THE DETERMINATION OF THE PHYSICAL CHARACTERISTICS OF AN INDIVIDUAL FROM BIOLOGICAL STAINS

FINAL REPORT

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EXECUTIVE SUMMARY

1. It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. Potential contributors of the stain must either be known to investigators (i.e. a developed suspect) or the questioned profile must be searched against a database of DNA profiles such as those maintained in the CODIS National DNA database. However, in those instances where there is no developed suspect and no match is obtained after interrogation of appropriate DNA databases, the DNA profile *per se* presently provides no meaningful information to investigators, with the notable exception of gender determination. In these situations it would be advantageous to the investigation, if additional probative information could be obtained from the biological stain. A useful biometric that could provide important probative information, and one that may be amenable to molecular genetic analysis, is the biological age of an individual. The ability to provide investigators with information as to whether a DNA donor is a newborn, infant, toddler, child, adolescent, adult, middle-aged or elderly individual could be useful in certain cases, particularly those involving young children such as kidnappings or in providing additional intelligence during terrorist investigations. Currently no validated molecular assays exist for age determination.

2. In the the work described herein we investigated whether determination of an individual's age is feasible in dried physiological stains. We sought to identify a number of potential RNA 'molecular clocks' that could provide investigators with information as to whether a DNA donor is a newborn, infant, toddler, child, adolescent, adult, middle-aged individual or old-aged

individual. The strategy was to identify genes that are differentially expressed (at the RNA level) during the various phases of human development. Also, since progressive reduction in telomere length in somatic tissues appears to be correlated with the 'biological age' of the cell we examined whether such telomere length changes were detectable in dried bloodstains.

3. The specific aims of the project were as follows:

Aim 1. To identify and develop sensitive assays for genes which are expressed in an age-specific manner in dried physiological stains.

Aim 1A Identify human developmentally-regulated genes that are expressed at the RNA level in blood and/or saliva in an age specific manner

Aim 1B Determine the abundance, stability and persistence of candidate genes identified in 1A in dried physiological stains

Aim 1C Develop rapid, quantitative assays for the genes identified in 1A and 1B

Aim 2. To develop sensitive assays for the determination of telomere length and to correlate the length with age using material extracted from dried physiological stains.

Aim 2A Apply TRF-Southern blot technique and STELA to forensic type samples for comparison with qPCR methods

Aim 2B Develop rapid and sensitive real time PCR assays for the determination of telomere length

Aim 2C Using the assays developed, correlate telomere length with age in samples taken from dried biological stains

4. We screened 319 potential age specific mRNA biomarkers (messenger RNA transcripts) and identified 7 that appeared to be expressed in an age-dependent in dried bloodstains for human biological age determination. These include:

a. HBG1n1 (expressed at elevated levels in newborns).

b. HBG1n2 (expressed at elevated levels in newborns).

c. HBG2n2 (expressed at elevated levels in newborns).

d. HBG2n3 (expressed at elevated levels in newborns).

e. HBE1 (expressed at elevated levels in newborns).

f. COL1A2 (expressed at elevated levels in younger individuals ($\leq$12 years))

g. IGFBP3 (expressed at elevated levels in post-pubertal individuals ($\geq$12 years)).

5. Duplex real-time PCR assays were designed and developed for two of the newborn-specific biomarkers (HBG1n1 and HBG2n3) that incorporate a housekeeping gene (the ribosomal protein S15) as an internal positive control (IPC). Individual qRT-PCR assays were developed to measure both of these transcripts in forensic specimens. Adjustment of the primer concentrations in the qRT-PCR reaction permitted the establishment of two temporally delimited assays, one of which was specific to blood from newborns 4-months or under ($\leq$4 months) and the other to newborns who were hours old (<24 hours). Both assays may be useful in a variety of child kidnapping, assault and criminal abortion investigations with the latter (<24 hours) being of particular use for those cases involving hospital abductions. A series of specificity performance checks carried out on the qRT-PCR assays revealed that the HBG(1/2)n transcripts appear to be restricted to blood from newborns in the human (or at least, primate) lineage. The assays appear to be sensitive and robust enough for forensic use in that only a few cell equivalents of total

RNA are required (i.e. 50 pg) and >100ng of total RNA is recoverable from typical sized (50-μl) bloodstains. The sensitivity of the assay is thus 50-500 cells assuming 0.1-1.0 pg total RNA per cell. The newborn blood-specific transcripts were detectable at least up to 15 months in the dried state.

6. A triplex real-time PCR assay has been designed and developed for two other age specific biomarkers (COL1A2 and IGFBP3) that also includes the housekeeping gene S15. The conditions of the assay are such that the relative expression of these three transcripts differs in an age dependent manner. Consequently the triplex qRT-PCR assay can be used to categorize bloodstain donors as likely originating from an individual belonging to one of four different age classes, namely 1 hour-3 months, 4 months-4 years, 5 -18 years and > 18 years. The triplex appears to have a high level of species specificity being confined to primates. The assay appears to be sensitive and robust enough for forensic use in that as little as 3 ng of input DNA can be used. The assay can be used to predict the bloodstain donor's age in stains left at room temperature for up to 18 months.

7. We were unable to demonstrate a correlation between telomere length and age in dried bloodstains using a variety of different analytical approaches.

ABSTRACT

The ability to determine the physical characteristics of an individual depositing a bloodstain at a crime scene would be an invaluable tool to investigators, akin to eyewitness information. One useful biometric that may be amenable to molecular genetic analysis is the biological age of an individual. In theory it may be possible to determine patterns of gene expression that are age-specific thus permitting the distinction between tissue sample originating from a individuals of different ages (e.g. newborn, adolescent, middle-age or elderly). We have discovered two novel isoforms of gamma hemoglobin messenger RNA, designated HBG1n and HBG2n, which exhibit an extremely restricted pattern of gene expression, being confined to newborn individuals. Multiplex qRT-PCR assays incorporating these novel mRNAs have been designed, tested and evaluated for their potential forensic use. The results indicate that the assays provide the ability to determine whether a bloodstain originated from a newborn baby.

A triplex real-time PCR assay has been designed and developed for two other age specific biomarkers (COL1A2 and IGFBP3) that also includes the housekeeping gene S15. The conditions of the assay are such that the relative expression of these three transcripts differs in an age dependent manner. Consequently the triplex qRT-PCR assay can be used to categorize bloodstain donors as likely originating from newborns (1-hour to 3-months), infants and toddlers (4-months to 4-years), children, juveniles, adults, middle-aged, and elderly individuals (>5-years). The latter age category (>5-years) may be further differentiated into '5-18 years' and '>18 years' categories.

We were unable to demonstrate a correlation between telomere length and age in dried bloodstains using a variety of different analytical approaches.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS:

The Identification of Newborns Using Messenger RNA Profiling Analysis. Alvarez, M. and Ballantyne, J. *Anal Biochem* 357 21-34 (2006)

The Identification of Biological Age by qRT-PCR Analysis of the COL1A2 and IGFBP3 Gene Transcripts in Bloodstains. Alvarez, M. and Ballantyne, J. *In preparation* (2008)

The Identification of Four Novel Developmentally Regulated Gamma Hemoglobin mRNA Transcripts. Alvarez, M. and Ballantyne, J. *In preparation* (2008)

Long Term Ambient Temperature Storage, Stability, and Recovery Efficiency of RNA from a Reversible Porous Nanoparticle Matrix. Alvarez, M., Almazan, M., Hogan, M., Utermohlen, J. and Ballantyne, J. *In preparation* (2008)

PRESENTATIONS:

2005 Age Determination: The Identification of Newborns Using Messenger RNA Profiling Analysis. AAFS Annual Meeting, New Orleans

2005 mRNA Applications in Forensic Genetics. Applied Biosystems Seminars, Foster City, CA

2006 Age Identification by RNA Profiling: Validation of a Newborn Child- Specific Real-Time PCR Assay. AAFS Annual Meeting, Seattle, WA

2006 The Determination of the Physical Characteristics of an Individual from Biological Stains: Age Determination.. Annual NIJ DNA Grantees Meeting, Washington DC

2006 The Determination of Physical Features of the Donor of a Crime Scene Sample. National Conference on Science and the Law. St Petersburg, FL.

2007 The Forensic Identification of Newborns using Messenger RNA Profiling Analysis. Cambridge Healthtech International Meeting on Quantitative PCR, San Diego, CA

2007 The Determination of the Physical Features of the Donor of a Crime Scene Sample. NIJ Applied Technology Conference, Orange County, CA.

2007 Getting Blood form a Rock: Getting More and More from Less and Less. International Society for Optical Engineering (SPIE) Defense and Security Symposium, Orlando, FL

2007 A Genetic Eyewitness: The Determination of Physical Characteristics of the Donor of a Body Fluid Stain. The NIJ Conference. Arlington, VA

2007 The Determination of the Physical Characteristics of an Individual from Bloodstains: Biological Age Determination. The NIJ Conference. Arlington, VA

2007 Long Term Ambient Temperature Storage, Stability, and Recovery Efficiency of RNA from a Reversible Porous Nanoparticle Matrix. Alvarez, M., Almazan, M., Hogan, M., Utermohlen, J. and Ballantyne, J. 18th International Symposium on Human Identification, Hollywood, CA

2007 Determining the Physical Characteristics of an Individual from Bloodstains: Biological Age Determination. 18th International Symposium on Human Identification, Hollywood, CA

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CHAPTER ONE: INTRODUCTION

It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. Potential contributors of the stain must either be known to investigators (i.e. a developed suspect) or the questioned profile must be searched against a database of DNA profiles such as those maintained in the CODIS National DNA database [1]. However, in those instances where there is no developed suspect and no match is obtained after interrogation of appropriate DNA databases, the DNA profile *per se* presently provides no meaningful information to investigators, with the notable exception of gender determination [2]. In these situations it would be advantageous to the investigation, if additional probative information could be obtained from the biological stain. Additional investigative parameters could include determining the physical characteristics of the individual depositing the biological stain. A number of physically recognizable characteristics of an individual are at least partly inherited and these include skin-, hair- and eye- color, stature (height and weight) and facial morphology [3-7]. Theoretically, and given sufficient knowledge of the genetics of complex polygenic traits, DNA analysis on a crime scene sample could provide investigators with information akin to eyewitness identification. Since, with few exceptions, our understanding of the genetics of these complex traits is somewhat rudimentary, development of significant forensic applications awaits further advances in our knowledge in this area. One exception may be skin and hair pigmentation since to a large degree the genetics of pigmentation has proved to be amenable to molecular genetic analysis [3, 4, 8]. An additional useful biometric that could provide important probative information, and one that may be amenable to molecular genetic analysis, is the biological age of an individual. The ability to provide investigators with

information as to whether a DNA donor is a newborn, infant, toddler, child, adolescent, adult, middle-aged or elderly individual [9] could be useful in certain cases, particularly those involving young children such as kidnappings or in providing additional intelligence during terrorist investigations. Currently no validated molecular assays exist for age determination. Two approaches have been evaluated for their ability to identify biomarkers associated with biological age; messenger RNA profiling and telomere length analysis.

The biological process of human ageing can looked at from two different perspectives. The first regards ageing as the inevitable degenerative processes that take place in individuals of post-reproductive age. The second, broader approach, regards ageing as part of the human developmental process that takes place from birth through old age. Thus postulated molecular symptoms of the degenerative ageing process include, inter alia, progressive damage to DNA, including mitochondrial DNA mutations, deletions, and insertions [10-13], the shortening of telomeric regions on the ends of chromosomes [14, 15], long-lived protein glycation [16], and reactive oxygen species (ROS)-mediated oxidative damage to macromolecules [17-19]. Studies of these processes often attempt to correlate specific molecular damage with increaseing age, particularly in post-reproductive individuals [20]. From a forensic standpoint however, it would be useful to be able to distinguish between individuals of all age groups, inclusing prepubertal children, teenagers and mature adults. Thus we have considered an alternative approach to age determination that is based upon the epigenetic and developmental control of gene expression that occurs during all stages of human development [9].

The developmental process of ageing is based on the theory that as individuals increase in chronological age, there will be subtle corresponding molecular based biological changes, each requiring genes to be expressed or silenced indicative of that particular stage of life. Using this

approach to biological age determination, every stage of the human lifecycle (birth through death) [9] can be defined by identifying sub-sets of the 20-25 thousand human genes [21] that will be differentially expressed [22]. Theoretically, a comparison of the gene expression profile from individuals of different ages could reveal constellations of candidate genes whose expression is correlated with a specific age. A number of recent reports have described age-associated differential gene expression profiles in skeletal muscle [23, 24], liver [25], brain [26], teeth [27, 28] and skin [29, 30].

Candidate genes for differential gene expression during human development were idenetified using PubMed literature searches. A clear example of developmental age related differential gene expression is that of hemoglobin gene switching [31, 32]. The human β-hemoglobin locus is located on the short arm of chromosome 11 (11p15.5), and encodes five functional β-like globin genes, ε, $^{G}\gamma$, $^{A}\gamma$, δ, and β, and a non-functional β-pseudogene ($^{\beta}\psi$) [33, 34]. The expression of embryonic hemoglobin (ε-globin) commences in the yolk sac in the early stages of gestational development, approximately during week two and continues until six weeks (37 days) postconception [35]. During the next six weeks of gestation (days 37-79), the newly developed fetal liver and fetal spleen begin to produce the fetal specific gamma globin chains ($^{A}\gamma$ and $^{G}\gamma$) of fetal hemoglobin [35]. This increased production of γ-globin is accompanied by a shutdown of ε-globin synthesis. Beginning at approximately 20 weeks gestation and continuing throughout life, adult β-globin gene expression commences in the bone marrow and γ-globin expression is down regulated [35]. This biological process of hemoglobin switching was investigated by us for the possibility that the detection of gamma (γ)-globin messenger RNA (mRNA) in a bloodstain would be indicative of a newborn baby. During these studies, we serendipitously discovered four truncated mRNA transcripts, which we have termed HBG1n1,

HBG1n2, HBG2n2 and HBG2n3, whose expression was restricted to newborn blood and fetal tissues involved in hematopoiesis. To aid in the forensic identification of newborn blood, duplex real-time PCR assays were developed for two of these isoforms [22].

Other genes expressed in an age-specific manner in blood were identified by screening >300 candidate mRNA transcripts. We have identified two additional genes namely- COL1A2 and IGFBP3, which exhibited elevated levels of expression in younger- and older-aged individuals, respectively. A triplex quantitative real-time PCR assay which can separate humans into three biological ages: newborns (1-hour to 3-months), infants and toddlers (4-months to 4-years), and children, juvenile, adults, middle-aged, and elderly individuals (>5-years) was designed and optimized. This assay contains the potential for subcategorizing the latter age group into pre- (5-years to 18-years) and post-pubertal (>18-years) age groups.

CHAPTER TWO: PROTOCOLS

Sample Preparation

Human blood samples were obtained from donors from Florida Hospital (Orlando, FL) after receiving exemption from the Hospital's Institutional Review Board and in accordance with procedures approved by the University of Central Florida's Institutional Review Board. Bloodstains were made by dispensing 50-µL aliquots onto sterile cotton gauze, allowed to air-dry overnight at room temperature and stored at -45ºC until needed.

Other body fluid samples were collected from volunteers in accordance with guidelines approved by the University of Central Florida's Institutional Review Board. Saliva and semen samples were obtained from healthy individuals and 50-µL stains prepared. Buccal swabs, vaginal secretion swabs and menstrual blood swabs were obtained from healthy individuals and allowed to air-dry overnight at room temperature. Venous blood, saliva and vaginal secretion swabs obtained from an expectant mother at various time points throughout the pregnancy and breast milk swabs (1-month post delivery) were air dried overnight. All stains were stored at -45ºC until needed.

For stability studies, venous blood (50-µL) was prepared on sterile cotton gauze and allowed to sit at room temperature (~25ºC) for one, three, six, nine, twelve and fifteen months. Animal blood (with biological age, if known) for species specificity testing was collected from two Pigtailed Macaques (22-days and 5-years), two Rhesus Macaques (24-days and 12-years) (Yerkes National Primate Research Center, Atlanta, GA); calf (10-months), sheep (3-years), lamb (4-months) (Innovative Research, Southfield, MI); cat, dog (Tuscawilla Oaks Animal

Hospital, Oviedo, FL); cow, horse (HemoStat Laboratories, Dixon, CA); deer (Charles R. Daniels, DeLand, FL); spider monkey (Coriell Cell Repository, Camden, NJ); African crown cranes (2- and 3-years), gopher tortoise (20-years), and patagonian cavy (1-year) (Wuesthoff Reference Laboratory, Melbourne, FL). One buccal swab from a Chinese Muntjac (12-years) (Wuesthoff Reference Laboratory, Melbourne, FL) was also tested for specificity. All stains were stored at -45ºC until needed.

RNA Isolation

A guanidine isothiocyanate-phenol:chloroform extraction method was used [22, 36, 37]. Briefly, 500-µL denaturing solution (4M guanidine isothiocyanate, 0.02M sodium citrate, 0.5% sarkosyl, 0.1M β-mercaptoethanol) was preheated in a Spin-Ease™ extraction tube (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD) at 56ºC for 10 minutes. Prepared stains were then added and incubated at 56ºC for 30 minutes. The stain was removed into a Spin-Ease™ extraction tube filter insert, placed back inside the extraction tube and centrifuged for 5 min at 16,000g, after which the filter and the fabric remnants were discarded. Fifty microliters of 2 M sodium acetate and 600-µL of acid phenol:chloroform 5:1, pH 4.5 (Ambion Inc., Austin, TX) were added to the extract, and incubated at 4ºC until two phases were resolved (~20 minutes), then centrifuged at 16,000g for 20 minutes. The RNA-containing aqueous phase was transferred to a sterile microcentrifuge tube, along with 30-µg GlycoBlue™ glycogen carrier (Ambion Inc., Austin, TX) and precipitated with 500-µL isopropanol overnight, at -20ºC. Samples were then centrifuged at 16,000g for 20 minutes to pellet the RNA. The supernatant was carefully removed and the pellet washed once with 1-mL 75% ethanol/25% DEPC-treated water and re-centrifuged at 16,000g for 10 minutes. The supernatant was discarded, the pellet dried in a vacuum

centrifuge for 3-5 minutes and re-solubilized in 12-17-µL of RNAsecure Resuspension Solution (Ambion Inc., Austin, TX) at 60ºC for 10 minutes. RNA samples were treated with DNase I immediately or subsequent to storage at -20ºC.

DNase I Digestion

Total RNA was treated with six units of TURBO^{TM} DNase (RNase-Free) (2 U/µL) (Ambion Inc., Austin, TX) at 37ºC for 1-2 hours. The TURBO^{TM} DNase was inactivated at 75ºC for 10 minutes, the samples chilled on ice and then stored at -20ºC until needed [38, 39].

DNA Extraction

Genomic DNA was extracted from 50-µL bloodstains by an organic solvent extraction method [40] followed by Centricon Filter Purification (Millipore Corp., Bedford, MA). Briefly, samples were incubated overnight at 56ºC in stain extraction buffer (0.1M NaCl, 10mM Tris–HCl pH 8.0, 25mM EDTA pH 8.0, 20mM SDS) supplemented with 0.5 mg/ml proteinase K. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, pH 6.6) was added to the extract, mixed gently by inversion and centrifuged for 5 min at 16,000g to separate the phases. The DNA containing aqueous layer was transferred to a prewet Centricon filter and purified by washing with 2-mL TE^{-4} (10 mM Tris, 0.1 mM EDTA) and centrifugation at 2000g. Finally, DNA was removed by inverted centrifugation at 1000g with 100-µL TE^{-4}.

Quantification of Nucleic Acids

RNA was quantified using a sensitive fluorescence assay based upon the binding of the unsymmetrical cyanine dye RiboGreen® (Molecular Probes, Eugene, OR) [41]. The manufacturer's instructions were followed for the high-range assay, which detects from 20-ng/mL to 1-µg/mL. Briefly, 200-µL assays comprised of 2-µL TURBO™ DNase treated RNA extract, 98-µL TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5, in nuclease-free water), and 100-µL 750 nM RiboGreen® reagent in a 96-well plate format. After RiboGreen® addition and a three minute incubation at room temperature protected from light, fluorescence emission at 535 nm (excited at 485 nm) was determined using a Wallac Victor2 microplate reader (Perkin Elmer Life Sciences, Boston, MA). RNA concentration was calculated using an appropriate standard curve as described by the manufacturer [41]. All RNA samples were diluted to 5ng/uL (saliva and buccal swabs were diluted to 10ng/uL) with nuclease-free water (Ambion Inc., Austin, TX).

DNA was quantified by the real-time PCR Human Quantifiler™ Kit (Applied Biosystems, Foster City, CA) was used for quantification. Extracted DNA was diluted to a final working concentration of 10ng/uL with TE^{-4}, after comparison to a standard curve which was generated by running DNA samples of known concentrations from 25 to 0.63 ng/uL [42].

Reverse Transcription (cDNA Synthesis)

For all blood and tissue RNA samples 6-µL of RNA (30-ng), and for saliva/buccal 6-µL of RNA (60-ng), was heated at 75ºC for 3 minutes, snap cooled. For the newborn duplex qPCR assays a mixture study of total RNA from three newborns (<24-hours) and three juvenile/adult females (16-, 22-, and 31-years) were combined in different ratio combinations (1:1, 1:5, 5:1, 1:10 and 10:1) to yield the 6-uL (30-ng) necessary for the amplification. To the RNA, 4-µL of a

10 mM dNTP mix (Applied Biosystems, Foster City, CA), 2-µL of 10X first-strand buffer (500 mM Tris–HCl pH 8.3, 750 mM KCl, 30 mM $MgCl_2$, 50 mM DTT), 2-µL Random Decamer primers (50-µM), 20-units SUPERase-In™ RNase Inhibitor (20 U/µL) (Ambion Inc., Austin, TX), 100-units Moloney Murine Leukemia Virus-Reverse Transcriptase (100 U/µL) (Ambion Inc., Austin, TX) and nuclease-free water (Ambion Inc., Austin, TX) were added to yield a final reaction volume of 20-µL. For the (–RT) reaction tubes the Moloney Murine Leukemia Virus-Reverse Transcriptase was replaced with Nuclease-free water. Reaction mixtures were incubated at 42ºC for 1 hour and 95ºC for 10 minutes to inactivate the reverse transcriptase [43, 44].

Candidate Gene Screening: Polymerase Chain Reaction

All single gene amplification reactions were conducted in a total volume of 25-µL. Three nanograms of cDNA was amplified with a standard reaction mix containing 1x PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.125 mM each dNTP, 0.4 µM primers (**APPENDIX D: CANDIDATE GENE PRIMER SEQUENCES FOR RT-PCR**) and 1.25-units AmpliTaq Gold™ DNA polymerase (5 U/µL) (Applied Biosystems, Foster City, CA). Nuclease-free water (Ambion Inc., Austin, TX) was added to yield the final reaction volume.

Standard PCR conditions consisted of an 11 minute denaturing step at 95ºC followed by 35 cycles at (1) 94ºC; 0:20 (2) 55ºC or 60ºC; 0:30 (3) 72ºC; 0:40 and a final extension step (72ºC; 10:00) [45, 46].

Gamma Hemoglobin Isoforms: Polymerase Chain Reaction

Amplimer sizes for all genes tested are included in

Table 14. All amplification reactions were conducted in a total volume of 25-µL containing genomic DNA (2-ng) or mRNA/cDNA (3-ng) (except for HBG1 and HBG2 singleplex reactions which contained 5-ng mRNA/cDNA). A standard reaction mix containing 1x PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.125 mM each dNTP, 0.4 µM primers and 1.25-units AmpliTaq Gold™ DNA polymerase (5 U/µL) (Applied Biosystems, Foster City, CA). Nuclease-free water (Ambion Inc., Austin, TX) was added to yield the final reaction volume.

For the newborn mRNA duplex HBG1n1-S15 and HBG2n3-S15 RT-PCR reactions, 3ng of cDNA was amplified with the following changes to the standard reaction mix: 0.6 µM S15 primers and 0.05 µM HBG1n1 or 0.05 µM HBG2n3 primers. The ribosomal protein gene transcript, S15, was included as an internal positive control for the reverse-transcription and amplification reactions.

Standard PCR conditions were used for all amplifications and consisted of an initial incubation step (95ºC; 11:00) followed by repeating cycles of [denaturation (94ºC; 0:20), annealing (60ºC; 0:30), and extention (72ºC; 0:40)] with a final incubation of (72ºC; 10:00) [45, 46]. Amplification cycle numbers are as follows: singleplex HBG, HBG1 and HBG2 (32 cycles; 55ºC annealing); HBG1n1, HBG1n2, HBG2n2 and HBG2n3 (28 cycles).

Post Amplification Electrophoresis

PCR and RT-PCR amplified products were visualized on 4% NuSieve® GTG® Agarose gels (Cambrex Bio Science Rockland, Inc., Rockland, ME). Electrophoresis was carried out at 100V for 1.25 hours in TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer. Gels were stained with SYBR® Gold nucleic acid stain (Molecular Probes, Eugene, OR), visualized on the

Omega10 Chemiluminescence Imaging System (MLTRA-LUM, Inc., Claremont, CA) and analyzed with ONE-Dscan 2.05, 1-D Gel Analysis Software for Windows (Scanalytics, Inc., Fairfax, VA).

Gamma Hemoglobin Isoforms: Cloning and Sequencing of the Identified Amplimers

Newly identified hemoglobin products were excised from agarose gels and purified using MERmaid® SPIN columns, which specifically isolate low molecular weight DNA products (10-200 bp) (Q-BIOgene, Carlsbad, CA). Purified products were cloned into TOP10F' One Shot® chemically competent cells using the TOPO TA Cloning® Kit (pCR®2.1-TOPO®) (Invitrogen, Carlsbad, CA). Positive colonies were isolated and plasmids purified using the RapidPURE™ Plasmid Mini Kit (Q-BIOgene, Carlsbad, CA). Plasmids which contained the inserted product were sent to Lark Technologies for sequencing analysis (Lark Technologies, Inc., Houston, TX).

Candidate Gene Screening: Real-Time PCR

All singleplex qRT-PCR assays were performed in a 25-µL total reaction volume consisting of a standard reaction mix containing: three nanograms of cDNA, 12.5-µL 2x Taqman® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.40-µM each forward and reverse primer, 0.25-µM of each probe and nuclease-free water (Ambion Inc., Austin, TX). Primer and probe sequences are listed in **APPENDIX F: CANDIDATE GENE PRIMER SEQUENCES FOR qRT-PCR**.

Three optimized duplex real-time PCR reactions consisted of the following changes to the standard reaction mix: COL1A2 0.9-µM primers to S15 0.025-µM primers; HBE1 0.2-µM primers to S15 0.4-µM primers, and IGFBP3 1.2-µM primers to S15 0.05-µM primers.

Real-Time PCR reactions were carried out on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA). Amplification conditions consisted of: (1) 1 cycle of 50˚C; 2:00 (2) 1 cycle of 95˚C; 10:00 (3) 50 cycles of 95˚C; 0:15 and 60˚C; 1:00. Data was collected at stage 3, step 2 (60˚C; 1:00). Delta cycle threshold (dCt) values were calculated by subtracting the Ct value generated from the age specific gene of interest (GOI) from the Ct value of the housekeeping gene (i.e. dCt = Ct (S15) – Ct (GOI)) [47]. Samples which fail to amplify the GOI are given a default Ct value of 40.0 or 50.0 (the amount of qPCR cycles used).

Triplex Real-Time PCR (qPCR) Amplification

All primer and probe sequences are listed in **Table 12**. Quantitative PCR assays were performed in a 25-µL total reaction volume consisting of a standard reaction mix containing: three nanograms of cDNA (blood, semen, vaginal secretions, menstrual blood) or six nanograms of cDNA (saliva/buccal), 12.5-µL Taqman® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 1.8-µM each COL1A2 primer, 1.5-µM each IGFBP3 primer, 0.1-µM each S15 primer, 0.25-µM of each probe and nuclease-free water (Ambion Inc., Austin, TX).

Real-Time PCR reactions were carried out on a 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). Amplification conditions consisted of: (1) 1 cycle of 50˚C; 2:00 (2) 1 cycle of 95˚C; 10:00 (3) 50 cycles of 95˚C; 0:15 and 60˚C; 1:00. Data was collected at stage 3, step 2 (60˚C; 1:00). Delta cycle threshold (dCt) values were calculated by subtracting the Ct value generated from the COL1A2 or IGFBP3 genes from the Ct value of the housekeeping gene (i.e. dCt = Ct (S15) – Ct (COL1A2 or IGFBP3)) and ddCt values calculated and plotted by (ddCt = dCt (S15-COL1A2) – dCt (S15-IGFBP3)) [47]. Samples which fail to

amplify any of the genes are given a default Ct value of 50.00 (the amount of qPCR cycles used) for that specific gene.

Gamma Hemoglobin Isoforms: Duplex Real-Time PCR (qPCR)

All primer and probe sequences are listed in **Table 15**. All qPCR assays were performed in a 25-µL total reaction volume consisting of a standard reaction mix containing: three nanograms of cDNA (blood, semen, vaginal secretions, menstrual blood and breast milk) or six nanograms of cDNA (saliva/buccal), 12.5-µL Taqman® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.25-µM of each probe and nuclease-free water (Ambion Inc., Austin, TX).

For the newborn assays (≤4 months old), 0.6-µM (S15), and 0.1-µM (HBG1n1) or 0.05-µM (HBG2n3) primers were added to the standard reaction mix. For the newborn assays (<24 hours old), 0.9-µM S15 primer and 0.05-µM HBG1n1 primer or 0.05-µM HBG2n3 primer were added to the standard reaction mix.

Real-Time PCR reactions were carried out on a 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Amplification conditions consisted of: (1) 1 cycle of 50˚C; 2:00 (2) 1 cycle of 95˚C; 10:00 (3) 40 cycles of 95˚C; 0:15 and 60˚C; 1:00. Data was collected at stage 3, step 2 (60˚C; 1:00). Delta cycle threshold (dCt) values were calculated by subtracting the Ct value generated from the newborn specific gene from the Ct value of the housekeeping gene (i.e. dCt = Ct (S15) – Ct (HBG1n1 or HBG2n3) [47]. Samples which fail to amplify the newborn genes are given a default HBG1n1 or HBG2n3 Ct value of 40.00 (the amount of qPCR cycles used).

Telomere Length Analysis: Delta Cycle Threshold Determination by Real-Time PCR – SYBR Green I Assay

Primer sequences are listed in **Table 19**. All qPCR assays were performed with a standard reaction mix containing: 17.5 nanograms of DNA, 1x SYBR® Green I PCR Buffer containing Passive Reference 1, 25-mM $MgCl_2$, 12.5-mM dNTPs, and 1.25-U AmpliTaq Gold DNA Polymerase (5U/µL) (Applied Biosystems, Foster City, CA). $T_{10}E_{0.1}$ (1M Tris-HCl pH 8.0, 0.5M Na_2EDTA) was used to bring the final volume to 25-µL. Primer concentrations for the telomere amplification were 270-nM tel 1 and 900-nM tel 2, while the single copy gene (36B4) amplification required 300-nM 36B4u and 500-nM 36B4d [48].

Real-Time PCR reactions were carried out on a 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Real-time PCR amplification conditions consisted of: (1) 1 cycle of 95˚C; 10:00 and either (2T) 22 cycles of 95˚C; 0:15 and 54˚C; 2:00 or (2S) 30 cycles of 95˚C; 0:15 and 58˚C; 1:00, for the telomere (2T) and single-gene (2S) amplifications, respectively. Data was collected at stage 2, step 2 (54˚C; 2:00 or 58˚C; 1:00). Each standard or DNA extract was performed in duplicate and average cycle threshold values were determined. The delta Ct calculation was determined by the difference in amplification rates of the single-gene, 36B4, to the telomere repeats, $dCt = S_{Ct} - T_{Ct}$.

Telomere Length Analysis: Real-Time PCR Amplification of Telomeres – TaqMan Assay

Primer and probe sequences are listed in **Table 19**. Genomic DNA (10.0 nanograms) was amplified in a standard reaction containing: 1x Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 250-nM probe (tel 3 or tel 6), 500-nM primers (tel 1 and tel 2 or tel 4 and tel 5), and an additional 5.0-U AmpliTaq Gold DNA Polymerase (5U/µL) (Applied

Biosystems, Foster City, CA). Nuclease-free water was used to yield the final reaction volume of 25-µL.

Real-Time PCR reactions were carried out on a 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Real-time PCR amplification conditions consisted of: (1) 1 cycle of 95˚C; 10:00 and (2) 40 cycles of 95˚C; 0:15 and 50˚C; 2:00. Data was collected at stage 2, step 2 (50˚C; 2:00). The cycle number at which the amplification curve reaches a pre-set threshold, the cycle threshold (Ct) value, was plotted against the biological age of each individual tested.

Telomere Length Analysis: STELA Telorette Ligation Reaction

Genomic DNA (200ng) was ligated with 0.9uM each telorette linker (**Table 19**) in six separate reactions containing 1x manufacturers ligation buffer and 10-units T4 DNA Ligase (USB Corp., Cleveland, OH) for 12-hours at 35°C [49]. The T4 ligase was inactivated by heating at 65°C for 15 minutes. Ligated DNA was re-purified using Centricon Filters and re-quantified using the Human Quantifiler Kit as described in the DNA Extraction and Quantification sections, respectively, and diluted to a final concentration of 1 ng/uL with TE^{-4}.

Telomere Length Analysis: STELA PCR Amplification

Forward (XpYpE2) and reverse (teltail) primer sequences are listed in **Table 19**. Telorette ligated genomic DNA (3-ng) was amplified in a 25-µL reaction volume containing 1x PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.3 mM each dNTP, 0.5uM telomere primer XpYpE2, 0.5uM teltail primer, 2.5-units AmpliTaq Gold™ DNA polymerase (5 U/µL) (Applied Biosystems, Foster City, CA) and nuclease-free water (Ambion

Inc., Austin, TX) [49]. PCR conditions for all six telorette amplifications consisted of an initial incubation step (95ºC; 11:00) followed by 35 cycles of [denaturation (94ºC; 0:15), annealing (65ºC; 0:30), and extention (68ºC; 10:00)] and a final incubation of (68ºC; 10:00) [45, 46, 49].

Telomere Length Analysis: STELA Post-Amplification Electrophoresis

PCR amplified products were visualized on 1% Agarose gels. Electrophoresis was carried out at 170V for 1.5 hours in TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer. Gels were stained with SYBR® Gold nucleic acid stain (Molecular Probes, Eugene, OR), visualized on the Omega10 Chemiluminescence Imaging System (MLTRA-LUM, Inc., Claremont, CA) and analyzed with ONE-Dscan 2.05, 1-D Gel Analysis Software for Windows (Scanalytics, Inc., Fairfax, VA).

CHAPTER THREE: RESULTS AND DISCUSSION

Messenger RNA Profiling Analysis for Biological Age Determination

Generating Candidate Genes from *a priori* Knowledge of Biochemistry and Physiology

Potential age dependent genes were identified by searching the NCBI PubMed literature database for (i) genes that would be expected to be expressed at different times during human development based upon their supposed biochemical/physiological function and (ii) genes that have empirically been shown to be differentially expressed at different times during development.

Physiological candidate genes for newborns (birth – 3-months) and infants (4-months – 9-months) included those proteins or transcripts which were specific to fetuses or fetal tissues [50-53], including fetal specific protein isoforms [50, 51] and cellular immune responses [54]. Pre-pubertal developmental changes were examined to develop candidate genes for toddlers (10-months – 3-years) and children (4-years – 12-years) [53]. For example, the *N*-methyl-D-aspartate receptor gene (GRIN1, with transcripts NR1-1, NR1-2 and NR1-3, GRIN2A and GRIN2B) exhibits increased expression in pre-pubertal mammals [55]. All juvenile or adolescent (13-years – 18-years) candidates involved pubertal developmental changes, mainly hormones which regulate sexual maturation. Female and male hormones, receptors, and activators are known to be upregulated in this age group and include estrogen [56], testosterone [57], and various sex steroids or endorphins [56-69]. Potential adult specific candidates included those from known protein isoforms such as the p45 adult specific form of the AUF1/hnRNP D (AU-rich element

RNA binding protein-1/heterogeneous nuclear ribonucleoprotein D) gene [51]. The majority of our candidate genes were targets for the middle-aged (46-years – 64-years) and elderly (>65-years) age groups. Such candidates include those involved in an increase in the production of DNA damage machinery [70, 71] and other factors which are induced upon increased oxidative stress [72] and generalized DNA damage [23, 73]. At the present time it is unclear how apoptosis affects ageing, although apoptosis has been implicated in numerous diseases, which have been shown to correlate with increased biological age [74, 75]. Additionally, it has been determined that increased bone loss is evident in older ages [76].

Finally, a significant number of age related gene candidates were obtained by published literature which illustrated an alteration in gene expression patterns during different developmental phases. Examples include increases in cyclins D1 and E [50], the insulin growth factor binding proteins [77-79], various pro-inflammatory mediators [80], the tumor suppressor genes p53 and p21 [50, 81, 82], regulators of telomere length [83], and other various factors regulating transcription and gene expression [50, 81, 84]. A list of all candidate genes tested, along with the NCBI gene description, Nucleotide Accession number, and target age group is included in **APPENDIX C: CANDIDATE GENE DATABASE**.

Initial Screening of 319 Potential Candidate Genes by RT-PCR Gel Based Gene Expression Profiling Analysis

To determine the expression profiles of the potential candidate age related genes, PCR primers were designed using Primer3 design software (http://frodo.wi.mit.edu/). The input DNA sequence was obtained from the NCBI Nucleotide database and all mRNA sequences were BLASTed against the human genome to identify precise exon/intron boundaries. Primers were then designed to land in separate exons for facile separation of DNA and mRNA species (**Figure**

1). **APPENDIX D: CANDIDATE GENE PRIMER SEQUENCES FOR RT-PCR** lists all individual candidate primer sequences.

Potential age correlated candidate genes are tested by a series of RT-PCR amplifications with a sample set of bloodstains comprising different biological ages (n = 4-10), ranging from 1-hour old newborns to elderly individuals. Genomic DNA was amplified with every candidate gene as a control to verify that any signal detected by RT-PCR was due to RNA and not contaminating genomic DNA. (**Figure 1**). **Figure 2** illustrates the basic protocol of candidate gene testing by RT-PCR analysis. Initially, a first-round 35-cycle PCR amplification reaction was performed and based on the obtained results; a candidate gene was either rejected outright or passed into a second round of PCR amplification. After this first-round amplification reaction candidates can still be rejected for two reasons. First, candidates that have no amplified mRNA/cDNA product, yet show amplification of the genomic DNA control were rejected, mainly because if more than 35-cycles are required for visual product amplification, it is inferred that the transcript was present at extremely low levels. Second, candidates that amplified an mRNA/cDNA and genomic DNA control product of the same size were rejected, due to the fact that mRNA specific detection could not be easily verified. Candidates could successfully pass this initial round of testing by generating one of two possible expression profiles. First, a candidate could exhibit amplification which showed a pattern of differential expression, termed sporadic expression or secondly, a candidate gene could amplify product in all ages tested. Candidates which were amplified in all ages tested were passed onto the next round but the amount of PCR cycles was decreased. PCR is an end-point analysis method and at 35-cycles, some high abundance transcripts appear saturated in all samples, but may actually be present in different copy numbers in varying ages. Therefore, a decrease in the amount of PCR cycles may

illustrate a subtle sporadic expression pattern between different ages. Based on the results obtained in the first-round of testing the second-round of screening consisted of either amplification at 35-cycles or at a decreased cycle number, usually 30, and regardless of cycle number, all candidates were assayed with a new sample representing different biological ages (n=8). Combined analysis of rounds-one and -two determined if a candidate had passed onto the third and final round of screening. To pass into the third-round of amplification a candidate must have exhibited a pattern of expression which was differentially expressed between ages and consistently expressed within a particular age group. The final amplification reaction was performed with a larger sample set representing all age groups (n>30), with the purpose of verifying that amplification of the target age groups was specific to that group. At this juncture candidates were either accepted and transferred to the quantitative RT-PCR analysis platform or rejected due to sporadic amplification within the target age group (in the sense that an amplicon was present in only a subset of the target age samples), or if expression was observed in samples fraom all age groups.

Using PubMed literature searches, 319 potential candidate genes were tested using the protocol described above and illustrated in **Figure 2**. A summary of the RT-PCR expression results is provided in **Table 1**, where the amount and percentage of accepted and rejected candidate genes is arranged by target age group, either newborns, juveniles, adults, or elderly. Of the 319 initial candidates, a total of 26 (8.15%) were accepted as potential biomarkers of biological age determination. Of these candidates; nine were from newborns, seven from juveniles and ten were from older age groups. These genes and their expression profiles are: AFP (fetal liver), COL1A2 (5-year), FLJ20344a (1-hour and 86-years), HBE1 (<3-months), and LOC151194 (1-hour and >68-years) for newborns (**Figure 3**). Additionally, four hemoglobin

transcripts, HBG1n1, HBG1n2, HBG2n2 and HBG2n3 were determined to be specific to newborn blood and are shown in **Figure 26**. Juvenile candidates: ASL (>84-years), PPOX (sporadic), PRL (7-months), SPTRX-1 (3-years), SPTRX-2 (7-months – 3-years), TBC1 (14-years – 15-years), and TEKT2 (7-months – 3-years) are shown in **Figure 4**; while the 'older' candidates: AGGF1 (<15-years), CDC2 (5-year), IGFBP3 (>29-years), LOH11CR2A (>79-years), MAD1L1 (<5-years), PDCD6 (5-years – 41-years), POLM (<13-years), POLQ (<5-years and 91-years), PPARD (<5-years), and SRC (<45-years) can be seen in **Figure 5** (see **APPENDIX C: CANDIDATE GENE DATABASE** for individual gene descriptions). These candidate genes were then transferred to the real-time PCR platform, which is described in the next section. Alternatively, of the original 319 candidates, 105 (32.92%) which originated from the Affymetrix GeneChip® and 188 (58.93%) of the literature candidates, were rejected for a total of 293 (91.85%) rejected candidates. Rejected literature candidates are categorized as such in **Table 2**. After the first-round of RT-PCR analysis 15.4% (49/319) of the literature candidates were rejected because no amplified mRNA/cDNA product was detected (data not shown), whilst 0.9% (3/319) were rejected due to the mRNA/cDNA and genomic DNA product amplifying at the same molecular size (data not shown). The majority of rejected candidates from amplification rounds-two and -three consisted of those, which, even after decreased cycle number, amplified an mRNA/cDNA product in all biological ages tested, with no apparent difference in expression levels, specifically, 28.5% (91/319) of candidates (data not shown). The final group of rejected candidates, 14.1% (45/319), were those which exhibited sporadic expression when multiple samples of the target age range were amplified (data not shown). **APPENDIX E: CANDIDATE GENE RT-PCR RESULTS** lists all candidates with their corresponding accepted age groups or rejection categories.

Quantitative Real-Time RT-PCR Gene Expression Profiling Analysis for 23 Potential Age Specific Biomarkers

Candidate genes that had passed the gel based screens described above were then transferred to a qPCR format. Quantitative RT-PCR assay precision can be improved by the inclusion of a co-amplified internal positive control (IPC). Real-time PCR primers were designed, along with a sequence specific minor groove binding (MGB) probe, using ABI Primer Express software (version 2.0.0). To inhibit signal fluorescence from genomic DNA, primers were designed to land in separate exons, while the sequence specific probe was targeted to bind directly across the exon/exon boundary (**Figure 6**). All candidate gene probes were 5′ 6-FAM labeled, while the housekeeping gene, S15, was 5′ VIC labeled, and all probes were 3′ labeled with a non-fluorescent quencher (NFQ). The qPCR primers and probes are listed in **APPENDIX F: CANDIDATE GENE PRIMER SEQUENCES FOR qRT-PCR**.

Only 23 of the 26 potential candidates were taken to real-time PCR. The newborn biomarkers, HBG1n2 and HBG2n2 were omitted from real-time assay development because two hemoglobin derived newborn candidates (HBG1n1 and HBG2n3) had previously been described and assays had already been developed (see the **Fetal Specific Isoforms** section). The alpha-fetoprotein (AFP) gene was not pursued at the qPCR level, because, although it was specific for the fetal liver, it was not detected in newborn blood (**Figure 3**).

Initially, all genes were amplified with a range of biological ages (n>10), from 1-hour old newborns to elderly individuals, along with a genomic DNA control and a non-template control (NTC), the latter being one that has nuclease-free water substituted for the nucleic acid. This first reaction verified if the primer and probe set were mRNA/cDNA specific, and if there was any primer/probe interaction, based on amplification results of the genomic DNA and NTC, respectively. Amplification of DNA from a variety of biological ages was important for two

reasons; first, differential amplification of the target age group must be verified by examining the cycle threshold (Ct) values generated against the non-target ages and second, the cycle threshold baseline of the target age group must be determined.

Results from the initial round of qPCR rejected 14 of the 23 candidates. Firstly, the polymerase mu subunit gene, POLM, was the only candidate rejected due to the Ct threshold never being crossed in any biological sample. The other 13 candidates were rejected because of non-differential expression across the age groups. These rejected genes included CDC2, POLQ, SRC, LOH11CR2A, ASL, FLJ20344a, LOC151194, SPTRX-1, SPTRX-2, PPOX, TBC1, TEKT2, and PRL.

Four of the original 23 candidates that were transferred to real-time PCR produced differential Ct values in the first-round of screening which allowed them to be pursued further. These genes AGGF1, MAD1L1, PDCD6, and PPARD, along with their first-round amplification results and Ct values are shown in **Figure 7.** As illustrated in these figures and tables, the younger aged individuals generated lower Ct values in the AGGF1 and PPARD amplifications, and increasing biological age yielded increased Ct values. With the MAD1L1 and PDCD6 candidates, lower Ct values were also generated with younger individuals, however the results were non-uniform, whereby some younger ages exhibited Ct values that were consistent with older aged individuals, >50-years. Second-round qPCR screening consisted of designing and developing duplex reactions, incorporating the IPC and subsequent testing of a larger number of samples of different biological ages (n=96). Analysis of duplex reactions was conducted by calculating Δ cycle threshold (dCt) values for each biological age, where the difference in amplification efficiency is determined by subtracting the Ct value of the gene of interest (GOI) from the Ct value of S15, ($dCt = Ct_{S15} - Ct_{GOI}$). This dCt metric is a measure of the relative

amount of GOI transcript. All four candidate genes, although producing differential amplification results in singleplex reactions, yielded similar dCt values in duplex reactions. As illustrated in **Figure 8** the results for AGGF1, MAD1L1, PDCD6, and PPARD, showed positive dCt values in all biological ages tested, from 1-hour to 102-years. These candidates were then rejected because of non-differential amplification of the GOI in the qPCR duplex reactions.

Discussed in the following sections are three candidate genes namely- COL1A2, HBE1 and IGFBP3, which showed target age specificity and were optimized into duplex reactions with the IPC housekeeping gene, S15. Additionally, two newborn candidates, HBG1n1 and HBG2n3, were optimized into duplex assayss with the S15 housekeeping gene and validation studies have been completed (see the **Fetal Specific Isoforms** section).

COL1A2, A Biomarker for Age Determination of Younger Aged Individuals

When searching the literature for candidate genes, an article by K. Kerschan-Schindl *et al*., revealed that the c-terminal telopeptide of type I collagen was increased in elderly subjects [76]. This led us to investigate genes known to be associated with bone development. Subsequently the collagen, type I, alpha 2 (COL1A2) gene was identified as a potential biomarker of ageing. First-round singleplex amplification results showed that COL1A2 expression was increased in younger individuals, 1-hour to 12-years old, and that all other age groups >12-years generated undetermined Ct values (default value of 50.0, the number of qPCR cycles used) (**Figure 9** and **Table 3**). A duplex reaction was then optimized and amplification results with samples from all age groups (n=96) are shown in **Figure and Table 4**. The dCt values demonstrated that the expression of COL1A2 was higher in younger individuals (1-hour – 5-months).

Initial validation specificity results, where 109 different blood samples were amplified in triplicate, are shown in **Table 5**, where each sample is listed with its corresponding average COL1A2 and S15 Ct value and the dCt values (±SD). **Figure** provides a summary of results comparing the target age group (newborns and infants) with the non-target ages: toddlers, children, juveniles, adults, middle-age, and elderly individuals. Additionally, the overall average COL1A2 and S15 Ct values ±SD, and average dCt values are illustrated; specifically, the newborn and infant age group dCt value was +6.748 (±4.185 SD), compared to –2.980 (±5.020) in toddlers and children, and –4.830 (±3.681) in juveniles, adults, mid-age and elderly individuals.

HBE1, A Biomarker for Age Determination of Newborns

The Homo sapiens hemoglobin, epsilon 1 gene (HBE1), was selected as a potential newborn specific gene, due to its restricted protein expression in embryonic blood and certain embryonic tissues. First-round singleplex amplification results showed that HBE1 expression was increased in younger individuals, specifically 1-hour old newborns, and all other ages >17-days, generated at a minimum a 4 cycle higher Ct value (**Figure 12** and **Table 6**). A duplex reaction was then optimized and the amplification results with all biological ages (n=96) is illustrated in **Figure 13** and **Table 7**. The dCt values demonstrate that the expression of HBE1 is higher in younger biological ages (1-hour – 3-months) compared to older individuals (>3-months in biological age).

Initial validation specificity results, where 139 different blood samples were amplified in triplicate, are shown in **Table 8** and **Figure 14**. Each sample is listed with its corresponding average HBE1 (GOI) and S15 Ct value and their standard deviations, as well as the calculated

dCt values (±SD). **Figure 14** gives a summary of the target age group (newborns), in comparison to the non-target ages: infants, toddlers, children, juveniles, adults, middle-age, and elderly individuals. Additionally, the overall average HBE1 and S15 Ct values ±SD, and average dCt values are illustrated; specifically, the newborn age group dCt value was +3.088 (±2.523 SD), compared to –2.978 (±0.302) in infants, toddlers, children, juveniles, and adults; and –1.348 (±0.330) in mid-age and elderly individuals.

IGFBP3, A Biomarker for Age Determination of Post-Pubertal Individuals

The Homo sapiens insulin-like growth factor binding protein 3 gene was identified as a age candidate when literature searches of human ageing revealed that mutations in Lamin A were responsible for premature ageing and that the levels of IGFBP3 decreased with lamin A splicing inhibition [73]. Additionally, a separate publication by Wang *et al.*, listed IGFBP3, in addition to numerous others, as a gene with increased expression in senescent cells [50].

First-round singleplex amplification results of IGFBP3 showed that Ct values were obtained with all samples in the post-pubertal (>15-years old) age range (19/19), while IGFBP3 was unamplifiable in 71% (5/7) of samples aged 1-hour to 12-years old **Table 9**. **Figure 15** shows this initial round of amplification results, where undetermined +RT Ct values are given a default value of 40.000, the amount of qPCR cycles used.

After the favorable singleplex amplification results, a duplex reaction was optimized and amplification results with all biological ages (n=96) is illustrated in **Figure 16** and **Table 10**. The dCt values demonstrate that amplification of IGFBP3 is at a higher level in older ages groups, as seen by positive dCt values in individuals > 35-years old. Significantly, 96% (44/46) of the younger biological ages, those from 1-hour to 34-years old, produced negative dCt values. Only

two samples a 14- and a 24-year old, produced positive dCt results of +2.126 and +2.054, respectively. Initial validation specificity results, where 123 different blood samples were amplified in triplicate, are shown in **Table 11**. Each sample is listed with its corresponding average IGFBP3 (GOI) and S15 Ct values and their standard deviations, as well as the calculated dCt values (±SD). **Figure 17** gives a summary of the target age group (adults, middle-aged and elderly), in comparison to the non-target ages: newborns, infants, toddlers, children and to a lesser extent juveniles. The overall average IGFBP3 and S15 individual Ct values ±SD, are listed for each age group and the calculated average dCt values are illustrated. Individual results from the IGFBP3-S15 duplex amplification yielded an average dCt value of –0.725 (±2.637), in the target age groups, compared to –9.914 (±5.402) in newborns, infants, and toddlers, and –4.208 (±4.260) in the children and juvenile age range.

The Development of a Triplex Quantitative Real-Time PCR Assay for Biological Age Determination

Quantitaive RT-PCR (qRT-PCR) Assay for Biological Age Determination

The two candidate genes, namely- COL1A2 and IGFBP3, which exhibited elevated levels of expression in younger- and older-aged individuals, respectively, were combined with an internal positive control (IPC) housekeeping gene, the ribosomal protein S15, to develop a triplex assay to determine biological age from human blood. The resulting prototype triplex qRT-PCR assay, was expected to be able to distinguish between blood samples originating from younger-aged and older-aged individuals. In order to accomplish this, the amount of COL1A2 and IGFBP3 expression in different age groups was characterized by a ddCt metric ([Ct S15 – Ct

COL1A2] vs. [Ct S15 – Ct IGFBP3]) which measures the relative quantity of each transcript in relation to the S15 internal positive control.

During the design and development of the qPCR triplex we found that younger-aged (i.e. ≤12-years) and older-aged (i.e. ≥12-years) individuals could actually be separated into four distinct age groups: newborns (≤3-months), infants and toddlers (4-months to 4-years), pre-adolescent/juvenile (5-years to 18-years) and post-adolescence (≥19-years). This categorization is possible by analysis of the relative amplification of all three transcripts and graphing the corresponding ddCt results. Samples from newborn individuals can generate two possible response curves and hence two types of ddCt results. The first occurs when a Ct value is obtained for the COL1A2 gene, and both S15 and IGFBP3 are undetermined (default Ct value 50.00); the ddCt metric for these samples would be +/0 (**Figure 18A**). The second outcome is when a Ct value is generated for the COL1A2 and S15 genes, whereby the S15 Ct value is always greater than that of COL1A2 (indicating the relatively low level of amplification of S15); and the ddCt for these newborns is +/– (**Figure 18B**). In contrast to the newborn ddCt results, when mRNA/cDNA from infants and toddlers is amplified, a Ct value is produced for S15, however the other two genes, COL1A2 and IGFBP3 fail to reach the threshold and therefore are provided with default Ct values of 50.00, leading to –/– ddCt metric values (**Figure 18C**). After developing and optimizing the triplex reaction, we found that the ability of children, juveniles, adults, middle-age, and elderly individuals to amplify the COL1A2 candidate gene was diminished, due to competing effects by the S15 and IGFBP3 genes. Samples belonging to these age groups can therefore generate two possible response curves; one in which both IGFBP3 and S15 amplify at levels sufficient to reach the threshold, yielding ddCt values of –/+ (**Figure 18D**), and one where only IGFBP3 yields a Ct value (S15 and COL1A2 are undetermined, Ct = 50.00)

producing a ddCt result of 0/+ (**Figure 18E**). It should be noted that the latter result was only obtained with biological ages ≥19-years, allowing us to sub-categorize this age group into pre- (5 to 18-years) and post- (≥19-years) adolescence.

Age Specificity of the Triplex qRT-PCR Assay

The ability of the qRT-PCR triplex assay to successfully sub-categorize individuals as belonging to certain age groups was tested by analyzing 140 blood samples from multiple donors varying in age from 1-hour to 102-years (1-hour to 3-months (n=17); 4- to 9-months (n=12); 10-months to 4-years (n=15); 5- to 12-years (n=9); 13- to 18-years (n=15) ; 19- to 45-years (n=28) ; 46- to 65-years (n=20) ; 66- to 102-years (n=24)). All samples were amplified in duplicate and the results are summarized in the form of a two-dimensional scatter plot in which each sample's ddCt value (dCt (S15-COL1A2), dCt (S15-IGFBP3)) is displayed (**Figure 19A**). Positive results from newborns are expected to be confined to the positive x-axis (ddCt = +/0) and lower right quadrant (ddCt = +/–), whereas positive results for infants and toddlers would be found in the lower left quadrant (ddCt –/–). Results for children, juveniles, adults, middle-aged, and elderly individuals are expected to be plotted in the upper left quadrant (ddCt = –/+) or specifically post-adolescent individuals (≥19-years) can be found on the positive y-axis (ddCt = 0/+).

As illustrated in **Figure 19A** and **Figure 19B** the ability to reliably separate a blood sample into one of four biological age groups was evaluated and the results listed in **Table 13**. For the newborn age group (1-hour to 3-months), 77% (13/17) of samples yielded ddCt metrics of +/0 or +/–. Infants and toddlers (4-months to 4-years) yielded –/– ddCt metric values in 82% (22/27) of samples. For the children, juvenile, adult, middle-age, and elderly age group (5-years to 102-years), 93% (89/96) of samples yielded ddCt metrics of –/+ or 0/+. In addition to

evaluating the specific location (quadrant, axis) of an individual "known" sample by its generated ddCt value, data can be extrapolated based on where an "unknown" samples ddCt metric lands. Eighty-one percent (13/16) of samples located on the positive x-axis or in the lower right quadrant were newborn in age, while 96% (44/46) of children, juveniles, adults, middle-age, and elderly individuals were plotted in the upper left quadrant. Additionally, 93% (42/45) of the samples which generated a 0/+ ddCt value were derived from the post-adolescent age group (≥19-years).

Body Fluid Specificity of the Triplex qRT-PCR Assay

Saliva (n=35), semen (n=2), vaginal secretions (n=2) and menstrual blood (n=7) from healthy donors; as well as saliva (n=8) and vaginal secretions (n=8) from a pregnant female were assayed with the qPCR triplex (**Figure 20**). All menstrual blood samples generated Ct values for the COL1A2 and IGFBP3 genes (S15 undetermined); yielding +/+ ddCt values. All semen samples gave Ct values for all three genes, with S15 being amplified the least efficiently and also generating +/+ ddCt values. All vaginal secretion samples generated Ct values for IGFBP3 and were undetermined for COL1A2 and S15; yielding 0/+ ddCt values (except for one sample that generated a COL1A2 Ct value and is plotted +/+). The majority of saliva samples (i.e. 26) failed to amplify any of the triplex genes (ddCt 0/0), however we did find one sample which amplified both COL1A2 and IGFBP3 (ddCt +/+), and one sample that amplified S15 only (ddCt –/–). Surprisingly, we also found that 15 saliva samples were able to amplify only the IGFBP3 gene (undetermined COL1A2 and S15); yielding 0/+ ddCt values, and all of these samples were ≥14-years in biological age.

Human Specificity of the Triplex qRT-PCR Assay

RNA was extracted from bloodstains from a variety of animal species including two Pigtailed Macaques (22-days and 5-years), two Rhesus Macaques (24-days and 12-years), a calf (10-months), cow, lamb (4-months), sheep (3-years), cat, dog, horse, deer, spider monkey, three African crown cranes (2-, 2- and 3-years), gopher tortoise (20-years), and a patagonian cavy (1-year), and tested with the qPCR triplex assay. One buccal swab from a Chinese Muntjac (12-years) was also tested. S15 Ct values were undetermined for all animal samples tested, while the deer and pigtailed macaque (5-years) amplified the COL1A2 and IGFBP3 genes, respectively (data not shown).

Mixture Study

Total RNA from the blood of a newborn (<24-hours old), a toddler (4-years) a juvenile (16-years) and an elderly (66-years) individual were combined to simulate blood mixtures that may be obtained in certain situations. Each separate admixed pair (i.e. newborn-toddler, newborn-juvenile, newborn-elderly, toddler-juvenile, toddler-elderly and juvenile-elderly) was analyzed (in duplicate) with each pair comprising a sample set of the same admixture ratios (10:1, 5:1, 1:1, 1:5, and 1:10). The mixed RT (reverse transcribed) reactions were amplified with the triplex assay and the results shown in **Figure** .

For all mixtures with an uneven contribution from both donors (ie. 5:1 and 10:1), the triplex qPCR assay gave results indicative of the age of the major contributor. For mixtures in which both donors contributed equally (i.e. 1:1) the ddCt value was located directly between the other mixture ddCt points (major and minor donor ratios). For the newborn-toddler mixtures, all ddCt values were located in the lower right and left quadrants, as predicted (**Figure 21A**). For

the newborn-juvenile mixtures, ddCt metrics were plotted in the upper left (juvenile major donor) or lower/upper right (newborn major donor) and the equal mixture (1:1) was located in the juvenile quadrant, however shifted more towards the origin (**Figure 21B**). For the newborn-elderly admixes, all ddCt points were found in the upper right quadrant, with the major elderly donor samples in the upper region and the newborn major donor in the lower region (**Figure 21C**). For the toddler-juvenile admixtures, the toddler major donor samples were located in the expected quandrant (left lower) and the juvenile major donor samples located in the upper left quadrant (**Figure 21D**). For the toddler-elderly samples the elderly ddCt were found on the positive y-axis, and due to the presence of an increase in IGFBP3 mRNA species, even if the major donor was younger in age the ddCt metric for these samples was pulled into the upper left quadrant, where we would expect to find ddCt vaules originating from children and juveniles (**Figure 21E**). Finally, the juvenile-elderly mixtures were found to be located in the upper left (juvenile major donor) and on the y-axis (elderly major donor) (**Figure 21F**).

Sensitivity

The sensitivity of the qRT-PCR triplex assay was determined by varying the amount of total RT input, into the real-time amplification, from reverse transcribed RNA isolated from bloodstains from a newborn (<24-hours), a toddler (4-years), a child (9-years), a juvenile (16-years), an adult (35-years) and an elderly (66-years) individual. Three nanograms to 25 femtograms of total RT input was amplified from each individual and the ddCt results analyzed. We found that reliable ddCt metric results were only obtained with the 3 ng input concentration. At lower concentrations (≤1.5-ng) the ability of younger-aged samples to amplify the COL1A2

transcript was diminished and most ages generated ddCt values of 0/+ and hence, found on the y-axis (data not shown).

Based upon this sensitivity study, an input of 3 ng of RT reaction into the qRT-PCR triplex assay is recommended.

Stability of COL1A2, IGFBP3, and S15 Transcripts in Aged Bloodstains

In order to be useful in forensic casework, the biological age specific transcripts should be stable over time in dried stains. In order to perform a preliminary assessment of these mRNA species in the dried state, blood from a newborn (1-hour old), two juveniles (14- and 15-years old) and two elderly individuals (84- and 86-years old) was deposited on cloth, allowed to air dry and stored at room temperature (~25˚C) for various time points (1, 3, 6, 9, 12, 15 and 18 months). Total RNA was then isolated from the bloodstains and assayed by the triplex qRT-PCR reaction to detect the temporal stability of the desired transcripts. The results are displayed in a two dimensional scatter plot as shown before (**Figure 22**). In all time points tested, the one-hour old newborn individual generated a ddCt metric of either +/0 or +/–, as expected. The ddCt values generated for both juveniles and both elderly individuals were found in the upper left (–/+) or on the y-axis (0/+) at all time points tested. The ability of the qRT-PCR triplex to detect mRNA transcripts associated with specific developmental stages was successful for up to 18-months of ambient temperature storage.

Fetal Specific Isoforms of Gamma Hemoglobin as Biomarkers for Biological Age Determination

Expression Analysis of the Standard Hemoglobin Gamma Transcripts, HBG1 and HBG2

The tetrameric fetal and adult hemoglobin protein complexes are composed of two alpha and either two gamma ($\alpha_2\gamma_2$) or two beta ($\alpha_2\beta_2$) hemoglobin chains, respectively. This well characterized variation in fetal versus adult hemoglobin was the basis for the initial design of a newborn specific assay. The gamma hemoglobin locus was analyzed by a reverse transcription-polymerase chain reaction (RT-PCR) using three different sets of primers. The universal set amplified both gamma hemoglobin genes simultaneously (HBG), while two sets of gene specific primers amplified either the HBG1 (A-gamma) or HBG2 (G-gamma) genes individually (**Figure 23**). All forward and reverse primers were designed to land in exons two and three (flanking intron two), respectively, for separation of cDNA and genomic DNA amplified products in agarose gels. To test the expression of the gamma hemoglobin transcripts over different biological ages, total RNA was extracted from venous bloodstain samples donated from individuals aged 1-hour to 91-years. Messenger RNA was reverse-transcribed and the corresponding cDNA, along with a genomic DNA control, was amplified using primers designed to specifically recognize total hemoglobin (HBG) or the individual HBG1 or HBG2 gene transcripts (**Figure 24**).

Contrary to the initial hypothesis, a gamma hemoglobin messenger RNA amplified product corresponding to total HBG (154 bp) (**Figure 24A**) or individual HBG1 (277 bp) (**Figure 24B**) or HBG2 (274 bp) (**Figure 24C**) genes was amplified in all ages tested. Detection

of these fetal hemoglobin chains ($^{A}\gamma$ and $^{G}\gamma$) in non-newborn blood samples was unexpected, based on our knowledge that expression of the fetal hemoglobin protein falls to ~3% within five months after birth and is completely replaced by adult hemoglobin after two years of biological age [35, 85]. These results demonstrate that, in contrast to the expression pattern for the fetal hemoglobin protein, HBG mRNA production is not solely restricted to the fetal and newborn stages of development. While evaluating the results from the standard hemoglobin amplification reactions in **Figure 24**, we serendipitously detected additional lower molecular weight bands in only the younger aged individuals aged 1-hour, 13-days and 3-months (+RT) (illustrated with asterisks) at approximately 65bp and 100bp, for the HBG1 (**Figure 24B**) and HBG2 (**Figure 24C**) amplifications, respectively.

Sequence Determination and Alignment of the Newborn Specific Gamma Hemoglobin Isoforms

To determine the molecular sequence of the newly identified low molecular weight amplimers; amplified products were excised from agarose gels, purified, cloned into chemically competent cells and sequenced (see **Gamma Hemoglobin Isoforms:** Cloning and Sequencing). The sequencing results for the HBG1 and HBG2 low molecular weight products revealed that each band was actually composed of two separate amplimers, which seemed to be of the same size or only slightly different. Once all four low molecular weight amplimers had been sequenced, we utilized the (NCBI) human genome BLAST alignment tool to determine the origin of the amplified products. Alignment results illustrated that both low molecular weight sequences obtained from the HBG1 reaction and both low molecular weight sequences from the HBG2 reaction, only aligned with regions of the original HBG1 and HBG2 transcripts, and did not exhibit sequence similarity to any other part of the human genome. The specificity of the

forward and reverse primers for both hemoglobin genes was also tested using the NCBI nucleotide BLAST (search for short, nearly exact matches). The results illustrated that although the primers are not human specific, they are specific for the HBG1 and HBG2 transcripts, within the human transcriptome.

After determining that these amplimers originated from the standard hemoglobin gamma genes, MegAlign software from DNAstar Lasergene was used to align the lower molecular weight sequences to the corresponding standard hemoglobin sequences. This was necessary in order to determine the regions of similarity and dissimilarity within the transcripts. Alignment analysis showed that all four of the low molecular weight amplimers contained identical sequences to the standard hemoglobin sequences, beginning with the forward primer binding site (located in exon two) and extending to the reverse primer binding site (located in exon three). More importantly, alignment analysis illustrated that the middle of the amplified sequence was deleted in all four low molecular weight products, specifically, the 3′ end of exon two and the 5′ end of exon three, was missing from all four of the sequences. Further evaluation of each specific deleted region (all four transcripts had a deleted region, however the size of the deleted region as well as the first and last nucleotides in the deletion was different) exhibited the presence of either a penta- or octanucleotide direct repeat sequence at the beginning and the end of each deletion. These direct repeat sequences were located in both exons two and three of each transcript and seemed to be the breakpoints between the aligned regions within the standard hemoglobin sequences and each of the four low molecular weight sequences. **Figure 25** illustrates the exact locations of these direct repeat breakpoints for the HBG1 and HBG2 genes and their low molecular weight products. Direct repeat sequences and their locations within the gene for HBG1 [Genbank: NM_000559] are ATGAT (292-296, 509-513) and AGATGCCA (272-279, 486-493),

and the corresponding low molecular weight amplimers have been named HBG1n1 and HBG1n2, respectively. The direct repeats for the HBG2 [Genbank: NM_000184] gene are TGCCC (311-315, 473-477) and CACTG (330-334, 492-496) and the corresponding low molecular weight amplimers have been named HBG2n2 and HBG2n3, respectively. After the sequencing and alignment results were interpreted, the deleted regions and amplicon sizes for each of these transcripts was determined. For HBG1n1, HBG1n2, HBG2n2 and HBG2n3 the number of deleted bases was 217, 214, 162, and 162 bp, which produced amplicons of 60, 63, 112 and 112 bp, respectively. It should also be noted that although the sequence of the breakpoints is known, the actual position within the direct repeat where the break occurs is unknown.

RT-PCR Amplification of the Individual Newborn Gamma Hemoglobin Isoforms

Based on the sequencing results for the four newborn transcripts, gel based RT-PCR assays were developed for amplification of the individual transcripts. Forward primers for the HBG1n1, HBG1n2, HBG2n2 and HBG2n3 assays were designed to span the breakpoints in each of the two isoforms, therefore precluding the amplification of the standard HBG genes (**Table 14** underlined sequences). Total RNA from bloodstains from three individuals aged 8-days, 15-years and 84-years were tested, along with a genomic DNA control. As expected an amplified product consistent with the detection of the four transcripts was detected only in the 8-day old newborn (**Figure 26**).

An internal positive control (IPC), the ribosomal protein, S15, was incorporated into two of the newborn assays resulting in two duplex RT-PCR reactions. S15 was chosen as the IPC instead of either of the commonly-used housekeeping genes, GAPDH or Beta-Actin, since S15

exhibited significantly fewer processed pseudogene derived artifacts in RNA isolates containing trace quantities of genomic DNA (data not shown). Each duplex reaction contained primers for the housekeeping gene, S15 [46], and one of the two newborn gamma isoforms, either HBG1n1 and HBG2n3. The S15-HBG1n1 (**Figure 27A**) and S15-HBG2n3 (**Figure 27B**) duplexes demonstrated the presence of S15 mRNA in all ages tested, while the newborn gamma hemoglobin gene transcripts were only found in individuals aged 1 hour to 3- and 4-months, respectively.

Quantitative Real-Time PCR Analysis of the HBG1n1 and HBG2n3 Newborn Specific Gamma Isoforms

The two gel-based duplex RT-PCR assays for the identification of HBG1n1 and HBG2n3 were re-configured for analysis using a real-time, quantitative PCR (qPCR) platform. The resulting prototype qRT-PCR assays as formulated, should detect the HBG1n1 and HBG2n3–derived amplicons at a significantly higher level in newborn individuals (≤4 months) compared to those of older age groups (>4 months). In order to accomplish this, the amount of HBG1n1 and HBG2n3 expression in different age groups was characterized by a dCt metric [Ct (S15) – Ct (HBG1n1 or HBG2n3)] which measures the expression of HBG1n1 and HBG2n3 isoforms in relation to the S15 internal positive control. Samples from newborns (≤4 months) typically generated Ct (HBG1n1 and HBG2n3) values less than that of S15, indicating the relatively high level of expression of HBG1n1 and HBG2n3 in newborns compared to the S15 housekeeping gene (**Figure 28A** and **Figure 28B**, left panels). In contrast, Ct (HBG1n1 and HBG2n3) values from non-newborns (>4 months old) were greater than generated S15 Ct values (**Figure 28A** and **Figure 28B**, right panels). In some non-newborn individuals (>4 months old) the HBG1n1 and HBG2n3 transcripts were present in insufficient quantity to reach the Ct threshold (**Figure**

28B2). In these instances the Ct (HBG1n1 and HBG2n3 was given a default value of 40.00 which represents the total number of PCR cycles used (i.e. 40). Therefore, the qRT-PCR assays, as configured, should produce positive dCt results for newborn blood samples whereas all other age groups should produce negative dCt results. For example, the dCt values of the newborns illustrated in **Figure 28A** and **Figure 28B** were +1.60 (HBG1n1) and +5.60 (HBG2n3) whereas the non-newborns were –2.61 (HBG1n1, 72-years old) and –8.03 (HBG2n3, 15-years old).

In certain circumstances (e.g. newborns whom have been illegally removed from the hospital) it would be useful to determine whether an individual was <24 hours old. It was possible (see below), by altering the primer concentrations, to modify the two duplex qRT-PCR assays described above such that they were predictive (i.e. based upon a positive dCt metric) of blood from a child <24 hours old (**Figure 28C** and **Figure 28D**). Examples of the results from the <24 hours newborn assays are provided in **Figure 28C** (HBG1n1) and **Figure 28D** (HBG2n3). The corresponding dCt values for a 1-hour newborn were +3.75 (HBG1n1) and +5.89 (HBG2n3), whereas an 8-day old newborn produced values of –1.40 and –1.66, respectively.

The precise Ct that an amplified gene product attains is dependent on two factors, the amount of target gene present in the sample and the concentration of primer and probe used in the PCR reaction. The two newborn duplex real-time PCR assays (≤4 months and <24 hours) illustrate the effect these two factors have in real-time PCR amplification (**Figure 29**). Ubiquitously expressed genes (i.e. housekeeping genes) are expressed at relatively the same levels in all cell types. Differentially expressed genes have regulated expression patterns and are either turned on/off (i.e. present/not-present) or are expressed at different levels (i.e. increased/decreased) in a tissue or developmental stage specific manner. In both newborn assays

the concentration of the newborn gamma hemoglobin isoform primers is similar (HBG1n1= 100nM (≤4 month) and 50nM (<24 hour); HBG2n3= 50nM (≤4 month) and 50nM (<24 hour)). Therefore, since the primer/probe concentrations are the same in both assays amplification of these hemoglobin isoforms is dependent on the initial gene copy number. **Figure 29** illustrates that a sample amplified with all four duplex reactions from both assays should produce a relatively constant HBG1n1 and HBG2n3 Ct value. Since the S15 housekeeping gene exhibits a constant level of expression (constant initial copy number), all samples should reach the threshold at relatively the same cycle number. Thus, increasing or decreasing the S15 primer/probe concentration will shift all amplification response plots to the left or right, respectively. The S15 primer concentrations vary significantly between the two newborn assays. In the ≤4 month assay 600nM is used, compared to 900nM in the <24 hour assay. Therefore, by increasing the S15 primer concentration all amplification plots (Ct values) are leftward shifted in the <24 hour assay when compared to the ≤4 month assay, and with the hemoglobin genes remaining constant, this allows older newborns to now produce negative dCt values (more S15 product than HBG1n1 and HBG2n3) when compared to younger newborns (more HBG1n1 and HBG2n3 product than S15) (**Figure 29**).

Biological Age Specificity of the qPCR Newborn Hemoglobin Biomarkers

The ability of the qRT-PCR assays to identify newborn individuals (≤4 months or <24 hours) was tested by analyzing 132 blood samples from multiple donors varying in biological age from 1-hour to 92-years (<24h (n=10); 1 day-1 month (n=19); 2-4 months (n=22); 5 months-3 years (n=37); 4-18 years (n=20); 19-92 years (n=24)). The results are summarized in the form of two-dimensional scatter plots in which each sample's dCt (S15-HBG1n1) and dCt (S15-

HBG2n3) are displayed (**Figure 30**). Positive results from newborns are expected to be confined to the upper right quadrant (positive dCt HBG1n1 and dCt HBG2n3) whereas negative results from non-newborns would be found in the lower left quadrant (negative dCt HBG1n1 and dCt HBG2n3).

In the ≤4 month assay, 98% (i.e. 50) of the 51 newborn (≤4 month old) samples yielded at least one positive dCt value, while 96% (i.e. 78) of the 81 non-newborns yielded two negative dCt values (**Figure 30A** and **Table 16**). Indeed the vast majority of ≤4 month old newborn samples (90% (46/51)), gave two positive dCt values (upper right quadrant). The one non-newborn sample that appears in the upper right quadrant in **Figure 30A** originates from a 7-month old infant. Subsequent repeat analysis (x2) places it in the lower right quadrant (i.e. one positive and one negative dCt). Of the samples that generated one positive and one negative dCt value, four of the six individuals were 4-months old. This is consistent with the occurrence of a transitional developmental state that occurs about 4-months after birth in which transcription of the HBG1n1 and HBG2n3 isoforms is curtailed.

For the <24 hour assay, all newborn samples aged from 1-hour to 24-hours generated positive dCt values (10/10) for each duplex (**Figure 30B** and **Table 16**). Ninety-seven percent (100/103) of individuals biologically aged greater than one-month generated two negative dCt values as expected.

No sex-specific differences were observed with either the <24 hour or ≤4 month assays (data not shown).

Body Fluid Specificity of the qPCR Newborn Hemoglobin Biomarkers

Saliva (n=18), semen (n=2), vaginal secretions (n=2) and menstrual blood (n=7) from healthy donors; as well as venous blood, saliva, vaginal secretions from a pregnant female and breast milk (1-month post delivery) were assayed with the ≤4 month and <24 hour duplexes. HBG1n1 and HBG2n3 Ct values were undetermined for all body fluids tested, illustrating that the two duplexes are specific for venous newborn blood (**Figure 31**).

Human Specificity of the qPCR Newborn Hemoglobin Biomarkers

RNA was extracted from bloodstains from a variety of animal species including two Pigtailed Macaques (one newborn and one adult), two Rhesus Macaques (one newborn and one adult), calf (newborn), cow (adult), lamb (newborn), sheep (adult), cat, dog, horse, deer, spider monkey, two African crown cranes, gopher tortoise, and a patagonian cavy, and tested with the newborn qPCR assays. One buccal swab from a Chinese Muntjac was also tested. HBG1n1 and HBG2n3 cycle threshold (Ct) values were undetermined for all animal samples tested, illustrating that the two duplexes are specific for human newborn blood (**Figure 32**).

Mixture Study of the qPCR Newborn Hemoglobin Biomarkers

The newborn assays are expected to be of use in the investigation of criminal abortion cases. In such instances putative products of conception are sometimes recovered and expected to comprise mixed samples, typically the newborn (or fetus) and that of an adult. Therefore, to ensure the detectability of newborn blood in the presence of adult blood, controlled mixture studies were carried out. Total RNA from the blood of newborns (<24-hours old) and either

juvenile (16-years) or adult (22- or 31-years) individuals was combined to simulate mixtures from criminal abortion cases. Three separate newborn/non-newborn admixed pairs were studied with each pair comprising a sample set of the same admixture ratios (1:1, 1:5, 5:1, 1:10 and 10:1). The 15 mixed RNAs were reverse-transcribed and amplified with both newborn duplexes, using the ≤4 month and <24 hours assay formats.

In the ≤4 month assay all 15 mixtures generated two positive dCt values, except for one of the 1:10 mixtures (24-hour newborn: 31-year adult) (**Figure 33A**). This latter sample generated one positive (S15-HBG1n1) and one negative (S15-HBG2n3) dCt value. In the <24 hour assay all of the 1:1, 5:1 and 10:1 mixtures generated two positive dCt values (**Figure 33B**). The three 1:5 mixtures and two of the three 1:10 mixtures generated one positive and one negative dCt value in the S15-HBG2n3 and S15-HBG1n1 assays, respectively. The other 1:10 mixture (24-hour newborn to 31-year adult) generated two negative dCt values (**Figure 33B**).

The above results indicate that the assays can detect newborn/non-newborn admixed samples and are likely to be of use to demonstrate the presence of newborn blood in putative products of conception.

Real-Time PCR Sensitivity of the Newborn Hemoglobin Biomarkers

The sensitivities of the qRT-PCR newborn assays were determined by varying the amount of total RNA input into the assays using RNA isolated from bloodstains from two newborns (both 1-hour old) and two non newborns (a 13-year old and a 53-year old). The average dCt values from both newborns and both adults are shown for each duplex reaction (**Figure 34**, **Table 17**, and **Table 18**). The ≤4 month newborn assay generated positive dCt values with ≥ 5 pg RNA with the newborn samples while the adult samples generated negative

dCt values with ≥ 50 pg RNA (**Figure 34A** and **Table 17**). Input RNA less than these concentrations did not produce detectable housekeeping gene or newborn gene products that reached the Ct threshold. With the <24 hour newborn assay, the S15-HBG1n1 duplex generated the expected positive and negative dCt values (newborns and adults, respectively) with ≥ 25 pg RNA input. (**Figure 34B1** and **Table 18**). With the S15-HBG2n3 assay, newborns generated positive dCt values with ≥ 5 pg of input RNA, while the adult samples generated negative dCt values with ≥ 50 pg input RNA (**Figure 34B2** and **Table 18**).

Based upon these sensitivity studies, a minimum input of 50 pg RNA is recommended for the qRT-PCR newborn assays.

Stability of HBG1n1 and HBG2n3 Transcripts in Aged Bloodstains

In order to be useful in forensic casework, the HBG1n1 and HBG2n3 transcripts should be stable over time in dried stains. In order to assess the stability of the newborn transcripts in the dried state, blood from two newborns (1-hour and 2-months old), two juveniles (14- and 15-years old) and two elderly individuals (84- and 86-years old) were deposited on cloth, allowed to air dry and stored at room temperature (~25˚C) for various time points (1, 3, 6, 9, 12 and 15 months). Total RNA was isolated from the bloodstains and then assayed for HBG1n and HBG2n transcripts by qRT-PCR. The results are displayed in a two dimensional scatter plot as before (**Figure**). In both newborn assays (i.e. ≤4 months and <24 hours), the one-hour old newborn individual generated two positive dCt values in all aged samples, while the juvenile and elderly individuals generated two negative dCt values at all time points tested.

Despite the excellent specificity exhibited by the assays with 15-month aged bloodstains (i.e. aged newborn bloodstains cluster separately from aged bloodstains from other

developmental age groups), caution must be exercised in aged samples from older newborns. While the two-month old newborn sample produced two positive dCt values when stored at room temperature up to one month with the ≤4 month assay, it produced one positive and one negative dCt value for the S15-HBG2n and S15-HBG1n duplexes respectively with stains aged 3-15 months (**Figure 35A**). In the <24 hour assay, the same two-month old newborn produced one positive and one negative dCt value when aged for 1, 3, 6 and 9 months but two negative dCts after 12 and 15 months of storage (**Figure 35B**).

Telomere Length Analysis for Biological Age Determination

One highly studied molecular process is the shortening of telomeric chromosomal regions with increasing chronological age [14, 86-92]. Telomeres are short tandem repeat sequences located at the ends of chromosomes and range from 100 to 280 nucleotides. They function to maintain chromosomal end integrity and stability by preventing exonucleolytic DNA degradation, inappropriate chromosomal fusions and protecting the ends of linear chromosomes from being mis-recognized by the DNA damage repair machinery. The telomere repeat core sequence in humans is TTAGGG and is present at the tips of chromosomes both as a block of contiguous perfect repeats and, more distally, as a block of imperfect tandem repeats. A subtelomeric region comprising additional sequences separates the telomere from the rest of the chromosome. Telomeres play an essential role in DNA replication in that after each cell division a number of tandem repeats are lost due to the inability of replicative DNA polymerases to synthesize DNA in the 5′ to 3′ direction (**Figure**) [89, 93]. This "end replication problem" results in dividing cells of a loss of ~50-200 bp of DNA and a progressive reduction in telomere length and generation of a G-rich 3′ overhang [89, 93, 94]. The current paradigm is that the structural integrity of the telomere is regularly monitored by the cellular machinery and a number of telomere-specific protein sensors (e.g. TRF1, TRF2, POT1, TIN1, TIN2) have been identified [95]. Although telomerase is an enzyme with reverse transcriptase activity that can reconstitute the lost repeats, its expression is normally restricted to germ and stem cells. Thus somatic cells exhibit a progressive reduced telomere length as cells divide and eventually the protective effect of the telomere structure is overcome and genomic instability or reproductive senescence results [96]. Progressive reduction in telomere length in somatic tissues is thus correlated with the

'biological age' of the cell and its use as a predictor of organismal 'chronological age' has been suggested [88, 95, 97].

Empirical observations in humans support the hypothesis that the average telomere length is inversely correlated with age [95]. Moreover reactive oxygen species (ROS) also cause telomere repeat loss and, since exposure to ROS is accumulative with age, telomere length might even be exacerbated in older individuals [98]. A number of other factors could potentially confound the use of telomere length. Nevertheless preliminary investigations by Ikeda and colleagues using bloodstains and teeth indicated that telomerase length estimation as an age indicator might be possible with forensic specimens [14, 28].

The generally accepted approach for telomere length determination involves terminal restriction fragment (TRF) analysis, in which DNA is enzymatically digested and segments detected using Southern hybridization to a probe containing the telomeric repeat. However, this method offers low resolution and suffers from a lack of sensitivity, requiring approximately 0.5 - 1 µg of human genomic DNA or more than 10^5 cells as well as the reduced ability to detect shorter telomeres [95, 99]. In addition, TRF represents the mean telomere length of all chromosomes and includes the unknown length of the subtelomeric region, where the TRF value is dependent on the restriction site of the subtelomeric region by the restriction enzyme [99]. Thus TRF does not provide information on actual telomere length. To overcome the downfalls of TRF analysis, two novel experimental approaches aimed at telomere length determination were investigated. The first approach was based on real-time PCR amplification, and utilized either the absolute quantification SYBR® Green I [48, 100] or the relative quantification Taqman® platforms, while the second, a single telomere length analysis (STELA), used a novel telomere-telorette ligation reaction [49].

Assessing Total Telomere Length by Delta Cycle Threshold Determination using Real-Time PCR and Telomere Specific Primers – SYBR Green I Assay

In real-time 'absolute' quantitative PCR, detection of product is often monitored by measuring the increase in fluorescence caused by the binding of the SYBR Green dye to double-stranded DNA [101, 102]. Quantitative PCR determines, for each sample well, the Cycle threshold (Ct) value, i.e. the fractional cycle number at which the well's accumulating fluorescence crosses a set threshold that is several standard deviations above baseline fluorescence [48, 103]. A recent paper by R. Cawthon measured the relative telomere length of an individual using a quantitative PCR approach [48]. This method of telomere length determination is based on measuring, for each DNA sample, the factor by which the "unknown" DNA sample differs from a reference DNA sample in its ratio of telomere repeat copy number (T), to a single copy gene number (S), thereby generating a final T/S ratio. The acidic ribosomal phosphoprotein PO gene, 36B4, was chosen as the single copy number gene due to its equal amplification in all DNA samples tested [48, 104]. In theory, all samples should amplify the 36B4 single copy gene, at the same rate and hence, generate similar Ct values. In contrast, unknown DNA telomere lengths should vary between biological ages and therefore generate varying Ct values, corresponding to that specific sample's telomere length (i.e. the younger the individual, longer the telomere, the lower the generated Ct value). The calculated difference in amplification rates between the telomere and single gene assays, the delta Ct ($dCt = S_{Ct} - T_{Ct}$), should reveal the quantity (length) of telomeres in relation to the single copy gene, assuming there is greater then one telomere present in each DNA sample. The calculated dCt values might then correlate with the biological age of the individual, whereby younger individuals generate larger dCt values, due to lower telomere Ct values; conversely, elderly individuals might generate smaller dCt values, because of increased telomere shortening. In our approach to

relative telomere length determination, Ct values were generated according to the published primer and amplification specifications; however the data analysis consisted of calculating delta cycle threshold (dCt) values, instead of T/S ratios, for the standards and unknown DNA samples.

Genomic DNA was extracted from individuals of various ages ranging from hour old neonates to a 91-year old elderly individual. Real-time PCR amplification was performed in duplicate, for both serially diluted DNA standards and the unknown samples of various biological ages. The average telomere length (T) and single copy gene number (S) values were calculated and an average delta Ct value was determined. **Figure** illustrates the dCt values for each diluted standard (50 to 6.3ng) and all biological ages assayed (1-hour to 91-years).

The single copy gene, 36B4, was amplified in all samples as an internal reference for the delta Ct calculations. **Figure 37** illustrates that for the diluted DNA standards the 36B4 and telomere Ct values increase, in correlation with decreased input DNA. Delta Ct analysis of the standards verified the correlation of 36B4 and telomere amplifications, independent of input DNA, the dCt values are relatively similar, ranging from 0.875 to 1.620 (average 1.302 ± 0.2722). Analysis of the biologically aged blood samples revealed that the single gene amplification was consistent throughout, generating a range of Ct values from 26.580 to 32.240 (average 28.006 ± 1.4715), however, we did not detect any additional variation in the telomere amplification with Ct values ranging from 23.650 to 29.395 (average 25.224 ± 1.4629). **Figure** illustrates the calculated dCt values from the various biologically aged individuals and in contrast to our expectations of decreasing delta Ct values with increasing biological age, we actually find that the highest dCt value was generated by a 91-year old individual (dCt = 3.360), while a 45-year old generated the lowest dCt value of 2.050, while all other biological ages had dCt values in between the 45- and 91-year olds.

Telomere Length Determination by Real-Time PCR Amplification using a Telomere Specific Probe – TaqMan Assay

We developed a novel quantitative real-time PCR (qPCR) based assay for telomere length analysis utilizing a TaqMan qPCR approach. This method utilizes a set of gene specific primers and a gene specific probe to determine the rate of amplification of a target gene sequence. Briefly, the fluorescently labeled oligonucleotide probe binds initially to the target DNA sequence due to its high annealing temperature, followed by binding of the forward and reverse primers. As amplification occurs the probe is cleaved by the 5′ exonuclease activity of the polymerase enzyme, thereby releasing the fluorescent signal attached to the 5′ end of the probe, which is interpreted by the analysis software of the real-time PCR instrument. As amplification continues a Ct value (i.e. the fractional cycle number at which a samples accumulating fluorescence crosses a set threshold that is several standard deviations above baseline fluorescence) is generated [48, 103]. We designed two different primer/probe sets to specifically land-on and amplify the telomere repeats (**Table 19**). The first combination set of real-time primers and probe consisted of longer sequences (tel 1=37bp, tel2=39bp, tel3=31bp) and yielded a higher overall annealing temperature of 68ºC, when compared to a second primer/probe set (tel 4=25bp, tel5=27bp, tel6=19bp), which was shorter in length and annealed at a lower temperature of 53ºC. Our rationale was that all biological ages have telomeres and all ages would amplify the telomeres, however if telomere length is correlated with age, then younger individuals would have longer telomeres, which would yield more fluorescent signal (due to more potential sites for primer and probe binding) and these samples would reach the predetermined threshold value at a lower fractional cycle number when compared to individuals of increasing biological age.

Real-time PCR amplification of genomic DNA obtained from various biologically aged individuals yielded cycle threshold (Ct) values ranging from 8.438 to 11.243, primer set 1 and 5.261 to 6.911, primer set 2 (**Figure 38**).

The results in **Figure** show that newborns and elderly individuals (1-hour, 89-years and 91-years) had the highest Ct values (mean = 9.75), while all ages in between (4-, 14-, 47-, and 63-years) consistently generated lower Ct values (mean = 8.63). These results looked very promising, until we tested the primer/probe set with a non-template control (NTC) sample. It was shown that the NTC was able to generate a Ct value similar to the ones obtained with our DNA samples. It was determined that this amplification was due to primer and probe interactions, whereby the probe was binding to one of the primers and the TaqMan polymerase was cleaving the probe to release a fluorescent signal. Multiple reaction parameters were tested to try and overcome the NTC amplification, without success.

Assessing the Length of Individual Telomeres using the STELA Telomere Amplification Reaction

The single telomere length analysis (STELA) assay, was originally developed for sizing the XpYp telomere, but has the potential of determining accurate telomere lengths for chromosomes 12q, 7q, 16q and 16p [49]. This method can reportedly successfully size telomeres, of all lengths, with as little as 250 pg template DNA. Briefly, six linker 'telorettes' comprising the seven bases with telomeric repeat homology (TTAGGG, TAGGGT, AGGGTT, GGGTTA, GGTTAG, and GTTAGG), followed by a 20-basepair segment (TGCTCCGTGCATCTGGCATC) non-complementary to the 3′ overhang are annealed and ligated to all telomere ends. The downstream 20-basepair non-complementary 'telorette' segment can then serve as a target for a PCR primer ('teletail'). Chromosomal specificity is

generated by using an upstream primer which is designed to bind in the subtelomeric region of the target chromosome. Post-amplified fragments are electrophoresed and visualized with nucleic acid staining on agarose gels.

To determine the length of the XpYp telomere in DNA samples from individuals of varying ages, we extracted DNA from sixteen different individuals aged 1-day (male), 1-day (female), 4-months, 9-months, 15-months, 21-months, 8-years, 12-years, 17-years (male), 17-years (female), 29-years, 43-years, 56-years, 56-years, 91-years, and 92-years. We ligated each of the six linkers to the DNA samples and amplified each aliquot with the 'teltail' and XpYp chromosome specific primer.

The results obtained from these experiments did not allow us to conclude that telomere length is correlated with biological age, based on the following results (**Figure**). First, we found that there was inconsistent amplification of the samples between the linkers. For example, two of the linkers amplified fourteen of the sixteen samples (telorettes 1 and 6), one amplified twelve of the sixteen samples (telorette 3), while the three other linkers amplified only seven of the samples (telorettes 2, 4, 5). Second, the lengths of the amplified DNA samples did not correlate with the biological ages of the individuals. In linker two, a 4-month old individual had a longer telomere length then both of the 1-day old samples, and in linker three, the 91- and 92-year old individuals had longer telomeres then the 15- and 21-month old individuals. The only result that was somewhat consistent with all of the six linkers was that, if multiple amplified products were produced during amplification (in a single DNA sample), their occurrence was restricted to the younger individuals. Overall both 1-day old, as well as the 4-month and 21-month old samples produced multiple amplimers, while other ages produced a single product.

CHAPTER FOUR: CONCLUSIONS

We have identified seven biomarkers that may be useful for the prediction of the biological age of the donor of a human bloodstain. Through triplicate specificity studies, three of these candidate genes COL1A2, HBE1 and IGFBP3, were shown to be expressed at elevated levels in younger aged individuals, newborns, or post-pubertal individuals, respectively. Duplex real-time PCR amplifications were designed and developed for the individual age specific biomarkers with the incorporation of the ribosomal protein, S15 as an internal positive control (IPC) housekeeping gene. We have also identified four novel gamma hemoglobin transcripts (HBG1n1, HBG1n2, HBG2n2 and HBG2n3) that exhibit restricted expression in the blood of (human) newborn children. Individual qRT-PCR assays were developed to measure two of these transcripts in forensic specimens. Adjustment of the primer concentrations in the qRT-PCR reaction permitted the establishment of two temporally delimited assays, one of which was specific to blood from newborns 4-months or under (≤4 months) and the other to newborns who were hours old (<24 hours). Both assays may be useful in a variety of child kidnapping, assault and criminal abortion investigations with the latter (<24 hours) being of particular use for those cases involving hospital abductions. Validation studies on these qRT-PCR assays revealed that the HBG1n1 and HBG2n3 transcripts appear to be restricted to blood from newborns in the human (or at least, primate) lineage. The assays appear to be robust enough for forensic use, in that the newborn blood-specific transcripts are detectable at least up to 15 months in the dried state. Additionally, the sensitivity of the reactions are compatible with forensic applications, where only a few cell equivalents of total RNA are required (i.e. 50 pg) and >100ng of total RNA is recoverable from typical sized (50-ul) bloodstains [105]. The sensitivity of the assay is thus 50-500 cells assuming 0.1-1.0 pg total RNA per cell [106-108].

In summary, we report the detection of seven mRNA transcripts whose expression levels are increased during specific developmental stages of the human lifecycle. Forensically useful real-time PCR assays have been designed, developed and optimized for facile sample analysis and biological age determination of newborns, younger aged individuals and post-pubertal populations. These assays could therefore therefore provide investigators with additional probative information from a crime scene stain, namely an estimate of the donor's age.

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APPENDIX A: FIGURES

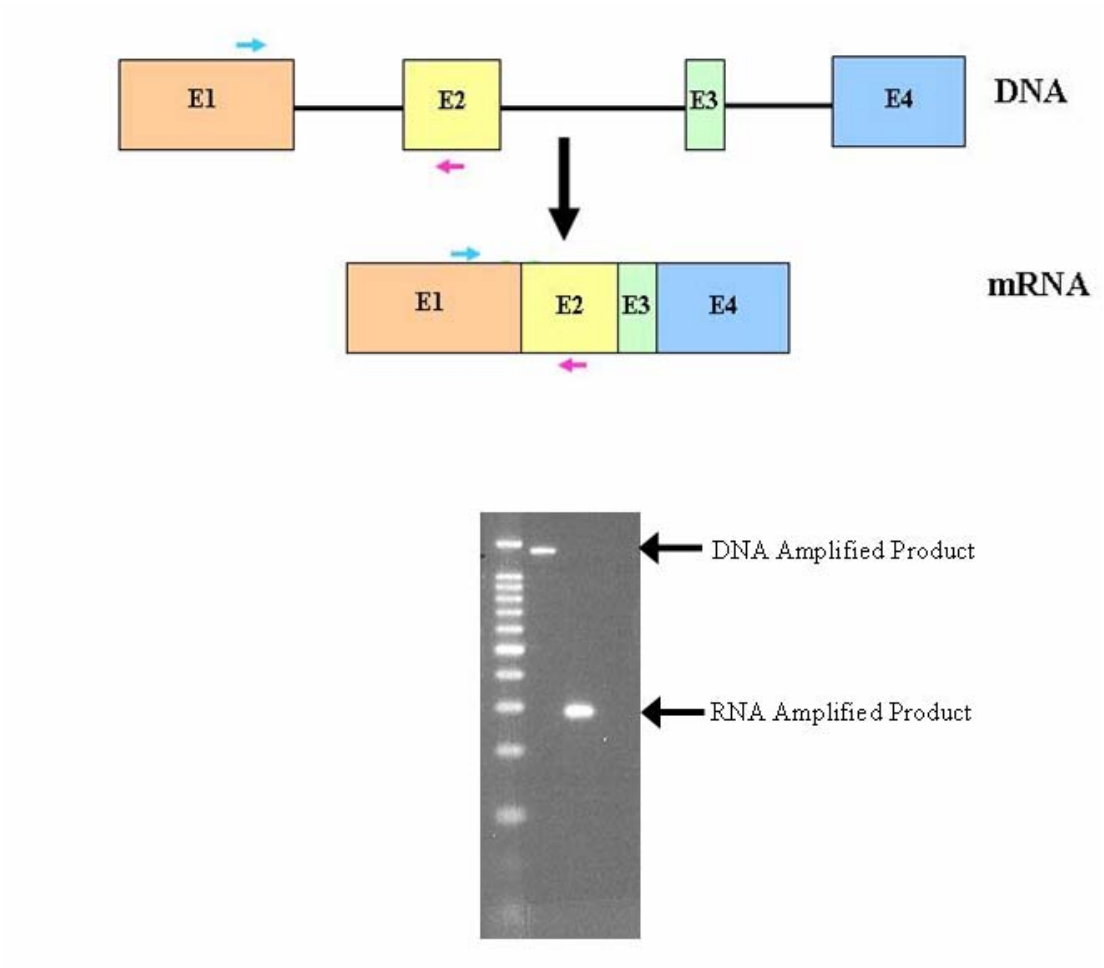


Figure 1: RT-PCR Primer Design.

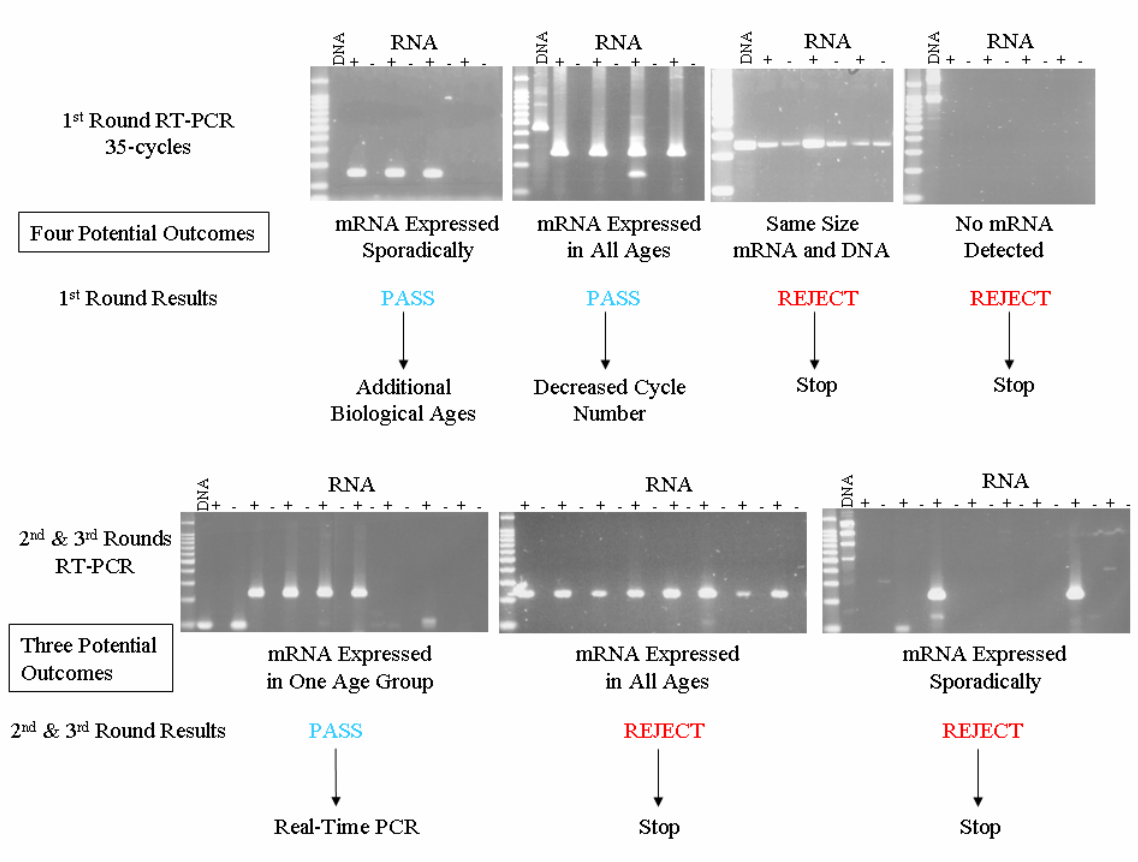


Figure 2: RT-PCR Procedure for Candidate Gene Testing.

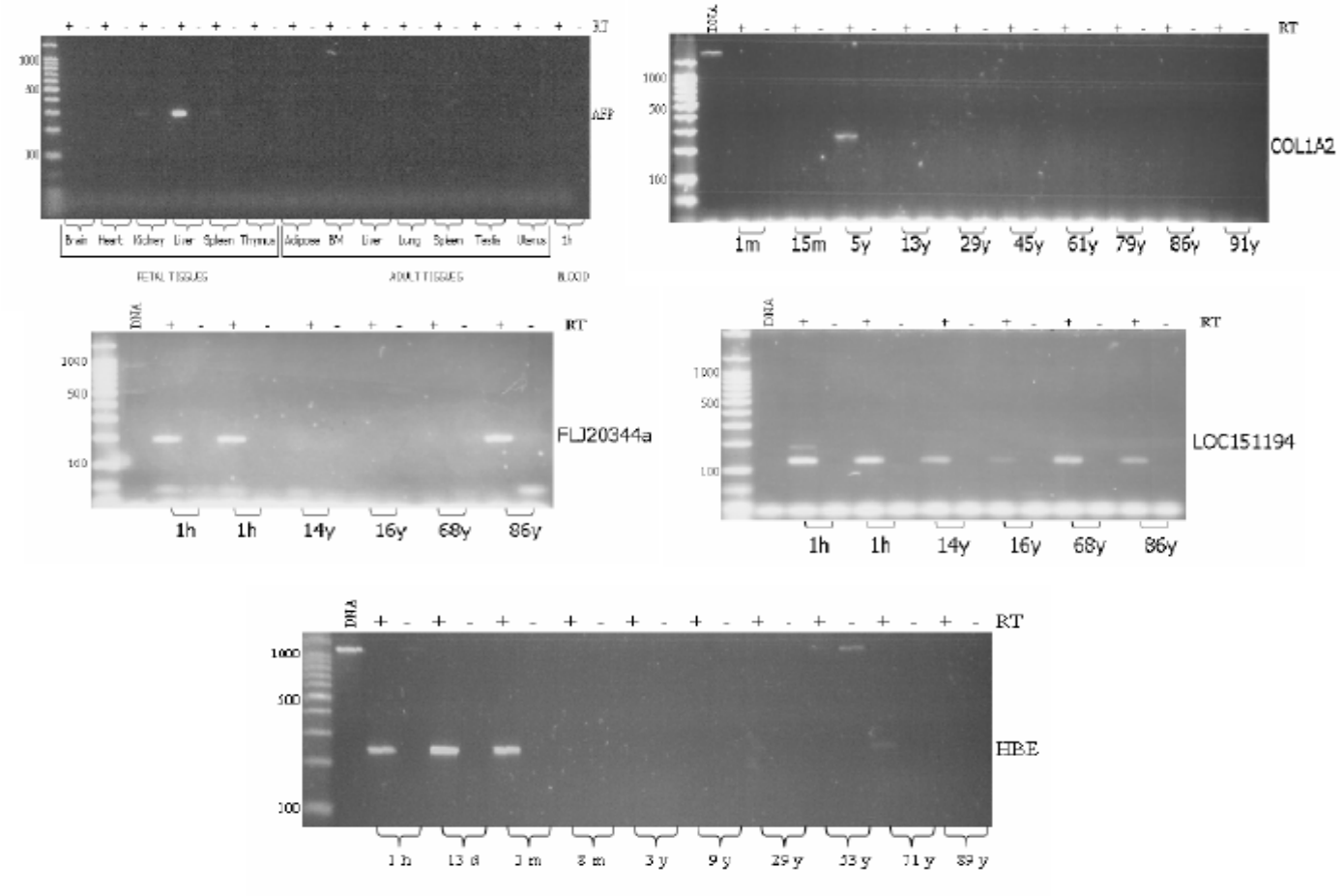


Figure 3: RT-PCR Newborn Candidates Taken to Real-Time PCR.

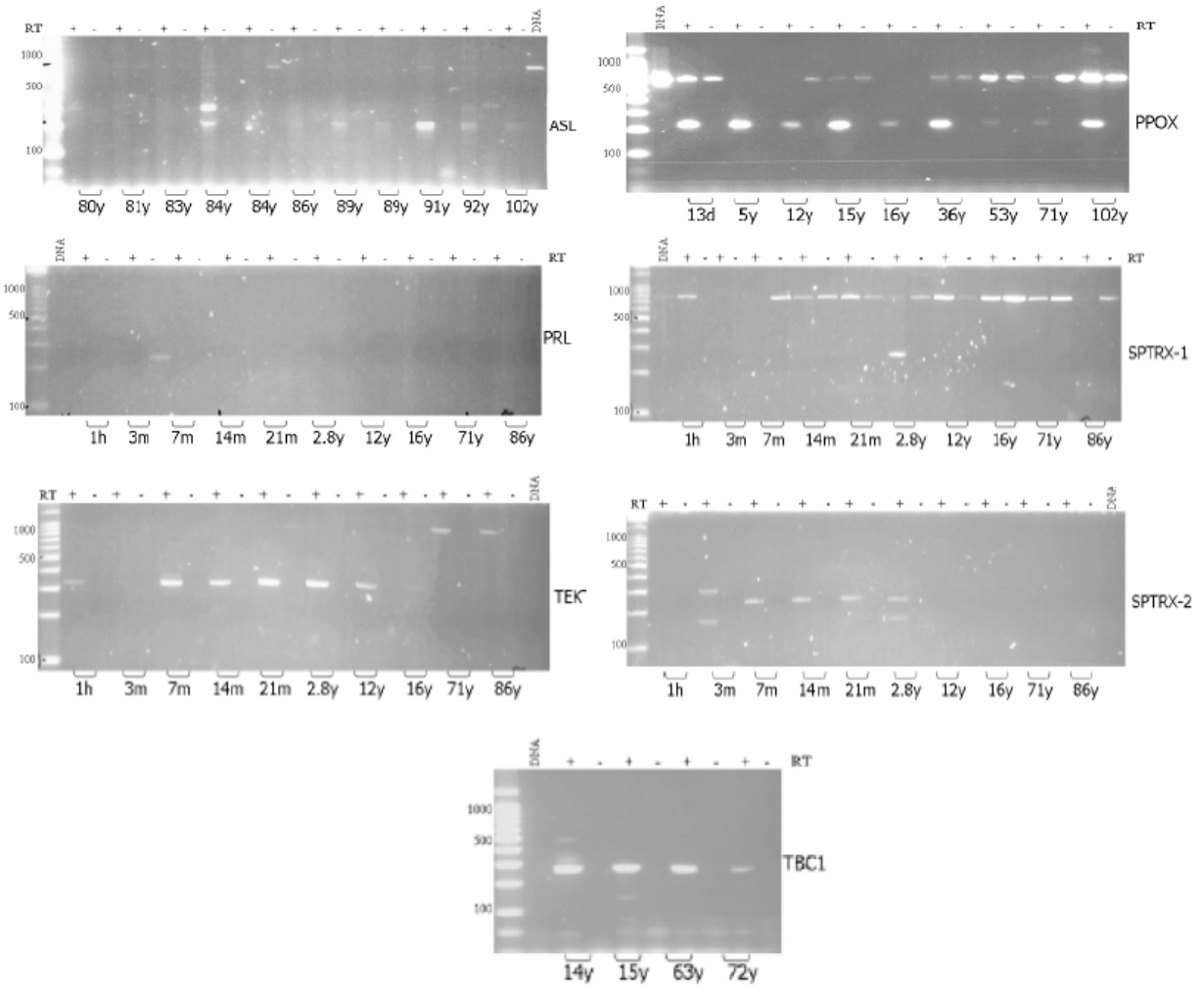


Figure 4: RT-PCR Juvenile Candidates Taken to Real-Time PCR.

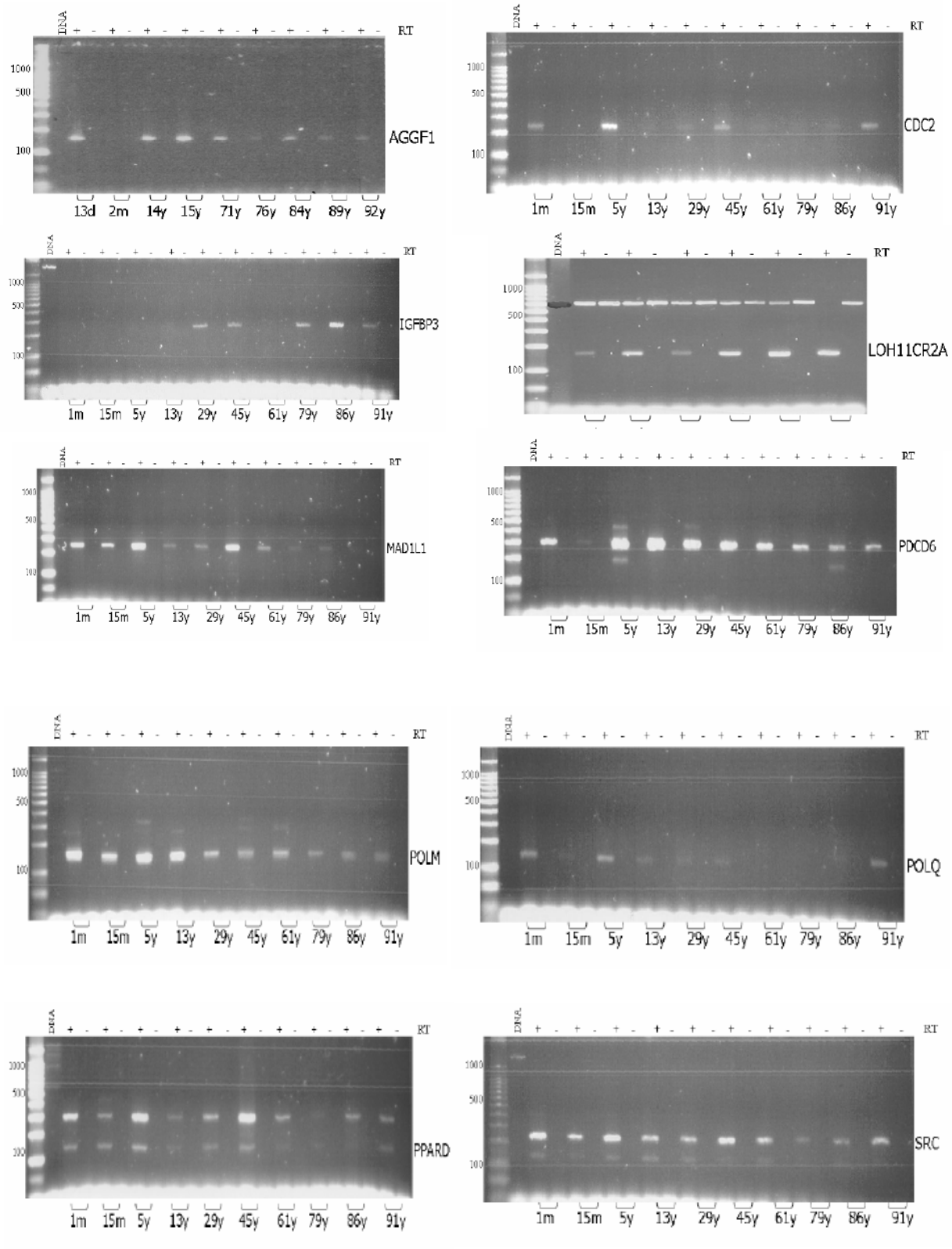


Figure 5: RT-PCR Elderly Candidates Taken to Real-Time PCR.

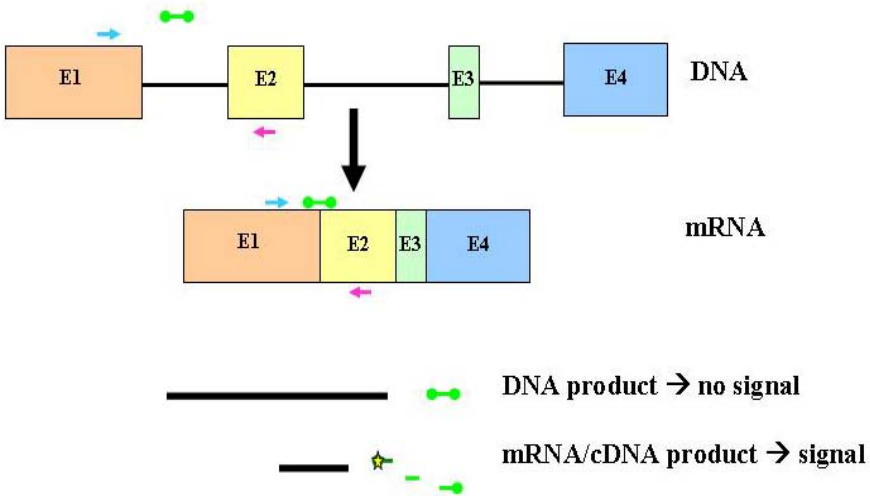
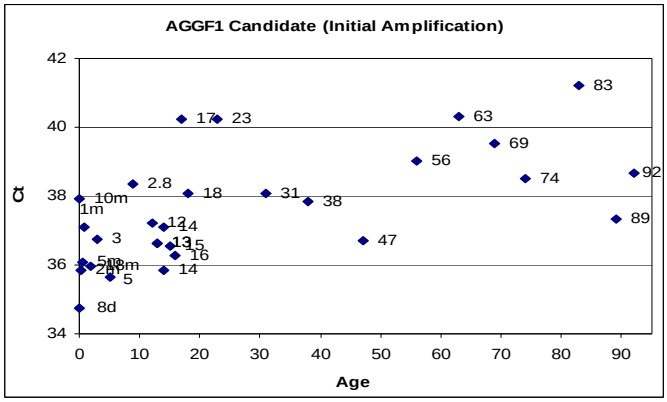


Figure 6: Real-Time PCR Primer Design.



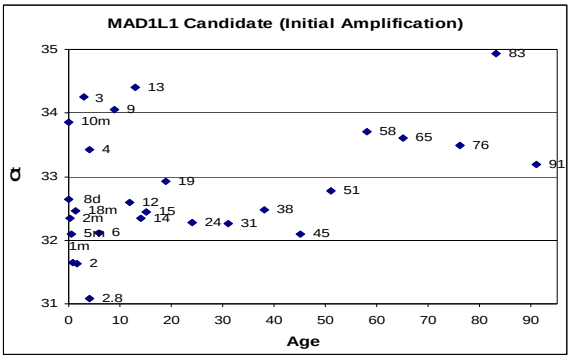
AGGF1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	37.910
Newborn -RT				Undet
Newborn +RT	F	8d	0.022	34.738
Newborn -RT				Undet
Newborn +RT	F	3m	0.250	35.838
Newborn -RT				Undet
Infant +RT	F	6m	0.500	36.086
Infant -RT				Undet
Toddler +RT	M	10m	0.833	37.095
Toddler -RT				Undet
Toddler +RT	F	21m	1.750	35.942
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	36.747
Toddler -RT				Undet
Child +RT	M	5y	5.000	35.635
Child -RT				Undet
Child +RT	F	9y	9.000	38.361
Child -RT				Undet
Child +RT	M	12y	12.000	37.210
Child -RT				Undet
Juvenile +RT	M	13y	13.000	36.632
Juvenile -RT				Undet
Juvenile +RT	M	13y	13.000	36.616
Juvenile -RT				Undet
Juvenile +RT	F	14y	14.000	35.855
Juvenile -RT				Undet
Juvenile +RT	M	14y	14.000	37.084
Juvenile -RT				Undet
Juvenile +RT	F	15y	15.000	36.552
Juvenile -RT				Undet
Juvenile +RT	M	16y	16.000	36.262
Juvenile -RT				Undet

AGGF1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	M	17y	17.000	40.226
Juvenile -RT				Undet
Juvenile +RT	M	18y	18.000	38.078
Juvenile -RT				Undet
Adult +RT	M	23y	23.000	40.220
Adult -RT				Undet
Adult +RT	F	31y	31.000	38.075
Adult -RT				Undet
Adult +RT	F	38y	38.000	37.850
Adult -RT				Undet
Middle-Age +RT	F	47y	47.000	36.699
Middle-Age -RT				Undet
Middle-Age +RT	F	56y	56.000	39.024
Middle-Age -RT				Undet
Middle-Age +RT	M	63y	63.000	40.325
Middle-Age -RT				Undet
Elderly +RT	F	69y	69.000	39.531
Elderly -RT				Undet
Elderly +RT	M	74y	74.000	38.497
Elderly -RT				Undet
Elderly +RT	M	83y	83.000	41.200
Elderly -RT				Undet
Elderly +RT	F	89y	89.000	37.350
Elderly -RT				Undet
Elderly +RT	M	92y	92.000	38.659
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Figure 7: Real-Time PCR First-Round Candidate Results.



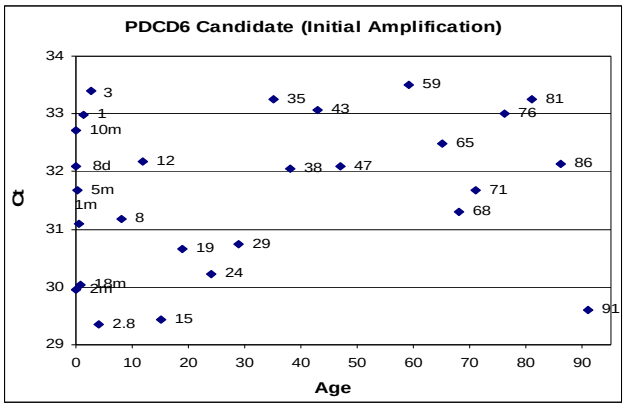
MAD1L1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	33.859
Newborn -RT				Undet
Newborn +RT	M	1m	0.083	32.639
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	32.352
Newborn -RT				Undet
Infant +RT	M	5m	0.417	32.101
Infant -RT				Undet
Infant +RT	F	9m	0.750	31.655
Infant -RT				Undet
Toddler +RT	M	15m	1.250	32.464
Toddler -RT				Undet
Toddler +RT	F	21m	1.750	31.636
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	34.260
Toddler -RT				Undet
Child +RT	M	4y	4.000	31.075
Child -RT				Undet
Child +RT	F	4y	4.000	33.428
Child -RT				Undet
Child +RT	M	6y	6.000	32.105
Child -RT				Undet
Child +RT	F	9y	9.000	34.052
Child -RT				Undet
Child +RT	M	12y	12.000	32.586
Child -RT				Undet
Juvenile +RT	M	13y	13.000	34.398
Juvenile -RT				Undet
Juvenile +RT	M	14y	14.000	32.349
Juvenile -RT				Undet
Juvenile +RT	M	15y	15.000	32.451
Juvenile -RT				Undet

MAD1L1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	M	17y	17.000	38.530
Juvenile -RT				Undet
Adult +RT	M	19y	19.000	32.931
Adult -RT				Undet
Adult +RT	M	24y	24.000	32.286
Adult -RT				Undet
Adult +RT	F	31y	31.000	32.262
Adult -RT				Undet
Adult +RT	M	38y	38.000	32.485
Adult -RT				Undet
Adult +RT	M	45y	45.000	32.100
Adult -RT				Undet
Middle-Age +RT	M	51y	51.000	32.777
Middle-Age -RT				Undet
Middle-Age +RT	F	58y	58.000	33.705
Middle-Age -RT				Undet
Elderly +RT	M	65y	65.000	33.602
Elderly -RT				Undet
Elderly +RT	F	71y	71.000	36.551
Elderly -RT				Undet
Elderly +RT	F	76y	76.000	33.497
Elderly -RT				Undet
Elderly +RT	M	83y	83.000	34.930
Elderly -RT				Undet
Elderly +RT	F	91y	91.000	33.187
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Figure 7 (continued): Real-Time PCR First-Round Candidate Results.



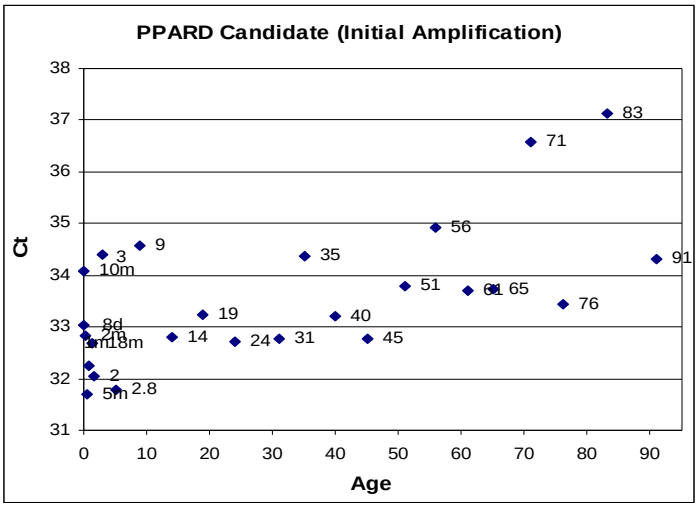
PDCD6-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	32.705
Newborn -RT				Undet
Newborn +RT	M	13d	0.036	32.089
Newborn -RT				Undet
Newborn +RT	M	1m	0.083	29.960
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	31.676
Newborn -RT				Undet
Infant +RT	M	5m	0.417	31.088
Infant -RT				Undet
Infant +RT	F	9m	0.750	30.036
Infant -RT				Undet
Toddler +RT	M	15m	1.250	32.980
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	33.405
Toddler -RT				Undet
Child +RT	M	4y	4.000	29.344
Child -RT				Undet
Child +RT	M	8y	8.000	31.188
Child -RT				Undet
Child +RT	M	12y	12.000	32.183
Child -RT				Undet
Juvenile +RT	M	15y	15.000	29.442
Juvenile -RT				Undet
Adult +RT	M	19y	19.000	30.664
Adult -RT				Undet
Adult +RT	M	24y	24.000	30.230
Adult -RT				Undet
Adult +RT	M	29y	29.000	30.743
Adult -RT				Undet

PDCD6-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Adult +RT	F	35y	35.000	33.250
Adult -RT				Undet
Adult +RT	M	38y	38.000	32.048
Adult -RT				Undet
Adult +RT	F	43y	43.000	33.073
Adult -RT				Undet
Middle-Age +RT	M	47y	47.000	32.093
Middle-Age -RT				Undet
Middle-Age +RT	F	53y	53.000	38.182
Middle-Age -RT				Undet
Middle-Age +RT	M	59y	59.000	33.505
Middle-Age -RT				Undet
Elderly +RT	M	65y	65.000	32.479
Elderly -RT				Undet
Elderly +RT	F	68y	68.000	31.293
Elderly -RT				Undet
Elderly +RT	F	71y	71.000	31.672
Elderly -RT				Undet
Elderly +RT	F	76y	76.000	33.004
Elderly -RT				Undet
Elderly +RT	F	81y	81.000	33.258
Elderly -RT				Undet
Elderly +RT	M	86y	86.000	32.128
Elderly -RT				Undet
Elderly +RT	F	91y	91.000	29.600
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Figure 7 (continued): Real-Time PCR First-Round Candidate Results.



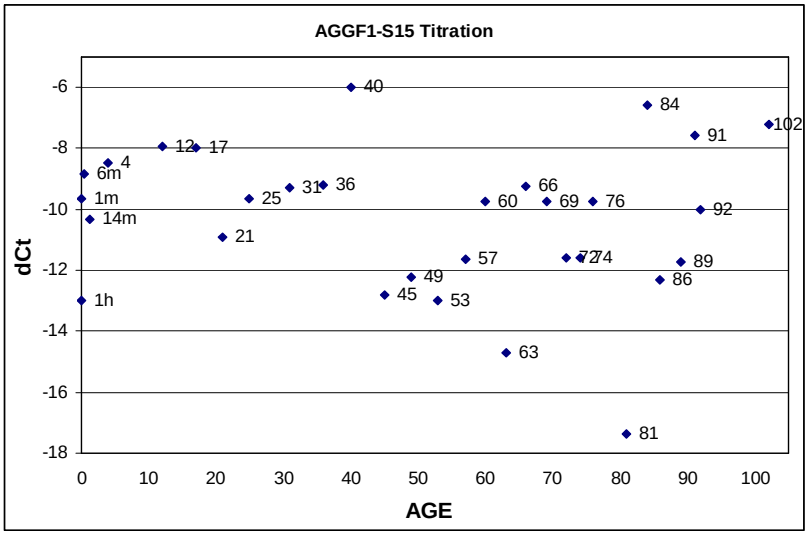
PPARD-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	34.078
Newborn -RT				Undet
Newborn +RT	M	1m	0.083	33.044
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	32.827
Newborn -RT				Undet
Infant +RT	M	5m	0.417	31.710
Infant -RT				Undet
Infant +RT	F	9m	0.750	32.254
Infant -RT				Undet
Toddler +RT	M	15m	1.250	32.690
Toddler -RT				Undet
Toddler +RT	F	21m	1.750	32.060
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	34.412
Toddler -RT				Undet
Child +RT	M	5y	5.000	31.771
Child -RT				Undet
Child +RT	F	9y	9.000	34.561
Child -RT				Undet
Child +RT	M	14y	14.000	32.793
Child -RT				Undet
Child +RT	M	19y	19.000	33.232
Child -RT				Undet
Child +RT	M	24y	24.000	32.705
Child -RT				Undet
Juvenile +RT	F	31y	31.000	32.767
Juvenile -RT				Undet

PPARD-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	F	35y	35.000	34.372
Juvenile -RT				Undet
Juvenile +RT	M	40y	40.000	33.203
Juvenile -RT				Undet
Juvenile +RT	M	45y	45.000	32.760
Juvenile -RT				Undet
Adult +RT	M	51y	51.000	33.782
Adult -RT				Undet
Adult +RT	M	56y	56.000	34.921
Adult -RT				Undet
Adult +RT	M	61y	61.000	33.688
Adult -RT				Undet
Adult +RT	M	65y	65.000	33.723
Adult -RT				Undet
Adult +RT	F	71y	71.000	36.565
Adult -RT				Undet
Middle-Age +RT	F	76y	76.000	33.450
Middle-Age -RT				Undet
Middle-Age +RT	M	83y	83.000	37.131
Middle-Age -RT				Undet
Elderly +RT	F	91y	91.000	34.315
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

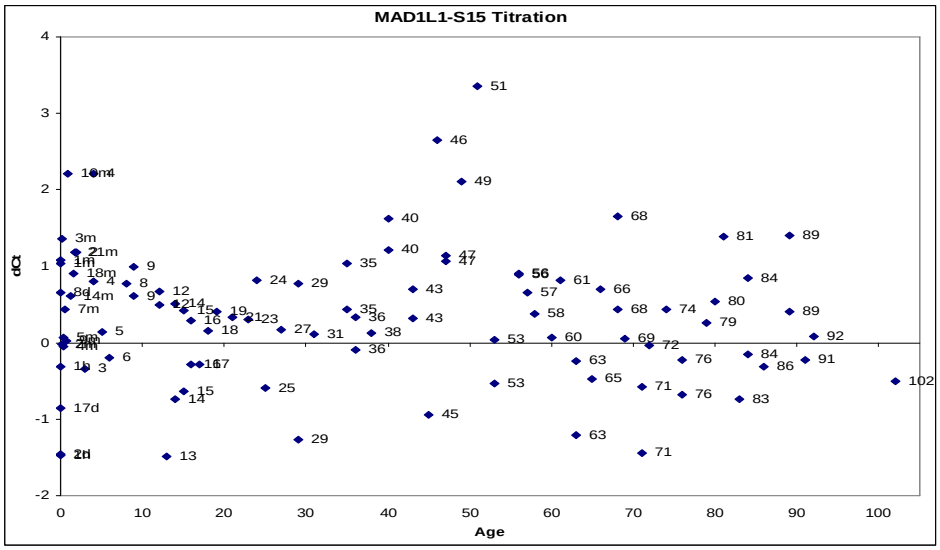
Figure 7 (continued): Real-Time PCR First-Round Candidate Results.



AGGF1-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	47.528	34.531	-12.997
Newborn +RT	1m	42.358	32.725	-9.633
Infant +RT	6m	42.544	33.715	-8.829
Toddler +RT	14m	44.931	34.623	-10.308
Child +RT	4y	45.015	36.543	-8.472
Child +RT	12y	41.466	33.554	-7.912
Juvenile +RT	17y	43.514	35.548	-7.966
Adult +RT	21y	45.017	34.108	-10.909
Adult +RT	25y	45.128	35.476	-9.652
Adult +RT	31y	42.238	32.956	-9.282
Adult +RT	36y	41.610	32.429	-9.181
Adult +RT	40y	40.757	34.752	-6.005
Adult +RT	45y	47.069	34.282	-12.787
Middle-Age +RT	49y	50.000	37.761	-12.239
Middle-Age +RT	53y	46.929	33.922	-13.007
Middle-Age +RT	57y	44.332	32.686	-11.646
Middle-Age +RT	60y	43.396	33.649	-9.747
Middle-Age +RT	63y	48.022	33.334	-14.688
Elderly +RT	66y	43.165	33.929	-9.236
Elderly +RT	69y	44.958	35.218	-9.740
Elderly +RT	72y	44.568	32.998	-11.570
Elderly +RT	74y	47.145	35.558	-11.587
Elderly +RT	76y	45.374	35.630	-9.744
Elderly +RT	79y	48.204	21.633	-26.571
Elderly +RT	81y	44.638	27.282	-17.356
Elderly +RT	84y	40.969	34.405	-6.564
Elderly +RT	86y	45.564	33.270	-12.294
Elderly +RT	89y	44.987	33.242	-11.745
Elderly +RT	91y	39.628	32.059	-7.569
Elderly +RT	92y	44.128	34.138	-9.990
Elderly +RT	102y	40.475	33.270	-7.205

Figure 8: Real-Time PCR Duplex Delta Ct Results.



MAD1L1-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	34.657	33.184	-1.473
Newborn +RT	1h	33.066	32.755	-0.311
Newborn +RT	2d	36.374	34.912	-1.462
Newborn +RT	8d	31.072	31.727	0.655
Newborn +RT	17d	31.434	30.577	-0.857
Newborn +RT	1m	32.413	33.488	1.075
Newborn +RT	1m	30.697	31.740	1.043
Newborn +RT	2m	33.176	33.156	-0.020
Newborn +RT	3m	32.052	33.406	1.354
Infant +RT	4m	33.452	33.402	-0.050
Infant +RT	5m	31.633	31.706	0.073
Infant +RT	7m	32.866	33.304	0.438
Infant +RT	9m	34.490	34.519	0.029
Toddler +RT	10m	31.316	33.520	2.204
Toddler +RT	14m	33.411	34.015	0.604
Toddler +RT	18m	32.456	33.363	0.907
Toddler +RT	21m	32.736	33.922	1.186
Toddler +RT	2y	32.733	33.921	1.188
Toddler +RT	3y	32.592	32.248	-0.344
Child +RT	4y	31.477	32.272	0.795
Child +RT	4y	32.245	34.458	2.213
Child +RT	5y	31.107	31.242	0.135
Child +RT	6y	34.500	34.311	-0.189
Child +RT	8y	34.194	34.962	0.768
Child +RT	9y	34.143	34.754	0.611
Child +RT	9y	33.163	34.156	0.993
Child +RT	12y	33.774	34.448	0.674
Child +RT	12y	32.587	33.080	0.493
Juvenile +RT	13y	33.190	31.698	-1.492
Juvenile +RT	14y	33.060	32.323	-0.737
Juvenile +RT	14y	32.396	32.899	0.503
Juvenile +RT	15y	32.993	33.420	0.427
Juvenile +RT	15y	32.454	31.812	-0.642

MAD1L1-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Adult +RT	36y	33.486	33.820	0.334
Adult +RT	36y	33.289	33.195	-0.094
Adult +RT	38y	32.470	32.599	0.129
Adult +RT	40y	32.029	33.245	1.216
Adult +RT	40y	32.114	33.744	1.630
Adult +RT	43y	34.106	34.803	0.697
Adult +RT	43y	33.830	34.155	0.325
Adult +RT	45y	33.346	32.395	-0.951
Elderly +RT	46y	35.252	37.904	2.652
Elderly -RT	47y	35.807	36.876	1.069
Middle-Age +RT	47y	33.711	34.854	1.143
Middle-Age +RT	49y	37.889	40.000	2.111
Middle-Age +RT	51y	35.641	39.000	3.359
Middle-Age +RT	53y	35.006	34.468	-0.538
Middle-Age +RT	53y	32.477	32.521	0.044
Middle-Age +RT	56y	34.712	35.610	0.898
Middle-Age +RT	56y	32.791	33.674	0.883
Middle-Age +RT	57y	33.684	34.341	0.657
Middle-Age +RT	58y	33.345	33.724	0.379
Middle-Age +RT	60y	32.946	33.010	0.064
Middle-Age +RT	61y	32.485	33.306	0.821
Middle-Age +RT	63y	33.063	31.862	-1.201
Middle-Age +RT	63y	32.158	31.917	-0.241
Elderly +RT	65y	33.872	33.400	-0.472
Elderly +RT	66y	32.819	33.518	0.699
Elderly +RT	68y	32.226	33.876	1.650
Elderly +RT	68y	33.649	34.080	0.431
Elderly +RT	69y	33.235	33.290	0.055
Elderly +RT	71y	33.436	32.865	-0.571
Elderly +RT	71y	34.692	33.247	-1.445
Elderly +RT	72y	34.417	34.376	-0.041
Elderly +RT	74y	33.026	33.457	0.431
Elderly +RT	76y	33.171	32.944	-0.227

Juvenile +RT	16y	33.574	33.288	-0.286	Elderly +RT	76y	35.582	34.907	-0.675
Juvenile +RT	16y	32.666	32.948	0.282	Elderly +RT	79y	34.132	34.388	0.256
Juvenile +RT	17y	33.963	33.686	-0.277	Elderly +RT	80y	32.481	33.025	0.544
Juvenile +RT	18y	33.975	34.138	0.163	Elderly +RT	81y	35.783	37.165	1.382
Adult +RT	19y	33.151	33.562	0.411	Elderly +RT	83y	35.448	34.711	-0.737
Adult +RT	21y	37.224	37.556	0.332	Elderly +RT	84y	31.489	32.333	0.844
Adult +RT	23y	32.736	33.045	0.309	Elderly +RT	84y	34.094	33.945	-0.149
Adult +RT	24y	31.290	32.101	0.811	Elderly +RT	86y	32.868	32.549	-0.319
Adult +RT	25y	33.915	33.319	-0.596	Elderly +RT	89y	33.992	35.399	1.407
Adult +RT	27y	33.964	34.138	0.174	Elderly +RT	89y	33.943	34.345	0.402
Adult +RT	29y	33.441	32.180	-1.261	Elderly +RT	91y	32.281	32.060	-0.221
Adult +RT	29y	32.816	33.583	0.767	Elderly +RT	92y	32.169	32.246	0.077
Adult +RT	31y	32.110	32.224	0.114	Elderly +RT	102y	32.414	31.917	-0.497
Adult +RT	35y	35.361	35.799	0.438	DNA		40.000	40.000	0.000
Adult +RT	35y	33.675	34.706	1.031	NTC		40.000	40.000	0.000

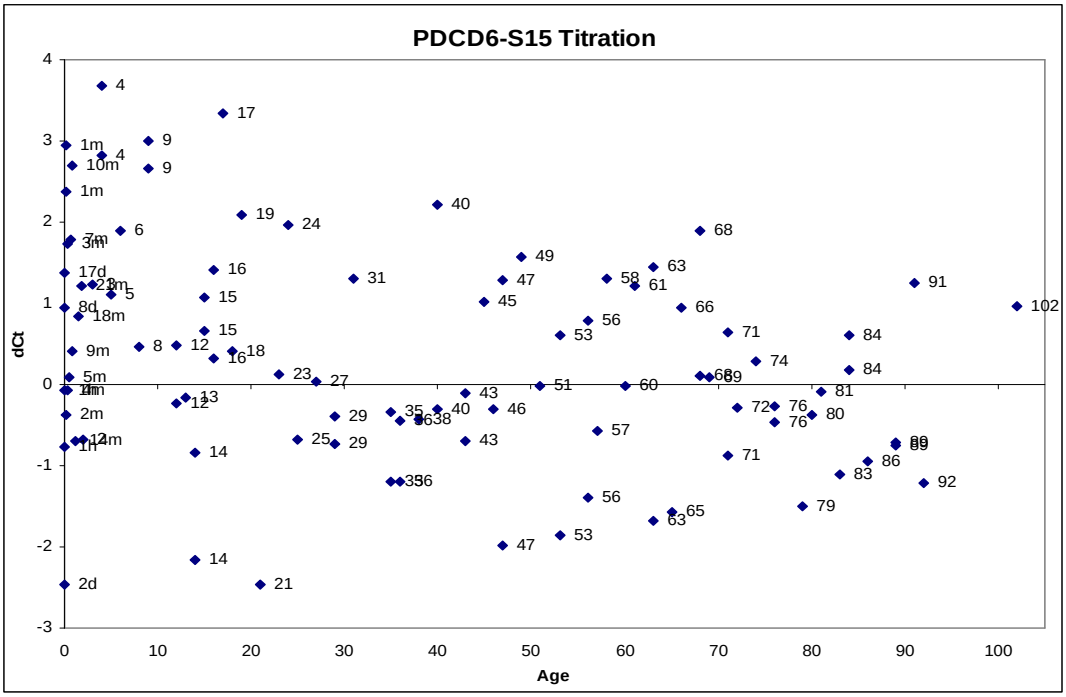


Figure 8 (continued): Real-Time PCR Duplex Delta Ct Results.

PDCD6-S15 Titration					PDCD6-S15 Titration				
Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	34.342	34.266	-0.076	Adult +RT	36y	35.388	34.184	-1.204
Newborn +RT	1h	34.692	33.921	-0.771	Adult +RT	36y	35.932	35.482	-0.450
Newborn +RT	2d	37.965	35.505	-2.460	Adult +RT	38y	33.189	32.762	-0.427
Newborn +RT	8d	31.603	32.549	0.946	Adult +RT	40y	32.015	34.236	2.221
Newborn +RT	17d	33.914	35.286	1.372	Adult +RT	40y	33.884	33.578	-0.306
Newborn +RT	1m	32.438	34.809	2.371	Adult +RT	43y	34.821	34.720	-0.101
Newborn +RT	1m	32.918	35.856	2.938	Adult +RT	43y	35.741	35.048	-0.693
Newborn +RT	2m	33.641	33.266	-0.375	Adult +RT	45y	32.372	33.387	1.015
Newborn +RT	3m	32.339	34.069	1.730	Elderly +RT	46y	34.858	34.562	-0.296
Infant +RT	4m	34.348	34.272	-0.076	Elderly -RT	47y	34.272	35.563	1.291
Infant +RT	5m	32.652	32.741	0.089	Middle-Age +RT	47y	35.365	33.391	-1.974
Infant +RT	7m	32.871	34.650	1.779	Middle-Age +RT	49y	35.881	37.456	1.575
Infant +RT	9m	35.446	35.856	0.410	Middle-Age +RT	51y	36.429	36.413	-0.016
Toddler +RT	10m	31.657	34.360	2.703	Middle-Age +RT	53y	36.811	34.946	-1.865
Toddler +RT	14m	35.100	34.404	-0.696	Middle-Age +RT	53y	33.509	34.125	0.616
Toddler +RT	18m	32.384	33.219	0.835	Middle-Age +RT	56y	35.961	34.567	-1.394
Toddler +RT	21m	32.843	34.050	1.207	Middle-Age +RT	56y	33.675	34.466	0.791
Toddler +RT	2y	34.142	33.457	-0.685	Middle-Age +RT	57y	34.749	34.178	-0.571
Toddler +RT	3y	32.026	33.264	1.238	Middle-Age +RT	58y	32.724	34.022	1.298
Child +RT	4y	31.664	34.477	2.813	Middle-Age +RT	60y	33.282	33.259	-0.023
Child +RT	4y	33.540	37.211	3.671	Middle-Age +RT	61y	32.480	33.699	1.219
Child +RT	5y	31.020	32.129	1.109	Middle-Age +RT	63y	34.973	33.287	-1.686
Child +RT	6y	33.472	35.373	1.901	Middle-Age +RT	63y	31.341	32.784	1.443
Child +RT	8y	35.501	35.969	0.468	Elderly +RT	65y	35.832	34.261	-1.571
Child +RT	9y	35.913	38.910	2.997	Elderly +RT	66y	33.469	34.411	0.942
Child +RT	9y	33.854	36.516	2.662	Elderly +RT	68y	33.699	33.802	0.103
Child +RT	12y	34.478	34.962	0.484	Elderly +RT	68y	33.303	35.199	1.896
Child +RT	12y	33.784	33.559	-0.225	Elderly +RT	69y	33.738	33.833	0.095
Juvenile +RT	13y	33.601	33.435	-0.166	Elderly +RT	71y	34.927	34.045	-0.882
Juvenile +RT	14y	33.785	32.942	-0.843	Elderly +RT	71y	33.130	33.765	0.635
Juvenile +RT	14y	32.669	30.514	-2.155	Elderly +RT	72y	34.257	33.965	-0.292
Juvenile +RT	15y	32.462	33.528	1.066	Elderly +RT	74y	35.264	35.555	0.291
Juvenile +RT	15y	31.584	32.249	0.665	Elderly +RT	76y	33.765	33.498	-0.267
Juvenile +RT	16y	33.227	33.546	0.319	Elderly +RT	76y	35.292	34.823	-0.469
Juvenile +RT	16y	32.204	33.615	1.411	Elderly +RT	79y	35.382	33.889	-1.493
Juvenile +RT	17y	32.306	35.640	3.334	Elderly +RT	80y	34.087	33.718	-0.369
Juvenile +RT	18y	34.445	34.859	0.414	Elderly +RT	81y	35.635	35.539	-0.096
Adult +RT	19y	32.412	34.506	2.094	Elderly +RT	83y	36.555	35.441	-1.114
Adult +RT	21y	37.960	35.500	-2.460	Elderly +RT	84y	33.128	33.305	0.177
Adult +RT	23y	33.679	33.807	0.128	Elderly +RT	84y	33.061	33.669	0.608
Adult +RT	24y	30.666	32.623	1.957	Elderly +RT	86y	34.494	33.554	-0.940
Adult +RT	25y	34.548	33.863	-0.685	Elderly +RT	89y	34.224	33.479	-0.745
Adult +RT	27y	34.569	34.606	0.037	Elderly +RT	89y	34.818	34.106	-0.712
Adult +RT	29y	33.321	32.925	-0.396	Elderly +RT	91y	31.701	32.947	1.246
Adult +RT	29y	34.837	34.101	-0.736	Elderly +RT	92y	34.167	32.953	-1.214
Adult +RT	31y	31.355	32.654	1.299	Elderly +RT	102y	32.567	33.526	0.959
Adult +RT	35y	35.770	34.575	-1.195	DNA		50.000	50.000	0.000

Figure 8 (continued): Real-Time PCR Duplex Delta Ct Results.

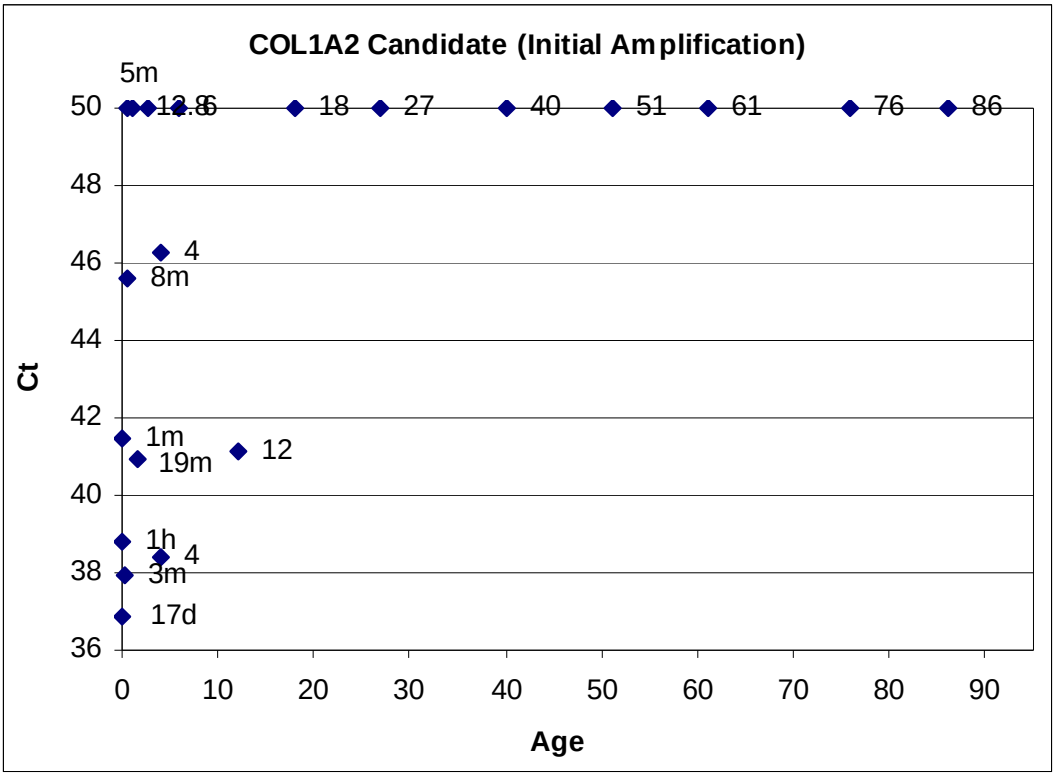


Figure 9: COL1A2 Real-Time PCR Singleplex Candidate Results.

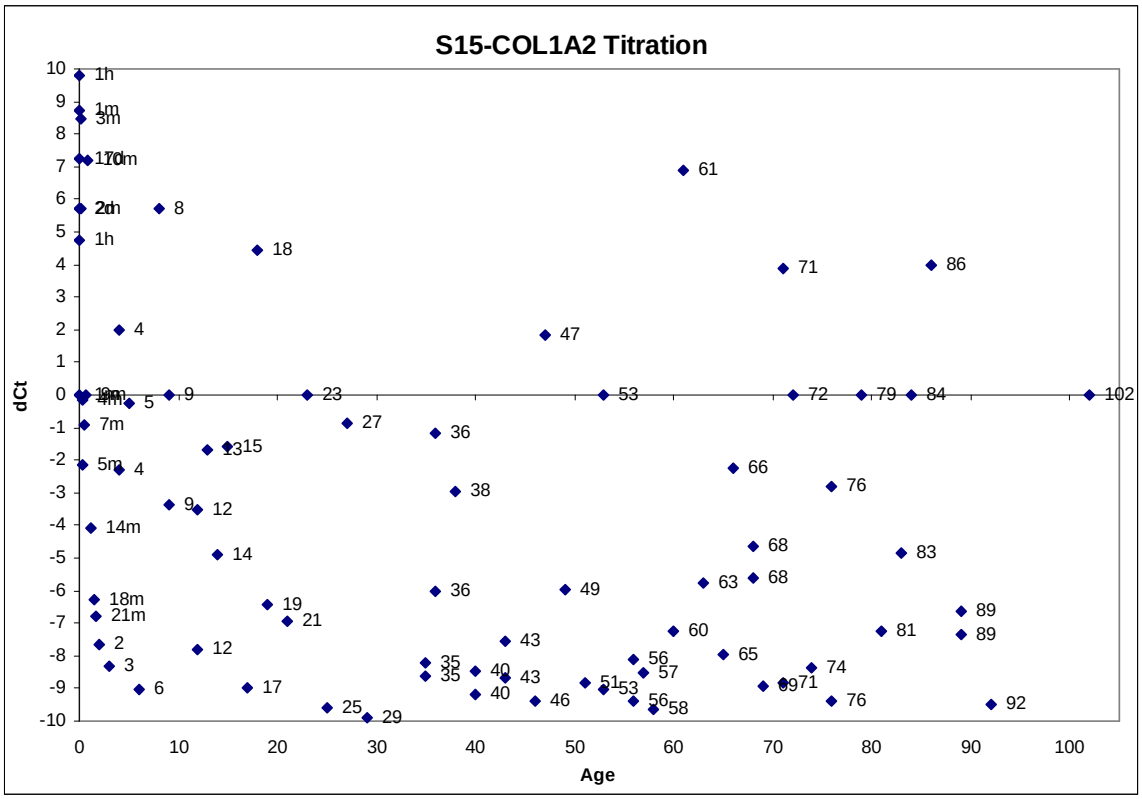
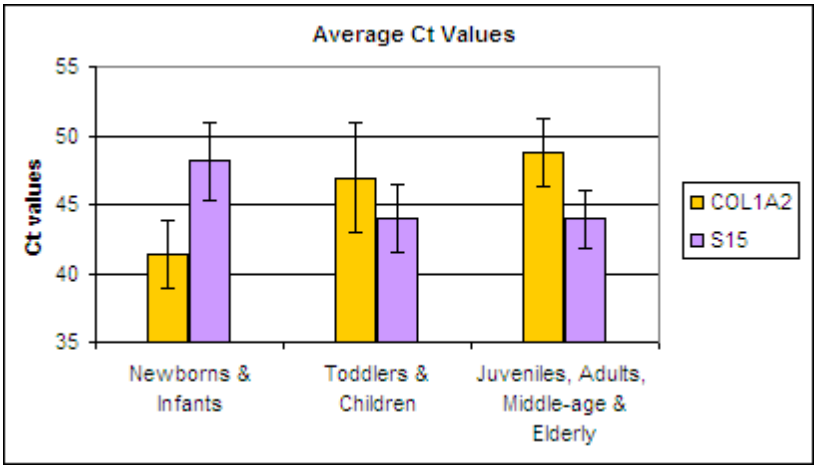


Figure 10: COL1A2 Real-Time PCR Duplex Delta Ct Results.

Average of all Samples per age group

Age Group	Average (GOI)	SD (GOI)	Average (S15)	SD (S15)	Average (dCt)	SD (dCt)	n=
Newborns & Infants	41.378	2.489	48.126	2.882	6.748	4.185	20
Toddlers & Children	46.928	4.008	43.948	2.497	-2.980	5.020	31
Juveniles, Adults, Middle-age & Elderly	48.751	2.491	43.921	2.052	-4.830	3.681	58



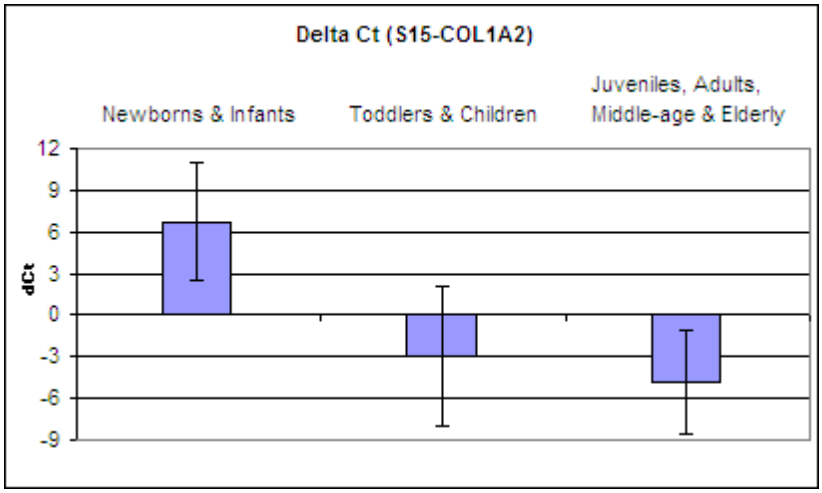


Figure 11: Newborn Candidate COL1A2 qPCR Duplex Biological Age Specificity.

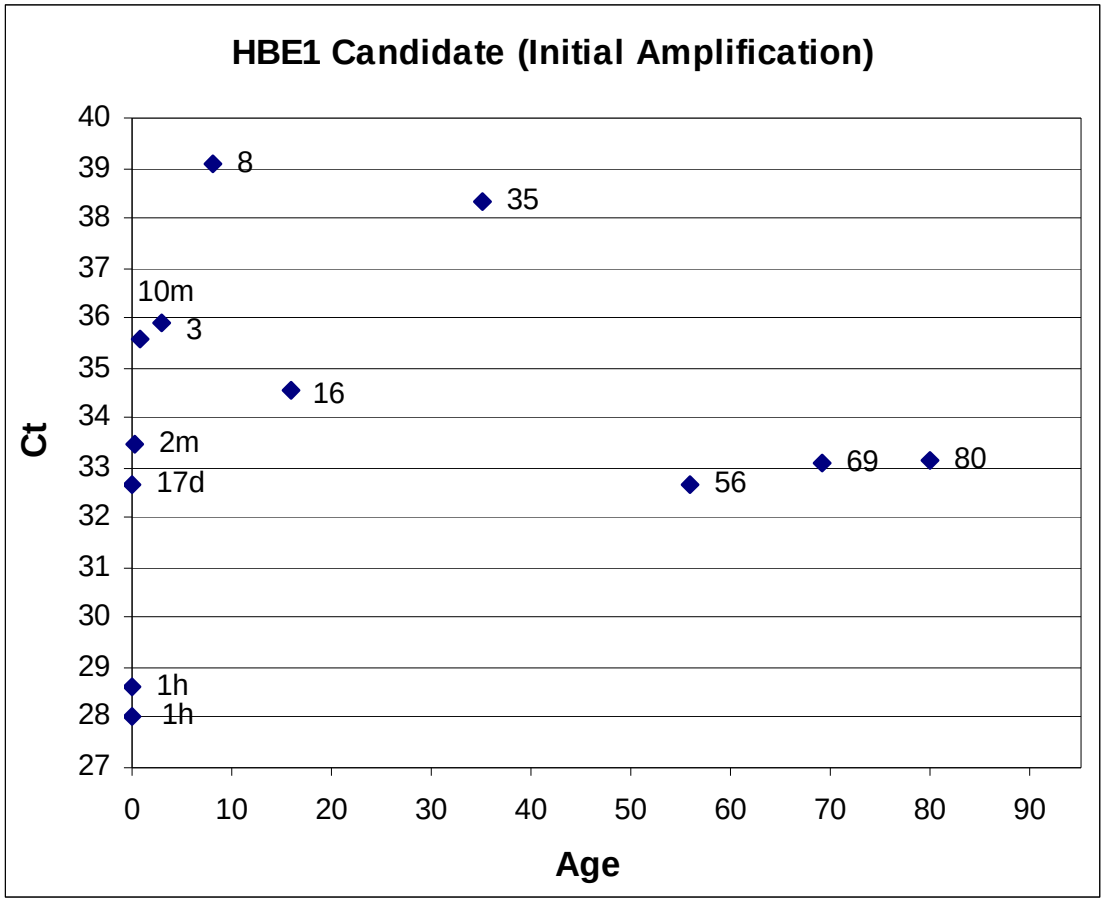


Figure 12: HBE1 Real-Time PCR Singleplex Candidate Results.

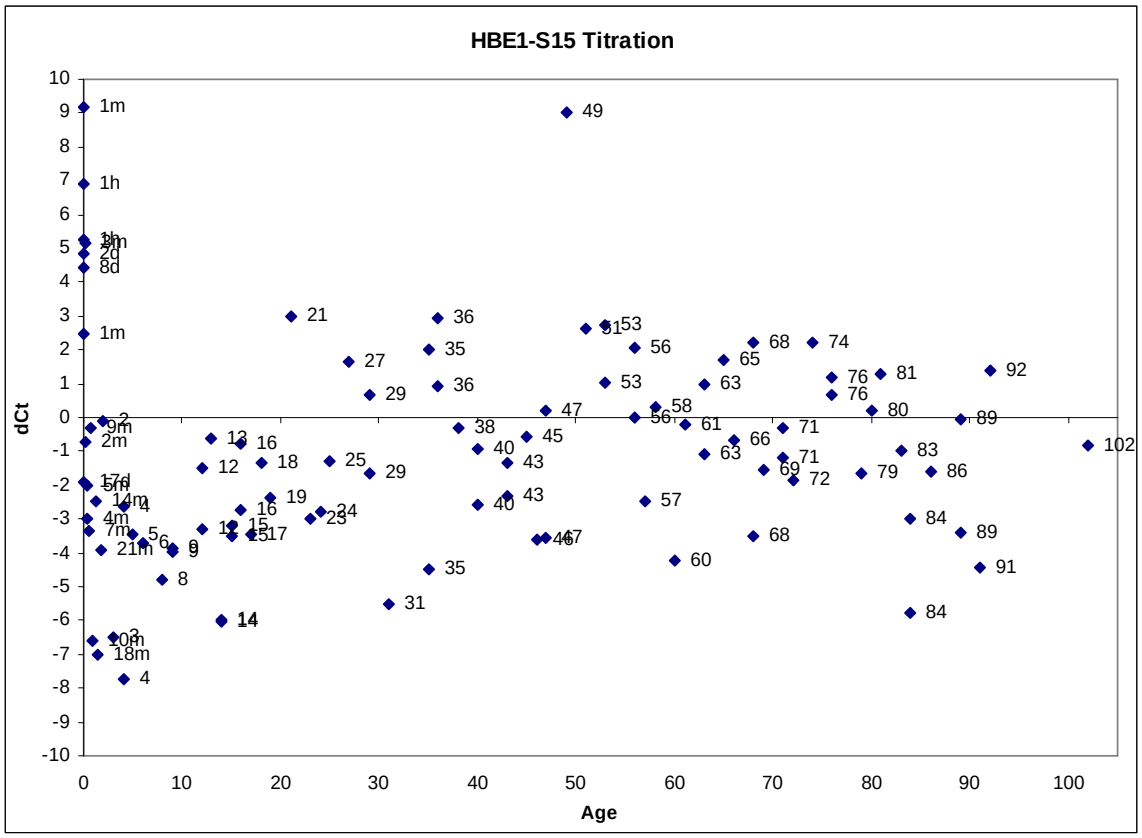
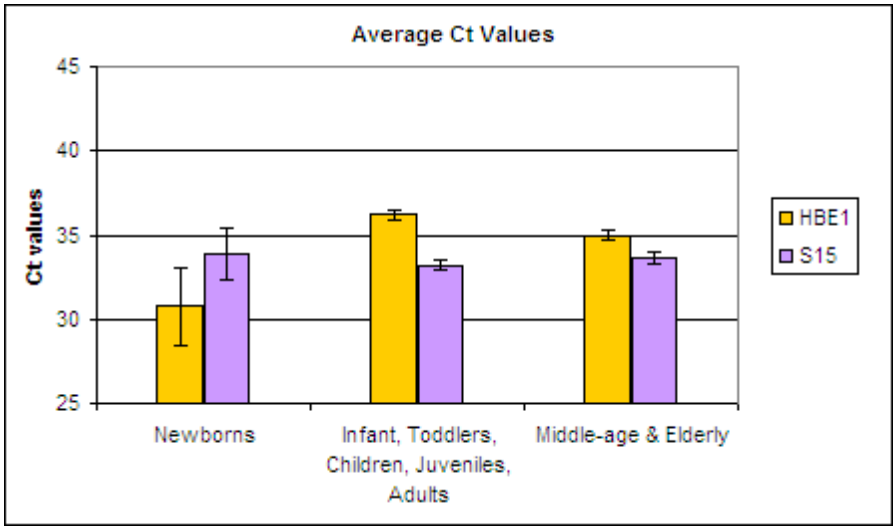


Figure 13: HBE1 Real-Time PCR Duplex Delta Ct Results.

Average of all Samples per age group

Age Group	Average (GOI)	SD (GOI)	Average (S15)	SD (S15)	Average (dCt)	SD (dCt)	n=
Newborns	30.750	2.270	33.838	1.531	3.088	2.523	17
Infant, Toddlers, Children, Juveniles, Adults	36.189	0.338	33.210	0.275	-2.978	0.302	81
Middle-age & Elderly	34.994	0.317	33.646	0.324	-1.348	0.330	41



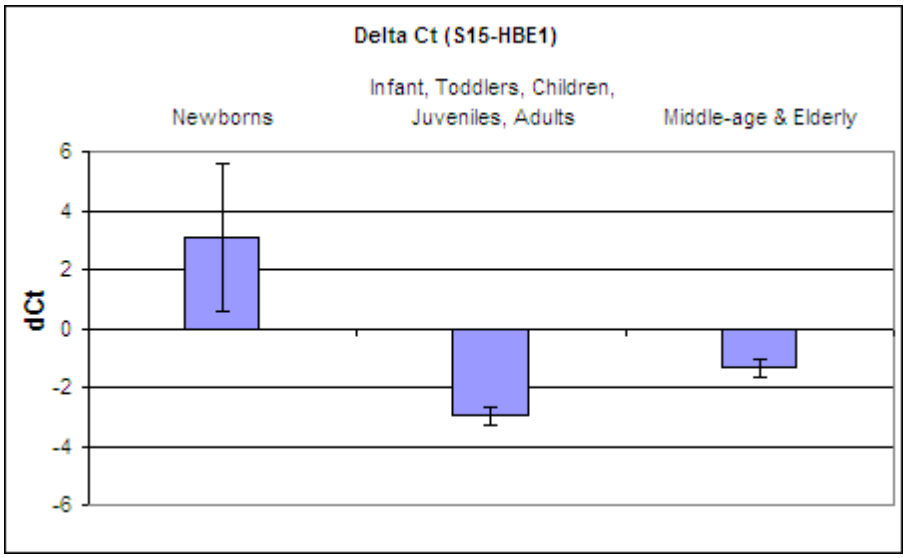


Figure 14: Newborn Candidate HBE1 qPCR Duplex Biological Age Specificity.

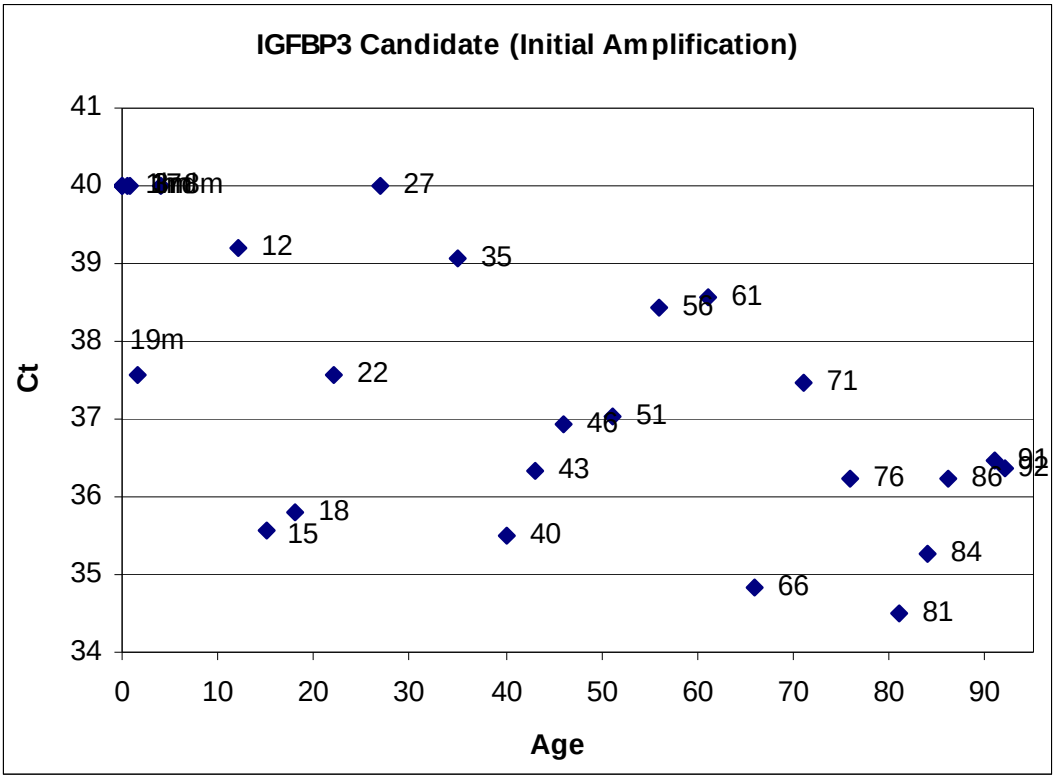


Figure 15: IGFBP3 Real-Time PCR Singleplex Candidate Results.

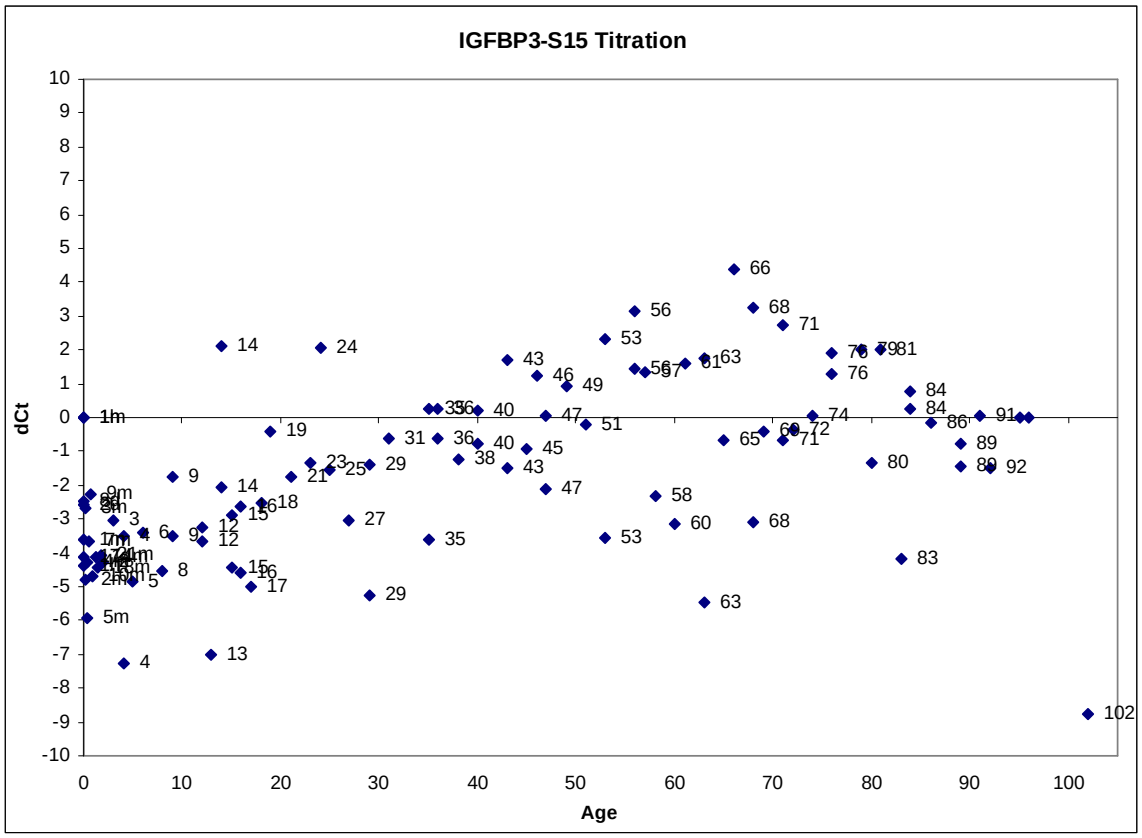
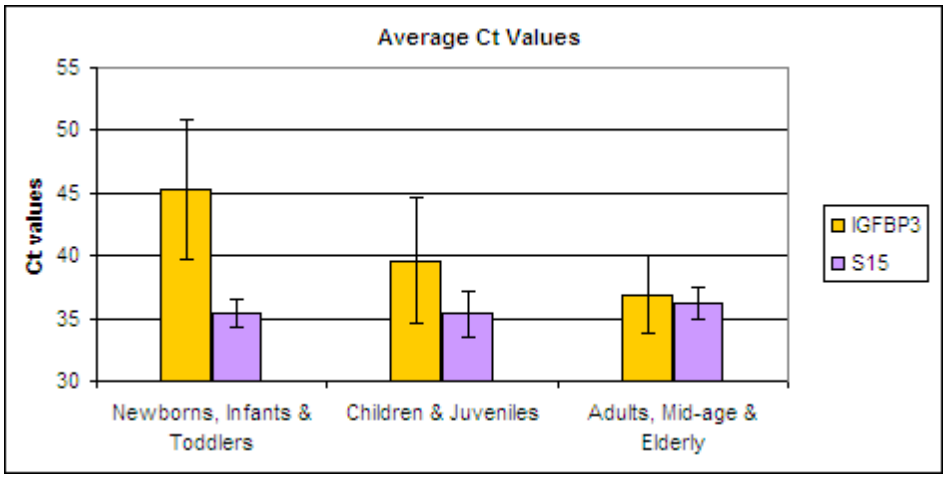


Figure 16: IGFBP3 Real-Time PCR Duplex Delta Ct Results.

Average of all Samples per age group

Age Group	Average (GOI)	SD (GOI)	Average (S15)	SD (S15)	Average (dCt)	SD (dCt)	n=
Newborns, Infants & Toddlers	45.298	5.576	35.384	1.131	-9.914	5.402	33
Children & Juveniles	39.604	5.033	35.396	1.818	-4.208	4.260	23
Adults, Mid-age & Elderly	36.914	3.143	36.189	1.331	-0.725	2.637	67



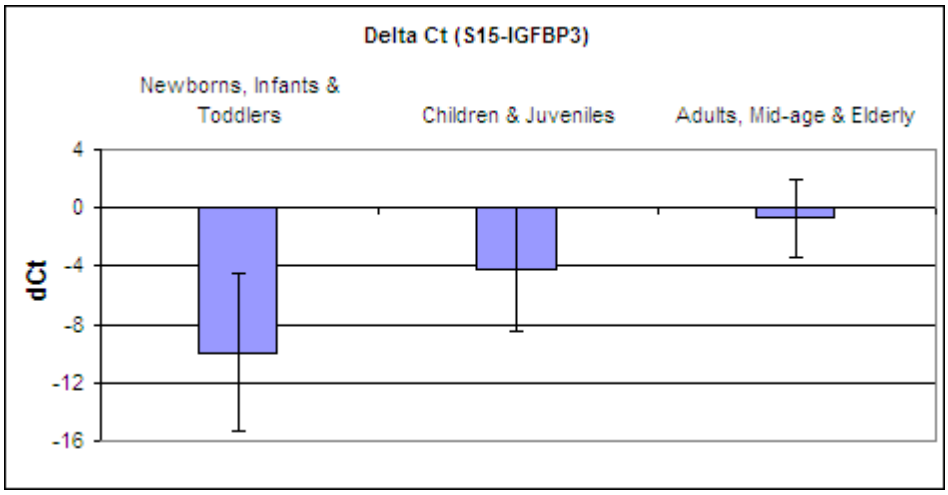


Figure 17: Post-pubertal Candidate IGFBP3 qPCR Duplex Biological Age Specificity.

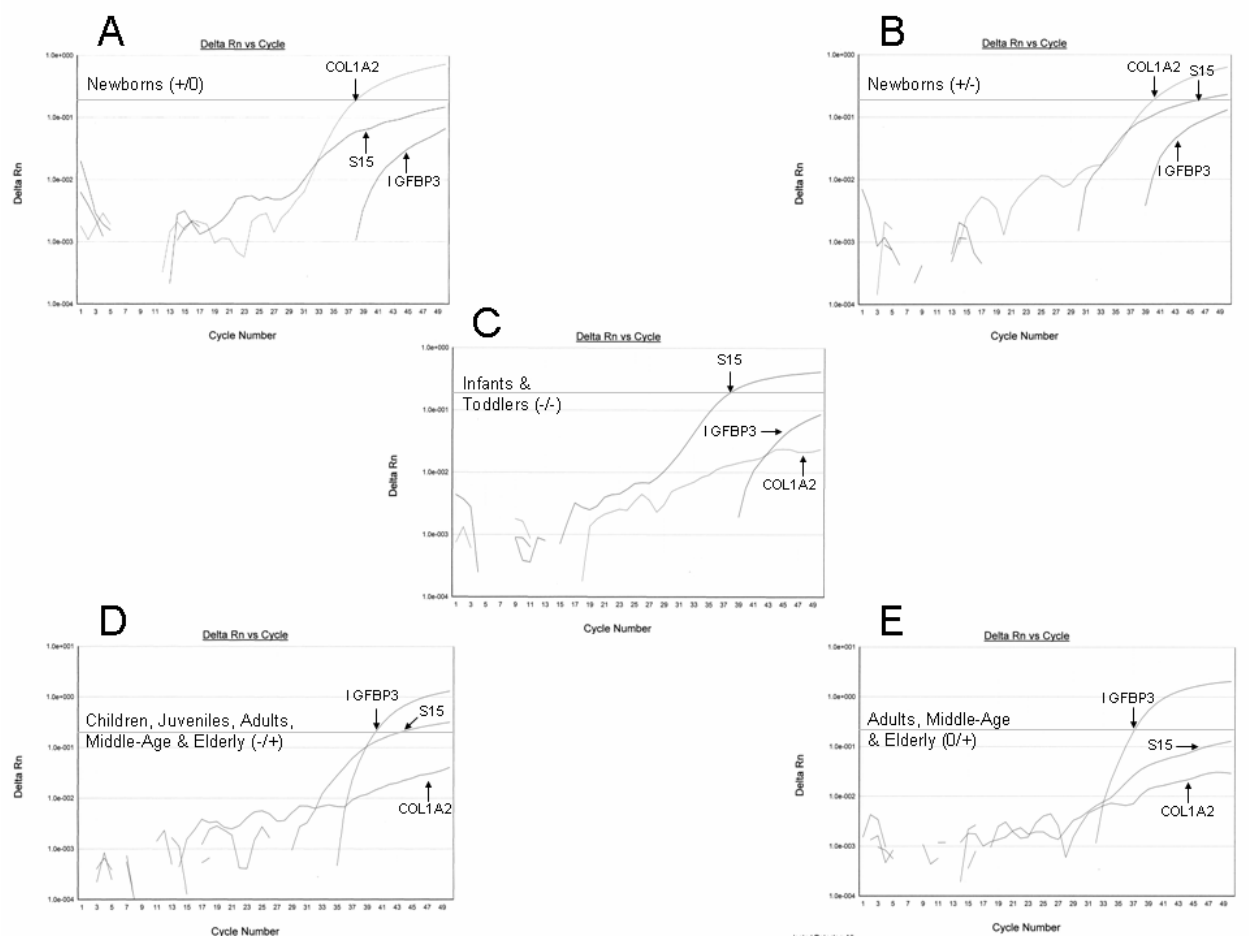
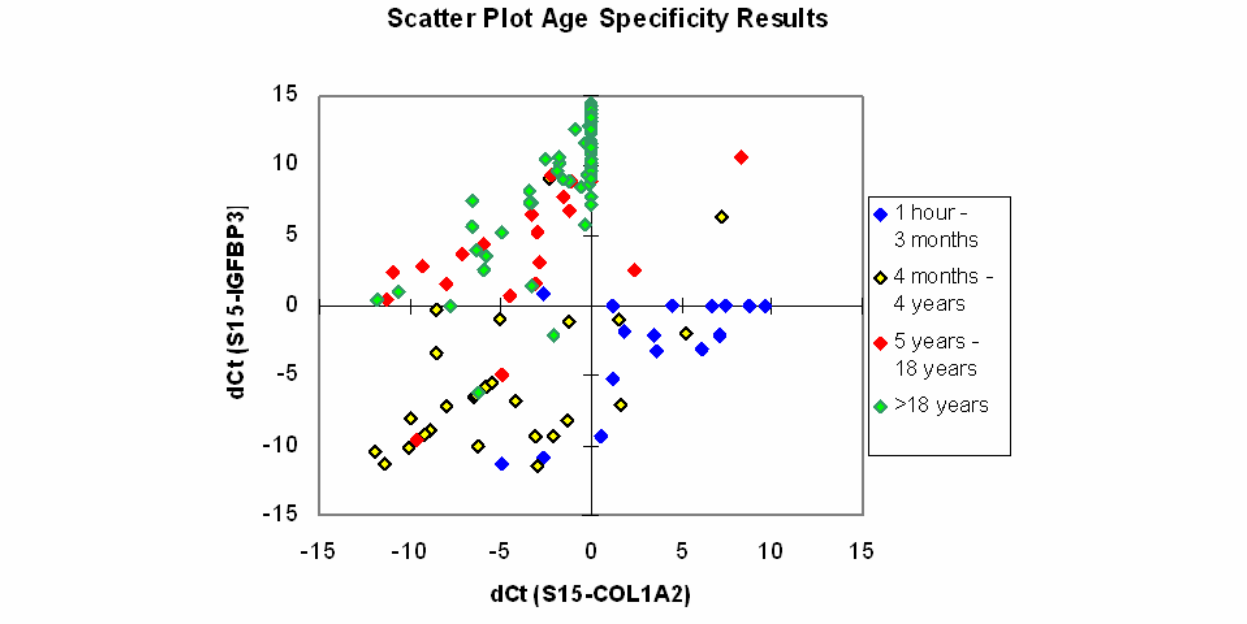


Figure 18: Real-time PCR Triplex for Biological Age Determination.

A



B

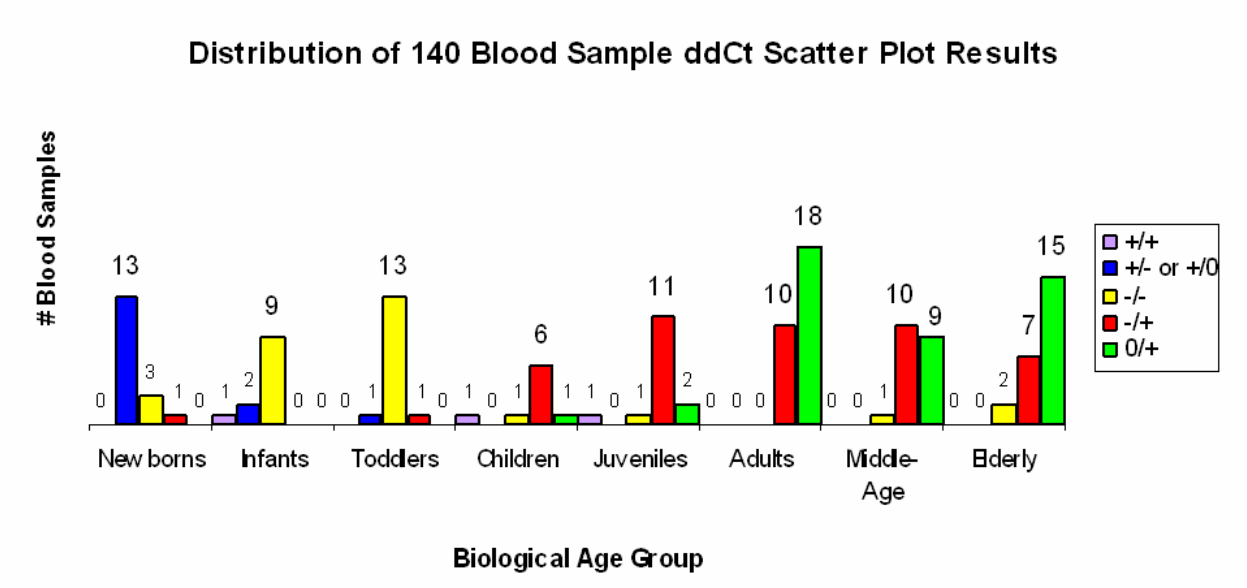


Figure 19: Biological Age Specificity of the Triplex Assay.

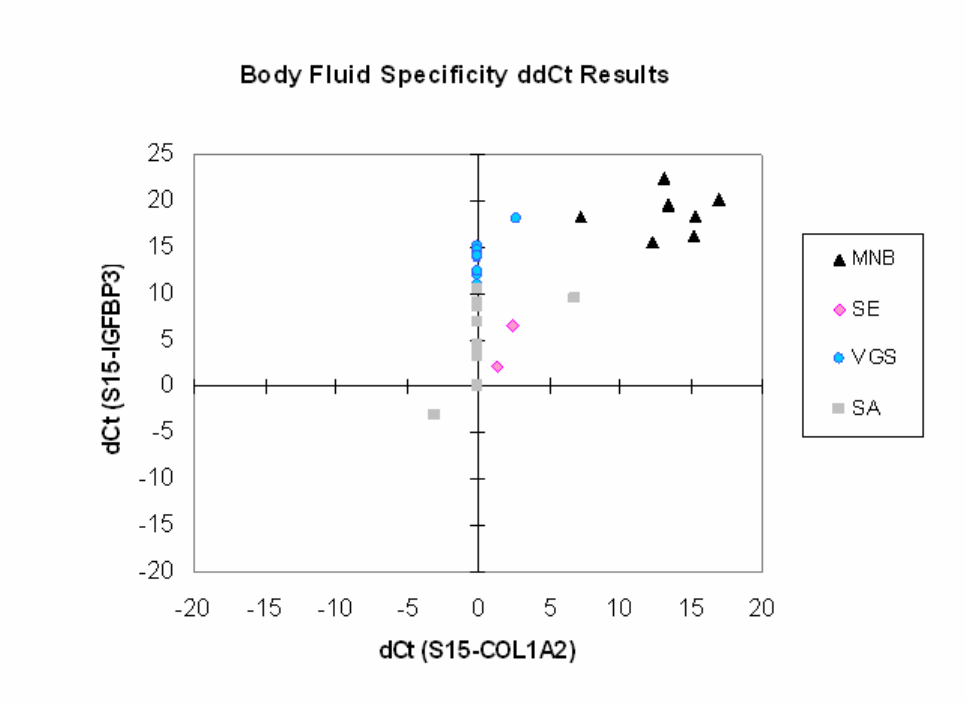


Figure 20: Body Fluid Specificity of the Triplex Assay.

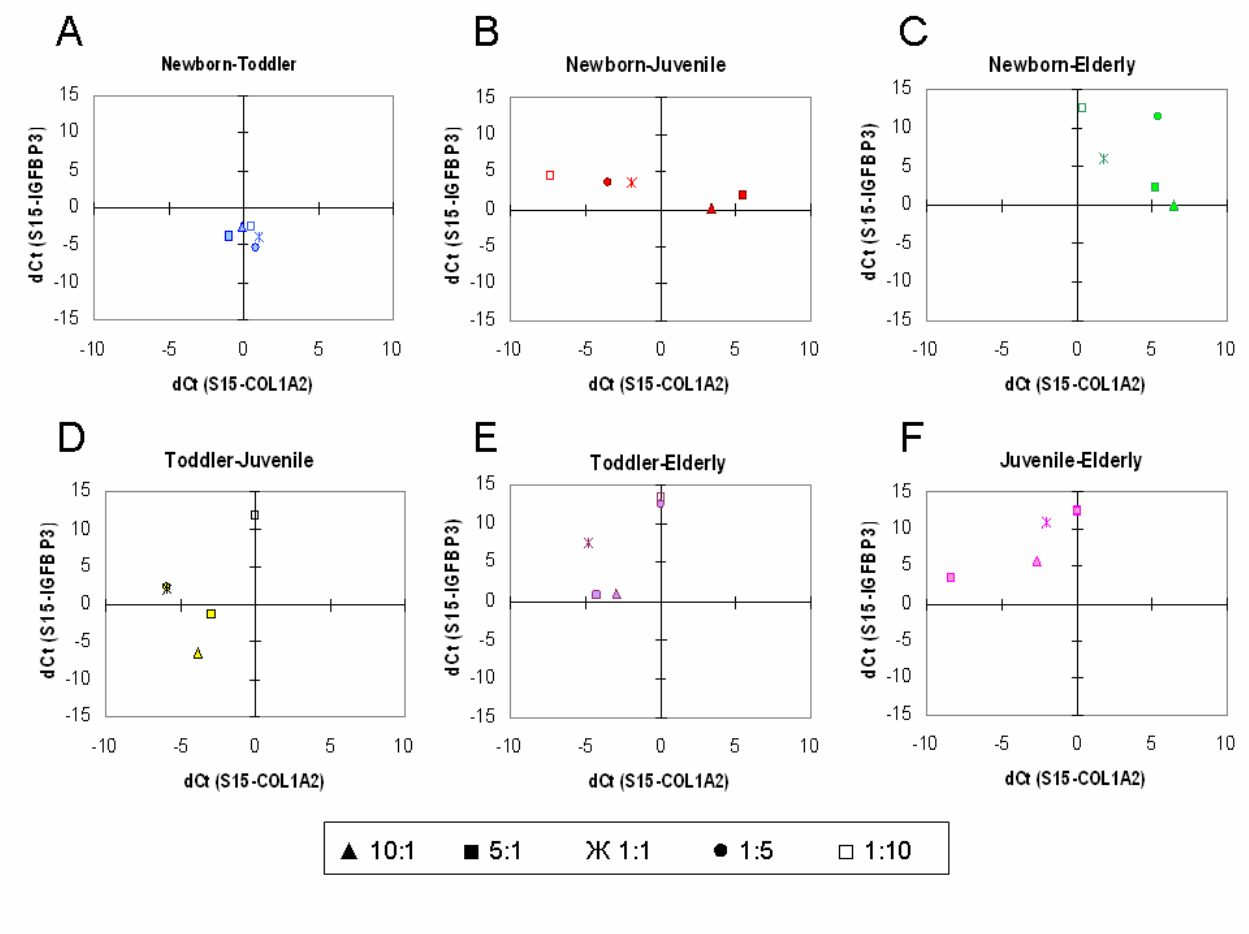


Figure 21: Mixture Study of the qRT-PCR Triplex Assay.

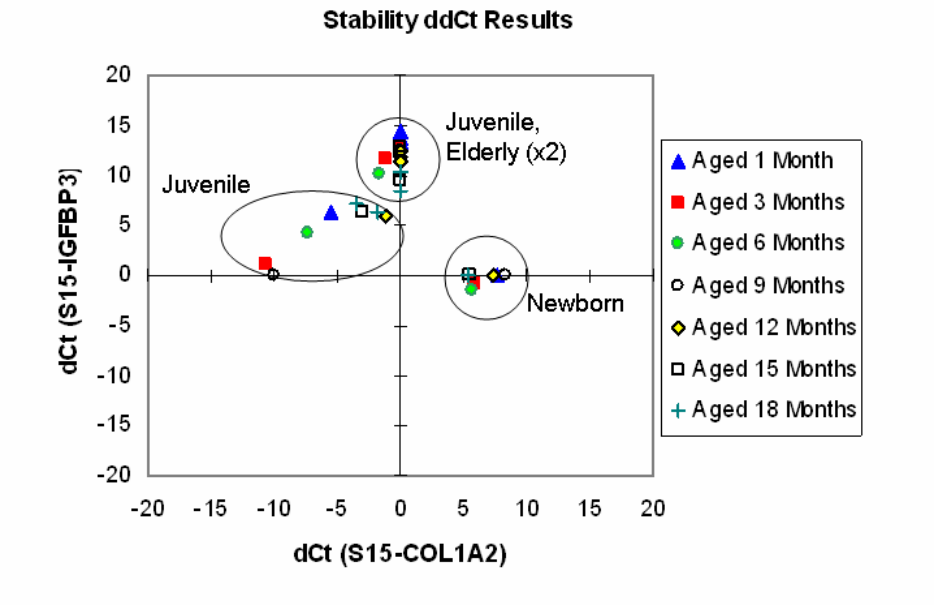


Figure 22: Temporal Stability of the COL1A2, IGFBP3 and S15 transcripts in bloodstains.

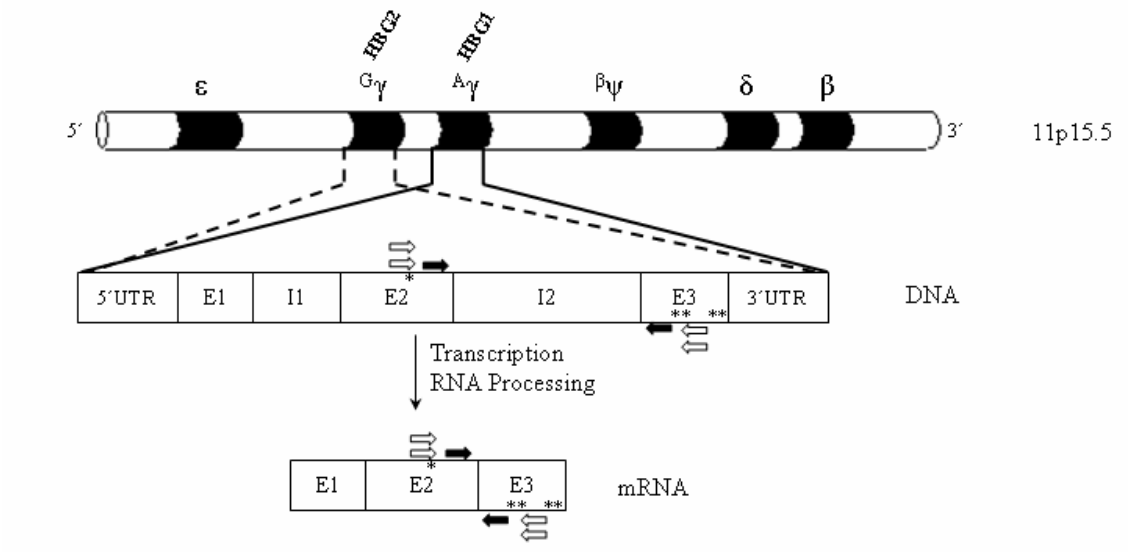


Figure 23: Structure of the Human Beta-Hemoglobin Locus.

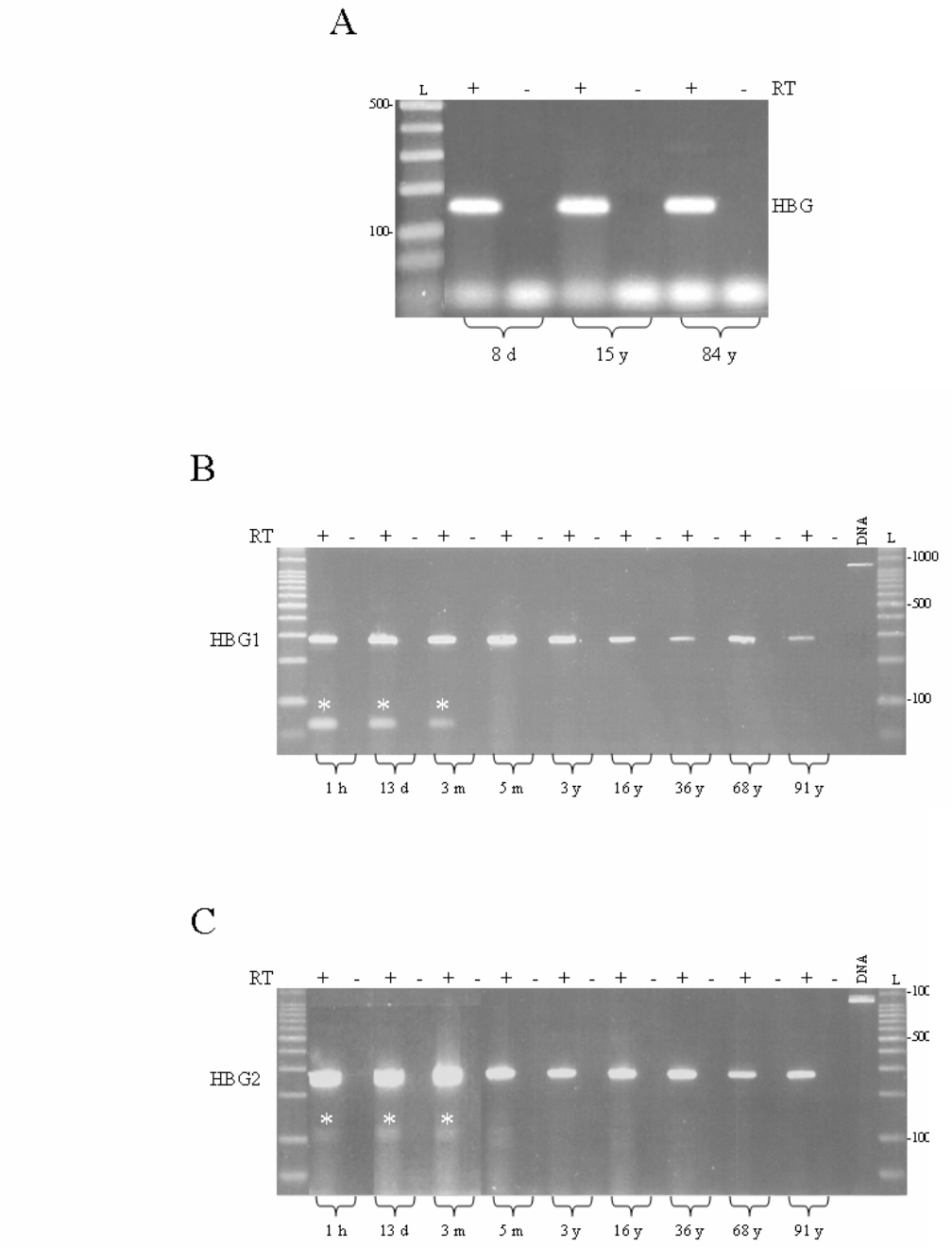


Figure 24: Identification of Gamma Hemoglobin Transcripts in Blood from Different Age Groups.

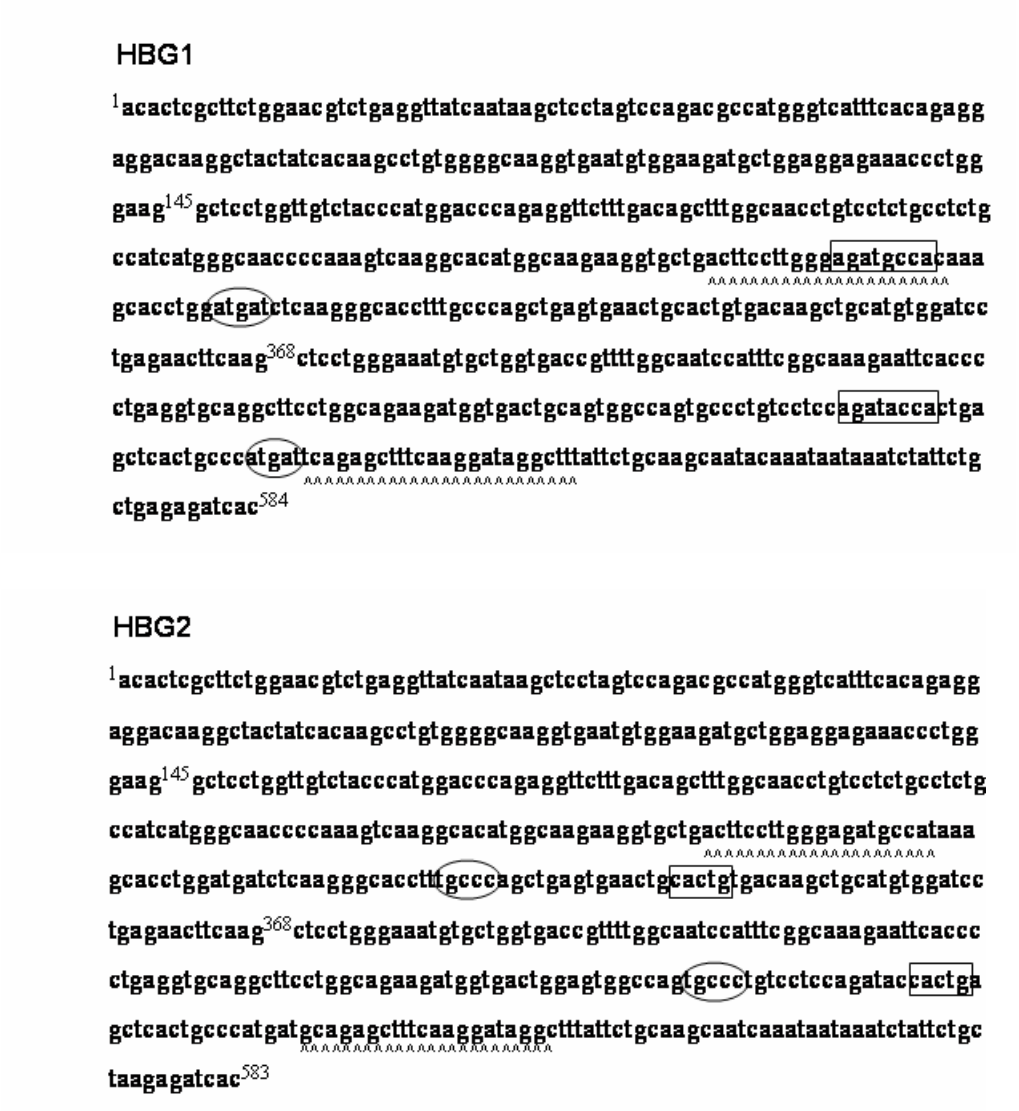


Figure 25: Standard mRNA Hemoglobin Sequences Identifying Newborn Specific Breakpoints.

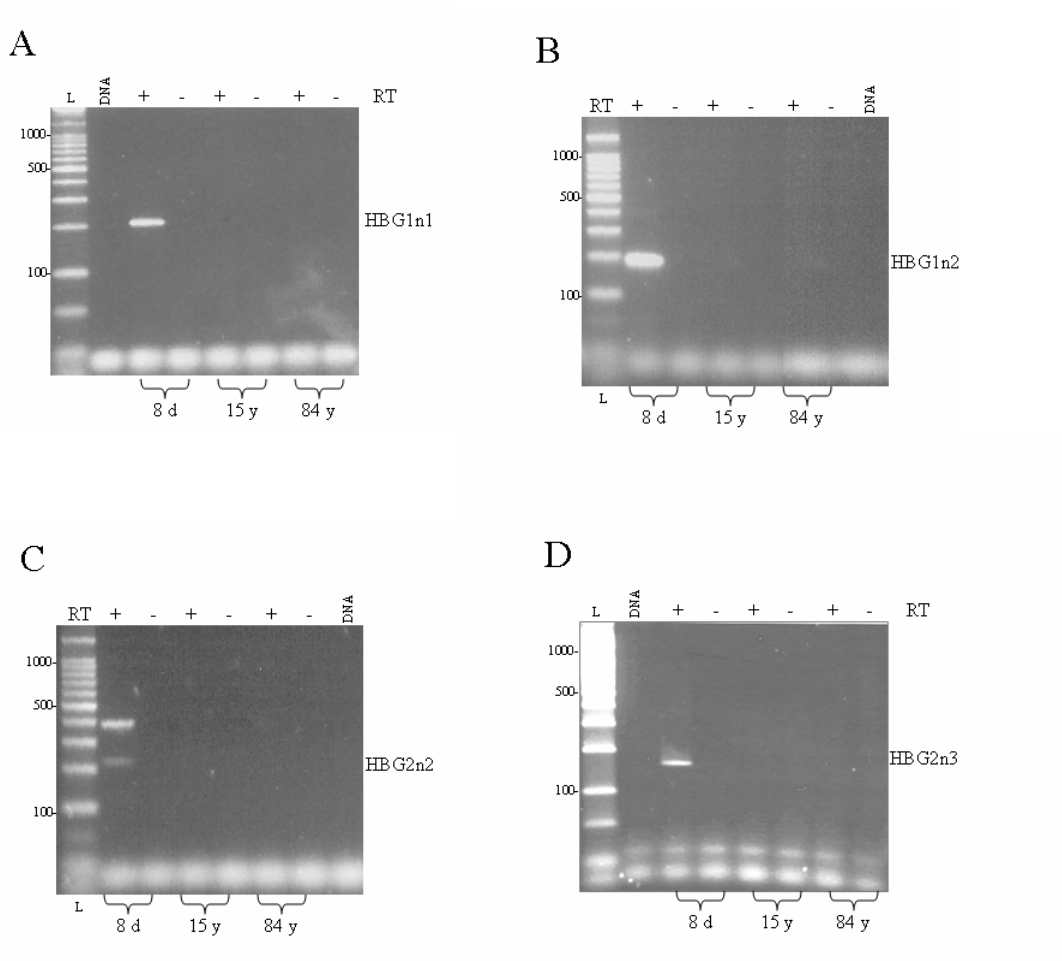


Figure 26: RT-PCR Amplification of Four Newborn-Specific Gene Transcripts.

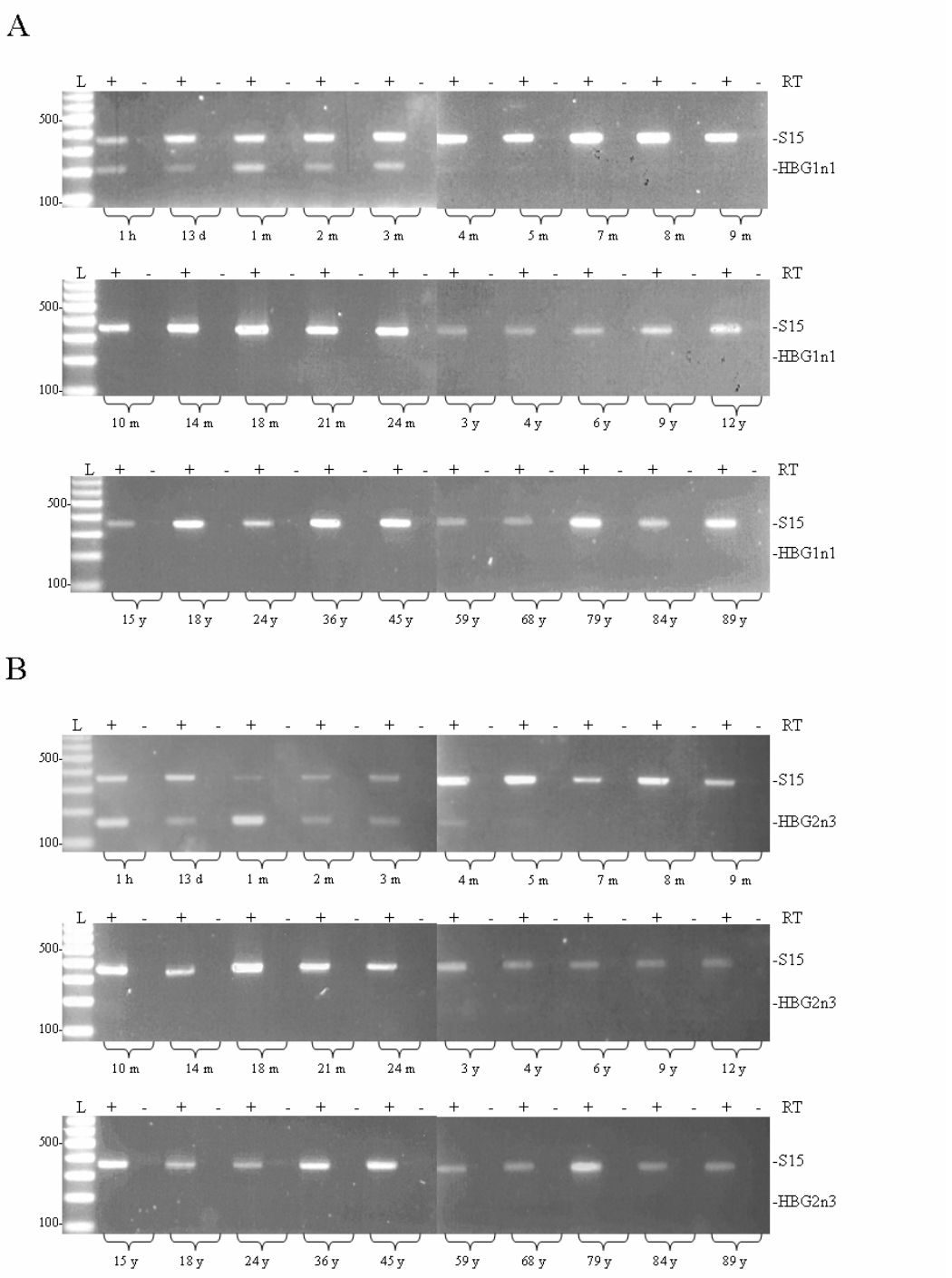


Figure 27: RT-PCR Based Age Specificity of the HBG1n1 and HBG2n3 Transcripts.

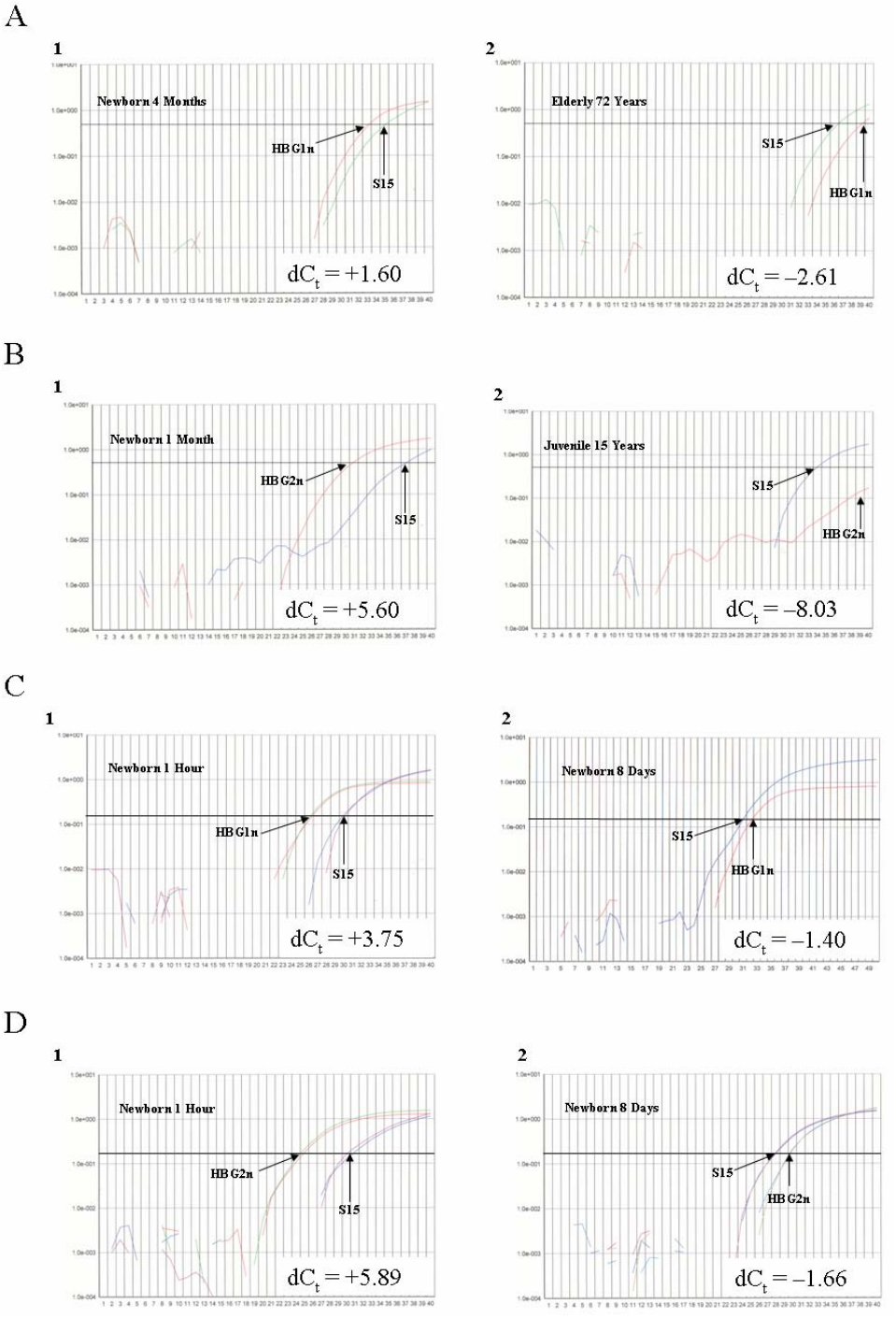


Figure 28: Quantitative Real-Time PCR Assays for the Identification of Newborns.

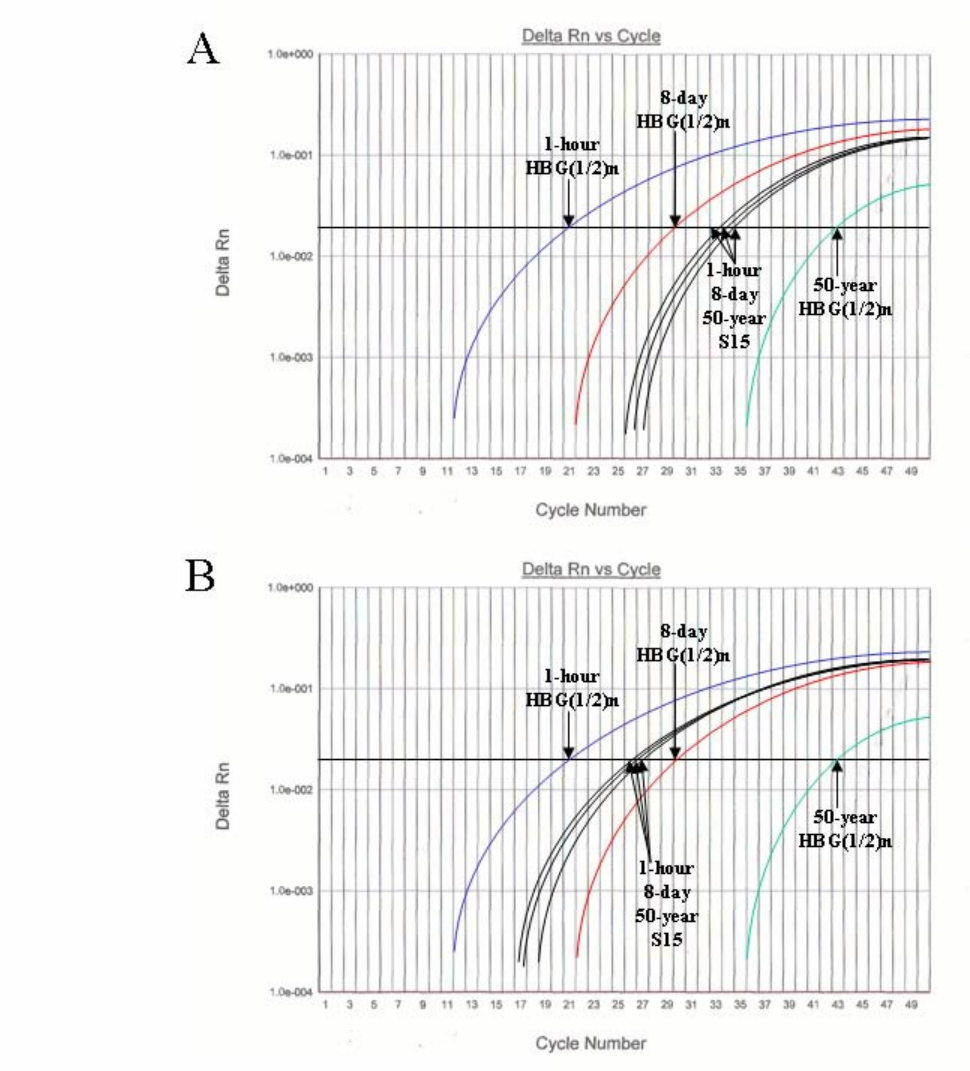
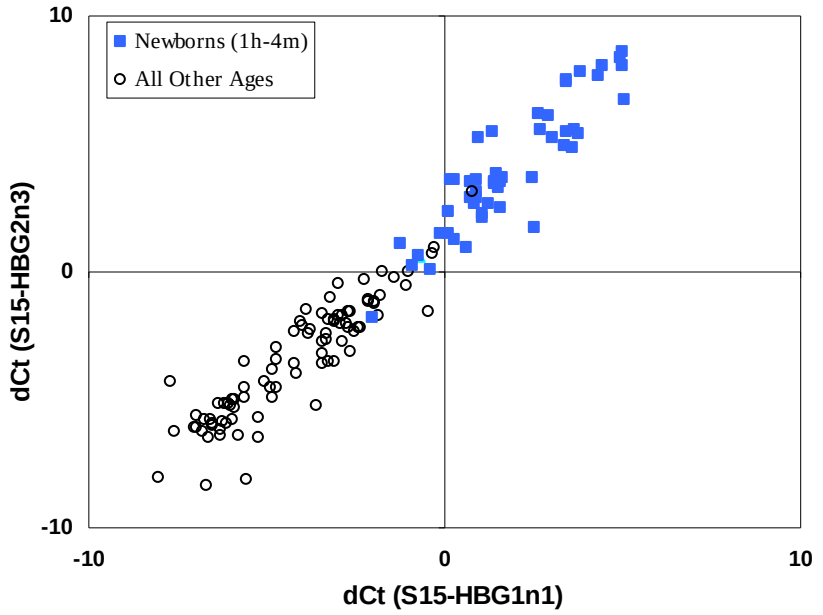


Figure 29: Delta Cycle Threshold Determination for Both Newborn Specific qPCR Assays.

A



B

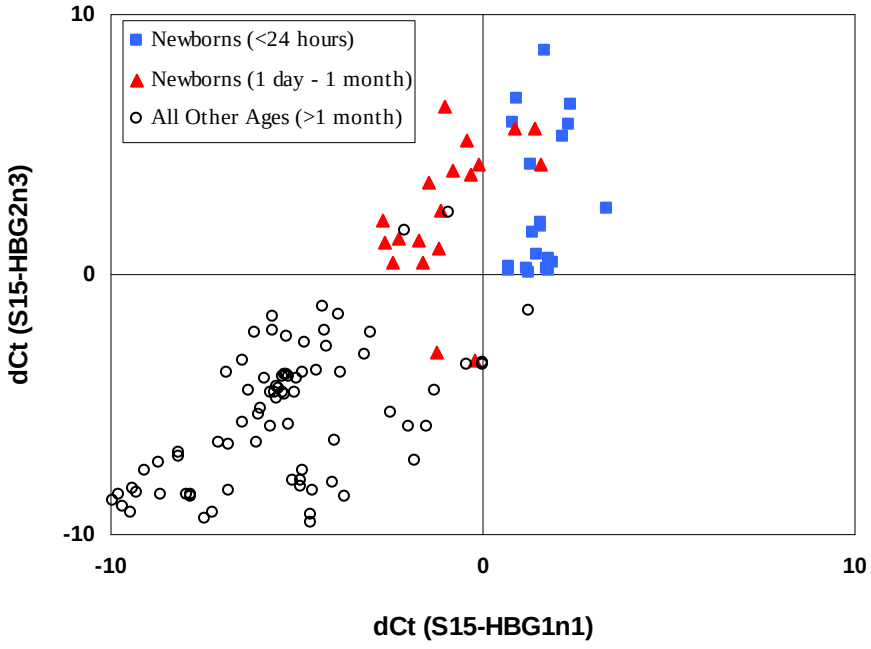
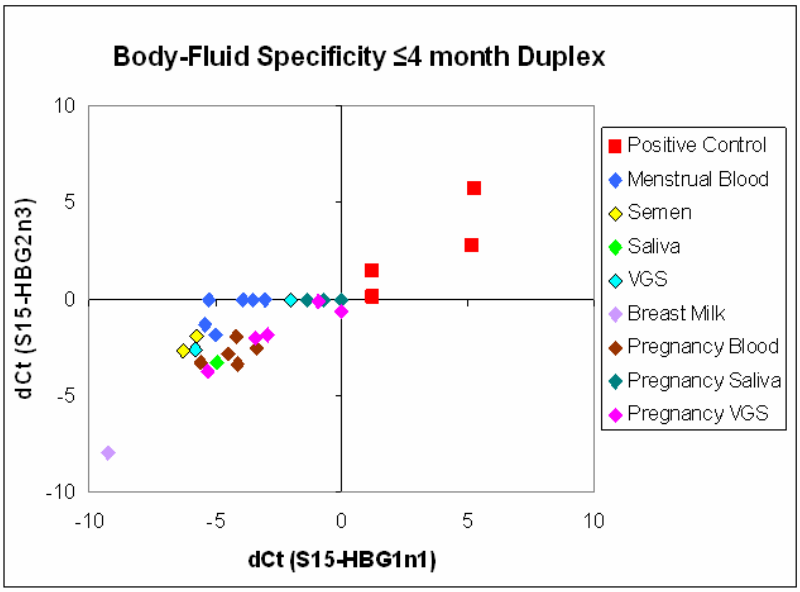


Figure 30: Biological Age Specificity of the HBG1n1 and HBG2n3 qRT-PCR Assays.

A



B

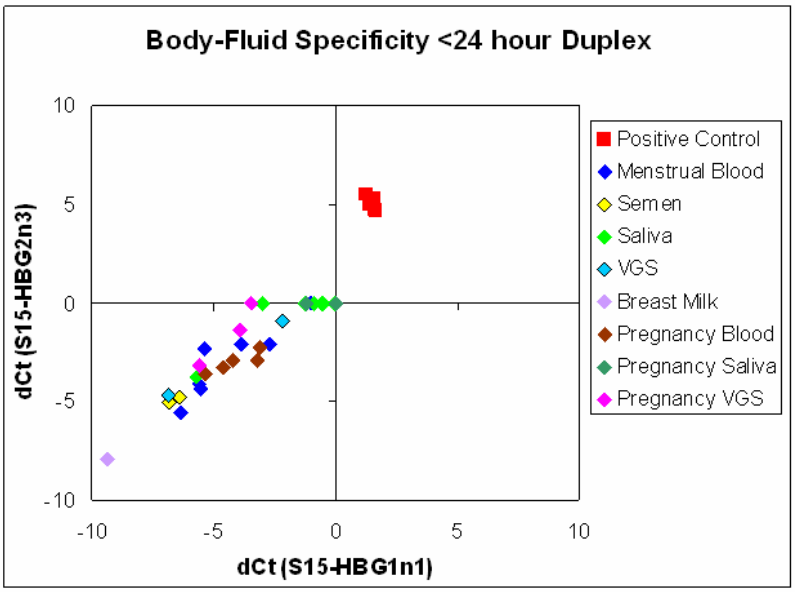
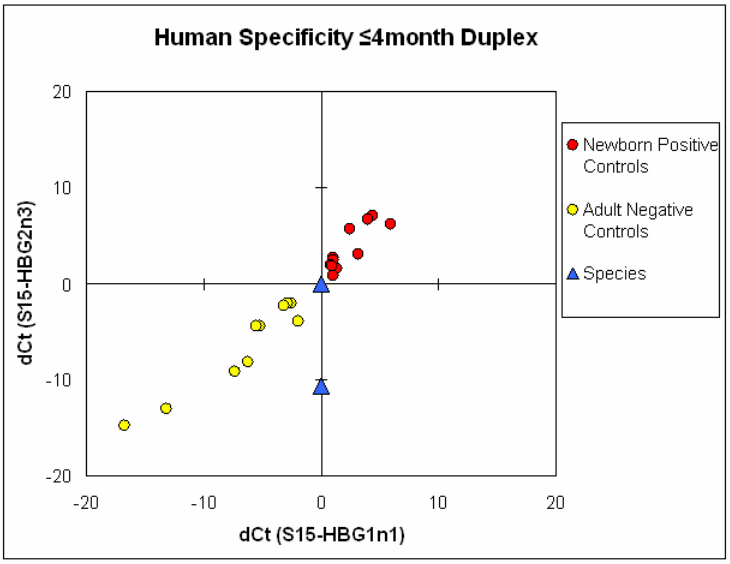


Figure 31: Body-Fluid Specificity for the Newborn Duplex Assays.

A



B

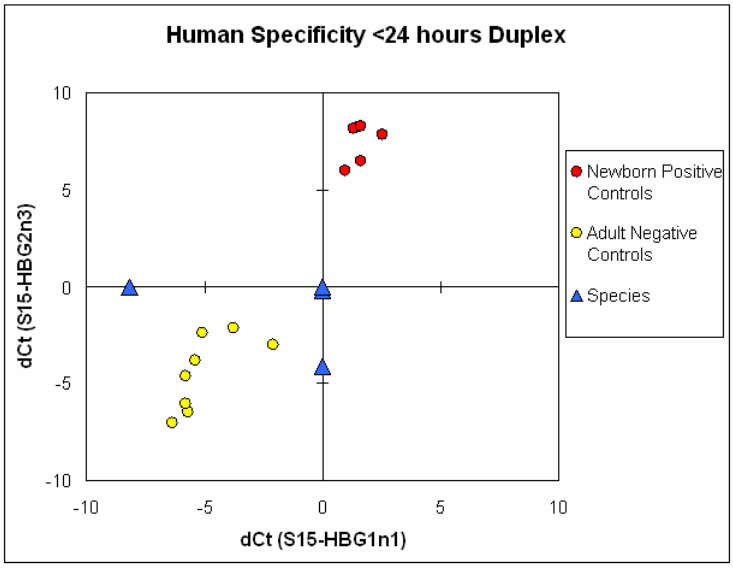
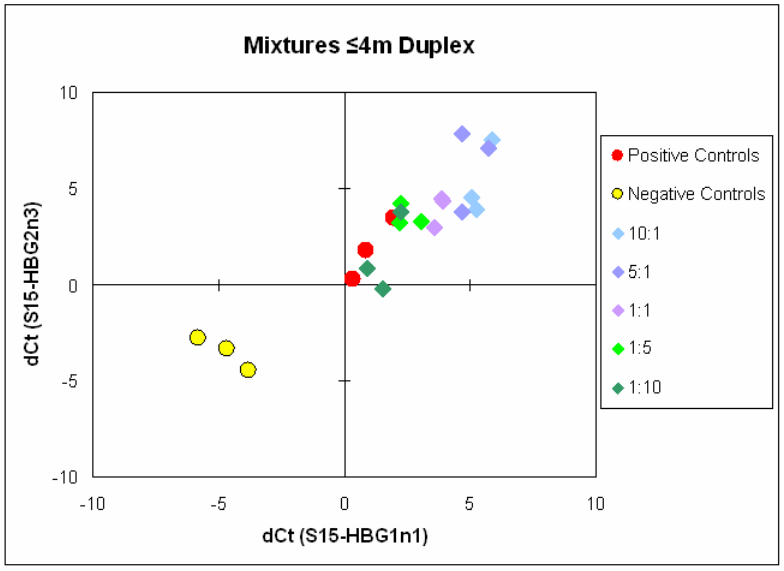


Figure 32: Human Specificity for the qPCR Newborn Duplexes

A



B

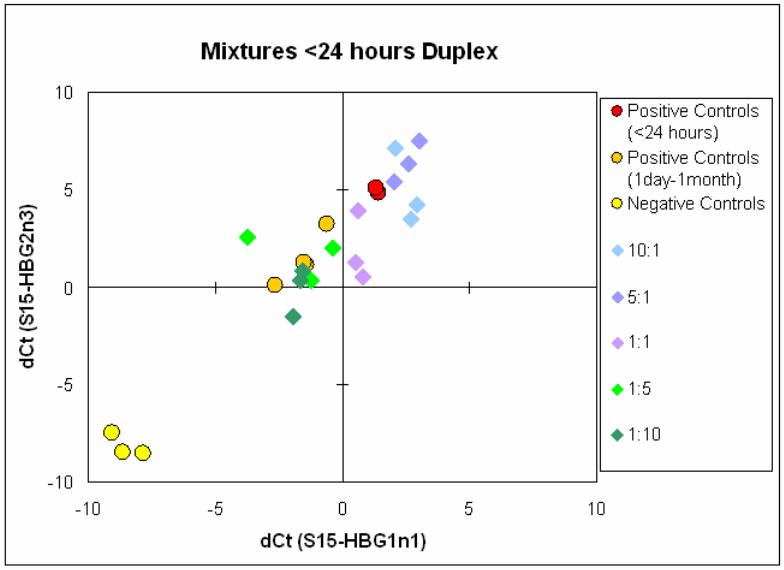


Figure 33: Mixture Study for qPCR Newborn Duplexes.

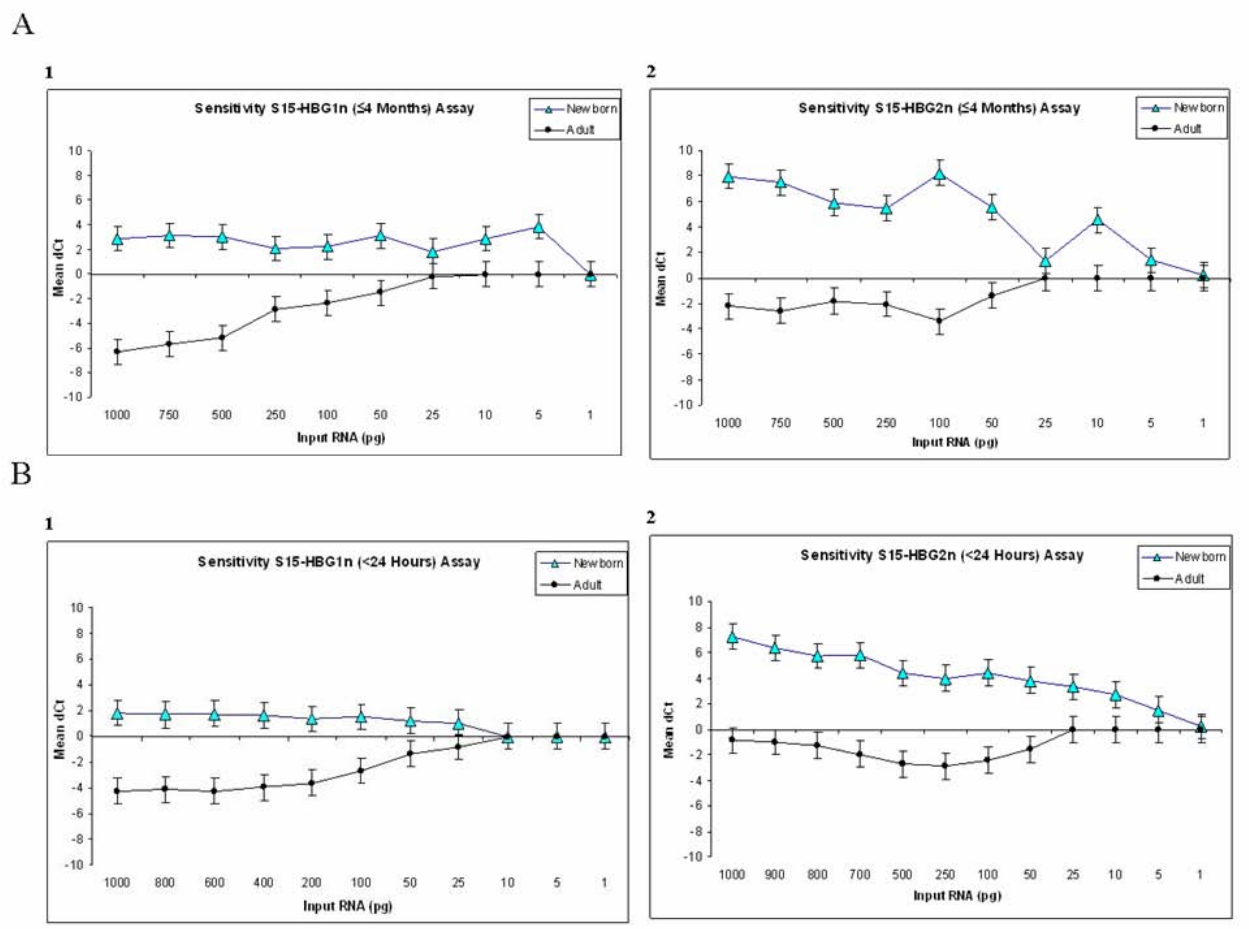


Figure 34: Sensitivity of the HBG1n1 and HBG2n3 qRT-PCR Assay.

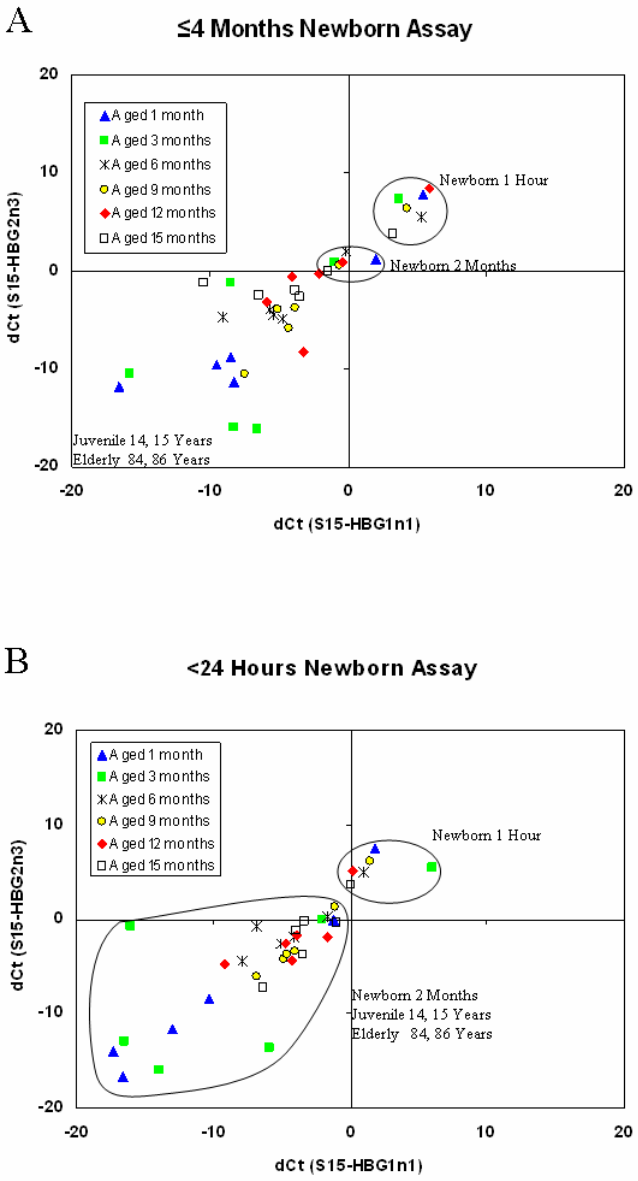


Figure 35: Temporal Stability of the HBG1n1 and HBG2n3 Transcripts in Bloodstains.

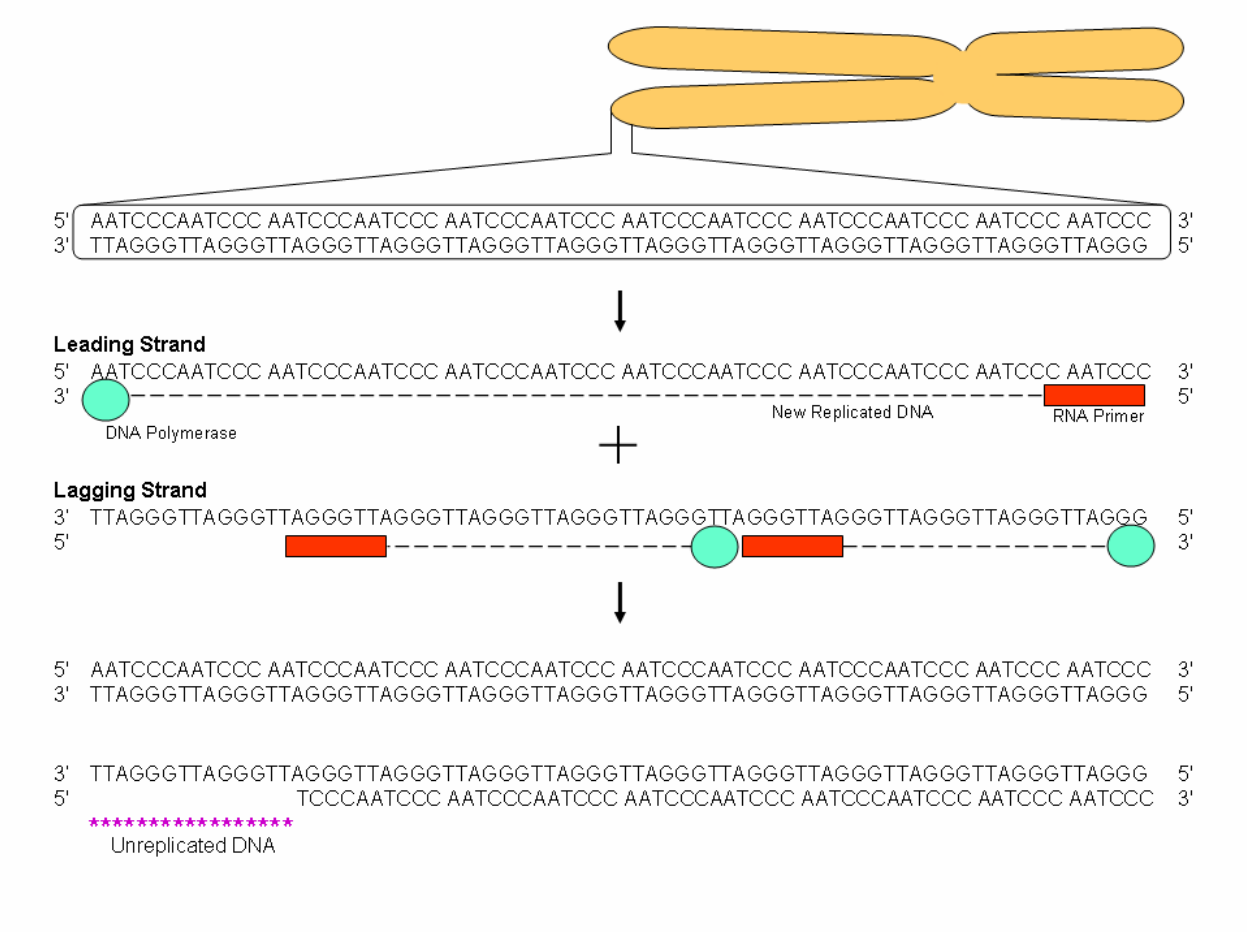
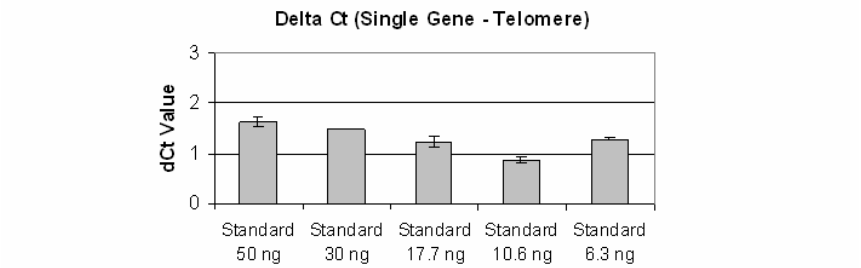
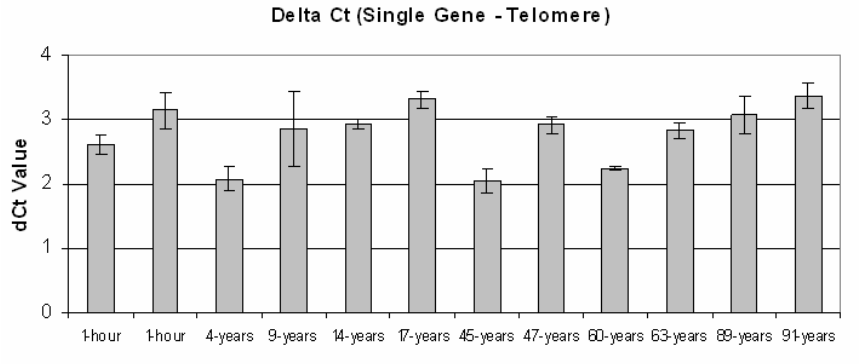


Figure 36: "End Replication Problem" of Telomeres.

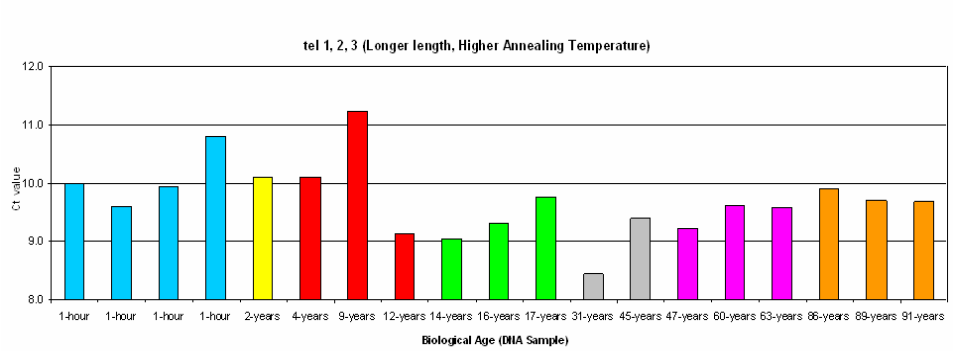


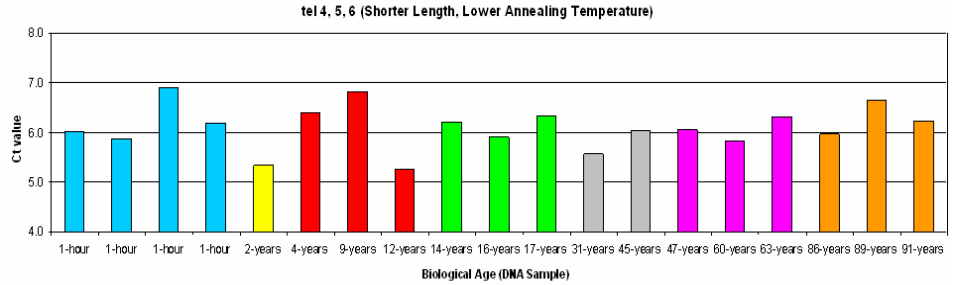


DNA Sample	Total DNA added to reaction (ng)	Reaction volume (uL)	Average Single Copy Gene Ct value (S)*	Average Telomere Ct value (T)	Delta Ct Value (S – T)	±SD
Standards						
STD1	50.0	25.0	22.405	20.785	1.620	0.085
STD2	30.0	25.0	23.270	21.780	1.490	0.000
STD3	17.7	25.0	24.085	22.850	1.235	0.092
STD4	10.6	25.0	25.045	24.170	0.875	0.064
STD5	6.3	25.0	26.065	24.775	1.290	0.014
Unknowns						
1-hour	20.0	25.0	27.520	24.915	2.605	0.148
1-hour	20.0	25.0	26.785	23.650	3.135	0.290
4-years	20.0	25.0	27.320	25.240	2.080	0.184
9-years	20.0	25.0	32.240	29.395	2.845	0.587
14-years	20.0	25.0	27.490	24.570	2.920	0.071
17-years	20.0	25.0	28.765	25.455	3.310	0.127
45-years	20.0	25.0	27.410	25.360	2.050	0.198
47-years	20.0	25.0	27.480	24.555	2.925	0.134
60-years	20.0	25.0	27.955	25.705	2.250	0.028
63-years	20.0	25.0	26.580	23.745	2.835	0.120
89-years	20.0	25.0	28.630	25.555	3.075	0.290
91-years	20.0	25.0	27.900	24.540	3.360	0.198

*The single copy gene is the acidic ribosomal phosphoprotein PO gene, 36B4

Figure 37: Telomere Delta Cycle Threshold Determination by Real-time PCR.





DNA Sample	Total DNA added to reaction (ng)	Reaction volume (uL)	tel 1, 2, 3 Ct value	tel 4, 5, 6 Ct value
1-hour	10.0	25.0	9.992	6.018
1-hour	10.0	25.0	9.587	5.872
1-hour	10.0	25.0	9.945	6.911
1-hour	10.0	25.0	10.811	6.189
2-years	10.0	25.0	10.096	5.348
4-years	10.0	25.0	10.098	6.391
9-years	10.0	25.0	11.243	6.805
12-years	10.0	25.0	9.115	5.261
14-years	10.0	25.0	9.040	6.223
16-years	10.0	25.0	9.307	5.901
17-years	10.0	25.0	9.760	6.321
31-years	10.0	25.0	8.438	5.560
45-years	10.0	25.0	9.397	6.038
47-years	10.0	25.0	9.215	6.058
60-years	10.0	25.0	9.616	5.822
63-years	10.0	25.0	9.579	6.298
86-years	10.0	25.0	9.893	5.958
89-years	10.0	25.0	9.704	6.645
91-years	10.0	25.0	9.687	6.244

Figure 38: Quantitative Amplification of Telomeres using TaqMan Real-time PCR.

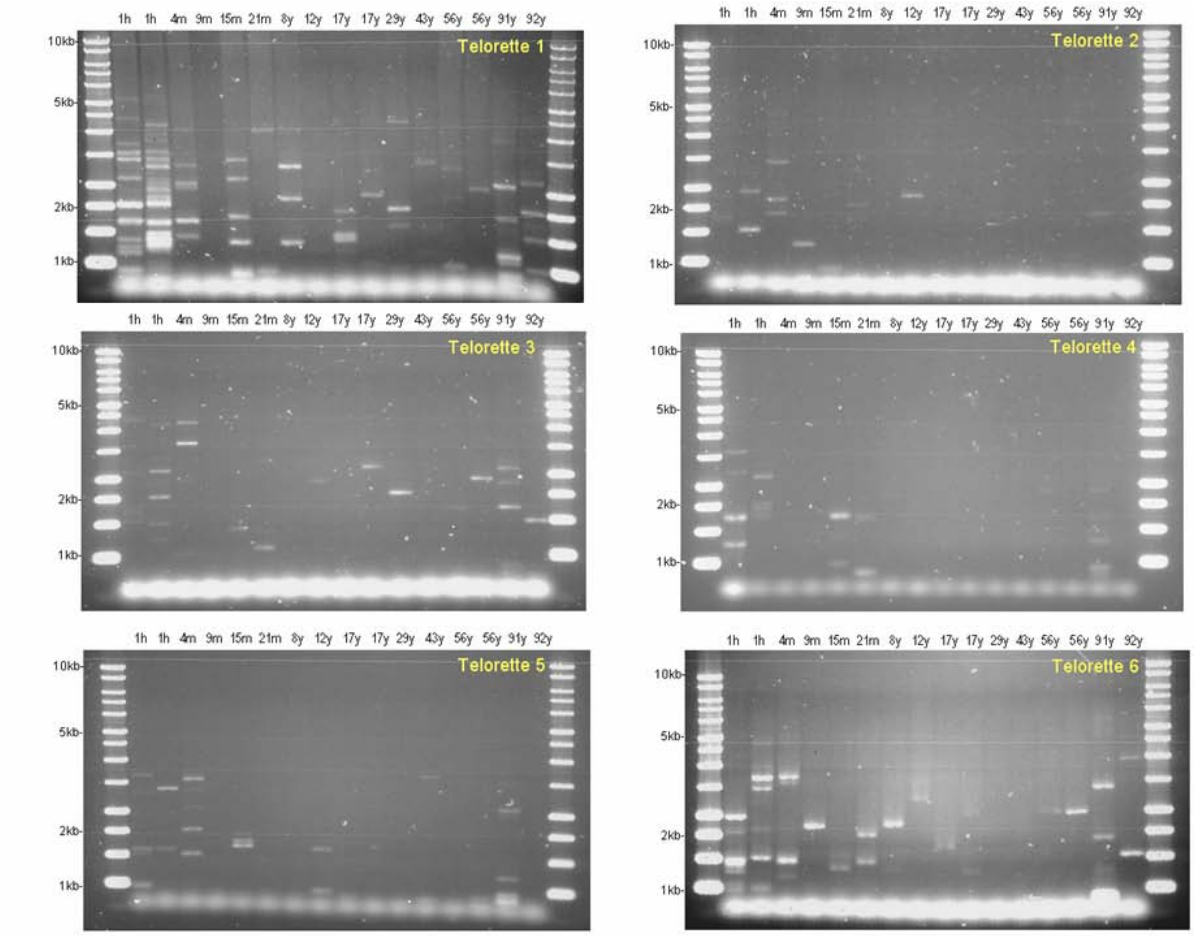


Figure 39: STELA Telomere Amplification.

APPENDIX B: TABLES

Table 1: Summary of Results from RT-PCR mRNA Profiling Analysis.

Target Age Group	Candidates Tested	Accepted Candidates		Rejected Candidates	
		#	%	#	%
Newborns	16	7	43.75	9	56.25
Juveniles	43	5	11.63	38	88.37
Adults	6	0	0.00	6	100.00
Elderly	143	8	5.59	135	94.41
		26	8.15	293	91.85

Table 2: Summary and Explanation of Rejected Candidates from RT-PCR Analysis.

Target Age Group	Total Candidates Tested	Number Rejected Candidates	Rejected Explaination							
			No mRNA Detected		Same Size mRNA and DNA		mRNA Expressed in All Ages		mRNA Expressed Sporadically	
			#	%	#	%	#	%	#	%
Newborns	16	9	3	33.3	0	0.0	3	33.3	3	33.3
Juveniles	43	38	19	50.0	3	7.9	3	7.9	13	34.2
Adults	6	6	0	0.0	0	0.0	4	66.7	2	33.3
Elderly	143	135	27	20.0	0	0.0	81	60.0	27	20.0
			49	15.4	3	0.9	91	28.5	45	14.1

Table 3: COL1A2 Real-Time PCR Singleplex Candidate Results.

COL1A2-Initial Amplification

Sample #	Sex	Age	Ct value
Newborn +RT	M	1h	38.791
Newborn -RT			Undet
Newborn +RT	F	17d	36.887
Newborn -RT			Undet
Newborn +RT	F	1m	41.468
Newborn -RT			Undet
Newborn +RT	F	3m	37.954
Newborn -RT			Undet
Infant +RT	F	5m	50.000
Infant -RT			Undet
Infant +RT	M	8m	45.571
Infant -RT			Undet
Toddler +RT	F	14m	50.000
Toddler -RT			Undet
Toddler +RT	F	19m	40.946
Toddler -RT			Undet
Toddler +RT	M	2.8y	50.000
Toddler -RT			Undet
Child +RT	M	4y	38.423
Child -RT			Undet
Child +RT	F	4y	46.277
Child -RT			Undet

COL1A2-Initial Amplification

Sample #	Sex	Age	Ct value
Child +RT	F	6y	50.000
Child -RT			Undet
Child +RT	F	12y	41.147
Child -RT			Undet
Juvenile +RT	M	18y	50.000
Juvenile -RT			Undet
Adult +RT	M	27y	50.000
Adult -RT			Undet
Adult +RT	M	40y	50.000
Adult -RT			Undet
Middle-Age +RT	M	51y	50.000
Middle-Age -RT			Undet
Middle-Age +RT	M	61y	50.000
Middle-Age -RT			Undet
Elderly +RT	M	76y	50.000
Elderly -RT			Undet
Elderly +RT	M	86y	50.000
Elderly -RT			Undet
DNA			Undet
DNA			Undet
NTC			Undet
NTC			Undet

Table 4: COL1A2 Real-Time PCR Duplex Delta Ct Results.

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	45.256	50.000	4.744	Adult +RT	36y	50.000	48.825	-1.175
Newborn +RT	1h	40.197	50.000	9.803	Adult +RT	36y	50.000	43.970	-6.030
Newborn +RT	2d	44.296	50.000	5.704	Adult +RT	38y	42.611	39.645	-2.966
Newborn +RT	8d	36.656	50.000	13.344	Adult +RT	40y	50.000	41.516	-8.484
Newborn +RT	17d	41.478	48.714	7.236	Adult +RT	40y	50.000	40.792	-9.208
Newborn +RT	1m	39.251	47.951	8.700	Adult +RT	43y	50.000	41.325	-8.675
Newborn +RT	1m	50.000	50.000	0.000	Adult +RT	43y	50.000	42.427	-7.573
Newborn +RT	2m	40.341	46.056	5.715	Adult +RT	45y	50.000	38.497	-11.503
Newborn +RT	3m	38.706	47.180	8.474	Elderly +RT	46y	50.000	40.595	-9.405
Infant +RT	4m	43.222	43.090	-0.132	Elderly -RT	47y	41.897	43.741	1.844
Infant +RT	5m	43.497	41.358	-2.139	Middle-Age +RT	47y	50.000	38.891	-11.109
Infant +RT	7m	43.332	42.439	-0.893	Middle-Age +RT	49y	50.000	44.039	-5.961
Infant +RT	9m	50.000	50.000	0.000	Middle-Age +RT	51y	50.000	41.160	-8.840
Toddler +RT	10m	41.271	48.489	7.218	Middle-Age +RT	53y	50.000	40.988	-9.012
Toddler +RT	14m	50.000	45.904	-4.096	Middle-Age +RT	53y	50.000	50.000	0.000
Toddler +RT	18m	50.000	43.718	-6.282	Middle-Age +RT	56y	50.000	41.875	-8.125
Toddler +RT	21m	50.000	43.215	-6.785	Middle-Age +RT	56y	50.000	40.611	-9.389
Toddler +RT	2y	50.000	42.368	-7.632	Middle-Age +RT	57y	50.000	41.501	-8.499
Toddler +RT	3y	50.000	41.687	-8.313	Middle-Age +RT	58y	50.000	40.335	-9.665
Child +RT	4y	41.864	39.589	-2.275	Middle-Age +RT	60y	50.000	42.757	-7.243
Child +RT	4y	40.689	42.658	1.969	Middle-Age +RT	61y	43.118	50.000	6.882
Child +RT	5y	39.318	39.088	-0.230	Middle-Age +RT	63y	50.000	38.031	-11.969
Child +RT	6y	50.000	40.970	-9.030	Middle-Age +RT	63y	42.383	36.639	-5.744
Child +RT	8y	44.289	50.000	5.711	Elderly +RT	65y	50.000	42.035	-7.965
Child +RT	9y	50.000	50.000	0.000	Elderly +RT	66y	50.000	47.742	-2.258
Child +RT	9y	50.000	46.608	-3.392	Elderly +RT	68y	42.262	37.598	-4.664
Child +RT	12y	50.000	46.480	-3.520	Elderly +RT	68y	50.000	44.386	-5.614
Child +RT	12y	50.000	42.175	-7.825	Elderly +RT	69y	50.000	41.053	-8.947
Juvenile +RT	13y	43.040	41.343	-1.697	Elderly +RT	71y	46.120	50.000	3.880
Juvenile +RT	14y	50.000	35.288	-14.712	Elderly +RT	71y	50.000	41.181	-8.819
Juvenile +RT	14y	45.600	40.692	-4.908	Elderly +RT	72y	50.000	50.000	0.000
Juvenile +RT	15y	41.540	39.939	-1.601	Elderly +RT	74y	50.000	41.608	-8.392
Juvenile +RT	15y	50.000	38.983	-11.017	Elderly +RT	76y	43.599	40.798	-2.801
Juvenile +RT	16y	50.000	39.755	-10.245	Elderly +RT	76y	50.000	40.631	-9.369
Juvenile +RT	16y	50.000	39.832	-10.168	Elderly +RT	79y	50.000	50.000	0.000
Juvenile +RT	17y	50.000	40.995	-9.005	Elderly +RT	80y	50.000	38.305	-11.695
Juvenile +RT	18y	42.170	46.590	4.420	Elderly +RT	81y	50.000	42.766	-7.234
Adult +RT	19y	50.000	43.591	-6.409	Elderly +RT	83y	50.000	45.156	-4.844
Adult +RT	21y	50.000	43.065	-6.935	Elderly +RT	84y	50.000	38.506	-11.494
Adult +RT	23y	50.000	50.000	0.000	Elderly +RT	84y	50.000	50.000	0.000
Adult +RT	24y	50.000	39.744	-10.256	Elderly +RT	86y	46.010	50.000	3.990
Adult +RT	25y	50.000	40.392	-9.608	Elderly +RT	89y	50.000	43.349	-6.651
Adult +RT	27y	43.390	42.501	-0.889	Elderly +RT	89y	50.000	42.636	-7.364
Adult +RT	29y	50.000	38.550	-11.450	Elderly +RT	91y	50.000	39.317	-10.683
Adult +RT	29y	50.000	40.104	-9.896	Elderly +RT	92y	50.000	40.486	-9.514
Adult +RT	31y	50.000	39.025	-10.975	Elderly +RT	102y	50.000	50.000	0.000
Adult +RT	35y	50.000	41.378	-8.622	DNA		50.000	50.000	0.000
Adult +RT	35y	50.000	41.799	-8.201	NTC		50.000	50.000	0.000

Table 5: COL1A2 Triplicate qPCR Results.

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Newborn +RT	1h	43.886	1.290	50.000	0.000	6.114	1.290
Newborn +RT	1h	41.552	0.193	50.000	0.000	8.448	0.193
Newborn +RT	1h	42.532	0.790	46.961	1.715	4.429	1.160
Newborn +RT	1h	41.648	1.889	50.000	0.000	8.352	1.889
Newborn +RT	1h	40.896	0.621	50.000	0.000	9.104	0.621
Newborn +RT	1h	43.963	0.384	50.000	0.000	6.037	0.384
Newborn +RT	1h	40.668	0.493	50.000	0.000	9.332	0.493
Newborn +RT	1d	38.139	0.311	50.000	0.000	11.861	0.311
Newborn +RT	2d	43.854	1.189	49.848	0.263	5.994	1.078
Newborn +RT	8d	37.388	0.244	50.000	0.000	12.612	0.244
Newborn +RT	13d	40.033	1.135	50.000	0.000	9.967	1.135
Newborn +RT	17d	40.004	1.114	43.424	2.341	3.420	1.439
Infant +RT	1m	40.222	0.630	50.000	0.000	9.778	0.630
Infant +RT	1m	44.994	4.372	43.056	1.175	-1.938	3.385
Infant +RT	2m	39.650	0.616	48.100	1.286	8.449	1.892
Infant +RT	3m	38.037	0.280	41.981	0.694	3.945	0.431
Infant +RT	3m	40.706	1.246	50.000	0.000	9.294	1.246
Infant +RT	4m	47.282	4.708	44.045	1.226	-3.237	5.204
Infant +RT	4m	39.991	1.852	50.000	0.000	10.009	1.852
Infant +RT	5m	42.121	1.174	45.112	1.271	2.991	0.378
Toddler +RT	6m	50.000	0.000	45.677	1.030	-4.323	1.030
Toddler +RT	7m	50.000	0.000	41.693	1.283	-8.307	1.283
Toddler +RT	7m	50.000	0.000	41.922	1.318	-8.078	1.318
Toddler +RT	7m	42.385	0.670	50.000	0.000	7.615	0.670
Toddler +RT	8m	44.901	4.426	46.547	2.214	1.646	2.356
Toddler +RT	8m	50.000	0.000	47.171	0.518	-2.829	0.518
Toddler +RT	8m	45.496	4.061	42.334	1.998	-3.162	3.044
Toddler +RT	9m	40.543	1.171	48.134	1.807	7.592	2.970
Toddler +RT	9m	50.000	0.000	43.834	0.681	-6.166	0.681
Toddler +RT	10m	50.000	0.000	46.348	2.491	-3.652	2.491
Toddler +RT	10m	39.394	0.536	44.314	0.992	4.920	0.459
Toddler +RT	14m	50.000	0.000	43.159	0.617	-6.841	0.617
Toddler +RT	15m	50.000	0.000	43.057	1.172	-6.943	1.172
Toddler +RT	18m	50.000	0.000	43.093	2.356	-6.907	2.356
Toddler +RT	19m	45.367	4.036	48.678	1.170	3.311	4.813
Toddler +RT	21m	50.000	0.000	43.164	0.770	-6.836	0.770
Toddler +RT	2y	50.000	0.000	42.657	0.418	-7.343	0.418
Toddler +RT	2.8y	50.000	0.000	43.486	2.171	-6.514	2.171
Toddler +RT	3y	41.396	0.547	41.065	0.583	-0.331	1.094
Toddler +RT	3y	50.000	0.000	42.255	0.690	-7.745	0.690

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Child +RT	4y	41.201	1.019	43.054	1.020	1.853	0.395
Child +RT	4y	42.100	0.724	43.757	0.360	1.658	0.618
Child +RT	4y	40.839	0.594	43.962	1.713	3.123	2.140
Child +RT	5y	39.351	0.687	39.363	2.269	0.011	1.961
Child +RT	6y	50.000	0.000	41.293	3.938	-8.707	3.938
Child +RT	8y	44.933	4.405	47.211	2.459	2.278	6.719
Child +RT	9y	50.000	0.000	43.840	0.935	-6.160	0.935
Child +RT	12y	50.000	0.000	45.390	1.553	-4.610	1.553
Child +RT	12y	50.000	0.000	40.897	1.515	-9.103	1.515
Child +RT	12y	50.000	0.000	43.476	0.786	-6.524	0.786
Child +RT	12y	46.861	5.437	41.560	2.573	-5.301	5.369
Juvenile +RT	13y	50.000	0.000	41.881	2.569	-8.119	2.569
Juvenile +RT	13y	50.000	0.000	42.489	0.726	-7.511	0.726
Juvenile +RT	14y	50.000	0.000	42.667	4.225	-7.333	4.225
Juvenile +RT	14y	50.000	0.000	38.952	2.576	-11.048	2.576
Juvenile +RT	14y	50.000	0.000	43.646	1.057	-6.354	1.057
Juvenile +RT	15y	50.000	0.000	42.970	2.163	-7.030	2.163
Juvenile +RT	15y	41.335	1.673	44.333	1.620	2.998	3.282
Juvenile +RT	15y	50.000	0.000	42.818	1.550	-7.182	1.550
Juvenile +RT	16y	45.302	4.078	45.432	1.115	0.130	3.578
Juvenile +RT	17y	50.000	0.000	44.379	2.201	-5.621	2.201
Juvenile +RT	17y	50.000	0.000	44.544	2.009	-5.456	2.009
Juvenile +RT	17y	50.000	0.000	45.018	1.796	-4.982	1.796
Juvenile +RT	18y	50.000	0.000	43.583	1.020	-6.417	1.020
Juvenile +RT	18y	45.147	4.248	43.758	2.022	-1.389	5.280
Adult +RT	19y	48.024	3.423	47.186	2.761	-0.838	6.002
Adult +RT	24y	50.000	0.000	45.658	1.423	-4.342	1.423
Adult +RT	26y	50.000	0.000	44.300	1.074	-5.700	1.074
Adult +RT	29y	50.000	0.000	43.917	2.233	-6.083	2.233
Adult +RT	29y	50.000	0.000	45.175	0.642	-4.825	0.642
Adult +RT	35y	50.000	0.000	44.768	0.613	-5.232	0.613
Adult +RT	35y	50.000	0.000	46.108	1.467	-3.892	1.467
Adult +RT	36y	50.000	0.000	44.930	1.783	-5.070	1.783
Adult +RT	38y	50.000	0.000	41.480	0.415	-8.520	0.415
Adult +RT	38y	50.000	0.000	42.031	1.055	-7.969	1.055
Adult +RT	38y	47.561	4.224	45.297	4.075	-2.264	8.299
Adult +RT	40y	48.983	1.761	37.840	3.453	-11.144	2.237
Adult +RT	40y	50.000	0.000	42.796	0.507	-7.204	0.507
Adult +RT	43y	50.000	0.000	44.101	0.756	-5.899	0.756
Adult +RT	45y	44.696	4.642	42.578	1.399	-2.119	3.873
Adult +RT	45y	50.000	0.000	42.193	0.528	-7.807	0.528
Adult +RT	45y	41.079	0.315	43.518	1.719	2.438	2.023

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Middle-Age +RT	47y	47.656	4.059	42.680	2.010	-4.976	5.906
Middle-Age +RT	51y	50.000	0.000	43.148	1.283	-6.852	1.283
Middle-Age +RT	53y	50.000	0.000	46.940	0.592	-3.060	0.592
Middle-Age +RT	53y	50.000	0.000	43.976	1.983	-6.024	1.983
Middle-Age +RT	56y	50.000	0.000	42.970	1.093	-7.030	1.093
Middle-Age +RT	57y	50.000	0.000	46.294	1.531	-3.706	1.531
Middle-Age +RT	58y	50.000	0.000	42.681	1.219	-7.319	1.219
Middle-Age +RT	59y	50.000	0.000	42.914	0.527	-7.086	0.527
Middle-Age +RT	60y	50.000	0.000	43.518	0.730	-6.482	0.730
Middle-Age +RT	61y	48.101	3.289	46.222	0.627	-1.880	3.916
Middle-Age +RT	61y	41.985	0.636	49.018	1.701	7.033	1.400
Middle-Age +RT	63y	50.000	0.000	44.614	0.734	-5.386	0.734
Middle-Age +RT	63y	42.738	1.602	42.804	0.414	0.066	1.225
Elderly +RT	65y	50.000	0.000	44.799	4.573	-5.201	4.573
Elderly +RT	68y	42.180	0.244	50.000	0.000	7.820	0.244
Elderly +RT	71y	47.878	3.675	42.159	0.920	-5.719	4.532
Elderly +RT	71y	50.000	0.000	44.437	2.132	-5.563	2.132
Elderly +RT	76y	50.000	0.000	45.017	1.156	-4.983	1.156
Elderly +RT	76y	50.000	0.000	46.260	1.787	-3.740	1.787
Elderly +RT	80y	50.000	0.000	42.367	1.702	-7.633	1.702
Elderly +RT	81y	50.000	0.000	42.744	1.182	-7.256	1.182
Elderly +RT	84y	50.000	0.000	42.006	1.987	-7.994	1.987
Elderly +RT	86y	47.308	4.663	44.190	0.133	-3.117	4.625
Elderly +RT	89y	50.000	0.000	43.455	2.898	-6.545	2.898
Elderly +RT	89y	47.564	4.219	47.109	3.505	-0.455	7.156
Elderly +RT	92y	50.000	0.000	41.896	1.166	-8.104	1.166
Elderly +RT	102y	50.000	0.000	42.832	1.240	-7.168	1.240

Table 6: HBE1 Real-Time PCR Singleplex Candidate Results.

HBE1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	F	1h	0.003	28.615
Newborn -RT				Undet
Newborn +RT	M	1h	0.003	28.041
Newborn -RT				Undet
Newborn +RT	F	17d	0.047	32.659
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	33.464
Newborn -RT				Undet
Toddler +RT	M	10m	0.833	35.566
Toddler -RT				Undet
Toddler +RT	F	3y	3.000	35.926
Toddler -RT				Undet
Child +RT	M	8y	8.000	39.075
Child -RT				Undet

HBE1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	M	16y	16.000	34.536
Juvenile -RT				Undet
Adult +RT	F	35y	35.000	38.329
Adult -RT				Undet
Middle-Age +RT	M	56y	56.000	32.677
Middle-Age -RT				Undet
Elderly +RT	F	69y	69.000	33.112
Elderly -RT				Undet
Elderly +RT	M	80y	80.000	33.140
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Table 7: HBE1 Real-Time PCR Duplex Delta Ct Results.

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	30.334	35.600	5.266	Adult +RT	36y	33.663	34.571	0.908
Newborn +RT	1h	29.454	36.348	6.894	Adult +RT	36y	34.011	36.938	2.927
Newborn +RT	2d	31.497	36.344	4.847	Adult +RT	38y	33.939	33.635	-0.304
Newborn +RT	8d	28.742	33.151	4.409	Adult +RT	40y	36.087	33.531	-2.556
Newborn +RT	17d	36.419	34.528	-1.891	Adult +RT	40y	34.268	33.344	-0.924
Newborn +RT	1m	31.793	34.283	2.490	Adult +RT	43y	35.758	34.431	-1.327
Newborn +RT	1m	26.481	35.650	9.169	Adult +RT	43y	36.427	34.102	-2.325
Newborn +RT	2m	34.816	34.115	-0.701	Adult +RT	45y	31.847	31.266	-0.581
Newborn +RT	3m	31.477	36.650	5.173	Elderly +RT	46y	37.779	34.190	-3.589
Infant +RT	4m	36.739	33.756	-2.983	Elderly -RT	47y	39.020	35.466	-3.554
Infant +RT	5m	34.472	32.444	-2.028	Middle-Age +RT	47y	33.377	33.568	0.191
Infant +RT	7m	37.068	33.692	-3.376	Middle-Age +RT	49y	32.288	41.326	9.038
Infant +RT	9m	35.862	35.536	-0.326	Middle-Age +RT	51y	34.377	36.995	2.618
Toddler +RT	10m	39.769	33.174	-6.595	Middle-Age +RT	53y	33.496	36.227	2.731
Toddler +RT	14m	36.301	33.809	-2.492	Middle-Age +RT	53y	32.336	33.344	1.008
Toddler +RT	18m	40.080	33.092	-6.988	Middle-Age +RT	56y	32.820	34.858	2.038
Toddler +RT	21m	37.282	33.368	-3.914	Middle-Age +RT	56y	34.211	34.193	-0.018
Toddler +RT	2y	33.388	33.307	-0.081	Middle-Age +RT	57y	36.067	33.589	-2.478
Toddler +RT	3y	39.098	32.606	-6.492	Middle-Age +RT	58y	33.307	33.603	0.296
Child +RT	4y	40.236	32.509	-7.727	Middle-Age +RT	60y	36.616	32.399	-4.217
Child +RT	4y	32.761	30.136	-2.625	Middle-Age +RT	61y	33.694	33.490	-0.204
Child +RT	5y	34.820	31.375	-3.445	Middle-Age +RT	63y	35.233	34.140	-1.093
Child +RT	6y	38.535	34.825	-3.710	Middle-Age +RT	63y	32.970	33.969	0.999
Child +RT	8y	39.831	35.021	-4.810	Elderly +RT	65y	33.069	34.779	1.710
Child +RT	9y	38.202	34.347	-3.855	Elderly +RT	66y	34.380	33.692	-0.688
Child +RT	9y	39.585	35.636	-3.949	Elderly +RT	68y	34.162	36.358	2.196
Child +RT	12y	38.094	34.787	-3.307	Elderly +RT	68y	37.887	34.393	-3.494
Child +RT	12y	34.692	33.182	-1.510	Elderly +RT	69y	35.421	33.888	-1.533
Juvenile +RT	13y	33.955	33.331	-0.624	Elderly +RT	71y	34.532	33.328	-1.204
Juvenile +RT	14y	38.146	32.170	-5.976	Elderly +RT	71y	34.341	34.017	-0.324
Juvenile +RT	14y	38.379	32.333	-6.046	Elderly +RT	72y	36.528	34.682	-1.846
Juvenile +RT	15y	36.141	32.941	-3.200	Elderly +RT	74y	32.297	34.526	2.229
Juvenile +RT	15y	36.014	32.498	-3.516	Elderly +RT	76y	32.672	33.363	0.691
Juvenile +RT	16y	34.429	33.673	-0.756	Elderly +RT	76y	33.382	34.562	1.180
Juvenile +RT	16y	35.811	33.060	-2.751	Elderly +RT	79y	35.049	33.391	-1.658
Juvenile +RT	17y	37.634	34.156	-3.478	Elderly +RT	80y	33.648	33.877	0.229
Juvenile +RT	18y	36.059	34.722	-1.337	Elderly +RT	81y	34.027	35.334	1.307
Adult +RT	19y	36.039	33.675	-2.364	Elderly +RT	83y	35.877	34.875	-1.002
Adult +RT	21y	33.974	36.982	3.008	Elderly +RT	84y	38.792	33.043	-5.749
Adult +RT	23y	36.217	33.207	-3.010	Elderly +RT	84y	36.862	33.858	-3.004
Adult +RT	24y	35.201	32.410	-2.791	Elderly +RT	86y	35.139	33.535	-1.604
Adult +RT	25y	34.418	33.117	-1.301	Elderly +RT	89y	36.299	32.876	-3.423
Adult +RT	27y	32.107	33.770	1.663	Elderly +RT	89y	34.405	34.331	-0.074
Adult +RT	29y	33.602	31.966	-1.636	Elderly +RT	91y	37.291	32.856	-4.435
Adult +RT	29y	33.783	34.439	0.656	Elderly +RT	92y	31.672	33.053	1.381
Adult +RT	31y	37.976	32.440	-5.536	Elderly +RT	102y	34.552	33.734	-0.818
Adult +RT	35y	38.910	34.427	-4.483	DNA		50.000	50.000	0.000
Adult +RT	35y	33.125	35.161	2.036	NTC		50.000	50.000	0.000

Table 8: HBE1 Triplicate qPCR Results.

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Newborn +RT	1h	34.639	0.461	35.726	0.698	1.087	0.298
Newborn +RT	1h	30.023	0.337	33.593	0.221	3.571	0.123
Newborn +RT	1h	30.427	0.179	34.385	0.237	3.959	0.133
Newborn +RT	1h	28.889	0.177	32.478	0.284	3.589	0.111
Newborn +RT	1h	28.890	0.159	33.646	0.421	4.756	0.378
Newborn +RT	1h	29.318	0.114	35.112	0.233	5.793	0.303
Newborn +RT	1h	28.733	0.277	37.526	0.888	8.793	0.672
Newborn +RT	1d	31.367	0.148	32.956	0.141	1.590	0.240
Newborn +RT	2d	31.126	0.097	35.821	1.042	4.694	0.968
Newborn +RT	8d	28.776	0.181	32.911	0.373	4.136	0.193
Newborn +RT	13d	28.713	0.380	32.743	0.252	4.030	0.626
Newborn +RT	17d	33.336	0.355	32.688	0.255	-0.647	0.116
Newborn +RT	1m	32.845	0.363	33.944	0.574	1.099	0.264
Newborn +RT	1m	27.493	0.112	32.049	0.028	4.556	0.084
Newborn +RT	2m	33.575	0.145	32.343	0.118	-1.233	0.100
Newborn +RT	3m	30.002	0.110	32.469	0.082	2.467	0.096
Newborn +RT	3m	34.602	0.323	34.862	0.641	0.260	0.461
Infant +RT	4m	36.031	0.307	32.667	0.116	-3.364	0.422
Infant +RT	4m	34.593	0.456	31.943	0.084	-2.651	0.481
Infant +RT	5m	36.198	1.215	32.806	0.325	-3.392	0.955
Infant +RT	5m	37.554	0.949	33.829	0.774	-3.725	0.357
Infant +RT	6m	35.244	0.459	32.742	0.283	-2.501	0.405
Infant +RT	7m	36.819	0.745	31.405	0.246	-5.414	0.628
Infant +RT	7m	33.533	0.733	32.645	0.520	-0.887	1.094
Infant +RT	7m	34.485	0.080	33.470	0.128	-1.016	0.132
Infant +RT	8m	36.568	1.072	32.066	0.334	-4.502	1.233
Infant +RT	8m	34.552	0.487	35.270	0.248	0.718	0.378
Infant +RT	8m	35.368	0.451	31.318	0.129	-4.050	0.353
Infant +RT	9m	38.327	0.617	32.295	0.145	-6.031	0.593
Infant +RT	9m	36.243	0.241	33.606	0.382	-2.637	0.596
Toddler +RT	10m	37.774	0.376	32.868	0.453	-4.905	0.300
Toddler +RT	10m	39.078	0.351	32.596	0.225	-6.482	0.573
Toddler +RT	14m	36.184	0.330	33.664	0.329	-2.520	0.328
Toddler +RT	14m	36.721	0.377	34.908	0.252	-1.814	0.324
Toddler +RT	15m	35.537	0.540	33.814	0.521	-1.723	0.327
Toddler +RT	18m	37.507	0.550	32.686	0.288	-4.820	0.600
Toddler +RT	19m	35.122	0.347	32.914	0.349	-2.207	0.274
Toddler +RT	21m	36.986	0.522	33.089	0.322	-3.897	0.537
Toddler +RT	2y	35.672	0.668	33.690	0.460	-1.982	0.208
Toddler +RT	2.8y	34.992	0.119	33.458	0.298	-1.534	0.324
Toddler +RT	3y	40.691	0.881	32.838	0.170	-7.853	0.818
Toddler +RT	3y	36.095	0.234	31.764	0.249	-4.330	0.251

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Child +RT	4y	33.239	0.453	32.537	0.156	-0.702	0.317
Child +RT	4y	39.946	0.764	31.998	0.508	-7.948	0.513
Child +RT	4y	35.054	0.644	33.583	0.318	-1.471	0.357
Child +RT	5y	35.224	0.115	30.800	0.286	-4.424	0.342
Child +RT	6y	37.249	0.683	32.361	0.522	-4.888	0.279
Child +RT	6y	38.796	0.880	33.669	0.237	-5.127	0.670
Child +RT	8y	38.383	0.337	34.840	0.289	-3.544	0.614
Child +RT	9y	37.478	0.683	33.591	0.362	-3.887	0.787
Child +RT	9y	38.319	0.255	34.516	0.500	-3.803	0.704
Child +RT	12y	38.077	1.369	33.992	0.358	-4.085	1.353
Child +RT	12y	35.012	0.259	32.411	0.286	-2.601	0.230
Child +RT	12y	35.721	0.126	32.905	0.307	-2.816	0.264
Child +RT	12y	38.174	0.839	33.580	0.486	-4.594	0.353
Juvenile +RT	13y	36.579	0.280	34.183	0.430	-2.396	0.513
Juvenile +RT	13y	38.910	0.682	32.542	0.093	-6.368	0.670
Juvenile +RT	14y	41.041	0.400	33.374	0.449	-7.667	0.848
Juvenile +RT	14y	39.090	0.911	32.994	0.308	-6.096	0.926
Juvenile +RT	14y	42.575	1.481	33.743	0.320	-8.832	1.362
Juvenile +RT	15y	34.271	0.271	30.532	0.048	-3.739	0.283
Juvenile +RT	15y	34.957	0.184	32.950	0.183	-2.007	0.151
Juvenile +RT	15y	36.975	0.799	32.968	0.755	-4.007	0.964
Juvenile +RT	16y	38.098	0.431	32.904	0.617	-5.194	0.416
Juvenile +RT	16y	36.090	0.359	33.343	0.150	-2.747	0.337
Juvenile +RT	17y	33.497	0.619	33.619	0.416	0.122	0.213
Juvenile +RT	17y	32.082	0.586	35.314	0.521	3.232	0.135
Juvenile +RT	17y	33.156	0.229	33.205	0.280	0.049	0.171
Juvenile +RT	18y	33.952	0.236	31.615	0.384	-2.337	0.192
Juvenile +RT	18y	34.323	0.476	32.079	0.796	-2.244	0.361

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Adult +RT	19y	37.761	0.408	34.610	0.399	-3.151	0.268
Adult +RT	19y	37.348	1.080	33.452	0.309	-3.896	0.947
Adult +RT	21y	32.957	0.570	33.217	1.370	0.260	0.902
Adult +RT	21y	33.743	0.286	32.924	0.639	-0.819	0.366
Adult +RT	22y	36.813	0.377	34.482	0.247	-2.331	0.131
Adult +RT	22y	36.532	0.144	33.301	0.093	-3.231	0.221
Adult +RT	23y	37.093	0.734	32.734	0.288	-4.359	0.448
Adult +RT	24y	36.691	0.341	32.084	0.226	-4.607	0.424
Adult +RT	25y	37.374	0.951	36.054	1.148	-1.320	0.224
Adult +RT	26y	34.100	0.356	32.009	0.288	-2.091	0.635
Adult +RT	26y	40.495	1.415	33.920	0.273	-6.576	1.295
Adult +RT	27y	32.948	0.193	33.828	0.326	0.881	0.145
Adult +RT	29y	36.890	1.238	33.546	1.173	-3.344	0.430
Adult +RT	29y	36.447	0.257	34.180	0.221	-2.267	0.140
Adult +RT	35y	37.427	0.645	34.180	0.022	-3.248	0.659
Adult +RT	35y	33.871	0.573	34.156	0.276	0.285	0.346
Adult +RT	36y	37.773	0.636	33.359	0.547	-4.414	0.719
Adult +RT	36y	32.424	0.315	33.371	0.285	0.947	0.097
Adult +RT	38y	32.911	0.102	31.941	0.365	-0.970	0.293
Adult +RT	38y	34.937	0.350	32.880	0.376	-2.057	0.207
Adult +RT	38y	36.152	0.350	33.664	0.173	-2.487	0.182
Adult +RT	40y	36.754	0.228	33.308	0.564	-3.446	0.372
Adult +RT	40y	34.180	0.584	32.989	0.340	-1.191	0.287
Adult +RT	43y	35.334	0.776	34.551	0.341	-0.783	0.444
Adult +RT	43y	34.337	0.682	34.247	0.283	-0.091	0.645
Adult +RT	45y	33.000	0.390	33.300	0.261	0.300	0.193
Adult +RT	45y	33.019	0.377	32.757	0.389	-0.262	0.763
Adult +RT	45y	37.822	1.606	34.479	1.697	-3.343	0.688

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Middle-Age +RT	46y	36.400	0.623	33.405	0.202	-2.995	0.769
Middle-Age +RT	47y	34.379	0.427	33.193	0.600	-1.186	0.354
Middle-Age +RT	47y	39.753	0.844	34.446	1.049	-5.307	0.205
Middle-Age +RT	51y	33.966	0.203	33.329	0.224	-0.638	0.124
Middle-Age +RT	53y	34.616	0.868	33.152	0.608	-1.464	0.352
Middle-Age +RT	53y	34.537	0.205	34.095	0.641	-0.442	0.762
Middle-Age +RT	56y	33.200	0.737	34.682	0.846	1.483	0.879
Middle-Age +RT	56y	34.232	0.523	33.719	0.679	-0.513	0.657
Middle-Age +RT	57y	36.640	0.572	34.428	0.384	-2.212	0.224
Middle-Age +RT	58y	35.420	0.533	35.219	0.943	-0.201	0.576
Middle-Age +RT	59y	34.343	0.084	33.297	0.285	-1.046	0.201
Middle-Age +RT	60y	36.811	0.356	32.951	1.291	-3.860	1.308
Middle-Age +RT	61y	29.949	0.182	32.494	0.518	2.545	0.381
Middle-Age +RT	61y	35.798	0.576	34.858	0.330	-0.940	0.621
Middle-Age +RT	63y	31.241	0.173	31.172	0.306	-0.069	0.140
Middle-Age +RT	63y	32.556	0.173	32.255	0.271	-0.301	0.165
Middle-Age +RT	65y	31.965	0.177	33.168	0.131	1.202	0.060
Elderly +RT	66y	37.121	0.312	35.042	0.140	-2.079	0.231
Elderly +RT	66y	38.308	0.353	33.882	0.168	-4.425	0.468
Elderly +RT	68y	36.408	0.562	34.371	0.391	-2.037	0.203
Elderly +RT	68y	36.392	0.987	33.782	0.395	-2.610	1.261
Elderly +RT	69y	35.221	0.280	33.547	0.473	-1.674	0.248
Elderly +RT	71y	34.893	0.579	35.971	0.431	1.078	0.168
Elderly +RT	71y	34.382	0.194	33.246	0.414	-1.136	0.249
Elderly +RT	71y	35.324	0.213	33.338	0.286	-1.985	0.459
Elderly +RT	72y	36.617	0.190	34.322	0.366	-2.295	0.177
Elderly +RT	74y	32.468	0.160	33.839	0.424	1.371	0.539
Elderly +RT	76y	34.318	0.397	33.890	0.431	-0.428	0.148
Elderly +RT	76y	35.705	0.610	34.568	0.323	-1.137	0.357
Elderly +RT	79y	34.739	0.143	34.091	1.088	-0.648	1.187
Elderly +RT	80y	33.629	0.216	32.656	0.064	-0.974	0.258
Elderly +RT	81y	31.964	0.189	32.923	0.216	0.959	0.220
Elderly +RT	83y	34.704	0.540	33.695	0.020	-1.008	0.555
Elderly +RT	84y	36.085	0.111	33.056	0.478	-3.029	0.368
Elderly +RT	84y	37.379	0.600	33.194	0.307	-4.185	0.326
Elderly +RT	86y	36.039	0.762	33.934	1.445	-2.105	0.689
Elderly +RT	89y	35.420	1.554	32.444	0.807	-2.976	0.834
Elderly +RT	89y	36.970	0.761	33.363	0.180	-3.607	0.648
Elderly +RT	91y	37.940	0.380	35.794	0.614	-2.146	0.983
Elderly +RT	92y	30.063	0.143	31.410	0.270	1.347	0.160
Elderly +RT	102y	36.843	1.094	33.264	0.382	-3.579	0.823

Table 9: IGFBP3 Real-Time PCR Singleplex Candidate Results.

Sample #	Sex	Age	Age (Yrs)	Ct value	Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000	Middle-Aged +RT	M	46y	46.000	36.918
Newborn -RT				Undet	Middle-Aged -RT				Undet
Newborn +RT	F	1m	0.083	40.000	Middle-Aged +RT	M	51y	51.000	37.031
Newborn -RT				Undet	Middle-Aged -RT				Undet
Infant +RT	F	5m	0.417	40.000	Middle-Aged +RT	F	56y	56.000	38.425
Infant -RT				Undet	Middle-Aged -RT				Undet
Toddler +RT	M	10m	0.833	40.000	Middle-Aged +RT	M	61y	61.000	38.577
Toddler -RT				Undet	Middle-Aged -RT				Undet
Toddler +RT	F	19m	1.583	37.562	Elderly +RT	M	66y	66.000	34.822
Toddler -RT				Undet	Elderly -RT				Undet
Child +RT	M	4y	4.000	40.000	Elderly +RT	F	71y	71.000	37.476
Child -RT				Undet	Elderly -RT				Undet
Child +RT	F	12y	12.000	39.208	Elderly +RT	M	76y	76.000	36.217
Child -RT				Undet	Elderly -RT				Undet
Juvenile +RT	M	15y	15.000	35.557	Elderly +RT	F	81y	81.000	34.500
Juvenile -RT				Undet	Elderly -RT				Undet
Juvenile +RT	M	18y	18.000	35.813	Elderly +RT	F	84y	84.000	35.280
Juvenile -RT				Undet	Elderly -RT				Undet
Adult +RT	M	22y	22.000	37.572	Elderly +RT	M	86y	86.000	36.229
Adult -RT				28.176	Elderly -RT				Undet
Adult +RT	M	27y	27.000	39.987	Elderly +RT	F	91y	91.000	36.459
Adult -RT				Undet	Elderly -RT				Undet
Adult +RT	M	35y	35.000	39.063	Elderly +RT	M	92y	92.000	36.382
Adult -RT				Undet	Elderly -RT				Undet
Adult +RT	M	40y	40.000	35.493	DNA				
Adult -RT				Undet	DNA				
Adult +RT	M	43y	43.000	36.318	NTC				
Adult -RT				Undet	NTC				

Table 10: IGFBP3 Real-Time PCR Duplex Delta Ct Results.

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	40.000	35.618	-4.382	Adult +RT	36y	36.329	35.712	-0.617
Newborn +RT	1h	40.000	40.000	0.000	Adult +RT	36y	36.693	36.955	0.262
Newborn +RT	2d	40.000	37.407	-2.593	Adult +RT	38y	35.831	34.602	-1.229
Newborn +RT	8d	36.605	34.137	-2.468	Adult +RT	40y	36.649	35.890	-0.759
Newborn +RT	17d	40.000	35.880	-4.120	Adult +RT	40y	34.720	34.943	0.223
Newborn +RT	1m	40.000	36.377	-3.623	Adult +RT	43y	37.365	35.865	-1.500
Newborn +RT	1m	40.000	40.000	0.000	Adult +RT	43y	38.017	39.724	1.707
Newborn +RT	2m	40.000	35.188	-4.812	Adult +RT	45y	35.068	34.117	-0.951
Newborn +RT	3m	37.728	35.044	-2.684	Elderly +RT	46y	35.485	36.726	1.241
Infant +RT	4m	38.677	34.410	-4.267	Elderly -RT	47y	39.947	40.000	0.053
Infant +RT	5m	40.000	34.060	-5.940	Middle-Age +RT	47y	36.340	34.229	-2.111
Infant +RT	7m	38.164	34.523	-3.641	Middle-Age +RT	49y	39.055	40.000	0.945
Infant +RT	9m	40.000	37.713	-2.287	Middle-Age +RT	51y	36.384	36.170	-0.214
Toddler +RT	10m	40.000	35.294	-4.706	Middle-Age +RT	53y	40.000	36.422	-3.578
Toddler +RT	14m	40.000	35.862	-4.138	Middle-Age +RT	53y	34.370	36.711	2.341
Toddler +RT	18m	40.000	35.584	-4.416	Middle-Age +RT	56y	36.037	37.489	1.452
Toddler +RT	21m	40.000	35.930	-4.070	Middle-Age +RT	56y	36.392	39.560	3.168
Toddler +RT	2y	40.000	35.701	-4.299	Middle-Age +RT	57y	35.489	36.810	1.321
Toddler +RT	3y	38.164	35.136	-3.028	Middle-Age +RT	58y	38.215	35.917	-2.298
Child +RT	4y	40.000	32.741	-7.259	Middle-Age +RT	60y	37.188	34.025	-3.163
Child +RT	4y	40.000	36.483	-3.517	Middle-Age +RT	61y	37.657	39.238	1.581
Child +RT	5y	38.097	33.272	-4.825	Middle-Age +RT	63y	37.585	39.318	1.733
Child +RT	6y	40.000	36.590	-3.410	Middle-Age +RT	63y	39.234	33.748	-5.486
Child +RT	8y	40.000	35.451	-4.549	Elderly +RT	65y	36.314	35.654	-0.660
Child +RT	9y	40.000	36.488	-3.512	Elderly +RT	66y	35.238	39.637	4.399
Child +RT	9y	38.375	36.635	-1.740	Elderly +RT	68y	34.411	37.674	3.263
Child +RT	12y	40.000	36.751	-3.249	Elderly +RT	68y	35.321	32.247	-3.074
Child +RT	12y	39.207	35.559	-3.648	Elderly +RT	69y	35.680	35.258	-0.422
Juvenile +RT	13y	40.000	32.970	-7.030	Elderly +RT	71y	37.262	40.000	2.738
Juvenile +RT	14y	36.370	34.298	-2.072	Elderly +RT	71y	36.505	35.812	-0.693
Juvenile +RT	14y	34.154	36.280	2.126	Elderly +RT	72y	37.797	37.428	-0.369
Juvenile +RT	15y	40.000	35.587	-4.413	Elderly +RT	74y	37.054	37.104	0.050
Juvenile +RT	15y	36.442	33.560	-2.882	Elderly +RT	76y	34.124	35.432	1.308
Juvenile +RT	16y	40.000	35.428	-4.572	Elderly +RT	76y	36.540	38.447	1.907
Juvenile +RT	16y	37.381	34.729	-2.652	Elderly +RT	79y	37.995	40.000	2.005
Juvenile +RT	17y	40.000	35.018	-4.982	Elderly +RT	80y	37.482	36.158	-1.324
Juvenile +RT	18y	34.484	31.980	-2.504	Elderly +RT	81y	35.630	37.650	2.020
Adult +RT	19y	35.387	34.980	-0.407	Elderly +RT	83y	40.000	35.812	-4.188
Adult +RT	21y	40.000	38.267	-1.733	Elderly +RT	84y	34.619	34.887	0.268
Adult +RT	23y	36.483	35.140	-1.343	Elderly +RT	84y	35.079	35.859	0.780
Adult +RT	24y	35.216	37.270	2.054	Elderly +RT	86y	35.056	34.884	-0.172
Adult +RT	25y	36.657	35.134	-1.523	Elderly +RT	89y	35.854	34.418	-1.436
Adult +RT	27y	38.964	35.944	-3.020	Elderly +RT	89y	34.860	34.106	-0.754
Adult +RT	29y	35.624	30.368	-5.256	Elderly +RT	91y	35.274	35.345	0.071
Adult +RT	29y	37.118	35.717	-1.401	Elderly +RT	92y	36.073	34.559	-1.514
Adult +RT	31y	35.212	34.600	-0.612	Elderly +RT	102y	31.403	22.654	-8.749
Adult +RT	35y	39.757	36.147	-3.610	DNA		50.000	50.000	0.000
Adult +RT	35y	36.306	36.550	0.244	NTC		50.000	50.000	0.000

Table 11: IGFBP3 Triplicate qPCR Results.

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Newborn +RT	1h	50.000	0.000	38.570	0.522	-11.430	0.522
Newborn +RT	1h	39.407	1.411	34.988	0.491	-4.419	1.871
Newborn +RT	1h	50.000	0.000	33.631	0.202	-16.369	0.202
Newborn +RT	1h	50.000	0.000	35.938	1.078	-14.062	1.078
Newborn +RT	1d	38.087	0.862	34.066	0.395	-4.021	0.590
Newborn +RT	2d	50.000	0.000	35.107	0.235	-14.893	0.235
Newborn +RT	8d	39.029	0.176	35.840	0.235	-3.189	0.233
Newborn +RT	17d	50.000	0.000	35.502	0.235	-14.498	0.235
Newborn +RT	1m	42.714	1.414	35.418	0.436	-7.296	0.993
Newborn +RT	1m	50.000	0.000	33.628	0.010	-16.372	0.010
Newborn +RT	2m	50.000	0.000	34.336	0.547	-15.664	0.547
Newborn +RT	3m	38.782	0.591	35.763	0.347	-3.018	0.284
Newborn +RT	3m	50.000	0.000	36.902	0.810	-13.098	0.810
Infant +RT	4m	38.146	0.690	35.441	0.444	-2.705	0.250
Infant +RT	4m	38.012	2.273	34.384	1.587	-3.628	1.165
Infant +RT	5m	50.000	0.000	34.733	0.256	-15.267	0.256
Infant +RT	5m	50.000	0.000	35.547	0.848	-14.453	0.848
Infant +RT	6m	50.000	0.000	35.691	0.396	-14.309	0.396
Infant +RT	7m	39.634	0.560	34.934	0.589	-4.701	0.064
Infant +RT	7m	38.247	0.439	35.108	0.201	-3.139	0.593
Infant +RT	7m	49.245	1.307	35.536	0.104	-13.710	1.405
Infant +RT	8m	50.000	0.000	34.021	0.317	-15.979	0.317
Infant +RT	8m	50.000	0.000	37.711	0.589	-12.289	0.589
Infant +RT	8m	37.256	0.360	33.955	0.295	-3.300	0.643
Toddler +RT	10m	39.289	0.168	35.597	0.281	-3.692	0.113
Toddler +RT	14m	50.000	0.000	36.456	1.170	-13.544	1.170
Toddler +RT	14m	50.000	0.000	34.656	0.533	-15.344	0.533
Toddler +RT	15m	50.000	0.000	37.107	0.709	-12.893	0.709
Toddler +RT	18m	38.590	0.978	35.428	0.252	-3.161	1.011
Toddler +RT	19m	39.169	0.508	34.943	0.505	-4.226	0.294
Toddler +RT	2y	50.000	0.000	36.797	0.952	-13.203	0.952
Toddler +RT	2.8y	50.000	0.000	34.670	0.920	-15.330	0.920
Toddler +RT	3y	39.224	0.563	35.263	0.209	-3.961	0.443
Child +RT	4y	38.398	0.431	35.682	0.764	-2.716	0.333
Child +RT	4y	50.000	0.000	34.535	0.607	-15.465	0.607
Child +RT	5y	36.425	0.346	32.738	0.333	-3.686	0.017
Child +RT	6y	50.000	0.000	36.892	0.147	-13.108	0.147
Child +RT	8y	36.771	0.984	35.901	0.392	-0.870	1.110
Child +RT	9y	38.757	0.305	35.561	0.584	-3.195	0.471
Child +RT	9y	38.836	0.541	38.401	0.628	-0.435	0.097
Child +RT	12y	37.708	0.780	35.979	0.163	-1.729	0.937
Child +RT	12y	38.628	0.655	36.171	0.175	-2.457	0.829
Child +RT	12y	38.528	1.005	35.136	0.359	-3.393	0.947

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Juvenile +RT	13y	38.718	0.405	35.536	0.215	-3.181	0.485
Juvenile +RT	14y	37.083	0.963	34.896	0.280	-2.187	0.961
Juvenile +RT	14y	34.963	0.298	34.661	0.608	-0.302	0.548
Juvenile +RT	14y	36.474	0.214	35.355	0.547	-1.120	0.590
Juvenile +RT	15y	37.191	1.536	32.871	0.527	-4.320	1.690
Juvenile +RT	15y	35.983	0.621	33.838	1.148	-2.145	0.896
Juvenile +RT	16y	36.094	0.127	32.933	0.249	-3.161	0.326
Juvenile +RT	16y	37.115	0.249	35.348	0.344	-1.767	0.440
Juvenile +RT	17y	50.000	0.000	36.925	0.374	-13.075	0.374
Juvenile +RT	17y	50.000	0.000	40.366	0.583	-9.634	0.583
Juvenile +RT	17y	39.957	1.932	36.517	0.362	-3.440	1.821
Juvenile +RT	18y	35.116	0.113	32.522	0.357	-2.594	0.259
Juvenile +RT	18y	38.153	2.200	35.351	0.869	-2.802	1.652
Adult +RT	19y	37.441	0.137	37.079	0.181	-0.361	0.068
Adult +RT	19y	36.567	0.697	35.985	0.287	-0.581	0.490
Adult +RT	21y	50.000	0.000	37.798	0.671	-12.202	0.671
Adult +RT	21y	38.887	1.142	35.585	1.127	-3.301	1.658
Adult +RT	22y	35.130	0.685	37.705	1.089	2.576	1.566
Adult +RT	22y	35.895	0.468	35.680	0.428	-0.215	0.825
Adult +RT	23y	37.117	0.578	36.050	0.558	-1.067	0.303
Adult +RT	24y	34.147	0.329	33.977	0.432	-0.171	0.220
Adult +RT	25y	38.488	0.851	37.699	0.233	-0.789	1.014
Adult +RT	26y	33.317	0.283	34.736	0.171	1.420	0.152
Adult +RT	26y	38.590	0.824	37.115	0.292	-1.475	0.692
Adult +RT	27y	37.427	0.733	35.920	0.285	-1.507	0.603
Adult +RT	29y	35.950	0.550	35.067	0.675	-0.883	0.987
Adult +RT	29y	35.837	0.469	35.680	0.049	-0.157	0.420
Adult +RT	35y	37.977	0.651	36.385	0.898	-1.592	1.174
Adult +RT	35y	35.787	0.223	36.768	1.040	0.981	1.050
Adult +RT	36y	36.175	0.137	36.608	0.184	0.433	0.227
Adult +RT	36y	36.768	0.607	35.465	0.234	-1.304	0.382
Adult +RT	38y	35.467	0.546	33.341	0.268	-2.126	0.685
Adult +RT	38y	36.300	0.164	36.247	0.139	-0.052	0.156
Adult +RT	38y	34.283	0.648	35.318	0.265	1.035	0.457
Adult +RT	40y	37.911	1.075	34.966	0.181	-2.945	1.086
Adult +RT	40y	34.112	0.309	36.223	1.173	2.111	0.975
Adult +RT	43y	36.559	0.610	36.939	0.728	0.380	0.588
Adult +RT	45y	34.969	0.207	33.670	1.421	-1.298	1.401
Adult +RT	45y	35.541	0.555	34.575	0.393	-0.966	0.905
Adult +RT	45y	33.149	0.233	34.542	0.573	1.393	0.343

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Middle-Age +RT	46y	36.191	0.863	36.864	0.672	0.673	1.176
Middle-Age +RT	47y	35.795	0.384	36.751	1.103	0.956	0.770
Middle-Age +RT	47y	37.376	0.893	36.643	0.439	-0.733	0.826
Middle-Age +RT	51y	36.298	0.356	35.528	0.021	-0.770	0.347
Middle-Age +RT	53y	50.000	0.000	41.367	0.611	-8.633	0.611
Middle-Age +RT	53y	34.138	0.649	35.843	1.067	1.704	0.680
Middle-Age +RT	56y	38.107	0.272	37.150	0.056	-0.957	0.292
Middle-Age +RT	56y	36.877	0.481	35.947	0.167	-0.930	0.598
Middle-Age +RT	57y	36.000	0.407	35.856	0.328	-0.143	0.265
Middle-Age +RT	58y	37.855	0.910	36.846	0.201	-1.009	0.921
Middle-Age +RT	59y	36.226	0.631	36.343	0.254	0.117	0.779
Middle-Age +RT	60y	36.856	0.177	35.514	0.465	-1.342	0.336
Middle-Age +RT	61y	36.764	0.713	36.297	1.603	-0.467	1.237
Middle-Age +RT	61y	36.284	0.398	35.662	1.467	-0.622	1.096
Middle-Age +RT	63y	38.499	0.686	37.161	0.210	-1.338	0.728
Middle-Age +RT	63y	37.668	0.782	35.015	0.248	-2.653	0.740
Elderly +RT	65y	38.641	0.486	37.875	0.419	-0.767	0.901
Elderly +RT	66y	36.362	0.200	40.077	0.515	3.715	0.316
Elderly +RT	66y	35.700	1.221	37.278	1.057	1.578	0.850
Elderly +RT	68y	35.378	0.626	37.045	0.518	1.666	0.771
Elderly +RT	68y	35.210	0.656	35.815	0.410	0.605	0.764
Elderly +RT	69y	36.190	0.752	35.968	0.287	-0.222	0.502
Elderly +RT	71y	50.000	0.000	37.466	0.842	-12.534	0.842
Elderly +RT	71y	37.558	1.188	36.608	0.253	-0.950	1.342
Elderly +RT	71y	37.155	1.963	35.963	0.688	-1.192	2.651
Elderly +RT	72y	37.689	0.424	37.167	0.815	-0.522	1.162
Elderly +RT	74y	36.982	1.253	38.339	0.679	1.357	1.009
Elderly +RT	76y	34.893	0.582	36.091	1.359	1.198	1.179
Elderly +RT	76y	36.849	1.373	36.681	0.287	-0.168	1.105
Elderly +RT	79y	37.504	0.614	37.753	0.983	0.249	0.576
Elderly +RT	80y	37.013	0.685	35.665	0.108	-1.347	0.579
Elderly +RT	81y	33.966	0.211	35.334	0.309	1.368	0.217
Elderly +RT	84y	35.291	0.272	35.880	0.269	0.588	0.480
Elderly +RT	84y	33.947	0.427	34.742	0.910	0.796	1.288
Elderly +RT	86y	35.380	0.293	34.175	0.319	-1.205	0.074
Elderly +RT	89y	36.909	0.505	35.011	0.656	-1.897	0.486
Elderly +RT	89y	35.134	0.375	35.417	1.039	0.282	1.388
Elderly +RT	91y	36.501	0.523	36.352	0.582	-0.149	1.002
Elderly +RT	92y	36.122	0.658	34.886	0.235	-1.236	0.824
Elderly +RT	102y	36.158	0.876	35.149	0.573	-1.009	1.140

Table 12: Primer and Probe Sequences for the qRT-PCR Triplex Assay for Age Determination.

	Gene	Accession #		Primer and Probe Sequences (5′→3′)
PCR				
	COL1A2	NM_000089	Forward	tggagtccgaggacctaatg
			Reverse	gcaagaccagcatgaccttt
	IGFBP3	NM_001013398	Forward	acagccagcgctacaaagtt
			Reverse	ggctgcccatacttatccac
qPCR				
	COL1A2	NM_000089	Forward	gcatccttggttagggtcaatc
			Reverse	catgccgtgacttgagactca
			Probe	6FAM agtagtaaccactgctcc MGBNFQ*
	IGFBP3	NM_001013398	Forward	agaacttctcctccgagtccaa
			Reverse	caggtgattcagtgtgtcttcca
			Probe	VIC acagaatatggtccctgcc MGBNFQ*
	S15	NM_001018	Forward	ccaaagcgatctcttctgaggat
			Reverse	acgccgcggtaggtgaa
			Probe	NED cggcaagatggcagaagtagagcagaa MGBNFQ*

* MGBNFQ, minor groove binding non-fluorescent quencher

Table 13: Biological Age Specificity Results for the Triplex Real-Time PCR assay.

Age Specificity Results of 140 Blood Samples

			ddCt Scatter Plot Results				
	Age Range	n=	+/+	+/- or +/0	-/-	-/+	0/+
Newborn	1h - 3m	17	0	13	3	1	0
Infants	4m - 9m	12	1	2	9	0	0
Toddlers	10m - 4y	15	0	1	13	1	0
Children	5y - 12y	9	1	0	1	6	1
Juveniles	13y - 18y	15	1	0	1	11	2
Adults	19y -45y	28	0	0	0	10	18
Middle-Age	46y - 65y	20	0	0	1	10	9
Elderly	66y - 102y	24	0	0	2	7	15

* h, hour; m, month; y, year

Table 14: Primer, Probe Sequences and Expected Product Sizes for the RT-PCR Newborn Assays.

RT-PCR Assay	Primer Sequences 5'→ 3'	DNA (bp)	RNA (bp)
S15			
Forward	5’ TTC-CGC-AAG-TTC-ACC-TAC-C 3’	361	361
Reverse	5’ CGG-GCC-GGC-CAT-GCT-TTA-CG 3’		
GNAS			
Forward	5’ AAG-ATC-GAC-GTG-ATC-AAG-CA 3’	855	371
Reverse	5’ CCA-GCA-AGG-ACT-TTC-TCA-GC 3’		
HBG			
Forward	5’ GTG-GAT-CCT-GAG-AAC-TTC-AA 3’	1040	154
Reverse	5’ GAG-CTC-AGT-GGT-ATC-TGG-AG 3’		
HBG1			
Forward	5’ ACT-TCC-TTG-GGA-GAT-GCC-AC 3’	1157	277
Reverse	5’ AAA-GCC-TAT-CCT-TGA-AAG-CTC-TGA 3’		
HBG2			
Forward	5’ ACT-TCC-TTG-GGA-GAT-GCC-AT 3’	1160	274
Reverse	5’ GCC-TAT-CCT-TGA-AAG-CTC-TGC 3’		
HBG1n1			
Forward	5’ GAA-AGC-TCT-GAA-TCA-TCC-AGG-TG 3’ a	0	207
Reverse	5’ GGG-CAA-GGT-GAA-TGT-GGA-AG 3’		
HBG1n2			
Forward	5’ AGT-GAG-CTC-AGT-GGC-ATC-TC 3' a	0	190
Reverse	5’ GGG-CAA-GGT-GAA-TGT-GGA-AG 3’		
HBG2n2			
Forward	5’ CTG-GAG-GAC-AGG-GCA-AAG-G 3’ a	0	225
Reverse	5’ GGG-CAA-GGT-GAA-TGT-GGA-AG 3’		
HBG2n3			
Forward	5’ GGC-AGT-GAG-CTC-AGT-GCA-GTT-C 3’ a	0	161
Reverse	5’ CAG-CTT-TGG-CAA-CCT-GTC-CT 3’		

a Underlined sequence identifies the location of the newborn hemoglobin isoform breakpoints

Table 15: Real-Time PCR primer and probe sequences for Forensic Newborn Identification.

qRT-PCR Assay	Primer & Probe Sequences 5'→ 3'
S15	
Forward	5’ CCA-AAG-CGA-TCT-CTT-CTG-AGG-AT 3’
Reverse	5' ACG-CCG-CGG-TAG-GTG-AA 3'
Probe	VIC CGG-CAA-GAT-GGC-AGA-AGT-AGA-GCA-GAA MGBNFQ b
HBG1n1	
Forward	5' GAA-AGC-TCT-GAA-TCA-TCC-AGG-TG 3' a
Reverse	5' AGT-CAA-GGC-ACA-TGG-CAA-GAA-G 3'
Probe	6FAM TTT-GTG-GCA-TCT-CCC-AAG-GAA-GTC-AGC MGBNFQ b
HBG2n3	
Forward	5' GCA-GTG-AGC-TCA-GTG-CAG-TTC 3' a
Reverse	5' TTC-CTT-GGG-AGA-TGC-CAT-AAA 3'
Probe	6FAM CAA-AGG-TGC-CCT-TGA-GAT-CAT-CCA-GG MGBNFQ b

a Underlined sequence identifies the location of the newborn hemoglobin isoform breakpoints

b MGBNFQ, minor groove binding non-fluorescent quencher

Table 16: Biological Age Specificity Results for the Two Newborn Duplex qPCR Assays.

		Assay Results					
		≤4 months			<24 hours		
Biological Age	n=	(+/+)[a]	(+/–)[b]	(–/–)[c]	(+/+)[a]	(+/–)[b]	(–/–)[c]
<24 hours	10	10	-	-	10	-	-
1 day – 1 month	19	19	-	-	3	14	2
2 months – 4 months	22	17	4	1	-	1	21
5 months – 3 years	37	1	2	34	-	2	35
4 years – 18 years	20	-	-	20	-	-	20
19 years – 92 years	24	-	-	24	-	-	24

[a] represents two positive delta Ct values (+/+)
[b] represents one positive delta Ct value and one negative delta Ct value (+/–)
[c] represents two negative delta Ct values (–/–)

Table 17: Sensitivity Data for ≤ 4 Month Newborn Duplex Assays.

Input RNA (picograms)	NEWBORN			ADULT		
	Ct HBG1n	Ct S15	dCt (S15-HBG1n)	Ct HBG1n	Ct S15	dCt (S15-HBG1n)
1000	28.460	31.336	+2.876	40.000	33.665	-6.336
750	28.132	31.259	+3.128	40.000	34.343	-5.657
500	28.878	31.881	+3.003	40.000	34.805	-5.195
250	31.132	33.190	+2.058	40.000	37.173	-2.827
100	32.873	35.084	+2.211	40.000	37.625	-2.376
50	34.116	37.213	+3.097	40.000	38.505	-1.496
25	35.732	37.576	+1.844	40.000	39.813	-0.187
10	35.285	38.146	+2.862	40.000	40.000	0
5	34.896	38.732	+3.836	40.000	40.000	0
1	40.000	40.000	0	40.000	40.000	0
	Ct HBG2n	Ct S15	dCt (S15-HBG2n)	Ct HBG2n	Ct S15	dCt (S15-HBG2n)
1000	29.849	37.829	+7.981	40.000	37.778	-2.222
750	28.624	36.094	+7.470	40.000	37.417	-2.584
500	29.710	35.589	+5.879	40.000	38.152	-1.849
250	30.602	36.095	+5.494	40.000	37.931	-2.069
100	30.165	38.382	+8.217	40.000	36.569	-3.432
50	32.143	37.720	+5.577	40.000	38.619	-1.381
25	37.952	39.295	+1.343	40.000	40.000	0
10	35.441	40.000	+4.560	40.000	40.000	0
5	38.616	40.000	+1.385	40.000	40.000	0
1	39.787	40.000	+0.213	40.000	40.000	0

Ct, cycle threshold; dCt, delta cycle threshold

Table 18: Sensitivity Data for < 24 Hour Newborn Duplex Assays.

Input RNA (picograms)	NEWBORN			ADULT		
	Ct HBG1n	Ct S15	dCt (S15-HBG1n)	Ct HBG1n	Ct S15	dCt (S15-HBG1n)
1000	32.651	34.466	+1.815	39.101	34.849	-4.252
800	32.810	34.504	+1.694	39.196	35.075	-4.121
600	32.900	34.631	+1.732	39.261	35.021	-4.240
400	33.892	35.531	+1.639	39.549	35.596	-3.953
200	34.053	35.414	+1.362	39.543	35.915	-3.628
100	35.664	37.211	+1.547	40.000	37.302	-2.698
50	36.577	37.809	+1.232	40.000	38.658	-1.342
25	37.382	38.430	+1.049	39.966	39.132	-0.834
10	38.663	39.824	+1.162	37.898	39.640	+1.742
5	39.635	40.000	+0.365	37.863	37.972	+0.109
1	40.000	40.000	0	40.000	40.000	0
	Ct HBG2n	Ct S15	dCt (S15-HBG2n)	Ct HBG2n	Ct S15	dCt (S15-HBG2n)
1000	28.166	35.389	+7.224	36.622	35.741	-0.881
800	29.007	34.724	+5.717	36.561	35.298	-1.263
600	29.132	34.921	+5.788	37.188	35.225	-1.963
400	30.959	35.381	+4.421	38.406	35.665	-2.741
200	32.203	36.192	+3.989	39.872	36.940	-2.933
100	33.191	37.636	+4.444	40.000	37.599	-2.401
50	34.441	38.250	+3.809	40.000	38.415	-1.585
25	31.307	34.646	+3.339	40.000	40.000	0
10	37.177	39.854	+2.677	40.000	40.000	0
5	38.467	39.969	+1.502	40.000	40.000	0
1	39.779	40.000	+0.221	40.000	40.000	0

Ct, cycle threshold; dCt, delta cycle threshold

Table 19: Telomere Real-time PCR and STELA primer, probe, and linker sequences.

Assay	Primer, Probe, & Linker Sequences
Real-Time PCR	
36B4u	5' CAG-CAA-GTG-GGA-AGG-TGT-AAT-CC 3' [a]
36B4d	5' CCC-ATT-CTA-TCA-TCA-ACG-GGT-ACA-A 3' [a]
tel 1	5' GGT-TTT-TGA-GGG-TGA-GGG-TGA-GGG-TGA-GGG-TGA-GGG-T 3' [a]
tel 2	5' TCC-CGA-CTA-TCC-CTA-TCC-CTA-TCC-CTA-TCC-CTA-TCC-CTA 3' [a]
tel 3	5' VIC-CCC-TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC-TAA-C-TAMRA 3'
tel 4	5' GGT-TTT-TGA-GGG-TGA-GGG-TGA-GGG-T 3'
tel 5	5' TCC-CGA-CTA-TCC-CTA-TCC-CTA-TCC-CTA 3'
tel 6	5' VIC-CCC-TAA-CCC-TAA-CCC-TAA-C-TAMRA 3'
STELA	
Telorette 1	5' TGC-TCC-GTG-CAT-CTG-GCA-TCC-CCT-AAC 3' [b]
Telorette 2	5' TGC-TCC-GTG-CAT-CTG-GCA-TCT-AAC-CCT 3' [b]
Telorette 3	5' TGC-TCC-GTG-CAT-CTG-GCA-TCC-CTA-ACC 3' [b]
Telorette 4	5' TGC-TCC-GTG-CAT-CTG-GCA-TCC-TAA-CCC 3' [b]
Telorette 5	5' TGC-TCC-GTG-CAT-CTG-GCA-TCA-ACC-CTA 3' [b]
Telorette 6	5' TGC-TCC-GTG-CAT-CTG-GCA-TCA-CCC-TAA 3' [b]
TelTail	5' TGC-TCC-GTG-CAT-CTG-GCA-TC 3' [b]
XpYpE2	5' TTG-TCT-CAG-GGT-CCT-AGT-G 3' [b]

[a] Cawthon R, Telomere measurement by quantitative PCR, Nucleic Acids Research, 2002, 30 (10).
[b] Baird D et al., Extensive allelic variation and ultrashort telomeres in senescent human cells, Nature Genetics, 2003, 33.

APPENDIX C: CANDIDATE GENE DATABASE

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
ABL1	Homo sapiens v-abl Abelson murine leukemia viral oncogene homolog 1, transcript variant b	Oncogene	Elderly	NM_007313
ACD	Homo sapiens adrenocortical dysplasia homolog (mouse), transcript variant 2	Disease	Elderly	NM_022914
ACTA2	Homo sapiens cDNA FLJ36021 fis, clone TESTI2016568	Affymetrix	Juvenile	AK093340
ACTN3	Homo sapiens actinin, alpha 3	Transcription & Gene Regulation	Elderly	NM_001104
ADAM12	AU145357 HEMBA1 Homo sapiens cDNA clone HEMBA1004611 3-	Affymetrix	Juvenile	AU145357
ADAM12a	AU145357 HEMBA1 Homo sapiens cDNA clone HEMBA1004611 3-	Affymetrix	Juvenile	AU145357
AFP	Homo sapiens alpha-fetoprotein	Fetal Protein	Newborn	NM_001134
AGGF1	Homo sapiens angiogenic factor with G patch and FHA domains 1	Affymetrix	Elderly	NM_018046
AIF1	Homo sapiens allograft inflammatory factor 1, transcript variant 3	Immunology & Interferons	Elderly	NM_001623
AKT1	Homo sapiens v-akt murine thymoma viral oncogene homolog 1, transcript variant 1	Oncogene	Elderly	NM_005163
AMID	Homo sapiens apoptosis-inducing factor, mitochondrion-associated, 2 (AIFM2)	Mitochondria	Elderly	NM_032797
ANKH	Homo sapiens ankylosis, progressive homolog (mouse)	Bone	Elderly	NM_054027
APEX1	Homo sapiens APEX nuclease (multifunctional DNA repair enzyme) 1, transcript variant 1	DNA Damage & Growth Arrest	Elderly	NM_001641

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
APOE (1)	Human apolipoprotein E mRNA, complete cds	Fetal Protein	Newborn	M12529
APOE (2)	Homo sapiens apolipoprotein E	Fetal Protein	Newborn	NM_000041
ARMC7	Homo sapiens armadillo repeat containing 7	Affymetrix	Elderly	NM_024585
Art3a	Human putative mono-ADP-ribosyltransferase (htMART)	Affymetrix	Juvenile	U47054
Art3b	Human putative mono-ADP-ribosyltransferase (htMART)	Affymetrix	Juvenile	U47054
ASL	Homo sapiens argininosuccinate lyase, transcript variant 2	Affymetrix	Juvenile	NM_000048
ATF7IP2	DCB Homo sapiens cDNA clone DCBBOG12 5'	Affymetrix	Newborn	AV7169647
ATPAF2	Homo sapiens ATP synthase mitochondrial F1 complex assembly factor 2, nuclear gene encoding mitochondrial protein	Affymetrix	Elderly	NM_145691
AUF1 hnRNPD p37	Homo sapiens heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) (HNRPD), transcript variant 4	Fetal Protein Isofrom	Newborn	NM_001003810
AUF1 hnRNPD p45	Homo sapiens heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) (HNRPD), transcript variant 1	Adult Isoform	Adult	NM_031370
BAX-(all)	Homo sapiens BCL2-associated X protein, transcript variant epsilon	Apoptosis	Elderly	NM_138764
BAX-a/d	Homo sapiens BCL2-associated X protein, transcript variant alpha	Apoptosis	Elderly	NM_138761

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
BAX-all(e)	Homo sapiens BCL2-associated X protein, transcript variant epsilon	Apoptosis	Elderly	NM_138764
BAX-b	Homo sapiens BCL2-associated X protein, transcript variant beta	Apoptosis	Elderly	NM_004324
BAX-d	Homo sapiens BCL2-associated X protein, transcript variant delta	Apoptosis	Elderly	NM_138763
BAX-e	Homo sapiens BCL2-associated X protein, transcript variant epsilon	Apoptosis	Elderly	NM_138764
BAX-s	Homo sapiens BCL2-associated X protein, transcript variant sigma	Apoptosis	Elderly	NM_138765
BCKDHA	Homo sapiens branched chain keto acid dehydrogenase E1, alpha polypeptide	Affymetrix	Juvenile	NM_000709
BCL2A1	Homo sapiens BCL2-related protein A1	Apoptosis	Elderly	NM_004049
BGLAP	Homo sapiens bone gamma-carboxyglutamate (gla) protein (osteocalcin)	Bone	Elderly	NM_199173
BIRC5	Homo sapiens baculoviral IAP repeat-containing 5 (survivin), transcript variant 3	Apoptosis	Elderly	NM_001012271
c5229134	Homo sapiens, clone IMAGE:5229134	Affymetrix	Juvenile	BC037976
c5286506	Homo sapiens cDNA clone IMAGE:5286506	Affymetrix	Juvenile	BC043160
CABP7	Homo sapiens calcium binding protein 7	Affymetrix	Juvenile	NM_182527
CABYR	Homo sapiens calcium binding tyrosine-(Y)-phosphorylation regulated (fibrousheathin 2), transcript variant 1	Hormone	Juvenile	NM_012189

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
CAMK2D	Homo sapiens calcium/calmodulin-dependent protein kinase (CaM kinase) II delta, transcript variant 1	Growth Factor	Elderly	NM_172127
CASP2	Homo sapiens caspase 2, apoptosis-related cysteine peptidase (neural precursor cell expressed, developmentally down-regulated 2), transcript variant 1	Apoptosis	Elderly	NM_032982
CBL	Homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence	Oncogene	Elderly	NM_005188
CCL5	Homo sapiens chemokine (C-C motif) ligand 5	Affymetrix	Elderly	NM_002985
CCM2	Homo sapiens cerebral cavernous malformation 2, transcript variant 1	Disease	Elderly	NM_001029835
CCND1	Homo sapiens cyclin D1	Cyclin	Elderly	NM_053056
CD200	Homo sapiens CD200 molecule, transcript variant 2	Immunology & Interferons	Juvenile	NM_001004196
CD28	Homo sapiens CD28 molecule	Affymetrix	Juvenile	NM_006139
CD28	Homo sapiens CD28 molecule	Immunology & Interferons	Elderly	NM_006139
CD86	Homo sapiens CD86 molecule, transcript variant 1	Immunology & Interferons	Elderly	NM_175862
CDC2	Homo sapiens cell division cycle 2, G1 to S and G2 to M, transcript variant 1	Cyclin	Elderly	NM_001786
CDC25C	Homo sapiens cell division cycle 25 homolog C (S. pombe), transcript variant 2	Cyclin	Elderly	NM_022809
CDKN1A	Homo sapiens cyclin-dependent kinase inhibitor 1A (p21, Cip1), transcript variant 2	Cyclin	Elderly	NM_078467

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
CDKN1B	Homo sapiens cyclin-dependent kinase inhibitor 1B (p27, Kip1)	Cyclin	Elderly	NM_004064
CDKN2C	Homo sapiens cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4), transcript variant 1	Cyclin	Elderly	NM_001262
CFIX	Homo sapiens coagulation factor IX (plasma thromboplastic component, Christmas disease, hemophilia B) (F9)	Growth Factor	Juvenile	NM_000133
CGI-96	Novel human gene mapping to chomosome 22	Affymetrix	Juvenile	AL157851
CHR1orf28	zw89h01.r1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:784177 5- similar to contains Alu repetitive element.	Affymetrix	Newborn	AA447464
CIITA	Homo sapiens class II, major histocompatibility complex, transactivator (CIITA)	Transcription & Gene Regulation	Elderly	NM_000246
CLEC2	Homo sapiens C-type lectin domain family 1, member B (CLEC1B)	Affymetrix	Elderly	NM_016509
CLEC2a	Homo sapiens C-type lectin domain family 1, member B (CLEC1B)	Affymetrix	Elderly	NM_016509
CLEC2b	Homo sapiens C-type lectin domain family 1, member B (CLEC1B)	Affymetrix	Elderly	NM_016509
COL1A1 CTx	Homo sapiens collagen, type I, alpha 1	Bone	Elderly	NM_000088
COL1A2	Homo sapiens collagen, type I, alpha 2	Bone	Newborn	NM_000089
COL6A1a	Homo sapiens, alpha-1 (VI)	Affymetrix	Juvenile	M20776
COL6A1b	Homo sapiens, alpha-1 (VI)	Affymetrix	Juvenile	M20776

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
CTBP1	Homo sapiens C-terminal binding protein 1, transcript variant 2	Transcription & Gene Regulation	Elderly	NM_001012614
CTSB	Homo sapiens cathepsin B, transcript variant 2	Bone	Elderly	NM_147780
CTSK	Homo sapiens cathepsin K (pycnodysostosis)	Bone	Elderly	NM_000396
CTSL	Homo sapiens cathepsin L1 (CTSL1), transcript variant 1	Bone	Elderly	NM_001912
CXorf22	Homo sapiens chromosome X open reading frame 22 (CXorf22)	Affymetrix	Juvenile	NM_152632
CYP17A1	Homo sapiens cytochrome P450, family 17, subfamily A, polypeptide 1	Hormone	Juvenile	NM_000102
CYP1B1	Homo sapiens cytochrome P450, family 1, subfamily B, polypeptide 1	Hormone	Juvenile	NM_000104
CYP7B1	Homo sapiens cytochrome P450, family 7, subfamily B, polypeptide 1 (CYP7B1),	Hormone	Juvenile	NM_004820
CYTBC2	Homo sapiens mRNA for cytochrome b large subunit of complex II, complete cds	Affymetrix	Elderly	D49737
DDB2	Homo sapiens damage-specific DNA binding protein 2, 48kDa	DNA Damage & Growth Arrest	Elderly	NM_000107
DHEA	Homo sapiens sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 (SULT2A1)	Hormone	Juvenile	NM_003167
DNCL2A-1	Homo sapiens dynein, light chain, roadblock-type 1 (DYNLRB1), transcript variant 1	Affymetrix	Juvenile	NM_014183
DNCL2A-2	Homo sapiens dynein, cytoplasmic, light polypeptide 2A, transcript variant 2	Affymetrix	Juvenile	NM_177953

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
DNCL2A-3	Homo sapiens dynein, cytoplasmic, light polypeptide 2A, transcript variant 3	Affymetrix	Juvenile	NM_177954
DNPEP	Homo sapiens aspartyl aminopeptidase	Affymetrix	Juvenile	NM_012100
DUSP6	Homo sapiens dual specificity phosphatase 6, mRNA (cDNA clone MGC:12852 IMAGE:3954486), complete cds	Affymetrix	Newborn	BC005047
DYRK2	Homo sapiens dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2, transcript variant 2	Growth Factor	Elderly	NM_006482
E2F1	Homo sapiens E2F transcription factor 1	Transcription & Gene Regulation	Elderly	NM_005225
E2IG2	Homo sapiens coiled-coil-helix-coiled-coil-helix domain containing 8	Hormone	Juvenile	NM_016565
ECGF1	Homo sapiens endothelial cell growth factor 1 (platelet-derived)	Growth Factor	Elderly	NM_001953
ELAVL1 HuR	Homo sapiens ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	Transcription & Gene Regulation	Elderly	NM_001419
EMD	Homo sapiens emerin (Emery-Dreifuss muscular dystrophy)	Disease	Elderly	NM_000117
ERBP	Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2)	Hormone	Juvenile	NM_014597
EREG	Homo sapiens epiregulin	Growth Factor	Elderly	NM_001432
ERF	Homo sapiens Ets2 repressor factor	Tumor Suppressor Genes	Elderly	NM_006494
ESR1	Homo sapiens estrogen receptor 1	Hormone	Juvenile	NM_000125

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
ESR2	Homo sapiens estrogen receptor 2 (ER beta), transcript variant a	Hormone	Juvenile	NM_001437
FACL6	AV727634 HTC Homo sapiens cDNA clone HTCAYH08 5-	Affymetrix	Newborn	AV727634
FKBP11	Homo sapiens FK506 binding protein 11, 19 kDa	Affymetrix	Juvenile	NM_016594
FKLF	Homo sapiens kruppel-like fetal and embryonic globin gene activator, complete cds	Fetal Protein	Newborn	AF272830
FLJ11078	Homo sapiens kelch-like 26 (Drosophila) (KLHL26)	Affymetrix	Juvenile	NM_018316
FLJ20245	Homo sapiens chromosome 9 open reading frame 167 (C9orf167)	Affymetrix	Elderly	NM_017723
FLJ20344a	Homo sapiens zinc finger protein 673 (ZNF673)	Affymetrix	Newborn	NM_017776
FLJ20344b	Homo sapiens zinc finger protein 673 (ZNF673)	Affymetrix	Newborn	NM_017776
FLJ20421	602136866F1 NIH_MGC_83 Homo sapiens cDNA clone IMAGE:4273120 5-	Affymetrix	Elderly	BF674724
FLJ21901	Homo sapiens FAST kinase domains 1 (FASTKD1)	Affymetrix	Newborn	NM_024622
FLJ22175	Homo sapiens chromosome 17 open reading frame 70 (C17orf70)	Affymetrix	Juvenile	NM_025161
FLJ22672 PAQR6	Homo sapiens progestin and adipoQ receptor family member VI, transcript variant 1	Affymetrix	Juvenile	NM_024897
FLJ30658	hn54d06.x1 NCI_CGAP_Co17 Homo sapiens cDNA clone IMAGE:3027467 3-	Affymetrix	Newborn	AW770868

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
FLJ35119	Homo sapiens chromosome 19 open reading frame 39 (C19orf39)	Affymetrix	Juvenile	NM_175871
FLJ35954	yi35b09.s1 Soares placenta Nb2HP Homo sapiens cDNA clone IMAGE:141209 3-	Affymetrix	Newborn	R66534
FLJ35982	Homo sapiens cDNA FLJ35982 fis, clone TESTI2013604	Affymetrix	Juvenile	AK093301
FLJ35982a	Homo sapiens cDNA FLJ35982 fis, clone TESTI2013604	Affymetrix	Juvenile	AK093301
FLJ35984	Homo sapiens cDNA FLJ35984 fis, clone TESTI2014097, highly similar to V_segment translation product	Affymetrix	Elderly	AK093303
FLJ37440	Homo sapiens hypothetical protein FLJ37440	Affymetrix	Juvenile	NM_153214
FLJ38628	Homo sapiens ring finger protein 185 (RNF185)	Affymetrix	Elderly	NM_152267
FLJ38745	Homo sapiens cDNA FLJ38745 fis, clone KIDNE2012291	Affymetrix	Juvenile	AK096064
FLJ43159	wr63b05.x1 NCI_CGAP_Ut1 Homo sapiens cDNA clone IMAGE:2492337 3-	Affymetrix	Juvenile	AI972146
GADD45A	Homo sapiens growth arrest and DNA-damage-inducible, transcript variant alpha	DNA Damage & Growth Arrest	Elderly	NM_001924
GADD45B	Homo sapiens growth arrest and DNA-damage-inducible, transcript variant beta	DNA Damage & Growth Arrest	Elderly	NM_015675
GAL	Homo sapiens galanin	Hormone	Juvenile	NM_015973
GFPT2	Homo sapiens glutamine-fructose-6-phosphate transaminase 2	Affymetrix	Juvenile	NM_005110

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
GGT1	Homo sapiens gamma-glutamyltransferase 1, transcript variant 1	Affymetrix	Juvenile	NM_005265
GHRH	Homo sapiens growth hormone releasing hormone	Hormone	Juvenile	NM_021081
GLO1	Homo sapiens glyoxalase I	Immunology & Interferons	Juvenile	NM_006708
GNAS	Homo sapiens GNAS complex locus, transcript variant 4	Housekeeping Gene	HSK	NM_016592
GNAS2	Homo sapiens GNAS complex locus, transcript variant 4	Housekeeping Gene	HSK	NM_016592
GNRH1	Homo sapiens gonadotropin-releasing hormone 1 (luteinizing-releasing hormone), transcript variant 1	Hormone	Juvenile	NM_000825
GNRH2	Homo sapiens gonadotropin-releasing hormone 2, transcript variant 1	Hormone	Juvenile	NM_001501
GNRHR	Homo sapiens gonadotropin-releasing hormone receptor, transcript variant 1	Hormone	Juvenile	NM_000406
GPR54	Homo sapiens KISS1 receptor (KISS1R)	Hormone	Juvenile	NM_032551
GRIN1 NR1-1	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 1, transcript variant NR1-1	Hormone	Juvenile	NM_000832
GRIN1 NR1-2	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 1, transcript variant NR1-2	Hormone	Juvenile	NM_021569
GRIN1 NR1-3	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 1, transcript variant NR1-3	Hormone	Juvenile	NM_007327
GRIN2A	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 2A	Hormone	Juvenile	NM_000833

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
GRIN2B	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 2B	Hormone	Juvenile	NM_000834
GSTP1	Homo sapiens glutathione S-transferase pi	Affymetrix	Elderly	NM_000852
H17	Homo sapiens FAD-dependent oxidoreductase domain containing 1 (FOXRED1)	Affymetrix	Juvenile	NM_017547
HBA1	Homo sapiens hemoglobin, alpha 1	Hemoglobin	Adult	NM_000558
HBA2	Homo sapiens hemoglobin, alpha 2	Hemoglobin	Adult	NM_000517
HBB	Homo sapiens hemoglobin, beta	Hemoglobin	Adult	NM_000518
HBD	Homo sapiens hemoglobin, delta	Hemoglobin	Adult	NM_000519
HBE1	Homo sapiens hemoglobin, epsilon 1	Hemoglobin	Newborn	NM_005330
HBG1	Homo sapiens hemoglobin, gamma A	Hemoglobin	Newborn	NM_000559
HBG1n1	Novel transcript isoform of hemoglobin, gamma A	Hemoglobin	Newborn	
HBG1n2	Novel transcript isoform of hemoglobin, gamma A	Hemoglobin	Newborn	
HBG2	Homo sapiens hemoglobin, gamma G	Hemoglobin	Newborn	NM_000184
HBG2n2	Novel transcript isoform of hemoglobin, gamma G	Hemoglobin	Newborn	
HBG2n3	Novel transcript isoform of hemoglobin, gamma G	Hemoglobin	Newborn	

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
HBZ	Homo sapiens hemoglobin, zeta	Hemoglobin	Newborn	NM_005332
HBQ	Homo sapiens hemoglobin, theta 1	Hemoglobin	Adult	NM_005331
HIC2	Homo sapiens hypermethylated in cancer 2	Affymetrix	Elderly	NM_015094
HIF1A	Homo sapiens hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor), transcript variant 1	Transcription & Gene Regulation	Elderly	NM_001530
HOMER3	Homo sapiens homer homolog 3 (Drosophila)	Affymetrix	Juvenile	NM_004838
HPCAL4	Homo sapiens hippocalcin like 4	Affymetrix	Elderly	NM_016257
HRAS	Homo sapiens v-Ha-ras Harvey rat sarcoma viral oncogene homolog, transcript variant 2	Oncogene	Elderly	NM_176795
HRG	Homo sapiens histidine-rich glycoprotein	Affymetrix	Juvenile	NM_000412
HTATIP	Homo sapiens HIV-1 Tat interacting protein, 60kDa, transcript variant 1	DNA Damage & Growth Arrest	Elderly	NM_182710
HTR1E	Homo sapiens 5-hydroxytryptamine (serotonin) receptor 1E	Affymetrix	Juvenile	NM_000865
HTR7	Homo sapiens 5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled), transcript variant a	Affymetrix	Juvenile	NM_000872
IFNG	Homo sapiens interferon, gamma	Immunology & Interferons	Elderly	NM_000619
IGF1	Homo sapiens insulin-like growth factor 1 (somatomedin C)	Growth Factor	Elderly	NM_000618

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
IGF2	Homo sapiens insulin-like growth factor 2 (somatomedin A), transcript variant 1	Growth Factor	Elderly	NM_000612
IGFBP3	Homo sapiens insulin-like growth factor binding protein 3, transcript variant 1	Growth Factor	Elderly	NM_001013398
IGFBP5	Homo sapiens insulin-like growth factor binding protein 5	Growth Factor	Elderly	NM_000599
IL1A	Homo sapiens interleukin 1, alpha	Immunology & Interferons	Elderly	NM_000575
INHA	Homo sapiens inhibin, alpha	Bone	Juvenile	NM_002191
IRF1	Homo sapiens interferon regulatory factor 1	Immunology & Interferons	Elderly	NM_002198
ITIH4	Homo sapiens inter-alpha (globulin) inhibitor H4 (plasma Kallikrein-sensitive glycoprotein)	Affymetrix	Juvenile	NM_002218
ITSN2	Homo sapiens intersectin 2, transcript variant 1	Affymetrix	Newborn	NM_006277
KIAA0276	Homo sapiens mRNA for KIAA0276 gene, partial cds	Affymetrix	Newborn	D87466
KIAA0894	Homo sapiens KIAA0894 protein	Affymetrix	Juvenile	NM_014896
KIAA1265	Homo sapiens mRNA for KIAA1265 protein, partial cds	Affymetrix	Newborn	AB033091
KIAA2022	Homo sapiens mRNA for KIAA2022 protein	Affymetrix	Juvenile	AB095942
KISS-1	Homo sapiens KiSS-1 metastasis-suppressor	Hormone	Juvenile	NM_002256

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
KITLG	Homo sapiens KIT ligand, transcript variant b	Fetal Protein	Newborn	NM_000899
KL	Homo sapiens klotho, transcript variant 2	Bone	Elderly	NM_153683
KLF13	Homo sapiens Kruppel-like factor 13	Transcription & Gene Regulation	Elderly	NM_015995
LASS5	Homo sapiens LAG1 homolog, ceramide synthase 5 (S. cerevisiae)	Affymetrix	Elderly	NM_147190
LATS1	Homo sapiens LATS, large tumor suppressor, homolog 1 (Drosophila)	Affymetrix	Juvenile	NM_004690
LEP	Homo sapiens leptin (obesity homolog, mouse)	Hormone	Juvenile	NM_000230
LHB	Homo sapiens luteinizing hormone beta polypeptide	Hormone	Juvenile	NM_000894
LHCGR	Homo sapiens luteinizing hormone/choriogonadotropin receptor	Hormone	Juvenile	NM_000233
LMNA (norm)	Homo sapiens lamin A/C, transcript variant 1	Transcription & Gene Regulation	Elderly	NM_170707
LMNA (RT)	Homo sapiens lamin A/C, transcript variant 1	Transcription & Gene Regulation	Elderly	NM_170707
LMNA (spec)	Homo sapiens lamin A/C, transcript variant 1	Transcription & Gene Regulation	Elderly	NM_170707
LOC151194	Homo sapiens family with sequence similarity 119, member A (FAM119A)	Affymetrix	Newborn	NM_145280
LOC152274	Homo sapiens cDNA FLJ31836 fis, clone NT2RP7000041	Affymetrix	Juvenile	AK056398

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
LOC284242	Homo sapiens, clone IMAGE:5745916	Affymetrix	Juvenile	BC035844
LOH11CR2A	Homo sapiens loss of heterozygosity, 11, chromosomal region 2, gene A, mRNA (cDNA clone MGC:4904 IMAGE:3461486), complete cds	Affymetrix	Elderly	BC001234
LZTFL1	Homo sapiens leucine zipper transcription factor-like 1	Affymetrix	Newborn	NM_020347
MAD1L1	Homo sapiens MAD1 mitotic arrest deficient-like 1 (yeast), transcript variant 1	Cyclin	Elderly	NM_003550
MCPH1	Homo sapiens microcephaly, primary autosomal recessive 1	Tumor Suppressor Genes	Elderly	NM_024596
MDM2	Homo sapiens Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse), transcript variant MDM2	Tumor Suppressor Genes	Elderly	NM_002392
MEPE	Homo sapiens matrix, extracellular phosphoglycoprotein with ASARM motif (bone)	Bone	Elderly	NM_020203
MET	Homo sapiens met proto-oncogene (hepatocyte growth factor receptor)	Oncogene	Elderly	NM_000245
MGC14288	Homo sapiens chromosome 12 open reading frame 62 (C12orf62)	Affymetrix	Elderly	NM_032901
MGC20460	Homo sapiens proline rich 8 (PRR8)	Affymetrix	Juvenile	NM_053043
MGC39650	Homo sapiens mRNA; cDNA DKFZp434F0919 (from clone DKFZp434F0919)	Affymetrix	Juvenile	AL137531
MIF	Homo sapiens macrophage migration inhibitory factor (MIF) gene, complete cds	Fetal Protein	Newborn	L19686
MLL	Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	Disease	Elderly	NM_005933

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
MMP-13	Homo sapiens matrix metallopeptidase 13 (collagenase 3)	Bone	Elderly	NM_002427
MMP-14	Homo sapiens matrix metallopeptidase 14 (membrane-inserted)	Bone	Elderly	NM_004995
MMP-9	Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	Bone	Elderly	NM_004994
MS4A4A	Homo sapiens membrane-spanning 4-domains, subfamily A, member 4, transcript variant 1	Affymetrix	Elderly	NM_024021
MS4A4Aa	Homo sapiens membrane-spanning 4-domains, subfamily A, member 4, transcript variant 1	Affymetrix	Elderly	NM_024021
MS4A4Ab	Homo sapiens membrane-spanning 4-domains, subfamily A, member 4, transcript variant 1	Affymetrix	Elderly	NM_024021
MT1X	Homo sapiens metallothionein 1X	Affymetrix	Elderly	NM_005952
MYC	Homo sapiens v-myc myelocytomatosis viral oncogene homolog (avian)	Oncogene	Elderly	NM_002467
NALP14	Homo sapiens NLR family, pyrin domain containing 14 (NLRP14)	Affymetrix	Juvenile	NM_176822
NBN	Homo sapiens nibrin, transcript variant 2	Disease	Elderly	NM_001024688
NDE1	Homo sapiens nudE nuclear distribution gene E homolog 1 (A. nidulans)	Affymetrix	Juvenile	NM_017668
NMI	Homo sapiens N-myc (and STAT) interactor	Oncogene	Elderly	NM_004688
NPPB	Homo sapiens natriuretic peptide precursor B	Bone	Elderly	NM_002521

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
NRAS	Homo sapiens neuroblastoma RAS viral (v-ras) oncogene homolog	Oncogene	Elderly	NM_002524
NTS	Homo sapiens neurotensin	Hormone	Juvenile	NM_006183
OGG1	Homo sapiens 8-oxoguanine DNA glycosylase (OGG1), nuclear gene encoding mitochondrial protein, transcript variant 1b	Mitochondria	Elderly	NM_016819
OPG TNFRSF11B	Homo sapiens tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	Bone	Elderly	NM_002546
OPGL RANKL TNFSF11	Homo sapiens tumor necrosis factor (ligand) superfamily, member 11, transcript variant 1	Bone	Elderly	NM_003701
OSGEP	Homo sapiens O-sialoglycoprotein endopeptidase	Affymetrix	Juvenile	NM_017807
OSM	Homo sapiens oncostatin M	Growth Factor	Elderly	NM_020530
OXTR	Homo sapiens oxytocin receptor	Hormone	Juvenile	NM_000916
PAQR6	Homo sapiens progestin and adipoQ receptor family member VI, transcript variant 1	Affymetrix	Juvenile	NM_024897
PDCD1	Homo sapiens programmed cell death 1	Programmed Cell Death	Elderly	NM_005018
PDCD10 CCM3	Homo sapiens programmed cell death 10, transcript variant 1	Programmed Cell Death	Elderly	NM_007217
PDCD11	Homo sapiens programmed cell death 11	Programmed Cell Death	Elderly	NM_014976
PDCD1LG2	Homo sapiens programmed cell death 1 ligand 2	Programmed Cell Death	Elderly	NM_025239

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
PDCD2L	Homo sapiens programmed cell death 2-like	Programmed Cell Death	Elderly	NM_032346
PDCD4	Homo sapiens programmed cell death 4 (neoplastic transformation inhibitor), transcript variant 2	Programmed Cell Death	Elderly	NM_145341
PDCD5	Homo sapiens programmed cell death 5	Programmed Cell Death	Elderly	NM_004708
PDCD6	Homo sapiens programmed cell death 6	Programmed Cell Death	Elderly	NM_013232
PDCD6IP	Homo sapiens programmed cell death 6 interacting protein	Programmed Cell Death	Elderly	NM_013374
PDCD7	Homo sapiens programmed cell death 7	Programmed Cell Death	Elderly	NM_005707
PDE6D	Homo sapiens phosphodiesterase 6D, cGMP-specific, rod, delta	Affymetrix	Juvenile	NM_002601
PGR	Homo sapiens progesterone receptor	Hormone	Juvenile	NM_000926
PIK3CA	Homo sapiens phosphoinositide-3-kinase, catalytic, alpha polypeptide	Oncogene	Elderly	NM_006218
PITPNC1	wc05c10.x1 NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2314290 3-	Affymetrix	Newborn	AI676095
PLEKHA8	Homo sapiens pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 8	Affymetrix	Juvenile	NM_032639
POLA1	Homo sapiens polymerase (DNA directed), alpha 1	Polymerase	Elderly	NM_016937
POLA2	Homo sapiens polymerase (DNA directed), alpha 2 (70kD subunit)	Polymerase	Elderly	NM_002689

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
POLB	Homo sapiens polymerase (DNA directed), beta	Polymerase	Elderly	NM_002690
POLD1	Homo sapiens polymerase (DNA directed), delta 1, catalytic subunit 125kDa	Polymerase	Elderly	NM_002691
POLE1	Homo sapiens polymerase (DNA directed), epsilon	Polymerase	Elderly	NM_006231
POLE2	Homo sapiens polymerase (DNA directed), epsilon 2 (p59 subunit)	Polymerase	Elderly	NM_002692
POLE3	Homo sapiens polymerase (DNA directed), epsilon 3 (p17 subunit)	Polymerase	Elderly	NM_017443
POLG	Homo sapiens polymerase (DNA directed), gamma	Polymerase	Elderly	NM_002693
POLH	Homo sapiens polymerase (DNA directed), eta	Polymerase	Elderly	NM_006502
POLI	Homo sapiens polymerase (DNA directed) iota	Polymerase	Elderly	NM_007195
POLK	Homo sapiens polymerase (DNA directed) kappa	Polymerase	Elderly	NM_016218
POLM	Homo sapiens polymerase (DNA directed), mu	Polymerase	Elderly	NM_013284
POLN	Homo sapiens polymerase (DNA directed) nu	Polymerase	Elderly	NM_181808
POLQ	Homo sapiens polymerase (DNA directed), theta	Polymerase	Elderly	NM_199420
POLR3F	Homo sapiens polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa	Polymerase	Elderly	NM_006466

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
POLR3K	Homo sapiens polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa	Polymerase	Elderly	NM_016310
POLS	Homo sapiens polymerase (DNA directed) sigma	Polymerase	Elderly	NM_006999
POMC	Homo sapiens proopiomelanocortin (adrenocorticotropin/ beta-lipotropin/ alpha-melanocyte stimulating hormone/ beta-melanocyte stimulating hormone/ beta-endorphin), transcript variant 2	Hormone	Juvenile	NM_000939
POT1	Homo sapiens POT1 protection of telomeres 1 homolog (S. pombe), transcript variant 2	Telomeres	Elderly	NR_003102
PPARD	Homo sapiens peroxisome proliferator-activated receptor delta	Bone	Elderly	NM_006238
PPAT	Homo sapiens glutamine PRPP amidotransferase (GPAT)	Affymetrix	Newborn	U00238
PPOX	Homo sapiens protoporphyrinogen oxidase, nuclear gene encoding mitochondrial protein	Mitochondria	Juvenile	NM_000309
PRDX5	Homo sapiens peroxiredoxin 5, nuclear gene encoding mitochondrial protein, transcript variant 1	Mitochondria	Elderly	NM_012094
PRKCA	Homo sapiens protein kinase C, alpha	Cyclin	Elderly	NM_002737
PRL	Homo sapiens prolactin	Hormone	Juvenile	NM_000948
PTGER4	Homo sapiens prostaglandin E receptor 4 (subtype EP4)	Bone	Elderly	NM_000958
PTH	Homo sapiens parathyroid hormone	Hormone	Juvenile	NM_000315

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
PTMS	Homo sapiens parathymosin	Affymetrix	Juvenile	NM_002824
PTPN18	Homo sapiens protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	Affymetrix	Elderly	NM_014369
RAD50	Homo sapiens RAD50 homolog (S. cerevisiae), transcript variant 1	DNA Damage & Growth Arrest	Elderly	NM_005732
RAF1	Homo sapiens v-raf-1 murine leukemia viral oncogene homolog 1	Oncogene	Elderly	NM_002880
RaI	wq65b01.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:2476105 3-	Affymetrix	Newborn	AW003297
RaIGPS2	Homo sapiens Ral GEF with PH domain and SH3 binding motif 2, transcript variant 1	Affymetrix	Newborn	NM_018037
RANK	Homo sapiens receptor activator of nuclear factor-kappa B	Growth Factor	Elderly	AF018253
RAPA-2 TRERF1	Homo sapiens mRNA for rapa-2 (rapa gene), transcript variant 3	Hormone	Juvenile	AJ277276
RARA	Homo sapiens retinoic acid receptor, alpha, transcript variant 1	Affymetrix	Juvenile	NM_000964
RB1	Homo sapiens retinoblastoma 1 (including osteosarcoma)	Tumor Suppressor Genes	Elderly	NM_000321
RBL1	Homo sapiens retinoblastoma-like 1 (p107), transcript variant 1	Tumor Suppressor Genes	Elderly	NM_002895
RBL2	Homo sapiens retinoblastoma-like 2 (p130)	Tumor Suppressor Genes	Elderly	NM_005611
REA	Homo sapiens prohibitin 2 (PHB2)	Hormone	Juvenile	NM_007273

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
RELA	Homo sapiens v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	Oncogene	Elderly	NM_021975
RUNX2	Homo sapiens runt-related transcription factor 2, transcript variant 3	Transcription & Gene Regulation	Elderly	NM_004348
S15	Homo sapiens ribosomal protein S15 (RPS15)	Housekeeping Gene	HSK	NM_001018
SEMA4A	Homo sapiens sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	Affymetrix	Elderly	NM_022367
SH3GL1	Homo sapiens SH3-domain GRB2-like 1	Affymetrix	Juvenile	NM_003025
SHBG	Homo sapiens sex hormone-binding globulin	Hormone	Juvenile	NM_001040
SLC20A1	Homo sapiens solute carrier family 20 (phosphate transporter), member 1	Immunology & Interferons	Elderly	NM_005415
SLC39A4	Homo sapiens solute carrier family 39 (zinc transporter), member 4, transcript variant 1	Affymetrix	Juvenile	NM_017767
SMG5	Homo sapiens Smg-5 homolog, nonsense mediated mRNA decay factor (C. elegans)	Telomeres	Elderly	NM_015327
SMG6	Homo sapiens Smg-6 homolog, nonsense mediated mRNA decay factor (C. elegans)	Telomeres	Elderly	NM_017575
SMG7	Homo sapiens Smg-7 homolog, nonsense mediated mRNA decay factor (C. elegans), transcript variant 1	Telomeres	Elderly	NM_173156
SNCA	Homo sapiens synuclein, alpha (non A4 component of amyloid precursor) (SNCA), transcript variant NACP140	Disease	Elderly	NM_000345
SPATA1 SP2	Homo sapiens spermatogenesis associated 1	Hormone	Juvenile	NM_022354

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
SPINK5L3	Homo sapiens serine PI Kazal type 5-like 3	Affymetrix	Juvenile	XM_376433 Replaced by NM_001040129
SPINKa	Homo sapiens cDNA FLJ30191 fis, clone BRACE2001313	Affymetrix	Juvenile	AK054753
SPINKb	Homo sapiens cDNA FLJ30191 fis, clone BRACE2001313	Affymetrix	Juvenile	AK054753
SPP1	Homo sapiens secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1), transcript variant 1	Bone	Elderly	NM_001040058
SPTRX-1	Homo sapiens thioredoxin domain containing 2 (spermatozoa) (TXNDC2)	Hormone	Juvenile	NM_032243
SPTRX-2	Homo sapiens thioredoxin domain containing 3 (spermatozoa) (TXNDC3)	Hormone	Juvenile	NM_016616
SRC	Homo sapiens v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian), transcript variant 1	Oncogene	Elderly	NM_005417
SRPX	Homo sapiens sushi-repeat-containing protein, X-linked	Affymetrix	Juvenile	NM_006307
SST	Homo sapiens somatostatin	Hormone	Juvenile	NM_001048
STAF42	wi67g12.x1 NCI_CGAP_Kid12 Homo sapiens cDNA clone IMAGE:2398438 3-	Affymetrix	Newborn	AI760812
STK16	Homo sapiens serine/threonine kinase 16, transcript variant 1	Affymetrix	Elderly	NM_003691
TBC1	Homo sapiens TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1, mRNA (cDNA clone IMAGE:5211948), with apparent retained intron	Affymetrix	Juvenile	BC028196

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
TEKT2	Homo sapiens tektin 2 (testicular)	Hormone	Juvenile	NM_014466
TEP1	Homo sapiens telomerase-associated protein 1	Telomeres	Elderly	NM_007110
TERF2	Homo sapiens telomeric repeat binding factor 2	Telomeres	Elderly	NM_005652
TERT	Homo sapiens telomerase reverse transcriptase, transcript variant 1	Telomeres	Elderly	NM_198253
TFAP2BL1	Human DNA sequence from clone RP3-336H9 on chromosome 6p12.1-21.1, complete sequence	Affymetrix	Juvenile	AL031224
TINF2	Homo sapiens TERF1 (TRF1)-interacting nuclear factor 2	Telomeres	Elderly	NM_012461
TNFAIP3	Homo sapiens tumor necrosis factor, alpha-induced protein 3	Apoptosis	Elderly	NM_006290
TNFRSF11A	Homo sapiens tumor necrosis factor receptor superfamily, member 11a, NFKB activator	Bone	Elderly	NM_003839
TNFSF10	Homo sapiens tumor necrosis factor (ligand) superfamily, member 10	Apoptosis	Elderly	NM_003810
TNIP1	Homo sapiens TNFAIP3 interacting protein 1	Apoptosis	Elderly	NM_006058
TNKS1BP1	Homo sapiens tankyrase 1 binding protein 1, 182kDa	Telomeres	Elderly	NM_033396
TP53	Homo sapiens tumor protein p53 (Li-Fraumeni syndrome)	Tumor Suppressor Genes	Elderly	NM_000546
TP53BP1	Homo sapiens tumor protein p53 binding protein, 1	Tumor Suppressor Genes	Elderly	NM_005657

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
TP53BP2	Homo sapiens tumor protein p53 binding protein, 2, transcript variant 2	Tumor Suppressor Genes	Elderly	NM_005426
TP53I3	Homo sapiens tumor protein p53 inducible protein 3, transcript variant 1	Tumor Suppressor Genes	Elderly	NM_004881
TP73	Homo sapiens tumor protein p73	Disease	Elderly	NM_005427
TPST1	Homo sapiens tyrosylprotein sulfotransferase 1	Affymetrix	Elderly	NM_003596
TRPC1	Homo sapiens transient receptor potential cation channel, subfamily C, member 1	Affymetrix	Juvenile	NM_003304
TSLL2	Homo sapiens cell adhesion molecule 4 (CADM4)	Affymetrix	Juvenile	NM_145296
TUFT1	Homo sapiens tuftelin 1	Affymetrix	Juvenile	NM_020127
UNQ501	Homo sapiens MBC3205	Affymetrix	Elderly	NM_198536
VSIG2	Homo sapiens V-set and immunoglobulin domain containing 2	Immunology & Interferons	Elderly	NM_014312
WHSC1L1	ny99e02.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:1286426 3-	Affymetrix	Newborn	AA741074
WRN	Homo sapiens Werner syndrome	DNA Damage & Growth Arrest	Elderly	NM_000553
XTP3TPA	Homo sapiens XTP3-transactivated protein A	Affymetrix	Elderly	NM_024096

APPENDIX D: CANDIDATE GENE PRIMER SEQUENCES FOR RT-PCR

NEWBORN CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
AFP	tcctcagcttgctgtctcag	gctgccatttttctggtgat	NM_001134
APOE (1)	ggtcgcttttgggattacct	tccagttccgatttgtaggc	M12529
APOE (2)	aggaagatgaaggttctgtg	ctcagttcttgggtgacttg	NM_000041
ATF7IP2	ccagtaaatgacctgcgaca	aaggcaaggaaagcagaaca	AV7169647
AUF1 hnRNPD (p37)	F1 aacgaggaggatgaaggga	R1 ccataaccactctgctggtca	NM_001003810
CHR1orf28	gcctgaatcttgattcccatt	gggatggtctagtgcaaagg	AA447464
COL1A2	tggagtccgaggacctaatg	gcaagaccagcatgacctttt	NM_000089
DUSP6	ctgtcccagtttttccctga	tcacagtgactgagcggcta	BC005047
FACL6	tgtaggcctagccccatgta	tgtgcttcatacatttgcacag	AV727634
FKLF	tgcagccacacctgaactac	tgtgtcggatcacgctagtc	AF272830
FLJ20344a	agtggcagcaactggactct	tcaacttgccagacttctgc	NM_017776
FLJ20344b	ctcccaaagtgctgggatta	tgcaaattgccaacatcact	NM_017776
FLJ21901	gacccgctagttgaagcact	acgttggcctcagaagaatc	NM_024622
FLJ30658	cctgggaaatgccaaaaata	attttgaagccaggtgatgc	AW770868
FLJ35954	tcaatgcaatagcaacttcctc	tccaattgtcccagtttgaa	R66534
HBE1	aacatggacaacctcaagcc	cacctgcaaactggaagagaa	NM_005330
HBG1	acttccttgggagatgccac	aaagcctatccttgaaagctctga	NM_000559
HBG1n1	gaaagctctgaatcatccaggtg	gggcaaggtgaatgtggaag	N/A
HBG1n2	agtgagctcagtggcatctc	gggcaaggtgaatgtggaag	N/A
HBG2	acttccttgggagatgccat	gcctatccttgaaagctctgc	NM_000184
HBG2n2	ctggaggacagggcaaagg	gggcaaggtgaatgtggaag	N/A
HBG2n3	ggcagtgagctcagtgcagttc	cagctttggcaacctgtcct	N/A
HBZ	catgtctctgaccaagactgaga	ggatacgaccgataggaacttgt	NM_005332
ITSN2	gggagtgctagcaagtctgg	atgactggcaggaaaccatc	NM_006277
KIAA0276	gactttgcacgggaaaagg	actggaaaaaggggccag	D87466
KIAA1265	cagggtggacatgatcacag	ggcttgagttgaagccagtc	AB033091
KITLG	gctttgcttttggagcctta	tgtggtctgtcactccagaca	NM_000899
LOC151194	tacctggagatgggagctgt	tgctacttttcgatccgtga	NM_145280
LZTFL1	gggctagtgtggccttcag	tgcttggcatagttggtttt	NM_020347
MIF	ttcatcgtaaacaccaacg	ttgctgtaggagcggttc	L19686
PITPNC1	cttcagcagtggcagtggta	tgttgggaaattttcagatgc	AI676095
PPAT	cagaggcaataccatctcacc	cccttcttgtacagatgaaacca	U00238
RaI	cgtggtggtttaaacactgg	gcagcctgttgatcttttgg	AW003297
RaIGPS2	catgcacttatggcagtggt	agggaatgcaaggtgtcatc	NM_018037
STAF42	cccaaaaggtttatttgtca	tgcttggtaattctccagtt	AI760812
WHSC1L1	caacagaaacgcttttataagataca	gtgattttgccagctggttc	AA741074

JUVENILE CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
ACTA2	gtggttctggtttgcctgat	ctggccctgtaacaccagat	AK093340
ADAM12	gtgcttttggctaccacaca	agttcttccccaccgagttt	AU145357
ADAM12a	gtgcttttggctaccacaca	aattataacagtgaacaagattggtgtt	AU145357
Art3a	tgcagccattatgagtgtgc	tatttgggtgggttcaagga	U47054
Art3b	actttgggggcaaaagaag	acttcagccttcacctggaa	U47054
ASL	aacatgggacaggctctcag	tagtcccacacgcagatcac	NM_000048
BCKDHA	cttgagtgccccatcatctt	cctcctttgtggcgttgtat	NM_000709
c5229134	acgtcactgtccaactcgtg	ctgtgacctggaggttggtt	BC037976
c5286506	ctcacagacacacccagaaac	aaaattccaggtgccaacag	BC043160
CABP7	ggctgctctacgacaccttc	ccaggtggcgtctacttcat	NM_182527
CABYR	gagctgttctcaaaaccaac	tgtcctcgtctgtgtctgta	NM_012189
CD28	gaaacacctttgtccaagtc	ggggagtcatgttcatgtag	NM_006139
CD200	ggggactgtgaccgacttta	tcaggtcctttggagaatgg	NM_001004196
CFIX	atgcattctgtggaggctct	cagttccagaagggcaatgt	NM_000133
CGI-96	agagagccaccctgtgaaga	ctggtcatatgcctccatga	AL157851
COL6A1a	ctggccctatcggacctaa	aagccctcggtgccattt	M20776
COL6A1b	gatgggagaaaggggagaag	gggtgcaatgtcgttgttatc	M20776
CXorf22	tgttttcgggggacagttag	tagcttcaacgcgtttcctt	NM_152632
CYP17A1	tgatggacgcctttatcctc	cataaaggaaggccaggaca	NM_000104
CYP1B1	gtggagaccaccacctctgt	gctgaaacccacattctggt	NM_000102
CYP7B1	aggcaagatgtcctggagaa	gggtgccgcagaagataata	NM_004820
DHEA	ggtttgaccacattcatgg	gggccactgtgaagtgattt	NM_003167
DNCL2A-1	atcggtcggaaatggca	cgaattcgaaggaaggtgag	NM_014183
DNCL2A-2	ccaggaggtcaaggctacag	gatgggaatgccttctgtgt	NM_177953
DNCL2A-3	agggaatcatcgtcgtgaac	agagcaagaagtgcccaaaa	NM_177954
DNPEP	gtcggtgtggagacctatgg	tatttcgctgcagatggatg	NM_012100
E2IG2	tgtacagaggatccccaacc	ggtcctcctcctcatcgtct	NM_016565
ERBP	ggttgccattgaggaagaaa	tgctgctgcttgtcaacttc	NM_014597
ESR1	gtgcctggctagagatcctg	agagacttcagggtgctgga	NM_000125
ESR2	tggagtctggtcgtgtgaag	gtcggcacttctctgtctcc	NM_001437
FKBP11	cctatggaaaacggggattt	catccctaccagaggcaaaa	NM_016594
FLJ11078	tcctcgatgttgtgctgact	agtccaggtccagtgtcacc	NM_018316
FLJ22175	acatctgcagtgtcgtctcg	aggcttgtcagtgccttgtt	NM_025161
FLJ22672 PAQR6	cacctgcaccagttctttgt	aagaggaagccagtgagcag	NM_024897
FLJ35119	aaacagcgctgctatttgct	ttgagggtgggtactggaag	NM_175871

JUVENILE CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
FLJ35982	gccctgaatctcaggcact	ggacagggaaggggatttta	AK093301
FLJ35982a	tcaggcactggaaggttacc	ggacagggaaggggatttta	AK093301
FLJ37440	agccttgtcaaaatggtggt	ctgcccgtagagctcacact	NM_153214
FLJ38745	ttgtgtggatgacgtcctgt	cagcctccatgaggttgatt	AK096064
FLJ43159	gcctgcattgccttatgaat	cagctgctttacccaggaac	AI972146
GAL	tcattcagcgacaagaatgg	tgcataaattggccgaagat	NM_015973
GFPT2	cagggatgacgtttgctttt	gatctcaagccacggatgat	NM_005110
GGT1	gtgttctgccgggatagaaa	caggtcctcagctgtcacaa	NM_005265
GHRH	aattggagagcatcctggtg	ccagttgcattttggctaca	NM_021081
GLO1	atgcgacccagagttaccac	ttcaatccagtagccatcagg	NM_006708
GNRH1	ctactgacttcgtgcgtgga	cttctggcccaatggattta	NM_000825
GNRH2	gatccccagaatgcccttag	cttcctgtgaagggaccact	NM_001501
GNRHR	ctggcctggatcctcagtag	ggcagctgaaggtgaaaaag	NM_000406
GPR54	ctcgctggtcatctacgtca	actcatggcggtcagagtg	NM_032551
GRIN1 NR1-1	cgggatcttcctgattttca	ggatggtactgctgcaggtt	NM_000832
GRIN1 NR1-2	cgggatcttcctgattttca	caccccggtgctctg	NM_021569
GRIN1 NR1-3	cgggatcttcctgattttca	tgtctttggaggacctacgc	NM_007327
GRIN2A	caagtgggagaaccatacgc	cattcatcccctcattggtt	NM_000833
GRIN2B	gcatgcctacatgggaaagt	tctccaaagagctgcaggat	NM_000834
H17	aaggtccagtccttgggagt	ctcggctccacaggtagctt	NM_017547
HOMER3	ccaggaagtgaaggaagcag	gtcctgcagtgcgaaaaact	NM_004838
HRG	gcccgaaaaaccttgtcata	ctagatccatggggcttgaa	NM_000412
HTR1E	cctcccaaagtgctggaat	tgtagcctcgaaggtttctca	NM_000865
HTR7	ccctccaactacctgatcgt	aagccagacggagagaatca	NM_000872
INHA	ctctgagcccgaggaagag	gagctattggaggctgctgt	NM_002191
ITIH4	ggacctcctgatgttcctga	agggtctgagagcaggttca	NM_002218
KIAA0894	ggtgaactcttttcgcaagc	agagcacacacagtccaacg	NM_014896
KIAA2022	cagccaacggagaaaacact	gctctgcatacagggcttct	AB095942
KISS-1	tggcagctactgcttttcct	cagtagcagctggcttcctc	NM_002256
LATS1	gctgtcgatgtggagacaga	ggttgtcccaccaacatttc	NM_004690
LEP	ggctttggccctatcttttc	accggtgactttctgtttgg	NM_000230
LHB	gtcaacaccaccatctgtgc	ggaagaggaggcctgagagt	NM_000894
LHCGR	aggctaattgccacgtcatc	gggtgtcttgggtaagcaga	NM_000233
LOC152274	aggaggagagaagggagcag	tcaactcctcgggaatgaac	AK056398
LOC284242	gctgaggagagggaagtgaa	gtggctctcagctctgctct	BC035844

JUVENILE CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
MGC20460	cagcagcccaagaacataca	actgttgggaacaggtccag	NM_053043
MGC39650	catctacccttcgcctctg	agatcatctgccccacactc	AL137531
NALP14	gtcttgggtgatggtggagt	agatgcgtcaggctcttgtt	NM_176822
NDE1	gagtccaaactcgcttcctg	ccagcagtacgaggagaagg	NM_017668
NTS	gcatgctactcctggctttc	ccaagagggaacatgtgctt	NM_006183
OSGEP	attggtgggtgtgaaccact	cctggacttgggtcgttaga	NM_017807
OXTR	ttcttcgtgcagatgtggag	ggacgagttgctcttttgc	NM_000916
PAQR6	ggggctcttctgggaaaata	agagcctcccctcaccag	NM_024897
PDE6D	gacctgtctgtccctggtgt	ggtgctgcctctatcaagga	NM_002601
PGR	gtcagtgggcagatgctgta	tgtgagctcgacacaactcc	NM_000926
PLEKHA8	ggcatcatgttatgctgtgg	gcttcagtcgctgagttcct	NM_032639
POMC	aggacctcaccacggaaag	gaagtggcccatgacgtact	NM_000939
PPOX	tctagccatggacagtctct	ctctagacctccacgaagtg	NM_000309
PRL	tccataacctctcctcagaaa	ataccacgtacttccgtgac	NM_000948
PTH	gggtctgcagtccaattcat	gcttcttacgcagccattct	NM_000315
PTMS	ctgaagagagctgccgaaga	aggctggggagaaagaagag	NM_002824
RAPA-2 TRERF1	ggctcttcagcaatgtcctc	cagggcttccataccagtgt	AJ277276
RARA	gggagctcattgagaaggtg	gtccgagaaggtcatggtgt	NM_000964
REA	gagctgagctttagccgaga	gggttcttgctcagtgcttc	NM_007273
SH3GL1	ctggcagaggtgaaggactc	gactcacctgctcgatgtca	NM_003025
SHBG	tcttggctcagtctccacct	ctcaagaccaccctggacat	NM_001040
SLC39A4	ttcgtggactttgtgttcca	acacactggagctgttgctg	NM_017767
SPATA1 SP2	caacctgttctttcttcagg	ttttgttaaaacctcctcca	NM_022354
SPINK5L3	tttggcacacacacacacac	agccttgagaagagctgctg	XM_376433
SPINKa	cagaagcagaagcccctatg	gccttcctctctgtcagtgg	AK054753
SPINKb	tgtgcttgcttccttgtcac	atctttgaggtcgtccatgc	AK054753
SPTRX-1	acagagagggaaaaccaact	tggtttcttctgaggacttg	NM_032243
SPTRX-2	gagcaatgcaacctttattc	tgcaatttttctctcctcat	NM_016616
SRPX	tcaagtgcccaagtgtgaag	ttctctggggcattgagttt	NM_006307
SST	cccagactccgtcagtttct	ccatagccgggtttgagtta	NM_001048
TBC1	tcacaacagtcatgacccaag	ggccactgggatgaactaga	BC028196
TEKT2	tgacacagatgaaggagtca	acagcctctgtcgatctcta	NM_014466
TFAP2BL1	ctagagaccaggctgccatc	gcagtgggttcagggagtag	AL031224
TRPC1	tgcttaccaaactgctggtg	tggtgagggaatgatgttga	NM_003304
TSLL2	cggataacggcacctacact	aaccgacgtctgagcctcta	NM_145296
TUFT1	agaggaacttcggagcaaca	gctcttgagcatgtcatcca	NM_020127

ADULT CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
AUF1 hnRNPD (p45)	F2 cccacgacactctgaagcag	R2 tccctggttccagttttgac	NM_031370
HBA1	gttaagggccacggcaag	ccaaggggcaagaagcat	NM_000558
HBA2	gttaagggccacggcaag	cagcgggcaggaggaac	NM_000517
HBB	tcctttggggatctgtcca	aaggaacctttaatagaaattggacag	NM_000518
HBD	ctgaggagaagactgctgtcaa	gaattccttgccaaagttgc	NM_000519
HBQ	cggctcctcacaagtcaga	agttcagcggtactcggaaac	NM_005331
ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
ABL1	gagggcgtgtggaagaaata	agtccaggaggttcccgtag	NM_007313
ACD	cagctcaatgctgtgcatct	ggtaccactttcctcggatg	NM_022914
ACTN3	gattcggctttgctacagga	agctggtcaatggtctccag	NM_001104
AGGF1	cacagaacggctgtaccaga	agattgaccaaggagcatgg	NM_018046
AIF1	ttggagtccccaagactcac	ccttcaaatcagggcaactc	NM_001623
AKT1	atggcaccttcattggctac	aaggtgcgttcgatgacagt	NM_005163
AMID	ggggatagacctgaagaacca	aatctctgctgccatctcca	NM_032797
ANKH	ctgtgcctgggctactacaa	ggccgactgattctctgtgt	NM_054027
APEX1	caaacctgccacactcaaga	gctgttaccagcacaaacga	NM_001641
ARMC7	gagaatgagaccctggtgga	agacagcaccgtctcctcat	NM_024585
ATPAF2	gagatcagctcctccaccag	actcaatgttgccccacttc	NM_145691
BAX-(all)	tgatggacgggtccgg	cccctgtcttcatgatctgc	NM_138764
BAX-a/d	aactggtgctcaaggccc	ggcgtcccaaagtaggaga	NM_138761
BAX-all(e)	tctgacggcaacttcaactg	ggaggaagtccaatgtccag	NM_138764
BAX-b	tctgacggcaacttcaactg	cactgtgacctgctccagaa	NM_004324
BAX-d	ccctttttgcttcagggga	ggaggaagtccaatgtccag	NM_138763
BAX-e	tctgacggcaacttcaactg	aatcgcttgaacccaggag	NM_138764
BAX-s	tctgacggcaacttcaactg	aaagatggtcacggtccaa	NM_138765
BCL2A1	ggcatcattaactggggaag	tccagccagatttaggttcaa	NM_004049
BGLAP	ggcagcgaggtagtgaagag	agcagagcgacaccctagac	NM_199173
BIRC5	ggaccaccgcatctctacat	gtctggctcgttctcagtgg	NM_001012271
CAMK2D	actatcaaccctgccaaacg	ccccattgttgatagcttcg	NM_172127
CASP2	agactgatcgtggggttgac	caggaacctcgtttggtgtt	NM_032982
CBL	tctaatgccagctcctcctt	ggccatctcgatgttgttct	NM_005188
CCL5	tacaccagtggcaagtgctc	tgtactcccgaacccatttc	NM_002985
CCM2	tgtttacacggagtccacca	accacccacatccacagat	NM_001029835
CCND1	tcctctccaaaatgccagag	tgaggcggtagtaggacagg	NM_053056

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
CD28	cggaccttctaagccctttt	atagggctggtaatgcttgc	NM_006139
CD86	agacgcggcttttatcttca	ttaaaaacacgctgggcttc	NM_175862
CDC2	ccatggggattcagaaattg	ccattttgccagaaattcgt	NM_001786
CDC25C	ggcacctgattggtgatttt	ctggaacttccccgacagta	NM_022809
CDKN1A	ggaagaccatgtggacctgt	ggattagggcttcctcttgg	NM_078467
CDKN1B	ccggctaactctgaggacac	cgagctgtttacgtttgacg	NM_004064
CDKN2C	acgtcaatgcacaaaatgga	cgaaaccagttcggtctttc	NM_001262
CIITA	gatgtggaagacctgggaaa	cacccaggtcagtgatgttg	NM_000246
CLEC2	tgatggctttgattctgctg	acaggggctgcatttatgac	NM_016509
CLEC2a	agctctcgtctccgttgg	cgctttgctaattgttgcag	NM_016509
CLEC2b	gcacaggaactctgcaacaa	gcctgaagaacccatagcag	NM_016509
COL1A1 CTx	atggctctcctggcaaagat	atcaccaggttcgcctttag	NM_000088
CTBP1	ccttcctggtgaacacagc	ggctgtcagatggtccttgt	NM_001012614
CTSB	ggccgagatctacaaaaacg	gccaccacttctgattcgat	NM_147780
CTSK	ccttgaggcttctcttggtg	tccacagccatcattctcag	NM_000396
CTSL	acagtggaccaagtggaagg	tgggcttacggttttgaaag	NM_001912
CYTBC2	gatggagcggttctggaata	ccagacacagggacttcaca	D49737
DDB2	cgatggaaactcagggaaga	aaatcaccacctctgcttgc	NM_000107
DYRK2	gccatgttaaccaggaaacc	cgacatgcaggtgatcattc	NM_006482
E2F1	agctggaccacctgatgaat	ctcagggcacaggaaaacat	NM_005225
ECGF1	acaaggtcagcctggtcctc	ctctgacccacgatacagca	NM_001953
ELAVL1 HuR	acaaaaacgtggcactcctc	gccccaggttgtagatgaaa	NM_001419
EMD	gccgcctcctcttatagctt	tgatgctctggtaggcactg	NM_000117
EREG	cgtgtggctcaagtgtcaat	agtgttcacatcggacacca	NM_001432
ERF	gggaaacggttcacctacaa	agatgaagagcaggctggtg	NM_006494
FLJ20245	gctgctcctggagtcttgg	gctcctgggacagatactcg	NM_017723
FLJ20421	gcatttaaagccatggagga	ctgaaaccatggggagagaa	BF674724
FLJ35984	accaggggtccatcctctac	ggaggtgctgggtttcataa	AK093303
FLJ38628	ctcaaggacagaggccagag	cagggccacaaataggaaga	NM_152267
GADD45A	ggaggaagtgctcagcaaag	atctctgtcgtcgtcctcgt	NM_001924
GADD45B	tgctgtgacaacgacatcaa	tttgtttgtggcagcaactc	NM_015675
GSTP1	gacctccgctgcaaatacat	ggctaggacctcatggatca	NM_000852
HIC2	ctgctgctcacatggtgtct	gatgacgtcacacaggaagc	NM_015094
HIF1A	tccatgtgaccatgaggaaa	ccaagcaggtcataggtggt	NM_001530
HPCAL4	caactgggcctttgagatgt	tggtcgtccttatcctggtc	NM_016257
HRAS	gagggcttcctgtgtgtgtt	agccaggtcacacttgttcc	NM_176795

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
HTATIP	catcctccaggcaatgagat	agtagcccacgatgtggaag	NM_182710
IFNG	agatgaccagagcatccaaaa	cagttcagccatcacttgga	NM_000619
IGF1	tggatgctcttcagttcgtg	cctgcactccctctacttgc	NM_000618
IGF2	acaccctccagttcgtctgt	cggaaacagcactcctcaac	NM_000612
IGFBP3	acagccagcgctacaaagtt	ggctgcccatacttatccac	NM_001013398
IGFBP5	tgcacctgagatgagacagg	gaatcctttgcggtcacaat	NM_000599
IL1A	aatgacgccctcaatcaaag	ccgtgagtttcccagaagaa	NM_000575
IRF1	ccaggctacatgcaggactt	gtaggtacccccttcccatcc	NM_002198
KL	aatggctggtttgtctcagg	tgtaacctctgtgccactcg	NM_153683
KLF13	gatcctagcggacctcaacc	attcccgggtggaagttg	NM_015995
LASS5	aaaatccaatgctggtttcg	ccaatagaaggccaattcca	NM_147190
LMNA (norm)	ggtggtgacgatctgggct	ccagtggagttgatgagagc	NM_170707
LMNA (RT)	gtggaaggcacagaacacct	gtgaggaggacgcaggaa	NM_170707
LMNA (spec)	gcgtcaggagccctgagc	gacgcaggaagcctccac	NM_170707
LOH11CR2A	ggcaccactccagaacattt	tcacccggaaatcacatttt	BC001234
MAD1L1	gagcagatccgttcgaagtc	gcatccaagttctgctgaca	NM_003550
MCPH1	agcccagagtgaacatgagc	aggtccttaaagccgtcaca	NM_024596
MDM2	ggtgctgtaaccacctcaca	tttttgtgcaccaacagacttt	NM_002392
MEPE	aactaagcaaagctgtgtgg	attctcactggcttcagaaa	NM_020203
MET	agcctgattgtgcatttcaa	gatgattccctcggtcagaa	NM_000245
MGC14288	gggaagttgcgtagacagtg	agctagctgcttgccagttg	NM_032901
MLL	taagcccaagtttggtggtc	cttctgcaggtaggctttgg	NM_005933
MMP-13	aacatccaaaaacgccagac	atgcagcatcaatacggttg	NM_002427
MMP-14	cactgcctacgagaggaagg	tcccttcccagactttgatg	NM_004995
MMP-9	gacaagctcttcggcttctg	gccattcacgtcgtccttat	NM_004994
MS4A4A	ggaatgaaattacgtctttggaa	cctgatgcagccagtacaga	NM_024021
MS4A4Aa	tctgtactggctgcatcagg	gccatgtgagaatgtgatgg	NM_024021
MS4A4Ab	aggagagagattcgagcacct	ggcagtcagaatctgcacaa	NM_024021
MT1X	tcctgcaaatgcaaagagtg	acagctgtcctggcatcag	NM_005952
MYC	cctaccctctcaacgacagc	ctctgacctttgccagga	NM_002467
NBN	gaaaaaggccaaggatggat	gccagatggatttctggaag	NM_001024688
NMI	cgcgtggactatgacagaca	gcccgttgaaagtgaatgtt	NM_004688
NPPB	accgcaaaatggtcctctac	gttgaggaaaaagcccctg	NM_002521
NRAS	gcgaaggcttcctctgtgta	agttcgtgggcttgttttgt	NM_002524
OGG1	atggggcatcgtactctagc	cgatgttgttgttggaggaa	NM_016819
OPG TNFRSF11B	ggcaacacagctcacaagaa	gtgtcttggtcgccatttt	NM_002546

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
OPGL RANKL TNFSF11	gcttgaagctcagccttttg	cgaaagcaaatgttggcata	NM_003701
OSM	agctgctcgaaagagtaccg	ctgctctaagtcggccagtc	NM_020530
PDCD1	aaggcgcagatcaaagagag	aatccagctccccatagtcc	NM_005018
PDCD10 CCM3	tgaagctgagaccacatcca	tgccatacgaagaagggact	NM_007217
PDCD11	gggcaagaagagtgtcaagc	gtggcagaaaagctctggtc	NM_014976
PDCD1LG2	atccaacttggctgcttcac	aagtgcaaatggcaagctct	NM_025239
PDCD2L	ctggtcgtgcaggtgtattg	gaaggcccctcctcagtatc	NM_032346
PDCD4	tggattaactgtgccaacca	tctcaaatgccctttcatcc	NM_145341
PDCD5	cttgaggcgctgaggagac	ccgactgatccagaacttgg	NM_004708
PDCD6	ctccgggatgatcgataaga	tccatgttgtgctgctcttc	NM_013232
PDCD6IP	ctgttgggaccctcagtctt	ttctgctgttttgccaggat	NM_013374
PDCD7	tgaagtgtgtgcaggaggtg	gtcgctgaagatgatgcgta	NM_005707
PIK3CA	cagacgcatttccacagcta	gcaaatggaaaggcaaagtc	NM_006218
POLA1	ccatcacagttttgcattgg	cagtgaggagctttgcacac	NM_016937
POLA2	cgaaagccaggcatagtacc	ggggctacgagttgacacac	NM_002689
POLB	attcggcaggatgatacgag	ccaattcgctgatgatggtt	NM_002690
POLD1	ggtgcagagctacgagaagg	atgaagagtcccggatgttg	NM_002691
POLE1	tggcatttgacattgagacg	gtttggtctcctggacgtgt	NM_006231
POLE2	ttgaacgatctgtggtggaa	aaatttggtgcagggtggt	NM_002692
POLE3	agaggcccgaggacctaaa	agcacatcactggcattcag	NM_017443
POLG	tgaggccaagatggagaact	tacgtttatgggcgttcctc	NM_002693
POLH	tggactaaacaagcccaacc	gttgcctgggtttaactgga	NM_006502
POLI	cagttgctcagcgtatccaa	aaggaatagggcactgacga	NM_007195
POLK	ccatgccaggatttattgct	ggatcgttcatgctcactca	NM_016218
POLM	ttccccactttggagaacac	gtaccaccggtcagcagtct	NM_013284
POLN	ccaagcacccaattcagatt	acaccaccttcttggtttgc	NM_181808
POLQ	gccttcaggactggactctg	agtagaagttgccgccaaga	NM_199420
POLR3F	tgcaaaagaaggcacagttg	aaaattcgagccactctgtc	NM_006466
POLR3K	atcgtggaggagggacaac	caccaagcacatcatccact	NM_016310
POLS	cccaccacttccagaacact	gctttcaaagacgcagttcc	NM_006999
POT1	tgggtattgtacccctccaa	ttgatgaagcattccaacca	NR_003102
PPARD	aagtggcagaggcagaag	ctgcgctcacacttctcgta	NM_006238
PRDX5	cgctcagcgggctatatact	aaagatggacaccagcgaat	NM_012094
PRKCA	caggatgatgacgtggagtg	gttccttgcacatcccaaag	NM_002737
PTGER4	ctggtggtgctcatctgct	tcacagaagcaattcggatg	NM_000958

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
PTPN18	ccagatgatcccacctgact	gaagagggcatcgtcgtaga	NM_014369
RAD50	cttggatatgcgaggacgat	ccagaagctggaagttacgc	NM_005732
RAF1	ggctggtagctgactgtgtg	ccggttgatcttcggtagag	NM_002880
RANK	ctctgatgccttttcctcca	agctggcagagaagaactgc	AF018253
RB1	aggaccgagaaggaccaact	cagacagaaggcgttcacaa	NM_000321
RBL1	ttgatggcttgttgtttgga	tgtttcaccatgtcccttga	NM_002895
RBL2	agaacctggaaagggcagat	tggggagctgtacctatcgt	NM_005611
RELA	ccacgagcttgtaggaaagg	ctgatagcctgctccaggtc	NM_021975
RUNX2	cggaatgcctctgctgttat	atgcgccctaaatcactgag	NM_004348
SEMA4A	tctgctcctgagtggtgatg	aaaccaggacacggatgaag	NM_022367
SLC20A1	ctatgcctgcacagttggaa	accagacgataagggcacag	NM_005415
SMG5	ctgcagttcaacccagaggt	aggtagggagacatggctga	NM_015327
SMG6	tgcctccactactgcaaaga	ggcatcttccgtgctacact	NM_017575
SMG7	caggagtcttccgtccagag	tgagagagaatccggtgagg	NM_173156
SNCA	aaaaccaaggagggagtggt	cccaactggtccttttgac	NM_000345
SPP1	gccgaggtgatagtgtggtt	attcaactcctcgctttcca	NM_001040058
SRC	ggctacatccccagcaacta	tgcggatcttgtagtgcttc	NM_005417
STK16	gggttccatgaatcaagcat	ccctttggaacaccatgtc	NM_003691
TEP1	gccgcactgtcttggtctat	ggagcttgatggcagtcttc	NM_007110
TERF2	gaccttccagcagaagatgc	cctgtgcaccagacagagtc	NM_005652
TERT	gcaaactctttggggtcttg	gggttcttccaaacttgctg	NM_198253
TINF2	tcctgaaagccctgaatcac	ctgcatccaactcagcacat	NM_012461
TNFAIP3	atagaaatccccgtccaagg	tgggcgtttcacattttaca	NM_006290
TNFRSF11A	catgtttacttgcccggttt	cctgacagacaccaccttga	NM_003839
TNFSF10	gagtatgaacagcccctgct	tccttgatgattcccaggag	NM_003810
TNIP1	tgagcaatggcaacaaagag	gctccagcatcttcaccttc	NM_006058
TNKS1BP1	ggaggggccagtaaagtctc	ctcttatcaggcgggtgaag	NM_033396
TP53	gcgcacagaggaagagaatc	cctcattcagctctcggaa	NM_000546
TP53BP1	cccatacttgggagtggaaa	cctcacttcgagcctcattc	NM_005657
TP53BP2	tccttggtcattcaggcttc	cggacgcactttcttctctt	NM_005426
TP53I3	gcttcaaatggcagaaaagc	aacccatcgaccatcaagag	NM_004881
TP73	gaccgaaaagctgatgagga	tcagctccaggctctctttc	NM_005427
TPST1	cccacctaactacggaaaacc	aagaggctcctggttctgct	NM_003596
UNQ501	atgcaaatgtgggtgacctt	aggctcaggaacagcaggta	NM_198536
VSIG2	tgcgtcttggaacttttcct	cccctctctttctggaacct	NM_014312
WRN	ggactttggtccacaagcat	tctttggtgcccgaagatac	NM_000553
XTP3TPA	cctccatgctgagtttgctg	atgccaccaggtagatgagg	NM_024096

APPENDIX E: CANDIDATE GENE RT-PCR RESULTS

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
ABL1	Elderly	Literature		Expressed in All Ages
ACD	Elderly	Literature		No mRNA Detected
ACTA2	Juvenile	Affymetrix		No mRNA Detected
ACTN3	Elderly	Literature		No mRNA Detected
ADAM12	Juvenile	Affymetrix		Same Size mRNA/DNA
ADAM12a	Juvenile	Affymetrix		Same Size mRNA/DNA
AFP	Newborn	Literature	Fetal Liver	
AGGF1	Elderly	Affymetrix	Elderly	
AIF1	Elderly	Literature		Expressed in All Ages
AKT1	Elderly	Literature		Expressed in All Ages
AMID	Elderly	Literature		Expressed Sporadically
ANKH	Elderly	Literature		No mRNA Detected
APEX1	Elderly	Literature		Expressed in All Ages
APOE (1)	Newborn	Literature		No mRNA Detected
APOE (2)	Newborn	Literature		Expressed Sporadically
ARMC7	Elderly	Affymetrix		Expressed Sporadically
Art3a	Juvenile	Affymetrix		No mRNA Detected
Art3b	Juvenile	Affymetrix		No mRNA Detected
ASL	Juvenile	Affymetrix	Juvenile	
ATF7IP2	Newborn	Affymetrix		Expressed in All Ages
ATPAF2	Elderly	Affymetrix		Expressed Sporadically
AUF1 hnRNPD p37	Newborn	Literature		Expressed Sporadically
AUF1 hnRNPD p45	Adult	Literature		Expressed Sporadically
BAX-(all)	Elderly	Literature		Expressed in All Ages
BAX-a/d	Elderly	Literature		Expressed in All Ages
BAX-all(e)	Elderly	Literature		Expressed in All Ages
BAX-b	Elderly	Literature		Expressed Sporadically
BAX-d	Elderly	Literature		Expressed Sporadically
BAX-e	Elderly	Literature		Expressed in All Ages
BAX-s	Elderly	Literature		No mRNA Detected
BCKDHA	Juvenile	Affymetrix		Expressed in All Ages
BCL2A1	Elderly	Literature		Expressed in All Ages
BGLAP	Elderly	Literature		Expressed Sporadically
BIRC5	Elderly	Literature		Expressed in All Ages
c5229134	Juvenile	Affymetrix		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
c5286506	Juvenile	Affymetrix		Same Size mRNA/DNA
CABP7	Juvenile	Affymetrix		No mRNA Detected
CABYR	Juvenile	Literature		No mRNA Detected
CAMK2D	Elderly	Literature		Expressed in All Ages
CASP2	Elderly	Literature		Expressed in All Ages
CBL	Elderly	Literature		Expressed in All Ages
CCL5	Elderly	Affymetrix		Expressed in All Ages
CCM2	Elderly	Literature		Expressed in All Ages
CCND1	Elderly	Literature		Expressed Sporadically
CD200	Juvenile	Literature		Expressed Sporadically
CD28	Juvenile	Affymetrix		Expressed in All Ages
CD28	Elderly	Literature		Expressed in All Ages
CD86	Elderly	Literature		Expressed in All Ages
CDC2	Elderly	Literature	Elderly	
CDC25C	Elderly	Literature		Expressed Sporadically
CDKN1A	Elderly	Literature		Expressed in All Ages
CDKN1B	Elderly	Literature		Expressed in All Ages
CDKN2C	Elderly	Literature		Expressed in All Ages
CFIX	Juvenile	Literature		No mRNA Detected
CGI-96	Juvenile	Affymetrix		Expressed in All Ages
CHR1orf28	Newborn	Affymetrix		Same Size mRNA/DNA
CIITA	Elderly	Literature		Expressed in All Ages
CLEC2	Elderly	Affymetrix		Expressed in All Ages
CLEC2a	Elderly	Affymetrix		Expressed in All Ages
CLEC2b	Elderly	Affymetrix		Same Size mRNA/DNA
COL1A1 CTx	Elderly	Literature		Expressed Sporadically
COL1A2	Newborn	Literature	Newborns	
COL6A1a	Juvenile	Affymetrix		Expressed Sporadically
COL6A1b	Juvenile	Affymetrix		No mRNA Detected
CTBP1	Elderly	Literature		Expressed in All Ages
CTSB	Elderly	Literature		Expressed in All Ages
CTSK	Elderly	Literature		Expressed in All Ages
CTSL	Elderly	Literature		Expressed Sporadically
CXorf22	Juvenile	Affymetrix		No mRNA Detected
CYP17A1	Juvenile	Literature		Expressed Sporadically
CYP1B1	Juvenile	Literature		No mRNA Detected
CYP7B1	Juvenile	Literature		Expressed Sporadically
CYTBC2	Elderly	Affymetrix		Same Size mRNA/DNA
DDB2	Elderly	Literature		Expressed in All Ages
DHEA	Juvenile	Literature		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
DNCL2A-1	Juvenile	Affymetrix		Expressed in All Ages
DNCL2A-2	Juvenile	Affymetrix		Expressed in All Ages
DNCL2A-3	Juvenile	Affymetrix		Expressed in All Ages
DNPEP	Juvenile	Affymetrix		Expressed in All Ages
DUSP6	Newborn	Affymetrix		Expressed in All Ages
DYRK2	Elderly	Literature		Expressed in All Ages
E2F1	Elderly	Literature		Expressed in All Ages
E2IG2	Juvenile	Literature		Expressed Sporadically
ECGF1	Elderly	Literature		No mRNA Detected
ELAVL1 HuR	Elderly	Literature		Expressed in All Ages
EMD	Elderly	Literature		Expressed in All Ages
ERBP	Juvenile	Literature		Expressed in All Ages
EREG	Elderly	Literature		No mRNA Detected
ERF	Elderly	Literature		Expressed in All Ages
ESR1	Juvenile	Literature		No mRNA Detected
ESR2	Juvenile	Literature		Expressed Sporadically
FACL6	Newborn	Affymetrix		No mRNA Detected
FKBP11	Juvenile	Affymetrix		Expressed in All Ages
FKLF	Newborn	Literature		Expressed in All Ages
FLJ11078	Juvenile	Affymetrix		Expressed in All Ages
FLJ20245	Elderly	Affymetrix		Same Size mRNA/DNA
FLJ20344a	Newborn	Affymetrix	Newborn	
FLJ20344b	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ20421	Elderly	Affymetrix		Same Size mRNA/DNA
FLJ21901	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ22175	Juvenile	Affymetrix		Expressed in All Ages
FLJ22672 PAQR6	Juvenile	Affymetrix		Expressed Sporadically
FLJ30658	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ35119	Juvenile	Affymetrix		Expressed in All Ages
FLJ35954	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ35982	Juvenile	Affymetrix		Same Size mRNA/DNA
FLJ35982a	Juvenile	Affymetrix		No mRNA Detected
FLJ35984	Elderly	Affymetrix		Same Size mRNA/DNA
FLJ37440	Juvenile	Affymetrix		No mRNA Detected
FLJ38628	Elderly	Affymetrix		Expressed Sporadically
FLJ38745	Juvenile	Affymetrix		Same Size mRNA/DNA
FLJ43159	Juvenile	Affymetrix		Same Size mRNA/DNA
GADD45A	Elderly	Literature		Expressed in All Ages
GADD45B	Elderly	Literature		Expressed in All Ages
GAL	Juvenile	Literature		Expressed Sporadically

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
GFPT2	Juvenile	Affymetrix		Expressed Sporadically
GGT1	Juvenile	Affymetrix		Expressed in All Ages
GHRH	Juvenile	Literature		Same Size mRNA/DNA
GLO1	Juvenile	Literature		Expressed in All Ages
GNRH1	Juvenile	Literature		Expressed Sporadically
GNRH2	Juvenile	Literature		Same Size mRNA/DNA
GNRHR	Juvenile	Literature		No mRNA Detected
GPR54	Juvenile	Literature		Expressed Sporadically
GRIN1 NR1-1	Juvenile	Literature		Expressed Sporadically
GRIN1 NR1-2	Juvenile	Literature		No mRNA Detected
GRIN1 NR1-3	Juvenile	Literature		No mRNA Detected
GRIN2A	Juvenile	Literature		No mRNA Detected
GRIN2B	Juvenile	Literature		No mRNA Detected
GSTP1	Elderly	Affymetrix		Expressed in All Ages
H17	Juvenile	Affymetrix		Expressed in All Ages
HBA1	Adult	Literature		Expressed in All Ages
HBA2	Adult	Literature		Expressed in All Ages
HBB	Adult	Literature		Expressed in All Ages
HBD	Adult	Literature		Expressed in All Ages
HBE1	Newborn	Literature	Newborn	
HBG1	Newborn	Literature		Expressed in All Ages
HBG1n1	Newborn	Literature	Newborn	
HBG1n2	Newborn	Literature	Newborn	
HBG2	Newborn	Literature		Expressed in All Ages
HBG2n2	Newborn	Literature	Newborn	
HBG2n3	Newborn	Literature	Newborn	
HBZ	Newborn	Literature		No mRNA Detected
HBQ	Adult	Literature		Expressed Sporadically
HIC2	Elderly	Affymetrix		No mRNA Detected
HIF1A	Elderly	Literature		Expressed in All Ages
HOMER3	Juvenile	Affymetrix		Expressed Sporadically
HPCAL4	Elderly	Affymetrix		Expressed Sporadically
HRAS	Elderly	Literature		No mRNA Detected
HRG	Juvenile	Affymetrix		Expressed Sporadically
HTATIP	Elderly	Literature		Expressed in All Ages
HTR1E	Juvenile	Affymetrix		No mRNA Detected
HTR7	Juvenile	Affymetrix		Expressed Sporadically
IFNG	Elderly	Literature		Expressed Sporadically
IGF1	Elderly	Literature		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
IGF2	Elderly	Literature		No mRNA Detected
IGFBP3	Elderly	Literature	Elderly	
IGFBP5	Elderly	Literature		No mRNA Detected
IL1A	Elderly	Literature		Expressed Sporadically
INHA	Juvenile	Literature		No mRNA Detected
IRF1	Elderly	Literature		Expressed in All Ages
ITIH4	Juvenile	Affymetrix		Expressed Sporadically
ITSN2	Newborn	Affymetrix		Expressed in All Ages
KIAA0276	Newborn	Affymetrix		Expressed in All Ages
KIAA0894	Juvenile	Affymetrix		No mRNA Detected
KIAA1265	Newborn	Affymetrix		Expressed in All Ages
KIAA2022	Juvenile	Affymetrix		Expressed Sporadically
KISS-1	Juvenile	Literature		Expressed Sporadically
KITLG	Newborn	Literature		Expressed Sporadically
KL	Elderly	Literature		Expressed Sporadically
KLF13	Elderly	Literature		No mRNA Detected
LASS5	Elderly	Affymetrix		Expressed Sporadically
LATS1	Juvenile	Affymetrix		Expressed in All Ages
LEP	Juvenile	Literature		Expressed Sporadically
LHB	Juvenile	Literature		No mRNA Detected
LHCGR	Juvenile	Literature		No mRNA Detected
LMNA (norm)	Elderly	Literature		Expressed in All Ages
LMNA (RT)	Elderly	Literature		Expressed Sporadically
LMNA (spec)	Elderly	Literature		No mRNA Detected
LOC151194	Newborn	Affymetrix	Newborn	
LOC152274	Juvenile	Affymetrix		No mRNA Detected
LOC284242	Juvenile	Affymetrix		No mRNA Detected
LOH11CR2A	Elderly	Affymetrix	Elderly	
LZTFL1	Newborn	Affymetrix		Same Size mRNA/DNA
MAD1L1	Elderly	Literature	Elderly	
MCPH1	Elderly	Literature		Expressed in All Ages
MDM2	Elderly	Literature		Expressed in All Ages
MEPE	Elderly	Literature		No mRNA Detected
MET	Elderly	Literature		Expressed Sporadically
MGC14288	Elderly	Affymetrix		Expressed in All Ages
MGC20460	Juvenile	Affymetrix		Expressed in All Ages
MGC39650	Juvenile	Affymetrix		Expressed Sporadically
MIF	Newborn	Literature		No mRNA Detected
MLL	Elderly	Literature		No mRNA Detected
MMP-13	Elderly	Literature		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
MMP-14	Elderly	Literature		Expressed Sporadically
MMP-9	Elderly	Literature		Expressed Sporadically
MS4A4A	Elderly	Affymetrix		Expressed Sporadically
MS4A4Aa	Elderly	Affymetrix		Expressed in All Ages
MS4A4Ab	Elderly	Affymetrix		Expressed Sporadically
MT1X	Elderly	Affymetrix		Expressed in All Ages
MYC	Elderly	Literature		Expressed in All Ages
NALP14	Juvenile	Affymetrix		Expressed Sporadically
NBN	Elderly	Literature		No mRNA Detected
NDE1	Juvenile	Affymetrix		Expressed in All Ages
NMI	Elderly	Literature		Expressed in All Ages
NPPB	Elderly	Literature		No mRNA Detected
NRAS	Elderly	Literature		Expressed in All Ages
NTS	Juvenile	Literature		No mRNA Detected
OGG1	Elderly	Literature		Expressed Sporadically
OPG TNFRSF11B	Elderly	Literature		No mRNA Detected
OPGL RANKL TNFSF11	Elderly	Literature		Expressed Sporadically
OSGEP	Juvenile	Affymetrix		Expressed in All Ages
OSM	Elderly	Literature		Expressed Sporadically
OXTR	Juvenile	Literature		Expressed Sporadically
PAQR6	Juvenile	Affymetrix		No mRNA Detected
PDCD1	Elderly	Literature		Expressed Sporadically
PDCD10 CCM3	Elderly	Literature		Expressed in All Ages
PDCD11	Elderly	Literature		Expressed in All Ages
PDCD1LG2	Elderly	Literature		Expressed Sporadically
PDCD2L	Elderly	Literature		Expressed Sporadically
PDCD4	Elderly	Literature		Expressed in All Ages
PDCD5	Elderly	Literature		Expressed in All Ages
PDCD6	Elderly	Literature	Elderly	
PDCD6IP	Elderly	Literature		Expressed in All Ages
PDCD7	Elderly	Literature		Expressed in All Ages
PDE6D	Juvenile	Affymetrix		Expressed in All Ages
PGR	Juvenile	Literature		No mRNA Detected
PIK3CA	Elderly	Literature		Expressed in All Ages
PITPNC1	Newborn	Affymetrix		Same Size mRNA/DNA
PLEKHA8	Juvenile	Affymetrix		Same Size mRNA/DNA

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
POLA1	Elderly	Literature		Expressed in All Ages
POLA2	Elderly	Literature		Expressed in All Ages
POLB	Elderly	Literature		Expressed in All Ages
POLD1	Elderly	Literature		Expressed in All Ages
POLE1	Elderly	Literature		Expressed in All Ages
POLE2	Elderly	Literature		Expressed in All Ages
POLE3	Elderly	Literature		Expressed in All Ages
POLG	Elderly	Literature		Expressed in All Ages
POLH	Elderly	Literature		Expressed in All Ages
POLI	Elderly	Literature		Expressed in All Ages
POLK	Elderly	Literature		Expressed in All Ages
POLM	Elderly	Literature	Elderly	
POLN	Elderly	Literature		No mRNA Detected
POLQ	Elderly	Literature	Elderly	
POLR3F	Elderly	Literature		Expressed in All Ages
POLR3K	Elderly	Literature		Expressed in All Ages
POLS	Elderly	Literature		Expressed in All Ages
POMC	Juvenile	Literature		No mRNA Detected
POT1	Elderly	Literature		Expressed in All Ages
PPARD	Elderly	Literature	Elderly	
PPAT	Newborn	Affymetrix		Same Size mRNA/DNA
PPOX	Juvenile	Literature	Juvenile	
PRDX5	Elderly	Literature		Expressed in All Ages
PRKCA	Elderly	Literature		Expressed in All Ages
PRL	Juvenile	Literature	Juvenile	
PTGER4	Elderly	Literature		Expressed in All Ages
PTH	Juvenile	Literature		No mRNA Detected
PTMS	Juvenile	Affymetrix		Same Size mRNA/DNA
PTPN18	Elderly	Affymetrix		Expressed Sporadically
RAD50	Elderly	Literature		Expressed in All Ages
RAF1	Elderly	Literature		No mRNA Detected
RaI	Newborn	Affymetrix		Same Size mRNA/DNA
RaIGPS2	Newborn	Affymetrix		Expressed in All Ages
RANK	Elderly	Literature		Expressed Sporadically
RAPA-2 TRERF1	Juvenile	Literature		Expressed in All Ages
RARA	Juvenile	Affymetrix		Expressed in All Ages
RB1	Elderly	Literature		Expressed in All Ages
RBL1	Elderly	Literature		Expressed in All Ages
RBL2	Elderly	Literature		Expressed in All Ages

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
REA	Juvenile	Literature		Same Size mRNA/DNA
RELA	Elderly	Literature		Expressed in All Ages
RUNX2	Elderly	Literature		Expressed in All Ages
SEMA4A	Elderly	Affymetrix		Expressed in All Ages
SH3GL1	Juvenile	Affymetrix		Expressed in All Ages
SHBG	Juvenile	Literature		No mRNA Detected
SLC20A1	Elderly	Literature		Expressed in All Ages
SLC39A4	Juvenile	Affymetrix		Same Size mRNA/DNA
SMG5	Elderly	Literature		Expressed in All Ages
SMG6	Elderly	Literature		Expressed in All Ages
SMG7	Elderly	Literature		Expressed in All Ages
SNCA	Elderly	Literature		Expressed in All Ages
SPATA1 SP2	Juvenile	Literature		Expressed Sporadically
SPINK5L3	Juvenile	Affymetrix		Same Size mRNA/DNA
SPINKa	Juvenile	Affymetrix		Same Size mRNA/DNA
SPINKb	Juvenile	Affymetrix		No mRNA Detected
SPP1	Elderly	Literature		Expressed Sporadically
SPTRX-1	Juvenile	Literature	Juvenile	
SPTRX-2	Juvenile	Literature	Juvenile	
SRC	Elderly	Literature	Elderly	
SRPX	Juvenile	Affymetrix		No mRNA Detected
SST	Juvenile	Literature		No mRNA Detected
STAF42	Newborn	Affymetrix		Same Size mRNA/DNA
STK16	Elderly	Affymetrix		Expressed Sporadically
TBC1	Juvenile	Affymetrix	Juvenile	
TEKT2	Juvenile	Literature	Juvenile	
TEP1	Elderly	Literature		No mRNA Detected
TERF2	Elderly	Literature		No mRNA Detected
TERT	Elderly	Literature		No mRNA Detected
TFAP2BL1	Juvenile	Affymetrix		No mRNA Detected
TINF2	Elderly	Literature		Expressed in All Ages
TNFAIP3	Elderly	Literature		Expressed in All Ages
TNFRSF11A	Elderly	Literature		Expressed Sporadically
TNFSF10	Elderly	Literature		Expressed in All Ages
TNIP1	Elderly	Literature		Expressed in All Ages
TNKS1BP1	Elderly	Literature		Expressed Sporadically

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
TP53	Elderly	Literature		Expressed in All Ages
TP53BP1	Elderly	Literature		No mRNA Detected
TP53BP2	Elderly	Literature		No mRNA Detected
TP53I3	Elderly	Literature		No mRNA Detected
TP73	Elderly	Literature		No mRNA Detected
TPST1	Elderly	Affymetrix		Expressed Sporadically
TRPC1	Juvenile	Affymetrix		Expressed Sporadically
TSLL2	Juvenile	Affymetrix		Expressed in All Ages
TUFT1	Juvenile	Affymetrix		Expressed in All Ages
UNQ501	Elderly	Affymetrix		Expressed in All Ages
VSIG2	Elderly	Literature		Expressed Sporadically
WHSC1L1	Newborn	Affymetrix		Same Size mRNA/DNA
WRN	Elderly	Literature		Expressed Sporadically
XTP3TPA	Elderly	Affymetrix		Expressed Sporadically

APPENDIX F: CANDIDATE GENE PRIMER SEQUENCES FOR qRT-PCR

Candidate Gene	Target Age Group	Primer and MGB Probe Sequences 5' → 3'	NCBI Genbank Accession Number
AGGF1	Elderly	502F aagctgctgcatcacacagaac 568T 6FAMcaggtggaagaacMGBNFQ 604R tcccacgttggagtattttactga	NM_018046
ASL	Juvenile	594F ctgcagtgacagctggttgag 676T 6FAMaatggcatccctttgcMGBNFQ 727R acatgctggccactgacctt	NM_001024943
CDC2	Elderly	881F acctggaatcctgcataagca 904T 6FAMtcctgaagactgactatatMGBNFQ 948R tctattaaaggaacttcgtcatccaa	NM_001786
COL1A2	Newborn	1383F gcatccttggttagggtcaatc 1406T 6FAMagtagtaaccactgctccMGBNFQ 1456R catgccgtgacttgagactca	NM_000089
FLJ20344a	Newborn	318F gcgaagcctgatgtgatcttc 395T 6FAMctgtgcagaagtctggMGBNFQ 455R tttgtcttggctttccttgtagtg	NM_017776
HBE1	Newborn	671F attgccctggcccataagta 697T 6FAMagttctcttccagtttgcagMGBNFQ 743R aggagggtgtcagggtcaca	NM_005330
HBG1n1	Newborn	61F gaaagctctgaatcatccaggtg 85T 6FAMtttgtggcatctcccaaggaagtcagcMGBNFQ 134R agtcaaggcacatggcaagaag	N/A
HBG2n3	Newborn	78F gcagtgagctcagtgcagttc 110T 6FAMcaaaggtgcccttgagatcatccaggMGBNFQ 159R ttccttgggagatgccataaa	N/A
IGFBP3	Elderly	743F agaacttctcctccgagtccaa 772T 6FAMacagaatatggtccctgccMGBNFQ 822R caggtgattcagtgtgtcttcca	NM_001013398
LOC151194	Newborn	400F gggctggtgggcatagtg 423T 6FAMcctgctgggtgctcaMGBNFQ 461R acttttcgatccgtgatagtcaca	NM_145280
LOH11CR2A	Elderly	1178F cttggcaccactccagaaca 1227T 6FAMcccctacagcttttMGBNFQ 1277R actaaacgtgtctgtaacttctccatct	BC001234
MAD1L1	Elderly	696F caggcagtgtcagcagaacttg 767T 6FAMctggcgagaccatcaaMGBNFQ 805R ccgagatcctccccttcagt	NM_003550

Candidate Gene	Target Age Group	Primer and MGB Probe Sequences 5' → 3'	NCBI Genbank Accession Number
PDCD6	Elderly	429F tcgataagaacgagctgaagca 459T 6FAMcaggtttcggctaccgMGBNFQ 498R atgtcgtggaactggtcagaga	NM_013232
POLM	Elderly	1095F ggcccaggtgtctgaagatg 1140T 6FAMatgtcttctgctccgggtMGBNFQ 1176R aacagccatgggctgtttg	NM_013284
POLQ	Elderly	6374F cagcccagacggttggaa 6403T 6FAMcatccctgcataaacMGBNFQ 6459R tgcaatcgtgggcagattc	NM_199420
PPARD	Elderly	906F catcctcaccggcaaagc 929T 6FAMacacggcgcccttMGBNFQ 963R tgtctcgatgtcgtggatcac	NM_006238
PPOX	Juvenile	791F aaacccatcgttccatattactgg 826T 6FAMtccgccctgccccMGBNFQ 869R tggcgaatgagtgctgagtc	NM_000309
PRL	Juvenile	983F ttctagagggcatggagctgat 1007T 6FAMtcagccaggttcatcMGBNFQ 1049R gggtagatctcattttctttggtttc	NM_000948
SPTRX-1	Juvenile	244F gagggaaaaccaactgtaacgtg 268T 6FAMcacccaaataaagctcaMGBNFQ 308R cacgttgctggacaggactagt	NM_032243
SPTRX-2	Juvenile	531F aaatgaactgaacgaagacgaaatt 564T 6FAMtgctgtcgcagaagcMGBNFQ 607R taaatggctgcaaagtcacaatg	NM_016616
SRC	Elderly	893F tgaggagtggtattttggcaaga 995T 6FAMcacgaaaggtgcctactMGBNFQ 1040R ggcgttgtcgaagtcagaca	NM_005417
TBC1	Juvenile	729F gctatgtgttcaaagccgatga 752T 6FAMcaaacaaaatgctcatcatcMGBNFQ 803R ctccggcagctctttcaaag	BC028196
TEKT2	Juvenile	700F tctcaacctcagatccccaaa 752T 6FAMcctgatggctccaccaMGBNFQ 808R gtccttgttgaaccgactgaagt	NM_014466

Candidate Gene	Target Age Group	Primer and MGB Probe Sequences 5' → 3'	NCBI Genbank Accession Number
GNAS	All Ages	1653F ggacaaagtcaacttccacatgttt 1690T NEDcagcgcgatgaacgccgcaaMGBNFQ 1749R gaagatgatggcagtcacatcgt	NM_016592
S15	All Ages	16F ccaaagcgatctcttctgaggat 40T VICcggcaagatggcagaagtagagcagaaMGBNFQ 105R acgccgcggtaggtgaa	NM_001018

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