panel shows the results of the primer set used in the initial Y-STR 20plex. The data in this top panel indicated the presence of a non-specific amplicon that sized at approximately 187 bp. The bottom panel shows the

GeneScan result for the new primer sets that were more specific to the DYS19 locus. The non-specific amplicon, which was present in the top panel has been eliminated.

As was the case for DYS391, new primers for DYS19 were designed to exploit sequence differences between the DYS19 locus and a newly discovered Y-homolog. The sequence of the Y-homolog was discovered by performing a BLAST search using the primer set responsible for the amplification of this non-specific amplicon. The new sequence contained within GenBank Accession number AC006335, not found in the initial search, revealed that almost the entire flanking region of the original DYS19 locus has been duplicated on a separate area of the Y chromosome. Both the initial and final BLAST search using identical parameters including the same DNA sequence. The sequences within Accessions AC017019 and AC006335 were both inputted into GenBank approximately at the same time (December 1999). However, AC006335 was overlooked during the initial search.

The DYS19 primers used in the final version of the Y-STR 20plex were carefully selected to avoid this duplication. Figure 4-5, is an illustration of the sequence alignment of AC017019 and AC006335. The DYS19 primers used in the Y STR 20plex contained a 4 base pair difference in the forward and a single base pair difference in the reverse relative to the AC006335 sequence. The original primers labeled in 6FAM were homologous to both AC017019 and AC006335. This new primer set labeled in NED is more specific to the DYS19 locus than the pair originally designed (see Figure 4-4).

The identification of polymorphic binding sites within primer binding regions could be illustrated by a close examination of the DYS460 locus. A BLAST search using a

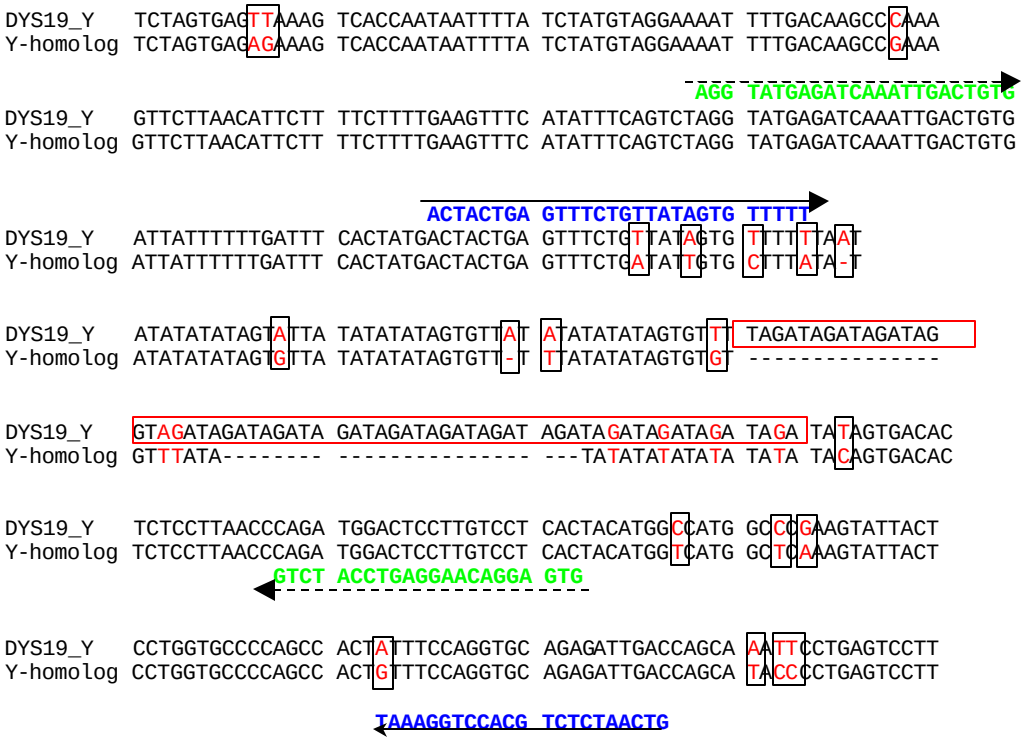



Figure 4-5
Sequence Alignment of Top Strands from DYS19 and Y Homolog
The DYS19 sequence comes from GenBank® Accession number AC017019 while the Y homolog is from AC006335. The Y homolog is missing 43 nucleotides indicated by dashes. The boxed sequence contains the repeat motif for DYS19 (see Table 1-1). The polymorphisms between DYS19 and its Y homolog are highlighted in red and boxed. The primers shown in green (dotted arrows) amplify both regions of the Y chromosome. The primers shown in blue (solid arrows) are the ones used in the Y STR 20plex. These new DYS19 primers contain a 4 base pair difference in the forward and a single base pair difference in the reverse relative to theAC006335 sequence to make them DYS19 specific.

previously published GATA A7.1 (DYS460) forward primer[57] indicated exact homology within a sequence given in GenBank accession BAC Clone AC009235. A sequence alignment was then performed using the Baylor College of Medicine (BCM) search launcher located at http://searchlauncher.bcm.tmc.edu:9331/multi-align/multi-align.html, comparing the Y-GATA-A7.1 sequence contained within BAC Clone AC009235 and the

sequence G42675. Sequence G42675 also contains the Y-GATA-A7.1 (DYS460) locuspreviously published GATA A7.1 (DYS460) forward primer[57] indicated exact homology within a sequence given in GenBank accession BAC Clone AC009235. A sequence alignment was then performed using the Baylor College of Medicine (BCM) search launcher located at http://searchlauncher.bcm.tmc.edu:9331/multi-align/multi-align.html, comparing the Y-GATA-A7.1 sequence contained within BAC Clone AC009235 and the sequence G42675. Sequence G42675 also contains the Y-GATA-A7.1 (DYS460) locus and was first deposited into GenBank by White et al.[57] Figure 4-6 shows the sequence alignment for sequences contained within AC009235 and G42675. From this alignment any sequence differences between the two sequences were easily determined. In the case of A7.1 (DYS460) there is a T deletion (boxed sequence in Figure 4-6) at position 29 for AC009235 that contains 10 AGAT repeats while the sequence within G42675 has 11 AGAT repeats. The T deletion is at a point in the sequence where the forward primer provided by White anneals. However, the T deletion is in the middle of the primer and not at or near the 3' end of the primer, so as to impact the PCR amplification.

There have been cases were mutations at or near the 3' end of a primer have produced little or extension during PCR. In one case an allele 19 for the autosomal STR marker VWA was present but failed to be amplified by a particular primer set. It was later reported that the lack of amplification resulted from a rare A-T nucleotide change in the DNA template at the second base from the 3' end of the AmpF*l*STR® VWA forward primer.[114] The White et al.[57] primers were not tested in this work, in order to see if the

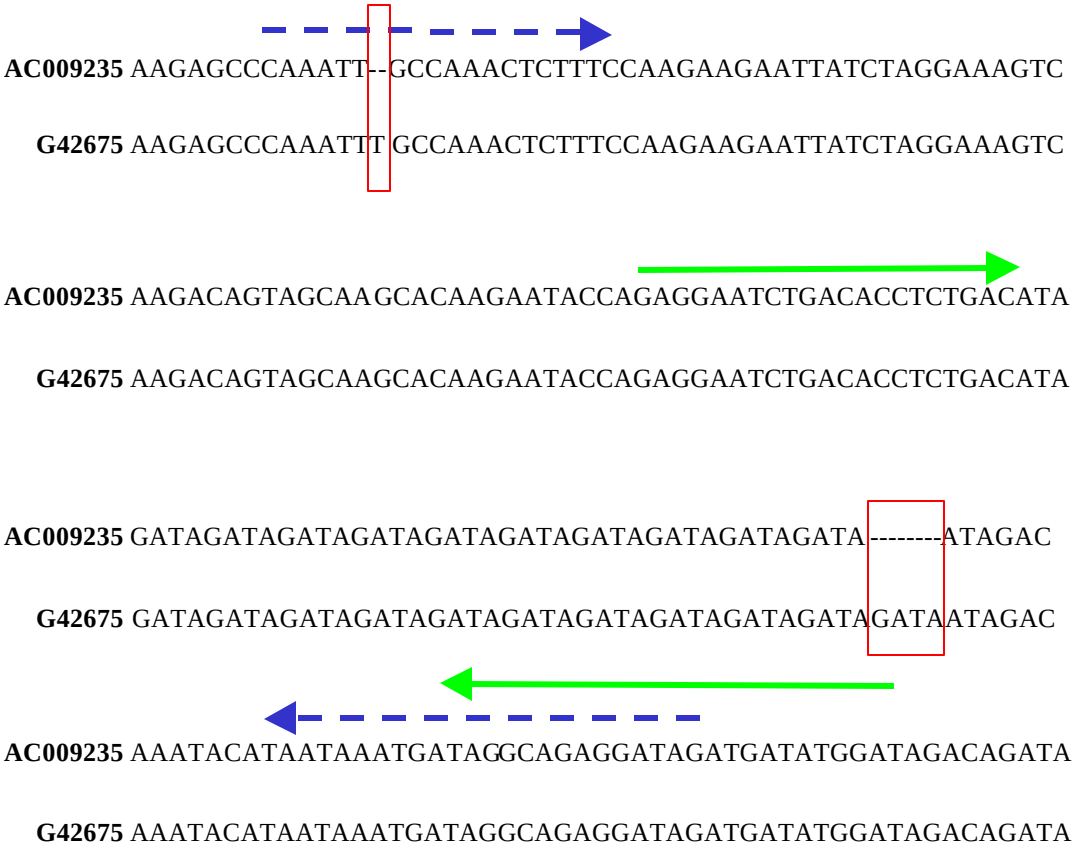



Figure 4-6
Sequence Alignment of the Top Strand from Y STR marker GATA A7.1 (DYS460)
The top strands of the sequences from GenBank® accession numbers AC009235 (10 GATA repeats) and G42675 (11 GATA repeats) were aligned. An extra "T" was observed in G42675 (see the boxed region). The dotted line arrows represent the primers from the original reference describing this marker.[57] The boxed areas represent differences in sequence between the sequences. Illustration taken from Schoske et al.[22]

presence of this T deletion in a sample could result in the lack of amplification. This polymorphism was presented as an illustration of how to avoid these sites altogether, and thus eliminate the need to empirically test primers that may be binding to sites where polymorphisms have been known to be reported.

Fourth, if necessary, primers were redesigned to avoid excessive regions of complementarity between primers. Primer complementarity should be avoided to prevent

the formation of primer-dimers, where the primers bind to one another instead of the template DNA. Primer3 does not currently have the capability for multiplex comparisons. The comparisons were accomplished using an algorithm developed by Dr. Peter M. Vallone that enabled a pair wise comparison of each primer in the multiplex PCR primer mixture.[19] The total number of possible primer comparisons is equal to ($2n^2$ + n) where n is the number of primer pairs to be tested.[19] In the case of the Y-STR 20plex, there were 595 possible primer interactions, including self interactions.

Table 4-3 shows two sets of interactions from the Y-STR 20plex primers with the highest degree of cross-reactivity. The "alignment" score reflects the number of complementary base pairs between the two primers. The G-C and A-T pairs are given equal weight. From previously reported work an alignment score of greater than 8 can lead to significant primer-dimer formation depending upon the PCR amplification conditions.[115] Based on that study, it was decided that if an alignment score of 8 was

Table 4-3
Select Primer Interactions
Illustration showing cross-checking performed on Y-STR 20plex primers. The primers shown below are from the literature that were not redesigned.

| Sequence Information | Potential Interaction[a] |
|---|---|
| 393-R vs. 390-R<br>Matches = 11<br>Alignment score = 7 | 3-CCGGGTTTTTACACATTTTATAT-5<br>\| \|\| \|\|\|\|\|\|\| \|<br>5-AACTCAAGTCCAAAAAATGAGG-3 |
| 385-F vs. 437-F<br>Matches = 10<br>Alignment score = 7 | 3-CCTCTGACTCCATCCTCCTAGT-5<br>\|\| \|\|\| \|\|\| \|\|<br>5-AGCATGGGTGACAGAGCTA-3 |

[a] Level of interaction based on an alignment score. This score is defined as the number of potential complementary base pairs minus the number of mismatched base pairs between two primers.[19]

reported for a pair of primers, the affected primer sets would have to be redesigned. Of the 595 possible interactions, none of the alignment scores were greater than 7 and only 24 had alignment scores of 5 or greater.

## Initial Testing of the Y-STR 20plex

According to the outlined design and testing protocol in Figure 4-1, all of the primer sets in the Y-STR 20plex should have been tested in a singleplex fashion. Ideally, this testing should be performed on three different DNA samples, two from male donors and one from a female. Two males were chosen in case amplification of one of the male templates failed. Variations in amplification between two males can arise from differences between the sequences in the primer binding region or differences in DNA template amounts. The female was chosen as a negative control to ensure that the primer set does not amplify any non-Y-chromosome regions.

Primer pairs should be tested under identical amplification conditions including the same DNA template concentration. If amplification of a particular locus was poor, the primer concentration for that locus was increased and the amplification repeated. Primer pairs that fail to amplify the male samples or indicated amplification in the female sample were eliminated. Ideally, these primers would have been eliminated during the design process through sequence homology checks. However, these searches are only as good as the databases. Databases may not have complete information and/or the information within them may be overlooked. Therefore empirical testing is still necessary.

In an attempt to speed up the construction of the Y-STR 20plex not all of the primer pairs used in the Y-STR 20plex were tested in a singleplex fashion. The Y-homolog to

DYS19 for example was only discovered after the newly designed primer set was already introduced into the Y-STR 20plex mix. If this set had been originally tested in singleplex fashion, the homolog would have been easily identifiable and not included in the Y-STR 20plex.

After the Y-STR 20plex was initially constructed and run, concentration adjustments were made based on relative peak height of the amplicons. If one PCR product was higher in peak height relative to the other amplicons in the multiplex, the appropriate primer pair concentrations were decreased to try to generate a more balanced yield between the various PCR products. Conversely, if one PCR product was lower in relative peak height to the other amplicons in the multiplex, the primer pair in question would be increased to generate an improved balance. The primer concentrations in some cases are higher than those in other multiplex work. Primer concentrations were used as the primary means to increase the amount of PCR product balance. The higher primer concentration permits for more robust amplification without increasing such items as cycle number, $MgCl_2$ concentration, buffer concentration, annealing temperature, and *Taq* concentration.[20]

The concentrations of DYS390 and DYS388 primer sets for example had to be adjusted in order to achieve a more balanced PCR product yield for the Y-STR 20plex. The two graphs given in figures 4-7 and 4-8 are plots of the peak heights in relative fluorescence units vs. the concentration (µM) of the primer set of interest. In figure 4-7, the DYS388 PCR amplicon peak heights are plotted versus their relative concentration in the Y-STR 20plex PCR reaction. It was evident that the amplification of the DYS388

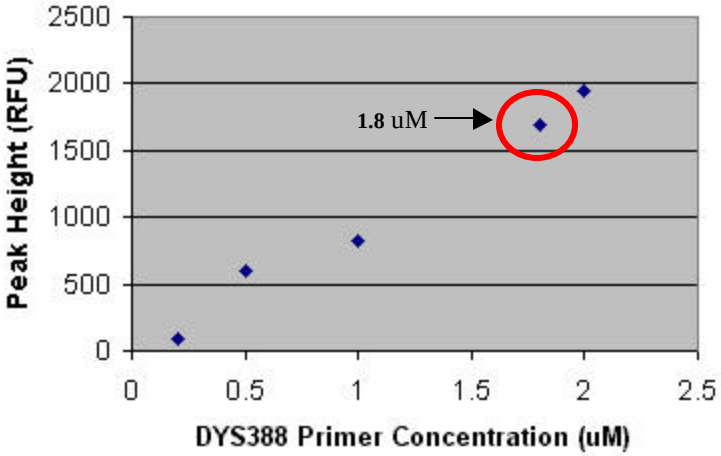



Figure 4-7
Plot of DYS388 primer set concentration (uM) vs. peak height
Concentrations are for DYS388 primer set in 20.0 uL PCR vessel. Peak height is measured in relative fluorescent units (RFUs). All samples were measured using the same amplication conditions and identical male templates (See materials and methods). The sample amount was held constant at 5.0 ng.

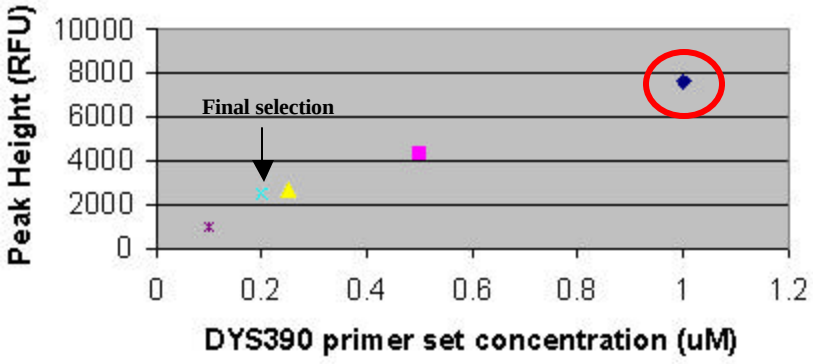



Figure 4-8
Plot of DYS390 primer set concentration (uM) vs. peak height
Concentrations are for DYS390 primer set in 20.0 uL PCR vessel. Peak height is measured in relative fluorescent units (RFUs). All samples were measured using the same amplication conditions and identical male templates (See materials and methods). The sample amount was held constant at 5.0 ng. The data point that is circled was off-scale according to the GeneScan® analysis software.

locus at lower concentration of primers produced significantly smaller PCR product yields than those at larger concentrations. A concentration of 1.8 µM was chosen for the DYS388 primer set because at higher primer concentration the increase in relative peak height was not drastic. In figure 4-8, the data for DYS390 primers showed that at the original concentration of 1.0 µM the peak height for the VIC labeled amplicon was off-scale at almost 8000 RFU and resulted in pull-up. Pull-up is the result of color bleeding from one spectral channel into another, often due to off-scale peaks. The primer concentrations for DYS390 was lowered to eliminate this off-scale phenomenon and thus

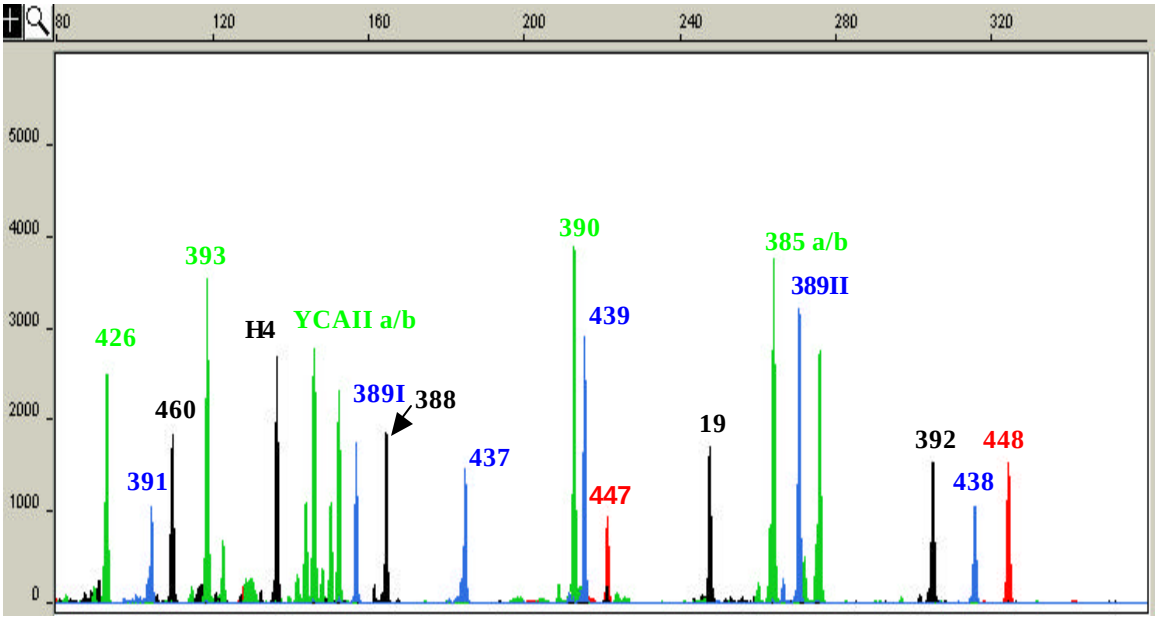



Figure 4-9
GeneScan® Result From a Male DNA Sample Amplified with the Y-STR 20plex
The male sample (1.0 ng) was amplified according to the procedures outlined in the materials and methods. The y-axis is labeled in relative fluorescence units (RFUs) and the x-axis is labeled in size (bp). The markers labeled in 6FAM (Blue) are DYS19, DYS389I/II, DYS391, DYS437, DYS438, and DYS439. The markers labeled in VIC (Green) are DYS426, DYS393, YCAII a/b, DYS390, and DYS385 a/b. The markers labeled in NED (Yellow) are DYS460, GATA H4, DYS388, DYS19 and DYS392. The markers labeled in PET (Red) are DYS447, and DYS448.

achieve a more balanced yield with respect to the other amplicons in the Y-STR multiplex. The concentration of the DYS390 primer set was set at 0.2 µM (Table 3-1) for two reasons. One, the reduction in primer concentration eliminated the pull-up phenomenon due to off-scale peaks, and two there was no appreciable difference seen between the peak heights when 0.25 µM of primer of 0.20 uM was used. An off-scale peak, diminishes the ability to quantitatively measure the PCR product yield based on relative fluorescent units. After several iterations and adjustments of primer concentrations for the Y-STR 20plex a final primer mix was decided upon with the concentrations listed in Table 3-1, which generates a set of fairly balanced PCR products (Figure 4-9).

## Y-STR 11plex Multiplex Design

### Selection of loci to be included in the Y-STR 11plex

The markers chosen for inclusion in the Y-STR 11plex stemmed from information provided to NIST by Dr. Michael Hammer and Dr. Alan Redd from the University of Arizona. They provided information on new polymorphic, and up until that point unpublished Y-STR markers. Information regarding the Y-STR 11plex markers was later published by Redd et al.[39] In turn, a new Y-STR multiplex would be designed at NIST that would incorporate these novel polymorphic markers. Originally, Dr. Hammer and Dr. Redd wanted a Y-STR multiplex to include markers DYS464, DYS446, DYS449, DYS447, DYS448, DYS449, DYS456 and DYS459 a/b. Later, due to some primer design issues (see Primer Design Issues for Y-STR 11plex) markers DYS446, DYS449, and DYS 459 a/b were excluded from the consideration for inclusion into the

multiplex and DYS450 was substituted in the place of DYS446. Lastly, DYS385 a/b was added because it was the second most polymorphic marker next to the newly discovered DYS464 a/b/c/d. Thus, the Y-STR 11plex consists of DYS385 a/b, DYS447, DYS448, DYS450, DYS456, DYS458 and DYS464 a/b/c/d.

Allele and Size Range Determination for Loci in the Y-STR 11plex

The allele ranges of the Y-STR 11plex loci listed in table 4-4 were originally defined through information provided by Redd et al.[39] and the population data presented in this work (see population studies). The accompanying size ranges in (Table 4-4) for the alleles were determined using the GenBank® accession sequence as the standard reference point for each locus as previously outlined for the Y-STR 20plex.

A schematic for the Y-STR 11plex was prepared using the size ranges in Table 4-4 and is shown in figure 4-10. Upon examination of the Y-STR 11plex schematic it is obvious that the size ranges for the amplicons are not packed as tightly as the Y-STR 20plex. The reason for this layout becomes evident after looking at schematic in figure 4-11. The Y-STR 11plex multiplex was initially designed so at some point the rest of the minimal haplotype primer sets (DYS19, DYS389I/II, DYS390, DYS391, DYS392, and DYS393) could be added and a new Y-STR 18plex could be tested (See Table 4-1 for size ranges of minimal haplotype). The size ranges of the alleles for the respective Y-STR 11plex loci accommodated the size ranges of the remaining European “minimal haplotype” loci (circled in Figure 4-11) without having to redesign any of these newly added primer sets. This demonstrates how closely packing size ranges together can reduce the need to redesign primers due to overlapping size ranges within color.

Table 4-4
Allele and estimated size ranges for Y STR 11plex Markers.
Allele ranges based on information provided by Redd et al.[39], and running of the population samples (see population studies). Size ranges given below are based on new allele ranges. The nomenclature for DYS439 and DYS448 have been changed since the original publication of the Y-STR 20plex.[53]

| STR Locus | Allele Range | Size Range | Previous Size Range | Reference to locus |
|---|---|---|---|---|
| DYS385 | **7-28** | **311-395 bp** | 242-326 bp | 38 |
| DYS447 | **19-33** | **192-261 bp** | 192-261 bp | 39 |
| DYS448 | **19-27** | **330-378 bp** | 283-331 bp | 39 |
| DYS450 | **6-11** | **185-210 bp** | 346-371bp | 39 |
| DYS456 | **12-18** | **90-114 bp** | 137-161 bp | 39 |
| DYS458 | **13-20** | **132-160 bp** | 111-139 bp | 39 |
| DYS464 | **11-20** | **250-286 bp** | 250-286 bp | 39 |

Primer Design Issues for Y-STR 11plex Primers

The previously published primers set was used for DYS447 locus.[39] The design of the Y-STR 11plex was focused around the primer sets for the loci circled in figure 4-11. This was due to the fact that the minimal haplotype is widely accepted in the forensic community and thus a new multiplex that contained the minimal haplotype and new markers could become useful. Second, the minimal haplotype primer sets that could be added to the Y-STR 11plex were successfully amplified with each of these markers.[53]

Primers used in the Y-STR 11plex were redesigned for the some of the same reasons as outlined above in the Y-STR 20plex. First, PCR product size ranges had to be adjusted from previous studies in order to optimize their size in each dye color with respect to the size ranges for the markers circled in figure 4-11. For example, the original designed primer sets for DYS456 and DYS458 could not be labeled with the same dye color if the original primers from the literature were used (Table 4-4). The size

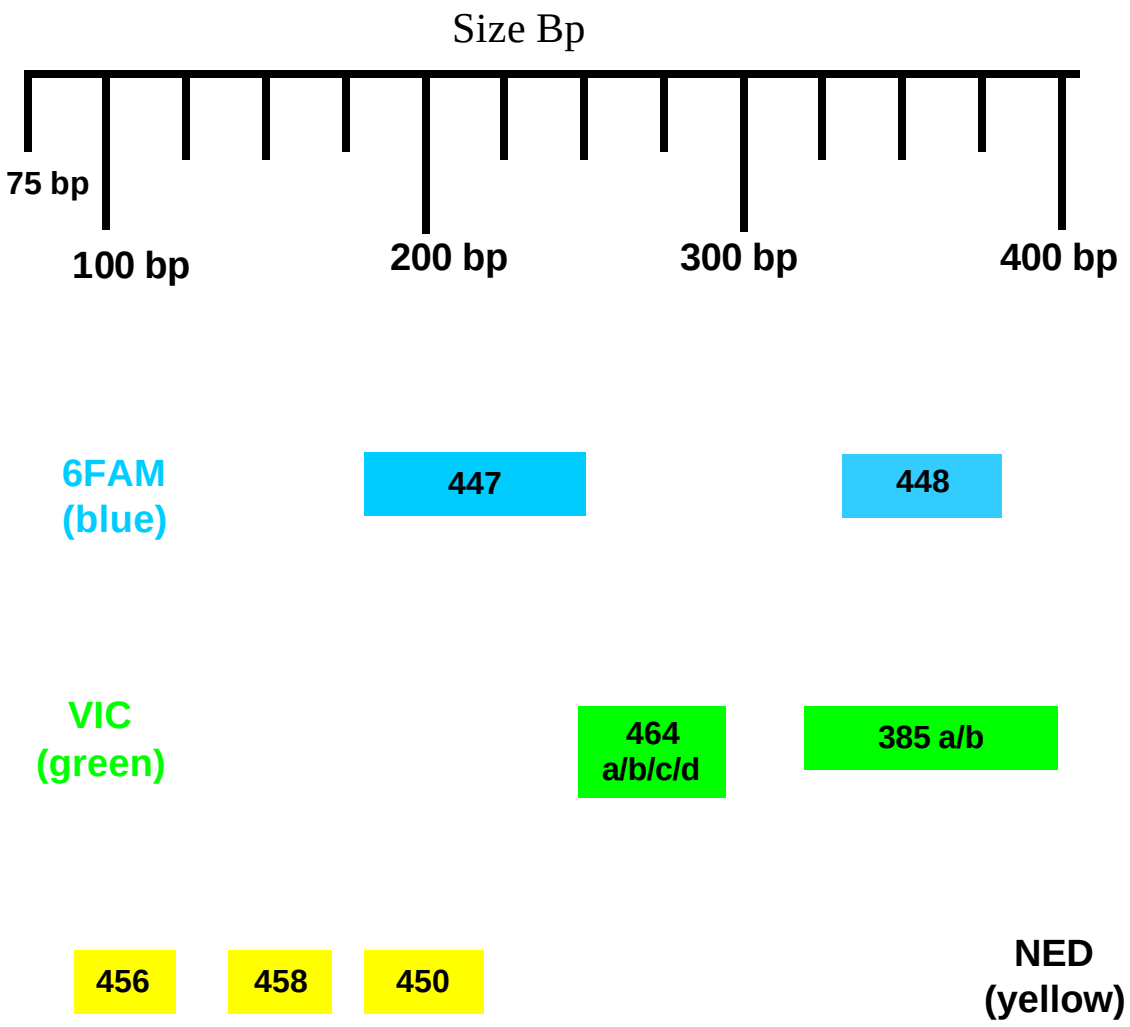



Figure 4-10
Schematic for the Y-STR 11plex.
Schematic of PCR product sizes produced with the known allele size ranges for the loci in the Y-STR 11plex. Markers names have been abbreviated (e.g. DYS456 is listed as 456). The size ranges illustrated here come from values in Table 4-4 for the Y-STR 11plex markers.

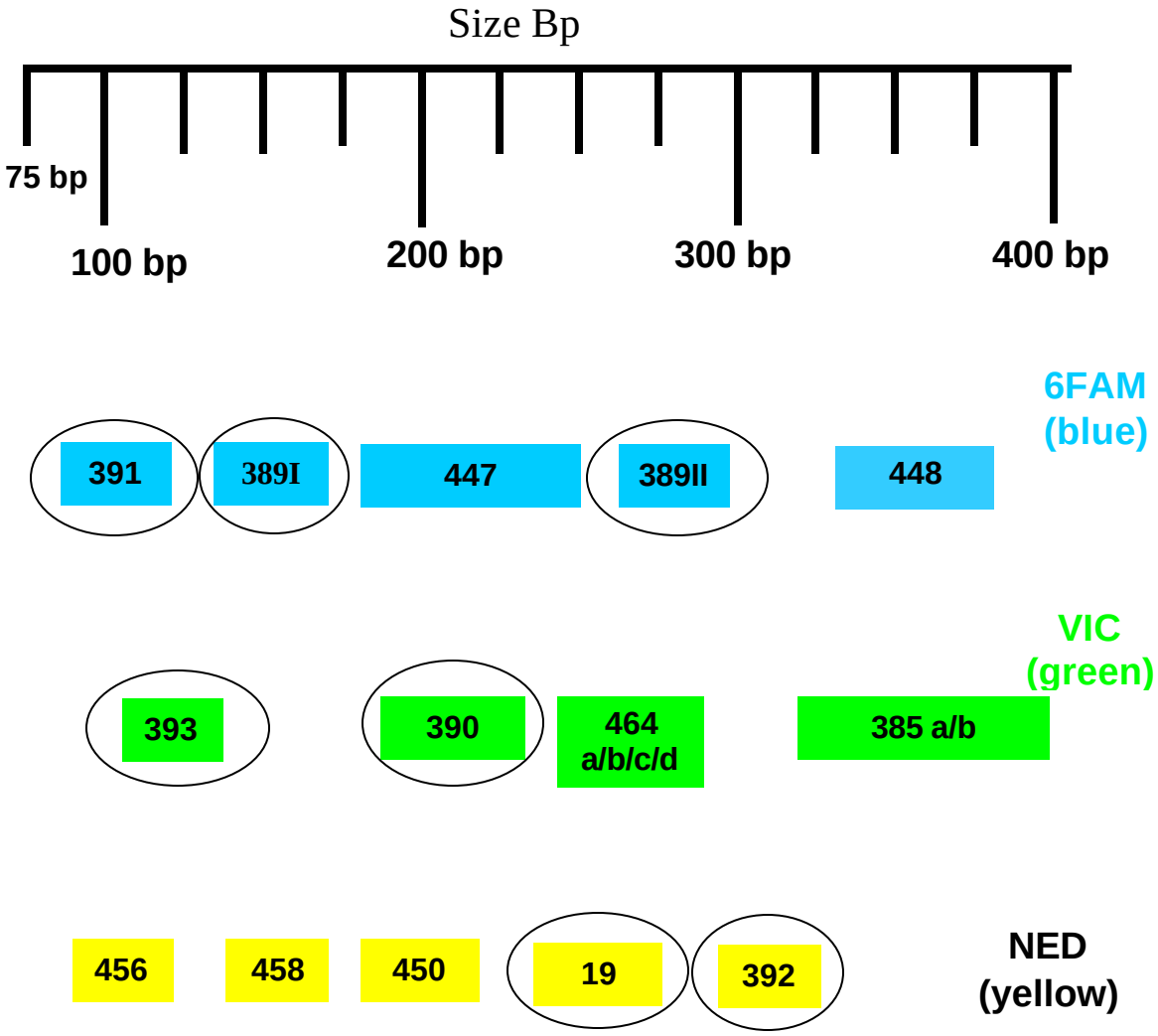



Figure 4-11
Schematic for the Y-STR 18plex.
Schematic of PCR product sizes produced with the known allele size ranges for the loci in the Y-STR 11plex. Markers names have been abbreviated (e.g. DYS456 is listed as 456). Circled markers indicate loci (accompanying size ranges) that could be added to Y-STR 11plex without having to redesign any of the primer sets. The size ranges illustrated here come from values in Table 4-1 for markers DYS19, DYS389II, DYS390, DYS391, DYS392, and DYS393) and Table 4-4 for the Y-STR 11plex markers.

ranges for DYS456 and DYS458 are 137-161 and 111-139 bp respectively when previous primers were used to perform PCR. Thus, an allele in the same dye color sizing at 137 bp could be possibly be identified as either a DYS456 or DYS458 allele.

Second, as was the case for the Y-STR 20plex primers the latest information about chromosome homology or polymorphic nucleotides in the primer binding regions was applied to avoid regions homologous to the Y-STR markers of interest or impact PCR amplification. A summary of sequence alignments that were performed for loci within the Y-STR 11plex and for markers originally slated for inclusion into the Y-STR11 plex is presented in Table 4-5. The table contains the GenBank accession numbers for each locus, any homologs and the results of the sequence alignments.

Three examples of chromosome homology were noted in the construction of the Y-STR 11plex, two involved X chromosome homology and the other involved homology with chromosome 19. Figure 4-12 illustrates the individual sequences for X and Y homologs of the DYS456 locus along with sequence alignment of these two regions. The newly designed reverse primer targeted sequence differences in order to maximize primer binding potential to only the Y homolog and make the amplification Y chromosome specific. The previously published reverse primer targeted only a single base deletion at the 3' of the primer.[39] The new reverse primer designed in this study targets 17 sequence differences. It is important to note that Redd et al.[39] did not indicate the presence of any non-specific amplicons using his DYS456 primer set. The reverse primer was redesigned as indicated in Figure 4-12 because it was assumed that an X homolog

Table 4-5
Summary of Sequence Alignments of Loci within the Y-STR 11plex
Sequence alignments are of top strands. The reference sequence listed first is compared to the other GenBank® accessions. Positions of alignment differences are given in relation to the repeat motif. Polymorphisms are designated Upstream (US) of 5' end of repeat or Downstream (DS) of 3' end of repeat.

| Locus | Sequences aligned | Seq. differences Relative to Repeat | |
|---|---|---|---|
| **385** | **AC0022486 (R&C) v. AC007379** | **No differences** | |
| **464** | **AC006338 v. AC006983**<br>**AC006338 v. AC010088**<br>**AC006338 v. AC025735** | **No differences** | |
| **459** | **AC073893 v. AC010682** | **No differences** | |
| | **AC073893 v. AC000100 (Chromosome 19 homolog)** | **Exactly homologous with exception of 4 base pair deletion in the Chromosome 19 homolog** | |
| **456** | **AC010106 v. AL162723 (X-homolog)** | **A/G 1 bp DS** | **T/C 44 bp DS** |
| | | **T/A 2 bp DS** | **-/C 53 bp DS** |
| | | **C/G 4 bp DS** | **T/C 51 bp US** |
| | | **C/T 5 bp DS** | |
| **446** | **AC006152 v. AL133512 (X-homolog)** | **-/T 178 bp DS** | **C/T 196 bp DS** |
| | | | **C/T 205 bp DS** |
| | | **T/G 188 bp DS** | **T/C 7 bp US** |

would likely result if the Redd et al.[39] primer set was used (See Initial Testing of Y-STR 11plex).

DYS446 was originally chosen for inclusion into the Y-STR 11plex due to the fact it was one of the most polymorphic STRs (STR Diversity 0.836) found in the study presented by Redd et al.[39] Unfortunately, during the primer design process a second example of X chromosome homology was found. Figure 4-13 illustrates the individual sequences for X and Y homologs of the DYS446 locus along with sequence alignment of these two regions. The sequence alignment indicated that both regions had almost the exact same sequence (Figure 4-13). The X sequence was missing 42 nucleotides with respect to the DYS446 locus (indicated by dashes) however 41 of them are within the region of the repeat motif. Additionally, there were only four polymorphic sites found in

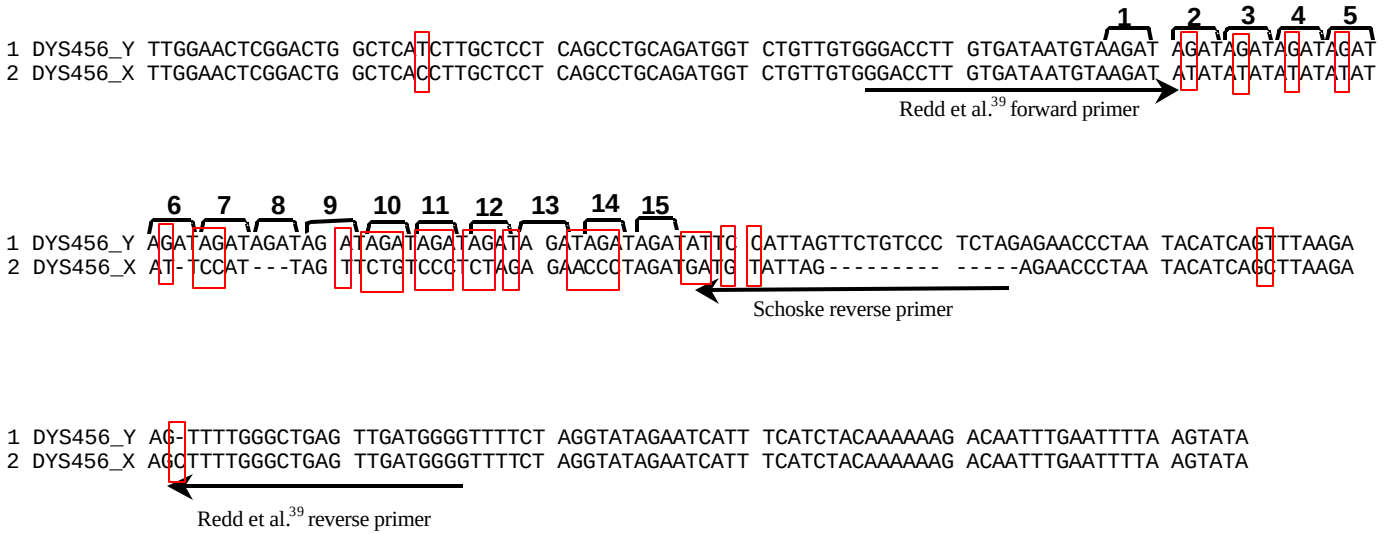



Figure 4-12
Alignment of Top Strands from DYS456 X and Y Homologous Sequences
The Y sequence comes from GenBank accession AC010106 while the X sequence is from AL162723. The X sequence is missing 18 nucleotides (indicated by dashes) compared to the Y sequence for DYS456. Sequence differences between the X and Y homlogos are boxed. The Redd et al.[39] forward primer was used in the Y-STR 11plex. Only the reverse primer was redesigned.


Figure 4-13
Alignment of Top Strands from DYS446 X and Y Homologous Sequences
The Y sequence comes from GenBank accession AC006152 while the X sequence is from AL33512.The X sequence is missing 42 nucleotides (indicated by dashes) compared to the Y sequence for DYS456. Sequence differences between the X and Y homlogs are boxed.

the sequence alignment (Table 4-5 and Figure 4-13). Redd et al.[39] targeted primers to exploit these sequence differences. The primer set targets one sequence difference in the forward primer and three sequence differences in the reverse primer.

By making the reverse primer longer to exploit these sequence differences, the annealing characteristics (i.e. predicted melting temperatures and primer-primer interactions) became suspect. First, the forward and reverse primers were quite different. The predicted melting temperatures ($T_m$) for the forward was 52.0$^o$ C and 67.0$^o$ C for the reverse. Secondly, the software used to calculate the melting temperature (Primer3) stated that the reverse primer had high self complementarity (i.e. high alignment score) and was unacceptable. For these reasons, Redd et al.[39] primers were never ordered or tested.

Attempts were made to design primers in order to exploit these sequence differences, however as was the case with the Redd et al.[39] primers, the annealing characteristics of these new primer sets were not acceptable. Thus, DYS446 was removed from consideration and DYS450 was substituted in its place. DYS450 was chosen for two reasons. One, no homologous sequences to the DYS450 sequence were found during the BLAST search (see materials and methods) using Redd et al.[39] Two primer sets could be designed that would generate amplicons in the size range laid out in the schematic (Table 4-4 and Figure 4-10).

The last example of chromosome homology was seen for the DYS459 a/b locus. A search of homologous sequences was performed using Redd et al.[39] forward primer according to the procedure in the materials and methods. The resulting BLAST search

indicated that the forward primer was exactly homologous to sequence within three GenBank accession numbers (Table 4-5). The first two accession numbers contain sequences on the Y chromosome. This finding is consistent with the information provided by Redd et al.[39] which stated DYS459 has two physical locations on the Y-chromosome, hence the DYS459 a/b designation. Thus, as many as two polymorphic amplicons can be generated per PCR amplification. The last accession number contained a sequence within chromosome 19 (Table 4-5).

The subsequent sequence alignment (not shown) indicated that with the exception of a 4 bp deletion, the DYS459 a/b locus was completely homlogous to a sequence on chromosome 19. Thus, DYS459 a/b was not considered for the Y-STR multiplex because it was speculated that any primer set would generate amplicons not only for the DYS459 a/b locus but for the chromosome 19 homolog as well.

Finally, if necessary, primers were redesigned to avoid excessive regions of complementarity between primers. There were 7 primer sets included in the Y-STR 11plex that target 11 different physical locations of the Y chromosome. The comparisons were accomplished using the same algorithm developed by Dr. Peter M. Vallone that is described above for the Y-STR 20plex.[19] In the case of the Y-STR 11plex, there were 100 possible primer interactions, including self interactions. Of the 100 possible interactions, none of the alignment scores were greater than 7 and only 5 had alignment scores of 5 or greater.

## Initial Testing of the Y-STR 11plex

According to the outlined design and testing protocol in Figure 4-1, all of the primer sets in the Y-STR 11plex were tested in a singleplex fashion. This testing was performed on three different DNA samples, two from male donors and one from a female. The singleplex data for DYS456 and DYS449 are presented to illustrate the point that no matter how good the primer design is, empirical testing is still needed to ensure the primers amplify the loci specified and that subsequent PCR reactions are free of non-specific amplicons. The PCR products for DYS456 and DYS449 serve as an example of the importance of empirically testing these primer sets in singleplex before combining them into a multiplex.

The GeneScan® results for three samples, two male and one female, amplified with the DYS456 primer set in singleplex fashion is shown in Figure 4-14. The results showed that the primer set was not Y-specific as was originally hoped. It was originally assumed that the non-specific amplicon at 166 bp in the female sample was in fact the X-homolog of DYS456 whose sequence is shown in Figure 4-12. However, this sequence would only be approximately 100 bps in length, if the DYS456 primer set used in this study amplified it. The non-specific amplicon sizing at 166 bp in Figure 4-14 is most likely an X-homolog but is not due to the X homolog sequence of DYS456 shown in Figure 4-12.

Even though there was evidence of an X-homolog in PCR amplification using DYS456 primer sets, it was kept in the Y-STR 11plex for two reasons. First, it was successfully amplified in the male samples. Second, even if the 166 bp peak became evident in other male samples and/or mixture of male and female samples it should not

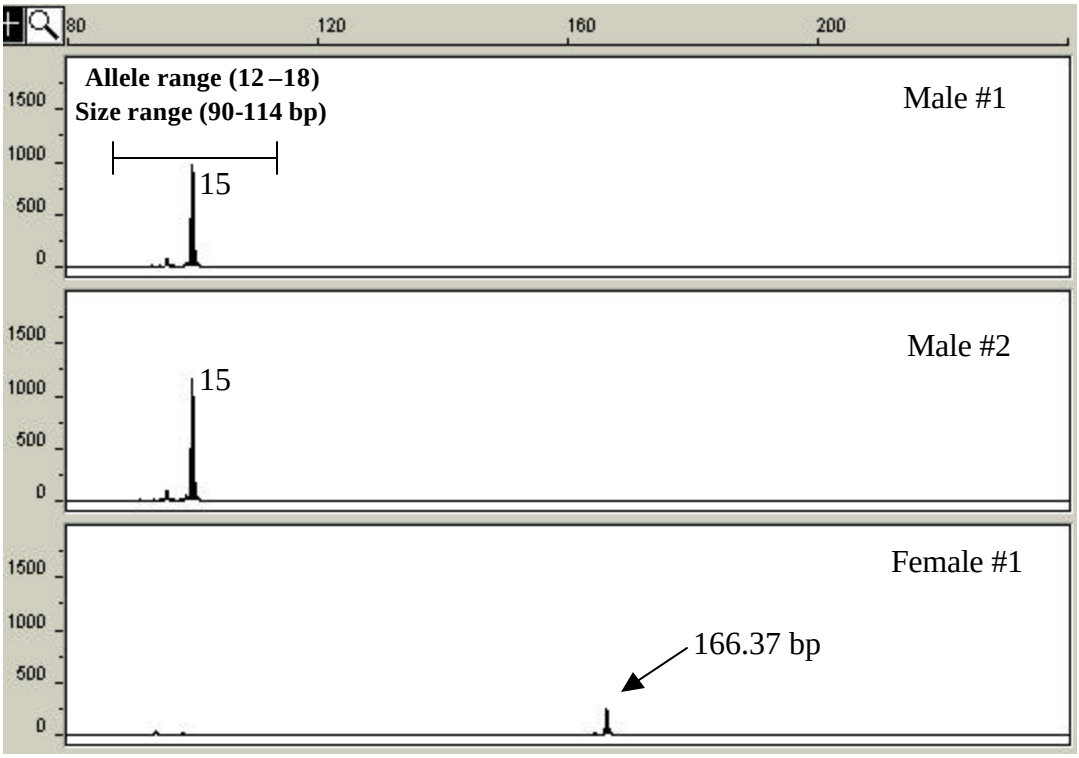



Figure 4-14
GeneScan® Result of Singlplex PCR of DYS456
PCR was performed in singleplex according to procedures outlined in materials and methods using 1.0 ng of template. Panels A and B are for two different male samples and Panel C is for a female sample. The primer set used is labeled in NED (Yellow).

impact proper allele calling. The 166 bp size of the non-specific amplicon falls between the estimated size range of the DYS456 and DYS458 loci (Table 4-4). Regardless, the DYS456 primer set should be redesigned at some point in order to avoid this homology with the X chromosome.

During the primer design process, regions homologous to the DYS449 locus were not found. However, the results of the singleplex PCR amplification of male and female

samples during the testing phase indicated the presence of a non-specific amplicon (Figure 4-15). This non-specific amplicon (209 bp) was only evident in the male samples as the female sample tested showed no amplified PCR products. Furthermore, it was invariant in the male specimens tested. These results taken together most likely indicate that this amplicon is due to presence of a homolog on the Y chromosome itself. Since only one female was tested during the initial testing phase, it can't be taken for granted that the peak at 209 bp is not an X-homolog. This particular female sample may have a polymorphism in its binding site that prohibited it from being amplified using the

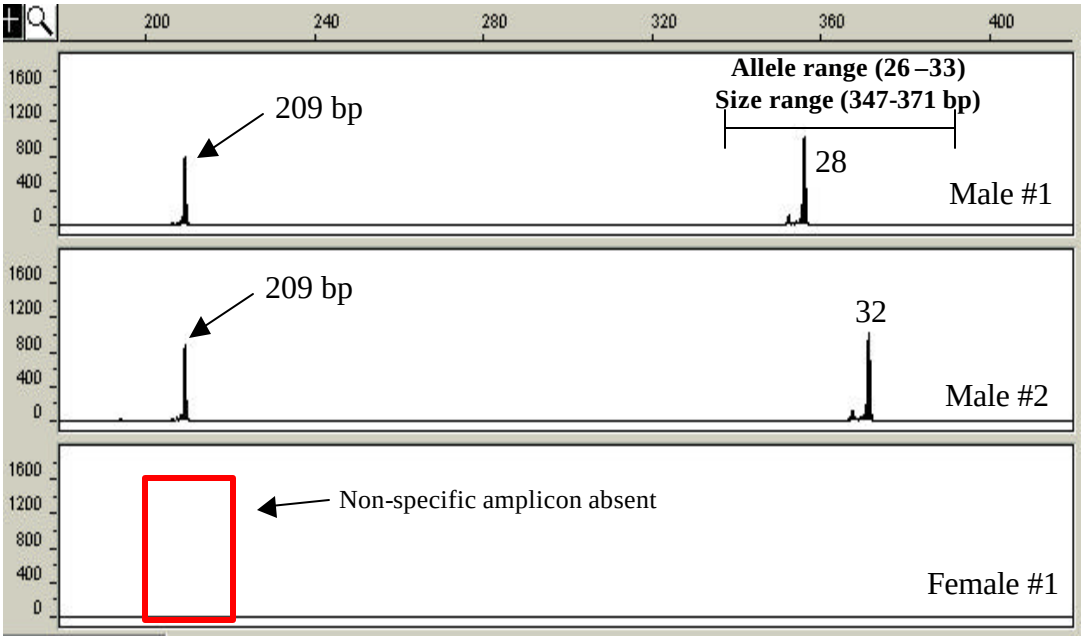



Figure 4-15
GeneScan® Result of Singleplex PCR of DYS449
PCR was performed in singleplex according to procedures outlined in materials and methods using 1.0 ng of template. Panels A and B are for two different male samples and Panel C is for a female sample. Neither the non-specific amplicon or an amplicon in the size range of the DYS449 locus is seen in the female sample. The primer set used is labeled in NED (Yellow).

DYS449 primer set. Thus, numerous female samples should be tested in order to prove that this amplicon is not the result of an X-homolog. The DYS449 primer set was removed from the Y-STR 11plex because its non-specific amplicon (209 bp) falls within the expect size range of amplicons for DYS450 (Table 4-5).

After the Y-STR 11plex was constructed and run, concentration adjustments that were made for the Y-STR 20plex were not necessary based on the analysis of GeneScan® results. The Y-STR 11plex generated a set of fairly balanced PCR products (Figure 4-16). All of the primer concentrations within the Y-STR 11plex were kept at 0.4 uM. At this concentration none of the peaks were off-scale as was the case for DYS390 in the Y-STR 20plex and none of the amplicons had relative peak heights of less than 1000 RFUs.

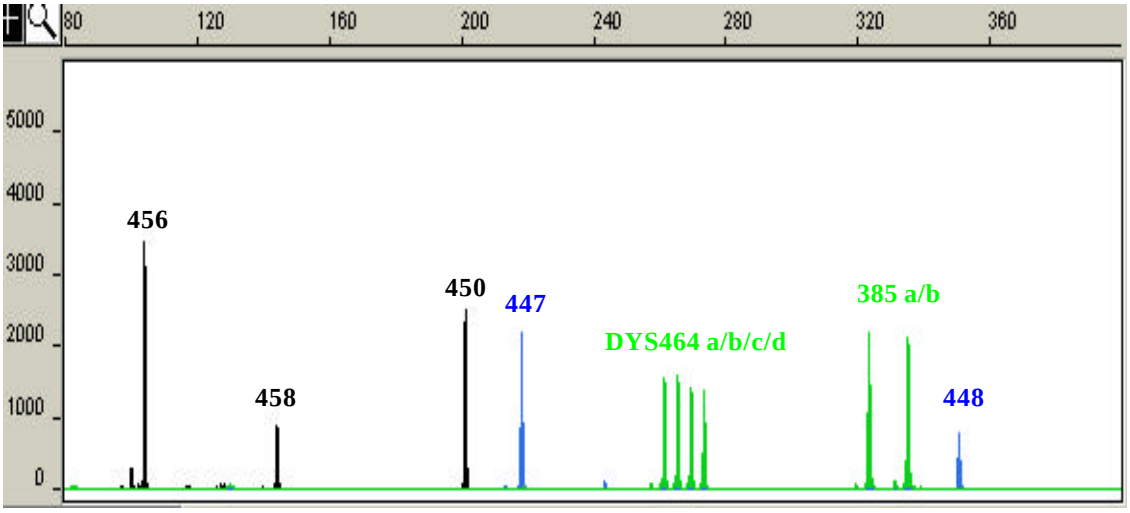



Figure 4-16
GeneScan® Result from a Male DNA Sample Amplified with the Y-STR 11plex
The male sample (1.0 ng) was amplified according to the procedures outlined in the materials and methods. The y-axis is labeled in relative fluorescence units (RFUs) and the x-axis is labeled in size (bp). The markers labeled in 6FAM (Blue) are DYS447, and DYS448. The markers labeled in VIC (Green) are DYS464 a/b/c/d, and DYS385 a/b. The markers labeled in NED (Yellow) are DYS450, DYS456 and DYS458.

## Y-STR Multiplex Assay Performance

Automated fluorescence analysis of PCR amplified short tandem repeat amplicons using capillary electrophoresis instrumentation such as the ABI 310 and ABI 3100 has become a widely used tool in forensic casework and databanking applications. One of the reasons for its wide acceptance is that it has been successfully validated by a number of laboratories. Validation refers to a the process of demonstrating that a certain laboratory procedure is robust, reliable and reproducible in the hands of the personnel performing the test in that laboratory.[5]

Validation is a crucial part of forensic DNA typing. Defense attorneys often challenge the process by which a laboratory performs DNA analysis. Therefore, forensic scientists must thoroughly document the validity of the assays performed in their laboratory. To aid the forensic community in these validation efforts, the Scientific Working Group on DNA Analysis Methods (SWGDAM) group published guidelines in 1995 on what studies should be performed if an STR assay is to become "SWGDAM" validated. There are examples offered in the literature describing the validation of both Y-STR and autosomal STR typing systems utilizing capillary electrophoresis as the method of analysis.[13,14,116]

A series of validation experiments were performed on the Y-STR multiplexes in this study. These experiments included an evaluation of the assay's concordance with commercially available Y-STR kits, precision, male-specificity, and sensitivity. The results of these experiments indicated that the ABI 3100 Genetic Analyzer and POP-6 sieving material together with the newly designed Y-STR multiplexes and Genotyper® macros provided a platform for the reliable analysis of Y-STR markers.

## GeneScan®, and Genotyper®: Software used for measurement of STR systems

There were two software programs used in the STR analysis of all samples in this study. They were GeneScan® version 3.7 and Genotyper® version 3.7 from Applied Biosystems. First, GeneScan® analyzed all of the data by comparing the fluorescently labeled PCR amplicons to an internal sizing standard within each sample well. The internal sizing standard used in this study was the GeneScan®-500 LIZ (orange labeled) standard. In the case of the ABI 3100 and the Y-STR 20plex, the LIZ standard was used because the Y-STR 20plex contained PET (red) labeled PCR amplicons. Hence, the traditional GS-500-ROX (red) standard could not be used. Both of these size standards contain 16 DNA fragments, ranging in size from 35 bp to 500 bp, labeled with either LIZ or ROX dyes. In order to maintain consistency between the Y-STR 20plex and Y-STR 11plex, the GS500-LIZ standard was used for both assays. Alternatively GS500-ROX could have been used for the Y-STR 11plex because none of its primer sets are labeled with the PET (red) fluorescent label.

DNA fragment peaks are sized based on the sizing curve produced from the points on the internal size standard (Figure 4-17). Each point on the graph corresponds to a peak in the GS-500 LIZ (orange) sizing standard. The internal size standard contains 16 peaks ranging from 35 to 500 bp. Studies involving sizing algorithms have described the Local Southern Method as a precise tool for sizing DNA.[13,14,117] As a result, the manufacturer of the GeneScan® software, Applied Biosystems, recommends that Local Southern Sizing be the method of choice for sizing short tandem repeat amplicons.

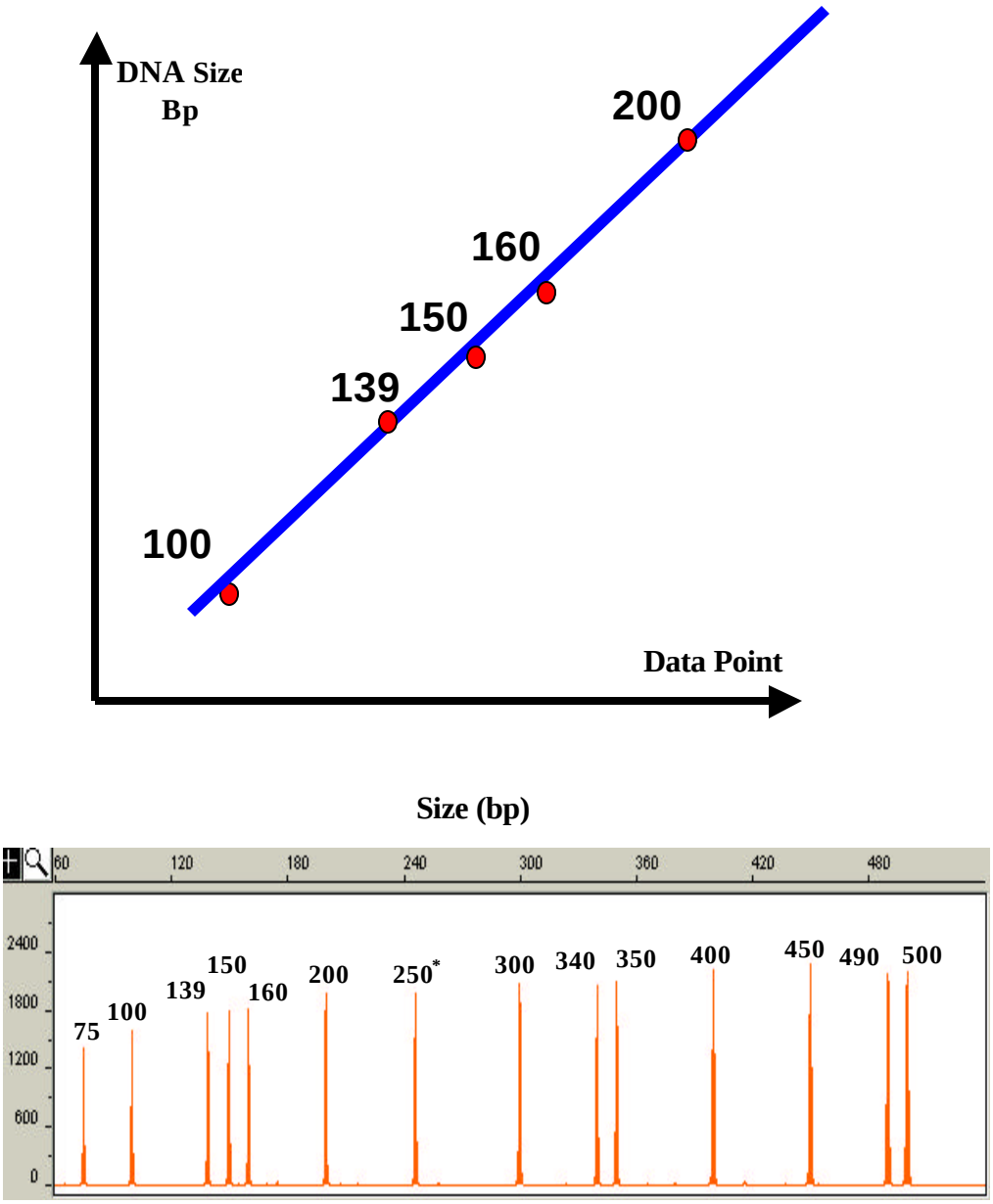



Figure 4-17
Sizing Calibration Curve
Sizing calibration curve is determined by the running of an internal sizing standard. The electropherogram above shows peaks ranging from 75 to 500 bp. The points on the curve are determined from the peaks shown in the electropherogram. GS-500 LIZ (orange labeled peaks) was the size standard used for all the analysis presented in this work. The 250* bp peak is not used in size calculations because of mobility issues. Sequence effects cause this peak to migrate anomalously. Graph above adapted from Butler.[5]

Local Southern sizing uses two peaks above and two peaks below the unknown amplicon to calculate its DNA size (Figure 4-18). In figure 4-18 both size standard peaks below (75 and 100 bp) and above (139 and 150 bp) were used to size the unknown amplicons. Even though the Local Southern method is Applied Biosystems preferred sizing algorithim, they have issued a warning that size estimates may be imprecise if any of the standard fragments run anomalously. Studies have shown that the GeneScan-500 internal standard fragments of 250 and 340 base pairs in length can run anomalously.[14,117] The migration of these fragments can be effected by differences in temperature, buffer, and urea concentration in the sieving material.[13] It is also important to note that in order to accurately assess the precision of this analytical system the same internal size standards and identical sizing algorithm must be used for all the sizing data if information from these runs are to be compared. Using different sizing algorithms and/or different internal standards can result in different sizes for the same peak.[5]

After the internal size standard was defined and applied to the sample data, the electropherogram can be displayed with the base pair size on the x-axis. These GeneScan® data files can then be imported into Genotyper® in order to determine the Y-STR haplotype for each sample. Genotyper® version 3.7 was used to convert allele sizes obtained from GeneScan® analysis software into allele designations and to build tables containing the genotype information. In the Genotyper® software, each allele is defined by a category. Each category contains information about the the size range, and dye color for each STR allele. Genotypes are assigned by comparing PCR product sizes obtained from unknown samples with these defined category sizes. With commercial

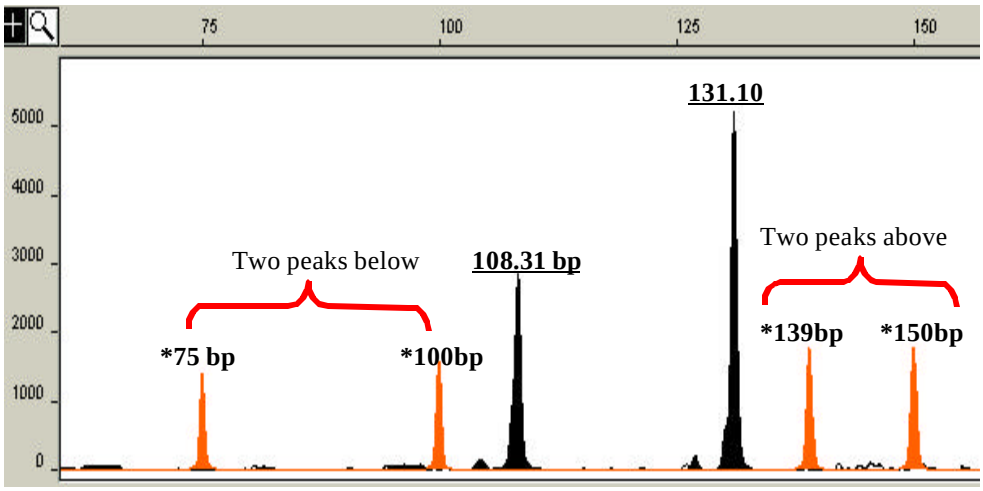



Figure 4-18
Local Southern Sizing Method
Local Southern sizing uses two peaks above and two peaks below the unknown amplicon to calculate its size. In this illustration the sizing standard peaks are labeled are in LIZ (orange) and their sizes are marked with an asterisk. The unknown amplicon peaks are labeled in NED (yellow) depicted in black and their respective calculated DNA sizes are underlined.

STR kits, these category sizes are defined through the use of allelic ladders. An allelic ladder is an artificial mixture of common alleles that are present in the human population for a particular STR marker.[118] They serve as the measuring stick for each STR locus.

The DYS391 allelic ladder in the Y-PLEX™ 6 kit contains NED (Yellow) labeled amplicons for alleles 9-12 and has an average size range from 245 bp to 257 bp (Figure 4-19). The Genotyper® software automatically uses the base pair size obtained for the injection of this allelic ladder as the center of the category or bin. For example, if allele 12 in the DYS391 allelic ladder was sized at 257.40 bp relative to the internal sizing standard, the category would be set at (257.40 ± 0.5 bp). It is also important to note that this size bin for the alleles in an allelic ladder are floating in the sense that size bins sizes

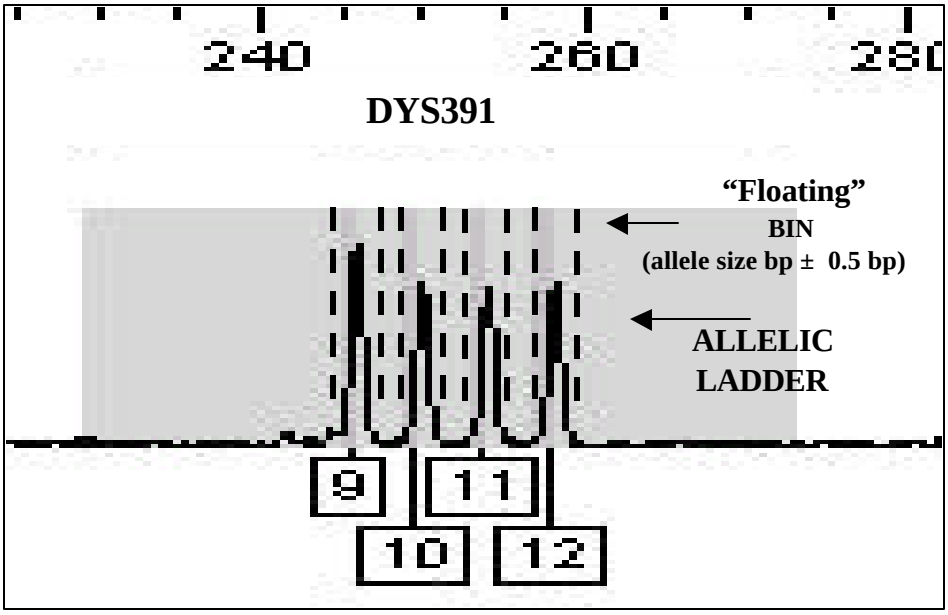



Figure 4-19
Y-PLEX™ 6 Kit DYS391 Allelic Ladder for ABI 310 Run
Category or bin (indicated by dashes in this example) is defined as the allelic ladder allele size ± 0.5 bp. Any DYS391 allele, 12 repeats in length, having a size outside the established bin would not be identified by the Genotyper software. The bin is "floating." The size bins float in a sense that size bins are redetermined based on every new injection of the ladder.[13]

are redetermined based on the size of the allelic ladder included in each new set of capillary injections (Figure 4-19).[13] For example, if the DYS391 allelic ladder is reinjected into a capillary and sized as 257.10 bp instead of the original 257.40, then the bin will be shifted down by 0.3 base pairs.

Alleles that fall outside of the category bins established by running the allelic ladder are designated as being off-ladder. Off-ladder alleles can occur for two reasons. First, the sample many contain an allele that does not correspond to one in the allelic ladder. For example, there have been DYS391 alleles with 6 repeats discovered.[98] A DYS391

allele with 6 repeats would be considered off-ladder if the Y-PLEX$^{TM}$ 6 kit was used. Second, the DYS391 allele could correspond to one of the alleles in an allelic ladder but whose size falls just outside the category window because due to measurement error.

The use of allelic ladders in the analysis of autosomal and later Y-STR systems has been recommended for a variety of reasons. First, it is been known for many years that DNA migration in electrophoresis is dependent not only on the length of DNA sample, but upon its nucleotide sequence.[13] Therefore, it was postulated that internal size standards can only guarantee accurate readings of DNA length when the standards and the unknown fragments have the same sequence and the same size. Second, allelic ladders take into account the different sizing measurements obtained from different instruments and conditions used by different laboratories. Finally, frequent running of allelic ladders (e.g. every 10 to 20 injections) helps take into account sizing differences seen due to changes in temperature. Temperature fluctuations of as little as 2 to 3$^{o}$ C have been reported to effect the sizing measurement of allelic ladders run on the ABI 310.[5]

It has been shown that through the use allelic ladders, a laboratory can obtain reliable STR typing results.[13,14] However, what if allelic ladders are not available for the loci you are studying and/or the allele range for a particular locus is not fully known yet (i.e. DYS391 above)? Even if a complete range of alleles is available are allelic ladders necessary if a laboratory uses the identical analytical platform from run to run? Can reliable genotyping results be obtained for a system that does not rely on allelic ladder but relies on sizing data obtained using the identical STR assay, CE instrument, analysis

software, sizing standard, sieving polymer, and using a unchanging set of electrophoretic parameters (i.e. temperature, separation voltage)?

## Y-STR 20plex and Y-STR 11plex Genotyper® Macro Construction

All of the Y-STR 20plex, Y-STR 11plex and Y-STR 9plex haplotypes shown in this work were run on an ABI 3100 Genetic Analyzer, utilizing POP-6™ sieving material, and electrophoretic conditions as described in the material and methods. These samples were subsequently analyzed in GeneScan® in order to determine the respective amplicon sizes. Finally, the GeneScan® results were imported into and analyzed using the Genotyper® Macro described below.

Instead of allelic ladders, allele calls for the Genotyper® results like the one shown in figure 4-20 were made with two Genotyper® macro that used categories with allele bin windows of ± 0.75 bp to ± 1.50 bp. The bin window sizes were arbitrarily set depending on the repeat size for the particular Y-STR locus. Allele bin windows of ± 0.75 were set for dinucleotide repeats, ± 1.00 bp for trinucleotide repeats, ± 1.25 bp for tetra and pentanucleotide repeats, and ± 1.50 bp for hexanucleotide repeats. Larger bin windows were initially set for two reasons. First, large bin sizes account for the sizing differences that were initially seen when samples were run on different instrumentation and/or run using a different sieving material, i.e. POP-4 instead of POP-6. For example, a sample run on the ABI 310 using POP-4 returned a base pair size of 218.66 bp for a DYS439 allele with 13 repeats. That same sample run on the ABI 3100 returned a size of 219.67 base pairs. Using the categories shown in Table 4-6, the marker sized on the 310 would

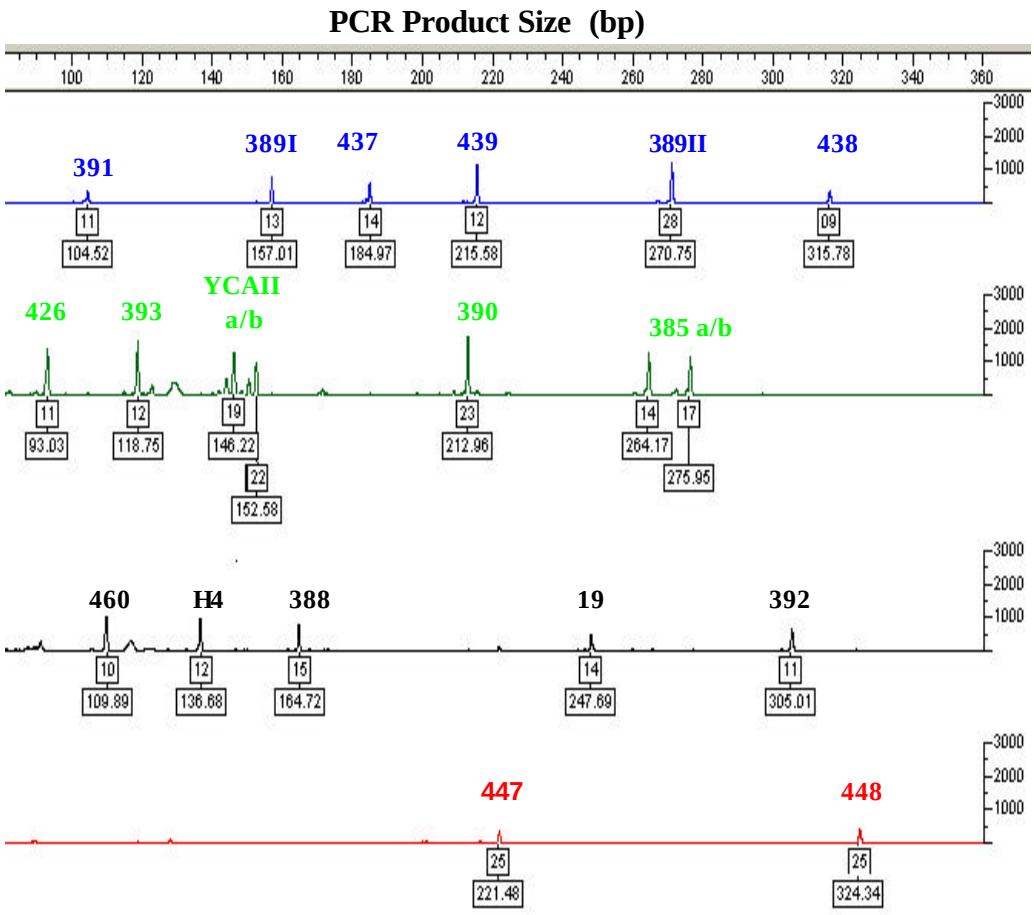



Figure 4-20
Genotyper® Result for a Male DNA Sample Obtained Using the Y-STR 20plex
The Genotyper result shown above breaks up amplicons by their respective fluorescent dye. The top panel contains amplicons with the FAM (Blue) dye label, the 2nd panel has VIC (Green) labeled amplicons, the 3rd panel has NED (Yellow) PCR products, and the bottom panel has PET (Red) labeled PCR prorducts. The allele calls generated for each Y-STR marker are based on a size comparison of each amplicon to a previously defined category size.

have fallen outside the category if the size of the bin window had been reduced. Second, as previously stated allelic bin windows sizes change or "float" every time allelic ladders are injected in order to account for the run to run variability of the instrument. The category sizes for these new macros were fixed, hence the bin windows were made larger than would have been used with allelic ladders to account for this variability.

Category sizes for the Y-STR 20plex and Y-STR 11plex (Tables 4-6 and 4-7) were determined in a number of ways. First, some of the category sizes for the alleles were based directly on the sequence from a single allele available in the Protoype Y-SRM standard and the allele sizing data provided by an internal sizing standard. In the 5 male SRM components (A-E) there were four different alleles for DYS19. They were 14, 15, 16 and 17. These allele calls were confirmed through DNA sequencing of these markers (See Prototype Y SRM 2395 for further information). Each of these SRM components were run on the ABI 3100 in POP-6 polymer under identical electrophoretic conditions. An average size was calculated by taking the sizing data from two different runs.

Second, for alleles which DNA sequencing was not performed, its size was inferred based on the size of its closest allele. The DYS19 locus had 10 categories ranging from allele 10 to allele 19. As stated above, the only DYS19 alleles sequenced were 14,15,16, and 17. So, the base pair size of allele 13 for DYS19 was inferred to be exactly four repeats smaller than allele 14. Admittedly, this may have not been the most accurate way to assign these values. For example, in DYS390, DNA sequencing data and subsequent sizing data was obtained for alleles 20-25 (Table 4-6). Upon closer examination of the

Table 4-6
Categories Used for the Y-STR 20plex and Y-STR 9plex Genotyper® Macro
Blue = FAM, Green = VIC, NED = Yellow, and PET = Red. These category sizes are unique to the ABI3100 run using POP-6™ sieving material. Changes in instrumentation and type of sieving material could change category sizes. Sequenced alleles are in bold.

**DYS19**

10 Highest peak at 231.69 ± 1.25 bp in yellow
11 Highest peak at 235.69 ± 1.25 bp in yellow
12 Highest peak at 239.69 ± 1.25 bp in yellow
13 Highest peak at 243.69 ± 1.25 bp in yellow
**14 Highest peak at 247.69 ± 1.25 bp in yellow**
**15 Highest peak at 251.69 ± 1.25 bp in yellow**
**16 Highest peak at 255.70 ± 1.25 bp in yellow**
**17 Highest peak at 259.69 ± 1.25 bp in yellow**
18 Highest peak at 263.61 ± 1.25 bp in yellow
19 Highest peak at 267.61 ± 1.25 bp in yellow

**DYS388**

10 Highest peak at 149.40 ± 1.00 bp in yellow
11 Highest peak at 152.40 ± 1.00 bp in yellow
**12 Highest peak at 155.40 ± 1.00 bp in yellow**
**13 Highest peak at 158.45 ± 1.00 bp in yellow**
14 Highest peak at 161.73 ± 1.00 bp in yellow
**15 Highest peak at 164.73 ± 1.00 bp in yellow**
16 Highest peak at 167.73 ± 1.00 bp in yellow
17 Highest peak at 170.73 ± 1.00 bp in yellow
18 Highest peak at 173.73 ± 1.00 bp in yellow

**DYS389II**

24 Highest peak at 254.49 ± 1.25 bp in blue
25 Highest peak at 258.49 ± 1.25 bp in blue
26 Highest peak at 262.49 ± 1.25 bp in blue
27 Highest peak at 266.49 ± 1.25 bp in blue
**28 Highest peak at 270.49 ± 1.25 bp in blue**
**29 Highest peak at 274.73 ± 1.25 bp in blue**
30 Highest peak at 278.73 ± 1.25 bp in blue
**31 Highest peak at 282.66 ± 1.25 bp in blue**
**32 Highest peak at 286.97 ± 1.25 bp in blue**
33 Highest peak at 290.97 ± 1.25 bp in blue
34 Highest peak at 294.97 ± 1.25 bp in blue

**DYS391**

06 Highest peak at 84.77 ± 1.25 bp in blue
07 Highest peak at 88.47 ± 1.25 bp in blue
08 Highest peak at 92.47 ± 1.25 bp in blue
09 Highest peak at 96.47 ± 1.25 bp in blue
**10 Highest peak at 100.47 ± 1.25 bp in blue**
**11 Highest peak at 104.52 ± 1.25 bp in blue**
**12 Highest peak at 108.44 ± 1.25 bp in blue**

**DYS385**

07 Highest peak at 236.03 ± 1.25 bp in green
08 Highest peak at 240.03 ± 1.25 bp in green
09 Highest peak at 244.30 ± 1.25 bp in green
10 Highest peak at 248.30 ± 1.25 bp in green
11 Highest peak at 252.30 ± 1.25 bp in green
**12 Highest peak at 256.30 ± 1.25 bp in green**
**13 Highest peak at 260.14 ± 1.25 bp in green**
**14 Highest peak at 264.09 ± 1.25 bp in green**
**15 Highest peak at 267.98 ± 1.25 bp in green**
16 Highest peak at 271.98 ± 1.25 bp in green
**17 Highest peak at 275.85 ± 1.25 bp in green**
18 Highest peak at 279.85 ± 1.25 bp in green
19 Highest peak at 283.85 ± 1.25 bp in green
**20 Highest peak at 287.67 ± 1.25 bp in green**
21 Highest peak at 291.67 ± 1.25 bp in green
22 Highest peak at 295.67 ± 1.25 bp in green
23 Highest peak at 299.67 ± 1.25 bp in green
24 Highest peak at 303.67 ± 1.25 bp in green
28 Highest peak at 319.67 ± 1.25 bp in green

**DYS389I**

09 Highest peak at 140.63 ± 1.25 bp in blue
10 Highest peak at 144.63 ± 1.25 bp in blue
11 Highest peak at 148.63 ± 1.25 bp in blue
**12 Highest peak at 152.63 ± 1.25 bp in blue**
**13 Highest peak at 157.00 ± 1.25 bp in blue**
**14 Highest peak at 161.05 ± 1.25 bp in blue**
15 Highest peak at 165.05 ± 1.25 bp in blue
16 Highest peak at 169.05 ± 1.25 bp in blue

**DYS390**

17 Highest peak at 188.88 ± 1.25 bp in green
18 Highest peak at 192.88 ± 1.25 bp in green
19 Highest peak at 196.88 ± 1.25 bp in green
20 Highest peak at 200.88 ± 1.25 bp in green
**21 Highest peak at 204.88 ± 1.25 bp in green**
**22 Highest peak at 208.90 ± 1.25 bp in green**
**23 Highest peak at 212.92 ± 1.25 bp in green**
**24 Highest peak at 216.75 ± 1.25 bp in green**
**25 Highest peak at 220.98 ± 1.25 bp in green**
26 Highest peak at 224.98 ± 1.25 bp in green
27 Highest peak at 228.98 ± 1.25 bp in green
28 Highest peak at 232.98 ± 1.25 bp in green

Table 4-6 (Continued)

**DYS392**

06 Highest peak at 289.88 ± 1.00 bp in yellow
07 Highest peak at 292.88 ± 1.00 bp in yellow
08 Highest peak at 295.88 ± 1.00 bp in yellow
09 Highest peak at 298.88 ± 1.00 bp in yellow
10 Highest peak at 301.88 ± 1.00 bp in yellow
**11 Highest peak at 304.88 ± 1.00 bp in yellow**
**12 Highest peak at 308.08 ± 1.00 bp in yellow**
**13 Highest peak at 311.62 ± 1.00 bp in yellow**
14 Highest peak at 314.62 ± 1.00 bp in yellow
15 Highest peak at 317.62 ± 1.00 bp in yellow
16 Highest peak at 320.62 ± 1.00 bp in yellow

**DYS393**

09 Highest peak at 106.78 ± 1.25 bp in green
10 Highest peak at 110.78 ± 1.25 bp in green
11 Highest peak at 114.78 ± 1.25 bp in green
**12 Highest peak at 118.78 ± 1.25 bp in green**
**13 Highest peak at 122.82 ± 1.25 bp in green**
**14 Highest peak at 126.87 ± 1.25 bp in green**
15 Highest peak at 130.87 ± 1.25 bp in green
16 Highest peak at 134.87 ± 1.25 bp in green
17 Highest peak at 138.87 ± 1.25 bp in green

**DYS437**

13 Highest peak at 180.85 ± 1.00 bp in blue
**14 Highest peak at 184.85 ± 1.00 bp in blue**
**15 Highest peak at 188.98 ± 1.25 bp in blue**
**16 Highest peak at 192.88 ± 1.25 bp in blue**
17 Highest peak at 196.88 ± 1.25 bp in blue
18 Highest peak at 200.88 ± 1.25 bp in blue

**DYS426**

09 Highest peak at 87.06 ± 1.00 bp in green
10 Highest peak at 90.06 ± 1.00 bp in green
**11 Highest peak at 93.06 ± 1.00 bp in green**
**12 Highest peak at 96.15 ± 1.00 bp in green**
13 Highest peak at 99.15 ± 1.00 bp in green

**DYS438**

06 Highest peak at 300.81 ± 1.25 bp in blue
07 Highest peak at 305.81 ± 1.25 bp in blue
08 Highest peak at 310.81 ± 1.25 bp in blue
**09 Highest peak at 315.81 ± 1.25 bp in blue**
**10 Highest peak at 320.88 ± 1.25 bp in blue**
**11 Highest peak at 326.23 ± 1.25 bp in blue**
**12 Highest peak at 331.39 ± 1.25 bp in blue**
13 Highest peak at 336.39 ± 1.25 bp in blue
14 Highest peak at 341.39 ± 1.25 bp in blue

**DYS439**

08 Highest peak at 199.48 ± 1.25 bp in blue
09 Highest peak at 203.40 ± 1.25 bp in blue
10 Highest peak at 207.40 ± 1.25 bp in blue
**11 Highest peak at 211.40 ± 1.25 bp in blue**
**12 Highest peak at 215.59 ± 1.25 bp in blue**
13 Highest peak at 219.59 ± 1.25 bp in blue
14 Highest peak at 223.59 ± 1.25 bp in blue
15 Highest peak at 227.59 ± 1.25 bp in blue

**DYS447**

19 Highest peak at 201.37 ± 1.25 bp in red
22 Highest peak at 206.37 ± 1.25 bp in red
**23 Highest peak at 211.37 ± 1.25 bp in red**
**24 Highest peak at 216.46 ± 1.25 bp in red**
**25 Highest peak at 221.39 ± 1.25 bp in red**
**26 Highest peak at 226.24 ± 1.25 bp in red**
27 Highest peak at 231.24 ± 1.25 bp in red
28 Highest peak at 236.24 ± 1.25 bp in red
29 Highest peak at 241.24 ± 1.25 bp in red
33 Highest peak at 241.24 ± 1.25 bp in red

**DYS448**

19 Highest peak at 287.83 ± 1.50 bp in red
21 Highest peak at 299.83 ± 1.50 bp in red
22 Highest peak at 305.83 ± 1.50 bp in red
**23 Highest peak at 311.83 ± 1.50 bp in red**
**24 Highest peak at 317.90 ± 1.50 bp in red**
**25 Highest peak at 324.25 ± 1.50 bp in red**
26 Highest peak at 330.25 ± 1.50 bp in red
27 Highest peak at 336.25 ± 1.50 bp in red

Table 4-6 (Continued)

**DYS460**

07 Highest peak at 97.91 ± 1.25 bp in yellow
08 Highest peak at 101.91 ± 1.25 bp in yellow
**09 Highest peak at 105.91 ± 1.25 bp in yellow**
**10 Highest peak at 109.90 ± 1.25 bp in yellow**
**11 Highest peak at 113.84 ± 1.25 bp in yellow**
12 Highest peak at 117.84 ± 1.25 bp in yellow
13 Highest peak at 121.84 ± 1.25 bp in yellow

**H4**

08 Highest peak at 120.40 ± 1.25 bp in yellow
09 Highest peak at 124.40 ± 1.25 bp in yellow
10 Highest peak at 128.40 ± 1.25 bp in yellow
11 Highest peak at 132.40 ± 1.25 bp in yellow
**12 Highest peak at 136.60 ± 1.25 bp in yellow**
13 Highest peak at 140.60 ± 1.25 bp in yellow
14 Highest peak at 144.60 ± 1.25 bp in yellow

**YCAII**

11 Highest peak at 134.29 ± 0.75 bp in green
18 Highest peak at 144.29 ± 0.75 bp in green
19 Highest peak at 146.29 ± 0.75 bp in green
20 Highest peak at 148.46 ± 0.75 bp in green
21 Highest peak at 150.43 ± 0.75 bp in green
22 Highest peak at 152.60 ± 0.75 bp in green
23 Highest peak at 154.66 ± 0.75 bp in green
24 Highest peak at 156.66 ± 0.75 bp in green
25 Highest peak at 158.66 ± 0.75 bp in green

Table 4-7
Categories used for the Y-STR 11plex Genotyper® Macro.
Blue = FAM, Green = VIC, and NED = Yellow. These category sizes are unique to the ABI3100 run using POP-6™ sieving material. Changes in instrumentation and type of sieving material could change category sizes. Sequenced alleles are in bold.

**DYS385**

07 Highest peak at 307.46 ± 1.25 bp in green
08 Highest peak at 311.46 ± 1.25 bp in green
09 Highest peak at 315.46 ± 1.25 bp in green
10 Highest peak at 319.46 ± 1.25 bp in green
11 Highest peak at 323.46 ± 1.25 bp in green
**12 Highest peak at 327.46 ± 1.25 bp in green**
**13 Highest peak at 331.33 ± 1.25 bp in green**
**14 Highest peak at 335.21 ± 1.25 bp in green**
**15 Highest peak at 339.03 ± 1.25 bp in green**
16 Highest peak at 343.03 ± 1.25 bp in green
**17 Highest peak at 345.91 ± 1.25 bp in green**
18 Highest peak at 349.91 ± 1.25 bp in green
19 Highest peak at 353.91 ± 1.25 bp in green
**20 Highest peak at 357.41 ± 1.25 bp in green**
21 Highest peak at 361.41 ± 1.25 bp in green
22 Highest peak at 365.41 ± 1.25 bp in green
23 Highest peak at 369.61 ± 1.25 bp in green
24 Highest peak at 373.41 ± 1.25 bp in green
28 Highest peak at 389.41 ± 1.25 bp in green

**DYS447**

22 Highest peak at 203.31 ± 1.25 bp in blue
**23 Highest peak at 208.31 ± 1.25 bp in blue**
**24 Highest peak at 213.29 ± 1.25 bp in blue**
**25 Highest peak at 218.32 ± 1.25 bp in blue**
**26 Highest peak at 223.39 ± 1.25 bp in blue**
27 Highest peak at 228.39 ± 1.25 bp in blue
28 Highest peak at 233.39 ± 1.25 bp in blue
29 Highest peak at 238.39 ± 1.25 bp in blue
33 Highest peak at 258.39 ± 1.25 bp in blue

**DYS448**

19 Highest peak at 326.88 ± 1.50 bp in blue
22 Highest peak at 344.88 ± 1.50 bp in blue
**23 Highest peak at 350.88 ± 1.50 bp in blue**
**24 Highest peak at 356.35 ± 1.50 bp in blue**
**25 Highest peak at 362.02 ± 1.50 bp in blue**
26 Highest peak at 368.02 ± 1.50 bp in blue
27 Highest peak at 374.02 ± 1.50 bp in blue

Table 4-7 (Continued)

| **DYS450** | | | **DYS456** | | |
|---|---|---|---|---|---|
| 07 | Highest peak at 191.33 ± | 1.25 bp in yellow | 13 Highest peak at | 91.89 ± | 1.25 bp in yellow |
| 08 | Highest peak at 196.33 ± | 1.25 bp in yellow | 14 Highest peak at | 96.26 ± | 1.25 bp in yellow |
| 09 | Highest peak at 201.19 ± | 1.25 bp in yellow | 15 Highest peak at | 100.26 ± | 1.25 bp in yellow |
| 10 | Highest peak at 206.18 ± | 1.25 bp in yellow | 16 Highest peak at | 104.12 ± | 1.25 bp in yellow |
| 11 | Highest peak at 211.18 ± | 1.25 bp in yellow | 17 Highest peak at | 108.07 ± | 1.25 bp in yellow |
| | | | 18 Highest peak at | 112.07 ± | 1.25 bp in yellow |
| **DYS458** | | | **DYS464** | | |
| 13 | Highest peak at 128.09 ± | 1.25 bp in yellow | 11 Highest peak at | 249.27 ± | 1.25 bp in green |
| 14 | Highest peak at 132.09 ± | 1.25 bp in yellow | 12 Highest peak at | 253.51 ± | 1.25 bp in green |
| 15 | Highest peak at 136.09 ± | 1.25 bp in yellow | 13 Highest peak at | 257.35 ± | 1.25 bp in green |
| 16 | Highest peak at 140.06 ± | 1.25 bp in yellow | 14 Highest peak at | 261.42 ± | 1.25 bp in green |
| 17 | Highest peak at 144.31 ± | 1.25 bp in yellow | 15 Highest peak at | 265.56 ± | 1.25 bp in green |
| 18 | Highest peak at 148.31 ± | 1.25 bp in yellow | 16 Highest peak at | 269.52 ± | 1.25 bp in green |
| 19 | Highest peak at 152.31 ± | 1.25 bp in yellow | 17 Highest peak at | 273.53 ± | 1.25 bp in green |
| 20 | Highest peak at 156.31 ± | 1.25 bp in yellow | 18 Highest peak at | 277.53 ± | 1.25 bp in green |
| | | | 19 Highest peak at | 281.53 ± | 1.25 bp in green |
| | | | 20 Highest peak at | 285.53 ± | 1.25 bp in green. |

category sizes, there isn't exactly a four base pair size difference between each allele as one might hope. They are all close to 4 base pairs (DYS390 is a tetranucleotide repeat) but not exactly. A potential cause of this is that the sequence differences present in these alleles which could effect their migration with respect to the internal size standard.[13,117] Thus, precisely determining category sizes for alleles for which DNA sequencing is not available is challenging.

Third, allele sizes for some of the loci DYS450, DYS456, DYS458, DYS464 a/b/c/d) were indirectly determined by running samples whose DNA sequences were reported by Redd et al.[39] In this manuscript DNA sequencing information was provided on four YCC samples which we have available in our laboratory for each of these markers. The sizes of the alleles for adjacent categories for which DNA sequence information was not published was then inferred based on the size of the repeat for that particular locus.

A fourth way in which category sizes can be assigned is based on sequence information provided in GenBank. In some of the earlier Genotyper® macros, the YCAII a/b categories were based solely on sequence information provided in GenBank. Numerous attempts at sequencing this locus had failed (see Prototype Y-SRM 2395). Without DNA sequencing data or access to samples in which the YCAII a/b DNA sequence was known, only Genbank sequence information could be used.

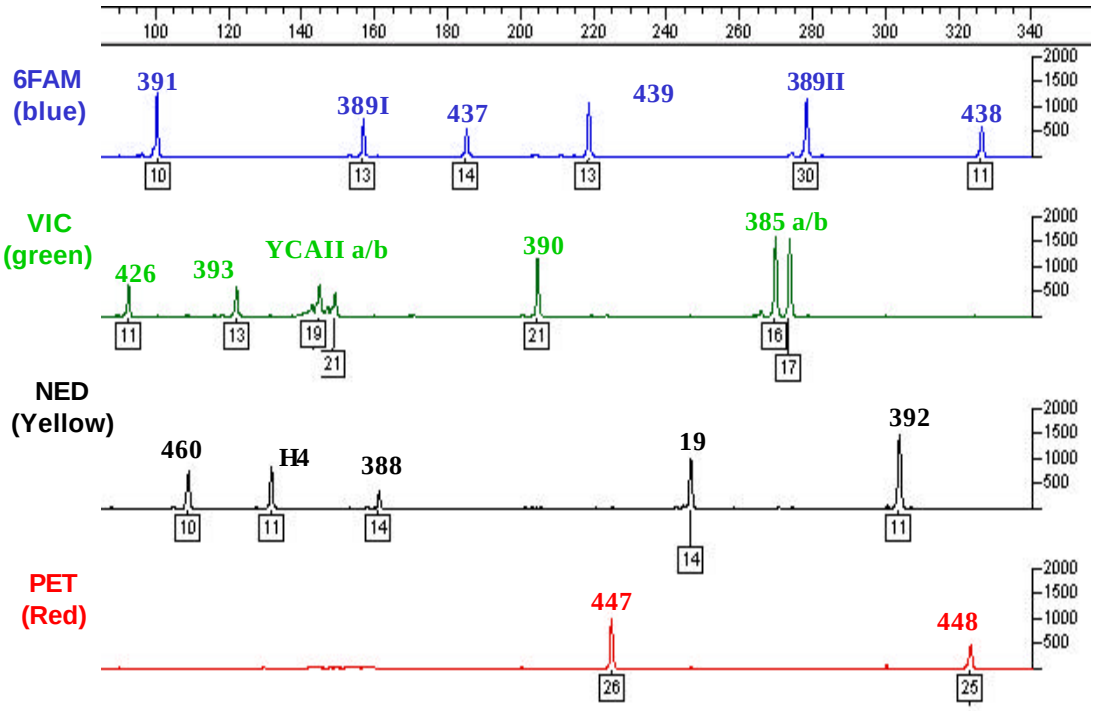
The original YCAII a/b categories sizes were set according to the allele and expected size range. YCAII a/b had category sizes ranging from 135 to 163 base pairs for the 11-25 allele range. YCAII a/b allele calls were then generated on samples including the Prototype Y SRM 2395 utilizing these category sizes. Thus, the YCAII a/b amplicons for Component A which sized at 146.38 and 154.56 for a particular run were originally typed as a 17,19. Through the results of an interlaboratory study, it was made evident that the original allele calls for the YCAII a/b locus were inaccurate. Dr. Debang Liu performed Y-STR analysis on the Prototype Y SRM 2395 and his results indicated that the actual allele calls for Component A of the SRM was 19,21 not 17,19. The original category sizes were off by 2 full dinucleotide repeats. Dr. Liu confirmed the YCAII a/b allele calls for the male SRM components by using male DNA samples whose YCAII a/b loci were sequenced by Dr. Lutz Roewer as a means of comparison. Based on Dr. Liu's results, the categories were adjusted by running the SRM and establishing a new category size based on the sizing results of each of the components.

## Concordance Studies

First, the concordance of allele designations with the Y-STR 20plex and other available primer sets was assessed. Haplotypes were generated on the same set of male samples (YCC panel) using the Y-STR 20plex and the Y-PLEX™ 6 kit.[53] These two assays have in common seven typing results from six different Y STR markers that may be compared. The Y-STR markers in common between the two assays are DYS19, DYS393, DYS389II, DYS390, DYS391, and DYS385 a/b. Panel A in Figure 4-21 depicts the result from a male DNA sample run on an ABI 3100 with the Y-STR 20plex assay while Panel B of figure 4-21 contains the result from the same male sample run on an ABI 310 with the Y-PLEX™ 6 kit. The allele calls were completely concordant even though they were generated on different instruments, by different primers and using different allele calling approaches. As mentioned above, allele calling for the Y-PLEX™ 6 kit relies on the use of allelic ladders, while the Y-STR 20plex allele calls were made with the Genotyper® macro described above that used categories with allele bin windows of +/0.75 to ± 1.50 bp (Table 4-6).

A comparison of Y chromosome haplotypes for 74 male samples in the YCC panel found that out of 1036 possible allele designations there was complete concordance between the two approaches with the exception of three calls.[53] The alleles that were subject of the discrepant calls were all deemed "off-ladder" alleles by the Y-PLEX™ 6 kit while the Y-STR 20plex assay identified them as full repeats. The Y-PLEX™ 6 kit produced a call of 9.3 at the DYS391 locus for one sample while the Y-STR 20plex result reported it as allele 10. The other two samples in question involved the DYS385 a/b

(A) Y-STR 20plex




(B) Y-PLEX™ 6 Kit

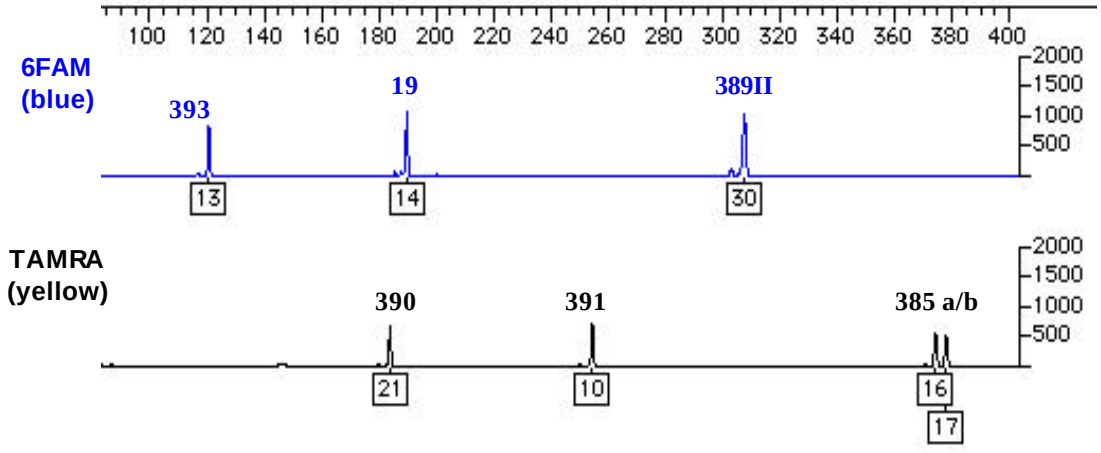



Figure 4-21
Genotyper® Results From the Same Male DNA Sample Using the Y-STR 20plex and the Y-PLEX™ 6 kit
(A) Results obtained using the Y STR 20plex and (B) using the Y-PLEX™ 6 kit from ReliaGene Technologies. Y chromosome haplotypes were generated as described in the Material and Methods section. Typing results were identical on the six loci (seven typing results) that overlapped. Adapted from Butler et al.[53]

locus. Both contained a 10.3 allele when compared to the Y-PLEX$^{TM}$ 6 kit allelic ladder but were deemed 11 repeats in the Y-STR 20plex assay. Amplicon sizes for the DYS391 and DYS385 a/b loci generated by the Y-STR 20plex were smaller than those generated by the Y-PLEX$^{TM}$ 6 kit (Figure 4-21). Thus, it is possible that these microvariants could be due to deletions that exist in regions lying outside of the Y-STR 20plex primer binding sites and therefore not detected with the new primer sets.[40] It is also possible that because the Y-STR 20plex bin windows are too large to confidently distinguish an allele containing a single base deletion from one with a full repeat, the three alleles in question were misclassified as the nearest full repeat instead of a variant.

A second study was performed comparing the allele designations of the Y-STR 20plex and Y-STR 11plex assays. Haplotypes were generated on the same set of male samples (A-2) using the Y-STR 20plex and the Y-STR 11plex. These two multiplexes have in common four typing results from three different Y STR markers that may be compared. The Y-STR markers in common between the two assays are DYS385 a/b, DYS447 and DYS448. The primer sets for the DYS447 locus for the Y-STR 20plex and Y-STR 11plex had the same sequence but used different fluorescent dyes to label the PCR amplicons. The DYS447 forward primer in the Y-STR 20plex was labeled in PET (red) while that same primer in the Y-STR 11plex was labeled in 6FAM (Blue). Different dyes on the DYS447 forward primer permit evaluation of signal height differences between 6FAM and PET labeled PCR products. The DYS385 a/b and DYS448 primer sets in the Y-STR 11plex both generated amplicons larger than the DYS385 a/b and DYS448 primer sets in the Y-STR 20plex. These different primer

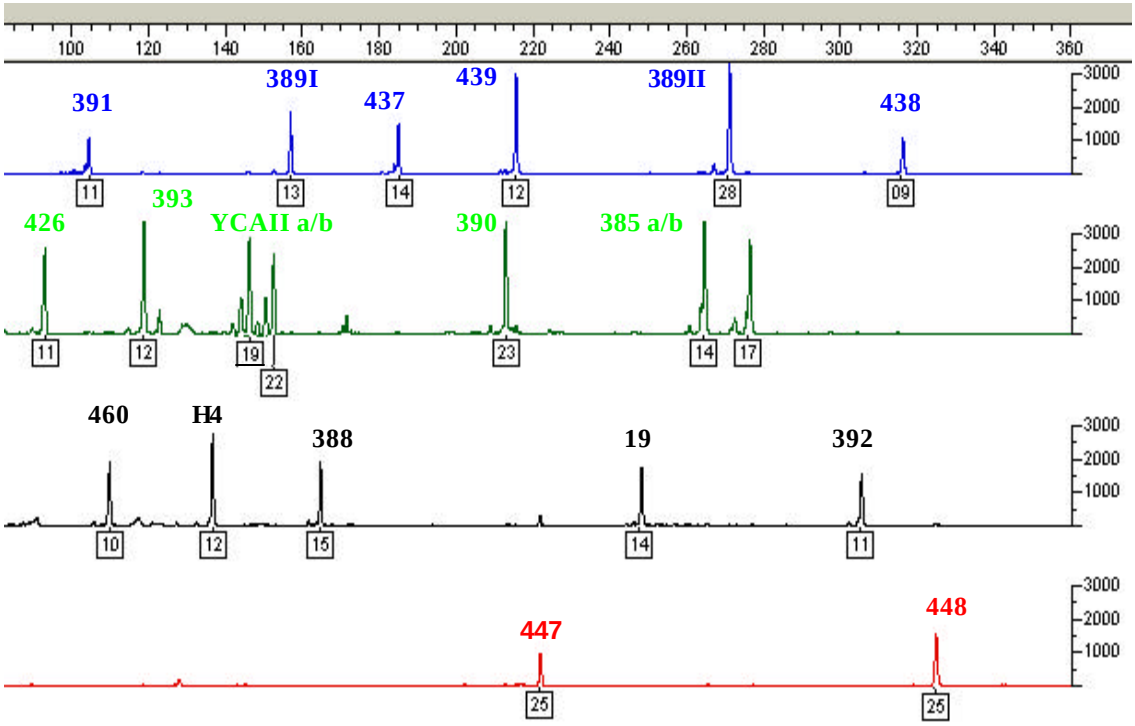
allowed for the evaluation of possible primer binding mutations as well as insertion/deletions in flanking regions outside of one primer set but inside another (e.g. DYS385[40,53]).

Panel A in Figure 4-22 depicts the results from a male sample run on an ABI 3100 with the Y-STR 20plex assay while Panel B of Figure 4-22 contains the result from the same male sample run on the same ABI 3100 with the Y-STR 11plex. The allele calls were generated using the same allele call approach relying on the Genotyper® macro (Table 4-6 and Table 4-7) described above. A comparison of Y chromosome haplotypes for the 647 male samples found that out of 2,588 possible allele designations there was complete concordance between the two assays.

## Precision Studies

In traditional precision studies involving STR assays, the calculated base pair size for STR allele amplification products are measured over the entire duration of a run and then compared to locus specific allelic ladders. All measured alleles should fall within a ± 0.5 bp window around the measured size for the corresponding allele in the allelic ladder.[5] This ± 0.5 bp window is based on previous studies involving ABI CE instrumentation which stated that the standard deviation for every allele sized in a run should measure to 0.16 base pairs or less.[13, 117] Thus, 99.7% of the allele calls should have base pair sizes within ± 0.48 bases (or three standard deviations from the mean). According to published validation studies, samples that size outside an allele size bin (± 0.5 bp) should be rerun to distinguish a true off-ladder allele from a sizing outlier.[13] The sizing data for the Y-STR 20plex and Y-STR 11plex was analyzed to see how adjustment of the category sizes

A)




(B)

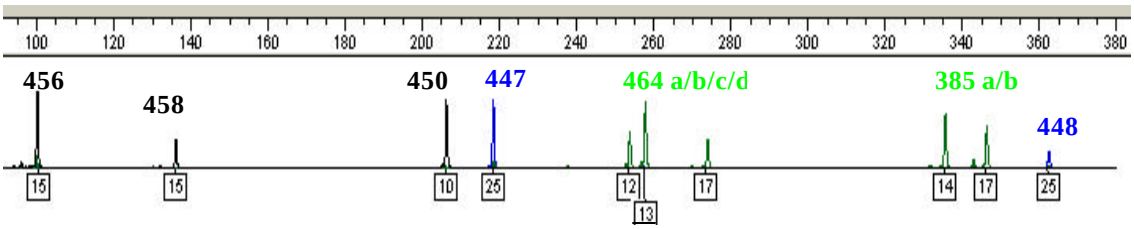



Figure 4-22
Genotyper® Results from the Same Male DNA Sample Using the Y-STR 20plex and theY-STR 11plex
Panel A, is for the Y-STR20plex and Panel B is for the Y-STR 11plex. Typing between the two multiplexes was identical on the three loci (four typing results) that overlapped. The overlapping loci were DYS385 a/b, DYS447 and DYS448.

and truncating of the bin windows to ± 0.5 bp affected the allele designations for the samples. By careful analysis of this sizing data, the question can be asked: can possible microvariants be identified that in previous concordance studies went undetected?

Precision of the Y-STR 20plex

The precision of the Y-STR 20plex was assessed using the ABI 3100 and POP-6 sieving material, GS-500 LIZ sizing standard, the Genotyper® macros described above, and the electrophoretic conditions described in the material and methods. The fixed category sizes, observed size ranges, mean size, and standard deviation for alleles contained within the data set are summarized in Table 4-8. The standard deviations values for 93 out of the 106 categories (alleles) studied were less than or equal to 0.16 with the largest deviation measuring 0.196 for allele 15 in the DYS392 locus. These results were similar to those presented for Y-STR validations run on commercially available Y-STR kits.[116] The Sinha et al.[116] validation study of the Y-PLEX™ 6 kit reported standard deviations of less than 0.1 except for two alleles and the largest recorded standard deviation (0.255) was reported for an allele within the DYS389II locus.

A direct comparison of these validation results cannot be made however because the Sinha et al.[116] precision data is the result of injecting an allelic ladder 25 times onto an ABI 310 instrument. The precision data presented in Table 4-8 is the result of injecting 647 unique samples collected during a six month period on an ABI 3100. However, the total allele number for each locus was greater than 647 because some samples were run in duplicate. The calculated means are compared to a fixed category size while other studies evaluate precision based on how a sample runs in regards to an allele in an allelic

Table 4-8
Precision of alleles in samples run on the ABI 3100 Genetic Analyzer.
Size difference is the difference between the originally established category size and the mean of the observed range for each allele. Sample runs were conducted over a six month period.

| Loci | Sample # (N) | Allele | Category Size | Observed Range | Mean | SD | Size* Difference |
|---|---|---|---|---|---|---|---|
| DYS19 | 47 | 13 | 243.69 | 243.36 – 243.81 | 243.62 | 0.096 | 0.07 |
| | 316 | 14 | 247.69 | 247.30 – 247.89 | 247.64 | 0.097 | 0.05 |
| | 198 | 15 | 251.64 | 251.38 – 251.88 | 251.68 | 0.079 | 0.04 |
| | 69 | 16 | 255.63 | 255.50 – 255.90 | 255.73 | 0.073 | 0.10 |
| | 50 | 17 | 259.61 | 259.65 – 260.02 | 259.81 | 0.068 | 0.20 |
| | | | | | | | |
| DYS385 | 10 | 10 | 248.30 | 248.20 – 248.55 | 248.33 | 0.092 | 0.03 |
| | 275 | 11 | 252.30 | 251.78 – 252.42 | 252.21 | 0.083 | 0.09 |
| | 46 | 12 | 256.30 | 255.90 – 256.33 | 256.13 | 0.082 | 0.17 |
| | 104 | 13 | 260.14 | 259.89 – 260.28 | 260.05 | 0.078 | 0.09 |
| | 302 | 14 | 264.09 | 263.71 – 264.19 | 263.95 | 0.083 | 0.14 |
| | 156 | 15 | 267.98 | 267.42 – 268.13 | 267.89 | 0.098 | 0.09 |
| | 138 | 16 | 271.98 | 271.60 – 272.03 | 271.82 | 0.079 | 0.16 |
| | 118 | 17 | 275.85 | 275.49 – 276.03 | 275.76 | 0.088 | 0.09 |
| | 69 | 18 | 279.85 | 279.51 – 279.96 | 279.72 | 0.084 | 0.13 |
| | 30 | 19 | 283.85 | 283.44 – 283.85 | 283.65 | 0.102 | 0.20 |
| | 9 | 20 | 287.67 | 287.35 – 287.59 | 287.50 | 0.117 | 0.08 |
| | | | | | | | |
| DYS388 | 10 | 10 | 149.40 | 148.96 – 149.13 | 149.04 | 0.055 | 0.36 |
| | 537 | 12 | 155.40 | 155.10 – 155.58 | 155.43 | 0.089 | 0.03 |
| | 55 | 13 | 158.45 | 158.29 – 158.71 | 158.58 | 0.088 | 0.13 |
| | 46 | 14 | 161.73 | 161.42 – 161.81 | 161.67 | 0.096 | 0.06 |
| | 19 | 15 | 164.73 | 164.63 – 164.86 | 164.76 | 0.059 | 0.03 |
| | 11 | 16 | 167.73 | 167.55 – 167.88 | 167.74 | 0.123 | 0.01 |
| | | | | | | | |
| DYS389I | 126 | 12 | 152.63 | 152.35 – 152.95 | 152.74 | 0.115 | 0.11 |
| | 421 | 13 | 157.00 | 156.53 – 157.22 | 157.00 | 0.103 | 0.00 |
| | 128 | 14 | 161.05 | 160.79 – 161.38 | 161.16 | 0.103 | 0.09 |
| | 8 | 15 | 165.05 | 165.22 – 165.36 | 165.28 | 0.049 | 0.23 |
| | | | | | | | |
| DYS389II | 3 | 26 | 262.49 | 262.23 – 262.54 | 262.44 | 0.179 | 0.05 |
| | 91 | 28 | 270.39 | 270.24 – 270.91 | 270.55 | 0.152 | 0.16 |
| | 230 | 29 | 274.73 | 274.21 – 275.03 | 274.63 | 0.147 | 0.10 |
| | 215 | 30 | 278.73 | 278.35 – 279.18 | 278.78 | 0.154 | 0.05 |
| | 108 | 31 | 282.66 | 282.52 – 283.20 | 282.90 | 0.155 | 0.24 |
| | 22 | 32 | 286.97 | 286.77 – 287.32 | 286.99 | 0.156 | 0.02 |
| | 4 | 33 | 290.97 | 291.11 – 291.22 | 291.17 | 0.046 | 0.20 |
| | | | | | | | |
| DYS390 | 5 | 20 | 200.88 | 200.76 – 200.93 | 200.83 | 0.071 | 0.05 |
| | 157 | 21 | 204.88 | 204.56 – 205.09 | 204.86 | 0.085 | 0.02 |
| | 70 | 22 | 208.90 | 208.63 – 209.12 | 208.84 | 0.104 | 0.06 |
| | 138 | 23 | 212.92 | 212.57 – 213.09 | 212.82 | 0.112 | 0.10 |
| | 243 | 24 | 216.75 | 216.54 – 217.13 | 216.83 | 0.127 | 0.12 |
| | 67 | 25 | 220.98 | 220.52 – 221.10 | 220.84 | 0.109 | 0.14 |

Table 4-8 (Continued)

| Loci | Sample # (N) | Allele | Category Size | Observed Range | Mean | SD | Size* Difference |
|---|---|---|---|---|---|---|---|
| DYS390 (Con't) | 3 | 26 | 224.98 | 224.83 – 225.07 | 224.96 | 0.121 | 0.02 |
| | | | | | | | |
| DYS391 | 21 | 9 | 96.47 | 96.41 – 96.66 | 86.53 | 0.061 | 0.06 |
| | 379 | 10 | 100.47 | 100.09 – 100.84 | 100.59 | 0.140 | 0.11 |
| | 267 | 11 | 104.52 | 104.01 – 104.79 | 104.56 | 0.134 | 0.04 |
| | 16 | 12 | 108.44 | 108.07 – 108.67 | 108.53 | 0.144 | 0.11 |
| | | | | | | | |
| DYS392 | 5 | 10 | 301.87 | 301.34 – 301.73 | 301.51 | 0.165 | 0.36 |
| | 321 | 11 | 304.87 | 304.37 – 305.25 | 304.85 | 0.164 | 0.02 |
| | 37 | 12 | 307.97 | 307.75 – 308.57 | 308.14 | 0.177 | 0.17 |
| | 256 | 13 | 311.62 | 310.84 – 311.91 | 311.41 | 0.194 | 0.20 |
| | 37 | 14 | 314.62 | 314.21 – 315.06 | 314.66 | 0.186 | 0.04 |
| | 3 | 15 | 317.62 | 317.58 – 317.97 | 317.79 | 0.196 | 0.21 |
| | 5 | 16 | 320.62 | 320.77 – 321.17 | 320.94 | 0.171 | 0.32 |
| | | | | | | | |
| DYS393 | 61 | 12 | 118.78 | 118.36 – 119.01 | 118.75 | 0.104 | 0.03 |
| | 471 | 13 | 122.82 | 122.40 – 123.03 | 122.79 | 0.106 | 0.03 |
| | 104 | 14 | 126.87 | 126.50 – 127.00 | 126.85 | 0.089 | 0.02 |
| | 44 | 15 | 130.87 | 130.64 – 131.05 | 130.92 | 0.102 | 0.05 |
| | 3 | 16 | 134.87 | 134.97 – 135.06 | 135.03 | 0.052 | 0.16 |
| | | | | | | | |
| DYS426 | 357 | 11 | 93.06 | 92.60 – 93.22 | 93.02 | 0.099 | 0.04 |
| | 312 | 12 | 96.15 | 95.81 – 96.41 | 96.21 | 0.105 | 0.06 |
| | 12 | 13 | 99.15 | 99.04 – 99.73 | 99.43 | 0.163 | 0.28 |
| | | | | | | | |
| DYS437 | 15 | 13 | 180.85 | 180.86 – 181.18 | 181.07 | 0.075 | 0.22 |
| | 312 | 14 | 184.85 | 185.04 – 185.23 | 185.04 | 0.079 | 0.19 |
| | 256 | 15 | 188.98 | 188.74 – 189.13 | 188.98 | 0.068 | 0.00 |
| | 92 | 16 | 192.88 | 192.73 – 193.11 | 192.95 | 0.076 | 0.07 |
| | 7 | 17 | 196.88 | 197.01 – 196.95 | 196.99 | 0.077 | 0.11 |
| | | | | | | | |
| DYS438 | 6 | 8 | 310.81 | 310.14 – 310.37 | 310.28 | 0.081 | 0.53 |
| | 32 | 9 | 315.81 | 315.37 – 315.91 | 315.64 | 0.160 | 0.17 |
| | 141 | 10 | 320.81 | 320.58 – 321.34 | 320.97 | 0.158 | 0.16 |
| | 216 | 11 | 326.13 | 325.72 – 326.59 | 326.13 | 0.137 | 0.06 |
| | 265 | 12 | 331.39 | 330.88 – 331.61 | 331.25 | 0.155 | 0.14 |
| | 10 | 13 | 336.39 | 336.10 – 336.47 | 336.26 | 0.142 | 0.13 |
| | | | | | | | |
| DYS439 | 37 | 10 | 207.40 | 207.43 – 207.67 | 207.53 | 0.055 | 0.13 |
| | 222 | 11 | 211.40 | 211.18 – 211.74 | 211.51 | 0.087 | 0.11 |
| | 315 | 12 | 215.59 | 215.20 – 215.73 | 215.49 | 0.086 | 0.10 |
| | 97 | 13 | 219.59 | 219.20 – 219.64 | 219.49 | 0.084 | 0.10 |
| | 11 | 14 | 223.59 | 223.30 – 223.63 | 223.51 | 0.095 | 0.08 |

Table 4-8 (Continued)

| Loci | Sample # (N) | Allele | Category Size | Observed Range | Mean | SD | Size* Difference |
|---|---|---|---|---|---|---|---|
| DYS447 | 4 | 21 | 201.37 | 201.56 – 201.72 | 201.61 | 0.072 | 0.24 |
| | 13 | 22 | 206.37 | 206.24 – 206.60 | 206.44 | 0.117 | 0.07 |
| | 73 | 23 | 211.37 | 211.12 – 211.16 | 211.41 | 0.090 | 0.04 |
| | 84 | 24 | 216.46 | 216.07 – 216.75 | 216.40 | 0.102 | 0.06 |
| | 292 | 25 | 221.39 | 221.03 – 221.64 | 221.37 | 0.112 | 0.02 |
| | 119 | 26 | 226.24 | 226.06 – 226.58 | 226.38 | 0.108 | 0.14 |
| | 70 | 27 | 231.24 | 231.04 – 231.60 | 231.38 | 0.099 | 0.14 |
| | 20 | 28 | 236.24 | 236.16 – 236.61 | 236.37 | 0.120 | 0.13 |
| | | | | | | | |
| DYS448 | 54 | 22 | 305.83 | 304.78 – 305.38 | 305.05 | 0.150 | 0.78 |
| | 285 | 23 | 311.83 | 310.84 – 312.03 | 311.54 | 0.183 | 0.31 |
| | 170 | 24 | 317.90 | 317.48 – 318.60 | 317.94 | 0.186 | 0.04 |
| | 147 | 25 | 324.25 | 323.79 – 324.49 | 324.19 | 0.157 | 0.06 |
| | 20 | 26 | 330.25 | 329.97 – 330.42 | 330.20 | 0.109 | 0.05 |
| | 7 | 27 | 336.25 | 335.98 – 336.40 | 336.19 | 0.124 | 0.06 |
| | | | | | | | |
| DYS460 | 13 | 9 | 105.91 | 105.55 – 106.09 | 105.90 | 0.145 | 0.01 |
| | 286 | 10 | 109.90 | 109.39 – 110.01 | 109.87 | 0.125 | 0.03 |
| | 343 | 11 | 113.85 | 113.41 – 114.09 | 113.86 | 0.124 | 0.01 |
| | 39 | 12 | 117.85 | 117.47 – 118.03 | 117.90 | 0.098 | 0.05 |
| | | | | | | | |
| H4 | 4 | 9 | 124.40 | 124.30 – 124.46 | 124.38 | 0.066 | 0.02 |
| | 35 | 10 | 128.40 | 128.10 – 128.54 | 128.39 | 0.115 | 0.01 |
| | 271 | 11 | 132.40 | 132.14 – 132.66 | 132.50 | 0.094 | 0.10 |
| | 328 | 12 | 136.60 | 136.29 – 136.78 | 136.63 | 0.082 | 0.03 |
| | 41 | 13 | 140.60 | 140.59 – 140.97 | 140.88 | 0.096 | 0.18 |
| | | | | | | | |
| YCAII | 5 | 11 | 130.29 | 129.82 – 130.00 | 129.90 | 0.065 | 0.39 |
| | 22 | 18 | 144.29 | 143.61 – 144.29 | 144.11 | 0.187 | 0.14 |
| | 593 | 19 | 146.29 | 145.71 – 146.56 | 146.27 | 0.162 | 0.02 |
| | 66 | 20 | 148.46 | 147.91 – 148.64 | 148.42 | 0.143 | 0.05 |
| | 197 | 21 | 150.43 | 149.95 – 150.73 | 150.52 | 0.156 | 0.09 |
| | 75 | 22 | 152.60 | 152.10 – 152.78 | 152.57 | 0.180 | 0.03 |
| | 267 | 23 | 154.66 | 154.16 – 154.92 | 154.67 | 0.143 | 0.01 |
| | 17 | 24 | 156.66 | 156.21 – 156.83 | 156.72 | 0.153 | 0.06 |

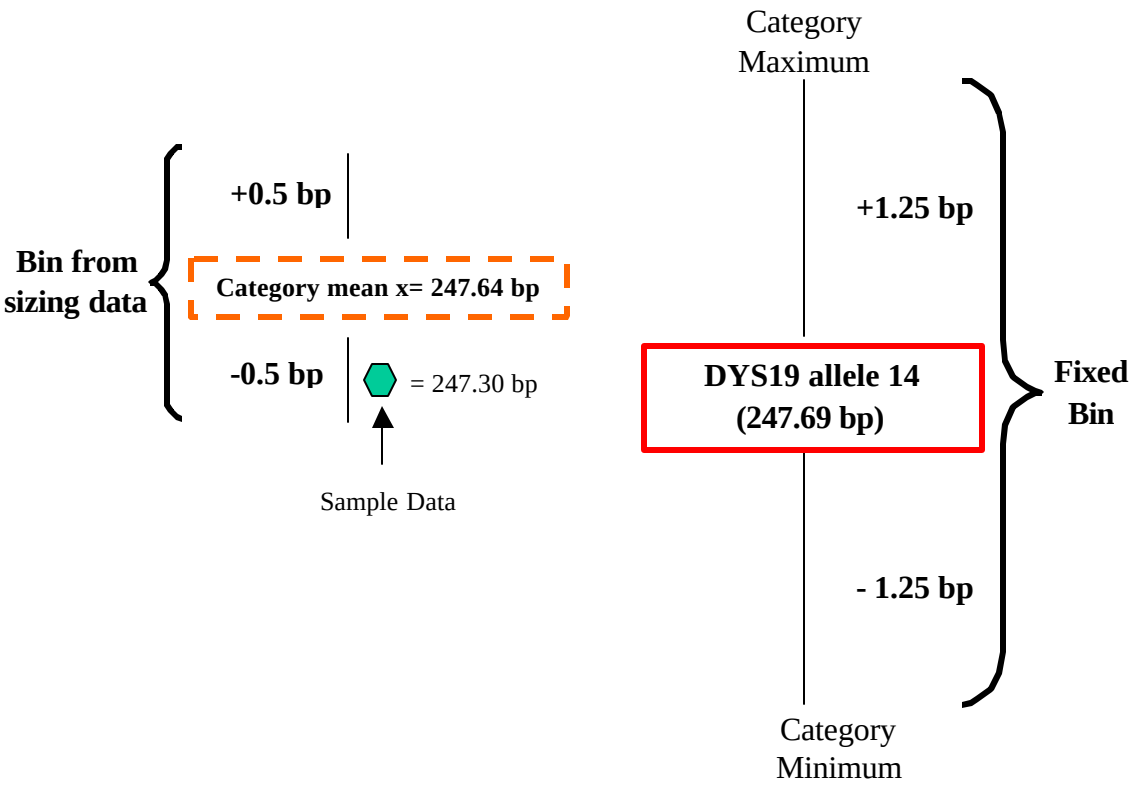



Figure 4-23
Precision Data Evaluation
The calculated sizes for a particular category (allele) are compared to the mean of numerous runs of that allele and to the fixed category size in the Genotyper® Macro. The original fixed category size is boxed in a solid line. The category mean is boxed in a dashed line

ladder. Remember an allelic ladder is run in order to take into account size variations that could be encountered between runs due to changing environmental parameters such as temperature fluctuations.[5] Based on these two facts, a sample allele in this study that did not fall with ± 0.5 bp of the category window cannot be properly designated as a true off-ladder or off-category allele (microvariant) or an anomalous result due to measurement error without first looking at the calculated mean and standard deviation for that particular category.

Figure 4-23 illustrates how precision data for each allele was evaluated. On the right hand side of the figure is the fixed category size for allele 14 of DYS19 and the resulting bin window sizes if the larger ± 1.25 bp spread was used. On the left side of Figure 4-23 results of precision data obtained for the allele 14 category shown in table 4-8 using the bin window spread of ± 0.5 bp. In this instance, the data point (247.30 bp) shown in figure 4-23 falls inside both of the proposed category windows. Shifting of the category size in this case of the allele 14 category would not have been necessary because the difference between the category window for allele 14 and the calculated mean was only 0.05 bp apart and all of the sample alleles fell within the ± 0.5 bp of the original fixed category size and the category mean (Figure 4-23).

The precision plot of DYS447 (Figure 4-24) is presented in order to show how precision data can be used to identify possible microvariants. The sizes for 677 of 678 DYS447 alleles examined were within ± 0.16 bp of their respective calculated mean and the size difference between these means and their respective category window was 0.24 base or less. The allele in question, when sized at 215.25 bp, (circled in Figure 4-24) was originally designated as having 24 repeats because it fell within the larger ± 1.25 bp window. If the 215.25 bp amplicon was removed from the 24 allele category the SD dropped to 0.10. This amplicon was over five standard deviations from the calculated mean of 216.40. The potential microvariant was missed because the original bin window size of ± 1.25 bp was too large. If a smaller bin window size of ± 0.5 bp had been used the suspect amplicon would have been classified “off-category”, not called by the

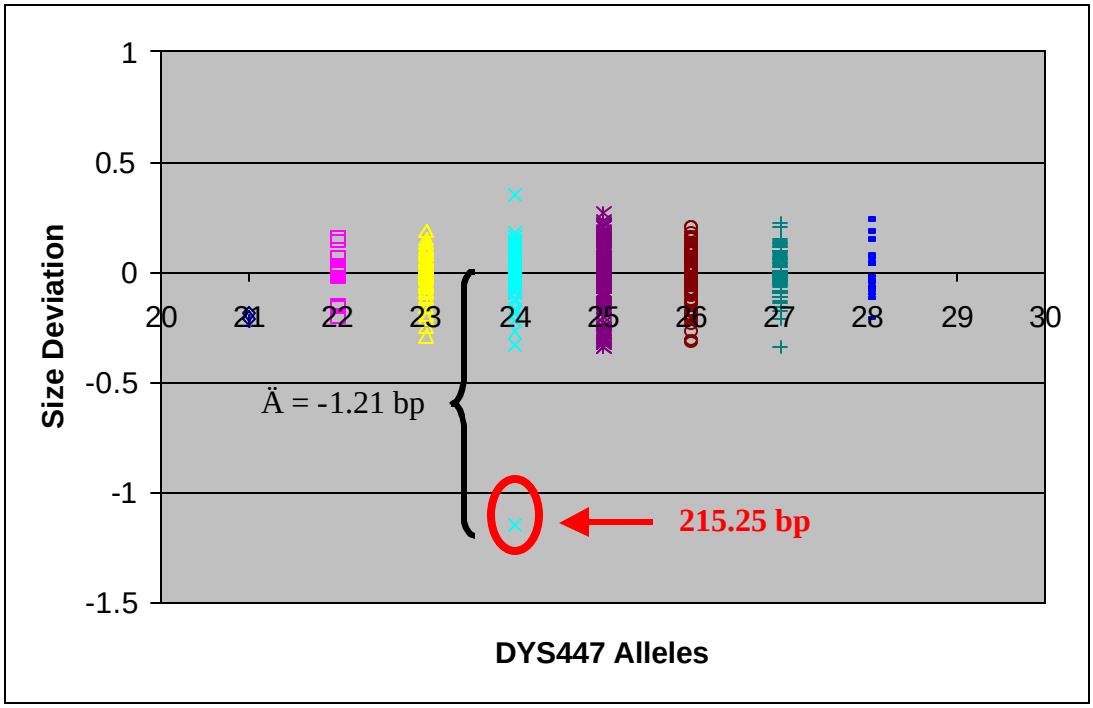


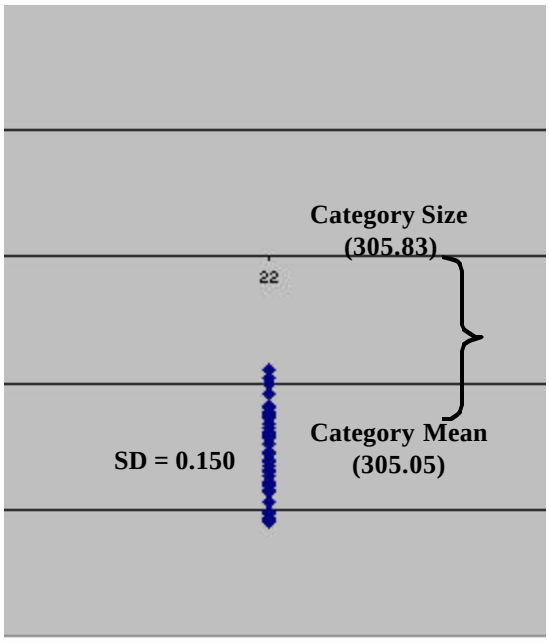

Figure 4-24
Precision plot for the DYS447 Locus (Y-STR 20plex)
The size difference of each sample compared to the category size is plotted vs. its respective category. The sample circled was originally called a 24 repeat. Its base pair size was 215.25 bp, or 1.21 bp less than established category size of 216.46 bp for allele 24. Data indicated that this sample is a possible microvariant (most likely a 23.4) The size difference between the category size and the calculated mean for allele 24 was 0.06 bp.

Genotyper® software, and easily identified as a possible microvariant (most likely a 23.4) allele instead of a 24 allele.

The precision data in Table 4-8 and subsequent precision plots also identified categories whose sizes should have been adjusted due to sizing anomalies. Figure 4-25 illustrates how the calculated mean for allele 22 of the DYS448 locus was more than 0.75 bp different than the category size (Table 4-8). However, the SD for this category was only 0.15 bp, indicating that these values were precise. If the category size for the 21 repeat allele was adjusted to the 305.05 bp, none of the samples would fall outside of the

± 0.5 bp window. If the smaller ± 0.5 base pair window was used instead of the larger ± 1.25 window without adjusting the original category size 51 out of the 54 alleles would have been labeled as off-category alleles.

With few exceptions, the Y-STR 20plex sizing data obtained using the ABI 3100 was very precise. If smaller bin windows (i.e. ± 0.5 bp) and the calculated means for the categories were used instead of the larger bin windows and original category (allele) sizes only 19 (0.14%) out of 13,345 alleles would have been classified as off-category. Conversely when the larger bin windows and original category sizes were used only one sample would have been considered off category and a possible microvariant. The




Allele 22 for DYS448 Locus

Figure 4-25
Precision Plot of Allele 22 for DYS448 Locus (Y-STR 20plex)
The size difference of each sample compared to the category size is plotted vs. the category size. The category size is 0.78 bp different than the calculated mean for that category. The category size should be shifted to reflect the category mean.

majority (13) of the off-category alleles were for the YCAII a/b locus. The other 6 off-category alleles were from DYS391 (2), DYS447 (1), and DYS448 (3). At some point these possible microvariants should be evaluated through either rerunning the PCR amplicon on the ABI 3100 to confirm their base pair size, DNA sequencing or by comparison to an allelic ladder to confirm the proper allele designations. It is clear that the Y-STR 20plex in concert with the ABI 3100 offered precise sizing and reliable Genotyper® results for over 13,000 alleles. These are the same allele calls that were evaluated in the population study presented in this work.

Precision of the Y-STR 11plex

The precision of the Y-STR 11plex and ABI 3100 was assessed in the same manner as described above for the Y-STR 20plex except that the Y-STR 11plex haplotypes were generated using the Genotyper® macro categories listed in Table 4-7. The fixed category sizes, observed size ranges, mean size, and standard deviation for alleles contained within the data set for the Y-STR 11plex are summarized in Table 4-9. The standard deviation values for 32 out of the 51 categories (alleles) studied were less than 0.16 with the largest deviation measuring 0.258 for allele 12 in the DYS385 a/b locus. Of the 17 categories with standard deviations of greater than 0.17, one was from the DYS447 locus, 8 from the DYS385 locus and 10 from the DYS464 locus.

A closer examination of these categories uncovered possible microvariants that greatly increased their respective standard deviations. If smaller bin windows (i.e. ± 0.5 bp) and the calculated means for the categories were used instead of the larger bin windows and original category (allele) sizes 74 (1.15%) out of 6,481 alleles would have

Table 4-9
Precision of Alleles in Samples Run with the Y-STR 11plex on the ABI 3100 Genetic Analyzer.
Size difference is the difference between the originally established category size and the mean of the observed range for each allele. Sample runs were conducted over a six month period.

| Loci | Sample # (N) | Allele | Category Size | Observed Range | Mean | SD | Size* Difference |
|---|---|---|---|---|---|---|---|
| DYS385 | 10 | 10 | 319.46 | 319.35 – 319.89 | 319.56 | 0.195 | 0.10 |
| | 265 | 11 | 323.46 | 323.19 – 324.66 | 323.65 | 0.247 | 0.19 |
| | 50 | 12 | 327.46 | 327.28 – 328.20 | 327.70 | 0.258 | 0.34 |
| | 100 | 13 | 331.33 | 330.90 – 332.38 | 331.59 | 0.244 | 0.26 |
| | 301 | 14 | 335.21 | 335.00 – 336.31 | 335.41 | 0.236 | 0.20 |
| | 146 | 15 | 339.03 | 338.84 – 339.85 | 339.25 | 0.240 | 0.22 |
| | 125 | 16 | 343.03 | 342.38 – 343.12 | 342.69 | 0.182 | 0.34 |
| | 112 | 17 | 345.91 | 345.00 – 346.36 | 346.00 | 0.126 | 0.09 |
| | 64 | 18 | 349.91 | 349.07 – 349.59 | 349.30 | 0.105 | 0.61 |
| | 31 | 19 | 352.91 | 352.68 – 352.96 | 352.79 | 0.067 | 0.12 |
| | 9 | 20 | 356.41 | 357.28 – 356.42 | 356.37 | 0.047 | 0.04 |
| | | | | | | | |
| DYS447 | 4 | 21 | 198.31 | 198.35 – 198.61 | 198.45 | 0.128 | 0.14 |
| | 13 | 22 | 203.31 | 203.32 – 203.55 | 203.44 | 0.056 | 0.13 |
| | 72 | 23 | 208.31 | 207.99 – 208.59 | 208.39 | 0.137 | 0.08 |
| | 81 | 24 | 213.29 | 213.05 – 213.29 | 213.39 | 0.138 | 0.10 |
| | 290 | 25 | 218.32 | 217.92 – 218.61 | 218.38 | 0.139 | 0.06 |
| | 109 | 26 | 223.39 | 222.92 – 223.94 | 223.39 | 0.156 | 0.00 |
| | 70 | 27 | 227.39 | 227.89 – 228.70 | 228.39 | 0.159 | 0.00 |
| | 17 | 28 | 233.39 | 233.16- 233.60 | 233.42 | 0.104 | 0.03 |
| | | | | | | | |
| DYS448 | 53 | 22 | 344.88 | 345.56 – 345.93 | 345.72 | 0.080 | 0.52 |
| | 275 | 23 | 350.88 | 350.69 – 351.02 | 350.85 | 0.052 | 0.03 |
| | 162 | 24 | 356.36 | 356.24 – 356.46 | 356.36 | 0.046 | 0.01 |
| | 140 | 25 | 362.02 | 361.81 – 362.09 | 361.95 | 0.063 | 0.07 |
| | 20 | 26 | 368.02 | 367.51 – 368.60 | 367.63 | 0.081 | 0.39 |
| | 6 | 27 | 374.02 | 373.21 – 373.53 | 373.74 | 0.123 | 0.68 |
| | | | | | | | |
| DYS450 | 203 | 8 | 196.33 | 196.01 – 196.65 | 196.38 | 0.114 | 0.05 |
| | 432 | 9 | 201.19 | 201.01 – 201.67 | 201.32 | 0.112 | 0.13 |
| | 25 | 10 | 206.18 | 206.04 – 206.54 | 206.32 | 0.132 | 0.14 |
| | | | | | | | |
| DYS456 | 15 | 13 | 91.89 | 91.55 – 92.15 | 91.93 | 0.160 | 0.23 |
| | 86 | 14 | 96.26 | 95.66 – 96.33 | 96.00 | 0.138 | 0.26 |
| | 285 | 15 | 100.26 | 99.65 –100.30 | 100.08 | 0.145 | 0.18 |
| | 197 | 16 | 104.12 | 103.64 – 104.29 | 104.03 | 0.140 | 0.09 |
| | 71 | 17 | 108.07 | 107.62 – 108.23 | 108.02 | 0.132 | 0.05 |
| | 18 | 18 | 112.07 | 111.71 – 112.72 | 112.03 | 0.124 | 0.04 |

Table 4-9 (Continued)

| Loci | Sample # (N) | Allele | Category Size | Observed Range | Mean | SD | Size* Difference |
|---|---|---|---|---|---|---|---|
| DYS458 | 20 | 14 | 132.09 | 131.67 – 132.14 | 131.94 | 0.129 | 0.15 |
| | 99 | 15 | 136.09 | 135.64 – 136.14 | 135.92 | 0.108 | 0.17 |
| | 174 | 16 | 140.06 | 139.66 – 140.93 | 139.99 | 0.137 | 0.07 |
| | 235 | 17 | 144.31 | 143.90 – 145.34 | 144.25 | 0.143 | 0.06 |
| | 108 | 18 | 148.31 | 148.14 – 148.69 | 144.48 | 0.127 | 0.17 |
| | 30 | 19 | 152.31 | 152.33 – 152.80 | 152.63 | 0.137 | 0.32 |
| | 8 | 20 | 156.31 | 156.71 – 156.95 | 156.80 | 0.072 | 0.49 |
| | | | | | | | |
| DYS464 | 26 | 11 | 249.27 | 249.24 – 249.84 | 249.48 | 0.166 | 0.21 |
| | 102 | 12 | 253.32 | 253.21 – 253.96 | 253.51 | 0.172 | 0.19 |
| | 189 | 13 | 257.35 | 257.18 – 258.40 | 257.54 | 0.189 | 0.19 |
| | 200 | 14 | 261.42 | 261.28 – 262.07 | 261.63 | 0.183 | 0.21 |
| | 458 | 15 | 265.56 | 265.27 – 266.90 | 265.68 | 0.189 | 0.12 |
| | 408 | 16 | 269.52 | 268.98 – 270.28 | 269.69 | 0.209 | 0.17 |
| | 366 | 17 | 273.53 | 273.34 – 274.57 | 273.77 | 0.207 | 0.24 |
| | 150 | 18 | 277.53 | 277.49 – 278.41 | 277.79 | 0.203 | 0.26 |
| | 23 | 19 | 281.53 | 281.53 – 282.33 | 281.92 | 0.224 | 0.39 |
| | 6 | 20 | 285.53 | 285.66 – 286.24 | 286.06 | 0.202 | 0.53 |

been classified as off-category. The possible microvariants found in the Y-STR 20plex for the DYS447 and DYS448 locus were correctly identified in the Y-STR 11plex. For example, the previous proposed 23.4 allele for DYS447 in the Y-STR 20plex work, sized at 212.4 bp with the Y-STR 11plex assay which was 1.03 bp smaller than the mean for that category. Thus, it was identified as a possible microvariant.

The majority of these possible microvariants (36 out of 76 off-category alleles) were connected to the DYS385 locus. The reason for this may be two fold. First, the Y-STR 11plex primer set resulted in amplicons approximately 95 base pairs larger than the Y-STR 20plex DYS385 primer set. An insertion or deletion may exist within the flanking region that was not amplified if the Y-STR 20plex primer set was used. This premise was confirmed by information published by Furedi et al.[40] In this study a thymine deletion was detected in a $T_7$ stretch within the 3' flanking region of each allele. A

DYS385 allele amplified with the Y-STR 11plex primers includes that $T_7$ stretch while the Y-STR 20plex reverse primer binds upstream of the $T_7$ stretch. Thus, the DYS385 primer set within the Y-STR 20plex assay would not detect such a deletion (Figure 4-26).

Second, the larger Y-STR 11plex primer sets generate amplicons in the size range (319 -357 bp). The sizing of these fragments could be affected by the anomalous running of the internal sizing standards. It has been reported that the 340-base fragment in the internal standard may migrate anomalously due to temperature fluctuations.[117] Temperature fluctuations have been known to affect other peaks within the internal sizing standard as well, particularly the 250 bp fragment. The anomalous migration of the 250 peak is a common occurrence and as a result it is not included in the generation of size standard curves.[117]

In summary, adjusting the category sizes and truncating the bin window sizes to ± 0.5 bp adequately identified possible microvariants that would have otherwise gone undetected. Only 93 (0.47%) out of 19,826 alleles were off-category and not given an allele designation after the Genotyper® Macro was adjusted. Both the Y-STR 20plex and Y-STR 11plex in concert with the ABI 3100 offers precise sizing and reliable allele designations even without allelic ladders with over 19,000 allele designations made on more than 640 male DNA samples.

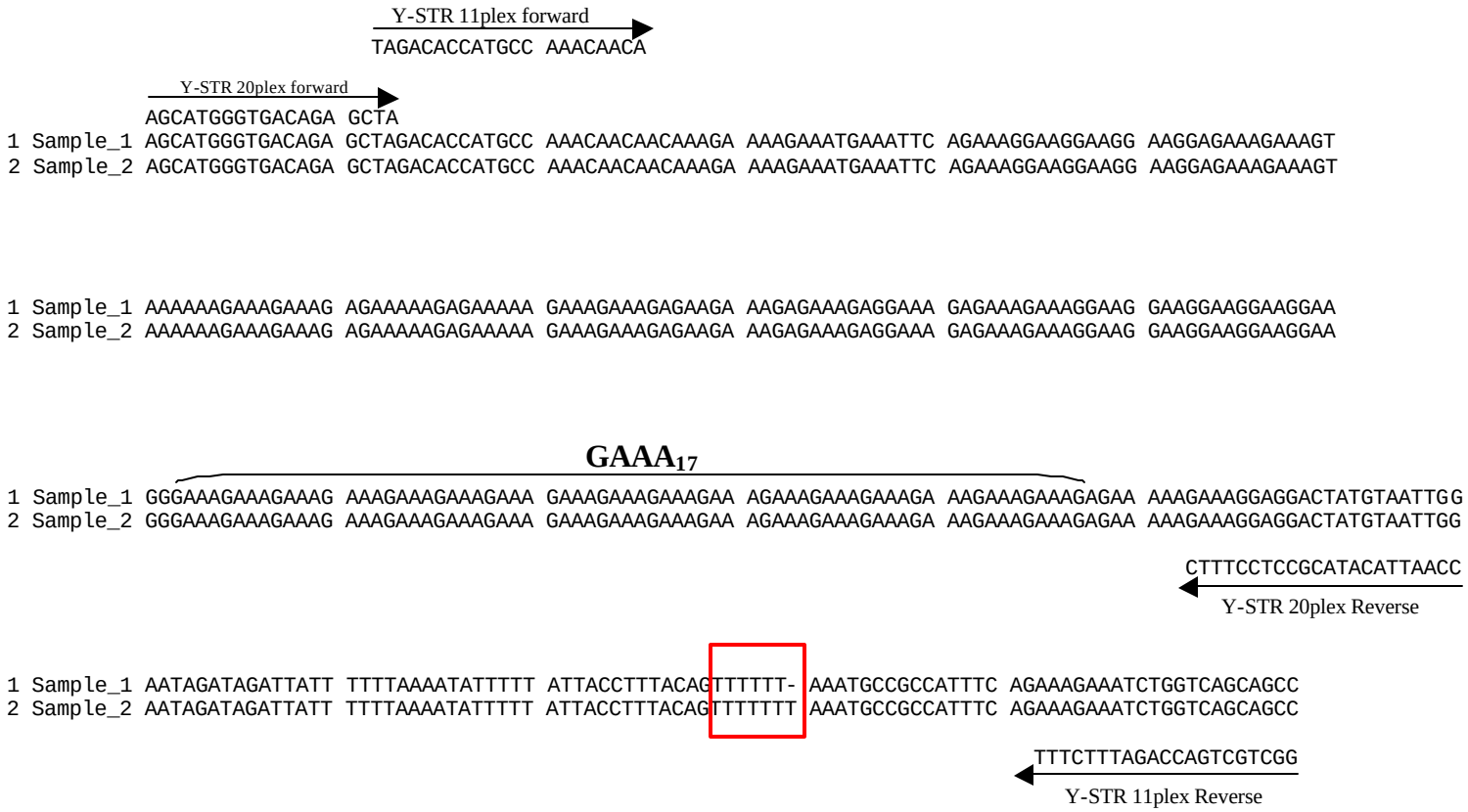



Figure 4-26
Alignment of top strands from two different male samples for the DYS 385 locus. Both samples contain 17 GAAA tetranucleotide repeats.
Sample 1 is missing a T resiude in a $T_7$ stretch (boxed) when compared to the sequence of sample 2. This stretch of sequence is not amplified when the Y-STR 20plex primers are used. Thus, both samples would be designated as having 17 repeats if the DYS385 primer set in the Y-STR 20plex was used. A sample with such a deletion (Sample 1) would be typed as 16.3 using either the Y-STR 11plex or Y-PLEX 6 kit. This T deletion was first reported by Furedi et al.[40] Note the exact sequence of the DYS385 a/b primer set for the Y-PLEX 6 kit is not known, however it generates PCR products larger than that of the Y-STR 11plex.

## Minimum Sensitivity

The minimum amount of sample DNA required to obtain a complete profile for the Y-STR 20plex, Y-STR 9plex and Y-STR 11plex PCR assay was investigated. Serial dilutions were prepared of a known DNA sample. The amount of DNA in the reactions was 1.0, 0.5 ng, 0.25 ng, 0.125, and 0.05 ng (50pg). These template amounts were chosen based on the validation guidelines for minimum sensitivity recommended by SWGDAM.[5,31,116] Additionally, these minimum sensitivity studies were performed at 32 cycles instead of the 28 cycle protocol that was used for all the population data and the precision studies. All other conditions (i.e. annealing temperature, primer concentration) were held constant. Initial sensitivity experiments were performed using 28 cycles instead of 32 cycles on the Y-STR 9plex. It was later noted that in a number of the Y-STR assays tested 30 cycles and in some cases 38 cycles were used.[27,31,116,119] Even though an increase in cycle number can improve sensitivity it can result in the formation of non-specific products, previously absent at lower cycle numbers.[5] At lower DNA levels (less than 0.25 ng), and 28 cycles of amplification, allele dropout for the DYS19 locus was observed. Based on this initial data and the fact that most Y-STR assays in the literature used 30 or more amplification cycles, all future sensitivity experiments were performed at 32 cycles of amplification.

Figure 4-27 shows the GeneScan result for the Y-STR 20plex, Y-STR 9plex, and Y-STR 11plex run with 50 pg of male sample amplified to 32 cycles. This figure demonstrates that each of these Y-STR multiplexes were sensitive to 50 pg with no allelic drop out detected. In a Y-PLEX$^{TM}$ 6 kit sensitivity study performed on an ABI 310 by

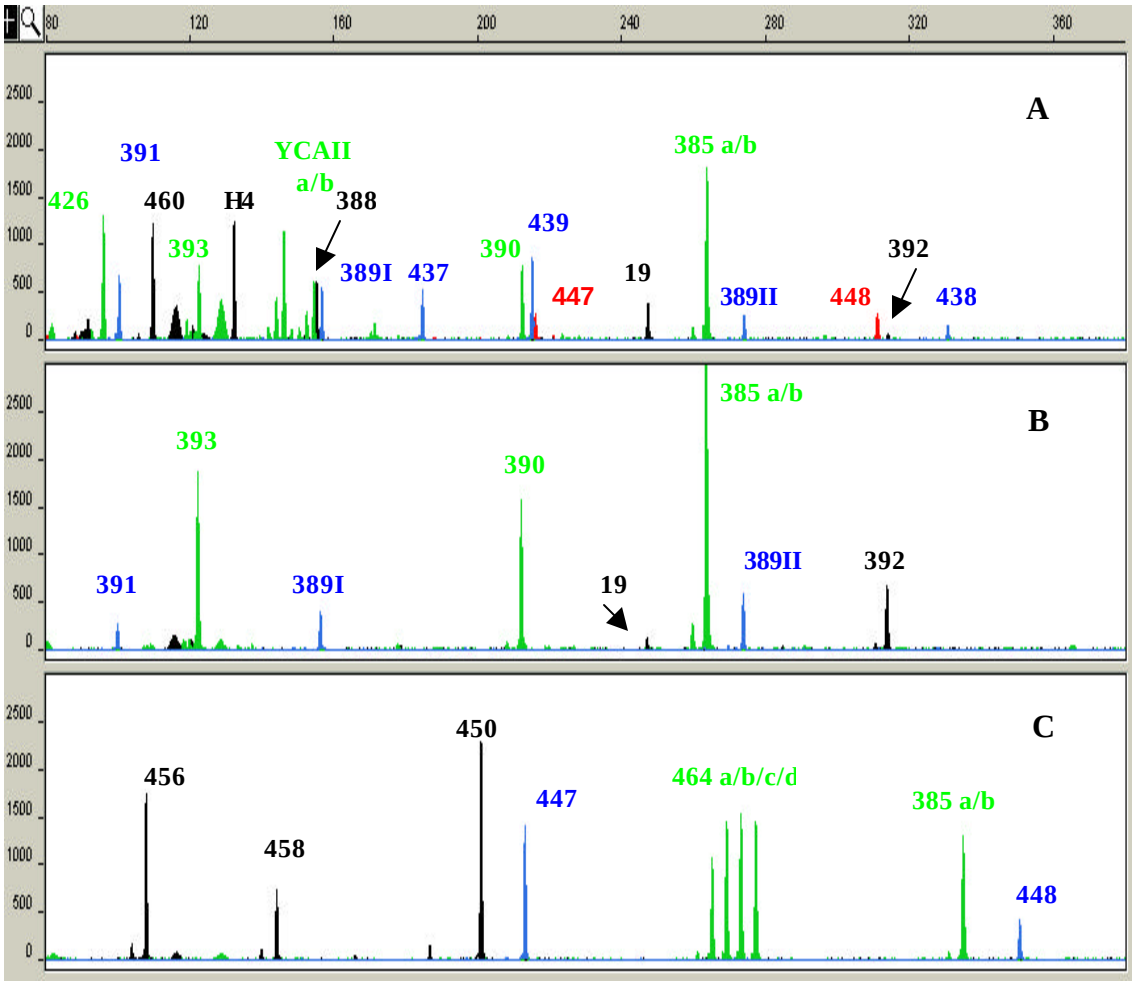



Figure 4-27
Minimum Sensitivity Results for Y-STR Multiplexes
Genescan® result for identical male samples run using three different Y-STR multiplexes. Each multiplex was run under identical PCR conditions (50pg at 32 PCR cycles). Panel A: Y-STR 20plex, Panel B: Y-STR 9plex, Panel C: Y-STR 11plex. No allelic drop out was seen in any of the multiplexes.

Sinha et al.[116], the data showed allelic drop out of DYS390, DYS391 and DYS385 a/b at levels of 0.l ng. The Sinha et al.[116] protocol included PCR to 30 cycles and a AmpliTaq Gold concentration of 2.5 U per PCR reaction. The PCR strategy used for the Y-STR 20plex, 11-plex, and 9-plex was 32 PCR cycles and 2U of AmpliTaq Gold per reaction.

It must be noted that a direct comparison cannot be made to the Sinha et al.[116] study work because of the differences in instrumentation, cycle number, and total number of loci simultaneously examined. However, this preliminary sensitivity does indicate that the Y-STR 20plex, Y-STR 11plex and Y-STR 9plex are as sensitive if not more so than this commercially available Y-PLEX™ 6 kit.

## Specificity and Male:Female Mixtures

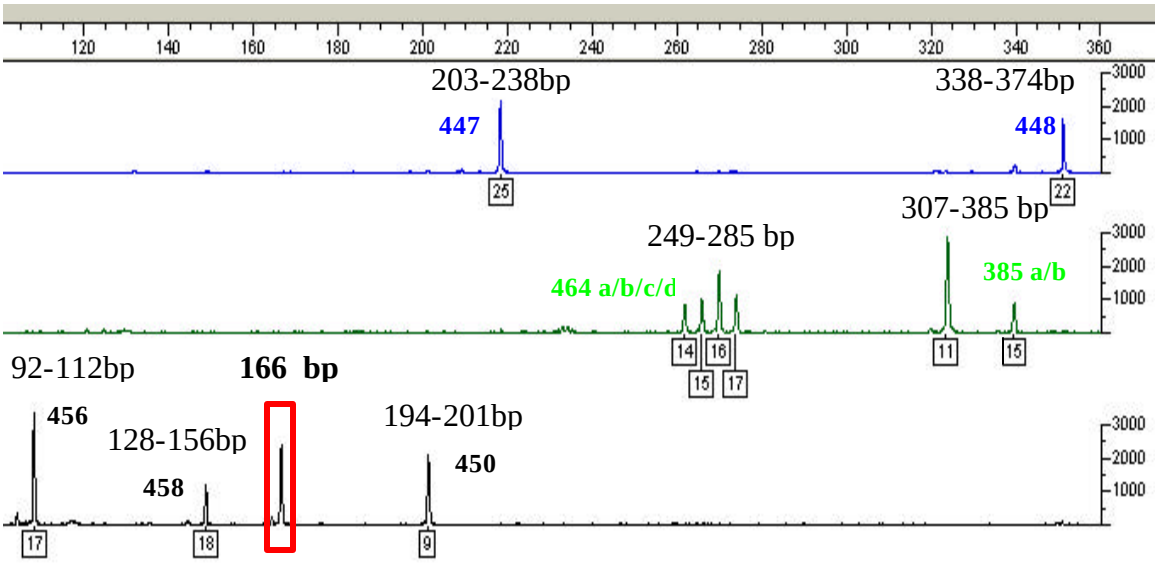
Mixtures of body fluids from different individuals are a common phenomenon in forensic casework. A vaginal swab submitted for criminal investigation in sexual assault cases could contain a combination of female epithelial cells and male semen. Extracting DNA from mixed vaginal/seminal samples can be difficult and is not always successful.[120,121] If the male committing the sexual assault is azospermic (does not ejaculate sperm), then the differential extraction which separates the sperm from the vaginal epithelial cells cannot be accomplished.[120] Thus, studying the male-specificity of any Y-STR in the presence of excess female DNA is very important.

All three Y-STR multiplexes were tested against DNA samples containing varying ratios of male:female template DNA. These mixture samples were designed to duplicate a scenario in which a vaginal swab containing a large female component with a very small male component. The amount of male sample was kept constant at 0.5 ng while the female sample was varied. The ratios ranged from a 1:100 to 1:1600. The 0.5 ng male template amount and male:female ratios were chosen based on previous male:female specificity analysis.[27,31,116]

Of the three multiplexes, only the Y-STR 11plex could obtain a complete Y-STR 11plex profile when the ratio of male:female DNA was 1:200 in a given sample. At levels above 1:200 the Y-STR 11plex had allelic dropout and/or had non-specific amplicons that interfered with proper allele calling for a particular Y-STR locus. Figure 4-28 is a Genotyper result for a 0.50 ng male DNA sample mixed with 50 ng of female DNA. Panel A represents the Y-STR loci broken up into their respective dye colors. The allele size ranges for each locus are given above the observed allele for that Y-STR marker. Panel B is the result of running 0.5 ng of the male standard template alone without any female template. The data in Figure 4-28 indicated that the non-specific amplicon at 166 bp labeled in NED would not impact the calling of alleles for the DYS450 or DYS456 locus. However, if any new alleles for the DYS458 locus are discovered this non-specific amplicon could be misrepresented as a DYS458 allele. Additionally, while a complete profile was obtained in the 1:100 mixture the phenomenon known as stochastic fluctuation was observed for the DYS385 a/b locus (Figure 4-28). Stochastic effects are an unequal sampling of two alleles present for a particular locus and result when only a few DNA molecules are used in the PCR amplification of the sample.[122] PCR reactions using DNA template below 100 pg, have been shown to exhibit allele dropout.[123]

While complete profiles were obtained for the Y-STR 20plex and Y-STR 9plex at the 1:100 mixture level, each had numerous nonspecific amplicons that made the identification of the Y-STR profile challenging. Figure 4-29 is a GeneScan result for the Y-STR 20plex run with 0.50 ng male DNA sample mixed with 50 ng of female. Panel A

Panel A: 1:100 Mixture of Male (0.5 ng) to Female (50.0 ng) DNA




Panel B: 0.50 ng of Male DNA

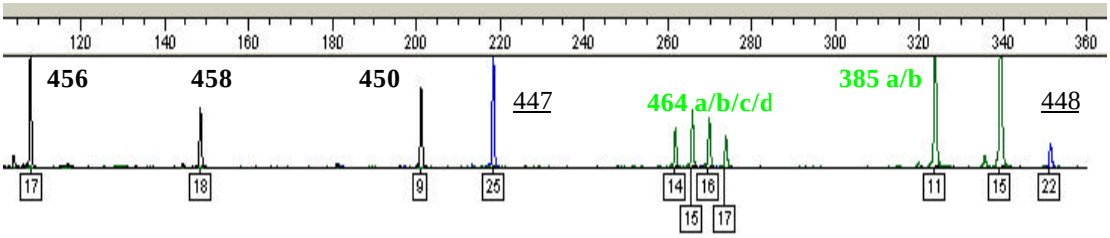



Figure 4-28
Genotyper® Results for Male:Female Mixture Sample Using the Y-STR 11plex
Panel A, is the result of the male:female sample in a 1:100 ratio. The size ranges in bp for each locus are given. The non-specific amplicon sized at 166 bp and did not impact allele calls for any of the locus. Panel B, is the male reference standard. All of the allele calls in Panel B were in concordance with Panel A. The results in Panel B did not show a NED labeled amplicon at 166 bp as in Panel A.

represents the Y-STR 20plex haplotype obtained by running 0.50 ng of the male sample. Panel B is the result for the Y-STR 20plex of a mixed sample of male:female in a 1:100 ratio. Panel C is the result for the Y-STR 20plex of a mixed sample of male:female in a 1:200 ratio. The data indicated that at 1:100 mixture level, a complete Y-STR 20plex profile can be seen but it obscured by the presence of numerous non-specific amplicons. The loci particularly affected by the presence of these non-specific amplicons (Boxed in Panel B) were DYS390, DYS393, DYS447, and DYS439. At the 1:200 mixture level, allelic drop out of numerous loci was seen. Only five loci could be detected at the 1:200 level. They were DYS426, DYS460, DYS393, DYS389I and H4 Similar results were obtained for the Y-STR 9plex. At the 1:100 mixture level a profile can be seen but at the ratio. Panel C is the result for the Y-STR 20plex of a mixed sample of male:female in a 1:200 ratio. The data indicated that at a 1:100 mixture level, a complete Y-STR 20plex profile can be seen but it obscured by the presence of numerous non-specific amplicons. The loci particularly affected by the presence of these non-specific amplicons (Boxed in Panel B) were DYS390, DYS393, DYS447, and DYS439. At the 1:200 mixture level, allelic drop out of numerous loci was seen. Only five loci could be detected at the 1:200 level. They were DYS426, DYS460, DYS393, DYS389I and H4.

Similar results were obtained for the Y-STR 9plex. At the 1:100 mixture level a profile can be seen but at the 1:200 level loci suffer from allelic dropout. This mixture data was compared to results published by Sinha et al.[116] for the Y-PLEX$^{TM}$ 6 kit. In this study a complete male profile was obtained down to a ratio of 1:125 male to female DNA

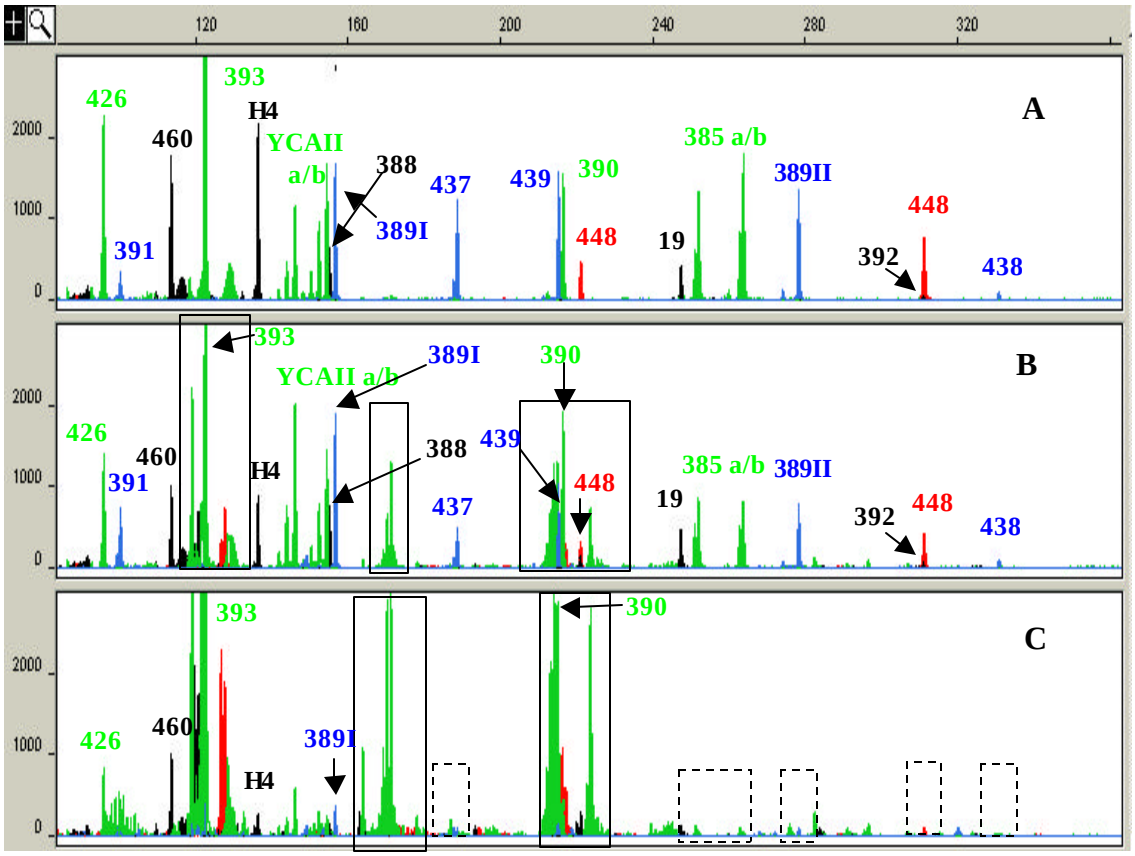



Figure 4-29
GeneScan® Result Obtained Using the Y-STR 20plex on Male:Female Mixture Samples
Panel A, 0.5 ng of male DNA. Panel B, Male:Female Mixture 1(0.5ng):100(50ng). Panel C, Male:Female Mixture 1(0.5 ng):200(100 ng). Solid Boxed areas indicate where non-specific amplicons could impact proper allele designations and dashed boxes indicate allelic drop-out

Additionally, Sinha et al.[116] indicated the presence of two non-specific products at high levels (> 10ng) of female DNA.

In summary, the Y-STR 20plex, Y-STR 9plex, and Y-STR 11plex were as sensitive as commercially available Y-STR kits when used with an increase in the number of PCR cycles. Both the Y-STR 20plex and Y-STR 9plex lacked the male specificity seen in the Y-PLEX™ 6 kit. The Y-STR 11plex was very male specific and had a small number of

non-specific amplicons at high female DNA levels. Higher cycle amplification numbers did increase the sensitivity of the assays, however the increase in non-specific amplicons with excess female DNA template is most likely the result of the increase in PCR amplification cycles.

## Population Studies

Studying the ability of Y-STR markers to differentiate between DNA samples from unrelated male donors is crucial to the forensic science community, particularly in cases of rape and in paternity testing. The primary drawback of Y-STRs in forensic applications is the lack of independence of these markers on the Y chromosome, due to the absence of recombination. Thus, while autosomal STR genotypes can differentiate any two individuals (excluding identical twins) with high statistical probability, Y-STR haplotypes are less likely to differentiate between unrelated males. There are two ways to increase the potential discriminatory capacity of a particular Y-STR haplotype. First, analyze as many Y-STRs as are currently available. Alternatively, one could combine only the most polymorphic markers into Y-STR multiplexes thereby increasing the discriminatory capacity of the resulting Y-STR haplotype. To study the diversity of Y-STR markers and various combinations of markers, Y-STR haplotype reference databases have been developed. Y-STR reference databases serve one main purpose: to offer reliable haplotype diversity estimates for the Y-STR markers.

The first Y-STR haplotype reference database was first made available to the public in February 2000 and is available at http://www.ystr.org.[41] As of February 1, 2003 it contained Y-STR DNA profiles on 12,675 male DNA samples from 83 different European populations. These profiles included information on the European minimal haplotype, with extended haplotype data also available on 3,413 of the 12,675 samples. The database is frequently updated and it takes continuous submissions through the guidance of the International Forensic Y-User Group.[41]

Currently there are two publicly internet available central databases that house Y-STR haplotype on samples from U.S. populations. The first is located at http://www.ystr.org/usa and the second can be found at http://www.reliagene.com. The former database constructed by Kayser et al.[124] had its last entry on June 26, 2002. The database consists of 1705 haplotypes (minimal haplotype markers only) from 30 regional U.S. populations. Of these 1705 haplotypes, 599 are African American, 628 Caucasian (European American) and 478 Hispanics.

More recently, ReliaGene Technologies has constructed a Y-STR database covering, the markers present in their Y-PLEX$^{TM}$ 6 and Y-PLEX$^{TM}$ 5 test kits. Their database as of January 29, 2003 contains Y-STR profiles on 2,563 U.S. samples. Like the www.ystr.org/usa database, ReliaGene's database has Y-STR profiles that include the minimal haplotype. ReliaGene also provides population data on two additional markers not found within the minimal haplotype - DYS438 and DYS439. Unfortunately, neither of these databases provided population information on any of the other markers evaluated in this study. A summary of the current state of U.S. population Y-STR haplotype databases including the data presented here is given in Table 4-10.

Allelic frequency distributions for loci DYS19, DYS388, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS447, DYS448, DYS450, DYS456, DYS458, DYS460, and H4 examined in this study are given in Appendix A-2. The phenotype frequencies for the multi-copy loci DYS385, YCAII, and DYS464 are also given in the Appendix A-2. This appendix contains allelic frequencies for Y-STR markers from three separate United States ethnic groups: African Americans,

Table 4-10
Summary of Y-STR Haplotype Databases of U.S. Populations
The minimal haplotype consists of DYS389I/II, DYS385, DYS390, DYS391, DYS392, and DYS393. The Y-PLEX 6 kit consists of DYS385, DYS389II, DYS390, DYS391, and DYS393. The 27 Y-STR markers include the minimal haplotype + DYS388, DYS426, DYS437, DYS438, DYS439, DYS447, DYS448, DYS450, DYS456, DYS458, DYS460, DYS464, GATA H4, and YCAII.

| Database | Ethnic groups | Sample number (N) | Y-STR markers |
|---|---|---|---|
| http://www.ystr.org/usa | African Americans | 599 | Minimal haplotype |
| | European Americans (Caucasians) | 628 | Minimal haplotype |
| | Hispanics | 478 | Minimal haplotype |
| | Total | 1,705 | 15,345 allele calls |
| http://www.reliagene.com | African Americans | 1,169 | Y-PLEX™ 6 kit |
| | Caucasians | 1,100 | Y-PLEX™ 6 kit |
| | Hispanics | 245 | Y-PLEX™ 6 kit |
| | Native Americans | 49 | Y-PLEX™ 6 kit |
| | Total | 2,563 | 17,941 allele calls* |
| U.S. Population in this study | African American | 260 | 27 Y-STRs |
| | Caucasians | 244 | 27 Y-STRs |
| | Hispanics | 143 | 27 Y-STRs |
| | Total | 647 | 17,469 allele calls |

* For 1,297 of the Reliagene samples Y-STR profiles also include information on DYS389I, DYS392, DYS438, and DYS439. This would increase the total number of allele calls by 5,188.

Caucasians, and Hispanics.

In this study the number of alleles found at each locus ranged from 5 to 11 with the exception of DYS385 a/b, YCAII a/b and DYS464 a/b/c/d. DYS385 a/b and YCAII a/b are duplicated on the Y-chromosome and can yield up to two different amplicons per primer set. DYS464 a/b/c/d is quadruplicated on the Y chromosome. As many as four

Table 4-11
STR Diversity among 22 Y-STRs in U.S. Populations
DYS385, DYS464 and YCAII are multicopy STRs

| Y-STR (N) | Pooled Populus STR diversity (647) | Rank | African American STR diversity (260) | Rank | Caucasian STR diversity (244) | Rank | Hispanic STR diversity (143) | Rank |
|---|---|---|---|---|---|---|---|---|
| DYS464 | 0.956 | 1 | 0.954 | 1 | 0.934 | 1 | 0.937 | 1 |
| DYS385 | 0.912 | 2 | 0.942 | 2 | 0.838 | 2 | 0.901 | 2 |
| YCAII | 0.790 | 3 | 0.797 | 3 | 0.701 | 5 | 0.772 | 4 |
| DYS458 | 0.765 | 4 | 0.758 | 5 | 0.743 | 3 | 0.793 | 3 |
| DYS390 | 0.764 | 5 | 0.664 | 10 | 0.701 | 5 | 0.665 | 13 |
| DYS447 | 0.747 | 6 | 0.767 | 4 | 0.683 | 7 | 0.748 | 5 |
| DYS389II | 0.736 | 7 | 0.722 | 6 | 0.675 | 8 | 0.734 | 6 |
| DYS448 | 0.721 | 8 | 0.722 | 6 | 0.595 | 11 | 0.704 | 8 |
| DYS456 | 0.700 | 9 | 0.671 | 9 | 0.731 | 4 | 0.695 | 9 |
| DYS438 | 0.691 | 10 | 0.560 | 15 | 0.594 | 12 | 0.690 | 10 |
| DYS19 | 0.676 | 11 | 0.722 | 6 | 0.498 | 19 | 0.672 | 12 |
| DYS439 | 0.656 | 12 | 0.636 | 11 | 0.639 | 9 | 0.717 | 7 |
| DYS437 | 0.637 | 13 | 0.499 | 17 | 0.583 | 13 | 0.624 | 14 |
| H4 | 0.611 | 14 | 0.612 | 12 | 0.562 | 14 | 0.609 | 15 |
| DYS392 | 0.609 | 15 | 0.434 | 20 | 0.596 | 10 | 0.673 | 11 |
| DYS460 | 0.570 | 16 | 0.568 | 14 | 0.555 | 15 | 0.556 | 18 |
| DYS389I | 0.549 | 17 | 0.531 | 16 | 0.538 | 17 | 0.596 | 16 |
| DYS391 | 0.534 | 18 | 0.447 | 19 | 0.552 | 16 | 0.577 | 17 |
| DYS426 | 0.519 | 19 | 0.375 | 21 | 0.482 | 20 | 0.522 | 19 |
| DYS450 | 0.489 | 20 | 0.487 | 18 | 0.177 | 22 | 0.414 | 21 |
| DYS393 | 0.485 | 21 | 0.586 | 13 | 0.363 | 21 | 0.448 | 20 |
| DYS388 | 0.365 | 22 | 0.246 | 22 | 0.501 | 18 | 0.312 | 22 |

peaks were observed with a single DYS464 primer pair. DYS385 a/b and YCAII a/b exhibited 56 and 27 phenotypes respectively for the three populations combined. The DYS464 a/b/c/d locus was even more variable with 110 phenotypes being found. The STR diversity values for each of the 22 Y-STR markers in each population is shown in Table 4-11. The higher the STR diversity value, the more polymorphic the marker. All of the markers were ranked according to their STR diversity value by each individual population and then as the total or "pooled" population. It is not generally recommended to pool allelic frequencies from different ethnic groups (i.e. African Americans and

Caucasians) due to statistically significant differences that may exist between these ethnic groups.[124] However, a pooled ranking was given to illustrate the point that even though a marker may not be particularly diverse for a given population it may be valuable in differentiating between samples of different ethnicity.

An example of different allelic frequency distributions that could exist between ethnic groups was seen when the data for DYS390 was examined. DYS390 is one of the markers currently within the minimal haplotype and was in a majority of the Y-STR multiplex assays used in this study. It was the only marker studied that was not one of the ten most polymorphic markers for each population but jumped into the top ten when the allelic frequency data was pooled. When taken separately DYS390 ranked $10^{th}$, $6^{th}$, and $13^{th}$ for the African American, Caucasian and Hispanic population respectively. This ranking jumped to $5^{th}$ when the allelic frequencies were pooled (Table 4-11). The allelic frequency distribution of DYS390 for each ethnic group was plotted in Figure 4-30 to further illustrate this phenomenon. The most common allele for DYS390 in African Americans contains 21 repeats. While the most frequent DYS390 allele for the Caucasians and Hispanics is allele 24. One could theorize that when a DYS390 allele of 21 repeats is found that there was a strong likelihood that the sample analyzed was from an African American.

The STR diversity values for each Y-STR marker also showed that even if a marker had a sizeable allele range it may not have been that polymorphic. The Y-STR DYS392 marker provides a good example of that phenomenon. The allele range obtained in this population study for DYS392 was 7-16. However, for African Americans it is one of

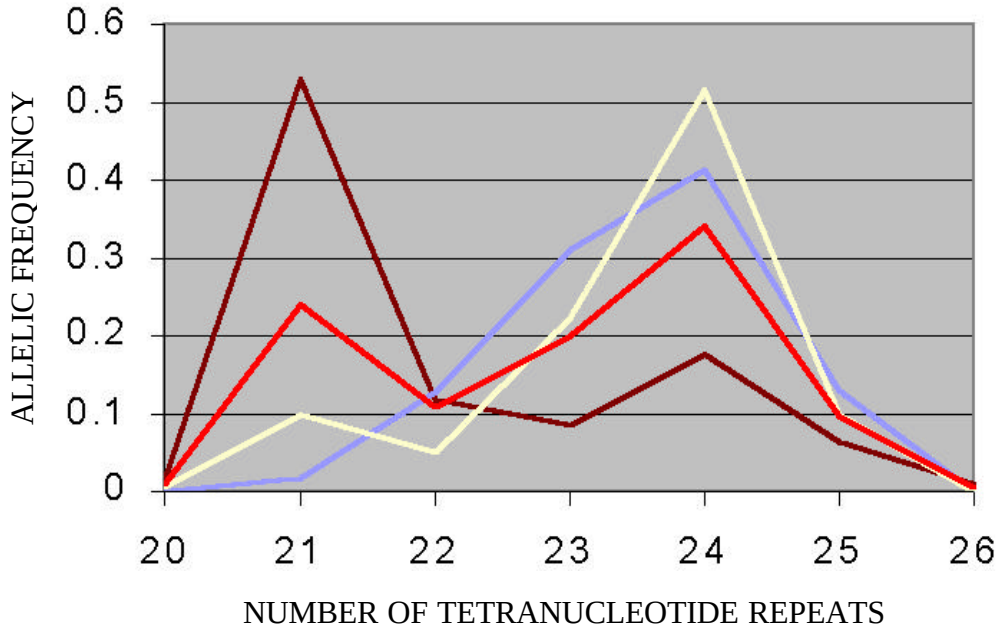



Figure 4-30
DYS390 Allelic Frequency Distribution
Lavender = African American, Blue= Caucasian, Yellow = Hispanic and Red = "pooled" population, allelic frequencies for all three ethnic groups were combined.

the least polymorphic, with a STR diversity value of 0.434. This was due to the fact that a majority of the alleles for DYS392 in African Americans are in the range of 11-13 with over 70% of the allele having the value of 11 (Appendix A-2). Thus, markers with both large allele ranges that are equally represented are the most polymorphic and often considered the most desirable. Unfortunately, even though a marker was polymorphic for one ethnic group, it may have been less polymorphic in another. For example, DYS392 was ranked as the 20$^{th}$ most polymorphic markers in African Americans but ranked 10$^{th}$ and 11$^{th}$ in the Caucasian and Hispanic populations respectively (Table 4-11). This STR diversity disparity was also seen in the case of DYS393. In the African American

population DYS393 was ranked 13th with a STR diversity value 0.586, but fell to a ranking of 21st and 19th in the Caucasian and Hispanic populations respectively.

The data indicated the most polymorphic markers (i.e. containing the highest STR diversity values) across the populations were the multi-copy loci DYS385, YCAII, and DYS464. The STR diversity values ranged from a low of 0.177 for DYS450 in Caucasians to a high of 0.956 for DYS464 in African Americans (Table 4-11). Of the top ten Y-STR markers, according to STR diversity values, in each ethnic group, seven were common to each population. These markers included DYS464 a/b/c/d, DYS385 a/b, YCAII a/b, DYS447, DYS456, DYS458, and DYS389II. Only two of these Y-STRs (DYS385 a/b and DYS389II) are in the European "minimal haplotype" and present in commercially available test kits.

The "complete" Y-STR haplotypes, consisting of the 22 Y-STR markers, for each U.S. population (African American, Caucasian, and Hispanic) are listed in Appendix A-3. Various combinations of Y-STR markers were studied in order to ascertain their respective discriminatory capacity. The various combinations included the Y-STR 20plex, Y-STR 9plex (minimal haplotype), extended haplotype, Y-STR 11plex, Y-PLEX™ 6 and Y-PLEX™ 5 kit markers, and the top ten most polymorphic markers minus YCAII a/b as ranked in Table 4-11 for the pooled population. These combinations of markers were chosen for comparison purposes for several reasons.

First, the "minimal haplotype" is used by Europeans in court cases.[42] Second, the extended haplotype was studied to see if YCAII could be replaced with a different polymorphic marker. YCAII was first included in the extended haplotype because it is

highly polymorphic and increased the number of unique haplotypes in the European database.[41] Unfortunately, YCAII is a dinucleotide repeat and is not an ideal marker when studying samples involving male:male mixtures due to a high degree of stutter.[39]

Third, the Y-PLEX$^{TM}$ 6 kit is one of only two commercially available Y test kits. Most forensic laboratories in the United States do not make their own primers and thus would only use what is commercially available. Fourth, during the January 2003 Scientific Working Group on DNA Analysis Methods (SWGDAM) meeting, the subcommittee on the Y chromosome decided that the core Y-STR loci to be used by the United States (U.S. haplotype) would be the minimal haplotype loci + DYS438 and DYS439. The reasoning behind this was two fold. One, the U.S. set of Y-STR markers should include the minimal haplotype markers widely used in Europe and second markers DYS438 and DYS439 that are in the commercially available Y-PLEX$^{TM}$ 5 kit should also be included. The discriminatory capacity of Y-PLEX$^{TM}$ 5 kit was not examined because it did not contain DYS385, the most polymorphic Y-STR marker currently in Y-STR Reference Haplotype Databases. Finally, the haplotype diversity of such combinations of markers as theY-STR 20plex, Y-STR 11plex, and the most polymorphic markers according to their STR diversity values were examined to see if any of the other markers not currently within the United States haplotype would make a potential Y-STR DNA profile more discriminatory.

The haplotype diversity and accompanying random match probability for each combination is shown in Table 4-12. The Y-PLEX$^{TM}$ 6 kit had the smallest haplotype diversity (HD) value for all three ethnic groups because it possessed the fewest markers.

Table 4-12
Haplotype Diversity and Random Match Probability for Y-STR Marker Combinations

| Y-STR Marker Combinations | African American (260) | | Caucasian (244) | | Hispanic (143) | |
|---|---|---|---|---|---|---|
| | HD* | RM@ Probability | HD* | RM@ Probability | HD* | RM@ Probability |
| Y-PLEX 6 kit | 0.9974 | 0.0026 | 0.9914 | 0.0086 | 0.9934 | 0.0066 |
| "minimal" haplotype | 0.9982 | 0.0018 | 0.9946 | 0.0053 | 0.9957 | 0.0043 |
| "extended" haplotype | 0.9988 | 0.0012 | 0.9971 | 0.0029 | 0.9975 | 0.0025 |
| "U.S. haplotype" | 0.9993 | 0.0007 | 0.9974 | 0.0026 | 0.9986 | 0.0014 |
| Y-STR 11plex | 0.9993 | 0.0007 | 0.9987 | 0.0013 | 0.9992 | 0.0008 |
| Y-STR 20plex | 0.9998 | 0.0002 | 0.9998 | 0.0002 | 0.9998 | 0.0002 |
| 22 Y-STR Markers | 0.9999 | 0.0001 | 0.9999 | 0.0001 | 0.9999 | 0.0001 |
| 10 Most Polymorphic | 0.9999 | 0.0001 | 0.9999 | 0.0001 | 0.9999 | 0.0001 |

*HD = Haplotype Diversity
@ RM = Random Match

Table 4-13
Discriminatory Capacity and Unique Number of Haplotypes for U.S. Populations

| Y-STR Marker Combinations | African American (260) | | Caucasian (244) | | Hispanic (143) | |
|---|---|---|---|---|---|---|
| | DC* | UH@ | DC* | UH@ | DC* | UH@ |
| Y-PLEX 6 kit | 82.3% | 188 | 68.9% | 136 | 78.3% | 97 |
| "minimal" haplotype | 88.5% | 213 | 75.8% | 161 | 81.1% | 100 |
| "extended" haplotype | 91.9% | 227 | 83.6% | 184 | 89.5% | 120 |
| "U.S. haplotype" | 91.9% | 222 | 82.3% | 176 | 93.3% | 121 |
| Y-STR 11plex | 93.1% | 227 | 88.5% | 198 | 94.4% | 127 |
| Y-STR 20plex | 98.5% | 252 | 97.2% | 230 | 98.6% | 139 |
| 22 Y-STR Markers | 98.9% | 254 | 99.6% | 242 | 99.3% | 141 |
| 10 Most Polymorphic | 96.9% | 244 | 97.5% | 232 | 99.3% | 141 |

* (DC)Discriminatory capacity (%) = Number of different Haplotypes observed/population (N)
@ (UH) Unique Haplotype is defined as a haplotype that occurs only once in a given population (N)

Its HD in African Americans was 0.9974 but only 0.9914 in Caucasians (Table 4-12). In general, the HD value and random match probability (RM) of various combinations grew in each population as the number of markers increased. When all 22 markers were included in the calculation of HD, its value reached 0.9999 for all populations with a RM of only 0.01%. By just looking at HD values an argument can be made that no significant benefit in discrimination is obtained by increasing the number of markers past those in the extended or U.S. haplotype. For example, in African Americans the HD value for the U.S. haplotype is 0.9933. This is 0.0056 less than the 0.9999 value for the

22 Y-STR markers. However, examining the discriminatory capacity and number of unique haplotypes observed per population illustrates the value of increasing the number of markers analyzed.

The discriminatory capacity and the number of unique haplotypes for each Y-STR marker combination studied are given in Table 4-13. The discriminatory capacity (DC) is a measure of how many different haplotypes were observed for a given population. For example in the case of Caucasians, there were 168 different Y-PLEX$^{TM}$ 6 kit haplotypes observed. Of those 168 different haplotypes; 136 occurred once, 20 occurred twice, 5 occurred 3 times, 1 occurred 4 times, 2 occurred 5 times, 2 occurred 8 times, 1 occurred 11 times and 1 occurred 12 times. For Caucasians, the Y-PLEX$^{TM}$ 6 kit markers had a DC of 68.9% (Table 4-13). While the Y-PLEX$^{TM}$ 6 kit had a 0.9914 HD value for Caucasians, a significant portion of the population was not resolved using the markers in the Y-PLEX$^{TM}$ 6 kit. When all 22 markers are included in the calculation of DC the number of different haplotypes moves from 168 to 242. With the exception of two samples, all of the Caucasians were distinguishable using the 22 marker haplotype.

The discriminatory capacity of the minimal haplotype for each population grew to over 88% in African Americans but was only 75.8% in Caucasians and 81.1% in Hispanics (Table 4-13). This difference in discriminatory capacity between the ethnic groups was most likely due to the phenotype frequency of DYS385. In Hispanics and Caucasians, the 11-14 phenotype accounted for 28.0% and 36.9% of the observed phenotypes. In the African American population studied, there wasn't one phenotype that showed up more than 14% of the time. Still, DYS385 is a marker that should be

included in any multiplex because of its large STR diversity value across populations. The addition of the dinucleotide repeat YCAII to the minimal haplotype, called the extended haplotype, improved the discriminatory capacity of some of the populations by over 8% (Table 4-13). The U.S. haplotype has substituted DYS438 and DYS439 for the dinucleotide repeat YCAII. Taken together, the Y-PLEX$^{TM}$ 6 and Y-PLEX$^{TM}$ 5 kits test for the U.S. haplotype.

The discriminatory capability of the European "extended" haplotype was compared to the U.S. haplotype for all three ethnic groups (Table 4-13). The U.S. haplotype was as discriminatory as the "extended haplotype" for African Americans. For African Americans (N=260), there were 239 U.S. haplotypes (222 of which were unique) and 239 extended haplotypes (227 of which were unique). The discriminatory capacity was identical (91.9%) for each population. For Hispanics (N=143), the U.S. haplotype was more discriminatory than the extended haplotype. There were 128 different extended haplotypes for Hispanics and 133 different U.S. haplotypes. The extended haplotype for Caucasians was slightly more diverse than the U.S. haplotype with 204 different "extended" haplotypes vs. 201 U.S. haplotypes. The data collected for this study appears to indicate that the U.S. haplotype offers a viable alternative to the European "extended" haplotype. The DYS438 and DYS439 could replace YCAII without any appreciable loss in discriminatory capability and without the stutter products seen in YCAII amplicons.

The Y-STR 20plex, which included the extended haplotype plus numerous other Y-STR markers (see section on selection of loci to be included in the Y-STR 20plex), was highly diverse. The discriminatory capacity ranged from 97.2% in Caucasians to 98.6%

in Hispanics (Table 4-13). However, the Y-STR 20plex contains markers such as DYS388 and DYS426 that are not highly polymorphic and did not aid in discriminating between individuals. The markers in the Y-STR 20plex other than those in the U.S. haplotype that were the most useful in discerning between unrelated individuals were DYS447 and DYS448. These markers are in both the Y-STR 20plex and the Y-STR 11plex.

The Y-STR 11plex contained some of the most polymorphic markers examined in this study. With the exception of DYS450, all of the Y-STR 11plex markers were ranked as one of the 10 most polymorphic markers for each ethnic group (See Table 4-11). Two of these markers, DYS464 and DYS385 are ranked 1 and 2 respectively for each population. Thus, the Y-STR 11plex generates highly diverse Y-STR profiles. The Y-STR 11plex had 227 unique haplotypes for African Americans out of 260, 198 out of 240 for Caucasians and 127 out of 143 for Hispanics (Table 4-13). Y-STR 11plex's discriminatory capability was better than that of the U.S. haplotype for all three U.S. populations. There were 198 unique Y-STR 11plex Caucasian haplotypes observed next to only 176 unique U.S. haplotypes. In African Americans the Y-STR 11plex exhibited 227 unique haplotypes next to 222 unique U.S. haplotypes. Finally, there were 127 unique Y-STR 11plex haplotypes for Hispanics compared to121 unique U.S. haplotypes (Table 4-13). The discriminatory power of the Y-STR 11plex could be directly linked to the new Y-STR marker DYS464. Its STR diversity value was 0.930 or greater for each population and it should be included in any future Y-STR multiplex development (Table 4-11).

As expected, the greatest diversity of Y-STR haplotypes was seen when all 22 markers were used. Out of 647 samples examined, six Y-STR haplotypes occurred twice, one profile occurred three times. Thus, there were a total of 632 unique Y-STR haplotypes in this data set. The haplotypes for the samples occurring more than once are shown in Table 4-14. Four of the six duplicates were population specific. The other two pairs matched an African American with a Caucasian, and a Hispanic with a Caucasian. The haplotype which occurred three times involved two Hispanics and one African American. The most common minimal haplotype[41] found in the pooled population was the one listed for sample PT84213 and Ca1 (Table 4-14). This particular minimal haplotype occurred 25 times in the population set listed in Appendix A-3. The other 23

Table 4-14
Y-STR Haplotype Data for Y-STR Profiles Occurring More than Once.
The minimal haplotype for each set is in bold print.
AA= African Americans, CAU=Caucasians, and HIS = Hispanics

| Sample ID | Ethnicity | Y haplotype (DYS19,DYS389I,DYS389II,DYS390,DYS391,DYS392, DYS393,DYS385 a/b,DYS426,DYS388,DYS437, DYS438, DYS439, DYS447,DYS448,DYS450,DYS456, DYS458,DYS460,H4,YCAII a/b, DYS464 a/b/c/d) |
|---|---|---|
| WA29612 | CAU | **14,13,29,23,11,13,12,12-14**,12,12,15,12,12,21,23,9,15,17,11, |
| WT62482 | CAU | 12,19-23,15-17 |
| PT84213 | AA | **14,14,31,22,10,11,12,12-15**,11,15,15,9,12,26,24,9,15,15,10, |
| TT51696 | CAU | 11,19-22,13-13.1-15-16 |
| PT84633 | HIS | **14,13,29,24,11,13,13,11-14**,12,12,14,12,11,25,23,9,15,17,11 |
| Ca1 | CAU | 12,19-23,15-16-17 |
| JT51499 | AA | **14,12,28,22,11,11,13,13-14**,11,14,16,10,11,24,24,9,15,14,10 |
| AA9 | AA | 11,19-21,12-14-14-16 |
| PT84253 | AA | **16,13,30,21,10,11,13,16-17**,11,12,14,11,11,25,26,8,15,18,10 |
| ZT79304 | AA | 12,19-19,13-15-16 |
| JT51484 | AA | **17,13,30,21,10,11,13,17-19**,11,12,14,11,13,27,25,8,16,16,11 |
| ZT79620 | AA | 11,19-21,13-15-16 |
| PT84348 | HIS | **13,15,31,24,9,11,13,13-14**,11,12,14,10,10,23,24,8,16,18,12 |
| ZT80369 | HIS | 12,19-24,14-16-17 |
| PT83904 | AA | |

samples were completely resolved using markers beyond the minimal haplotype. The PT84213 and Ca1 minimal haplotype was found to match 9 out of 599 African Americans, 25 out of 628 Caucasians and 19 out of 478 Hispanic samples in the U.S. Population database constructed by Kayser et al.[41] The discriminatory capacity percentage for the 22 Y-STR haplotype was over 98% for each population analyzed in this study (Table 4-13). It would be interesting to see if the non-minimal haplotype markers in this work could resolve any of the matches found in the Kayser et al.[30] database. Kasyer et al.[30] suggested in some of the earliest population studies that the only way to truly increase discriminatory capacity using Y-STR markers was to examine as many markers as possible. Unfortunately, many forensic laboratories do not possess the capabilities to study numerous Y-STR markers.

The top ten most diverse Y-STR markers for the pooled population minus the stutter prone YCAII were examined as a set. Included in this set are: DYS464, DYS447, DYS448, DYS456, DYS458, DYS390, DYS438, DYS389I, and DYS389II. DYS389I was included in this group because the primers used in this study to amplify DYS389II would also amplify DYS389I. The discriminatory capacity of these markers was similar to the 22 Y-STR profile. For Caucasians, there were only 10 more unique Y-STR 22 marker profiles than haplotypes originating from the top ten most diverse markers (Table 4-13). In African Americans this difference dropped to 9 and in Hispanics there was no difference between the Y-STR 22 marker profile and the profile obtained by using only the most polymorphic markers.

It has been shown that Y-STR 20plex and Y-STR 11plex designed in this study were highly discriminatory. The data also indicates that the new U.S. haplotype could be even more diverse than the extended haplotype in U.S. populations and that markers DYS438 and DYS439 were good alternatives to the stutter prone YCAII a/b. Additionally, future multiplex work should include the most polymorphic markers presented here such as DYS464 a/b/c/d, DYS456, DYS458, DYS447 and DYS448.

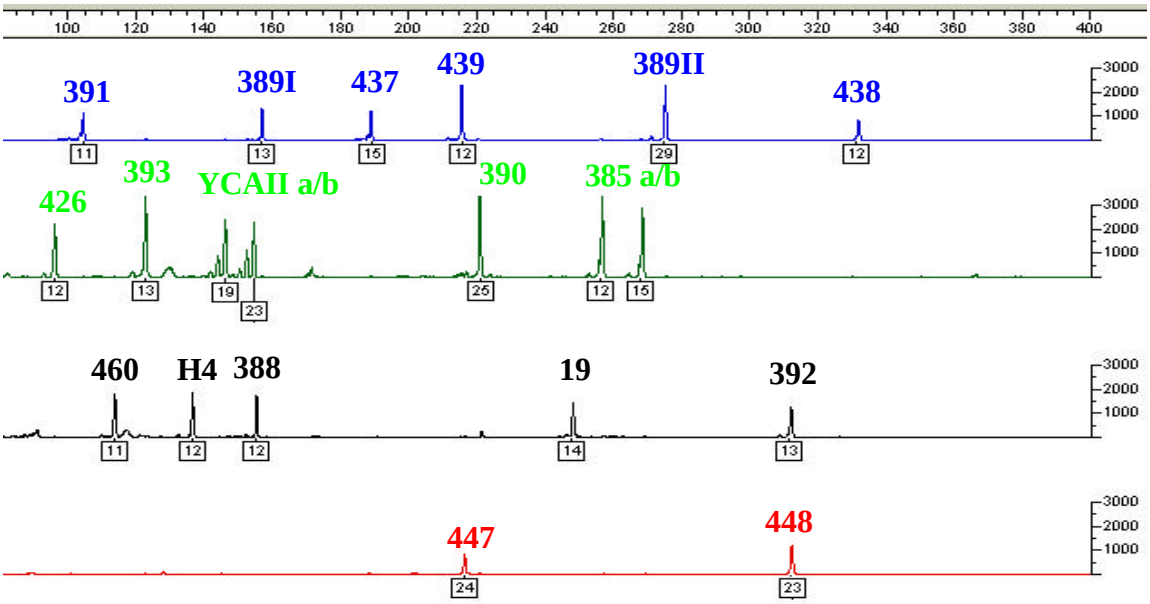
Prototype Standard Reference Material® 2395
Human Y-Chromosome DNA
Profiling Standard

The Standard Reference Material (SRM) 2395 is intended for use in the standardization and paternity quality assurance procedures for PCR-based genetic testing and for instructional law enforcement or non-clinical research purposes that involve the Y chromosome. This SRM can also be used for quality assurance when assigning values to in-house control materials.

Over 144 different DNA samples (66 genomic and 78 cell lines) were examined as part of the selection of six candidate samples for SRM 2395. Five male genomic DNA samples (labeled A-E) and one female genomic DNA sample (labeled F) were selected based on diversity of Y-STR haplotypes obtained. Following the initial screening, each of the proposed SRM components were characterized. This characterization was a two fold process. First, all of the samples were typed with 4 different Y STR multiplexes: ReliaGene Technologies Y-PLEX™ 6 kit, ReliaGene Y-PLEX™ 5 kit , the Y-STR 20plex and the Y-STR 11plex. Second, the Y-STR loci for each of the male samples were sequenced to provide certified values (number of repeats) for these same Y-STR markers.

Each one of the SRM components was tested numerous times with the Y-STR multiplex PCR assays mentioned above. The overlapping loci between Y-STR multiplexes permitted an evaluation of concordance in allele designations for Y-STR markers common to each multiplex. For example, the Y-PLEX™ 6 kit allele calls given in panel B of Figure 4-31 matched the allele calls for the common loci shown in panel A

A) Y-STR 20plex




B) Y-PLEX$^{TM}$ 6 kit

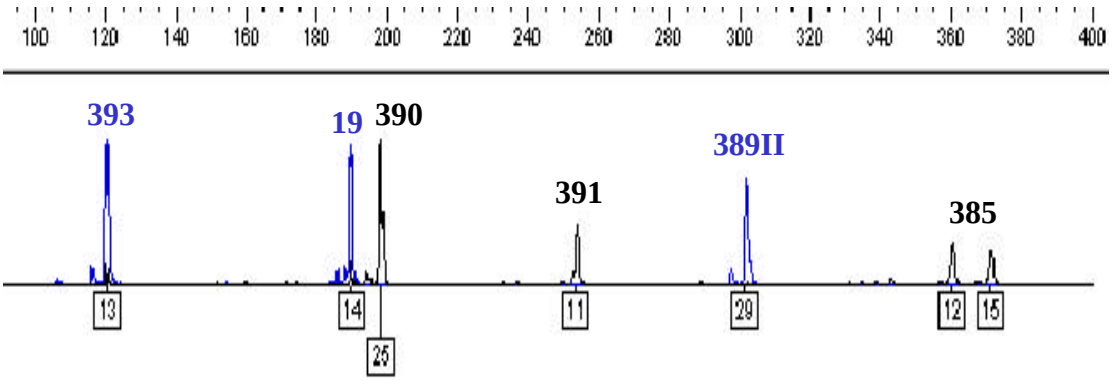



Figure 4-31
Genotyper® Result of SRM Component A Using the Y-STR 20plex and Y-PLEX$^{TM}$ 6 Kit Panel A is the result for the Y-STR 20plex. Panel B is the result of the Y-STR 11 plex. There is complete concordance between common markers in each multiplex.

using the Y-STR 20plex. All of the loci typed using both Reliagene Y-PLEX$^{TM}$ 6 and Y-PLEX$^{TM}$ 5 kits, can be found within the Y-STR 20plex assay. These markers included DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, and DYS439. A comparison of Y chromosome haplotypes for the five SRM components found out that out of 55 possible common allele designations there was complete concordance between the Y-PLEX$^{TM}$ 6 and 5 kit, and the Y-STR 20plex. Additionally, the allele calls for markers DYS385 a/b, DYS447 and DYS448 that are common to both the Y-STR 20plex and the Y-STR 11plex were also compared. Again, all were in complete concordance.

For a number of markers, primers used in the commercial kits were different than those in the Y-STR 20plex and Y-STR 11plex. This resulted in different sized amplicons for the same markers. Even though the base pair size was different for a particular amplicon it still had the same repeat number. For example, DYS391 in component A consists of 11 TCTA repeats. It has a base pair size of approximately 105 bp for the Y-STR 20plex and 255 bp for the Y-PLEX$^{TM}$ 6 kit from Reliagene (Figure 4-31).

The Y-STR haplotypes for each of the components of the SRM are given in Tables 4-15 and 4-16. Table 4-15 contains all the markers in the certified category and the Table 4-16 contains all the markers in the "informational only" category. A certified value indicated that a particular allele had been compared to an allelic ladder from a commercial kit (i.e., Y-PLEX$^{TM}$ 6 kit), and/or it has been sequenced. The markers with certified values include those within the European minimal haplotype, plus DYS426, DYS435, DYS436, DYS437, DYS438, DYS438, DYS439, DYS447, DYS448, DYS460,

Table 4-15
Y-STR Haplotypes for SRM 2395 Components A-E. “Certified” Category

| Component ID | Description | DYS19 | DYS389I | DYS389II | DYS390 | DYS391 | DYS392 | DYS393 | DYS385 a/b | DYS426 | DYS388 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Male 1 | 14 | 13 | 29 | 25 | 11 | 13 | 13 | 12-15 | 12 | 12 |
| B | Male 2 | 14 | 13 | 28 | 23 | 11 | 11 | 12 | 14-17 | 11 | 15 |
| C | Male 3 | 16 | 14 | 32 | 21 | 12 | 11 | 13 | 17-20 | 11 | 12 |
| D | Male 4 | 15 | 12 | 28 | 22 | 10 | 11 | 14 | 14-15 | 11 | 12 |
| E | Male 5 | 17 | 14 | 31 | 24 | 12 | 12 | 14 | 13-15 | 11 | 13 |
| F | Female | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

| Component ID | Description | DYS435 | DYS436 | DYS437 | DYS438 | DYS439 | DYS447 | DYS448 | DYS460 | DYS461 | DYS462 | H4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Male 1 | 12 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 11 | 12 |
| B | Male 2 | 11 | 12 | 14 | 9 | 12 | 25 | 25 | 10 | 13 | 11 | 12 |
| C | Male 3 | 11 | 12 | 14 | 11 | 11 | 25 | 25 | 9 | 13 | 12 | 12 |
| D | Male 4 | 11 | 12 | 16 | 11 | 11 | 23 | 25 | 11 | 11 | 13 | 12 |
| E | Male 5 | 11 | 12 | 14 | 10 | 11 | 26 | 24 | 11 | 12 | 12 | 12 |
| F | Female | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 4-16
Y-STR Haplotypes for SRM 2395 Components A-E. "Informational" Category

| Component ID | Description | DYS450 | DYS456 | DYS458 | DYS464 a/b/c/d | YCAII a/b |
|---|---|---|---|---|---|---|
| A | Male 1 | 10 | 15 | 16 | 14-15-17 | 19-23 |
| B | Male 2 | 10 | 15 | 15 | 12-13-17 | 19-22 |
| C | Male 3 | 8 | 15 | 17 | 13-16-18 | 19-19 |
| D | Male 4 | 9 | 15 | 16 | 13-14 | 20-20 |
| E | Male 5 | 10 | 15 | 16 | 11-14-15 | 19-21 |
| F | Female | -- | -- | -- | -- | -- |

DYS461, DYS462, DYS388 and GATA H4. An informational value indicates that an allele call has been based solely on sequence information provided in GenBank and cannot be confirmed through comparison to an allelic ladder or DNA sequencing of the Y-STR marker. The markers considered informational only are DYS450, DYS456, DYS458, DYS464 a/b/c/d, and YCAII. All loci present in the Y-PLEX$^{TM}$ 6 and Y-PLEX$^{TM}$ 5 kits have been certified through sequencing the 5 male components of SRM 2395. The forensic DNA typing community has become increasingly dependent on commercial kits. Thus, by using the Y SRM 2395, a forensic laboratory could validate a Y-STR protocol using commercially available Y-STR kits according to established validation guidelines.[5]

## Sequence Analysis

Currently, 22 markers including all of those in the European minimal haplotype have been sequenced for each of the components. The DNA sequencing included both the top and bottom strand of each Y-STR marker. This translated into over 27,000 separate base calls analyzed. The top and bottom strands for each marker were aligned and a consensus

sequence was constructed. Sequencing both the top and the bottom strand served as a method to confirm the DNA sequences obtained. For example, if the top strand of a Y-STR marker had 10 tetranucleotide repeats it would be expected that the reverse or bottom strand would have 10 tetranucleotide repeats as well. Additionally, if a polymorphism was identified in the top strand, its presence could be confirmed by looking for its complementary base in the opposing strand.

The only locus from the European extended haplotype that was unable to be sequenced was the YCAII locus. YCAII a/b is a dinucleotide repeat and typically produces a measurable amount of stutter product two or even four bases shorter than the corresponding main allele peak. As a result, consensus sequence analysis of PCR products can lead to inhomogeneous results. An attempt was made to separate the YCAII amplicon from its stutter products using gel electrophoresis. Alleles from the other loci with multiple amplicons including 389I/II and DYS385 a/b were successfully separated and eventually sequenced (see materials and methods). Unfortunately, attempts at separating alleles and the resulting stutter products from the duplicated YCAII locus using gel electrophoresis have been unsuccessful. This is most likely due to the fact that YCA II alleles and/or their corresponding stutter products are often separated by only two dinucleotide repeats, making their separation on a gel challenging. YCAII a/b alleles have only been successfully sequenced through the use of cloning.[110]

Sequence data for each of the markers is summarized in Table 4-17. The sequencing data helped to serve a number of purposes. First, it confirmed the allele calls made by each of the Y-STR multiplex assays. There was complete concordance between the

Table 4-17
Sequence Data for Y-STR Loci
Sequence Data obtained with Big Dye v. 3 sequencing kits. All sequencing results were generated at NIST with the exception of DYS462 from Leiden University. Peter de Knijff's group at Leiden also confirmed the NIST sequencing results at DYS19, DYS388, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS460, and DYS461.

| | | | |
|---|---|---|---|
| DYS19 | A | 14 | $[TAGA]_3$ tagg$[TAGA]_{11}$ |
| | B | 14 | $[TAGA]_3$ tagg$[TAGA]_{11}$ |
| | C | 16 | $[TAGA]_3$ tagg$[TAGA]_{13}$ |
| | D | 15 | $[TAGA]_3$ tagg$[TAGA]_{12}$ |
| | E | 17 | $[TAGA]_3$ tagg$[TAGA]_{14}$ |

| | | | |
|---|---|---|---|
| DYS438 | A | 12 | $[TTTTC]_{12}$ |
| | B | 9 | $[TTTTC]_9$ |
| | C | 11 | $[TTTTC]_{11}$ |
| | D | 11 | $[TTTTC]_{11}$ |
| | E | 10 | $[TTTTC]_{10}$ |

| | | | |
|---|---|---|---|
| DYS388 | A | 12 | $[ATT]_{12}$ |
| | B | 15 | $[ATT]_{15}$ |
| | C | 12 | $[ATT]_{12}$ |
| | D | 12 | $[ATT]_{12}$ |
| | E | 13 | $[ATT]_{13}$ |

| | | | |
|---|---|---|---|
| DYS439 | A | 12 | $[GATA]_{12}$ |
| | B | 12 | $[GATA]_{12}$ |
| | C | 11 | $[GATA]_{11}$ |
| | D | 11 | $[GATA]_{11}$ |
| | E | 11 | $[GATA]_{11}$ |

| | | | |
|---|---|---|---|
| DYS391 | A | 11 | $[TCTA]_{11}$ |
| | B | 11 | $[TCTA]_{11}$ |
| | C | 12 | $[TCTA]_{12}$ |
| | D | 10 | $[TCTA]_{10}$ |
| | E | 10 | $[TCTA]_{10}$ |

| | | | |
|---|---|---|---|
| DYS460 | A | 11 | $[ATAG]_{11}$ |
| | B | 10 | $[ATAG]_{10}$ |
| | C | 9 | $[ATAG]_9$ |
| | D | 11 | $[ATAG]_{11}$ |
| | E | 11 | $[ATAG]_{11}$ |

| | | | |
|---|---|---|---|
| DYS392 | A | 13 | $[TAT]_{13}$ |
| | B | 11 | $[TAT]_{11}$ |
| | C | 11 | $[TAT]_{11}$ |
| | D | 11 | $[TAT]_{11}$ |
| | E | 12 | $[TAT]_{12}$ |

| | | | |
|---|---|---|---|
| DYS461 | A | 12 | $[TAGA]_{11}$CAGA |
| | B | 13 | $[TAGA]_{12}$CAGA |
| | C | 13 | $[TAGA]_{12}$CAGA |
| | D | 11 | $[TAGA]_{10}$CAGA |
| | E | 12 | $[TAGA]_{11}$CAGA |

| | | | |
|---|---|---|---|
| DYS393 | A | 13 | $[AGAT]_{13}$ |
| | B | 12 | $[AGAT]_{12}$ |
| | C | 13 | $[AGAT]_{13}$ |
| | D | 14 | $[AGAT]_{14}$ |
| | E | 14 | $[AGAT]_{14}$ |

| | | | |
|---|---|---|---|
| DYS462 | A | 11 | $[TATG]_{11}$ |
| | B | 11 | $[TATG]_{11}$ |
| | C | 12 | $[TATG]_{12}$ |
| | D | 13 | $[TATG]_{13}$ |
| | E | 12 | $[TATG]_{12}$ |

| | | | |
|---|---|---|---|
| DYS426 | A | 12 | $[GTT]_{12}$ |
| | B | 11 | $[GTT]_{11}$ |
| | C | 11 | $[GTT]_{11}$ |
| | D | 11 | $[GTT]_{11}$ |
| | E | 11 | $[GTT]_{11}$ |

| | | | |
|---|---|---|---|
| DYS385a | A | 12 | $[GAAA]_{12}$ |
| | B | 14 | $[GAAA]_{14}$ |
| | C | 17 | $[GAAA]_{17}$ |
| | D | 14 | $[GAAA]_{14}$ |
| | E | 13 | $[GAAA]_{13}$ |

| | | | |
|---|---|---|---|
| DYS435 | A | 12 | $[TGGA]_{12}$ |
| | B | 11 | $[TGGA]_{11}$ |
| | C | 11 | $[TGGA]_{11}$ |
| | D | 11 | $[TGGA]_{11}$ |
| | E | 11 | $[TGGA]_{11}$ |

| | | | |
|---|---|---|---|
| DYS385b | A | 15 | $[GAAA]_{15}$ |
| | B | 17 | $[GAAA]_{17}$ |
| | C | 20 | $[GAAA]_{20}$ |
| | D | 15 | $[GAAA]_{15}$ |
| | E | 15 | $[GAAA]_{15}$ |

Table 4-17 (Continued)

| | | | |
|---|---|---|---|
| DYS389I | A | 13 | $[TCTG]_3\ [TCTA]_{10}$ |
| | B | 13 | $[TCTG]_3\ [TCTA]_{10}$ |
| | C | 14 | $[TCTG]_3\ [TCTA]_{11}$ |
| | D | 12 | $[TCTG]_3\ [TCTA]_9$ |
| | E | 14 | $[TCTG]_3\ [TCTA]_{11}$ |
| DYS389II | A | 29 | $[TCTG]_5\ [TCTA]_{11}$ … $[TCTG]_3\ [TCTA]_{10}$ |
| | B | 28 | $[TCTG]_5\ [TCTA]_{10}$ … $[TCTG]_3\ [TCTA]_{10}$ |
| | C | 32 | $[TCTG]_6\ [TCTA]_{12}$ … $[TCTG]_3\ [TCTA]_{11}$ |
| | D | 28 | $[TCTG]_5\ [TCTA]_{11}$ … $[TCTG]_3\ [TCTA]_9$ |
| | E | 31 | $[TCTG]_5\ [TCTA]_{12}$ … $[TCTG]_3\ [TCTA]_{11}$ |
| DYS390 | A | 25 | $[TCTG]_8\ [TCTA]_{12}\ [TCTG]_1\ [TCTA]_4$ |
| | B | 23 | $[TCTG]_8\ [TCTA]_{10}\ [TCTG]_1\ [TCTA]_4$ |
| | C | 21 | $[TCTG]_8\ [TCTA]_5$ ACTA $[TCTA]_2\ [TCTG]_1\ [TCTA]_4$ |
| | D | 22 | $[TCTG]_8\ [TCTA]_9\ [TCTG]_1\ [TCTA]_4$ |
| | E | 24 | $[TCTG]_8\ [TCTA]_{11}\ [TCTG]_1\ [TCTA]_4$ |
| DYS447 | A | 24 | $[TAATA]_6\ [TAAAA]\ [TAATA]_{10}\ [TAAAA]\ [TAATA]_6$ |
| | B | 25 | $[TAATA]_9\ [TAAAA]\ [TAATA]_8\ [TAAAA]\ [TAATA]_6$ |
| | C | 25 | $[TAATA]_7\ [TAAAA]\ [TAATA]_8\ [TAAAA]\ [TAATA]_8$ |
| | D | 23 | $[TAATA]_6\ [TAAAA]\ [TAATA]_9\ [TAAAA]\ [TAATA]_6$ |
| | E | 26 | $[TAATA]_7\ [TAAAA]\ [TAATA]_{11}\ [TAAAA]\ [TAATA]_6$ |
| DYS448 | A | 23 | $[AGAGAT]_{11}\ N_2\ [AGAGAT]_1\ N_2\ [AGAGAT]_3\ N_{14}\ [AGAGAT]_8$ |
| | B | 25 | $[AGAGAT]_{13}\ N_2\ [AGAGAT]_1\ N_2\ [AGAGAT]_3\ N_{14}\ [AGAGAT]_8$ |
| | C | 25 | $[AGAGAT]_{13}\ N_2\ [AGAGAT]_1\ N_2\ [AGAGAT]_3\ N_{14}\ [AGAGAT]_8$ |
| | D | 25 | $[AGAGAT]_{12}\ N_2\ [AGAGAT]_1\ N_2\ [AGAGAT]_3\ N_{14}\ [AGAGAT]_9$ |
| | E | 24 | $[AGAGAT]_{12}\ N_2\ [AGAGAT]_1\ N_2\ [AGAGAT]_3\ N_{14}\ [AGAGAT]_8$ |
| DYS437 | A | 15 | $[TCTA]_9\ [TCTG]_2\ [TCTA]_4$ |
| | B | 14 | $[TCTA]_8\ [TCTG]_2\ [TCTA]_4$ |
| | C | 14 | $[TCTA]_8\ [TCTG]_2\ [TCTA]_4$ |
| | D | 16 | $[TCTA]_{10}\ [TCTG]_2\ [TCTA]_4$ |
| | E | 14 | $[TCTA]_8\ [TCTG]_2\ [TCTA]_4$ |
| GATA-H4 | A | 12 | $[TAGA]_{12}$ |
| | B | 12 | $[TAGA]_{12}$ |
| | C | 12 | $[TAGA]_{12}$ |
| | D | 12 | $[TAGA]_{12}$ |
| | E | 11 | $[TAGA]_{11}$ |

sequencing data summarized in Table 4-17, and the corresponding allele calls for each SRM component given in Tables 4-15 and 4-16. Also, the sequence data presented here was compared to sequencing results generated by a research group at the University of Leiden headed by Peter de Knijff. Dr. de Knijff's group sequenced markers DYS19, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS460, and DYS461. All of Dr. de Knijff's results were in concordance with those presented in Table 4-17.

The second purpose of sequencing these Y-STR markers was to identify any possible polymorphisms that may exist within the flanking regions of the repeat motif and/or within the repeat motif itself. All of the sequences generated using the SRM components were compared to the reference sequences in GenBank (See Table 1-1) through sequence alignments. Only two polymorphisms relative to the GenBank reference sequence were found out of over 27,00 base calls. The first one was located within the DYS437 locus for component C. The polymorphism is a C→T substitution six bases down stream from the forward primer binding site of the Y-STR 20plex DYS437 primer set. Thus, the substitution did not affect PCR or the allele calling for component C at DYS437. This same DYS437 C→T point mutation was also seen in samples sequenced by Gusmao et al.[125]

The other polymorphism was found in sequence of DYS390 for component C. The variant nucleotide was located within the center of the repeat motif (Figure 4-32). DYS390 consists of a compound tetranucleotide repeat (see Table 4-17). In the middle of the repeat region of component C, an adenosine is substituted for a thymine. The repeat motif changed from a $[TCTG]_8[TCTA]_8[TCTG]_1[TCTA]_4$ to a $[TCTG]_8[TCTA]_5$

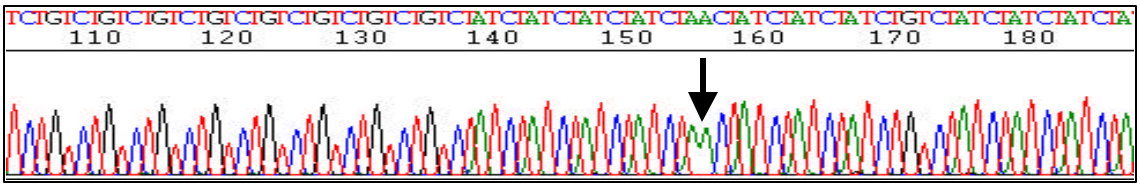



Figure 4-32
Sequence of DYS390 Repeat Region for Component C
This figure shows a variant nucleotide in the center of the repeat motif. In this sequence an Adenosine (indicated by the arrow) has replaced a thymine.

$[ACTA]_1$ $[TCTA]_2[TCTG]_1[TCTA]_4$. Even though, there was a polymorphism within the repeat motif, the number of repeats for that marker remained unaffected at 21 tetranucleotide repeat units.

Third, DNA sequencing data allowed for the identification of internal sequence variations that exist in certain loci. Internal sequence variation is a term used to describe a situation in which two alleles for the same loci have an identical number of repeats but are in a different order when the sequencing data is examined. For example, in Table 4-17, the sequencing of DYS448 for component B and D indicated that each had 25 AGAGAT repeats. However, there is an internal sequence variation between components B and D for the DYS448 locus. For component B, the repeat was in the order of 13 + 1 +3 +8 AGAGAT repeats. While component D's repeat was in the order of 12 +1 +3 +9 (Table 4-17). This internal sequence variation phenomenon was also seen for component B and D for DYS389I/II, and components B and C for DYS447 (Table 4-17).

Internal sequence variations seen in compound repeats can lead to an underestimation of the STR diversity of these markers making them actually more polymorphic than they seem based solely on fragment size analysis. Regardless of these internal variations

which are not all identified when analyzing PCR product sizes, DYS389I/II, DYS447 and DYS448 were some of the most highly polymorphic markers studied and should be included in future Y-STR multiplex work.

## Y-STR Nomenclature Issues

The analysis of of SRM 2395 components also permits an evaluation of Y-STR marker nomenclature used previously. Some locus nomenclatures take into account the total number of repetitive units (invariant plus variant) while others have taken into account only the variable repetitive stretches. In 2001, Gill et al.[35] issued guidance on how to assign the proper repeat designation of Y-STR markers. If a nomenclature is already in use, it is recommended that it should be continued. However, to encourage consistency for newly reported STRs, it is recommended that alleles should be named according to the total number of repeat units of the DNA that comprises both variant and invariant repeats.[35] Unfortunately, these guidelines are sometimes not followed and can lead to different allele calling for the same sample by different laboratories. For example, it can create problems in different proficiency testing (PT) trials. Quality control offices responsible for PT programs have reported problems certifying whether or not a result is correct or is a discrepancy due to differences in nomenclature.[126] There are a number of Y-STR markers that have been reported in the literature using different nomenclatures.

In the case of DYS439, three different descriptions of its tetranucleotide repeat motif (Figure 4-33) have been presented in the literature. DYS439 was first described by Ayub et al.[55] as a simple GATA repeat with an allele range of 9-14 repeats (panel A of figure 4-

Ayub et al.[55] nomenclature

$[GATA]_2N_4[GATA]_3N_{14}[GATA]_1N_3[GATA]_1N_7$ **$[GATA]_{9-14}$**

**Alleles 9-14**

Grignani et al.[56] nomenclature

**$[GATA]_2$** $N_4$**$[GATA]_3$** $N_{14}$**$[GATA]_1$** $N_3$ **$[GATA]_1$** $N_7$ **$[GATA]_{9-14}$**

**Alleles 16-21**

Gonzalez-Neira et al.[59] nomenclature

**$[ATCT]_2$** $N_{20}$**$[GATA]_2$**$N_3$ **$[AGAT]_3$** $N_{14}$**$[AGAT]_2$** $N_{10}$ **$[AGAT]_{9-14}$**

**Alleles 18-23**

Figure 4-33
Different Nomenclatures Previously Used in the Literature to Designate DYS439
The Ayub et al.[55] nomenclature was used in the final iteration of this work. The boxed portion of sequence is the invariant ATCT repeat, thus the allele range is +2 with respect to the nomenclature presented by Grignani et al.[56]

33). Later Grignani et al.[56], as shown in panel B of Figure 4-33, described the DYS439 repeat but this time included the invariant portion of the marker previously excluded by Ayub et al.[55] This increased the repeat number by seven and changed the allele range from 9-14 to 16-21 repeats. Finally, Gonzalez-Neira et al.[59] changed the repeat designation from a GATA to a AGAT and added two more invariant [ATCT] repeats (boxed in panel C of Figure 4-33), as compared to the Grignani et al.[56] sequence. This resulted in +9 repeat difference relative to the original allele range. Consequently, as indicated in panel C of Figure 4-33, a DYS439 allele designated a "9" by Ayub et al.[55] would be an 18 using the Gonzalez-Niera et al.[59] nomenclature. This could lead to confusion in laboratories that want to use DYS439 in their analysis of DNA samples and create DNA database incompatibilities.

In this study the nomenclature set forth by Ayub et al.[55] for DYS439 was finally chosen for four reasons. First, it follows the general guideline set forth by Gill et al.[35] that states that the nomenclature of the first to discover the repeat should be used. Second, the Ayub et al.[55] guidelines are currently being used in the commercially available Y-PLEX$^{TM}$ 5 kit. Third, a comparative analysis of the sequence structure of DYS439 was made in human and chimpanzees, in order to elucidate better the structure of this Y-STR.[127] It is thought that analyzing STR sequences in primates could provide insight on the evolution of STRs and help to prevent inconsistencies in nomeclature. The comparison indicated that in both humans and chimpanzees, no further variation was round relative to the tandem repeat originally considered by Ayub et al.[55] Thus, it is postulated that no variation is expected in the constant repeated units and Ayub et al.[55]

nomenclature should be maintained.[127] Finally, in all of the SRM 2395 components sequenced the invariant sections of the sequence did not change between components and no polymorphisms were found outside of the variable repeat block.

Another Y-STR marker that has been described differently in the literature has been DYS389I/II. The DYS389I/II marker was first reported by Kayser et al.[30] in 1997. The DYS389I/II nomenclature was originally described as tetranucleotide consisting of $[TCTG]_n[TCTA]_p[TCTA]_q$, designated A, B, and D in Figure 4-34. DYS389I consisted on segment D and DYS389II consisted of segment A + B + D. Later, the repeat segment C containing 3 [TCTG] repeats was added to the allele designations. Segment C is a invariant repeat within the DYS389I/II marker and makes the allele three repeats larger.[35] Thus, a DYS389II originally reported, as an 28 by Kayser et al.[30] would now be designated as a 31. In this work, the repeat designation for DYS389I/II that included the nonvariant C segment was the one used (Figure 4-34). This designation was chosen for two reasons. First, the newer DYS389I/II repeat designation is the one currently used by the Europeans in their central DNA database for both European and U.S. populations.[41, 124] Second, this is the repeat designation currently used by ReliaGene in their Y-PLEX™ 6 and Y-PLEX™ 5 kits.

Differing nomenclatures have also been used for DYS448. Figure 4-35 shows three different possible descriptions of DYS448 hexanucleotide repeat motif. DYS448 was first described in the literature by Butler et al.[53] as a loci containing two variable [AGAGAT] repeats interspersed with a non-varying intervening sequence 42 nucleotides in length (Panel A figure 4-35). Within this 42 base pair sequence was a invariant block of 3

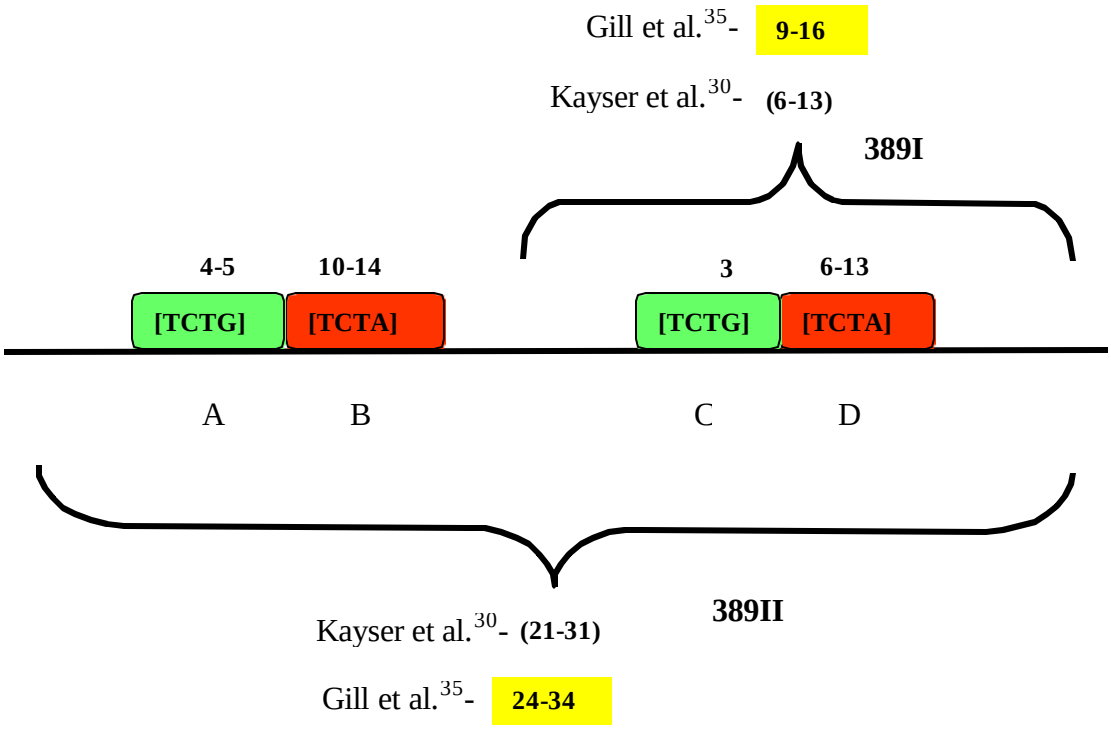



Figure 4-34
Different DYS389I/II Nomenclatures
Original paper, Kayser et al.[30] defines allele nomenclature without repeat segment "C"; it has now been added in more recent nomenclatures[35,41,124] thus making alleles +3 repeats larger. The more recent nomenclature is used in this work and is now widely accepted.

[AGAGAT] repeats. The nomenclature of this marker was postulated to be $[AGAGAT]_nN_{10}[AGAGAT]_3N_{14}[AGAGAT]_p$, where n and p represent the varying number of AGAGAT repeats. Second, Redd et al.[39] described DYS448 as a hexa-nucleotide repeat with two variable areas with 42 base pair sequence interspersed between both varying portions of the sequence. However, Redd et al.[39] omits the invariant $[AGAGAT]_3$ portion that was originally included by Butler et al.[53] (panel B figure 4-35). Thus, a DYS448 allele designated a "20" by Butler et al.[53] would be –3 or

**Butler et al.[53] nomenclature**

$[AGAGAT]_n N_2 [AGAGAT]_1 N_2 [AGAGAT]_3 N_{14} [AGAGAT]_p$

**Alleles 18-26**

**Redd et al.[39] nomenclature**

$[AGAGAT]_n N_2 [AGAGAT]_1 N_2 [AGAGAT]_3 N_{14} [AGAGAT]_p$

**Alleles 15-23**

**Schoske nomenclature**

$[AGAGAT]_n N_2 [AGAGAT]_1 N_2 [AGAGAT]_3 N_{14} [AGAGAT]_p$

**Alleles 19-27**

Figure 4-35
Different DYS448 Nomenclatures.
Boxed regions show repeats used in allele designations.

designated as a "17" if the Redd et al.[39] nomenclature was used. Finally, a third nomenclature was postulated in this study, which included another invariant [AGAGAT] repeat previously omitted by Butler et al.[53] There is another invariant [AGAGAT] repeat to go along the with the invariant $[AGAGAT]_3$ block within the 42 base pairs separating the two variable blocks of [AGAGAT] repeats. This increases the total number of invariant AGAGAT repeats to 4 (panel C Figure 4-35). If the extra invariant block is included the DYS448 nomenclature would be $[AGAGAT]_nN_2[AGAGAT]_1N_2$ $[AGAGAT]_3N_{14}[AGAGAT]_p$. This new nomenclature would be +1 with respect to the Butler et al.[53] nomenclature and +4 with respect to the Redd et al.[39] nomenclature. The nomenclature described in panel C of Figure 4-35 was used for DYS448 because it follows the spirit of the ISFG guidelines which state that for newly reported STRs the alleles should be named according to the total number of repeat units of the DNA that comprises both variant and invariant repeats.[35] The variation in nomenclatures for DYS448 demonstrate the challenges in standardizing STR allele nomenclature even within the same laboratory.

The last example of a Y-STR marker with more than one proposed nomenclature in the literature is GATA H4. The proposed repeat structure for these different nomenclatures and accompanying allele ranges is presented in Table 4-18. White et al.[57] first discovered the Y-STR GATA H4 in 1999. It was described as a tetranucleotide GATA repeat with both varying and non-varying segments. Later, Gonzalez-Neira et al.[59] following ISFG guidelines[35] introduced H4 nomenclature that included 18 invariant tetranucleotide repeats previously omitted by White et al.[57] and changed the variant

Table 4-18
Different H4 Nomenclatures in the Literature
The one proposed by Butler et al.[53] was used in this study. The variant part of the repeat motif is bolded in each case. Combining H4.1 and H4.2 sequences is equivalent to H4 locus.[127]

| Repeat Motif – are for total H4 locus unless otherwise noted | Allele Range | Reference |
|---|---|---|
| **$[GATA]_n$** | 7-13 | White et al.[57] |
| 5'-$[AGAT]_4$ $N_2$ $[ATAG]_3[GTAG]_3$**$[ATAG]_n$**$N_{13}$ $[GATG]_2N_1[ATAG]_4N_4[ATAG]_2$-3' | 25-31 | Gonzalez-Neira et al.[59] |
| 5'-$[AGAT]_4$ $N_4$ $[AGAT]_2[AGGT]_3$**$[AGAT]_n$**$N_{24}$ $[ATAG]_4N_1[ATAC]_1[ATAG]_2$-3' | 23-29 | Gusmao et al.[127] |
| 5'-$[AGAT]_4N_4$ $[AGAT]_2[AGGT]_3$**$[AGAT]_n$** (H4.1 locus) | 16-22 | |
| 5'$[ATAG]_4[ATAC]_1[ATAG]_2$-3' (H4.2 locus invariant 7 repeat block in humans) | N/A | |
| **$[TAGA]_n$** | 8-14 | Butler et al.[53] |

repeat motif from a [GATA] to a [ATAG]. As a result the allele range shifted from 7-13 to 25-31.

Gusmao et al.[127] has also proposed new nomenclature for H4 (Table 4-18). This recommendation was based in part on sequence variations found in GATA H4 when comparing humans and chimpanzees. The H4 nomenclature proposed by Gusmao et al.[127] also contained both the invariant and variant portions of the locus. However, when compared to the Gonzalez-Neira et al.[59] nomenclature, there are differences which result in a shift in the number of repeats and the sequence of the variant part of the repeat (Table 4-18). First, the variant repeat in the Gonzalez-Neira et al.[59] nomenclature is ATAG while in Gusmao et al.[127] the variant repeat is AGAT. A second difference between the Gonzalez-Neira and the Gusmao H4 nomenclature can be seen from examining the 5' end of the repeat motif. Both proposed nomenclatures start with a invariant $[AGAT]_4$, but in the Gonzalez Neira et al.[59] structure the next invariant repeat is $[ATAG]_3$ while in the Gusmao et al.[127] nomenclature the next invariant repeat is $[AGAT]_2$ (Table 4-18). Finally, the Gonzalez-Neira et al.[59] consensus structure for H4 includes 8 invariant tetranucleotide repeats occurring downstream of the variant [AGAT]

region while Gusmao et al.[127] only counted 7 invariant tetranucleotide repeats for after the variant repeat block (Table 4-18). The allele range for H4 if the Gusmao et al.[127] consensus sequence was selected would be 23 to 29 repeats, instead of 25 to 31 repeats if the Gonzalez-Neira et al.[59] nomenclature was chosen.

Gusmao et al.[127] also suggested that a smaller amplicon can be generated using different primer sets and thus the GATA H4 locus could be divided into two parts, H4.1 and H4.2. The consensus sequence of H4.1 is $[AGAT]_4N_4\,[AGAT]_2[AGGT]_3[AGAT]_n$ and H4.1 was polymorphic in humans (Table 4-18). The structure of H4.2 is $[ATAG]_4[ATAC]_1[ATAG]_2$ was not polymorphic in humans. In chimpanzees the opposite held true, the H4.1 locus was invariable while the H4.2 locus was variable. If just H4.1 was examined the allele range would be 16 to 22 repeats (Table 4-18). This represented a –7 shift from the 23-29 range if the entire Gusmao et al.[127] consensus sequence for H4 was used. The –7 shift represents the omission of H4.2, which contained 7 invariant tetranucleotide repeats (Table 4-18).

A new H4 nomenclature was introduced by Butler et al.[53] and used in this study. In the Butler et al.[53] nomenclature, just the variant portion of the H4 locus is counted. However, the variant repeat is now designated as TAGA instead of the AGAT presented by Gusmao et al.[127] (Table 4-18). Figure 4-36 illustrates the top strand of a DNA sequence alignment for the H4 sequence given by Gusmao et al.[127], and the full sequence for Component A of SRM 2395 generated as a part of this study. The $[TAGA]_n$ nomenclature for H4 was chosen for a number of reasons. First, the H4 primers used in the Y-STR 20plex generate small amplicons that do not encompass the full H4 repeat

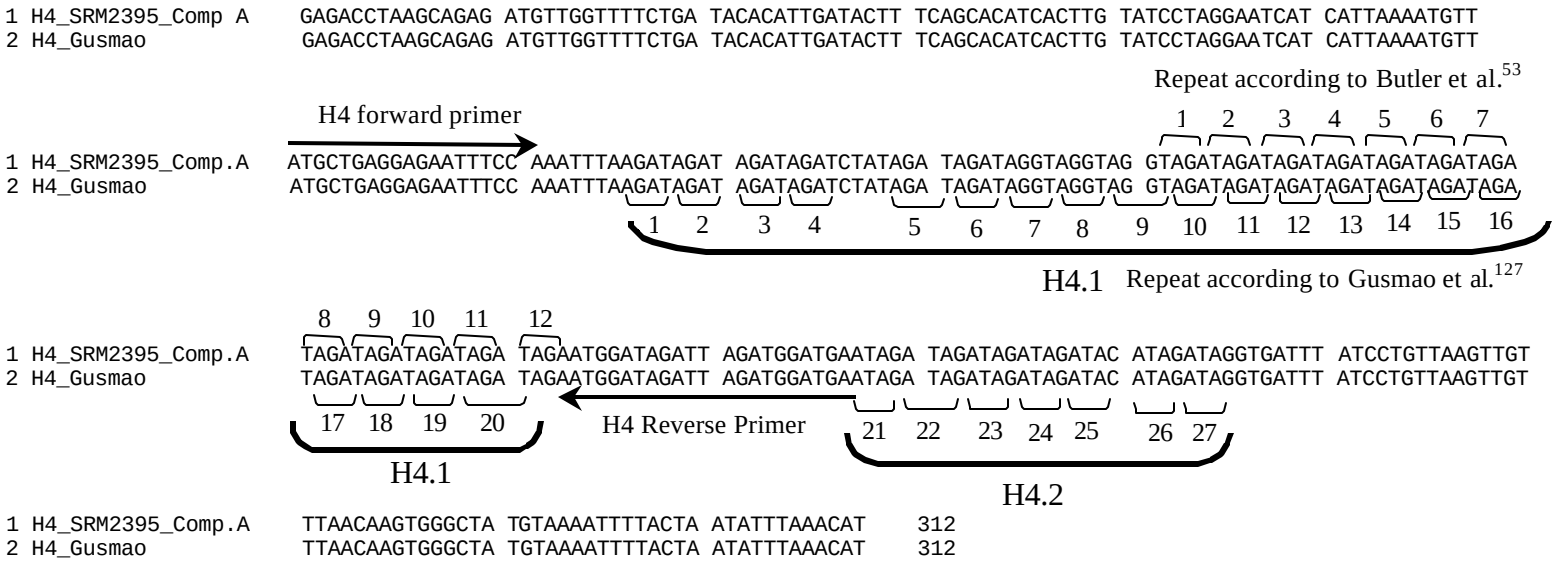



Figure 4-36
Alignment of Top Strands from H4 Locus from Gusmao et al.[127] and SRM Component A
The sequences are exactly homologous to one another. According to the Butler et al.[53] nomenclature $[TAGA]_n$ Component A would be designated as having 12 repeats. If the Gusmao et al.[127] nomenclature $[AGAT]_4N_4[AGAT]_2[AGGT]_3[AGAT]_n[ATAG]_4[ATAC]_1[ATAG]_2$ is used, the number of repeats changes to 27. See Table 4-18 for further information on different H4 nomenclatures. H4 can be split into H4.1 and H4.2 if primers for H4 (see arrows) that were used in the Y-STR 20plex[53] are used.

region. The reverse primer is internal to the H4.2 region (Figure 4-36), which has now been shown to be invariant in other studies and therefore not necessary for human population studies.[127] Second, an analysis of the sequences of all the male SRM components showed that with the exception of the variant repeat TAGA region no variation was seen in the other regions of the H4 locus. Finally, the TAGA repeat designation was chosen over the AGAT designation because the first noticeable repeat is TAGA if the invariant repeats are not included. Regardless of the nomenclature used there must be an official standard which is followed by laboratories performing and reporting Y-STR analysis.

Perhaps the most significant benefit from sequencing the SRM components is that these standards permitted accurate calibration of allele calls and construction of reliable genotyping macros. Furthermore, once characterized these SRM components provided a level of in-house quality control ensuring that both the Y-STR multiplexes and analysis instrumentation were providing amplicon base pair sizes that were both precise and accurate in terms of allele calls. This accuracy also impacts the population study efforts described here.

## Analysis of Forensic Casework Samples Using Y-STR Multiplexes

Semen traces are one of the most important evidence materials involving cases of sexual assault. Multiplex autosomal STR analysis combined with the gender typing marker amelogenin, currently represent the most popular approach to DNA forensic identification. There are a number of situations however where multiplex autosomal kits fail to detect the DNA profile of the semen contributor although a Y-chromosome peak at the amelogenin marker indicated the presence of male DNA. These situations include mixture samples containing semen from azospermic individuals.[27,120,128] There have also been documented situations were the amelogenin sex test failed to amplify the Y region of the DNA.[129-130] In the absence of the Y-amelogenin peak, a STR profile of a male could be mistaken as that of a female. An increasing popular method in the evaluation of these unique cases is Y-STR analysis. Y-STR DNA profiling has become a powerful tool for analysis of vaginal swabs from sexual assault cases having a mixed female and male fraction.[27-28, 31-32,121]

Y-STR DNA profiling was also used in a manhunt in Poland looking for a serially rapist.[131] In that particular case, Y-STR DNA profiles on 714 suspects were generated over a 15 month period. One of these male samples had an identical Y-STR DNA profile to that of the offender but his autosomal profile did not match that of the forensic evidence. A detailed analysis of the suspect sample to the evidence showed that 9 out of 10 autosomal loci displayed the same alleles. This finding suggested that the rapist could be a man closely related to the original suspect. Later it was established that the original suspect had a brother. A DNA sample was obtained from the brother and his Y-STR

DNA and autosomal DNA profiles matched that of the forensic evidence collected in the case. Y-STR assays were able to link the rapist to the crime by relying on the fact that Y-STRs follow a paternal lineage. This case further stressed the importance of the Y-chromosome in the analysis of forensic evidence.

Now that confidence had been established in the performance of our Y-STR multiplexes they were tested to see how they would work against some actual forensic casework samples. These samples were provided by and run at the United States Army Criminal Investigation Laboratory (USACIL) in Forest Park, Georgia. All of the information regarding the evidence was provided by USACIL. Evidence was not prepared in this study. All of the sample preparation was performed by USACIL personnel (See materials and methods). The forensic evidence in these cases were run on an ABI 3100 using POP-4 sieving material. Both cases involved sexual assault.

Case Number 2002-0513

This case involved the forced sodomy of a female by a male. There were seven exhibits collected in this case. Exhibits 1 and 2 were paper towels with stains. These two exhibits were suspected to contain the semen of the suspect and salvia of the victim as she related she had utilized the paper towel to wipe her mouth after the act. Exhibits 3 and 7 were blood stains taken from the victim and the suspect, respectively. Exhibits 4 and 5, both hairs, were collected from a toilet seat inside of the quarters, which was said to be used by the victim. Exhibit 6, another paper towel with stains, was discovered at the scene, however it was unknown if it was utilized by the suspect. Exhibit 6 was confirmed to not have been used by the victim. The requestor in this case originally asked

USACIL personnel to examine and identify the serological evidence (semen, saliva, hair) from Exhibits 1, 2, and 4-6 and compare any resulting DNA profiles to those found in Exhibits 3 and 7 (Reference samples from victim and suspect).

Amylase (an enzyme found in high concentration in salvia was found on both Exhibits 1 and 2. The immunological presence of semen was found on both Exhibits 1 and 2. The presence of the assailant's sperm mixed with salvia from the victim on both pieces of evidence warrants the need of performing differential extraction. Differential extraction is a method that separates the epithelial and sperm cells. Differential extraction was first described by Gill et al.[132] and is commonly used by forensic laboratories to isolate the female and male fractions in sexual assault cases that contain both a mixture of male and female DNA.

Following the differential extraction procedure Exhibits 1 and 2 were tested using autosomal STR kits. The autosomal DNA profiles from Exhibits 1 and 2 matched the autosomal DNA profile provided by the victim (Exhibit 3). Even though there was an immunological presence of semen on exhibits 1 and 2, no male autosomal STR profile were successfully developed for either piece of evidence. Neither semen nor salvia was found on Exhibit 6, thus DNA profiling was not performed. Exhibits 4 and 5 were not examined.

The lack of an autosomal DNA profile led USACIL personnel to investigate the use of Y-STR DNA profiling. A sample of Exhibit 1 was originally sent to ReliaGene Technologies by USACIL personnel in an attempt to obtain a Y-STR DNA profile for the suspect. ReliaGene Technologies analyzed Exhibit 1 (Y379.1) and a sample of the

suspect's blood (Y380.3) using the Y-PLEX$^{TM}$ 6 kit. The Y-PLEX$^{TM}$ 6 kit tests for six Y-STR loci: DYS389II, DYS385, DYS19, DYS390, DYS391 and DYS393. According to the report submitted to USACIL, ReliaGene Technologies were able to successfully obtain a Y-STR profile for both samples submitted. The Y-STR profile, provided by ReliaGene for each sample is given below in Table 4-19. The Y-STR profile obtained from sample Y374.1 matched that of the suspect. Thus, ReliaGene concluded that the suspect could not be excluded as the DNA donor in exhibit 1. The ability of the Y-PLEX$^{TM}$ 6 kit to discriminate between unrelated individuals is less than that of either of the Y-STR multiplex kits used in this study (see population studies). With that being said, USACIL personnel asked if any of the Y-STR multiplexes currently under development could be tested on the exhibits presented in this case.

Both the Y-STR 20plex and Y-STR 9plex were tested against 1.0 ng of DNA extracted from Exhibit 1 (Y374.1). At 28 cycles of PCR amplification neither the Y-STR 20plex or the Y-STR 9plex produced a profile. Next, samples were amplified but to 32 cycles instead of 28 cycles with hopes of increasing the sensitivity. The Y-STR 20plex was unable to amplify any of the loci. However, a partial Y-STR haplotype was obtained

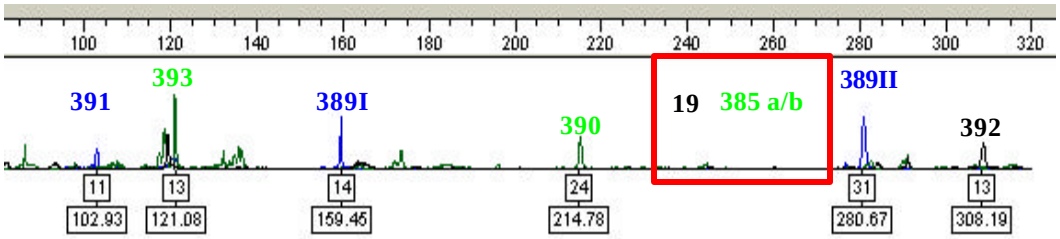
Table 4-19
Y-PLEX$^{TM}$ 6 Kit Haplotypes Obtained from ReliaGene Technologies
The DNA in sample Y374.1 was extracted from exhibit 1, Paper Towel. The DNA in sample Y380.3 was extracted from a blood sample provided by the suspect.

| Y-STR Markers | Sample Y380.3 | Sample Y 374.1 |
|---|---|---|
| DYS393 | 13 | 13 |
| DYS19 | 14 | 14 |
| DYS389II | 31 | 31 |
| DYS390 | 24 | 24 |
| DYS391 | 11 | 11 |
| DYS385 a/b | 11,14 | 11,14 |

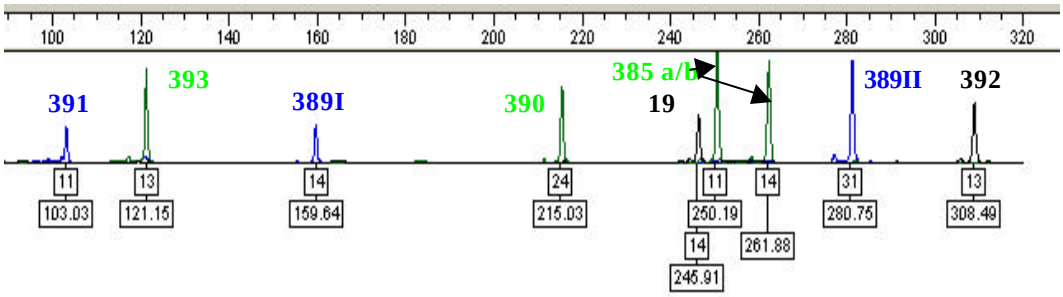
by the Y-STR 9plex. The Genotyper result of sample Y374.1 and the suspect reference sample Y380.3 is shown in Figure 4-37. The partial Y-STR 9plex haplotype was missing DYS385 a/b, and DYS19. The allele calls for the remaining loci in sample Y374 matched that of the reference sample. Also, the Y-STR haplotype obtained from the reference sample (Y380.3) matched the one obtained by ReliaGene Technologies for markers common to both the Y-PLEX 6 kit and Y-STR 9plex (Figure 4-37). The drop-out of amplicons for the DYS385 a/b and DYS19 locus seen in the testing of the Y-STR 9plex could be due to a small amount of male DNA in the Y374.1 sample. The 1.0 ng of DNA extracted from Y374.1 was a mixture of male and female DNA, thus the exact amount of male sample was not known. In previous sensitivity studies (see Y-STR multiplex performance) full profiles were obtained when 50 picograms of DNA was used and amplified to 32 cycles using PCR. Thus, it can be speculated that the amount of male DNA within this tested mixture could be lower than 50 picograms.

The Y-PLEX$^{TM}$ 6 kit was also tested at USACIL according to manufacturer's protocol. None of the loci amplified with 1.0 ng total DNA template. A closer examination of the Y-PLEX$^{TM}$ 6 kits protocol provided a possible explanation of why ReliaGene was able to obtain a full profile while the one done in this study failed. ReliaGene's protocol dictates that upwards of 5.0 ng of DNA can be used in its PCR amplification protocol. It is likely that ReliaGene used the largest amount of DNA they could extract from the sample they received. Unfortunately, the report provided by ReliaGene did not include a GeneScan® result nor the exact amount of DNA amplified. This case clearly illustrates the importance of Y-STR testing. The Y-STR 9plex was able

A)




B)




| Sample info | 19 | 385 a/b | 389I | 389II | 390 | 391 | 392 | 393 |
|---|---|---|---|---|---|---|---|---|
| 374.1 | NR | NR | 14 | 31 | 24 | 11 | 13 | 13 |
| 380.3 | 14 | 11-14 | 14 | 31 | 24 | 11 | 13 | 13 |
| 374.1 Reliagene | 14 | 11-14 | NT | 31 | 24 | 11 | NT | 13 |

NR = No Reaction
NT = Not Tested

Figure 4-37
Genotyper® Result for Samples Run Using the Y-STR 9plex
Panel A is from sample Y374.1. Y374.1 is a 1.0 ng mixture of male:female DNA. The ratio of male:female is not known. Panel B is from the male suspect Y380. Y380 is 1.0 ng of male suspect DNA. The profiles between the evidence and reference samples match for all observed alleles. DYS 385 a/b and DYS19 failed (Boxed in Red) in the Y374.1 sample. The Y-STR profiles are listed above as well.

to produce a partial STR profile for a sample where an autosomal profile could not be obtained.

Finally, the Y-PLEX 6$^{TM}$ and Y-STR 9plex haplotypes shown in Figure 4-37 were inputted into four different Y-STR databases to see how unique this sample's Y-PLEX$^{TM}$ 6 and Y-STR 9plex haplotypes were (Table 4-20). A match for samples with the Y-PLEX$^{TM}$ 6 kit haplotype for sample Y380.3 was found 0.31% to 0.70% of the time depending on the size and ethnicity of the database (Table 4-20). The Y-STR 9plex haplotype offers two additional Y-STR markers (DYS392, and DYS389I) that are not available in the Y-PLEX$^{TM}$ 6 kit. Thus, it is expected that the Y-STR 9plex will have a greater ability to discriminate two unrelated male samples. A match for the Y-STR 9plex

Table 4-20
Matches of Y380s Y-STR Haplotypes in Various Y-STR Databases

| Databases | Ethnicity | Y-PLEX 6 kit # of matches/ samples (% matched) | Minimal Haplotype # of matches/samples (% matched) |
|---|---|---|---|
| Y-STR Haplotype Reference Database http://ystr.chartie.de | Various European Populations | 39/12,675 (0.31 %) | 23/12,675 (0.18%) |
| Y-STR Haplotype Reference Database for U.S. Populations http://www.ystr.org/usa | African American | 1/628 (0.16%) | 1/628 (0.16%) |
| | Caucasian | 2/599 (0.33%) | 2/599 (0.33%) |
| | Hispanic | 2/478 (0.44%) | 2/478 (0.44%) |
| ReliaGene Technologies http://www.Religagene.com | African American | 5/1169 (0.45%) | 0/535 (0.00%) |
| | Caucasian | 5/1100 (0.43%) | 2/517 (0.39%) |
| | Hispanic | 3/245 (0.12%) | 0/245 (0.00%) |
| U.S. population* | African American | 0/260 (0.00%) | 0/260 (0.00%) |
| | Caucasian | 0/244 (0.00%) | 0/244 (0.00%) |
| | Hispanic | 1/143 (0.70%) | 1/143 (0.70%) |

*Population studies presented in this study

haplotype in sample Y380.3 was found in 0.18% to 0.70% of the time depending on the size and ethnicity of the database (Table 4-20). In the case of sample Y380.3, the addition of DYS392 and DYS389I in the Y-STR 9plex did offer a nominal increase in the uniqueness of the sample as expected.

## Case Number 2002-0080

This case involved the sodomy of a male victim by a male suspect. There were 18 separate exhibits collected from the crime scene. These exhibits included cuttings from boxer shorts, oral and penile swabs, and blood collected from the victim and the suspect. All of the exhibits that indicated the presence of saliva, semen or blood were tested with multiplex autosomal STR kits (See Table 4-21). The autosomal STR profiles obtained from Exhibit 8, 12, and 16 matched the DNA profile of the victim at all the genetic loci tested. The DNA profile developed from Exhibits 10, 14 and 15 matched the DNA

Table 4-21
List of Evidence Collected in USACIL Case 2002-0080
Exhibit 5 corresponds to sample 579.1. Samples 559 and 560 correspond to exhibits 17 and 18 respectively.

| Exhibit # | Evidence description |
|---|---|
| 5 | Underwear |
| 8 | Swab with stain |
| 10 | Swab with stain |
| 12 | Swab with stain |
| 14 | Socks |
| 15 | Shirt, |
| 16 | Underwear |
| 17 | Blood Sample, victim |
| 18 | Blood Sample, suspect |

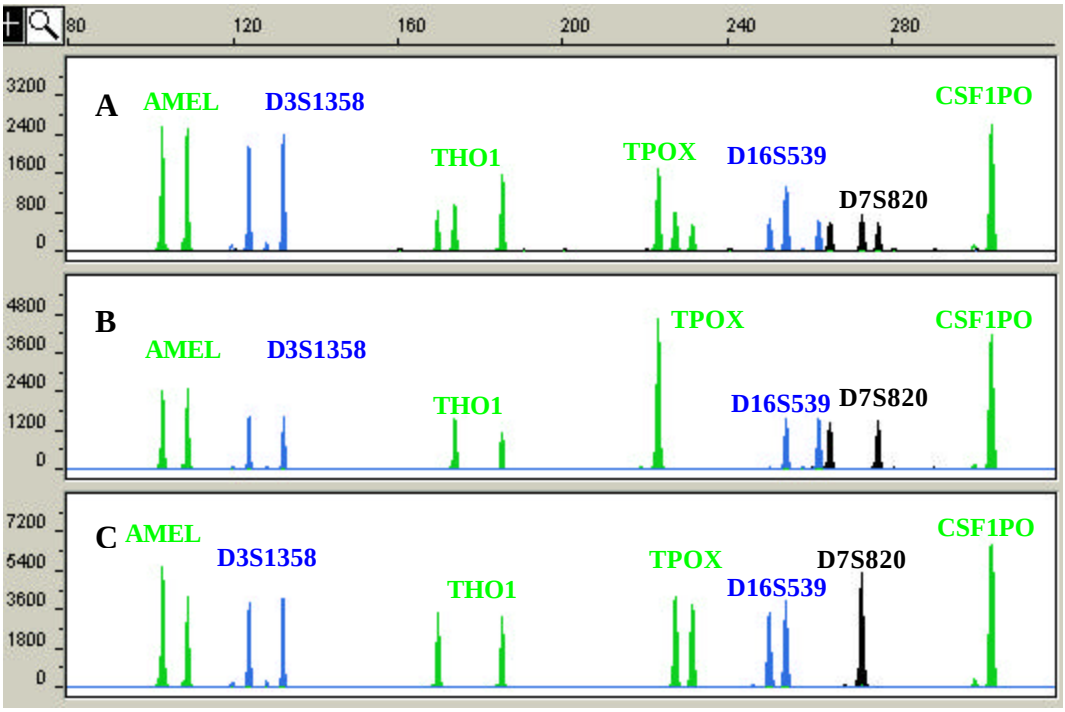



Figure 4-38
GeneScan® Results on Three Male Samples Using the AmpFlSTR® Cofiler™ STR Kit
Results were generated at USACIL on an ABI 310. Panel A, is a mixture of two male samples. Panel B, is a sample of male DNA from the victim and Panel C, is a mixture sample from the suspect. The AMEL, THO1, TPOX, and CSF1PO amplicons are labeled with JOE (Green). The D3S1358 and D16S539 amplicons are labeled with FAM (Blue). The D7S820 amplicons are labeled with NED (yellow).

profile of the suspect for all genetic loci tested. Two autosomal STR DNA profiles were detected on Exhibits 16 and 5. GeneScan® results obtained from Exhibit 5 (Sample 579.1) with the AmpFlSTR® Cofiler™ autosomal STR kit are shown in Figure 4-38. Panel A, contained a mixture of both the victim's and suspects DNA. Panel B, contained a sample of the victim's DNA while Panel C showed the STR profile of the suspect.

When two contributors to a mixed DNA sample share one or more alleles, the alleles are masked and genotypes from each contributor may not be easily distinguishable.[5] For example in Panel A of Figure 4-38, three peaks are evident for the D7S820 locus: alleles

8,10,11. Depending on the ratio of peak heights a three allele pattern can be the result of a heterozygote + heterozygote (one overlapping allele) or between a heterozygote + homozygote, no overlapping alleles. The data shown in panels B and C indicated the latter was the case for the D7S820 locus. Discerning DNA profiles for samples that containing materials from more than one contributor can be difficult and often tedious to analyze.

Even though the autosomal STR DNA kits adequately discerned the mixture found within sample 579.1, USACIL personnel wanted to see if the Y-STR 20plex and Y-STR 9plex could resolve Y-STR samples if the sample contained DNA from more than one contributor. Results were generated using both the Y-STR 20plex and Y-STR 9plex on sample 579.1 and indicated that the sample tested contained DNA from more than one male contributor. The GeneScan® result in Figure 4-39 is broken up by the four different fluorescent dye colors. All of the locus with multiple allele calls were boxed. There were multiple alleles found for DYS391, DYS385 a/b, DYS388, DYS389I, DYS392, DYS426, DYS437, DYS438, DYS439 and YCAII a/b. The Genotyper® macro for the Y-STR 20plex was able to generate a Y-STR haplotype that was concordant to the ones obtained from both the suspect's and victim's reference sample (Table 4-22).

The testing of the Y-STR 20plex and Y-STR 9plex on these two selected cases helped to illustrate that these Y-STR multiplexes could provide satisfactory results when tested against actual forensic casework samples. These results stressed the importance of using Y-STR profiling especially in areas where a male autosomal STR profile cannot be obtained. If a case such as 2002-0513 relied solely on DNA evidence, then the Y-STR

profile obtained may have been the only piece of evidence linking the suspect to the crime. The evidence evaluated in case sample 2002-0080 showed that Y-STR assays could easily demonstrate that a sample had more than one male contributor. Future casework samples should be evaluated using new Y-STR multiplexes that include only

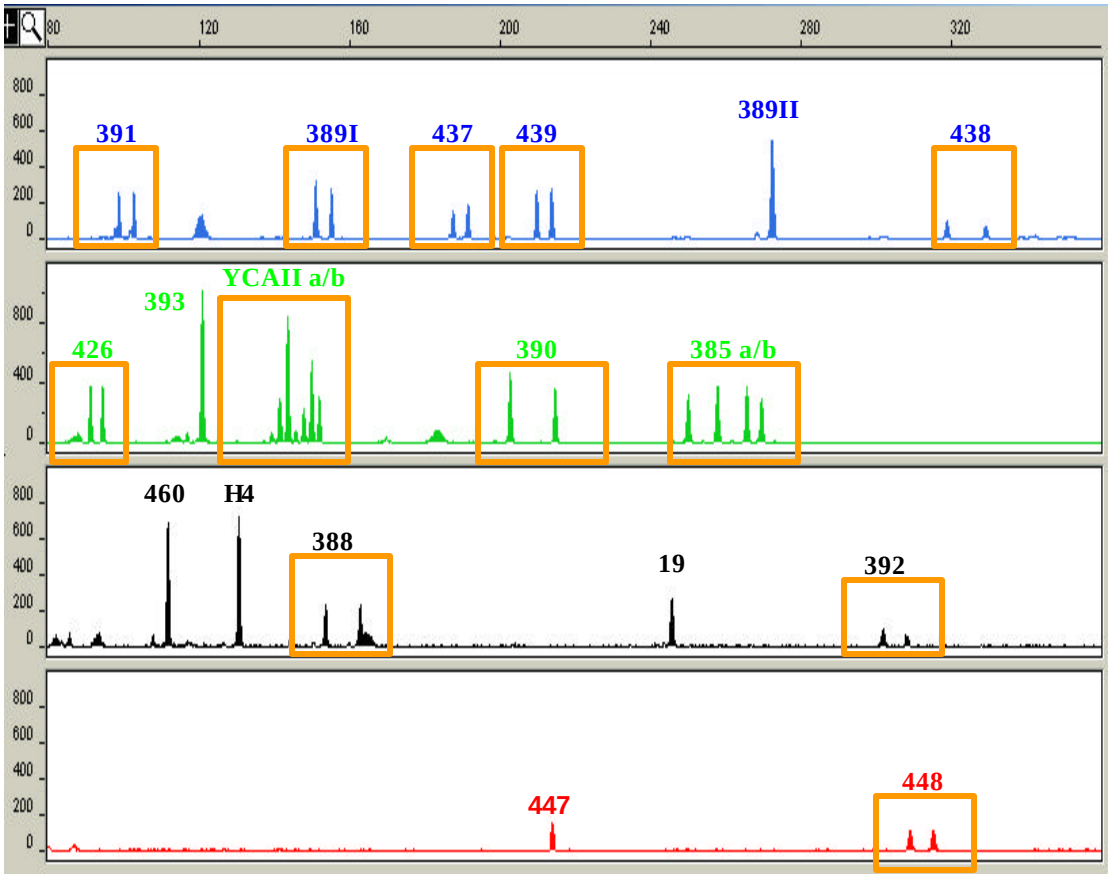



Figure 4-39
GeneScan® Result for Sample 579.1 Using the Y-STR 20plex
Sample 579.1 contains a mixture of two different male DNA profiles. The GeneScan® result is separated by respective dye color. Panel A, contains amplicons labeled in FAM (Blue). Panel B, contains amplicons labeled in VIC (Green). Panel C, contains amplicons labeled in NED (Yellow). Panel D, contains amplicons labeled in PET (Red). The boxed areas represent loci that had multiple alleles. The presence of multiple alleles at more than one locus indicates that the sample had more than one contributor.

Table 4-22
Y-STR Haplotypes for Samples T559, T560, and 579.1.
The profiles for samples T559 and T560 were obtained from blood samples from the victim and suspect respectively. Sample 579.1 contains a mixture of male DNA contributed by the victim and the suspect.

| Sample | Y-haplotype (19,385a/b,388,389I,389II,390,391,392,393,426,437,438,439,447, 448,460,H4, YCAII a/b) |
|---|---|
| T559 | 14,13-15,15,12,29,21,10,11,13,11,16,10,18,24,24,11,11,19-22 |
| T560 | 14,11-16,12,13,29,24,11,13,13,12,15,12,19,24,23,11,11,19-23 |
| 579.1 | 14,11-13-15-16,12-15,12-13,29,21-24,10-11,11-13,13,11-12,15-16,10-12, 18-19,24,23-24,11,19-22-23 |

the most polymorphic markers (see population studies) and are optimized to yield male specific results. These new multiplex assays could then increase the discriminatory capacity of the generated Y-STR Haplotype and benefit such cases as 2002-0513.

## Conclusion

During this research project novel Y-chromosome STR assays have been developed that offer the ability to obtain information from more than 20 different sites along the Y-chromosome in multiplex PCR amplifications. These multiplexes were constructed using a systematic approach that relied heavily on careful primer design. Two multiplexes were developed to provide a Y-STR haplotype consisting of 27 PCR products. A new U.S. population Y-STR database was generated as part of this study roughly equaling the size of previously publicly available U.S. population databases on Y-STR haplotypes. Precision data from multiplex Y-STR assays demonstrated that reliable haplotypes could be generated in the absence of allelic ladders using the multi-capillary ABI 3100 instrument platform. Calibration of PCR product sizes to appropriate allele designations was accomplished through sequencing Y-STR alleles that have become part of a NIST standard reference material, SRM 2395. SRM 2395 will aid future comparisons of different primer sets for commonly used Y-STR markers. A standard set of nomenclatures is presented that should help to standardize future allele designations for Y-STR markers. The multiplex assays developed as part of this study are sensitive and can provide Y-STR haplotypes in male:female mixtures. These Y-STR multiplex assays were successfully tested on DNA samples from forensic casework materials at the US Army Crime Laboratory.

## Future Research

This study laid out a plethora of information on Y-STR markers and multiplex assays, but there are areas that still need to be explored. First, the results of the specificity studies indicated that work still needs to be done before the Y-STR 20plex could be routinely used in forensic casework. Adaptation of these primer sets into a commercial male-specific Y-STR kit has already been done by the Promega Corporation (Madison, WI). Second, the possible microvariants identified during the precision studies should be revaluated to see if the alleles are actual microvariants or were sizing differences due to measurement error. Third, using the provided population information, new multiplexes should be constructed that include only the most polymorphic Y-STR markers across U.S. ethnic groups. Newly available Y-STR markers in the genome database could also be evaluated and put into additional multiplex PCR assays. Finally, SRM 2395 should be frequently updated to include new polymorphic Y-STR markers.

A-1
Summary of Published Allele Frequencies for Commonly Used Y-STR Markers.

| Locus | Population Ethnicity | Sample Size | Allele Range 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS19 | **EUROPE** | | | | | | | | | | | | |
| | Innsbruck (Austria) | 100 | | | | 0.140 | 0.560 | 0.230 | 0.070 | 0.010 | | | 71 |
| | Germany | 86 | 0.070 | | | 0.512 | 0.279 | 0.081 | 0.046 | 0.012 | | | 72 |
| | Bavaria (Germany) | 151 | | | | NL | NL | NL | NL | NL | | | 73 |
| | Cologne (Germany) | 163 | | | | 0.041 | 0.566 | 0.268 | 0.052 | 0.052 | | | 74 |
| | Wurrtemberg (Germany) | 218 | | | | 0.101 | 0.532 | 0.243 | 0.083 | 0.041 | | | 75 |
| | Munster (Germany) | 272 | | | | 0.040 | 0.570 | 0.230 | 0.120 | 0.040 | | | 71 |
| | Koln (Germany) | 100 | | | | 0.040 | 0.620 | 0.250 | 0.060 | 0.030 | | | 71 |
| | Jena (Germany) | 143 | | | | 0.060 | 0.550 | 0.180 | 0.160 | 0.060 | | | 71 |
| | Heidelberg (Germany) | 113 | | | | 0.070 | 0.500 | 0.290 | 0.090 | 0.040 | | | 71 |
| | Hanover (Germany) | 53 | | | | 0.070 | 0.490 | 0.230 | 0.130 | 0.070 | | | 71 |
| | Magdeberg (Germany) | 210 | | | | 0.050 | 0.470 | 0.250 | 0.170 | 0.060 | | | 71 |
| | Bradenburg (Germany | 233 | | | | 0.030 | 0.450 | 0.270 | 0.150 | 0.100 | | | 71 |
| | Munich 1 (Germany) | 125 | | | | 0.060 | 0.460 | 0.260 | 0.180 | 0.030 | | | 71 |
| | Munich 2 (Germany) | 259 | | | | 0.160 | 0.450 | 0.170 | 0.190 | 0.030 | | | 71 |
| | Berlin 1(Germany) | 233 | | | | 0.070 | 0.390 | 0.270 | 0.210 | 0.060 | | | 71 |
| | Bremen | 49 | | | | 0.100 | 0.590 | 0.160 | 0.140 | | | | 71 |
| | Leiden | 88 | | | | 0.040 | 0.700 | 0.190 | 0.030 | 0.020 | | | 71 |
| | Leicester, pooled | 339 | | | 0.010 | 0.040 | 0.460 | 0.260 | 0.160 | 0.070 | 0.010 | | 71 |
| | British | 41 | | | | 0.050 | 0.800 | 0.100 | 0.020 | 0.020 | | | 71 |
| | Bratislava | 57 | | | | 0.070 | 0.190 | 0.210 | 0.310 | 0.210 | | | 71 |
| | Norway | 300 | | | | 0.027 | 0.527 | 0.313 | 0.120 | 0.013 | | | 71 |
| | Athens (Greece) | 47 | | | | 0.255 | 0.255 | 0.319 | 0.128 | 0.043 | | | 72 |
| | Galicia (Spain) | 107 | | | | 0.150 | 0.626 | 0.140 | 0.075 | 0.009 | | | 72 |
| | London (UK) | 115 | | | | 0.043 | 0.704 | 0.191 | 0.061 | | | | 72 |
| | Rome (Italy) | 125 | 0.008 | | | 0.128 | 0.552 | 0.240 | 0.040 | 0.032 | | | 72 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS19 | **EUROPE** | | | | | | | | | | | | |
| | Roma | 100 | 0.010 | | | 0.130 | 0.520 | 0.260 | 0.050 | 0.030 | | | 71 |
| | Milano | 50 | | | | 0.060 | 0.640 | 0.200 | 0.060 | 0.040 | | | 71 |
| | Toscany (Central Italy) | 107 | | | | NL | NL | NL | NL | NL | | | 76 |
| | Trieste | 71 | | | | 0.010 | 0.390 | 0.250 | 0.310 | 0.030 | | | 71 |
| | Udine | 57 | | | | 0.070 | 0.560 | 0.300 | 0.050 | 0.020 | | | 71 |
| | Verona | 202 | | | | 0.070 | 0.510 | 0.310 | 0.100 | 0.010 | | | 71 |
| | Catalans | 25 | | | | 0.040 | 0.800 | 0.160 | | | | | 71 |
| | Basques 1 | 52 | | | | 0.080 | 0.790 | 0.100 | | 0.040 | | | 71 |
| | Basques 2 | 30 | | | 0.030 | | 0.900 | | | 0.070 | | | 71 |
| | Iceland | 28 | | | | 0.070 | 0.640 | 0.070 | 0.140 | 0.070 | | | 71 |
| | Coimbra (Portugal) | 119 | | | | 0.067 | 0.563 | 0.286 | 0.076 | 0.008 | | | 72 |
| | Northern Portugal | 212 | | | | 0.099 | 0.608 | 0.236 | 0.052 | 0.005 | | | 77 |
| | Brussels (Belgium) | 83 | | | | 0.084 | 0.627 | 0.229 | 0.060 | | | | 72 |
| | Holland | 99 | | | | 0.030 | 0.727 | 0.162 | 0.051 | 0.030 | | | 72 |
| | Finland | 67 | 0.030 | | | 0.807 | 0.104 | 0.059 | | | | | 72 |
| | Dublin (Ireland) | 38 | 0.026 | | | 0.711 | 0.158 | 0.105 | | | | | 72 |
| | Iberian Peninsula | 763 | | | 0.008 | 0.104 | 0.667 | 0.166 | 0.029 | 0.025 | | | 58 |
| | Valencia (Eastern Spain) | 140 | | | | NL | NL | NL | NL | NL | | | 78 |
| | Majorca (Eastern Spain) | 53 | | | | 0.038 | 0.623 | 0.245 | 0.094 | | | | 79 |
| | Minorca (Eastern Spain) | 40 | | | 0.025 | 0.100 | 0.650 | 0.125 | 0.100 | | | | 79 |
| | Valencia (Eastern Spain) | 24 | | | | 0.042 | 0.583 | 0.333 | | | | | 79 |
| | Cantabria (Northern Spain) | 107 | | | | 0.178 | 0.589 | 0.159 | 0.056 | 0.019 | | | 80 |
| | Caceres (Central Spain) | 94 | | | 0.011 | 0.128 | 0.585 | 0.202 | 0.053 | 0.021 | | | 81 |
| | Southwest Spain | 111 | | | | NL | NL | NL | NL | NL | | | 82 |
| | Bern (Switzerland) | 126 | | | | 0.126 | 0.559 | 0.236 | 0.063 | 0.016 | | | 83 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS19 | **EUROPE** | | | | | | | | | | | | |
| | Bern (Switzerland) | 100 | | | | 0.140 | 0.560 | 0.230 | 0.060 | 0.010 | | | 71 |
| | Norwegian | 300 | | | | 0.027 | 0.527 | 0.313 | 0.120 | 0.013 | | | 84 |
| | Budapest (Central Hungary) | 116 | | | NL | NL | NL | NL | NL | NL | NL | | 40 |
| | Baranya County (SW Hungary) | 78 | | | NL | NL | NL | NL | NL | NL | NL | | 40 |
| | Slovenia | 121 | | | | NL | NL | NL | NL | NL | | | 85 |
| | Lithuania | 152 | | | | 0.020 | 0.118 | 0.454 | 0.322 | 0.086 | | | 86 |
| | Latvia | 145 | | | | 0.035 | 0.145 | 0.421 | 0.310 | 0.090 | | | 86 |
| | Estonia | 133 | | | | 0.045 | 0.459 | 0.226 | 0.248 | 0.023 | | | 86 |
| | Maderia Archipelago | 111 | | | 0.009 | 0.135 | 0.613 | 0.207 | 0.036 | | | | 87 |
| | Strasbourg (Eastern France) | 100 | | | | 0.080 | 0.600 | 0.260 | 0.040 | 0.020 | | | 88 |
| | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | |
| | Inuit | 62 | | | | 0.770 | 0.160 | 0.030 | 0.030 | | | | 71 |
| | Surinam | 54 | | | 0.020 | 0.110 | 0.220 | 0.500 | 0.110 | 0.040 | | | 71 |
| | Rio de Janeiro, Brazil | 109 | | | | 0.156 | 0.459 | 0.266 | 0.064 | 0.055 | | | 89 |
| | Buenos Aires, Caucasians | 100 | | | | 0.220 | 0.560 | 0.190 | 0.030 | | | | 71 |
| | Belo Horizonte | 252 | | | | 0.160 | 0.540 | 0.230 | 0.060 | 0.010 | | | 71 |
| | Mapuches | 16 | | | | 0.440 | 0.180 | 0.380 | | | | | 71 |
| | Tehuelches | 12 | | | | 0.160 | 0.420 | 0.420 | | | | | 71 |
| | Wichis | 6 | | | | 1.000 | | | | | | | 71 |
| | Yanomami | 27 | | | | 0.810 | 0.180 | | | | | | 71 |
| | New York (NY), Asians (USA) | 25 | | | | | 0.160 | 0.520 | 0.280 | 0.040 | | | 71 |
| | NY, African Americans (USA) | 81 | | | | 0.050 | 0.230 | 0.380 | 0.180 | 0.150 | | | 71 |
| | NY, Hispanics (USA) | 88 | | | 0.010 | 0.180 | 0.480 | 0.180 | 0.090 | 0.060 | | | 71 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS19 | **AFRICA** | | | | | | | | | | | | |
| | Moroacan Arabs (NWA) | 44 | | | | 0.592 | 0.295 | 0.045 | 0.045 | 0.023 | | | 90 |
| | Southern Morccan (NWA) | 44 | | | | 0.682 | 0.205 | 0.045 | 0.068 | | | | 90 |
| | Saharawis (NWA) | 29 | | | | 0.760 | 0.172 | 0.034 | | 0.034 | | | 90 |
| | Mozabites (NWA) | 68 | | | | 0.822 | 0.074 | 0.059 | 0.015 | 0.015 | 0.015 | | 90 |
| | Equatorial New Guinea | 48 | | | | 0.021 | 0.083 | 0.542 | 0.146 | 0.208 | | | 91 |
| | Equatorial New Guinea | 57 | | | | 0.018 | 0.070 | 0.544 | 0.140 | 0.228 | | | 92 |
| | Pygmy | 31 | | | | | 0.060 | 0.420 | 0.260 | 0.260 | | | 71 |
| | Ovambo | 34 | | | | 0.030 | 0.120 | 0.470 | 0.290 | 0.090 | | | 71 |
| | Morocco | 50 | | | | 0.580 | 0.240 | 0.160 | 0.020 | | | | 71 |
| | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | |
| | Chengdu, China | N/A | | | | | | NL | | NL | NL | | 93 |
| | Chengdu, China | 63 | | | | 0.111 | 0.222 | 0.397 | 0.206 | 0.064 | | | 74 |
| | China | 36 | | | | | 0.250 | 0.280 | 0.390 | 0.050 | 0.030 | | 71 |
| | Mongolians | 40 | | | | | 0.270 | 0.320 | 0.300 | 0.050 | 0.050 | | 71 |
| | Osaka | 150 | | | | 0.130 | 0.050 | 0.490 | 0.220 | 0.110 | | | 71 |
| | Japan 1 | 221 | | | | 0.080 | 0.030 | 0.510 | 0.230 | 0.150 | 0.010 | | 71 |
| | Japan 2 | 172 | | | | 0.060 | 0.060 | 0.410 | 0.240 | 0.210 | | | 71 |
| | Japan | 117 | | | | NL | NL | NL | | | | | 94 |
| | Japan | 108 | | | | 0.040 | 0.060 | 0.390 | 0.300 | 0.200 | 0.010 | | 95 |
| | Japan | 72 | | | | NL | NL | NL | NL | NL | | | 96 |
| | Japan (Okinawa) | 87 | | | | 0.081 | 0.058 | 0.299 | 0.287 | 0.276 | | | 97 |
| | Japan (Honshu) | 207 | | | | 0.048 | 0.058 | 0.488 | 0.217 | 0.184 | 0.005 | | 97 |
| | India | 35 | | | | | 0.370 | 0.510 | 0.110 | | | | 71 |
| | South Korea | 316 | | | | 0.010 | 0.155 | 0.456 | 0.280 | 0.098 | 0.003 | | 98 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS19 | **ASIA** | | | | | | | | | | | | |
| | South Korea | 330 | | | | 0.024 | 0.182 | 0.446 | 0.294 | 0.052 | 0.003 | | 99 |
| | Korea | 33 | | | | | 0.240 | 0.450 | 0.210 | 0.100 | | | 71 |
| | Pakistani | 35 | | | | | 0.180 | 0.540 | 0.200 | 0.080 | | | 71 |
| | Ahom | 21 | | | | 0.050 | 0.570 | 0.330 | 0.050 | | | | 71 |
| | Kachari | 28 | | | | | 0.680 | 0.250 | 0.070 | | | | 71 |
| | NO-Thai | 42 | | | | | 0.070 | 0.690 | 0.140 | 0.100 | | | 71 |
| | SO-Thai | 12 | | | | | 0.420 | 0.500 | 0.140 | 0.100 | | | 71 |
| | Kampuchea | 11 | | | | | 0.180 | 0.360 | 0.450 | | | | 71 |
| | Taiwan | 14 | | | | 0.070 | 0.290 | 0.290 | 0.290 | 0.070 | | | 71 |
| | Taiwan | 582 | | 0.002 | 0.002 | 0.033 | 0.205 | 0.464 | 0.222 | 0.072 | 0.002 | | 100 |
| | Ami | 7 | | | | 0.140 | 0.140 | 0.570 | 0.140 | | | | 71 |
| | Turkey | 92 | | | | 0.087 | 0.294 | 0.435 | 0.174 | 0.011 | | | 101 |
| | South India | 64 | | | | | NL | NL | NL | NL | | | 102 |
| | Eastern India | 150 | | | | 0.020 | 0.227 | 0.573 | 0.140 | 0.040 | | | 103 |
| | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | |
| | Papua New Guinea 1 | 23 | | | | | 0.180 | 0.770 | | | 0.040 | | 71 |
| | Papua New Guinea 2 | 50 | | | | | 0.520 | 0.480 | | | | | 71 |
| | S-Borneo | 13 | | | | 0.080 | 0.150 | 0.540 | 0.230 | | | | 71 |
| | Trobriands | 63 | | | | 0.060 | 0.080 | 0.510 | 0.330 | 0.020 | | | 71 |
| | W-Samoa | 10 | | | | | | 0.200 | 0.800 | | | | 71 |
| | Austrailian Caucasian | 214 | | | | 0.051 | 0.650 | 0.182 | 0.084 | 0.033 | | | 104 |
| | | | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | | | |
| | Greece | 64 | | | | 0.188 | 0.391 | 0.188 | 0.217 | 0.015 | | | 105 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS388 | **EUROPE** | | | | | | | | | | | |
| | Iberian Peninsula | 763 | | 0.004 | 0.810 | 0.080 | 0.028 | 0.046 | 0.029 | 0.004 | | 58 |
| | Norway | 300 | 0.020 | | 0.590 | 0.063 | 0.300 | 0.020 | 0.007 | | | 84 |
| | Toscany (Central Italy) | 107 | | | | NL | NL | NL | NL | | | 76 |
| | | | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | | | |
| | Moroccan Arabs | 44 | | | 0.795 | 0.045 | 0.023 | | | 0.114 | 0.023 | 90 |
| | Southern Moroccan Berbers | 44 | | | 0.841 | 0.045 | | 0.045 | 0.023 | 0.023 | 0.023 | 90 |
| | Saharawis | 29 | | | 0.828 | | | | 0.034 | 0.138 | | 90 |
| | Mozabites | 68 | | | 0.926 | 0.059 | | | 0.015 | | | 90 |
| | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | |
| | Rio de Janeiro | 109 | | | | 0.661 | 0.083 | 0.073 | 0.184 | | | 89 |
| | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | |
| | Taiwan | 582 | 0.198 | 0.016 | 0.722 | 0.050 | 0.014 | 0.002 | | | | 100 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 9(6) | 10(7) | 11(8) | 12(9) | 13(10) | Allele 14(11) | Range 15(12) | 16(13) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS389I | **EUROPE** | | | | | | | | | | |
| | Bern (Switzerland) | 100 | | | 0.020 | 0.021 | 0.059 | 0.180 | | | 71 |
| | Munster (Germany) | 124/118 | | | | 0.180 | 0.690 | 0.120 | | | 71 |
| | Berlin 1 (Germany) | 70 | | | | 0.240 | 0.530 | 0.230 | | | 71 |
| | Germany | 86 | | | | 0.326 | 0.533 | 0.130 | 0.011 | | 72 |
| | Ledien | 88 | | | | 0.270 | 0.600 | 0.120 | | | 71 |
| | Roma (Italy) | 100 | | | | 0.240 | 0.570 | 0.180 | 0.010 | | 71 |
| | Rome (Italy) | 125 | | | | 0.232 | 0.544 | 0.216 | 0.008 | | 72 |
| | Basque 2 | 30 | | | | 0.030 | 0.430 | 0.530 | | | 71 |
| | Iceland | 28/22 | | | | 0.390 | 0.500 | 0.110 | | | 71 |
| | Valencia (Eastern Spain) | 140 | | | | NL | NL | NL | | | 78 |
| | Northern Spain | 107 | | 0.009 | | 0.131 | 0.523 | 0.290 | 0.037 | 0.009 | 80 |
| | Galicia (Spain) | 107 | | | | 0.140 | 0.626 | 0.224 | 0.009 | | 72 |
| | Caceres (Central Spain) | 94 | | 0.011 | | 0.117 | 0.713 | 0.160 | | | 81 |
| | Lithuania | 152 | | | | 0.105 | 0.665 | 0.211 | 0.013 | 0.007 | 86 |
| | Latvia | 145 | | | 0.014 | 0.083 | 0.690 | 0.207 | 0.007 | | 86 |
| | Estonia | 133 | | | 0.008 | 0.173 | 0.556 | 0.256 | 0.008 | | 86 |
| | Athens (Greece) | 47 | | | 0.021 | 0.149 | 0.617 | 0.213 | | | 72 |
| | London (UK) | 71 | | | 0.007 | 0.207 | 0.621 | 0.150 | 0.014 | | 72 |
| | Coimbra (Portugal) | 119 | | | | 0.143 | 0.664 | 0.193 | | | 72 |
| | Brussels (Belgium) | 83 | | | | 0.157 | 0.711 | 0.133 | | | 72 |
| | Holland | 99 | | | 0.010 | 0.243 | 0.626 | 0.121 | | | 72 |
| | Dublin (Ireland) | 38 | | | | 0.096 | 0.731 | 0.173 | | | 72 |
| | Finland | 67 | | | | 0.268 | 0.238 | 0.494 | | | 72 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 24(21) | 26(23) | 27(24) | 28(25) | 29(26) | 30(27) | 31(28) | 32(29) | 33(30) | 34(31) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS389II | **EUROPE** | | | | | | | | | | | | |
| | Bern (Switzerland) | 100 | | 0.010 | 0.040 | 0.130 | 0.410 | 0.250 | 0.120 | 0.040 | | | 71 |
| | Munster (Germany) | 124/118 | | | 0.020 | 0.360 | 0.330 | 0.230 | 0.050 | 0.010 | 0.010 | | 71 |
| | Berlin 1 (Germany) | 70 | | | | 0.210 | 0.290 | 0.330 | 0.100 | 0.070 | | | 71 |
| | Germany | 86 | | | | 0.187 | 0.351 | 0.308 | 0.110 | 0.022 | | 0.022 | 72 |
| | Ledien | 88 | | | | 0.310 | 0.410 | 0.250 | 0.030 | | | | 71 |
| | Roma (Italy) | 100 | | | | 0.310 | 0.410 | 0.250 | 0.030 | | | | 71 |
| | Rome (Italy) | 125 | | 0.008 | 0.024 | 0.144 | 0.360 | 0.312 | 0.120 | 0.032 | | | 72 |
| | Basque 2 | 30 | | | 0.030 | | 0.400 | 0.400 | 0.100 | 0.070 | | | 71 |
| | Iceland | 28/22 | | | 0.040 | 0.320 | 0.270 | 0.140 | 0.180 | 0.040 | | | 71 |
| | Valencia (Eastern Spain) | 140 | | | | NL | NL | NL | NL | NL | | | 78 |
| | Northern Spain | 107 | | 0.009 | | 0.122 | 0.441 | 0.290 | 0.122 | 0.047 | | | 80 |
| | Galicia (Spain) | 107 | | | 0.028 | 0.131 | 0.505 | 0.196 | 0.112 | 0.028 | | | 72 |
| | Caceres (Central Spain) | 94 | | | 0.011 | 0.149 | 0.532 | 0.298 | 0.011 | | | | 81 |
| | Lithuania | 152 | | | | 0.026 | 0.250 | 0.513 | 0.165 | 0.040 | 0.007 | | 86 |
| | Latvia | 145 | | | | 0.069 | 0.338 | 0.428 | 0.124 | 0.041 | | | 86 |
| | Estonia | 133 | | | | 0.188 | 0.308 | 0.990 | 0.083 | 0.015 | 0.008 | | 86 |
| | Athens (Greece) | 47 | | | 0.021 | 0.107 | 0.255 | 0.319 | 0.234 | 0.064 | | | 72 |
| | London (UK) | 71 | | | 0.014 | 0.141 | 0.465 | 0.324 | 0.042 | | 0.026 | | 72 |
| | Coimbra (Portugal) | 119 | | 0.008 | 0.008 | 0.059 | 0.555 | 0.244 | 0.109 | 0.017 | | | 72 |
| | Brussels (Belgium) | 83 | | | 0.012 | 0.048 | 0.614 | 0.229 | 0.084 | 0.012 | | | 72 |
| | Holland | 99 | | | 0.020 | 0.434 | 0.434 | 0.244 | 0.040 | 0.040 | 0.010 | | 72 |
| | Dublin (Ireland) | 38 | | | | 0.111 | 0.556 | 0.200 | 0.111 | 0.022 | | | 72 |
| | Finland | 67 | | | | 0.283 | 0.164 | 0.495 | 0.044 | 0.014 | | | 72 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 9(6) | 10(7) | 11(8) | 12(9) | 13(10) | Allele 14(11) | Range 15(12) | 16(13) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS389I | **EUROPE (Con't)** | | | | | | | | | | |
| | Budapest (Central Hungary) | 116 | | | | | NL | NL | NL | | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | NL | NL | NL | | 40 |
| | Strasbourg (Eastern France) | 100 | | | | 0.190 | 0.650 | 0.160 | | | 88 |
| | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | |
| | Inuit | 62 | | | | 0.080 | 0.160 | 0.600 | 0.140 | 0.020 | 71 |
| | Surinam | 54 | | | | 0.180 | 0.540 | 0.260 | 0.020 | | 71 |
| | Buenos Aries, Caucasians | 100 | | | | 0.110 | 0.610 | 0.280 | | | 71 |
| | Rio de Janiero (Brazil) | 109 | | | | 0.009 | 0.321 | 0.569 | 0.101 | | 71 |
| | Mapuches | 16 | | | | | 0.810 | 0.190 | | | 71 |
| | Tehuelches | 12 | | | | 0.080 | 0.670 | 0.250 | | | 71 |
| | Wichis | 6 | | | | | 0.830 | 0.170 | | | 71 |
| | Yanomami | 12 | | | | | 1.000 | | | | 71 |
| | New York, Asians | 25 | | | | 0.600 | 0.200 | 0.200 | | | 71 |
| | New York, African Americans | 81 | | | | 0.160 | 0.630 | 0.180 | | | 71 |
| | New York, Hispanics | 88 | | | | 0.140 | 0.570 | 0.280 | 0.010 | | 71 |
| | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | |
| | Chinese | 36 | | | 0.030 | 0.510 | 0.140 | 0.310 | | | 71 |
| | Taiwan | 582 | 0.002 | | 0.010 | 0.574 | 0.253 | 0.156 | 0.005 | | 100 |
| | Mongolians | 40 | | | 0.020 | 0.100 | 0.700 | 0.170 | | | 71 |
| | Osaka (Japan) | 150 | | | | 0.170 | 0.300 | 0.530 | | | 71 |
| | Ami | 7 | | | | 0.430 | 0.430 | 0.140 | | | 71 |
| | Korean | 316 | | | 0.019 | 0.402 | 0.241 | 0.335 | 0.003 | | 98 |
| | Turkey | 92 | | | | | | | | | 101 |
| | Eastern India | 150 | | 0.007 | 0.133 | 0.393 | 0.467 | | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 24 (21) | 26(23) | 27(24) | 28(25) | 29(26) | 30(27) | 31(28) | 32(29) | 33(30) | 34(31) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS389II | **EUROPE** | | | | | | | | | | | | |
| | Budapest (Central Hungary) | 116 | | | | NL | NL | NL | NL | NL | | | 40 |
| | Baranya County (SW Hungary) | 78 | | | | NL | NL | NL | NL | NL | | | 40 |
| | Strasbourg (Eastern France) | 100 | | | | 0.140 | 0.550 | 0.240 | 0.050 | 0.020 | | | 88 |
| | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | |
| | Inuit | 62 | | | | 0.060 | 0.140 | 0.340 | 0.240 | 0.190 | 0.020 | | 71 |
| | Surinam | 54 | | 0.020 | 0.020 | 0.060 | 0.240 | 0.390 | 0.200 | 0.070 | | | 71 |
| | Buenos Aries, Caucasians | 100 | | | | 0.130 | 0.370 | 0.320 | 0.140 | 0.040 | | | 71 |
| | Rio de Janiero (Brazil) | 109 | | | | 0.037 | 0.330 | 0.450 | 0.138 | 0.046 | | | 71 |
| | Mapuches | 16 | | | | 0.060 | 0.440 | 0.370 | 0.130 | | | | 71 |
| | Tehuelches | 12 | | | | 0.080 | 0.500 | 0.170 | 0.170 | 0.080 | | | 71 |
| | Wichis | 6 | | | | | 0.170 | 0.660 | 0.170 | | | | 71 |
| | Yanomami | 12 | | | | | 0.080 | 0.500 | 0.420 | | | | 71 |
| | New York, Asians | 25 | | | 0.120 | 0.320 | 0.480 | 0.040 | 0.040 | | | | 71 |
| | New York, African Americans | 81 | | | 0.010 | 0.060 | 0.200 | 0.430 | 0.260 | 0.040 | | | 71 |
| | New York, Hispanics | 88 | | | | 0.070 | 0.350 | 0.400 | 0.150 | 0.030 | | | 71 |
| | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | |
| | Chinese | 36 | | | 0.110 | 0.310 | 0.190 | 0.190 | 0.190 | | | | 71 |
| | Taiwan | 582 | | 0.016 | 0.101 | 0.330 | 0.277 | 0.182 | 0.057 | 0.031 | 0.005 | | 100 |
| | Mongolians | 40 | 0.002 | | 0.050 | 0.220 | 0.400 | 0.200 | 0.120 | | | | 71 |
| | Osaka (Japan) | 150 | | | | 0.130 | 0.270 | 0.220 | 0.370 | | | | 71 |
| | Ami | 7 | | | | 0.280 | 0.280 | 0.280 | 0.140 | | | | 71 |
| | Korean | 316 | | | 0.130 | 0.212 | 0.402 | 0.206 | 0.041 | 0.010 | | | 98 |
| | Turkey | 92 | | | | 0.130 | 0.283 | 0.391 | 0.130 | 0.065 | | | 101 |
| | Eastern India | 150 | | 0.100 | 0.247 | 0.280 | 0.253 | 0.107 | 0.013 | | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 9(6) | 10(7) | 11(8) | 12(9) | 13(10) | Allele 14(11) | Range 15(12) | 16(13) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS389I | **AFRICA** | | | | | | | | | | |
| | | | | | | | | | | | |
| | Pygmy | 31 | | | 0.130 | 0.320 | 0.420 | 0.060 | 0.060 | | 71 |
| | Ovambo | 23 | | | | 0.230 | 0.640 | 0.140 | | | 71 |
| | Morocco | 41 | | | | 0.170 | 0.320 | 0.440 | 0.050 | 0.020 | 71 |
| | Equatorial Guinea | 48 | | | | 0.083 | 0.667 | 0.229 | 0.021 | | 91 |
| | Equatorial Guinea | 57 | | | | 0.105 | 0.649 | 0.228 | 0.018 | | 92 |
| | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | |
| | Papua New Guinea 1 | 23 | | | | 0.260 | 0.560 | 0.170 | | | 71 |
| | Papua New Guinea 2 | 53 | | | | 0.270 | 0.530 | 0.180 | 0.020 | | 71 |
| | S-Borneo | 13 | | | | 0.610 | 0.300 | 0.080 | | | 71 |
| | Trobriands | 63 | | | 0.020 | 0.320 | 0.510 | 0.160 | | | 71 |
| | W-Samoa | 10 | | | | 0.200 | 0.600 | 0.200 | | | 71 |
| | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | |
| | Greece | 64 | | | | 0.1739 | 0.5942 | 0.2174 | 0.0145 | | 105 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 24(21) | 26(23) | 27(24) | 28(25) | 29(26) | 30(27) | 31(28) | 32(29) | 33(30) | 34(31) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS389II | **EUROPE** | | | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | | | | |
| | Pygmy | 31 | | | 0.030 | 0.350 | 0.260 | 0.160 | 0.060 | 0.060 | 0.060 | | 71 |
| | Ovambo | 23 | | | | 0.260 | 0.220 | 0.220 | 0.040 | 0.170 | 0.090 | | 71 |
| | Morocco | 41 | | | | 0.190 | 0.150 | 0.560 | 0.100 | | | | 71 |
| | Equatorial Guinea | 48 | | | | | 0.188 | 0.500 | 0.271 | 0.042 | | | 91 |
| | Equatorial Guinea | 57 | | | | | 0.175 | 0.509 | 0.281 | 0.035 | | | 92 |
| | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | |
| | Papua New Guinea 1 | 23 | | | | 0.040 | 0.520 | 0.300 | 0.090 | | 0.040 | | 71 |
| | Papua New Guinea 2 | 53 | | | | 0.080 | 0.450 | 0.280 | 0.080 | 0.110 | | | 71 |
| | S-Borneo | 13 | | | | 0.460 | 0.310 | 0.230 | | | | | 71 |
| | Trobriands | 63 | | | 0.020 | 0.160 | 0.270 | 0.130 | 0.360 | 0.060 | | | 71 |
| | W-Samoa | 10 | | | | 0.100 | | 0.600 | 0.200 | 0.100 | | | 71 |
| | | | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | | | |
| | Greece | 64 | | | | 0.087 | 0.349 | 0.304 | 0.203 | 0.058 | | | 105 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 9 11 | 9 12 | 9 13 | 9 13-14 | 9 14 | 9 15 | 9 16 | 9 17 | 9 18 | 9 19 | 10 10 | 10 11 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | | | | | | | | | | | 0.010 | | 71 |
| | Berlin 3, pooled (Germnay) | 136 | | | | | | | | 0.010 | | | | | 71 |
| | Iberian Peninsula | 763 | | | | | 0.001 | | | | | | | | 71 |
| | Norway | 300 | | | | | | | | | | | | | 71 |
| | Lithuania | 152 | | | 0.007 | | | | | | | | | 0.007 | 86 |
| | Budapest (Central Hungary) | 116 | | | | | | | | | | | | | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | | | | | | | | | 40 |
| | Northern Portugal | 212 | | | | | | | | | | | | | 77 |
| | Strasbourg (Eastern France) | 100 | | | | | | | | | | | | | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | | | | | | | | | | | 0.010 | | 71 |
| | Mapuches | 16 | | | | | | | | | | | | | 71 |
| | Tehuelches | 12 | | | | | | | | | | | | | 71 |
| | Yanomami | 27 | | | | | | | | | | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | | | | | | 0.110 | 0.020 | | | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | | | | | | | | | 0.003 | 0.019 | 0.003 | | 71 |
| | Taiwan | 582 | | | | | | | | | NL | | | NL | 100 |
| | Turkey | 92 | | | | | | | | | | | | | 101 |
| | Eastern India | 100 | 0.200 | 0.213 | 0.033 | 0.007 | 0.013 | | 0.007 | | | | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 10 12 | 10 13 | 10 14 | 10 15 | 10 16 | 10 17 | 10 18 | 10 19 | 10 20 | 10 22 | 11 11 | 11 12 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | 0.010 | 0.020 | 0.030 | | | | | | | | 0.010 | | 71 |
| | Berlin 3, pooled (Germnay) | 136 | | 0.010 | 0.030 | | | | | | | | 0.010 | 0.010 | 71 |
| | Iberian Peninsula | 763 | | 0.003 | 0.008 | 0.005 | | | | | | | 0.022 | 0.005 | 71 |
| | Norway | 300 | | | 0.013 | 0.003 | | | | | | | 0.007 | 0.010 | 71 |
| | Lithuania | 152 | 0.040 | 0.066 | 0.046 | | | | | | | | 0.020 | | 86 |
| | Budapest (Central Hungary) | 116 | | | NL | | | | | | | | | | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | | | | | | | | | 40 |
| | Northern Portugal | 212 | | 0.009 | 0.009 | 0.005 | | | | | | | 0.005 | 0.009 | 77 |
| | Strasbourg (Eastern France) | 100 | | 0.020 | 0.020 | 0.010 | | | | | | | 0.020 | | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | 0.030 | 0.130 | 0.050 | | | | | | | | | | 71 |
| | Mapuches | 16 | | 0.060 | | | | | | | | | | | 71 |
| | Tehuelches | 12 | | | 0.080 | | | | | | | | | | 71 |
| | Yanomami | 27 | | | | | | | | | | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | | | | | | | | | | | | 0.030 | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | 0.003 | | 0.003 | | | 0.054 | 0.098 | 0.092 | 0.019 | 0.003 | | 0.029 | 71 |
| | Taiwan | 582 | NL | NL | | | | NL | | | | | NL | NL | 100 |
| | Turkey | 92 | | | | | | | | | | | | 0.012 | 101 |
| | Eastern India | 100 | 0.027 | 0.027 | 0.033 | 0.013 | 0.027 | 0.013 | | | | 0.013 | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 11 13 | 11 14 | 11 15 | 11 16 | 11 17 | 11 18 | 11 19 | 12 12 | 12 13 | 12 14 | 12.2 14 | 12 15 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | 0.070 | 0.380 | 0.050 | 0.020 | | | | 0.010 | 0.040 | 0.040 | | 0.030 | 71 |
| | Berlin 3, pooled (Germnay) | 136 | 0.020 | 0.270 | 0.060 | 0.010 | 0.010 | | | | 0.010 | 0.040 | | 0.010 | 71 |
| | Iberian Peninsula | 763 | 0.050 | 0.425 | 0.087 | 0.013 | 0.004 | | | 0.028 | 0.008 | 0.060 | | 0.020 | 71 |
| | Norway | 300 | 0.117 | 0.350 | 0.053 | 0.007 | | | | 0.003 | 0.003 | 0.023 | | 0.007 | 71 |
| | Lithuania | 152 | 0.158 | 0.362 | 0.125 | | | | | | 0.013 | | | 0.007 | 86 |
| | Budapest (Central Hungary) | 116 | NL | NL | NL | | | | | NL | NL | NL | | NL | 40 |
| | Baranya County (SW Hungary) | 78 | | NL | | | | | | | | | | | 40 |
| | Northern Portugal | 212 | 0.009 | 0.382 | 0.071 | 0.014 | | | | 0.014 | 0.014 | 0.075 | 0.005 | | 77 |
| | Strasbourg (Eastern France) | 100 | 0.040 | 0.370 | 0.120 | 0.010 | 0.010 | | | | 0.020 | 0.110 | | 0.030 | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | 0.060 | 0.160 | 0.020 | 0.010 | | | | | | 0.050 | | 0.020 | 71 |
| | Mapuches | 16 | | | | | | | | | | 0.060 | | 0.180 | 71 |
| | Tehuelches | 12 | 0.250 | | | | | | | | | | | | 71 |
| | Yanomami | 27 | | | | | | | | | | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | | | 0.020 | | | | | 0.160 | 0.020 | | | 0.050 | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | 0.010 | | | 0.003 | 0.029 | | | 0.013 | 0.006 | 0.010 | | 0.003 | 71 |
| | Taiwan | 582 | NL | NL | | NL | NL | NL | NL | NL | NL | NL | | NL | 100 |
| | Turkey | 92 | 0.036 | 0.095 | 0.047 | 0.036 | | | | 0.012 | 0.036 | 0.024 | | 0.036 | 101 |
| | Eastern India | 100 | | 0.020 | 0.027 | 0.047 | 0.047 | 0.007 | 0.007 | 0.007 | 0.020 | 0.007 | | 0.020 | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 12 16 | 12 17 | 12 17.1 | 12 18 | 12 19 | 12 20 | 12 22 | 13 13 | 13 14 | 13 15 | 13 16 | 13 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | 0.020 | | | | | | | | 0.050 | 0.040 | 0.010 | 0.010 | 71 |
| | Berlin 3, pooled (Germnay) | 136 | 0.030 | 0.010 | | | | 0.010 | | 0.010 | 0.040 | 0.040 | 0.010 | 0.020 | 71 |
| | Iberian Peninsula | 763 | 0.010 | 0.004 | | 0.005 | | | | 0.004 | 0.046 | 0.024 | 0.021 | 0.008 | 71 |
| | Norway | 300 | 0.003 | | | | | | | 0.017 | 0.087 | 0.007 | 0.007 | 0.013 | 71 |
| | Lithuania | 152 | 0.007 | 0.007 | NL | | | | | | 0.040 | | | | 86 |
| | Budapest (Central Hungary) | 116 | NL | | | | | | | NL | NL | NL | NL | NL | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | | | | | NL | | NL | | 40 |
| | Northern Portugal | 212 | 0.014 | | | | | | | 0.099 | 0.052 | 0.033 | 0.028 | 0.019 | 77 |
| | Strasbourg (Eastern France) | 100 | 0.020 | | | | | | | | 0.050 | | 0.020 | 0.010 | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | 0.010 | | | | | | | 0.030 | 0.020 | | 0.020 | | 71 |
| | Mapuches | 16 | | | | | | | | 0.060 | | | 0.130 | | 71 |
| | Tehuelches | 12 | | | | | | | | | 0.080 | | | | 71 |
| | Yanomami | 27 | | | | | | | | | | | 0.080 | | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | 0.110 | | | | | | | 0.030 | 0.140 | 0.080 | | 0.060 | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | 0.020 | 0.035 | | 0.048 | 0.035 | 0.006 | | 0.041 | 0.006 | 0.006 | 0.022 | 0.010 | 71 |
| | Taiwan | 582 | NL | NL | | NL | NL | NL | NL | NL | NL | NL | NL | NL | 100 |
| | Turkey | 92 | | 0.048 | | | 0.012 | | | | 0.036 | 0.060 | 0.071 | 0.071 | 101 |
| | Eastern India | 100 | 0.027 | 0.033 | | 0.007 | | | | 0.020 | 0.027 | 0.053 | 0.060 | 0.020 | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 13 17.1 | 13 17.2 | 13 18 | 13 18.1 | 13 19 | 13 20 | 13 21 | 13 22 | 13 23 | 13 24 | 14 14 | 14 15 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | | | | | | | | | | | 0.010 | 0.030 | 71 |
| | Berlin 3, pooled (Germnay) | 136 | | | 0.040 | | | | | | | | 0.040 | 0.040 | 71 |
| | Iberian Peninsula | 763 | | | 0.009 | | | 0.001 | 0.001 | | | | 0.034 | 0.014 | 71 |
| | Norway | 300 | | | | | 0.003 | 0.007 | | | | | 0.110 | 0.090 | 71 |
| | Lithuania | 152 | | | | | | | | | | | 0.020 | 0.033 | 86 |
| | Budapest (Central Hungary) | 116 | NL | NL | NL | | NL | | | | | | NL | NL | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | | | | | | | NL | NL | 40 |
| | Northern Portugal | 212 | | | 0.014 | | 0.005 | | 0.005 | | | | 0.042 | 0.033 | 77 |
| | Strasbourg (Eastern France) | 100 | | | | | | | | | | | 0.040 | 0.010 | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | | | 0.030 | | 0.040 | | | | | | 0.030 | 0.040 | 71 |
| | Mapuches | 16 | | | 0.130 | | 0.060 | | | | | | 0.060 | 0.130 | 71 |
| | Tehuelches | 12 | | | | | | | | | | | 0.170 | 0.170 | 71 |
| | Yanomami | 27 | | | | | 0.080 | | | | | | | 0.080 | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | | | | | | | | | | | | 0.050 | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | | | 0.038 | | 0.067 | 0.032 | 0.003 | 0.003 | | | 0.003 | | 71 |
| | Taiwan | 582 | | | NL | | NL | NL | NL | NL | NL | NL | NL | NL | 100 |
| | Turkery | 92 | | | 0.060 | | | | | | | | | 0.024 | 101 |
| | Eastern India | 100 | | | 0.013 | | 0.020 | | | | | | 0.020 | 0.020 | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 14 16 | 14 17 | 14 17.2 | 14 17.1 | 14 18 | 14 19 | 14 20 | 14 21 | 14 22 | 15 15 | 15 16 | 15 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | 0.030 | 0.020 | | | | | | | | | | 0.010 | 71 |
| | Berlin 3, pooled (Germnay) | 136 | 0.010 | 0.030 | | | 0.010 | | | | | 0.020 | 0.010 | 0.020 | 71 |
| | Iberian Peninsula | 763 | 0.010 | 0.003 | | | 0.004 | | | | | 0.012 | 0.008 | 0.004 | 71 |
| | Norway | 300 | 0.007 | 0.003 | | | | 0.003 | | | | 0.033 | 0.007 | | 71 |
| | Lithuania | 152 | 0.013 | | | | | | | | | 0.020 | | | 86 |
| | Budapest (Central Hungary) | 116 | NL | | | NL | NL | | | | | NL | NL | NL | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | | | | | | NL | NL | NL | 40 |
| | Nothern Portugal | 212 | 0.005 | 0.005 | | | 0.009 | 0.005 | | | | 0.024 | 0.009 | | 77 |
| | Strasbourg (Eastern France) | 100 | 0.010 | | 0.01 | | | | | | | 0.010 | 0.010 | 0.010 | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | | 0.080 | | | 0.040 | | | | | | | | 71 |
| | Mapuches | 16 | | | | | | | | | | | | | 71 |
| | Tehuelches | 12 | | | | | | | | | | | | | 71 |
| | Yanomami | 27 | 0.150 | 0.080 | | | 0.230 | | | | | | | 0.310 | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | | 0.020 | | | | | | | | 0.100 | | | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | 0.006 | 0.013 | | | 0.029 | 0.016 | 0.013 | | | | | | 71 |
| | Taiwan | 582 | NL | NL | | | NL | NL | NL | | NL | | NL | | 100 |
| | Turkery | 92 | 0.048 | 0.048 | | | | | 0.012 | | | | 0.012 | 0.060 | 101 |
| | Eastern India | 100 | 0.013 | 0.007 | | | | | | | | | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 15 18 | 15 19 | 15 20 | 15 21 | 15 23 | 16 16 | 16 17 | 16 18 | 16 19 | 16 20 | 16 22 | 17 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | | | | |
| | Magdeburg | 164 | 0.010 | | | | | | | 0.010 | 0.020 | | | | 71 |
| | Berlin 3, pooled (Germnay) | 136 | 0.010 | 0.010 | | | | 0.010 | 0.010 | 0.010 | | | | | 71 |
| | Iberian Peninsula | 763 | 0.003 | 0.001 | | | | 0.003 | 0.008 | 0.012 | 0.001 | | | 0.003 | 71 |
| | Norway | 300 | | | | | | | | | | | | 0.003 | 71 |
| | Lithuania | 152 | | | | | | | 0.007 | | | | | | 86 |
| | Budapest (Central Hungary) | 116 | NL | | NL | | | | NL | | | | | NL | 40 |
| | Baranya County (SW Hungary) | 78 | | NL | | | | | | | NL | | | | 40 |
| | Nothern Portugal | 212 | | | | | | 0.009 | | 0.005 | 0.005 | | | 0.009 | 77 |
| | Strasbourg (Eastern France) | 100 | | | | | | 0.020 | | 0.010 | | | | | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | 0.030 | | 0.010 | | | | 0.010 | | 0.030 | | | 0.030 | 71 |
| | Mapuches | 16 | | | 0.130 | | | | | | | | | | 71 |
| | Tehuelches | 12 | 0.080 | | | | | | | | | | | 0.170 | 71 |
| | Yanomami | 27 | | | | | | | | | | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Trobriands | | | | | | | | | | | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Korean | 316 | | 0.019 | 0.013 | 0.013 | | | | 0.003 | 0.003 | | 0.006 | | 71 |
| | Taiwan | 582 | NL | NL | NL | | NL | NL | | | | NL | | | 100 |
| | Turkery | 92 | 0.012 | 0.012 | | | | | 0.036 | 0.012 | | | | 0.036 | 101 |
| | Eastern India | 100 | 0.007 | | | | | | | | | | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 16 22 | 17 17 | 17 18 | 17 19 | 17 20 | 18 18 | 18 19 | 18 21 | 19 19 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS385 | **EUROPE** | | | | | | | | | | | |
| | Magdeburg | 164 | | | 0.020 | 0.010 | | | 0.010 | | | 71 |
| | Berlin 3, pooled (Germnay) | 136 | | | 0.030 | 0.010 | | | | | | 71 |
| | Iberian Peninsula | 763 | | 0.003 | 0.001 | 0.003 | | | 0.001 | | | 71 |
| | Norway | 300 | | 0.003 | 0.003 | | | | | | | 71 |
| | Lithuania | 152 | | | 0.007 | | | | | | | 86 |
| | Budapest (Central Hungary) | 116 | | NL | | | | | | | | 40 |
| | Baranya County (SW Hungary) | 78 | | | NL | | | NL | | | | 40 |
| | Nothern Portugal | 212 | | 0.009 | | 0.005 | 0.005 | | | | 0.005 | 77 |
| | Strasbourg (Eastern France) | 100 | | | | | | | | | | 88 |
| | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | |
| | Buenous Aires, Caucasians | 100 | | 0.030 | | | | | 0.010 | | | 71 |
| | Mapuches | 16 | | | | | | | | | | 71 |
| | Tehuelches | 12 | | 0.170 | | | | | | | | 71 |
| | Yanomami | 27 | | | | | | | | | | 71 |
| | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | |
| | Trobriands | | | | | | | | | | | 71 |
| | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | |
| | Korean | 316 | 0.006 | | | | | | 0.003 | 0.003 | | 71 |
| | Taiwan | 582 | | | | | | | | | | 100 |
| | Turkery | 92 | | 0.036 | 0.012 | 0.012 | | | 0.012 | | | 101 |
| | Eastern India | 100 | | | | | | | | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS390 | **EUROPE** | | | | | | | | | | | | | | 71 |
| | Bern (Switzerland) | 64 | | | | | 0.030 | 0.140 | 0.280 | 0.390 | 0.160 | | | | 71 |
| | Bern (Switzerland) | 126 | | | | | 0.031 | 0.157 | 0.244 | 0.425 | 0.142 | | | | 83 |
| | Berlin 2 (Germany) | 59 | | | | | 0.030 | 0.190 | 0.150 | 0.390 | 0.240 | | | | 71 |
| | Berlin 1 (Germany) | 70 | | | | | | 0.090 | 0.360 | 0.260 | 0.230 | 0.070 | | | 71 |
| | Munster (Germany) | 114 | | | | | 0.030 | 0.160 | 0.260 | 0.370 | 0.170 | 0.010 | | | 71 |
| | Heidleberg (Germany) | 104 | | | | | | 0.090 | 0.200 | 0.330 | 0.280 | 0.110 | | | 71 |
| | Bavaria (Germany) | 151 | | | | | | NL | NL | NL | NL | NL | | | 73 |
| | Wurtemberg (Germany) | 257 | | | | | 0.012 | 0.241 | 0.284 | 0.339 | 0.113 | 0.012 | | | 75 |
| | Germany | 91 | | | | | | 0.209 | 0.268 | 0.337 | 0.163 | 0.023 | | | 72 |
| | Germany | 136 | | | | | 0.015 | 0.110 | 0.324 | 0.397 | 0.125 | 0.029 | | | 74 |
| | Leiden | 88 | | | | | 0.010 | 0.180 | 0.390 | 0.320 | 0.070 | 0.020 | 0.010 | | 71 |
| | Leicester, pooled | 337 | | | 0.010 | | 0.070 | 0.160 | 0.290 | 0.310 | 0.120 | 0.030 | 0.010 | | 71 |
| | Roma (Italy) | 100 | | | | | 0.010 | 0.150 | 0.390 | 0.390 | 0.050 | 0.010 | | | 71 |
| | Rome (Italy) | 125 | | | | | 0.016 | 0.160 | 0.684 | 0.368 | 0.064 | 0.008 | | | 72 |
| | Toscany (Central Italy) | 107 | | | | | NL | NL | NL | NL | NL | NL | NL | | 76 |
| | Catalans | 29 | | | | | | 0.070 | 0.170 | 0.690 | 0.070 | | | | 71 |
| | Basques 1 | 53 | | | | | | | 0.170 | 0.770 | 0.060 | | | | 71 |
| | Basques 2 | 30 | | | | | | | 0.170 | 0.730 | 0.100 | | | | 71 |
| | Iberian Peninsula | 768 | | | | | 0.005 | 0.048 | 0.248 | 0.595 | 0.098 | 0.005 | | | 58 |
| | Coimbra (Portugal) | 119 | | | | | 0.034 | 0.109 | 0.193 | 0.521 | 0.135 | | 0.008 | | 72 |
| | Northern Portugal | 212 | | | | | 0.024 | 0.052 | 0.212 | 0.599 | 0.108 | 0.005 | | | 77 |
| | Valencia (Eastern Spain) | 113 | | | | | | NL | NL | NL | NL | NL | | | 78 |
| | Northern Spain | 107 | | | | | | 0.056 | 0.159 | 0.626 | 0.159 | | | | 80 |
| | Majorca (Eastern Spain) | 53 | | | | | 0.038 | 0.038 | 0.226 | 0.566 | 0.132 | | | | 79 |
| | Minorca (Eastern Spain) | 40 | | | | | | 0.050 | 0.200 | 0.675 | 0.075 | | | | 79 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 17 | 18 | 19 | 20 | Allele 21 | Range 22 | 23 | 24 | 25 | 26 | 27 | 28 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS390 | **EUROPE** | | | | | | | | | | | | | | |
| | Valencia (Eastern Spain) | 24 | | | | | 0.017 | 0.043 | 0.222 | 0.624 | 0.094 | | | | 79 |
| | Galicia (Spain) | 107 | | | | | 0.009 | 0.056 | 0.271 | 0.533 | 0.131 | | | | 72 |
| | Southwest Spain | 111 | | | | | | NL | NL | NL | NL | | | | 82 |
| | Caceres (Central Spain) | 100 | | | | | 0.011 | 0.053 | 0.223 | 0.575 | 0.138 | | | | 81 |
| | Norway | 300 | | | | | 0.003 | 0.160 | 0.316 | 0.296 | 0.220 | 0.003 | | | 83 |
| | Athens (Greece) | 47 | | | | | 0.021 | 0.064 | 0.319 | 0.404 | 0.192 | | | | 72 |
| | London (UK) | 121 | | | | | 0.025 | 0.132 | 0.298 | 0.388 | 0.157 | | | | 72 |
| | Brussels (Belgium) | 83 | | | | | | 0.145 | 0.277 | 0.446 | 0.096 | 0.036 | | | 72 |
| | Holland | 99 | | | | | 0.010 | 0.182 | 0.414 | 0.253 | 0.141 | | | | 72 |
| | Finland | 67 | | | | | | 0.014 | 0.449 | 0.449 | 0.074 | 0.014 | | | 72 |
| | Dublin (Ireland) | 49 | | | | | | 0.041 | 0.204 | 0.490 | 0.224 | 0.041 | | | 72 |
| | Budapest (Hungary) | 116 | | | | | NL | NL | NL | NL | NL | NL | | | 40 |
| | Baranya county (SW Hungary) | 78 | | | | | NL | NL | NL | NL | NL | NL | | | 40 |
| | Slovenia | 121 | | | | | NL | NL | NL | NL | NL | NL | NL | | 85 |
| | Lithuania | 152 | | | | | | 0.026 | 0.290 | 0.204 | 0.428 | 0.053 | | | 86 |
| | Latvia | 145 | | | | | | 0.041 | 0.310 | 0.241 | 0.324 | 0.083 | | | 86 |
| | Estonia | 133 | | | | | | 0.098 | 0.308 | 0.286 | 0.226 | 0.083 | | | 86 |
| | Madeira Archipelago | 111 | | | | | | 0.072 | 0.279 | 0.550 | 0.081 | 0.018 | 0.613 | | 87 |
| | Strasbourg (Eastern France) | 100 | | | | | 0.020 | 0.080 | 0.240 | 0.510 | 0.140 | 0.010 | | | 89 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | 71 |
| | Inuit | 62 | | | | | | 0.050 | 0.060 | 0.860 | 0.030 | | | | 71 |
| | Surinam | 54 | | | | | 0.260 | 0.170 | 0.300 | 0.130 | 0.150 | | | | 71 |
| | Buenous Aires, Caucasians | 100 | | | | | 0.020 | 0.120 | 0.280 | 0.540 | 0.040 | | | | 71 |
| | de Janeiro | 109 | | | | | 0.064 | 0.055 | 0.229 | 0.339 | 0.275 | 0.037 | | | 89 |
| | Mapuches | 16 | | | | | | | 0.370 | 0.630 | | | | | 71 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS390 | **AMERICA** | | | | | | | | | | | | | | |
| | Tehuelches | 12 | | | | | 0.170 | | 0.250 | 0.580 | | | | | 71 |
| | Wichis | 6 | | | | | | | 0.170 | 0.830 | | | | | 71 |
| | Yanomami | 13 | | | | | | 0.850 | 0.080 | 0.080 | | | | | 71 |
| | New York, Asians | 25 | | | | | | | 0.680 | 0.200 | 0.120 | | | | 71 |
| | New York, African Americans | 81 | | | | 0.010 | 0.520 | 0.120 | 0.140 | 0.170 | 0.040 | | | | 71 |
| | New York, Hispanics | 88 | | | | | 0.160 | 0.040 | 0.250 | 0.480 | 0.070 | | | | 71 |
| | | | | | | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | | | | | | |
| | Pygmy | 31 | | | | | 0.320 | 0.060 | 0.290 | 0.060 | 0.260 | | | | 71 |
| | Ovambo | 34 | | | | | 0.760 | 0.090 | | 0.090 | 0.060 | | | | 71 |
| | Morocco | 51 | | | | | | 0.120 | 0.180 | 0.550 | 0.160 | | | | 71 |
| | Morocan Arabs (NWA) | 44 | | | | | 0.068 | | 0.318 | 0.546 | 0.068 | | | | 58 |
| | Southern Moroccan Berbs (NWA) | 44 | | | | | 0.023 | 0.068 | 0.250 | 0.545 | 0.114 | | | | 58 |
| | Saharawis (NWA) | 29 | | | | | 0.034 | 0.034 | 0.207 | 0.310 | 0.415 | | | | 58 |
| | Mozabites | 68 | | | | 0.015 | 0.029 | 0.029 | 0.132 | 0.604 | 0.176 | 0.015 | | | 58 |
| | Guinea Equatorial | 57 | | | | | 0.719 | 0.035 | 0.035 | 0.123 | 0.088 | | | | 92 |
| | | | | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | | | | |
| | Ami | 7 | | | | | | | 0.140 | 0.860 | | | | | 71 |
| | Chengdu (China) | N/A | | | | | | | NL | NL | | | | | 93 |
| | China | 36 | | | | | 0.030 | 0.050 | 0.530 | 0.250 | 0.130 | | | | 71 |
| | China | 63 | | | | | | 0.111 | 0.106 | 0.492 | 0.746 | 0.015 | | | 74 |
| | China | 113 | | | | | | 0.080 | 0.434 | 0.310 | 0.168 | 0.009 | | | 106 |
| | India | 112 | | | | | 0.036 | 0.402 | 0.205 | 0.152 | 0.188 | 0.018 | | | 106 |
| | Eastern India | 150 | | | | | 0.067 | 0.133 | 0.147 | 0.227 | 0.133 | 0.247 | 0.047 | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS390 | **ASIA** | | | | | | | | | | | | | | |
| | Japanese | 117 | | | | | | NL | NL | NL | NL | NL | NL | | 94 |
| | Japanese | 108 | | | | | 0.110 | 0.120 | 0.250 | 0.240 | 0.160 | 0.120 | | | 95 |
| | Japanese | 72 | | | | | NL | NL | NL | NL | NL | NL | | | 96 |
| | Korea | 330 | | | | | | 0.103 | 0.561 | 0.273 | 0.058 | 0.006 | | | 99 |
| | Korea | 316 | | | | 0.003 | 0.003 | 0.111 | 0.535 | 0.269 | 0.076 | 0.003 | | | 98 |
| | Malaysia | 113 | | | | 0.009 | 0.106 | 0.089 | 0.319 | 0.266 | 0.204 | 0.009 | | | 106 |
| | Mongolians | 40 | | | | | | 0.070 | 0.250 | 0.370 | 0.270 | | 0.020 | | 71 |
| | Osaka (Japan) | 150 | | | | | | 0.160 | 0.190 | 0.220 | 0.350 | 0.070 | | | 71 |
| | Taiwan | 582 | | | | 0.002 | 0.010 | 0.060 | 0.394 | 0.337 | 0.179 | 0.017 | 0.002 | | 100 |
| | Turkey | 92 | | | | | 0.044 | 0.120 | 0.380 | 0.228 | 0.217 | 0.011 | | | 101 |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | |
| | Papua New Guinea 1 | 23 | | | | 0.090 | | | 0.090 | 0.700 | 0.130 | | | | 71 |
| | Papua New Guinea 2 | 48 | | | | 0.150 | | 0.020 | 0.270 | 0.520 | 0.040 | | | | 71 |
| | S-Borneo | 13 | | | | | 0.310 | 0.150 | 0.080 | 0.310 | 0.150 | | | | 71 |
| | Trobriands | 63 | | | | 0.080 | 0.020 | 0.030 | 0.320 | 0.330 | 0.220 | | | | 71 |
| | W-Samoa | 10 | | | 0.100 | 0.500 | 0.100 | | 0.100 | 0.200 | | | | | 71 |
| | Australian Caucasian | 214 | | | | | | 0.103 | 0.304 | 0.453 | 0.121 | 0.019 | | | 104 |
| | Australian Caucasian | 130 | | | | | | 0.108 | 0.285 | 0.439 | 0.162 | 0.008 | | | 106 |
| | | | | | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | | | | | |
| | Greece | 64 | | | | | 0.087 | 0.058 | 0.290 | 0.333 | 0.174 | 0.058 | | | 105 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS391 | **EUROPE** | | | | | | | | | | | |
| | Bern (Switzerland) | 64 | | | | | 0.660 | 0.330 | 0.020 | | | 71 |
| | Bern (Switzerland) | 126 | | | | 0.008 | 0.619 | 0.357 | 0.016 | | | 83 |
| | Berlin 1 (Germany) | 70 | | | | 0.030 | 0.640 | 0.310 | 0.010 | | | 71 |
| | Koln (Germany) | 11 | | | | | 0.640 | 0.270 | 0.090 | | | 71 |
| | Leiden (Germany) | 88 | | | | 0.030 | 0.580 | 0.350 | 0.030 | | | 71 |
| | Bavaria (Germany) | 151 | | | | NL | NL | NL | | | | 71 |
| | Germany | 136 | | | | 0.031 | 0.586 | 0.363 | 0.015 | | | 74 |
| | Roma (Italy) | 100 | | | | 0.040 | 0.650 | 0.300 | 0.010 | | | 71 |
| | Toscany (Central Italy) | 107 | | | | NL | NL | NL | NL | | | 76 |
| | Apulia (Southern Italy) | 73 | | | | 0.136 | 0.589 | 0.219 | 0.013 | | | 107 |
| | Trieste | 71 | | | | | 0.460 | 0.540 | | | | 71 |
| | Udine | 57 | | | | | 0.560 | 0.380 | 0.030 | 0.020 | | 71 |
| | Catalans | 30 | | | | 0.030 | 0.400 | 0.570 | | | | 71 |
| | Basques 1 | 51 | | | | 0.040 | 0.390 | 0.530 | 0.040 | | | 71 |
| | Basques 2 | 30 | | | | 0.030 | 0.330 | 0.600 | 0.030 | | | 71 |
| | Norway | 300 | | | | 0.007 | 0.546 | 0.436 | 0.007 | 0.003 | | 84 |
| | Valencia (Eastern Spain) | 113 | | | | NL | NL | NL | NL | | | 78 |
| | Majorca (Eastern Spain) | 53 | | | | 0.019 | 0.283 | 0.679 | 0.019 | | | 79 |
| | Minorca (Eastern Spain) | 40 | | | | 0.025 | 0.350 | 0.625 | | | | 79 |
| | Valencia (Eastern Spain) | 24 | | | | 0.042 | 0.333 | 0.625 | | | | 79 |
| | Northern Spain | 107 | | | | 0.131 | 0.383 | 0.439 | 0.047 | | | 80 |
| | Southwest Spain | 111 | | | | NL | NL | NL | NL | | | 82 |
| | Caceres (Central Spain) | 100 | | | | 0.043 | 0.436 | 0.489 | 0.032 | | | 81 |
| | Iberian Peninsula | 763 | | | | 0.084 | 0.413 | 0.478 | 0.022 | 0.003 | | 58 |
| | Slovenia | 121 | | | | NL | NL | NL | | | | 85 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS391 | **EUROPE** | | | | | | | | | | | |
| | Lithuania | 152 | | | | 0.007 | 0.408 | 0.559 | 0.026 | | | 86 |
| | Latvia | 145 | | | | 0.007 | 0.359 | 0.628 | 0.007 | | | 86 |
| | Estonia | 133 | | | | | 0.414 | 0.556 | 0.030 | | | 86 |
| | Maderia Archipelago | 111 | | | | 0.054 | 0.514 | 0.027 | 0.369 | 0.036 | | 87 |
| | Budapest (Central Hungary) | 116 | | | | NL | NL | NL | NL | | | 40 |
| | Baranya County (SW Hungary) | 78 | | | | | NL | NL | NL | | | 40 |
| | Nothern Portugal | 212 | | | 0.005 | 0.052 | 0.486 | 0.448 | 0.009 | | | 77 |
| | Strasbourg | 100 | | | 0.010 | 0.040 | 0.330 | 0.610 | 0.010 | | | 88 |
| | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | |
| | Inuit | 62 | | | | | 0.810 | 0.190 | | | | 71 |
| | Surinam | 54 | | | | 0.060 | 0.680 | 0.240 | 0.020 | | | 71 |
| | Buenous Aires, Caucasians | 100 | | | | 0.050 | 0.550 | 0.380 | 0.020 | | | 71 |
| | de Janeiro (Brazil) | 109 | | | | 0.018 | 0.312 | 0.459 | 0.211 | | | 89 |
| | Mapuches | 16 | | | | | 0.440 | 0.500 | 0.060 | | | 71 |
| | Wichis | 6 | | | | | 1.000 | | | | | 71 |
| | Yanomami | 13 | | | | | 0.920 | 0.080 | | | | 71 |
| | | | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | | | |
| | Guinea Equatorial | 57 | | | | 0.053 | 0.719 | 0.228 | | | | 92 |
| | Morocan Arabs (NWA) | 44 | | | | 0.409 | 0.386 | 0.182 | 0.023 | | | 90 |
| | Mozabites | 68 | | | | 0.147 | 0.765 | 0.088 | | | | 90 |
| | Pygmy | 31 | | | 0.030 | 0.130 | 0.450 | 0.350 | 0.030 | | | 71 |
| | Saharawis (NWA) | 29 | | | | 0.794 | 0.034 | 0.172 | | | | 90 |
| | Southern Moroccan Berbs (NWA) | 44 | | | 0.023 | 0.613 | 0.250 | 0.114 | | | | 90 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS391 | **ASIA** | | | | | | | | | | | |
| | Ami | 7 | | | | | 0.570 | 0.290 | 0.140 | | | 71 |
| | Chengdu (China) | N/A | | | | | NL | NL | | | | 93 |
| | Chinese | 36 | | | | | 0.780 | 0.022 | | | | 71 |
| | Chinese | 62 | | | | 0.081 | 0.742 | 0.177 | | | | 74 |
| | Japanese | 117 | | | | NL | NL | NL | NL | | | 94 |
| | Japanese | 108 | | | | | 0.020 | 0.790 | 0.190 | | | 95 |
| | Korea | 330 | | | 0.003 | 0.058 | 0.718 | 0.221 | | | | 99 |
| | Korea | 316 | | | 0.003 | 0.035 | 0.741 | 0.222 | | | | 98 |
| | Mongolians | 40 | | | | 0.150 | 0.670 | 0.170 | | | | 71 |
| | Taiwan | 582 | 0.002 | | 0.002 | 0.034 | 0.751 | 0.205 | 0.007 | | | 100 |
| | Turkey | 92 | | | | 0.065 | 0.761 | 0.174 | | | | 101 |
| | South India | 64 | | | | | NL | NL | | | | 102 |
| | Western India | 98 | | | | | NL | NL | | | | 108 |
| | Eastern India | 100 | | | | 0.013 | 0.800 | 0.180 | 0.007 | | | 103 |
| | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | |
| | Austrailian Caucasian | 214 | | | | 0.009 | 0.551 | 0.416 | 0.023 | | | 104 |
| | Papua New Guinea 1 | 23 | | | | | 0.740 | 0.260 | | | | 71 |
| | S-Borneo | 13 | | | | 0.150 | 0.540 | 0.310 | | | | 71 |
| | Trobriands | 63 | | | | 0.060 | 0.860 | 0.080 | | | | 71 |
| | W-Samoa | 10 | | | | 0.100 | 0.800 | 0.100 | | | | 71 |
| | | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | | |
| | Greece | 100 | | | | 0.015 | 0.623 | 0.333 | 0.029 | | | 105 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS392 | **EUROPE** | | | | | | | | | | | | | | |
| | Bern (Swtizerland) | 64 | | | | | | 0.450 | 0.060 | 0.390 | 0.060 | 0.030 | | | 71 |
| | Bern (Swtizerland) | 126 | | | | | | 0.437 | 0.095 | 0.389 | 0.063 | 0.016 | | | 83 |
| | Berlin 1 (Germany) | 70 | | 0.010 | | | | 0.640 | 0.070 | 0.260 | 0.030 | 0.010 | | | 71 |
| | Cologne (Germnay) | 136 | | | | | | 0.482 | 0.037 | 0.420 | 0.037 | 0.015 | | | 74 |
| | Bavaria (Germmany) | 151 | | | | | | NL | NL | NL | NL | NL | | | 73 |
| | Leiden | 88 | | | | | | 0.390 | 0.060 | 0.540 | 0.010 | | | | 71 |
| | Roma | 100 | | | | | | 0.580 | 0.090 | 0.260 | 0.060 | | 0.010 | | 71 |
| | Toscany (Central Italy) | 107 | | | | | | NL | NL | NL | NL | NL | | | 76 |
| | Apulia (Southern Italy) | 73 | | | | | 0.013 | 0.575 | 0.301 | 0.095 | 0.013 | | | | 107 |
| | Trieste | 71 | | | | | | 0.700 | 0.270 | 0.030 | | | | | 71 |
| | Udine | 57 | | | | | | 0.440 | 0.420 | 0.140 | | | | | 71 |
| | Catalans | 32 | | | | | | 0.560 | 0.030 | 0.370 | 0.030 | | | | 71 |
| | Basques 1 | 52 | | | | | | 0.250 | | 0.750 | | | | | 71 |
| | Basques 2 | 30 | | | | | | 0.070 | | 0.930 | | | | | 71 |
| | Iceland | 28 | | | | | | 0.540 | 0.070 | 0.360 | 0.040 | | | | 71 |
| | Budapest (Hungary) | 116 | | | | | | NL | NL | NL | NL | NL | NL | | 40 |
| | Baranya (SW Hungary) | 78 | | | | | | NL | NL | NL | NL | NL | NL | | 40 |
| | Iberian Peninsula | 768 | | | | | | 0.275 | 0.037 | 0.649 | 0.035 | 0.004 | | | 58 |
| | Valencia (Eastern Spain) | 140 | | | | | NL | NL | NL | NL | NL | NL | | | 78 |
| | Northern Spain | 107 | | | | | 0.009 | 0.336 | 0.047 | 0.598 | 0.009 | | | | 80 |
| | Southwest Spain | 111 | | | | | | NL | NL | NL | NL | | | | 82 |
| | Norwegian | 300 | | | | | | | 0.050 | 0.246 | 0.070 | | | 0.633 | 84 |
| | Slovenia | 121 | | | | | | | NL | NL | NL | | | | 85 |
| | Lithuanina | 152 | | | | | 0.007 | 0.671 | 0.020 | 0.026 | 0.211 | 0.059 | 0.007 | | 86 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS392 | **EUROPE** | | | | | | | | | | | | | | |
| | Latvia | 145 | | | | | | 0.648 | 0.021 | 0.062 | 0.228 | 0.041 | | | 86 |
| | Estonia | 133 | | | | | | 0.556 | 0.015 | 0.053 | 0.361 | 0.015 | | | 86 |
| | Madeira Archipelago | 111 | | | | | | 0.414 | 0.036 | 0.441 | 0.099 | 0.009 | | | 87 |
| | Nothern Portugal | 212 | | | | | | 0.335 | 0.047 | 0.557 | 0.052 | 0.009 | | | 77 |
| | Strasbourg (Eastern France) | 100 | | | | | | 0.210 | 0.140 | 0.630 | 0.010 | 0.010 | | | 88 |
| | | | | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | | | | |
| | Inuit | | | | | | 0.020 | 0.110 | 0.020 | 0.560 | 0.270 | 0.020 | | | 71 |
| | Surinam | 54 | | | | | 0.070 | 0.630 | 0.020 | 0.240 | 0.040 | | | | 71 |
| | Buenous Aires, Caucasians | 100 | | | | | 0.050 | 0.280 | 0.030 | 0.450 | 0.030 | 0.110 | 0.050 | | 71 |
| | de Janeiro (Brazil) | 109 | | | | | | 0.202 | 0.321 | 0.349 | 0.128 | | | | 89 |
| | Mapuches | 16 | | | | | | 0.060 | | 0.250 | 0.560 | 0.430 | | | 71 |
| | Tehuelches | 12 | | | | | | 0.250 | | 0.250 | 0.420 | 0.080 | | | 71 |
| | Wichis | 6 | | | | | | 0.`7 | | 0.660 | 0.170 | | | | 71 |
| | Yanomami | 11 | | | | | | | | | 0.820 | 0.180 | | | 71 |
| | | | | | | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | | | | | | |
| | Africa, pooled | 30 | | | | | 0.130 | 0.670 | 0.070 | 0.130 | | | | | 71 |
| | Pygmy | 31 | | | | | | 0.970 | 0.030 | | | | | | 71 |
| | Morocan Arabs (NWA) | 44 | | | | | | 0.932 | | 0.068 | | | | | 90 |
| | Southern Moroccan Berbs (NWA) | 44 | | | | | 0.023 | 0.954 | | 0.023 | | | | | 90 |
| | Saharawis (NWA) | 29 | | | | | | 0.759 | 0.241 | | | | | | 90 |
| | Mozabites | 68 | | | | | | 0.956 | | 0.044 | | | | | 90 |
| | | | | | | | | | | | | | | | 90 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS392 | **ASIA** | | | | | | | | | | | | | | 71 |
| | Chinese | 36 | | | | | | 0.080 | 0.050 | 0.310 | 0.500 | 0.050 | | | 74 |
| | Chinese | 61 | | | | | | 0.082 | 0.098 | 0.377 | 0.393 | 0.048 | | | 93 |
| | Chengdu (China) | N/A | | | | | | NL | NL | NL | NL | | | | 100 |
| | Taiwan | 582 | | | | 0.002 | 0.003 | 0.038 | 0.070 | 0.416 | 0.421 | 0.043 | 0.003 | 0.003 | 71 |
| | Mongolians | 40 | | | | | | 0.720 | 0.020 | 0.070 | 0.150 | | 0.020 | | 71 |
| | Ami | 7 | | | | | | 0.140 | | 0.710 | | 0.140 | | | 98 |
| | Korea | 316 | | | | | | 0.146 | 0.142 | 0.410 | 0.187 | 0.032 | 0.003 | | 95 |
| | Japan | 108 | | | | | | 0.340 | 0.150 | 0.360 | 0.120 | 0.030 | | | 103 |
| | Eastern India | 100 | | | | | 0.033 | 0.247 | 0.540 | 0.040 | 0.093 | 0.040 | 0.007 | | |
| | | | | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | | | | 71 |
| | Papua New Guinea 1 | 23 | | | | | | 0.090 | | 0.870 | 0.040 | | | | 71 |
| | S-Borneo | 13 | | | | | | 0.380 | | 0.310 | 0.080 | 0.150 | 0.080 | | 71 |
| | Trobriands | 63 | | | | | | 0.080 | | 0.380 | 0.480 | 0.060 | | | 71 |
| | W-Samoa | 10 | | | | | | | 0.700 | 0.100 | 0.100 | | 0.100 | | |
| | | | | | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | | | | | 105 |
| | Greece | 100 | | | | | | 0.725 | 0.058 | 0.203 | 0.015 | | | | |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS393 | **EUROPE** | | | | | | | | | | | |
| | Bern (Switzerland) | 64 | | | 0.020 | 0.060 | 0.730 | 0.170 | 0.020 | | | 71 |
| | Bern (Switzerland) | 126 | | | 0.008 | 0.070 | 0.746 | 0.135 | 0.032 | | | 83 |
| | Berlin 1(Germany) | 70 | | | | 0.040 | 0.800 | 0.100 | 0.060 | | | 71 |
| | Heidelberg (Germany) | 128 | | | | 0.120 | 0.710 | 0.140 | 0.030 | | | 71 |
| | Koln (Germany) | 76 | | | | 0.080 | 0.770 | 0.120 | 0.030 | | | 71 |
| | South Wurttemberg (Germany) | 215 | | | 0.009 | 0.112 | 0.712 | 0.140 | 0.028 | | | 75 |
| | Bavaria (Germany) | 151 | | | | NL | NL | NL | | | | 73 |
| | Cologne (Germany) | 136 | | | | 0.125 | 0.721 | 0.132 | 0.021 | | | 74 |
| | Germany | 98 | | | | 0.102 | 0.704 | 0.174 | 0.020 | | | 72 |
| | Roma | 100 | | | 0.030 | 0.280 | 0.570 | 0.110 | 0.010 | | | 71 |
| | Rome (Italy) | 125 | | | 0.024 | 0.304 | 0.544 | 0.112 | 0.016 | | | 72 |
| | Toscany (Central Italy) | 107 | | | NL | NL | NL | NL | NL | | | 76 |
| | Trieste | 71 | | | 0.010 | 0.150 | 0.730 | 0.040 | 0.060 | | | 71 |
| | Udine | 57 | | | | 0.210 | 0.580 | 0.160 | 0.050 | | | 71 |
| | Leiden | 88 | | | | 0.110 | 0.750 | 0.110 | 0.020 | | | 71 |
| | Basques 2 | 30 | | | | 0.100 | 0.830 | 0.070 | | | | 71 |
| | Valencia (Eastern Spain) | 140 | | | | NL | NL | NL | | | | 78 |
| | Majorca (Eastern Spain) | 53 | | | | 0.113 | 0.736 | 0.132 | 0.019 | | | 79 |
| | Minorca (Eastern Spain) | 40 | | 0.025 | | 0.150 | 0.725 | 0.100 | | | | 79 |
| | Valencia (Eastern Spain) | 24 | | | | 0.125 | 0.750 | 0.125 | | | | 79 |
| | Calicia (Spain) | 107 | | | | 0.140 | 0.767 | 0.084 | 0.009 | | | 72 |
| | Northern Spain | 107 | | | 0.009 | 0.122 | 0.804 | 0.065 | | | | 80 |
| | Southwest Spain | 111 | | | NL | NL | NL | NL | NL | | | 82 |
| | Caceres (Central Spain) | 100 | | | | 0.138 | 0.723 | 0.128 | 0.011 | | | 81 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS393 | **EUROPE** | | | | | | | | | | | |
| | Iberian Peninsula | 763 | | | 0.001 | 0.123 | 0.772 | 0.092 | | | | 58 |
| | Coimbra (Portugal) | 119 | | | | 0.168 | 0.656 | 0.168 | 0.008 | | | 72 |
| | Nothern Portugal | 212 | | | | 0.170 | 0.703 | 0.108 | 0.019 | | | 77 |
| | Athens (Greece) | 47 | | | | 0.341 | 0.489 | 0.149 | 0.021 | | | 72 |
| | London (United Kingdom) | 139 | | 0.007 | | 0.065 | 0.827 | 0.086 | 0.014 | | | 72 |
| | Brussles (Belgium) | 83 | | | | 0.120 | 0.747 | 0.120 | 0.012 | | | 72 |
| | Holland | 99 | | | | 0.030 | 0.808 | 0.142 | 0.020 | | | 72 |
| | Finland | 67 | | | | 0.014 | 0.478 | 0.478 | 0.030 | | | 72 |
| | Dublin (Ireland) | 38 | | | | 0.079 | 0.816 | 0.079 | 0.026 | | | 72 |
| | Norwegian | 300 | | 0.003 | | 0.043 | 0.244 | 0.400 | 0.007 | | | 84 |
| | Baranya (Southwest Hungary) | 78 | | | | NL | NL | NL | NL | | | 40 |
| | Budapest (Central Hungary) | 116 | | | | NL | NL | NL | NL | | | 40 |
| | Madeira Archipelago | 111 | | | | 0.180 | 0.694 | 0.117 | 0.009 | | | 87 |
| | Lithuania | 152 | | | 0.013 | 0.033 | 0.691 | 0.237 | 0.026 | | | 86 |
| | Latvia | 145 | | | | 0.069 | 0.628 | 0.297 | 0.007 | | | 86 |
| | Estonia | 133 | | | | 0.015 | 0.549 | 0.406 | 0.023 | 0.008 | | 86 |
| | Slovenia | 121 | | | NL | NL | NL | NL | NL | | | 85 |
| | Strasbourg (Eastern France) | 100 | | | | 0.080 | 0.800 | 0.110 | | 0.010 | | 87 |
| | | | | | | | | | | | | |
| | **AMERICA** | | | | | | | | | | | 71 |
| | Inuit | 62 | | | | | 0.480 | 0.520 | | | | 71 |
| | Surinam | 54 | | | | 0.180 | 0.460 | 0.310 | 0.040 | | | 71 |
| | Buenos Aires, Caucasians | 100 | 0.020 | | | 0.160 | 0.660 | 0.140 | 0.020 | | | 71 |
| | Mapuches | 16 | | | | | 0.870 | 0.130 | | | | 71 |
| | Tehuelches | 12 | | | | 0.080 | 0.750 | 0.170 | | | | 71 |
| | Wichis | 6 | | | | | 1.000 | | | | | 71 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS393 | **AMERICA** | | | | | | | | | | | |
| | Yanomami | 27 | | | | | 1.000 | | | | | 71 |
| | | | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | | | |
| | Pygmy | 31 | | | | 0.060 | 0.420 | 0.350 | 0.160 | | | 71 |
| | Morocan Arabs (NWA) | 44 | | 0.023 | | 0.159 | 0.750 | 0.068 | | | | 58 |
| | Southern Moroccan Berbs (NWA) | 44 | | | | 0.114 | 0.818 | 0.068 | | | | 58 |
| | Saharawis (NWA) | 29 | | | | 0.172 | 0.828 | | | | | 58 |
| | Mozabites | 68 | | | | 0.015 | 0.941 | 0.015 | 0.029 | | | 58 |
| | Guinea Equatorial | 57 | | | | 0.018 | 0.509 | 0.246 | 0.228 | | | 92 |
| | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | |
| | Chinese | 36 | | | | 0.530 | 0.310 | 0.140 | 0.030 | | | 71 |
| | Chengdu, (China) | N/A | | | | NL | NL | NL | NL | | | 93 |
| | Chengdu, (China) | 63 | | | | 0.476 | 0.302 | 0.222 | | | | 74 |
| | Taiwanese | 582 | | | 0.003 | 0.519 | 0.306 | 0.143 | 0.029 | | | 100 |
| | Mongolians | 40 | | | | 0.220 | 0.620 | 0.150 | | | | 71 |
| | Ami | 7 | | | | 0.280 | 0.140 | 0.430 | 0.140 | | | 71 |
| | Koreans | 330 | | | 0.024 | 0.412 | 0.412 | 0.094 | 0.042 | 0.009 | | 99 |
| | Koreans | 316 | | | 0.010 | 0.424 | 0.418 | 0.791 | 0.057 | 0.010 | 0.003 | 98 |
| | Japanese | 117 | | | | NL | NL | NL | NL | | | 94 |
| | Japanese | 72 | | | | NL | NL | NL | NL | | | 96 |
| | Japanese | 108 | | | | 0.190 | 0.570 | 0.180 | 0.050 | 0.010 | | 96 |
| | Turkey | 92 | | | 0.011 | 0.348 | 0.489 | 0.120 | 0.033 | | | 101 |
| | South India | 64 | | NL | NL | NL | NL | | | | | 102 |
| | Western India | 98 | | NL | NL | NL | NL | | | | | 108 |
| | Eastern India | 100 | | | 0.013 | 0.240 | 0.507 | 0.220 | 0.020 | | | 103 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS393 | **OCEANIA** | | | | | | | | | | | |
| | Papua New Guinea 1 | 23 | | | | | 0.560 | 0.350 | 0.090 | | | 71 |
| | S-Borneo | 13 | | | 0.080 | 0.380 | 0.230 | 0.230 | 0.080 | | | 71 |
| | Trobriands | 63 | | | | 0.020 | 0.860 | 0.130 | | | | 71 |
| | W-Samoa | 10 | | | | | 0.200 | 0.800 | | | | 71 |
| | Austrailian Caucasians | 214 | | | | 0.093 | 0.743 | 0.131 | 0.033 | | | 104 |
| | | | | | | | | | | | | |
| | **MISCELLANEOUS** | | | | | | | | | | | |
| | Greece | 100 | | | | 0.2609 | 0.594 | 0.1014 | 0.044 | | | 105 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | 13 | 14 | 15 | 16 | 17 | 18 | References |
|---|---|---|---|---|---|---|---|---|---|
| DYS437 | **EUROPE** | | | | | | | | |
| | Northwest Italy | 131 | | 0.313 | 0.511 | 0.176 | | | 56 |
| | Iberian Peninsula | 763 | 0.003 | 0.341 | | 0.579 | 0.077 | | 58 |
| | Northern Portugal | 212 | | 0.316 | 0.557 | 0.127 | | | 77 |
| | Caceres (Central Spain) | 100 | | 0.3723 | 0.5213 | 0.1064 | | | 81 |
| | | | | | | | | | |
| | **AFRICA** | | | | | | | | |
| | Guinea Equatorial | 57 | | 0.807 | 0.1404 | 0.0175 | | | 92 |
| | | | | | | | | | |
| | **ASIA** | | | | | | | | |
| | Pakistan | 278 | | 0.3921 | 0.2698 | 0.3345 | 0.0036 | | 55 |
| | | | | | | | | | |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS438 | **EUROPE** | | | | | | | | | | | |
| | Northwest Italy | 131 | | | 0.008 | 0.206 | 0.298 | 0.061 | 0.374 | 0.053 | | 56 |
| | Iberian Peninsula | 763 | | 0.004 | 0.003 | 0.087 | 0.201 | 0.041 | 0.641 | 0.024 | 0.001 | 58 |
| | Northern Portugal | 212 | | | | 0.123 | 0.245 | 0.038 | 0.566 | 0.028 | | 77 |
| | | | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | | | |
| | Australian Caucasians | 130 | | | | 0.039 | 0.262 | 0.108 | 0.554 | 0.039 | | 106 |
| | | | | | | | | | | | | |
| | **ASIA** | | | | | | | | | | | |
| | Pakistan | 278 | 0.007 | | | 0.235 | 0.368 | 0.357 | 0.033 | | | 55 |
| | Malyasia | 113 | | | 0.009 | 0.106 | 0.655 | 0.221 | 0.009 | | | 106 |
| | China | 113 | | | | 0.009 | 0.770 | 0.212 | 0.009 | | | 106 |
| | India | 112 | | | 0.009 | 0.348 | 0.357 | 0.268 | 0.018 | | | 106 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 8 | 9 | 10 | 11 | 12 | 13 | 14 | References |
|---|---|---|---|---|---|---|---|---|---|---|
| DYS439 | **EUROPE** | | | | | | | | | |
| | Northwest Italy | 131 | | | 0.038 | 0.328 | 0.435 | 0.191 | 0.008 | 56 |
| | Iberian Peninsula | 763 | 0.001 | 0.003 | 0.064 | 0.295 | 0.467 | 0.153 | 0.017 | 58 |
| | Northern Portugal | 212 | | 0.009 | 0.108 | 0.377 | 0.292 | 0.108 | 0.005 | 77 |
| | Caceres (Central Spain) | 100 | | | 0.053 | 0.266 | 0.543 | 0.117 | 0.021 | 81 |
| | | | | | | | | | | |
| | **OCEANIA** | | | | | | | | | |
| | Australian Caucasians | 130 | | | 0.039 | 0.392 | 0.431 | 0.123 | 0.015 | 106 |
| | | | | | | | | | | |
| | **AFRICA** | | | | | | | | | |
| | Guinea Equatorial | 57 | | | 0.018 | 0.298 | 0.509 | 0.158 | 0.018 | 92 |
| | | | | | | | | | | |
| | **ASIA** | | | | | | | | | |
| | Pakistan | 278 | | 0.007 | 0.213 | 0.343 | 0.235 | 0.368 | 0.357 | 55 |
| | Malyasia | 113 | | | 0.0885 | 0.336 | 0.4602 | 0.106 | 0.009 | 106 |
| | China | 113 | | | 0.0531 | 0.319 | 0.5044 | 0.097 | 0.027 | 106 |
| | India | 112 | | | 0.2053 | 0.304 | 0.3393 | 0.152 | | 106 |
| | | | | | | | | | | |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 7 | 8 | 9 | 10 | 11 | 12 | 13 | References |
|---|---|---|---|---|---|---|---|---|---|---|
| DYS460 | **EUROPE** | | | | | | | | | |
| | Iberian Peninsula | 763 | | 0.003 | 0.018 | 0.408 | 0.535 | 0.035 | 0.001 | 58 |
| | Northern Portuguese | 208 | | | 0.043 | 0.433 | 0.495 | 0.029 | | 109 |
| | | | | | | | | | | |
| | **ASIA** | | | | | | | | | |
| | Japan (Okinawa) | 87 | | | 0.103 | 0.241 | 0.632 | 0.023 | | 97 |
| | Japan (Honshu) | 207 | | | 0.092 | 0.179 | 0.662 | 0.063 | 0.005 | 97 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 8 | 9 | 10 | 11 | 12 | 13 | References |
|---|---|---|---|---|---|---|---|---|---|
| GATA H4 | **EUROPE** | | | | | | | | |
| | Northern Portuguese | 208 | | 0.014 | 0.341 | 0.543 | 0.091 | 0.010 | 109 |
| | | | | | | | | | |
| | | | | | | | | | |
| | **ASIA** | | | | | | | | |
| | Japan (Okinawa) | 87 | 0.012 | 0.414 | 0.517 | 0.035 | 0.012 | 0.012 | 97 |
| | Japan (Honshu) | 207 | 0.014 | 0.425 | 0.512 | 0.048 | | | 97 |

A-1 (Continued)

| Locus | Population Ethnicity | Sample Size | Allele Range 11 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| YCAII | **EUROPE** | | | | | | | | | | | |
| | Germany | 265 | NL | NL | NL | NL | NL | NL | NL | NL | NL | 110 |

A-2
Allele frequencies for Y-STRs Examined in this Study
Y-STR markers included are DYS19, DYS388, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS426, DYS437, DYS438, DYS439, DYS447, DYS448, DYS450, DYS456, DYS458, DYS460, and GATA H4. Frequencies for multicopy loci (DYS385 a/b, YCAII a/b and DYS464 a/b/c/d represented as phenotypes. Allele frequency data presented for three separate U.S. ethnic groups.

| LOCUS | ALLELE | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS19 | 12 | | 0.004 | | 0.002 |
| | 13 | 0.023 | 0.045 | 0.161 | 0.062 |
| | 14 | 0.262 | 0.676 | 0.483 | 0.467 |
| | 15 | 0.408 | 0.201 | 0.259 | 0.297 |
| | 16 | 0.150 | 0.061 | 0.084 | 0.102 |
| | 17 | 0.154 | 0.012 | 0.014 | 0.070 |
| | 14-15-17 | 0.004 | | | 0.002 |
| DYS388 | 9 | | 0.004 | | 0.002 |
| | 10 | 0.023 | 0.016 | | 0.015 |
| | 11 | 0.004 | | 0.007 | 0.003 |
| | 12 | 0.865 | 0.689 | 0.825 | 0.790 |
| | 13 | 0.069 | 0.086 | 0.084 | 0.079 |
| | 14 | 0.035 | 0.123 | 0.028 | 0.066 |
| | 15 | 0.004 | 0.057 | 0.014 | 0.026 |
| | 16 | | 0.020 | 0.035 | 0.015 |
| | 17 | | 0.004 | 0.007 | 0.003 |
| DYS389I | 12 | 0.162 | 0.213 | 0.175 | 0.184 |
| | 13 | 0.166 | 0.627 | 0.566 | 0.618 |
| | 14 | 0.192 | 0.160 | 0.238 | 0.190 |
| | 15 | 0.008 | | 0.021 | 0.008 |
| DYS389II | 26 | | | 0.007 | 0.002 |
| | 27 | | 0.004 | 0.007 | 0.003 |
| | 28 | 0.062 | 0.184 | 0.154 | 0.128 |
| | 29 | 0.212 | 0.484 | 0.336 | 0.342 |
| | 30 | 0.408 | 0.234 | 0.350 | 0.329 |
| | 31 | 0.258 | 0.074 | 0.105 | 0.155 |
| | 32 | 0.042 | 0.020 | 0.042 | 0.034 |
| | 33 | 0.015 | | | 0.006 |
| | 34 | 0.004 | | | 0.002 |

A-2 (Continued)

| LOCUS | ALLELE | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS390 | 20 | 0.015 | | 0.007 | 0.008 |
| | 21 | 0.531 | 0.016 | 0.098 | 0.241 |
| | 22 | 0.119 | 0.127 | 0.049 | 0.107 |
| | 23 | 0.085 | 0.311 | 0.224 | 0.201 |
| | 24 | 0.177 | 0.414 | 0.517 | 0.342 |
| | 25 | 0.065 | 0.131 | 0.098 | 0.097 |
| | 26 | 0.008 | | 0.007 | 0.005 |
| DYS391 | 8 | | 0.004 | | 0.002 |
| | 9 | 0.008 | 0.020 | 0.070 | 0.026 |
| | 10 | 0.688 | 0.463 | 0.490 | 0.560 |
| | 11 | 0.285 | 0.484 | 0.427 | 0.391 |
| | 12 | 0.019 | 0.131 | 0.014 | 0.022 |
| DYS392 | 7 | 0.004 | | | 0.002 |
| | 8 | 0.004 | | | 0.002 |
| | 9 | 0.004 | | | 0.002 |
| | 10 | | | 0.021 | 0.005 |
| | 11 | 0.727 | 0.332 | 0.336 | 0.491 |
| | 12 | 0.046 | 0.057 | 0.077 | 0.057 |
| | 13 | 0.192 | 0.537 | 0.455 | 0.380 |
| | 14 | 0.023 | 0.061 | 0.077 | 0.049 |
| | 15 | | 0.008 | 0.007 | 0.005 |
| | 16 | | 0.004 | 0.028 | 0.008 |
| DYS393 | 12 | 0.054 | 0.102 | 0.112 | 0.085 |
| | 13 | 0.588 | 0.787 | 0.727 | 0.694 |
| | 14 | 0.231 | 0.094 | 0.098 | 0.150 |
| | 15 | 0.115 | 0.016 | 0.063 | 0.066 |
| | 16 | 0.012 | | | 0.005 |
| DYS426 | 9 | 0.004 | | | 0.002 |
| | 10 | | | 0.007 | 0.002 |
| | 11 | 0.758 | 0.340 | 0.420 | 0.526 |
| | 12 | 0.227 | 0.635 | 0.552 | 0.453 |
| | 13 | 0.012 | 0.025 | 0.021 | 0.019 |

A-2 (Continued)

| LOCUS | ALLELE | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS437 | 13 | 0.038 | 0.004 | 0.028 | 0.023 |
| | 14 | 0.677 | 0.193 | 0.483 | 0.451 |
| | 15 | 0.196 | 0.574 | 0.364 | 0.376 |
| | 16 | 0.062 | 0.230 | 0.119 | 0.138 |
| | 17 | 0.023 | | 0.007 | 0.011 |
| | 18 | 0.004 | | | 0.002 |
| DYS438 | 8 | 0.023 | | | 0.009 |
| | 9 | 0.027 | 0.066 | 0.042 | 0.045 |
| | 10 | 0.112 | 0.258 | 0.273 | 0.202 |
| | 11 | 0.619 | 0.086 | 0.210 | 0.328 |
| | 12 | 0.212 | 0.574 | 0.441 | 0.399 |
| | 13 | 0.008 | 0.016 | 0.028 | 0.015 |
| | 14 | | | 0.007 | 0.002 |
| DYS439 | 10 | 0.023 | 0.049 | 0.084 | 0.046 |
| | 11 | 0.323 | 0.316 | 0.336 | 0.323 |
| | 12 | 0.488 | 0.500 | 0.364 | 0.465 |
| | 13 | 0.150 | 0.119 | 0.189 | 0.147 |
| | 14 | 0.015 | 0.012 | 0.028 | 0.017 |
| | 15 | | 0.004 | | 0.002 |
| DYS447 | 19 | | 0.004 | | 0.002 |
| | 21 | | 0.016 | | 0.006 |
| | 22 | 0.012 | 0.029 | 0.021 | 0.020 |
| | 23 | 0.073 | 0.148 | 0.119 | 0.111 |
| | 23.4 | | 0.004 | | 0.002 |
| | 24 | 0.081 | 0.139 | 0.168 | 0.122 |
| | 25 | 0.365 | 0.516 | 0.413 | 0.433 |
| | 26 | 0.200 | 0.102 | 0.203 | 0.164 |
| | 27 | 0.219 | 0.025 | 0.049 | 0.108 |
| | 28 | 0.050 | 0.012 | 0.014 | 0.028 |
| | 29 | | 0.004 | | 0.002 |
| | 33 | | | 0.014 | 0.003 |

A-2 (Continued)

| LOCUS | ALLELE | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS448 | 19 | 0.008 | | | 0.003 |
| | 22-24 | 0.004 | | | 0.002 |
| | 23-24 | 0.012 | | | 0.005 |
| | 24-25 | 0.004 | | | 0.002 |
| | 22 | 0.027 | 0.103 | 0.133 | 0.079 |
| | 22.5 | | | 0.007 | 0.002 |
| | 23 | 0.231 | 0.572 | 0.448 | 0.407 |
| | 24 | 0.223 | 0.255 | 0.259 | 0.243 |
| | 25 | 0.415 | 0.062 | 0.126 | 0.218 |
| | 26 | 0.062 | 0.008 | 0.014 | 0.031 |
| | 27 | 0.015 | | 0.014 | 0.009 |
| DYS450 | 6 | 0.008 | | | 0.003 |
| | 7 | | 0.004 | | 0.002 |
| | 8 | 0.631 | 0.045 | 0.195 | 0.312 |
| | 9 | 0.342 | 0.906 | 0.756 | 0.643 |
| | 10 | 0.019 | 0.045 | 0.041 | 0.039 |
| | 11 | | | 0.008 | 0.002 |
| DYS456 | 12 | 0.004 | | | 0.002 |
| | 13 | 0.015 | 0.037 | 0.016 | 0.023 |
| | 14 | 0.088 | 0.168 | 0.138 | 0.130 |
| | 15 | 0.485 | 0.377 | 0.390 | 0.430 |
| | 16 | 0.281 | 0.295 | 0.333 | 0.295 |
| | 17 | 0.100 | 0.115 | 0.122 | 0.107 |
| | 18 | 0.027 | 0.008 | | 0.014 |
| DYS458 | 13 | | 0.008 | | 0.002 |
| | 14 | 0.023 | 0.037 | 0.028 | 0.029 |
| | 15 | 0.135 | 0.193 | 0.112 | 0.151 |
| | 16 | 0.304 | 0.164 | 0.252 | 0.240 |
| | 17 | 0.323 | 0.418 | 0.294 | 0.352 |
| | 17.2 | | | 0.007 | 0.002 |
| | 18 | 0.165 | 0.148 | 0.210 | 0.168 |
| | 19 | 0.042 | 0.025 | 0.084 | 0.043 |
| | 19.2 | | | 0.007 | 0.002 |
| | 20 | 0.008 | 0.008 | 0.014 | 0.009 |

A-2 (Continued)

| LOCUS | ALLELE | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS460 | 9 | 0.008 | 0.029 | 0.033 | 0.020 |
| | 10 | 0.485 | 0.311 | 0.447 | 0.413 |
| | 11 | 0.442 | 0.586 | 0.488 | 0.507 |
| | 12 | 0.062 | 0.074 | 0.033 | 0.059 |
| | 13 | 0.004 | | | 0.002 |
| GATA H4 | 9 | 0.008 | 0.004 | 0.008 | 0.008 |
| | 10 | 0.073 | 0.029 | 0.049 | 0.073 |
| | 11 | 0.485 | 0.316 | 0.398 | 0.485 |
| | 12 | 0.385 | 0.578 | 0.472 | 0.385 |
| | 13 | 0.046 | 0.074 | 0.073 | 0.046 |
| | 14 | 0.004 | | | 0.004 |

A-2 (Continued)

| LOCUS | Phenotype | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS385 | 8-16.3 | | 0.004 | | 0.002 |
| | 9-14 | | 0.004 | | 0.002 |
| | 10-13 | | 0.008 | | 0.003 |
| | 10-14 | 0.004 | 0.020 | 0.007 | 0.011 |
| | 10-15 | | 0.004 | | 0.002 |
| | 11-12 | 0.008 | 0.004 | 0.007 | 0.006 |
| | 11-13 | 0.012 | 0.066 | 0.042 | 0.039 |
| | 11-14 | 0.135 | 0.369 | 0.280 | 0.255 |
| | 11-15 | 0.038 | 0.107 | 0.077 | 0.073 |
| | 11-16 | 0.004 | 0.020 | | 0.009 |
| | 11-11 | 0.012 | 0.012 | 0.021 | 0.014 |
| | 12-13 | 0.004 | 0.012 | | 0.006 |
| | 12-14 | 0.012 | 0.037 | 0.056 | 0.031 |
| | 12-15 | 0.008 | 0.016 | | 0.009 |
| | 12-16 | 0.008 | | 0.007 | 0.005 |
| | 12-17 | | 0.004 | | 0.002 |
| | 12-12 | 0.012 | 0.012 | 0.014 | 0.012 |
| | 12.2-14 | | | 0.007 | 0.002 |
| | 13-14 | 0.027 | 0.074 | 0.063 | 0.053 |
| | 13-15 | 0.015 | 0.016 | 0.035 | 0.020 |
| | 13-16 | 0.004 | 0.004 | 0.028 | 0.009 |
| | 13-16.3 | | 0.012 | | 0.005 |
| | 13-17 | 0.004 | 0.004 | 0.007 | 0.005 |
| | 13-18 | | 0.008 | 0.007 | 0.005 |
| | 13-19 | | | 0.007 | 0.002 |
| | 13-20 | | | 0.007 | 0.002 |
| | 13-21 | | 0.004 | | 0.002 |
| | 13-13 | | 0.004 | | 0.002 |
| | 14-15 | 0.023 | 0.041 | 0.007 | 0.026 |
| | 14-15.3 | | 0.004 | | 0.002 |
| | 14-16 | 0.004 | 0.004 | 0.035 | 0.012 |
| | 14-16.3 | | 0.008 | | 0.003 |
| | 14-17 | 0.015 | 0.004 | 0.035 | 0.015 |
| | 14-18 | 0.004 | | 0.007 | 0.003 |
| | 14-19 | | | 0.028 | 0.006 |
| | 14-20 | 0.012 | | | 0.005 |
| | 14-14 | 0.027 | 0.041 | 0.014 | 0.029 |
| | 15-16 | 0.054 | 0.016 | 0.021 | 0.032 |
| | 15-17 | 0.027 | 0.008 | 0.014 | 0.017 |
| | 15-18 | 0.038 | | 0.021 | 0.020 |

A-2 (Continued)

| LOCUS | Phenotype | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS385 | 15-19 | 0.004 | | 0.007 | 0.003 |
| (Con't) | 15-21 | 0.004 | | | 0.002 |
| | 15-15 | 0.012 | 0.020 | 0.021 | 0.017 |
| | 16-17 | 0.135 | | 0.042 | 0.063 |
| | 16-18 | 0.027 | 0.008 | 0.021 | 0.019 |
| | 16-19 | 0.015 | 0.004 | 0.007 | 0.009 |
| | 16-20 | 0.004 | | | 0.002 |
| | 16-16 | 0.077 | 0.004 | 0.021 | 0.037 |
| | 17-18 | 0.054 | 0.008 | 0.014 | 0.028 |
| | 17-19 | 0.038 | 0.004 | | 0.017 |
| | 17-20 | 0.012 | | | 0.005 |
| | 17-17 | 0.046 | | 0.014 | 0.022 |
| | 18-19 | 0.019 | | | 0.008 |
| | 18-18 | 0.038 | | | 0.015 |
| | 19-20 | 0.004 | | | 0.002 |
| | 19-19 | 0.004 | | | 0.002 |
| YCAII | 18-18 | 0.004 | | | 0.002 |
| | 18-19 | 0.015 | | 0.007 | 0.008 |
| | 18-20 | 0.012 | 0.012 | 0.007 | 0.011 |
| | 18-21 | 0.019 | | 0.007 | 0.008 |
| | 18-22 | 0.004 | 0.004 | 0.007 | 0.005 |
| | 18-23 | 0.008 | | | 0.003 |
| | 19-19 | 0.231 | 0.049 | 0.070 | 0.127 |
| | 19-20 | 0.042 | 0.045 | 0.042 | 0.043 |
| | 19-21 | 0.335 | 0.197 | 0.140 | 0.240 |
| | 19-22 | 0.058 | 0.082 | 0.091 | 0.074 |
| | 19-23 | 0.181 | 0.506 | 0.441 | 0.359 |
| | 19-24 | 0.012 | 0.033 | 0.028 | 0.023 |
| | 19-25 | | 0.004 | 0.007 | 0.003 |
| | 20-20 | 0.004 | 0.020 | 0.035 | 0.017 |
| | 20-21 | 0.012 | 0.004 | 0.014 | 0.009 |
| | 20-22 | | | 0.007 | 0.002 |
| | 20-23 | 0.004 | 0.008 | 0.021 | 0.009 |
| | 21-21 | 0.031 | 0.016 | 0.021 | 0.023 |
| | 21-22 | 0.023 | 0.008 | | 0.012 |
| | 21-23 | | | 0.014 | 0.003 |
| | 22-22 | | 0.004 | 0.014 | 0.005 |
| | 22-23 | | 0.004 | 0.021 | 0.006 |
| | 22-24 | | | 0.007 | 0.002 |

A-2 (Continued)

| LOCUS | Phentoype | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| YCAII | 23-23 | | 0.008 | 0.003 | 0.003 |
| (Con't) | 23-24 | 0.004 | | | 0.002 |
| | 24-24 | 0.004 | | | 0.002 |
| | 11-21 | | | 0.007 | 0.002 |
| DYS464 | 14 | | 0.012 | | 0.005 |
| | 15 | | 0.004 | | 0.002 |
| | 16 | | 0.004 | | 0.002 |
| | 17 | | 0.004 | | 0.002 |
| | 11-12-14-15 | | | 0.007 | 0.002 |
| | 11-13-14-15 | | 0.004 | 0.007 | 0.003 |
| | 11-13-15-16 | | | 0.007 | |
| | 11-13-15.1-16 | | | 0.007 | 0.002 |
| | 11-13-16 | | | 0.007 | 0.002 |
| | 11-14 | | | 0.014 | 0.003 |
| | 11-14-15 | 0.004 | 0.016 | | 0.009 |
| | 11-14-16 | 0.004 | 0.012 | | 0.006 |
| | 11-15-16 | | | 0.007 | 0.002 |
| | 11-15-16-17 | 0.004 | | | 0.002 |
| | 11-15-18 | | 0.004 | | 0.002 |
| | 11-16 | 0.004 | | | 0.002 |
| | 11-16-18 | 0.008 | | | 0.003 |
| | 12-13-14 | 0.004 | 0.025 | 0.014 | 0.014 |
| | 12-13-14-15 | 0.004 | 0.004 | | 0.003 |
| | 12-13-14-16 | | 0.008 | 0.007 | 0.005 |
| | 12-13-15-17 | 0.004 | | | 0.002 |
| | 12-14 | | 0.004 | 0.007 | 0.003 |
| | 12-14.3 | | | 0.007 | 0.002 |
| | 12-14-15 | 0.008 | 0.033 | 0.007 | 0.017 |
| | 12-14-15-16 | 0.012 | 0.061 | 0.021 | 0.032 |
| | 12-14-15-17 | 0.004 | | | 0.002 |
| | 12-14-16 | | 0.008 | 0.007 | 0.005 |
| | 12-14-16-18 | 0.008 | | | 0.003 |
| | 12-14-17 | | 0.008 | 0.007 | 0.005 |
| | 12-15 | 0.008 | 0.008 | | 0.006 |
| | 12-15-16 | 0.004 | 0.045 | | 0.019 |
| | 12-15-16-17 | 0.012 | 0.008 | | 0.008 |
| | 12-15-16-18 | 0.023 | | | 0.009 |
| | 12-15-17 | 0.004 | | 0.007 | 0.003 |
| | 12-15-17-18 | 0.004 | | | 0.002 |

A-2 (Continued)

| LOCUS | Phenotype | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS464 (Con't) | 12-15-17-19 | 0.004 | | | 0.002 |
| | 12-15-18 | 0.004 | | | 0.002 |
| | 12-16 | 0.008 | | 0.007 | 0.005 |
| | 12-16-17 | 0.012 | | | 0.005 |
| | 12-16-19 | | | 0.014 | 0.003 |
| | 12-16-20 | 0.004 | | | 0.002 |
| | 13-13.1-15-16 | 0.004 | 0.004 | | 0.003 |
| | 13-14 | 0.004 | 0.012 | 0.014 | 0.009 |
| | 13-14-15 | | 0.008 | | 0.003 |
| | 13-14-15-16 | 0.008 | | | 0.003 |
| | 13-14-15-17 | 0.004 | | | 0.002 |
| | 13-14-15-18 | | | 0.007 | 0.002 |
| | 13-14-16 | 0.004 | | | 0.002 |
| | 13-14-16-17 | 0.004 | | | 0.002 |
| | 13-14-16-18 | | | 0.007 | 0.002 |
| | 13-14-17 | 0.004 | | 0.007 | 0.003 |
| | 13-14.3-15-17 | | | 0.007 | 0.002 |
| | 13-14.3-16-17 | 0.004 | | | 0.002 |
| | 13-15 | 0.004 | 0.004 | 0.007 | 0.005 |
| | 13-15-15.1 | | 0.004 | | 0.002 |
| | 13-15-16 | 0.042 | 0.004 | 0.014 | 0.022 |
| | 13-15-16-17 | 0.019 | 0.008 | 0.007 | 0.012 |
| | 13-15-16-18 | 0.038 | | | 0.015 |
| | 13-15-17 | 0.004 | 0.004 | 0.007 | 0.005 |
| | 13-15-17-18 | 0.012 | | | 0.005 |
| | 13-15-18 | 0.012 | 0.004 | 0.007 | 0.008 |
| | 13-15-19 | | 0.008 | | 0.003 |
| | 13-15.3-17 | | 0.004 | | 0.002 |
| | 13-16 | | 0.004 | 0.014 | 0.012 |
| | 13-16-17 | 0.146 | | 0.014 | 0.062 |
| | 13-16-17-18 | 0.031 | | | 0.012 |
| | 13-16-17-19 | 0.004 | | | 0.002 |
| | 13-16-18 | 0.092 | 0.004 | 0.021 | 0.043 |
| | 13-16-18-19 | 0.004 | 0.004 | | 0.003 |
| | 13-16-19 | 0.015 | | 0.007 | 0.008 |
| | 13-17-18 | 0.008 | | | 0.003 |
| | 13-17-20 | | | 0.007 | 0.002 |
| | 14-14.3-16-18 | 0.004 | | | 0.002 |
| | 14-14.3 | | 0.004 | | 0.002 |

A-2 (Continued)

| LOCUS | Phenotype | African Americans N=260 | Caucasians N=244 | Hispanics N=143 | Pooled N= 647 |
|---|---|---|---|---|---|
| DYS464 | 14.3-15-16-17 | 0.004 | | | 0.002 |
| | 14-15 | 0.004 | 0.025 | 0.021 | 0.015 |
| | 14-15-16 | 0.012 | 0.016 | 0.021 | 0.015 |
| | 14-15-16-17 | 0.004 | 0.016 | 0.021 | 0.012 |
| | 14-15-16-18 | 0.012 | | 0.014 | 0.008 |
| | 14-15-17 | 0.019 | 0.053 | 0.021 | 0.032 |
| | 14-15-17-18 | 0.012 | 0.016 | 0.014 | 0.014 |
| | 14-15-18 | 0.004 | 0.004 | 0.014 | 0.006 |
| | 14-15.3-17 | | 0.008 | 0.007 | 0.005 |
| | 14-16 | | | 0.021 | 0.005 |
| | 14-16-17 | 0.019 | 0.004 | 0.056 | 0.022 |
| | 14-16-17-18 | 0.015 | 0.004 | | 0.008 |
| | 14-16-18 | 0.008 | | | 0.003 |
| | 14-16-20 | 0.004 | | | 0.002 |
| | 14-17 | | 0.008 | 0.021 | 0.008 |
| | 14-17-18-19 | 0.004 | | | 0.002 |
| | 15-15.3-16 | | 0.004 | | 0.002 |
| | 15-15.3-17-18 | | | 0.007 | 0.002 |
| | 15-16 | 0.042 | 0.029 | 0.049 | 0.039 |
| | 15-16-17 | 0.077 | 0.152 | 0.147 | 0.121 |
| | 15-16-17-18 | 0.004 | 0.025 | 0.007 | 0.012 |
| | 15-16-17-19 | | 0.004 | | 0.002 |
| | 15-16-18 | 0.008 | 0.029 | 0.014 | 0.017 |
| | 15-16-18-20 | | 0.004 | | 0.002 |
| | 15-16-19 | | 0.004 | | 0.002 |
| | 15-16-20 | | 0.004 | | 0.002 |
| | 15-17 | 0.050 | 0.172 | 0.189 | 0.127 |
| | 15-17-18 | 0.027 | 0.037 | 0.021 | 0.029 |
| | 15-17-18-19 | | 0.004 | | 0.002 |
| | 15-17-19 | | 0.004 | | 0.002 |
| | 15-18 | 0.004 | 0.004 | 0.014 | 0.006 |
| | 15-18-19 | 0.004 | | 0.007 | 0.003 |
| | 16-17 | 0.019 | 0.008 | | 0.011 |
| | 16-18 | | 0.004 | | 0.002 |
| | 16-19 | 0.004 | | | 0.002 |
| | 17-18 | 0.004 | | | 0.002 |

A-3
Haplotypes for U.S. Population Samples Across 27 Regions of the Y Chromosome

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GT37019 | 17 | 16,19 | 12 | 13 | 31 | 21 | 10 | 11 | 16 | 11 | 14 | 11 | 12 | 28 | 25 | 10 | 11 | 19,21 | 8 | 16 | 18 | 13,16,17 |
| GT37020 | 16 | 16,19 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 12 | 27 | 25 | 10 | 11 | 19,21 | 8 | 16 | 16 | 13,16,18 |
| GT37026 | 15 | 16,17 | 12 | 13 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,16,17 |
| GT37027 | 14 | 11,15 | 12 | 13 | 29 | 23 | 12 | 13 | 13 | 12 | 14 | 12 | 12 | 24 | 23 | 11 | 11 | 19,23 | 9 | 17 | 15 | 15,16,17 |
| GT37032 | 15 | 15,18 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 27 | 25 | 12 | 12 | 18,19 | 9 | 15 | 16 | 13,16,17,18 |
| GT37047 | 15 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 10 | 12 | 19,23 | 9 | 17 | 20 | 14,15,17 |
| GT37166 | 15 | 18,19 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 17 | 13,16,18 |
| GT37168 | 15 | 16,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 12 | 12 | 26 | 26 | 11 | 11 | 19,21 | 9 | 16 | 17 | 13,15,16,18 |
| GT37169 | 17 | 18,19 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 13 | 11 | 11 | 27 | 25 | 11 | 11 | 21,21 | 8 | 16 | 16 | 13,15,16,18 |
| GT37170 | 14 | 12,14 | 14 | 13 | 29 | 22 | 11 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15 |
| GT37171 | 16 | 14,17 | 12 | 12 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 26 | 25 | 10 | 12 | 19,21 | 8 | 15 | 16 | 13,16,18 |
| GT37173 | 15 | 14,18 | 12 | 14 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 27 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,15,16 |
| GT37175 | 15 | 14,20 | 12 | 12 | 28 | 25 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 23 | 23 | 11 | 11 | 19,19 | 8 | 15 | 16 | 13,15,16 |
| GT37178 | 15 | 14,14 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 11 | 19,21 | 8 | 16 | 17 | 14,16,17,18 |
| GT37179 | 17 | 17,20 | 12 | 14 | 31 | 22 | 10 | 11 | 14 | 11 | 14 | 10 | 12 | 26 | 26 | 11 | 11 | 20,21 | 8 | 15 | 16 | 13,14,16,17 |
| GT37184 | 14 | 11,14 | 12 | 13 | 29 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,19 | 9 | 15 | 17 | 15,16,17 |
| GT37190 | 16 | 17,19 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 13 | 11 | 11 | 27 | 26 | 11 | 11 | 21,21 | 8 | 17 | 16 | 13,16,18 |
| JT51331 | 17 | 17,17 | 12 | 14 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 18 | 16 | 13,16,17 |
| JT51332 | 16 | 15,18 | 12 | 13 | 30 | 20 | 12 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 18,19 | 8 | 15 | 18 | 13,16,17 |
| JT51334 | 16 | 17,18 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 14 | 26 | 25 | 10 | 11 | 19,21 | 8 | 16 | 17 | 13,16,19 |
| JT51335 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 12 | 12 | 14 | 12 | 12 | 25 | 22 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| JT51336 | 13 | 15,17 | 12 | 12 | 30 | 25 | 10 | 11 | 14 | 11 | 14 | 9 | 11 | 24 | 22 | 10 | 11 | 19,22 | 8 | 15 | 18 | 15,16 |
| JT51338 | 17 | 17,19 | 12 | 14 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 27 | 24 | 10 | 11 | 19,21 | 8 | 15 | 15 | 12,16,17 |
| JT51462 | 17 | 15,17 | 13 | 14 | 31 | 22 | 11 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 24 | 10 | 10 | 19,21 | 8 | 15 | 16 | 12,14,16,18 |
| JT51463 | 15 | 16,16 | 12 | 13 | 32 | 21 | 10 | 11 | 14 | 11 | 14 | 10 | 11 | 25 | 24 | 10 | 11 | 19,21 | 8 | 15 | 18 | 13,15,16,18 |
| JT51464 | 15 | 16,18 | 12 | 14 | 32 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 12,16,17 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JT51465 | 16 | 16,17 | 13 | 14 | 31 | 22 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 26 | 25 | 10 | 10 | 19,21 | 8 | 13 | 17 | 12,14,16,18 |
| JT51467 | 15 | 11,11 | 10 | 14 | 34 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 | 27 | 12 | 13 | 21,22 | 9 | 13 | 17 | 14,16,17,18 |
| JT51468 | 15 | 11,14 | 14 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 11 | 19,23 | 9 | 16 | 17 | 15,17,18 |
| JT51469 | 15 | 16,18 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 24 | 25 | 10 | 13 | 19,19 | 8 | 15 | 18 | 14,16,18 |
| JT51471 | 15 | 18,18 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 25 | 10 | 13 | 19,19 | 8 | 15 | 16 | 15,18,19 |
| JT51472 | 14 | 11,14 | 12 | 14 | 32 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 23 | 24 | 12 | 14 | 19,23 | 9 | 16 | 15 | 12,15,16 |
| JT51475 | 15 | 16,17 | 12 | 14 | 32 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 24 | 10 | 11 | 19,19 | 8 | 14 | 17 | 13,16,17 |
| JT51476 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,23 | 9 | 17 | 17 | 15,16 |
| JT51477 | 16 | 16,16 | 12 | 12 | 30 | 22 | 11 | 12 | 13 | 11 | 17 | 8 | 11 | 24 | 24 | 10 | 9 | 18,20 | 9 | 15 | 18 | 13,15,17,18 |
| JT51478 | 15 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 13 | 27 | 25 | 11 | 11 | 19,21 | 8 | 17 | 17 | 12,16,20 |
| JT51481 | 17 | 17,19 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 14 | 27 | 25 | 11 | 11 | 19,21 | 8 | 15 | 16 | 13,15,16 |
| JT51482 | 15 | 15,17 | 12 | 14 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 26 | 26 | 11 | 12 | 19,19 | 8 | 15 | 16 | 16,17 |
| JT51483 | 13 | 15,16 | 12 | 13 | 31 | 23 | 10 | 14 | 13 | 12 | 14 | 11 | 10 | 26 | 23 | 10 | 11 | 19,24 | 9 | 17 | 15 | 14,15,18 |
| JT51484 | 17 | 17,19 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 13,15,16 |
| JT51485 | 15 | 16,18 | 12 | 12 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 24 | 10 | 12 | 19,19 | 8 | 15 | 15 | 16,19 |
| JT51486 | 15 | 17,17 | 12 | 15 | 33 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,19 | 8 | 16 | 16 | 13,16,19 |
| JT51487 | 17 | 15,16 | 13 | 13 | 30 | 22 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 25 | 10 | 10 | 19,21 | 8 | 15 | 15 | 12,15,16,18 |
| JT51488 | 15 | 16,16 | 12 | 14 | 33 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 11 | 19,21 | 8 | 15 | 18 | 13,15,16,18 |
| JT51489 | 15 | 17,18 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 11 | 19,19 | 8 | 16 | 16 | 14,17,18,19 |
| JT51493 | 17 | 18,18 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 13 | 27 | 25 | 10 | 11 | 19,21 | 8 | 18 | 16 | 13,16,18 |
| JT51494 | 15 | 17,20 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,19 | 9 | 15 | 17 | 12,15,17,18 |
| JT51495 | 15 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| JT51496 | 15 | 16,17 | 12 | 14 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 12 | 27 | 24 | 10 | 11 | 19,21 | 8 | 15 | 18 | 12,16,17 |
| JT51498 | 14 | 11,14 | 12 | 13 | 29 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| JT51499 | 14 | 13,14 | 14 | 12 | 28 | 22 | 11 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 11 | 19,21 | 9 | 15 | 14 | 12,14,15,16 |
| MT95085 | 16 | 12,12 | 12 | 12 | 30 | 22 | 10 | 11 | 12 | 11 | 14 | 10 | 11 | 27 | 23 | 11 | 12 | 21,22 | 9 | 15 | 16 | 13,15 |
| MT95087 | 14 | 11,15 | 13 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MT95095 | 15 | 16,16 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,17,18 |
| MT95098 | 14 | 14,15 | 12 | 12 | 28 | 25 | 11 | 11 | 13 | 11 | 15 | 11 | 11 | 23 | 23 | 10 | 11 | 19,20 | 8 | 15 | 18 | 15,16 |
| MT95102 | 16 | 16,17 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 25 | 24 | 11 | 12 | 18,19 | 8 | 15 | 17 | 13,16,18 |
| MT95104 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 11 | 11 | 19,23 | 9 | 16 | 19 | 15,16,18 |
| MT95105 | 15 | 16,20 | 10 | 13 | 30 | 22 | 11 | 11 | 14 | 12 | 14 | 8 | 11 | 23 | 24 | 11 | 11 | 19,19 | 9 | 15 | 15 | 13,14,17 |
| MT95106 | 17 | 18,18 | 12 | 13 | 30 | 21 | 12 | 11 | 13 | 11 | 13 | 11 | 11 | 28 | 25 | 11 | 11 | 21,21 | 8 | 16 | 18 | 13,16,17 |
| MT95114 | 14 | 11,14 | 12 | 13 | 30 | 23 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 10 | 12 | 19,23 | 9 | 15 | 16 | 15,16 |
| MT95124 | 15 | 11,14 | 12 | 12 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| MT95356 | 15 | 11,15 | 12 | 13 | 30 | 25 | 11 | 11 | 13 | 12 | 14 | 11 | 10 | 23 | 24 | 11 | 13 | 19,21 | 9 | 17 | 15 | 12,15 |
| MT95357 | 17 | 18,18 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 14 | 16 | 13,16,17 |
| MT95358 | 15 | 11,11 | 10 | 12 | 30 | 24 | 10 | 11 | 14 | 11 | 14 | 10 | 12 | 25 | 27 | 10 | 11 | 21,22 | 9 | 14 | 19 | 15,17 |
| MT95362 | 15 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 26 | 23 | 11 | 12 | 18,23 | 9 | 16 | 17 | 14,15,17 |
| MT95364 | 17 | 17,17 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 13,16,17 |
| MT95368 | 15 | 16,17 | 12 | 13 | 32 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 13 | 19,19 | 8 | 15 | 16 | 13,16,17 |
| MT95369 | 14 | 14,20 | 13 | 12 | 28 | 25 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 23 | 23 | 11 | 11 | 19,20 | 8 | 15 | 18 | 14,15,16 |
| MT95371 | 15 | 13,15 | 12 | 12 | 29 | 22 | 11 | 11 | 13 | 11 | 17 | 10 | 11 | 26 | 23,24 | 11 | 12 | 18,21 | 9 | 15 | 16 | 14,15,17 |
| MT95372 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 14 | 12 | 14 | 12 | 13 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| MT95373 | 16 | 17,17 | 12 | 13 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 14 | 27 | 24 | 10 | 11 | 19,21 | 8 | 15 | 16 | 13,15,16,17 |
| MT95379 | 17 | 16,16 | 13 | 14 | 31 | 22 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 26 | 24 | 11 | 10 | 19,21 | 8 | 16 | 16 | 12,15,17,19 |
| OT05560 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 12 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,16,17 |
| OT05562 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 24 | 12 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| OT05563 | 16 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 16 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 12 | 19,21 | 8 | 16 | 17 | 13,16,17 |
| OT05565 | 14 | 11,11 | 12 | 13 | 29 | 25 | 11 | 13 | 13 | 12 | 15 | 13 | 12 | 23 | 24 | 10 | 12 | 19,23 | 9 | 16 | 19 | 15,16,17 |
| OT05568 | 14 | 11,14 | 12 | 13 | 29 | 22 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 19 | 15,17,18 |
| OT05569 | 14 | 11,14 | 12 | 13 | 30 | 26 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,16 |
| OT05570 | 15 | 14,17 | 12 | 12 | 29 | 23 | 10 | 11 | 13 | 11 | 17 | 8 | 13 | 25 | 24 | 10 | 11 | 18,23 | 9 | 16 | 16 | 13,16,17 |
| OT05575 | 14 | 13,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 13 | 15 | 12 | 12 | 27 | 23 | 10 | 10 | 19,23 | 9 | 16 | 17 | 15,16,17,18 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OT05576 | 15 | 16,17 | 12 | 13 | 31 | 21 | 10 | 11 | 12 | 11 | 14 | 12 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,16,17 |
| OT05578 | 15 | 15,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 27 | 23 | 11 | 10 | 19,21 | 8 | 16 | 17 | 12,15,16,17 |
| OT05582 | 15 | 15,16 | 12 | 14 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,15,16,17 |
| OT05587 | 14 | 11,14 | 12 | 14 | 30 | 23 | 11 | 11 | 13 | 12 | 14 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 14,15,17,18 |
| OT05588 | 15 | 17,19 | 12 | 13 | 30 | 23 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 26 | 25 | 11 | 11 | 19,21 | 8 | 18 | 15 | 13,16,18 |
| OT05589 | 14 | 11,14 | 10 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 26 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| OT05591 | 14 | 13,14 | 14 | 13 | 29 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,16 |
| OT05592 | 13 | 16,17 | 12 | 13 | 30 | 23 | 10 | 11 | 13 | 11 | 14 | 10 | 13 | 26 | 24 | 10 | 12 | 18,22 | 8 | 15 | 14 | 13,14.3,16,17 |
| OT05593 | 14 | 11,16 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 22 | 10 | 12 | 23,23 | 9 | 16 | 16 | 15,17 |
| OT05594 | 14 | 14,14 | 12 | 12 | 28 | 25 | 11 | 11 | 13 | 11 | 15 | 12 | 11 | 23 | 23 | 10 | 11 | 19,20 | 8 | 15 | 17 | 15,16 |
| OT05597 | 15 | 12,15 | 12 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 25 | 12 | 12 | 20,21 | 9 | 15 | 16 | 12,13,14 |
| OT05598 | 16 | 17,19 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 12 | 19,21 | 8 | 15 | 16 | 13,16,18 |
| OT05599 | 15 | 13,15 | 12 | 12 | 29 | 22 | 10 | 12 | 13 | 11 | 16 | 9 | 12 | 25 | 22,24 | 11 | 11 | 19,20 | 10 | 15 | 16 | 13,15,18 |
| OT05600 | 14 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 15 | 15,17,18 |
| OT05601 | 17 | 17,17 | 12 | 13 | 30 | 22 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 27 | 25 | 11 | 11 | 19,20 | 8 | 18 | 16 | 13,16,17 |
| OT05603 | 15 | 15,18 | 12 | 13 | 31 | 21 | 10 | 9 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,15,16 |
| OT05888 | 16 | 16,17 | 14 | 14 | 31 | 22 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 26 | 24 | 12 | 10 | 19,21 | 8 | 15 | 17 | 14,15,17,18 |
| OT05890 | 15 | 14,15 | 12 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 17 | 8 | 12 | 26 | 24 | 10 | 12 | 18,21 | 9 | 15 | 18 | 14,16,17 |
| OT05892 | 14 | 14,14 | 14 | 12 | 28 | 23 | 10 | 11 | 12 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,17 |
| OT05893 | 17 | 16,18 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 11 | 26 | 25 | 11 | 11 | 19,21 | 8 | 17 | 16 | 13,15,17 |
| OT05894 | 15 | 16,17 | 13 | 14 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 27 | 24 | 10 | 11 | 19,22 | 8 | 14 | 17 | 12,15,16,18 |
| OT05896 | 16 | 16,18 | 12 | 13 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 11 | 27 | 25 | 11 | 10 | 19,21 | 8 | 16 | 16 | 13,16,17 |
| OT05897 | 14 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 13 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 13,16,17 |
| OT05898 | 15 | 12,13 | 11 | 13 | 30 | 22 | 10 | 12 | 13 | 12 | 16 | 10 | 12 | 24 | 25 | 10 | 11 | 21,21 | 10 | 15 | 17 | 11,16 |
| OT05899 | 15 | 16,17 | 12 | 13 | 31 | 22 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 11 | 12 | 19,19 | 8 | 15 | 15 | 13,16,17 |
| OT05901 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 11 | 11 | 19,23 | 9 | 17 | 18 | 14,16,17,18 |
| PT83859 | 15 | 15,18 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 14 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,16,17,18 |
| PT83860 | 16 | 18,18 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 10 | 11 | 19,21 | 8 | 15 | 16 | 13,16,17,18 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT83862 | 16 | 16,19 | 12 | 13 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,20 | 8 | 15 | 16 | 13,15,16,17 |
| PT83863 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 15 | 17 | 14,15,17 |
| PT83864 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 14 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| PT83865 | 15 | 17,17 | 12 | 13 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 26 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 13,16,17 |
| PT83866 | 14 | 11,15 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,22 | 9 | 16 | 17 | 15,17 |
| PT83867 | 16 | 16,17 | 12 | 14 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 24 | 10 | 11 | 19,19 | 8 | 14 | 17 | 13,16,18 |
| PT83868 | 17 | 17,18 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 17 | 13,16,17 |
| PT83869 | 17 | 17,18 | 12 | 13 | 30 | 21 | 10 | 11 | 16 | 11 | 14 | 11 | 13 | 27 | 25 | 11 | 11 | 18,19 | 8 | 16 | 16 | 13,16,17 |
| PT83870 | 15 | 15,18 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 27 | 10 | 11 | 19,19 | 8 | 14 | 16 | 13,14,15,16 |
| PT83871 | 15 | 16,16 | 12 | 14 | 31 | 21 | 11 | 11 | 14 | 11 | 14 | 11 | 12 | 25 | 25 | 11 | 11 | 19,21 | 8 | 15 | 16 | 13,15,18 |
| PT83872 | 17 | 17,19 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 18 | 17 | 13,16,17,18 |
| PT83873 | 16 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 11 | 19,21 | 8 | 16 | 16 | 15,16,17 |
| PT83874 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| PT83875 | 16 | 17,17 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 13,16,19 |
| PT83876 | 15 | 15,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 15 | 12 | 11 | 27 | 24 | 10 | 11 | 19,21 | 8 | 15 | 18 | 12,15,18 |
| PT83878 | 15 | 16,16 | 12 | 14 | 32 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 11 | 19,21 | 8 | 15 | 18 | 13,15,16,18 |
| PT83879 | 14 | 17,18 | 12 | 14 | 31 | 20 | 11 | 11 | 14 | 12 | 14 | 11 | 12 | 22 | 19 | 10 | 10 | 18,20 | 8 | 12 | 15 | 14,16,18 |
| PT83880 | 15 | 16,17 | 12 | 13 | 31 | 21 | 10 | 12 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 11 | 19,19 | 8 | 14 | 17 | 13,16,18 |
| PT83881 | 16 | 17,18 | 12 | 14 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 17 | 15 | 13,15,17,18 |
| PT83882 | 17 | 14,14 | 12 | 12 | 29 | 23 | 10 | 11 | 13 | 11 | 17 | 8 | 12 | 24 | 24 | 11 | 12 | 18,21 | 9 | 17 | 17 | 14,15,16,18 |
| PT83883 | 13 | 19,20 | 13 | 14 | 31 | 24 | 11 | 11 | 12 | 11 | 14 | 10 | 12 | 25 | 24 | 11 | 12 | 19,22 | 8 | 15 | 16 | 14,14.3,16,18 |
| PT83885 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 12 | 12 | 20,23 | 9 | 17 | 17 | 15,17 |
| PT83886 | 15 | 17,17 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 12,16 |
| PT83887 | 17 | 12,16 | 13 | 13 | 30 | 24 | 11 | 11 | 13 | 11 | 15 | 10 | 11 | 25 | 24 | 10 | 11 | 21,21 | 10 | 15 | 18 | 12,13,14,15 |
| PT83891 | 15 | 12,12 | 10 | 12 | 31 | 23 | 11 | 11 | 13 | 11 | 14 | 10 | 12 | 27 | 25 | 11 | 12 | 21,22 | 9 | 14 | 16 | 17,18 |
| PT83892 | 17 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 26 | 25 | 12 | 11 | 19,21 | 8 | 16 | 16 | 13,16,18 |
| PT83893 | 16 | 16,18 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 14 | 17 | 14,16,17,18 |
| PT83895 | 15 | 15,17 | 12 | 13 | 31 | 21 | 10 | 12 | 12 | 11 | 14 | 11 | 11 | 25 | 26 | 10 | 11 | 19,19 | 6 | 16 | 16 | 15,17,18 |
| PT83896 | 14 | 14,20 | 12 | 12 | 28 | 25 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 23 | 23 | 11 | 11 | 19,20 | 8 | 15 | 17 | 14,15,16 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT83897 | 15 | 16,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 11 | 19,19 | 8 | 15 | 17 | 13,16,17 |
| PT83898 | 16 | 14,16 | 13 | 13 | 29 | 25 | 11 | 11 | 13 | 11 | 15 | 10 | 12 | 27 | 25 | 10 | 10 | 19,19 | 9 | 14 | 16 | 14,15 |
| PT83899 | 14,15,17 | 13,14 | 12 | 14 | 30 | 22 | 10 | 11 | 14 | 11 | 17 | 10 | 10 | 25 | 25 | 11 | 11 | 20,20 | 9 | 14 | 19 | 13,14 |
| PT83902 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 22 | 11 | 11 | 19,23 | 9 | 15 | 17 | 14,16,17 |
| PT83903 | 14 | 11,14 | 12 | 13 | 30 | 24 | 11 | 13 | 14 | 12 | 15 | 13 | 13 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,16,17 |
| PT83904 | 13 | 13,14 | 12 | 15 | 31 | 24 | 9 | 11 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 12 | 12 | 19,24 | 8 | 16 | 18 | 14,16,17 |
| PT83907 | 15 | 15,19 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 15 | 13,15,16 |
| PT83909 | 15 | 15,17 | 13 | 13 | 30 | 23 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 25 | 11 | 10 | 19,21 | 8 | 14 | 15 | 13,15,16,17 |
| PT83910 | 16 | 15,18 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 18 | 13,16 |
| PT83912 | 14 | 13,16 | 12 | 14 | 31 | 23 | 10 | 13 | 13 | 11 | 14 | 9 | 12 | 27 | 23 | 10 | 11 | 23,24 | 9 | 16 | 18 | 13,16,17 |
| PT83913 | 15 | 15,16 | 12 | 12 | 29 | 22 | 10 | 11 | 12 | 11 | 18 | 10 | 12 | 25 | 24,25 | 10 | 11 | 18,21 | 9 | 16 | 15 | 14,16,17 |
| PT83916 | 15 | 16,17 | 12 | 14 | 32 | 22 | 10 | 8 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 16 | 16 | 13,16,17 |
| PT83918 | 16 | 15,18 | 12 | 14 | 32 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 24 | 26 | 12 | 12 | 19,19 | 8 | 15 | 20 | 13,16,17 |
| PT83919 | 15 | 16,17 | 12 | 13 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 10 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,16,18 |
| PT83920 | 14 | 11,14 | 12 | 13 | 30 | 26 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 18 | 15,16 |
| PT83978 | 15 | 14,17 | 12 | 13 | 30 | 22 | 10 | 11 | 13 | 11 | 16 | 9 | 12 | 25 | 23,24 | 10 | 11 | 18,21 | 9 | 15 | 15 | 14,16,17 |
| PT84177 | 15 | 17,18 | 12 | 14 | 32 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 26 | 25 | 11 | 11 | 19,19 | 8 | 15 | 18 | 13,16,17 |
| PT84178 | 17 | 14,15 | 12 | 12 | 29 | 23 | 10 | 12 | 13 | 11 | 16 | 8 | 11 | 25 | 23,24 | 10 | 12 | 21,21 | 9 | 15 | 19 | 14,16,20 |
| PT84179 | 17 | 15,18 | 12 | 12 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 28 | 25 | 10 | 11 | 19,21 | 8 | 18 | 16 | 14,15,16,18 |
| PT84180 | 15 | 15,21 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,15,17,18 |
| PT84181 | 15 | 13,17 | 13 | 13 | 29 | 24 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 23 | 23 | 10 | 9 | 19,20 | 8 | 14 | 15 | 15,16 |
| PT84182 | 16 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 10 | 25 | 25 | 10 | 11 | 19,21 | 8 | 18 | 16 | 13,16,18 |
| PT84183 | 15 | 13,15 | 13 | 14 | 31 | 22 | 10 | 11 | 12 | 11 | 14 | 9 | 12 | 26 | 22 | 11 | 10 | 19,22 | 9 | 15 | 16 | 12,13,15,17 |
| PT84184 | 15 | 16,17 | 12 | 13 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 15 | 13,16 |
| PT84185 | 14 | 16,16 | 12 | 12 | 29 | 21 | 11 | 11 | 14 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 11 | 19,19 | 8 | 15 | 17 | 13,16,17 |
| PT84187 | 14 | 12,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 14 | 16 | 14,15,16,17 |
| PT84189 | 15 | 14,17 | 12 | 14 | 31 | 21 | 10 | 12 | 14 | 11 | 14 | 11 | 11 | 26 | 25 | 9 | 11 | 19,21 | 8 | 16 | 17 | 16,17 |
| PT84190 | 15 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 16 | 11 | 13 | 26 | 23 | 11 | 11 | 19,21 | 9 | 16 | 18 | 14,15,17,18 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT84191 | 17 | 16,19 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 10 | 11 | 19,21 | 8 | 16 | 15 | 13,16,18 |
| PT84192 | 15 | 16,17 | 12 | 14 | 33 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,16,18 |
| PT84195 | 15 | 16,16 | 13 | 14 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 27 | 24 | 10 | 11 | 19,22 | 8 | 14 | 17 | 12,15,16,18 |
| PT84198 | 16 | 17,18 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 26 | 11 | 12 | 19,19 | 8 | 16 | 18 | 13,16,17 |
| PT84200 | 15 | 15,16 | 12 | 13 | 32 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,16,17,18 |
| PT84201 | 17 | 17,18 | 12 | 13 | 30 | 22 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 26 | 12 | 12 | 19,21 | 8 | 15 | 16 | 13,16,17 |
| PT84202 | 14 | 17,17 | 12 | 13 | 30 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 18 | 13,16,18 |
| PT84206 | 17 | 17,18 | 12 | 14 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 13 | 27 | 25 | 10 | 11 | 19,21 | 8 | 16 | 15 | 13,15,16,18 |
| PT84208 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 12 | 12 | 15 | 11 | 13 | 25 | 23 | 11 | 13 | 19,22 | 9 | 15 | 16 | 14,15,16 |
| PT84209 | 15 | 18,18 | 12 | 14 | 31 | 20 | 11 | 11 | 13 | 12 | 14 | 12 | 12 | 22 | 19 | 10 | 10 | 18,20 | 8 | 15 | 15 | 14,15,16,18 |
| PT84210 | 14 | 12,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 13 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| PT84211 | 15 | 12,16 | 12 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 9 | 12 | 28 | 22 | 11 | 11 | 18,18 | 9 | 15 | 16 | 13,14,15,17 |
| PT84212 | 15 | 15,18 | 12 | 13 | 30 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 24 | 10 | 11 | 19,21 | 8 | 15 | 16 | 13,15,16 |
| PT84213 | 14 | 14,15 | 15 | 14 | 31 | 22 | 10 | 11 | 12 | 11 | 15 | 9 | 12 | 26 | 24 | 10 | 11 | 19,22 | 9 | 15 | 15 | 13,13.1,15,16 |
| PT84214 | 17 | 17,18 | 12 | 14 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 26 | 25 | 10 | 11 | 19,21 | 8 | 16 | 17 | 13,17,18 |
| PT84215 | 15 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 9 | 11 | 19,19 | 8 | 15 | 16 | 13,15,16 |
| PT84216 | 13 | 16,16 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 11 | 15 | 10 | 12 | 26 | 24 | 11 | 13 | 19,22 | 8 | 15 | 15 | 14.3,15,16,17 |
| PT84222 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 22 | 24 | 11 | 10 | 19,22 | 9 | 15 | 14 | 12,14,15 |
| PT84223 | 15 | 15,15 | 12 | 13 | 31 | 21 | 10 | 12 | 13 | 11 | 14 | 11 | 12 | 24 | 24 | 10 | 12 | 19,19 | 8 | 16 | 17 | 13,16 |
| PT84224 | 15 | 16,17 | 12 | 13 | 30 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 25 | 10 | 11 | 19,19 | 8 | 15 | 16 | 13,15,16,18 |
| PT84225 | 15 | 13,15 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,16,17 |
| PT84226 | 15 | 16,17 | 12 | 14 | 31 | 20 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 23 | 24 | 10 | 13 | 19,19 | 8 | 17 | 18 | 13,16,17 |
| PT84227 | 15 | 16,16 | 13 | 13 | 29 | 24 | 10 | 12 | 15 | 11 | 15 | 10 | 12 | 26 | 24 | 11 | 11 | 19,21 | 10 | 14 | 16 | 11,14,15 |
| PT84228 | 14 | 12,15 | 12 | 13 | 28 | 24 | 11 | 13 | 13 | 13 | 15 | 12 | 11 | 25 | 23 | 12 | 12 | 19,22 | 9 | 15 | 16 | 15,16,18 |
| PT84230 | 16 | 16,17 | 12 | 14 | 32 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 26 | 12 | 12 | 19,19 | 8 | 17 | 16 | 13,16,17 |
| PT84231 | 16 | 18,18 | 12 | 12 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 25 | 25 | 10 | 13 | 19,19 | 8 | 15 | 17 | 16,17 |
| PT84232 | 15 | 11,15 | 12 | 13 | 29 | 25 | 10 | 13 | 12 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| PT84234 | 15 | 14,15 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 26 | 10 | 12 | 19,19 | 8 | 15 | 18 | 13,16,18 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT84236 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 11 | 13 | 19,23 | 9 | 16 | 17 | 15,16 |
| PT84239 | 15 | 16,16 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 27 | 24 | 11 | 10 | 19,21 | 8 | 17 | 17 | 12,15,16,18 |
| PT84240 | 16 | 11,12 | 10 | 13 | 31 | 24 | 10 | 7 | 13 | 11 | 14 | 10 | 12 | 27 | 27 | 10 | 11 | 21,22 | 9 | 13 | 18 | 15,16,17 |
| PT84241 | 16 | 11,13 | 12 | 13 | 30 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 12 | 24 | 23 | 10 | 12 | 19,23 | 9 | 15 | 14 | 12,15 |
| PT84242 | 15 | 15,18 | 12 | 13 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 28 | 24 | 10 | 11 | 19,21 | 8 | 15 | 19 | 15,17 |
| PT84243 | 16 | 16,16 | 12 | 12 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 26 | 25 | 11 | 12 | 19,21 | 8 | 15 | 17 | 13,15,16,18 |
| PT84244 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,21 | 9 | 15 | 17 | 14,15,17 |
| PT84245 | 14 | 14,14 | 14 | 13 | 30 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| PT84247 | 15 | 15,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 16 | 17 | 13,16,18 |
| PT84248 | 14 | 11,13 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 11 | 11 | 24 | 23 | 11 | 11 | 19,23 | 9 | 17 | 17 | 16,17 |
| PT84249 | 15 | 15,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 24 | 11 | 10 | 19,23 | 8 | 15 | 19 | 12,15,16,18 |
| PT84251 | 14 | 17,18 | 12 | 13 | 30 | 21 | 10 | 12 | 13 | 11 | 13 | 11 | 11 | 27 | 25 | 11 | 11 | 19,23 | 8 | 16 | 16 | 13,16,17 |
| PT84252 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,16,17 |
| PT84253 | 16 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 26 | 10 | 12 | 19,19 | 8 | 15 | 18 | 13,15,16 |
| PT84254 | 16 | 16,16 | 12 | 12 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 13 | 27 | 25 | 11 | 10 | 19,21 | 8 | 15 | 18 | 13,16,17 |
| PT84256 | 14 | 14,14 | 12 | 12 | 28 | 25 | 11 | 11 | 13 | 11 | 15 | 11 | 11 | 23 | 23 | 10 | 11 | 19,20 | 8 | 15 | 17 | 15,16 |
| TT50913 | 16 | 18,19 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 13 | 11 | 13 | 28 | 25 | 11 | 12 | 21,21 | 8 | 16 | 16 | 13,15,18 |
| TT50916 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 18 | 15,16,17 |
| TT50922 | 14 | 11,14 | 12 | 13 | 30 | 24 | 11 | 14 | 13 | 12 | 16 | 12 | 13 | 26 | 23 | 11 | 12 | 19,23 | 9 | 15 | 19 | 15,17 |
| TT50923 | 15 | 15,16 | 12 | 13 | 29 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 25 | 26 | 11 | 12 | 19,21 | 8 | 15 | 18 | 13,16,18,19 |
| WT51499 | 14 | 11,12 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 26 | 23 | 11 | 12 | 19,23 | 9 | 17 | 18 | 15,17,18 |
| WT51503 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 26 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,17,18 |
| WT51507 | 15 | 17,17 | 12 | 12 | 29 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,23 | 8 | 15 | 17 | 13,16,18 |
| WT51510 | 14 | 12,12 | 12 | 13 | 29 | 23 | 12 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,18 |
| WT51545 | 15 | 16,17 | 12 | 13 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 11 | 27 | 25 | 11 | 11 | 19,22 | 8 | 16 | 15 | 13,16,17 |
| WT51546 | 15 | 15,16 | 12 | 14 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 12 | 13 | 27 | 23 | 10 | 12 | 19,21 | 8 | 15 | 18 | 13,14,15,16 |
| WT51556 | 14 | 14,14 | 12 | 12 | 28 | 25 | 10 | 11 | 13 | 11 | 15 | 11 | 11 | 23 | 23 | 10 | 11 | 19,20 | 8 | 15 | 17 | 15,16 |
| ZT79303 | 15 | 17,18 | 12 | 13 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 26 | 26 | 10 | 11 | 19,19 | 8 | 15 | 19 | 13,16,17,19 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZT79304 | 16 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 26 | 10 | 12 | 19,19 | 8 | 15 | 18 | 13,15,16 |
| ZT79305 | 15 | 15,16 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 9 | 14 | 11 | 13 | 28 | 24 | 10 | 11 | 19,21 | 8 | 15 | 18 | 11,15,16,17 |
| ZT79306 | 15 | 16,17 | 12 | 12 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 25 | 10 | 11 | 19,19 | 8 | 15 | 17 | 13,16,17 |
| ZT79307 | 14 | 10,14 | 12 | 14 | 31 | 23 | 10 | 13 | 13 | 12 | 14 | 12 | 13 | 23 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| ZT79310 | 14 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 16 | 12 | 11 | 25 | 23 | 11 | 12 | 21,22 | 9 | 15 | 16 | 15,16,17 |
| ZT79311 | 17 | 15,15 | 12 | 13 | 33 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 11 | 19,23 | 8 | 15 | 15 | 13,16,18 |
| ZT79322 | 17 | 16,17 | 12 | 13 | 29 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 25 | 25 | 13 | 13 | 19,22 | 8 | 15 | 16 | 13,16,19 |
| ZT79327 | 17 | 17,19 | 12 | 14 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 12 | 11 | 19,21 | 8 | 17 | 17 | 13,16,17,18 |
| ZT79328 | 14 | 11,15 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 24 | 10 | 12 | 19,23 | 9 | 17 | 17 | 15,17 |
| ZT79330 | 15 | 17,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 26 | 24 | 12 | 11 | 19,23 | 8 | 17 | 15 | 13,16,18 |
| ZT79337 | 17 | 16,17 | 12 | 12 | 29 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,23 | 8 | 16 | 15 | 13,15,16,18 |
| ZT79338 | 17 | 18,18 | 12 | 13 | 30 | 21 | 11 | 11 | 14 | 11 | 14 | 11 | 12 | 28 | 25 | 10 | 11 | 19,23 | 8 | 17 | 16 | 13,16 |
| ZT79339 | 17 | 18,18 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 28 | 25 | 10 | 11 | 19,23 | 8 | 17 | 16 | 13,16 |
| ZT79340 | 16 | 16,16 | 12 | 13 | 30 | 21 | 9 | 11 | 14 | 11 | 14 | 11 | 11 | 26 | 25 | 10 | 11 | 19,23 | 8 | 15 | 17 | 13,15,16,17 |
| ZT79341 | 15 | 16,17 | 12 | 12 | 29 | 21 | 12 | 11 | 12 | 11 | 14 | 11 | 12 | 26 | 25 | 10 | 12 | 19,19 | 8 | 15 | 15 | 13,16,17 |
| ZT79617 | 15 | 15,17 | 12 | 14 | 31 | 21 | 10 | 12 | 12 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 11 | 19,19 | 6 | 16 | 16 | 15,17,18 |
| ZT79618 | 15 | 15,16 | 12 | 13 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 11 | 27 | 25 | 10 | 11 | 19,21 | 8 | 15 | 19 | 12,15,16,17 |
| ZT79619 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 24 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| ZT79620 | 17 | 17,19 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 13,15,16 |
| aa1 | 15 | 16,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 28 | 24 | 10 | 11 | 19,22 | 8 | 14 | 17 | 11,16,18 |
| aa2 | 15 | 17,17 | 12 | 13 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 10 | 12 | 26 | 26 | 10 | 11 | 19,19 | 8 | 15 | 18 | 13,16,17,18 |
| aa3 | 17 | 15,16 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 28 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 16,17 |
| aa4 | 15 | 15,15 | 13 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 25 | 25 | 11 | 10 | 19,21 | 8 | 14 | 18 | 12,15,17 |
| aa5 | 15 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 11 | 19,21 | 8 | 13 | 16 | 13,14,16 |
| aa6 | 17 | 17,19 | 12 | 14 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 17 | 13,16,18 |
| aa7 | 16 | 19,19 | 12 | 14 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 17 | 17 | 13,16,18 |
| aa8 | 15 | 15,16 | 13 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 24 | 10 | 11 | 19,22 | 8 | 16 | 18 | 12,15,16,18 |
| aa9 | 14 | 13,14 | 14 | 12 | 28 | 22 | 11 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 11 | 19,21 | 9 | 15 | 14 | 12,14,15,16 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aa5 | 15 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 26 | 24 | 11 | 11 | 19,21 | 8 | 13 | 16 | 13,14,16 |
| aa6 | 17 | 17,19 | 12 | 14 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 17 | 13,16,18 |
| aa7 | 16 | 19,19 | 12 | 14 | 31 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 17 | 17 | 13,16,18 |
| aa8 | 15 | 15,16 | 13 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 24 | 10 | 11 | 19,22 | 8 | 16 | 18 | 12,15,16,18 |
| aa9 | 14 | 13,14 | 14 | 12 | 28 | 22 | 11 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 11 | 19,21 | 9 | 15 | 14 | 12,14,15,16 |
| aa10 | 17 | 18,19 | 12 | 14 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 26 | 11 | 11 | 19,21 | 8 | 17 | 17 | 13,16,17,18 |
| aa11 | 15 | 16,18 | 12 | 13 | 31 | 22 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 11,16,18 |
| aa12 | 17 | 17,20 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 28 | 25 | 10 | 12 | 19,21 | 8 | 15 | 17 | 13,16,17 |
| aa13 | 14 | 11,14 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 13 | 19,24 | 9 | 15 | 18 | 15,16,17 |
| aa14 | 16 | 16,16 | 13 | 13 | 29 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 25 | 24 | 11 | 11 | 19,19 | 10 | 17 | 15 | 11,14,16 |
| aa15 | 15 | 18,18 | 12 | 13 | 29 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,16,17 |
| aa16 | 16 | 18,19 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 12 | 28 | 25 | 11 | 11 | 19,20 | 8 | 16 | 15 | 13,15,16,18 |
| aa17 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| aa18 | 15 | 16,16 | 12 | 12 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 25 | 24 | 11 | 10 | 20,21 | 8 | 15 | 16 | 13,16,18 |
| aa19 | 17 | 17,18 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 13 | 11 | 13 | 27 | 25 | 12 | 11 | 21,21 | 8 | 17 | 14 | 13,16,17 |
| aa20 | 15 | 14,15 | 12 | 13 | 31 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 12 | 27 | 24 | 10 | 11 | 19,21 | 8 | 14 | 17 | 12,15,16,17 |
| BC11352 | 15 | 15,17 | 12 | 14 | 30 | 22 | 10 | 11 | 12 | 11 | 14 | 9 | 11 | 23.4 | 23 | 11 | 12 | 19,21 | 9 | 15 | 18 | 12,14,15 |
| GC03394 | 15 | 13,16.3 | 15 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 15 | 9 | 12 | 28 | 23 | 11 | 11 | 19,20 | 9 | 13 | 16 | 13,15,18 |
| GT36864 | 14 | 13,16 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,22 | 9 | 14 | 15 | 12,14,16 |
| GT36866 | 14 | 13,18 | 15 | 13 | 29 | 23 | 10 | 11 | 12 | 11 | 15 | 9 | 11 | 25 | 25 | 10 | 11 | 19,23 | 9 | 15 | 14 | 14,15,16 |
| GT36877 | 15 | 13,15 | 12 | 12 | 30 | 21 | 10 | 11 | 14 | 11 | 16 | 10 | 12 | 23 | 26 | 11 | 11 | 19,20 | 9 | 17 | 16 | 12,13,14 |
| GT36878 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 11 | 19,23 | 9 | 16 | 18 | 13,15,16,17 |
| GT36886 | 16 | 10,13 | 12 | 13 | 30 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 11 | 23 | 24 | 12 | 12 | 19,23 | 9 | 16 | 16 | 12,15,16 |
| GT38065 | 16 | 14,16 | 14 | 13 | 30 | 24 | 11 | 11 | 13 | 11 | 14 | 10 | 11 | 24 | 23 | 11 | 10 | 19,19 | 9 | 14 | 15 | 14,15,16 |
| GT38066 | 14 | 11,13 | 12 | 13 | 29 | 24 | 11 | 13 | 12 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 16 | 14,15,17,18 |
| GT38067 | 16 | 11,14 | 12 | 13 | 29 | 25 | 11 | 11 | 13 | 12 | 14 | 11 | 10 | 23 | 24 | 11 | 13 | 19,22 | 9 | 15 | 15 | 14,15,16 |
| GT38069 | 14 | 14,14 | 14 | 13 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 12 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| GT38072 | 16 | 15,16 | 14 | 12 | 28 | 25 | 11 | 11 | 13 | 11 | 15 | 10 | 13 | 25 | 25 | 10 | 10 | 19,19 | 9 | 14 | 17 | 14,15, |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GT38075 | 15 | 13,14 | 14 | 13 | 30 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 11 | 11 | 19,21 | 9 | 14 | 15 | 12,15,16 |
| GT38076 | 15 | 14,14 | 13 | 12 | 28 | 22 | 10 | 11 | 14 | 11 | 15 | 11 | 11 | 22 | 25 | 9 | 12 | 19,19 | 9 | 15 | 18 | 13,14, |
| GT38078 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 12 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| GT38081 | 16 | 11,13 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 10 | 18 | 16 | 15,17,18 |
| GT38083 | 14 | 14,15 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 13 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| GT38086 | 14 | 14,14 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 12 | 23 | 25 | 10 | 11 | 19,21 | 9 | 14 | 14 | 12,14,15,16 |
| GT38089 | 14 | 11,14 | 12 | 13 | 29 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 16 | 16 | 14,16,17,18 |
| GT38091 | 15 | 15,16 | 14 | 13 | 29 | 23 | 10 | 12 | 14 | 11 | 15 | 10 | 11 | 25 | 24 | 12 | 11 | 19,19 | 10 | 13 | 15 | 14,15 |
| GT38094 | 15 | 11,12 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 15,16,17 |
| GT38095 | 14 | 13,13 | 15 | 14 | 31 | 23 | 10 | 12 | 12 | 11 | 15 | 9 | 12 | 26 | 24 | 11 | 11 | 22,22 | 9 | 17 | 15 | 12,13,14,15 |
| GT38097 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 11 | 10 | 19,21 | 9 | 15 | 15 | 12,14,15 |
| GT38098 | 15 | 15,16 | 13 | 14 | 32 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 25 | 24 | 10 | 11 | 19,21 | 10 | 14 | 14 | 11,14,16 |
| GT38100 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| GT38106 | 14 | 11,11 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 12,15,16,17 |
| GT38107 | 13 | 13,14 | 14 | 12 | 27 | 22 | 10 | 11 | 13 | 11 | 15 | 10 | 12 | 22 | 24 | 10 | 11 | 19,21 | 9 | 14 | 16 | 12,15,16 |
| GT38108 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 24 | 23 | 11 | 11 | 19,23 | 9 | 16 | 18 | 15,16,17,18 |
| GT38114 | 14 | 12,15 | 12 | 13 | 30 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 10 | 11 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| GT38119 | 14 | 11,14 | 12 | 13 | 30 | 24 | 10 | 13 | 14 | 12 | 14 | 12 | 11 | 25 | 23 | 11 | 11 | 19,22 | 9 | 16 | 17 | 15,17,18 |
| JA44327 | 14 | 12,14 | 12 | 13 | 29 | 24 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 14,17 |
| JM28315 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 14 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 14,15,17 |
| JT51178 | 14 | 11,16 | 12 | 13 | 29 | 24 | 11 | 14 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 14,15,16,17 |
| JT51211 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| JT52345 | 14 | 11,14 | 12 | 14 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,16,20 |
| JT52346 | 14 | 11,16 | 12 | 13 | 29 | 24 | 12 | 13 | 13 | 12 | 16 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| MT94803 | 14 | 11,14 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 13 | 19,24 | 9 | 17 | 19 | 15,16 |
| MT94807 | 14 | 12,12 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 13 | 13 | 25 | 23 | 11 | 12 | 19,19 | 9 | 16 | 18 | 15,17 |
| MT94815 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 14 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 19 | 14,15,17 |

A-3 (Contiuned)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MT94817 | 14 | 11,16 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 9 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 19 | 15,17,18 |
| MT94826 | 13 | 16,18 | 12 | 13 | 30 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 25 | 24 | 9 | 12 | 19,21 | 8 | 18 | 17 | 14,15.3,17 |
| MT94827 | 14 | 14,15 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| MT94829 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,24 | 9 | 15 | 18 | 15,16,17 |
| MT94833 | 15 | 14,14 | 14 | 13 | 30 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 12 | 19,21 | 9 | 14 | 15 | 12,14,16 |
| MT94835 | 14 | 11,14 | 12 | 14 | 30 | 23 | 11 | 16 | 14 | 11 | 14 | 11 | 10 | 25 | 23 | 10 | 12 | 18,20 | 8 | 15 | 14 | 14,14.3 |
| MT94838 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| MT94839 | 15 | 11,14 | 12 | 14 | 31 | 25 | 11 | 11 | 13 | 12 | 14 | 11 | 10 | 25 | 24 | 12 | 13 | 19,21 | 9 | 15 | 15 | 12,15,16 |
| MT94842 | 16 | 8,16.3 | 15 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 28 | 23 | 11 | 12 | 19,20 | 9 | 13 | 16 | 13,15,17 |
| MT94843 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 12 | 11 | 16 | 10 | 12 | 23 | 24 | 9 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15 |
| MT94846 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17 |
| MT94848 | 13 | 16,16 | 12 | 13 | 30 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 |  | 9 | 13 | 19,22 | 8 | 17 | 15 | 17 |
| MT94850 | 16 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 11 | 12 | 19,21 | 9 | 14 | 15 | 12,14,15 |
| MT94853 | 14 | 10,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 24 | 23 | 11 | 11 | 19,23 | 9 | 16 | 18 | 15,16,18 |
| MT94855 | 14 | 11,15 | 12 | 14 | 30 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 10 | 12 | 19,23 | 9 | 17 | 17 | 15,17 |
| MT94858 | 14 | 11,14 | 12 | 13 | 29 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 10 | 25 | 23 | 10 | 12 | 22,23 | 9 | 16 | 18 | 15,16,19 |
| MT94859 | 15 | 15,15 | 13 | 14 | 31 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 25 | 23 | 10 | 10 | 19,21 | 10 | 14 | 15 | 11,14,15 |
| MT94866 | 14 | 11,16 | 12 | 13 | 30 | 24 | 11 | 15 | 12 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 17 | 18 | 14,15,16,17 |
| MT94868 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 16 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| MT94869 | 14 | 11,14 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 13 | 19,24 | 9 | 15 | 18 | 15,17 |
| MT94875 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 11 | 11 | 19,23 | 9 | 17 | 17 | 15,16,18 |
| MT94877 | 15 | 11,14 | 10 | 13 | 31 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 25 | 24 | 12 | 12 | 19,23 | 9 | 15 | 15 | 14 |
| MT94882 | 14 | 11,15 | 12 | 13 | 28 | 25 | 10 | 13 | 13 | 13 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,22 | 9 | 16 | 18 | 15,17 |
| MT94883 | 14 | 11,13 | 12 | 13 | 29 | 25 | 12 | 14 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,23 | 9 | 16 | 17 | 14,16,17 |
| MT94884 | 16 | 12,17 | 13 | 13 | 30 | 25 | 11 | 12 | 13 | 11 | 15 | 10 | 11 | 25 | 24 | 10 | 12 | 21,22 | 9 | 16 | 16 | 13,14 |
| MT94886 | 15 | 11,14 | 10 | 13 | 32 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 15 | 12,14,17 |
| MT94892 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 11 | 19,21 | 9 | 14 | 14 | 12,15,16 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MT94893 | 15 | 11,15 | 13 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 11 | 12 | 25 | 23 | 12 | 11 | 19,23 | 9 | 15 | 17 | 15,17 |
| MT97121 | 14 | 11,13 | 12 | 14 | 30 | 25 | 9 | 14 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,23 | 9 | 17 | 16 | 15,16 |
| MT97122 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 13 | 25 | 22 | 11 | 12 | 19,23 | 9 | 17 | 18 | 15,16,17 |
| MT97123 | 14 | 11,14 | 12 | 13 | 30 | 22 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| MT97124 | 13 | 17,18 | 12 | 13 | 30 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 25 | 25 | 9 | 12 | 19,21 | 8 | 16 | 16 | 14,15.3,17 |
| MT97126 | 14 | 12,14 | 12 | 14 | 30 | 23 | 10 | 13 | 13 | 12 | 16 | 12 | 12 | 25 | 22 | 11 | 12 | 19,22 | 9 | 16 | 17 | 15,16,17 |
| MT97131 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 10 | 12 | 19,24 | 9 | 16 | 17 | 15,16,17 |
| MT97136 | 14 | 12,13 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 12 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 14 | 12,14,15,16 |
| MT97139 | 16 | 11,14 | 12 | 13 | 30 | 25 | 11 | 11 | 13 | 12 | 14 | 11 | 11 | 24 | 24 | 11 | 13 | 20,23 | 9 | 16 | 15 | 12,15,16 |
| MT97141 | 14 | 11,15 | 12 | 13 | 31 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 13 | 19,24 | 9 | 15 | 20 | 15,16,17 |
| MT97145 | 14 | 9,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 16 | 15,17 |
| MT97150 | 14 | 11,13 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| MT97152 | 14 | 12,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 11 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 16 | 15,16,17 |
| MT97155 | 14 | 13,14 | 14 | 13 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 12 | 19,21 | 9 | 14 | 16 | 12,14,15 |
| MT97156 | 14 | 10,13 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 14,15,17 |
| MT97158 | 15 | 11,14 | 12 | 14 | 31 | 25 | 11 | 11 | 13 | 12 | 14 | 11 | 10 | 24 | 24 | 12 | 13 | 19,21 | 9 | 15 | 15 | 12,15,16 |
| MT97159 | 14 | 11,15 | 12 | 13 | 29 | 25 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 13 | 19,19 | 9 | 15 | 17 | 15,17 |
| MT97163 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 13 | 19,23 | 9 | 15 | 17 | 15,17,18 |
| MT97164 | 14 | 11,14 | 12 | 14 | 30 | 24 | 12 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,24 | 9 | 16 | 17 | 15,17 |
| MT97165 | 14 | 11,14 | 12 | 12 | 28 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,22 | 9 | 16 | 19 | 15,17 |
| MT97166 | 14 | 12,14 | 12 | 13 | 29 | 24 | 11 | 12 | 13 | 13 | 15 | 12 | 11 | 25 | 23 | 11 | 11 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| MT97167 | 14 | 12,13 | 14 | 12 | 28 | 22 | 10 | 12 | 13 | 11 | 16 | 10 | 12 | 22 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15 |
| MT97173 | 14 | 13,14 | 14 | 12 | 28 | 22 | 9 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,13,15,16 |
| MT97176 | 14 | 11,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 17 | 16 | 15,17 |
| MT97177 | 14 | 13,15 | 16 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 11 | 11 | 19,21 | 9 | 15 | 15 | 12,15,16,17 |
| MT97178 | 15 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,23 | 9 | 14 | 14 | 12,14,15,16 |
| MT97179 | 15 | 14,15 | 13 | 12 | 28 | 23 | 11 | 11 | 14 | 11 | 16 | 10 | 11 | 24 | 25 | 10 | 12 | 20,20 | 9 | 15 | 16 | 12,13,14 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MT97180 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| MT97185 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 23,23 | 9 | 16 | 17 | 15,16,17 |
| MT97186 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 22 | 23 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,15 |
| MT97187 | 15 | 12,15 | 13 | 14 | 32 | 23 | 10 | 12 | 15 | 11 | 14 | 9 | 11 | 25 | 24 | 11 | 10 | 19,21 | 10 | 15 | 15 | 11,14,15 |
| MT97188 | 13 | 10,15 | 12 | 13 | 29 | 24 | 11 | 13 | 12 | 12 | 15 | 12 | 13 | 25 | 23 | 10 | 13 | 19,23 | 9 | 15 | 16 | 15,16,17 |
| MT97189 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17 |
| MT97192 | 14 | 11,15 | 12 | 13 | 28 | 24 | 11 | 13 | 13 | 13 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17,18 |
| MT97195 | 14 | 13,15 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 23 | 10 | 12 | 19,21 | 9 | 14 | 15 | 12,13,14,16 |
| MT97196 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 13 | 15 | 12 | 12 | 25 | 25 | 11 | 11 | 19,23 | 9 | 17 | 17 | 15,17 |
| MT97199 | 14 | 11,13 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 13 | 12 | 25 | 23 | 11 | 12 | 19,21 | 9 | 17 | 17 | 15,17 |
| MT97200 | 14 | 11,13 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 17 | 16 | 15,16,18 |
| OT07753 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 23 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| OT07760 | 15 | 12,13 | 13 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 11 | 12 | 26 | 22 | 11 | 13 | 18,22 | 9 | 15 | 15 | 14 |
| OT07767 | 14 | 10,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,19 | 9 | 15 | 16 | 15,16,17 |
| OT07776 | 14 | 13,21 | 16 | 13 | 31 | 23 | 10 | 11 | 12 | 11 | 14 | 10 | 11 | 26 | 24 | 10 | 11 | 21,22 | 9 | 15 | 13 | 13,16,18 |
| OT07785 | 14 | 11,14 | 12 | 12 | 28 | 23 | 11 | 13 | 14 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| PT83505 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,19 | 9 | 14 | 18 | 14,15,17 |
| PT83535 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 22 | 11 | 12 | 19,23 | 9 | 16 | 17 | 16 |
| PT83538 | 14 | 11,14 | 12 | 12 | 28 | 23 | 10 | 13 | 14 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,21 | 9 | 15 | 17 | 15,16,17,18 |
| PT86478 | 14 | 10,14 | 12 | 12 | 28 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 11 | 19,23 | 9 | 17 | 17 | 15,16,17,18 |
| PT86536 | 14 | 13,18 | 16 | 13 | 30 | 23 | 10 | 11 | 12 | 11 | 15 | 9 | 11 | 26 | 24 | 10 | 12 | 19,22 | 9 | 15 | 18 | 12,14,15,16 |
| TT50697 | 16 | 11,14 | 12 | 13 | 31 | 24 | 11 | 11 | 13 | 12 | 14 | 11 | 10 | 24 | 24 | 11 | 12 | 19,23 | 9 | 17 | 15 | 12,15,16 |
| TT50698 | 15 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 22 | 10 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17 |
| TT50700 | 14 | 19,19 | 12 | 13 | 29 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 | 23 | 9 | 12 | 19,21 | 8 | 16 | 15 | 13,15.3,17 |
| TT50701 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,16,17,19 |
| TT50705 | 15 | 11,13 | 14 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 17 | 15,17 |
| TT50708 | 14 | 13,14 | 14 | 12 | 28 | 23 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 22 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 14,15 |

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| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TT50720 | 14 | 11,11 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 26 | 23 | 11 | 12 | 19,22 | 9 | 15 | 18 | 15,16,17 |
| TT50721 | 14 | 15,15 | 13 | 13 | 31 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 25 | 24 | 10 | 11 | 19,21 | 10 | 15 | 15 | 11,14,16 |
| TT51669 | 14 | 12,14 | 12 | 13 | 29 | 24 | 10 | 13 | 14 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 13,14,15 |
| TT51675 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,22 | 9 | 15 | 17 | 15,17 |
| TT51676 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 14 | 12 | 15 | 13 | 15 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17 |
| TT51677 | 14 | 11,14 | 12 | 13 | 28 | 24 | 11 | 14 | 13 | 13 | 15 | 12 | 13 | 26 | 22 | 11 | 13 | 19,23 | 9 | 17 | 17 | 14,15,17 |
| TT51679 | 15 | 14,15.3 | 15 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 26 | 23 | 11 | 11 | 19,20 | 9 | 13 | 17 | 13,15,19 |
| TT51680 | 15 | 12,12 | 13 | 13 | 28 | 23 | 10 | 11 | 13 | 11 | 15 | 10 | 11 | 26 | 24 | 11 | 12 | 21,21 | 9 | 14 | 17 | 11,13,14,15 |
| TT51682 | 15 | 15,15 | 13 | 14 | 31 | 23 | 10 | 12 | 15 | 11 | 14 | 10 | 12 | 25 | 24 | 11 | 10 | 19,21 | 10 | 13 | 15 | 11,14,15 |
| TT51683 | 14 | 11,14 | 12 | 14 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,22 | 9 | 16 | 16 | 15,16,17 |
| TT51684 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 23,23 | 8 | 16 | 16 | 14,15,17 |
| TT51689 | 14 | 14,14 | 15 | 12 | 29 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 12 | 23 | 25 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| TT51693 | 14 | 12,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 24 | 11 | 12 | 19,23 | 9 | 14 | 16 | 15,16,17 |
| TT51694 | 14 | 13,14 | 13 | 12 | 29 | 22 | 10 | 11 | 12 | 11 | 16 | 10 | 11 | 23 | 25 | 12 | 12 | 20,20 | 9 | 15 | 17 | 12,13,14 |
| TT51696 | 14 | 14,15 | 15 | 14 | 31 | 22 | 10 | 11 | 12 | 11 | 15 | 9 | 12 | 26 | 24 | 10 | 11 | 19,22 | 9 | 15 | 15 | 13,13.1,15,16 |
| TT51698 | 13 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 24 | 23 | 12 | 11 | 19,23 | 9 | 17 | 18 | 15,16,18 |
| TT51702 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 14,15,16,17 |
| TT51703 | 13 | 15,17 | 12 | 14 | 32 | 24 | 9 | 12 | 14 | 11 | 14 | 10 | 12 | 21 | 24 | 10 | 12 | 19,22 | 8 | 15 | 17 | 14,15,16,17 |
| UA16891 | 14 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| UA16894 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 11 | 15 | 12 | 11 | 24 | 23 | 11 | 11 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| UA16895 | 14 | 11,14 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 9 | 19,23 | 9 | 16 | 18 | 15,17,18 |
| UA16896 | 14 | 13,14 | 15 | 13 | 30 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 12 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15 |
| UA16897 | 14 | 12,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 11 | 12 | 24 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| UA16899 | 14 | 11,14 | 12 | 14 | 30 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 18 | 15,16,18 |
| UA16903 | 17 | 16,19 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 14 | 27 | 25 | 11 | 11 | 19,21 | 8 | 15 | 18 | 13,16,18,19 |
| UA16908 | 14 | 14,15 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 10 | 14 | 15 | 12,14,15,16 |
| UA16918 | 14 | 13,14 | 16 | 12 | 28 | 22 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 23 | 24 | 11 | 11 | 19,21 | 9 | 15 | 16 | 12,14,15,16 |
| UA16921 | 17 | 15,15 | 13 | 13 | 30 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 25 | 25 | 10 | 11 | 21,21 | 8 | 15 | 17 | 12,14,15 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UA16924 | 14 | 11,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 22 | 11 | 11 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| UA16928 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 26 | 22 | 12 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| UA16929 | 14 | 11,14 | 12 | 14 | 30 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 16 | 15,16,17 |
| UC10177 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| UC10178 | 15 | 11,15 | 12 | 13 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17, |
| UT57281 | 13 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 14 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| UT57283 | 14 | 13,14 | 15 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 11 | 11 | 19,21 | 9 | 15 | 15 | 12,14,15,16 |
| UT57286 | 14 | 11,13 | 12 | 13 | 29 | 24 | 11 | 14 | 13 | 12 | 15 | 12 | 11 | 25 | 22 | 11 | 12 | 19,23 | 9 | 17 | 17 | 15,16,17 |
| UT57287 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 22 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| UT57288 | 14 | 13,14 | 14 | 13 | 29 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,15 |
| UT57292 | 14 | 11,15 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 26 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,15.3,16 |
| UT57293 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 14 | 12 | 15 | 12 | 13 | 23 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 14,15,16 |
| UT57299 | 15 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 10 | 12 | 19,22 | 9 | 17 | 17 | 15,16,17 |
| UT57300 | 15 | 14,14 | 13 | 13 | 30 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 25 | 23 | 11 | 10 | 19,21 | 10 | 14 | 15 | 11,14,15 |
| UT57302 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 11 | 19,24 | 9 | 16 | 17 | 13,16 |
| UT57303 | 15 | 14,16.3 | 15 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 26 | 23 | 12 | 11 | 19,20 | 9 | 13 | 17 | 13,14,15 |
| UT57310 | 15 | 11,14 | 10 | 12 | 31 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 25 | 23 | 12 | 12 | 19,23 | 9 | 15 | 14 | 14 |
| UT57312 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| UT57317 | 16 | 13,15 | 16 | 13 | 30 | 23 | 9 | 11 | 12 | 11 | 14 | 9 | 12 | 27 | 25 | 10 | 13 | 19,22 | 10 | 16 | 15 | 13,15,15.1 |
| UT57318 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| UT58295 | 15 | 10,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,24 | 9 | 15 | 17 | 15,16,17 |
| UT58298 | 15 | 13,16.3 | 15 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 28 | 23 | 11 | 11 | 19,20 | 9 | 14 | 16 | 11,15,18 |
| UT58299 | 14 | 15,16 | 15 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 22 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 11,14,16 |
| UT58300 | 14 | 11,14 | 12 | 14 | 29 | 24 | 11 | 13 | 13 | 11 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,25 | 9 | 16 | 17 | 15,16,17 |
| UT58301 | 15 | 12,15 | 15 | 14 | 30 | 23 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 | 22 | 10 | 11 | 21,21 | 9 | 14 | 19 | 14,15 |
| UT58302 | 14 | 11,14 | 12 | 13 | 29 | 24 | 12 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 12 | 12 | 19,23 | 9 | 17 | 17 | 16,17 |
| UT58303 | 16 | 13,17 | 13 | 13 | 31 | 22 | 10 | 12 | 13 | 11 | 14 | 10 | 11 | 25 | 24 | 11 | 11 | 19,19 | 10 | 13 | 15 | 14,15 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UT58305 | 14 | 11,14 | 12 | 13 | 29 | 23 | 8 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,17,18 |
| UT58310 | 14 | 11,15 | 12 | 13 | 29 | 23 | 10 | 14 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 11 | 12 | 19,20 | 9 | 15 | 17 | 15,16,17,18 |
| UT58314 | 15 | 14,14 | 13 | 12 | 29 | 22 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 23 | 26 | 10 | 13 | 20,20 | 9 | 15 | 17 | 13,15 |
| UT58315 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 16 | 12 | 12 | 25 | 23 | 11 | 12 | 19,22 | 9 | 15 | 18 | 15,16 |
| UT58317 | 15 | 14,15 | 13 | 12 | 31 | 22 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 23 | 25 | 11 | 12 | 20,20 | 9 | 15 | 16 | 13,14 |
| UT58318 | 14 | 14,15 | 17 | 14 | 31 | 21 | 11 | 11 | 12 | 11 | 15 | 9 | 10 | 29 | 24 | 10 | 11 | 19,22 | 9 | 15 | 16 | 13,15,16 |
| UT58319 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 10 | 13 | 19,23 | 9 | 16 | 20 | 14,15,17,18 |
| UT58320 | 14 | 11,16 | 12 | 13 | 29 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| UT58321 | 14 | 11,15 | 12 | 13 | 30 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 13 | 19,23 | 9 | 15 | 17 | 15,17,19 |
| UT58324 | 14 | 13,14 | 14 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 11 | 19,21 | 9 | 13 | 14 | 12,15,16 |
| UT58333 | 15 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| UT58334 | 15 | 11,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 16 | 15,16,17 |
| UT58335 | 12 | 11,14 | 12 | 14 | 30 | 23 | 11 | 13 | 13 | 12 | 16 | 12 | 11 | 24 | 23 | 11 | 13 | 19,23 | 9 | 15 | 18 | 15 |
| UT58336 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| UT58337 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 14 | 13 | 12 | 15 | 12 | 12 | 26 | 22 | 12 | 12 | 19,23 | 9 | 17 | 17 | 16,18 |
| WA29584 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 16 | 15,17 |
| WA29594 | 13 | 17,18 | 12 | 14 | 31 | 25 | 9 | 11 | 13 | 11 | 14 | 10 | 13 | 21 | 24 | 10 | 11 | 19,19 | 8 | 15 | 17 | 14,15,17 |
| WA29612 | 14 | 12,14 | 12 | 13 | 29 | 23 | 11 | 13 | 12 | 12 | 15 | 12 | 12 | 21 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| WT51342 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17,18 |
| WT51343 | 14 | 11,15 | 12 | 14 | 31 | 23 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 17 | 17 | 15,16,18 |
| WT51345 | 14 | 11,14 | 12 | 13 | 30 | 23 | 12 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 11 | 19,23 | 9 | 17 | 17 | 15,17,18 |
| WT51355 | 15 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 12 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17 |
| WT51358 | 16 | 11,14 | 9 | 13 | 29 | 23 | 10 | 13 | 13 | 12 | 16 | 11 | 12 | 25 | 22 | 11 | 12 | 19,23 | 9 | 16 | 15 | 15,16,17 |
| WT51359 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 18 | 15,16,18,20 |
| WT51362 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 27 | 23 | 12 | 11 | 19,23 | 9 | 16 | 16 | 15,16,17 |
| WT51373 | 14 | 11,14 | 12 | 14 | 30 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 18,20 | 9 | 15 | 17 | 15,17 |
| WT51378 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 12 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,23 | 9 | 17 | 16 | 14,15,17,18 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WT51381 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 11 | 13 | 12 | 14 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17,18 |
| WT51386 | 15 | 13,16.3 | 15 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 27 | 22 | 11 | 11 | 19,20 | 9 | 14 | 17 | 13,15,16,17 |
| WT52457 | 14 | 11,15 | 12 | 13 | 29 | 23 | 10 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| WT52470 | 14 | 14,15 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 24 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| WT52471 | 16 | 11,14 | 12 | 14 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 24 | 10 | 11 | 20,21 | 9 | 15 | 17 | 15,17 |
| WT52472 | 15 | 14,14 | 13 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 12 | 20,20 | 9 | 16 | 16 | 12,13,14 |
| WT52474 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 11 | 14 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 16 | 15,17 |
| WT52480 | 14 | 12,15 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,16 |
| WT52482 | 14 | 12,14 | 12 | 13 | 29 | 23 | 11 | 13 | 12 | 12 | 15 | 12 | 12 | 21 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| WT52485 | 15 | 14,15 | 13 | 12 | 29 | 22 | 10 | 11 | 15 | 11 | 16 | 10 | 12 | 23 | 25 | 10 | 12 | 18,20 | 9 | 14 | 16 | 12,13,14 |
| WT52486 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 14,17 |
| WT52490 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 16 | 12 | 12 | 25 | 23 | 11 | 12 | 19,22 | 9 | 17 | 17 | 15,17 |
| WT52493 | 13 | 11,14 | 12 | 13 | 29 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 24 | 10 | 12 | 19,22 | 9 | 15 | 17 | 15,18 |
| ZC08844 | 14 | 11,13 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 10 | 12 | 19,21 | 9 | 15 | 17 | 15,16,17,18 |
| ZT81337 | 17 | 10,14 | 12 | 14 | 32 | 25 | 12 | 11 | 13 | 12 | 14 | 11 | 10 | 23 | 24 | 11 | 12 | 19,23 | 9 | 16 | 18 | 12,15,16 |
| ZT81342 | 14 | 11,14 | 12 | 13 | 29 | 25 | 10 | 12 | 12 | 12 | 15 | 13 | 12 | 26 | 23 | 11 | 12 | 20,23 | 9 | 15 | 17 | 15,16,17 |
| ZT81372 | 14 | 11,11 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17 |
| ZT81377 | 15 | 13,14 | 14 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 11 | 11 | 19,21 | 9 | 14 | 13 | 12,14,15,16 |
| ZT81380 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 16 | 14,15,17 |
| ZT81381 | 15 | 14,15 | 12 | 13 | 29 | 23 | 11 | 11 | 13 | 11 | 16 | 10 | 13 | 23 | 25 | 11 | 11 | 21,21 | 9 | 15 | 17 | 12,13,14 |
| ZT81382 | 15 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,17 |
| ZT81387 | 15 | 11,14 | 10 | 13 | 30 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 24 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 12,14,17 |
| ca1 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| ca2 | 14 | 11,14 | 12 | 13 | 29 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 24 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17,18 |
| ca3 | 14 | 15,15 | 14 | 12 | 28 | 23 | 10 | 11 | 13 | 11 | 16 | 10 | 12 | 22 | 24 | 10 | 11 | 19,19 | 9 | 14 | 15 | 12,14,15,16 |
| ca4 | 14 | 12,12 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 16 | 12 | 11 | 24 | 23 | 11 | 11 | 19,23 | 9 | 16 | 17 | 15,16,18 |
| ca5 | 14 | 11,14 | 12 | 12 | 28 | 25 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 11 | 19,23 | 9 | 16 | 18 | 16,17 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ca6 | 15 | 14,16.3 | 15 | 12 | 28 | 23 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 27 | 23 | 12 | 11 | 19,20 | 9 | 13 | 16 | 13,15,19 |
| ca7 | 16 | 14,14 | 12 | 12 | 28 | 21 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 23 | 27 | 9 | 11 | 19,20 | 9 | 15 | 15 | 12,14 |
| ca8 | 14 | 11,14 | 12 | 13 | 30 | 25 | 10 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,16 |
| ca9 | 13 | 16,18 | 12 | 13 | 31 | 25 | 10 | 11 | 13 | 13 | 14 | 10 | 12 | 19 | 24 | 11 | 12 | 19,22 | 8 | 15 | 16 | 15,16 |
| ca10 | 14 | 11,14 | 12 | 13 | 29 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 22 | 11 | 11 | 19,20 | 9 | 15 | 18 | 15,17 |
| ca11 | 15 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 12 | 12 | 16 | 11 | 12 | 26 | 23 | 11 | 11 | 19,21 | 9 | 16 | 18 | 14,15,18 |
| ca12 | 15 | 11,14 | 12 | 13 | 30 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 11 | 23 | 24 | 11 | 12 | 19,19 | 9 | 17 | 16 | 12,15,16 |
| ca13 | 15 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 27 | 24 | 11 | 12 | 19,23 | 9 | 15 | 19 | 15,16,17 |
| ca14 | 15 | 11,13 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 17 | 17 | 15,17 |
| ca15 | 14 | 11,14 | 12 | 14 | 30 | 24 | 10 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 22 | 10 | 11 | 19,23 | 9 | 17 | 17 | 15,16 |
| ca16 | 14 | 11,14 | 12 | 13 | 30 | 24 | 11 | 14 | 13 | 12 | 15 | 12 | 13 | 24 | 22 | 12 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| ca17 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 14,15 |
| ca18 | 14 | 14,14 | 14 | 13 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| ca19 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 15 | 14 | 12 | 15 | 12 | 13 | 25 | 22 | 11 | 12 | 19,23 | 7 | 14 | 18 | 15,17 |
| ca20 | 14 | 11,15 | 12 | 13 | 29 | 24 | 12 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,17,18,19 |
| GA05070 | 13 | 15,19 | 12 | 14 | 30 | 24 | 10 | 15 | 13 | 12 | 14 | 11 | 12 | 25 | 24 | 11 | 12 | 19,23 | 9 | 17 | 15 | 14,17 |
| GA05071 | 14 | 12,14 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 13 | 24 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 15,17 |
| GT37306 | 14 | 11,14 | 12 | 13 | 28 | 24 | 11 | 13 | 13 | 13 | 16 | 12 | 13 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 18 | 15,17 |
| GT37351 | 15 | 15,17 | 13 | 13 | 31 | 23 | 10 | 12 | 15 | 11 | 14 | 10 | 13 | 24 | 24 | 10 | 10 | 19,21 | 10 | 14 | 16 | 11,12,14,15 |
| GT37402 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 22 | 10 | 11 | 19,23 | 9 | 16 | 18 | 15,16 |
| GT37420 | 14 | 13,20 | 12 | 14 | 29 | 23 | 10 | 10 | 14 | 12 | 14 | 11 | 11 | 26 | 23 | 10 | 11 | 20,20 | 9 | 15 | 16 | 12,16 |
| GT37463 | 13 | 14,17 | 12 | 13 | 30 | 24 | 10 | 14 | 13 | 12 | 14 | 11 | 12 | 26 | 25 | 10 | 11 | 19,23 | 9 | 16 | 16 | 13,15,16,17 |
| GT37483 | 13 | 15,15 | 12 | 13 | 31 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 | 24 | 9 | 12 | 19,21 | 8 | 17 | 15 | 15,16,17 |
| GT37542 | 15 | 12,14 | 12 | 13 | 30 | 26 | 11 | 13 | 12 | 12 | 15 | 11 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 19 | 14,15,17 |
| GT37590 | 13 | 17,18 | 12 | 13 | 30 | 23 | 10 | 11 | 13 | 11 | 14 | 10 | 11 | 26 | 24 | 9 | 13 | 19,21 | 8 | 14 | 16 | 14,16,17 |
| GT37607 | 14 | 11,15 | 12 | 13 | 29 | 25 | 10 | 13 | 14 | 12 | 15 | 11 | 11 | 24 | 23 | 11 | 13 | 19,22 | 9 | 16 | 18 | 15,17 |
| GT37692 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 13 | 19,23 | 9 | 16 | 16 | 14,15,16,18 |

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| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GT37700 | 14 | 11,15 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 16 | 12 | 13 | 25 | 23 | 11 | 12 | 19,22 | 9 | 17 | 17 | 15,17 |
| GT37713 | 15 | 11,12 | 12 | 13 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 14 | 25 | 23 | 11 | 12 | 19,22 | 9 | 15 | 18 | 15,18 |
| GT37732 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 15 | 17 | 12,14,15,16 |
| GT37765 | 13 | 14,17 | 12 | 13 | 30 | 24 | 10 | 16 | 13 | 12 | 14 | 11 | 11 | 25 | 24 | 10 | 12 | 19,23 | 9 | 15 | 15 | 13,17,20 |
| GT37767 | 15 | 13,16 | 12 | 14 | 30 | 25 | 11 | 11 | 13 | 11 | 15 | 10 | 11 | 24 | 25 | 11 | 10 | 20,20 | 9 | 17 | 17 | 13,14 |
| GT37778 | 16 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 27 | 25 | 11 | 11 | 19,21 | 8 | 17 | 16 | 13,16,18 |
| GT37812 | 15 | 11,14 | 12 | 14 | 30 | 23 | 11 | 14 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 12 | 19,19 | 9 | 17 | 18 | 15,17 |
| GT37828 | 15 | 15,16 | 12 | 13 | 30 | 23 | 11 | 13 | 13 | 11 | 13 | 9 | 11 | 27 | 23 | 11 | 11 | 21,23 | 9 | 14 | 16 | 11,13,15,16 |
| GT37853 | 15 | 13,15 | 12 | 12 | 29 | 22 | 10 | 11 | 14 | 11 | 16 | 10 | 11 | 23 | 25 | 11 | 12 | 20,21 | 9 | 17 | 16 | 12,13,14 |
| GT37862 | 13 | 14,18 | 12 | 13 | 30 | 25 | 10 | 16 | 13 | 13 | 14 | 11 | 11 | 27 | 24 | 11 | 12 | 19,23 | 9 | 16 | 17 | 13,14,16,17 |
| GT37864 | 14 | 12,16 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 26 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| GT37869 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 14 | 13 | 12 | 15 | 10 | 13 | 25 | 23 | 10 | 11 | 19,19 | 9 | 15 | 16 | 15,16,17 |
| GT37888 | 13 | 13,14 | 12 | 14 | 30 | 24 | 9 | 11 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 10 | 11 | 19,20 | 8 | 16 | 18 | 15,16,17 |
| GT37900 | 16 | 13,16 | 16 | 13 | 29 | 23 | 9 | 11 | 12 | 11 | 14 | 9 | 13 | 26 | 25 | 10 | 12 | 19,22 | 10 | 15 | 14 | 11,13,15.1,16 |
| GT37913 | 15 | 16,18 | 16 | 12 | 28 | 24 | 10 | 11 | 12 | 11 | 14 | 9 | 13 | 26 | 23 | 11 | 9 | 19,20 | 9 | 14 | 20 | 14,15,17 |
| JT51826 | 14 | 11,14 | 12 | 12 | 27 | 24 | 10 | 14 | 12 | 12 | 14 | 12 | 11 | 26 | 23 | 11 | 11 | 19,23 | 9 | 16 | 14 | 15,16 |
| JT52076 | 14 | 13,14 | 14 | 12 | 28 | 22 | 10 | 11 | 13 | 11 | 17 | 10 | 11 | 23 | 24 | 11 | 10 | 19,23 | 9 | 14 | 14 | 12,13,14,16 |
| MT95744 | 16 | 12,14 | 12 | 13 | 31 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 24 | 23 | 10 | 12 | 19,23 | 9 | 17 | 15 | 13,15 |
| MT95855 | 16 | 14,19 | 12 | 13 | 30 | 21 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 16 | 13,16,19 |
| MT96356 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 22 | 11 | 11 | 22,23 | 9 | 15 | 18 | 15,16,17 |
| OT07280 | 14 | 11,14 | 12 | 12 | 29 | 24 | 11 | 11 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 19 | 15,16,17 |
| PT84348 | 13 | 13,14 | 12 | 15 | 31 | 24 | 9 | 11 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 12 | 12 | 19,24 | 8 | 16 | 18 | 14,16,17 |
| PT84349 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 14 | 12 | 12 | 26 | 22 | 10 | 12 | 19,23 | 9 | 17 | 16 | 15,17 |
| PT84386 | 16 | 16,17 | 12 | 13 | 29 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 13 | 27 | 25 | 10 | 11 | 19,21 | 8 | 16 | 16 | 12,16,19 |
| PT84411 | 14 | 11,14 | 12 | 14 | 31 | 24 | 11 | 13 | 13 | 11 | 15 | 12 | 13 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 20 | 15,18,19 |
| PT84541 | 14 | 15,17 | 12 | 13 | 30 | 20 | 10 | 12 | 13 | 12 | 16 | 14 | 12 | 22 | 24 | 10 | 11 | 19,20 | 9 | 15 | 17 | 14,17 |
| PT84633 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 17 | 15,16,17 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT85056 | 15 | 16,17 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 12 | 27 | 23 | 11 | 11 | 19,21 | 8 | 16 | 15 | 14,15,17 |
| PT85089 | 15 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,25 | 9 | 15 | 18 | 15,16,17 |
| PT85278 | 15 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 22 | 10 | 11 | 19,23 | 9 | 14 | 17 | 15,16,17 |
| PT85300 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| PT85499 | 14 | 11,14 | 12 | 13 | 29 | 23 | 12 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,17 |
| PT85612 | 15 | 16,16 | 12 | 13 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 27 | 25 | 10 | 12 | 19,19 | 8 | 17 | 15 | 13,16,18 |
| PT85658 | 14 | 12,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 19,22 | 9 | 16 | 14 | 15,17 |
| TT51023 | 15 | 13,16 | 12 | 12 | 29 | 21 | 11 | 11 | 15 | 11 | 16 | 10 | 11 | 24 | 27 | 10 | 11 | 19,20 | 10 | 15 | 18 | 12,14,15 |
| TT51034 | 14 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 11 | 19,23 | 9 | 17 | 18 | 15,17 |
| TT51035 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 26 | 22 | 10 | 11 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| TT51151 | 14 | 11,15 | 12 | 14 | 30 | 24 | 11 | 14 | 13 | 12 | 14 | 12 | 12 | 24 | 22 | 10 | 11 | 19,23 | 9 | 16 | 18 | 15,17 |
| TT51208 | 15 | 11,14 | 12 | 13 | 30 | 25 | 11 | 13 | 14 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 16 | 17 | 15,16,17,18 |
| TT51279 | 14 | 14,17 | 17 | 13 | 29 | 23 | 10 | 11 | 12 | 11 | 14 | 10 | 12 | 25 | 24 | 12 | 11 | 21,23 | 9 | 15 | 17 | 14,16 |
| TT51304 | 14 | 12,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 25 | 22 | 10 | 11 | 19,23 | 9 | 15 | 17 | 14,15,16,17 |
| TT51328 | 14 | 17,18 | 12 | 13 | 30 | 24 | 10 | 11 | 13 | 11 | 14 | 11 | 11 | 22 | 23 | 11 | 10 | 18,19 | 8 | 15 | 19 | 13,16 |
| TT51345 | 14 | 12,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 24 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| TT51349 | 15 | 16,17 | 12 | 13 | 31 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 24 | 10 | 12 | 19,19 | 8 | 15 | 15 | 13,16,17 |
| TT51399 | 13 | 14,17 | 12 | 13 | 30 | 24 | 10 | 16 | 13 | 12 | 14 | 11 | 12 | 26 | 24 | 11 | 12 | 19,21 | 9 | 15 | 17 | 13,14,15,18 |
| TT51407 | 15 | 16,19 | 12 | 13 | 31 | 23 | 10 | 11 | 14 | 11 | 14 | 11 | 13 | 25 | 24 | 11 | 11 | 19,22 | 8 | 16 | 16 | 14,16,17 |
| TT51422 | 16 | 11,13 | 12 | 12 | 29 | 25 | 10 | 11 | 13 | 12 | 14 | 11 | 10 | 24 | 24 | 11 | 12 | 19,23 | 9 | 16 | 15 | 12,14,15,16 |
| TT51435 | 15 | 13,15 | 12 | 12 | 30 | 21 | 10 | 11 | 15 | 11 | 16 | 10 | 12 | 23 | 27 | 10 | 11 | 19,20 | 9 | 15 | 16 | 12,14 |
| TT51483 | 16 | 12,12 | 13 | 13 | 28 | 23 | 10 | 11 | 13 | 11 | 14 | 10 | 13 | 24 | 25 | 10 | 12 | 21,21 | 9 | 14 | 18 | 14,15 |
| TT51511 | 15 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 18 | 15,16,18 |
| TT51530 | 13 | 15,18 | 12 | 12 | 28 | 23 | 10 | 13 | 14 | 12 | 14 | 11 | 13 | 26 | 24 | 10 | 12 | 19,22 | 9 | 16 | 18 | 14,16,17 |
| WA29328 | 14 | 14,17 | 12 | 13 | 30 | 24 | 11 | 14 | 13 | 12 | 15 | 11 | 11 | 23 | 22 | 10 | 11 | 19,22 | 9 | 14 | 16 | 14,15,18 |
| ZA08588 | 14 | 11,14 | 13 | 13 | 28 | 23 | 12 | 13 | 13 | 12 | 15 | 12 | 14 | 26 | 22 | 10 | 12 | 19,23 | 9 | 15 | 16 | 15,16 |
| ZC08755 | 14 | 11,15 | 12 | 13 | 28 | 24 | 10 | 14 | 12 | 10 | 15 | 12 | 13 | 25 | 23 | 10 | 13 | 20,23 | 9 | 15 | 16 | 15,16,18 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZC08763 | 14 | 12,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 13 | 19,22 | 9 | 16 | 18 | 15,17 |
| ZC08773 | 14 | 11,15 | 12 | 14 | 30 | 25 | 11 | 13 | 13 | 12 | 14 | 12 | 13 | 25 | 23 | 11 | 12 | 20,23 | 9 | 16 | 16 | 15,17 |
| ZT79837 | 14 | 12.2,14 | 12 | 14 | 29 | 24 | 11 | 14 | 12 | 12 | 15 | 12 | 13 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,16 |
| ZT79848 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 11 | 33 | 22 | 10 | 11 | 19,23 | 9 | 16 | 18 | 15,17 |
| ZT79868 | 14 | 11,13 | 12 | 14 | 30 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 14,15,17,18 |
| ZT79874 | 15 | 15,16 | 12 | 12 | 30 | 22 | 10 | 10 | 14 | 11 | 16 | 10 | 12 | 24 | 25 | 10 | 11 | 20,21 | 9 | 14 | 19 | 13,14 |
| ZT79882 | 16 | 17,17 | 12 | 13 | 30 | 21 | 10 | 11 | 15 | 11 | 13 | 11 | 14 | 27 | 25 | 11 | 11 | 19,21 | 8 | 16 | 16 | 12,16,19 |
| ZT79899 | 15 | 11,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 24 | 10 | 12 | 19,19 | 9 | 16 | 18 | 15,17 |
| ZT79907 | 14 | 11,13 | 12 | 13 | 29 | 25 | 10 | 13 | 12 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 20,23 | 9 | 15 | 17 | 15,16,17 |
| ZT79976 | 15 | 17,17 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 12 | 24 | 25 | 10 | 12 | 19,19 | 8 | 14 | 19 | 13,16,17 |
| ZT79994 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 16 | 13 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| ZT79995 | 14 | 11,15 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 16 | 13 | 12 | 25 | 23 | 10 | 12 | 19,21 | 9 | 15 | 17 | 15,17 |
| ZT80028 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 16 | 12 | 11 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 17 | 15,17,18 |
| ZT80070 | 14 | 14,19 | 16 | 14 | 31 | 23 | 10 | 11 | 12 | 11 | 14 | 10 | 11 | 26 | 24 | 11 | 11 | 22,22 | 9 | 15 | 19.2 | 12,15,17 |
| ZT80090 | 14 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,17 |
| ZT80131 | 14 | 14,14 | 12 | 12 | 28 | 25 | 10 | 12 | 13 | 11 | 16 | 11 | 11 | 24 | 26 | 10 | 10 | 20,20 | 9 | 15 | 17 | 11,14 |
| ZT80133 | 14 | 11,14 | 12 | 14 | 32 | 24 | 10 | 13 | 13 | 12 | 14 | 12 | 13 | 26 | 22 | 10 | 11 | 19,23 | 9 | 15 | 17 | 15,17,18 |
| ZT80147 | 14 | 14,19 | 12 | 13 | 32 | 25 | 10 | 14 | 12 | 12 | 15 | 11 | 11 | 25 | 26 | 11 | 12 | 19,23 | 9 | 15 | 17 | 13,14,17 |
| ZT80154 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 24 | 23 | 10 | 12 | 19,23 | 9 | 15 | 15 | 15,16,17 |
| ZT80163 | 15 | 14,16 | 12 | 14 | 32 | 23 | 10 | 13 | 13 | 11 | 14 | 9 | 11 | 26 | 23 | 10 | 11 | 22,24 | 9 | 15 | 18 | 11,13,16 |
| ZT80245 | 14 | 11,14 | 12 | 13 | 30 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 11 | 13 | 19,24 | 9 | 16 | 18 | 15,16,17 |
| ZT80255 | 15 | 14,19 | 15 | 12 | 28 | 24 | 10 | 11 | 13 | 11 | 16 | 9 | 14 | 28 | 23 | 11 | 11 | 19,20 | 9 | 13 | 16 | 13,15,18 |
| ZT80317 | 16 | 15,15 | 13 | 13 | 29 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 26 | 24 | 11 | 11 | 19,21 | 10 | 16 | 16 | 11,14,15 |
| ZT80333 | 15 | 11,13 | 11 | 13 | 30 | 22 | 9 | 11 | 14 | 12 | 15 | 10 | 13 | 23 | 25 | 10 | 13 | 21,21 | 11 | 14 | 19 | 11,15,16 |
| ZT80334 | 14 | 11,14 | 12 | 14 | 29 | 23 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 22 | 11 | 12 | 22,23 | 9 | 15 | 15 | 15,16,17 |
| ZT80335 | 13 | 13,14 | 12 | 14 | 30 | 24 | 9 | 11 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 11 | 11 | 19,22 | 8 | 15 | 17 | 14,15,16,17 |
| ZT80358 | 16 | 13,19 | 16 | 12 | 29 | 23 | 10 | 11 | 12 | 11 | 14 | 10 | 11 | 24 | 22.5 | 10 | 11 | 20,22 | 9 | 16 | 17.2 | 14,16 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZT80369 | 13 | 13,14 | 12 | 15 | 31 | 24 | 9 | 11 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 12 | 12 | 19,24 | 8 | 16 | 18 | 14,16,17 |
| ZT80384 | 13 | 13,14 | 12 | 14 | 30 | 23 | 9 | 11 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 11 | 12 | 19,22 | 8 | 15 | 17 | 14,15,16,17 |
| ZT80427 | 14 | 11,14 | 12 | 13 | 29 | 23 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 26 | 22 | 11 | 11 | 19,23 | 9 | 17 | 17 | 15,16,17 |
| ZT80625 | 15 | 16,17 | 12 | 14 | 32 | 21 | 11 | 11 | 13 | 11 | 14 | 11 | 11 | 25 | 24 | 10 | 12 | 19,19 | 8 | 15 | 15 | 13,16,18 |
| ZT80626 | 13 | 16,18 | 12 | 13 | 29 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 | 24 | 9 | 12 | 19,21 | 8 | 15 | 15 | 14,15.3,17 |
| ZT80634 | 13 | 13,14 | 12 | 14 | 30 | 24 | 9 | 12 | 13 | 11 | 14 | 10 | 10 | 23 | 24 | 11 | 11 | 19,22 | 8 | 16 | 19 | 14,16,17 |
| ZT80635 | 14 | 11,14 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 14 | 16 | 15,17 |
| ZT80655 | 14 | 11,14 | 12 | 12 | 28 | 24 | 11 | 13 | 13 | 13 | 15 | 12 | 10 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 16 | 15,16,17 |
| ZT80656 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 24 | 23 | 11 | 12 | 19,24 | 9 | 14 | 17 | 15,17 |
| ZT80658 | 15 | 16,16 | 12 | 14 | 32 | 21 | 10 | 11 | 15 | 11 | 14 | 11 | 11 | 26 | 24 | 11 | 10 | 19,23 | 8 | 15 | 18 | 13,15,16 |
| ZT80659 | 13 | 14,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 12 | 19,23 | 9 | 17 | 16 | 15,16,17 |
| ZT80696 | 15 | 14,15 | 13 | 12 | 29 | 22 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 22 | 25 | 10 | 11 | 20,20 | 9 | 15 | 18 | 12,13,14 |
| ZT80718 | 13 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 17 | 15,16 |
| ZT80731 | 16 | 16,16 | 12 | 14 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 11 | 13 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 18 | 13,15,16 |
| ZT80737 | 14 | 11,13 | 12 | 13 | 29 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 24 | 10 | 13 | 19,23 | 9 | 16 | 17 | 15,17,18 |
| ZT80782 | 14 | 10,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 13 | 11 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 18 | 15,16,17 |
| ZT80786 | 14 | 13,18 | 16 | 12 | 30 | 23 | 11 | 11 | 12 | 11 | 14 | 10 | 11 | 25 | 24 | 12 | 11 | 22,22 | 9 | 16 | 17 | 12,14,17 |
| ZT80815 | 14 | 13,15 | 14 | 12 | 28 | 23 | 11 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 24 | 10 | 11 | 19,21 | 9 | 14 | 15 | 12,14,15,16 |
| ZT80826 | 14 | 11,14 | 12 | 14 | 30 | 23 | 10 | 13 | 13 | 12 | 14 | 12 | 12 | 33 | 22 | 10 | 11 | 19,21 | 9 | 16 | 17 | 15,16,17 |
| ZT80863 | 17 | 12,12 | 13 | 13 | 28 | 23 | 10 | 11 | 13 | 11 | 15 | 10 | 12 | 25 | 25 | 10 | 12 | 21,21 | 9 | 14 | 17 | 11,14 |
| ZT80865 | 15 | 11,14 | 12 | 13 | 30 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 11 | 19,23 | 9 | 16 | 19 | 15,18 |
| ZT80869 | 13 | 13,15 | 14 | 12 | 28 | 24 | 10 | 11 | 13 | 11 | 16 | 10 | 11 | 23 | 23 | 10 | 11 | 19,21 | 9 | 14 | 16 | 12,14,16 |
| ZT80870 | 15 | 13,16 | 15 | 12 | 29 | 24 | 10 | 11 | 12 | 11 | 16 | 9 | 12 | 28 | 23 | 11 | 11 | 20,20 | 9 | 13 | 16 | 13,15,17 |
| ZT80925 | 13 | 15,18 | 12 | 13 | 30 | 24 | 10 | 11 | 13 | 11 | 14 | 10 | 12 | 26 | 23 | 9 | 11 | 19,21 | 8 | 17 | 15 | 15,15.3,17,18 |
| ZT80932 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 12 | 12 | 15 | 12 | 12 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 16 | 14,15,17,18 |
| ZT80979 | 13 | 13,14 | 12 | 14 | 30 | 24 | 9 | 11 | 13 | 11 | 14 | 10 | 11 | 23 | 23 | 11 | 12 | 19,22 | 8 | 15 | 18 | 14,16,17 |
| ZT80984 | 14 | 11,11 | 12 | 14 | 30 | 23 | 10 | 13 | 13 | 12 | 15 | 12 | 13 | 25 | 23 | 11 | 13 | 19,23 | 9 | 17 | 19 | 15,17 |

A-3 (Continued)

| Sample | 19 | 385 a/b | 388 | 389I | 389II | 390 | 391 | 392 | 393 | 426 | 437 | 438 | 439 | 447 | 448 | 460 | H4 | YCAII a/b | 450 | 456 | 458 | 464 a/b/c/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZT81014 | 15 | 14,16 | 13 | 14 | 30 | 23 | 10 | 12 | 15 | 11 | 14 | 10 | 11 | 23 | 23 | 11 | 11 | 19,21 | 10 | 14 | 17 | 14,15,16 |
| ZT81068 | 14 | 12,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 12 | 12 | 24 | 22 | 10 | 11 | 19,23 | 9 | 15 | 17 | 15,16,17 |
| ZT81076 | 15 | 14,16 | 13 | 14 | 30 | 23 | 10 | 12 | 15 | 11 | 14 | 10 | 11 | 23 | 23 | 11 | 11 | 19,21 | 9 | 15 | 17 | 15,17 |
| H1 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 12 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 16 | 16 | 15,17 |
| H2 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 11 | 25 | 23 | 10 | 12 | 19,23 | 9 | 15 | 17 | 15,17 |
| H3 | 15 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 15,16 |
| H4 | 14 | 11,14 | 12 | 14 | 30 | 24 | 11 | 13 | 13 | 12 | 14 | 13 | 13 | 25 | 22 | 10 | 11 | 19,23 | 9 | 16 | 17 | 15,16,17, |
| H5 | 14 | 11,14 | 12 | 14 | 30 | 24 | 10 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 22 | 10 | 11 | 19,23 | 9 | 15 | 19 | 15,17 |
| H6 | 14 | 13,15 | 12 | 13 | 29 | 24 | 11 | 13 | 12 | 11 | 15 | 12 | 11 | 25 | 24 | 11 | 12 | 19,23 | 9 | 15 | 17 | 14,15,16 |
| H7 | 15 | 13,17 | 13 | 13 | 28 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 12 | 24 | 24 | 11 | 11 | 19,21 | 10 | 14 | 15 | 14,16 |
| H8 | 15 | 15,16 | 12 | 13 | 28 | 23 | 10 | 12 | 13 | 11 | 14 | 10 | 11 | 26 | 23 | 10 | 13 | 18,20 | 10 | 15 | 20 | 14,15,16 |
| H9 | 14 | 11,14 | 12 | 13 | 29 | 24 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 24 | 23 | 11 | 12 | 19,23 | 9 | 16 | 19 | 15,16,17 |
| H10 | 13 | 14,16 | 12 | 13 | 30 | 22 | 10 | 16 | 13 | 12 | 14 | 11 | 10 | 24 | 24 | 10 | 11 | 21,23 | 9 | 15 | 16 | 14,15 |
| H11 | 14 | 11,15 | 12 | 13 | 29 | 24 | 11 | 13 | 12 | 12 | 15 | 12 | 12 | 26 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 14,15,16,18 |
| H12 | 14 | 11,11 | 12 | 13 | 30 | 25 | 11 | 13 | 13 | 12 | 15 | 12 | 12 | 25 | 23 | 11 | 12 | 19,23 | 9 | 15 | 16 | 14,17 |
| H13 | 13 | 15,18 | 12 | 12 | 28 | 24 | 9 | 13 | 14 | 12 | 14 | 11 | 12 | 26 | 24 | 10 | 12 | 19,23 | 9 | 15 | 19 | 14,15,18 |
| H14 | 15 | 11,11 | 12 | 13 | 29 | 24 | 10 | 13 | 13 | 12 | 14 | 12 | 11 | 25 | 23 | 10 | 12 | 19,23 | 9 | 16 | 20 | 15,16 |
| H15 | 15 | 16,17 | 12 | 13 | 31 | 21 | 10 | 11 | 13 | 11 | 14 | 12 | 12 | 25 | 25 | 10 | 12 | 19,19 | 8 | 15 | 17 | 13,16 |
| H16 | 17 | 11,13 | 13 | 13 | 28 | 24 | 10 | 11 | 13 | 11 | 15 | 10 | 10 | 25 | 25 | 10 | 11 | 11,21 | 10 | 14 | 17 | 14,15 |
| H17 | 15 | 15,15 | 13 | 14 | 32 | 23 | 10 | 12 | 14 | 11 | 14 | 10 | 11 | 25 | 24 | 11 | 11 | 19,21 | 10 | 15 | 16 | 11,13,14,15 |
| H18 | 13 | 16,18 | 12 | 13 | 31 | 25 | 11 | 10 | 13 | 11 | 14 | 10 | 12 | 26 | 24 | 10 | 10 | 19,22 | 8 | 15 | 16 | 13, 14.3,15,17 |
| H19 | 16 | 14,16 | 13 | 12 | 26 | 24 | 10 | 14 | 13 | 12 | 14 | 11 | 11 | 26 | 23 | 11 | 12 | 19,23 | 9 | 16 | 15 | 14,16,17 |
| H20 | 14 | 11,14 | 12 | 15 | 31 | 23 | 11 | 14 | 14 | 11 | 14 | 10 | 10 | 24 | 23 | 11 | 12 | 18,20 | 8 | 14 | 16 | 12,14.3 |

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