# Examination of DNA Mixture Proportion Variability Using Multiple STR Typing Kits and NIST Standard Reference Material® 2391c Component D


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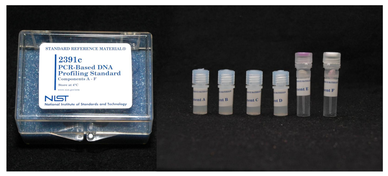
Standard Reference Material® 2391c (SRM 2391c) PCR-based DNA Profiling Standard is the third renewal of this SRM, originally released in 1995 (see http://www.nist.gov/srm/index.cfm). SRM 2391c consists of 6 components labeled A through F. Components A through D are supplied as genomic DNA solutions with component A as a single source female; component B as a single source male; component C as a single source male: and component D as a mixture of components A and C. Components E (single source female) and F (single source male) are cells deposited on 903 and FTA papers respectively, two 6 mm punches per component. Inclusion of two kinds of storage paper will enable laboratories to test direct PCR methods. The components of SRM 2391c are from different sources than used in SRM 2391, SRM 2391a, and SRM 2391b. Certified or Reference short tandem repeat (STR) genotypes for 68 loci (51 autosomal and 17 Y-STRs) across the six components are supplied. In order to avoid any potential null alleles, the SRM 2391c components were tested with twenty-two different genotyping kits: Applied Biosystems (Profiler, Profiler Plus, Profiler Plus ID, COfiler, Identifiler, Identifiler Plus, NGM, NGM Select, SGM Plus, SEfiler, MiniFiler, and Yfiler), Promega (PP 16, PP 16HS, PP ESX 17, PP ESI 17, PP ES, PP S5, PP Y, and FFFL), Qiagen (ESSplex and IDplex), plus additional primer sets developed by our group at NIST. Component D is a mixture prepared as a 3 parts component A and 1 part component C. This component has been tested with 20 of these kits. Estimates of the mixture ratio for Component D have been evaluated through the use of the DNA quantitation data, peak height, peak area data and the True Allele software.

Renewal of this SRM is critical for the forensic DNA human identification testing laboratories that adhere to the FBI issued Quality Assurance Standards of Forensic DNA testing (2011) Section 9.5.5. "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to a procedure against an appropriate and available NIST standard reference material or standard traceable to a NIST standard." [1]




The SRM delivers Certified Values for genotypes at loci when two or more kits use different primers to generate the Polymerase Chain Reaction (PCR) products or when the results obtained with a single set of primers have been confirmed by sequencing. It also delivers a Certified Value for the relative composition of Component D that was prepared from the source materials used for two of the other components.

## SRM 2391c Component Descriptions

Component A is extracted genomic DNA from Buffy coat white blood cells from an anonymous female.

Components B and C are two cell line DNAs purchased from Coriell Cell Repositories (Camden, NJ): NA03469 and NA10451.

Component D is a 3:1 mixture of the Components A and C materials.

Components A,B,C and D are solubilized in $TE^{-4}$ buffer (10 mmol/L Tris HCl, 0.1 mmol/L EDTA, pH 8.0)

Components E and F are "stains" created by depositing cells on to pre-punched paper spots.

Component E is a "stain" on 903 paper of a female cell line (CRL-1486).

Component F is a "stain" on FTA paper of a male cell line (HTB-157).

These two cell lines were purchased from the American Type Culture Collection (Manassas, VA) and grown at NIST.

"Conventional" DNA concentrations of the materials used to prepare the SRM 2391c components A, B, and C were estimated from optical densities at 260 nm determined using the BioCary 100 spectrophotometer. The conventional assertion is that an aqueous solution of 50 ng/µL of double-stranded DNA in a 1.0-cm pathlength cuvette at 260 nm has an optical density, $OD_{260}$, of 1.0 [2]. Dilutions of these materials were made based on the $OD_{260}$ measurements.

The DNA concentrations for the final dilutions of components A, B, C, and D were verified by qPCR using three commercial quantification kits and one in-house kit. The three commercial kits include Quantifiler Human DNA Quantification Kit (Life Technologies), Quantifiler Duo DNA Quantification Kit (Life Technologies), and Plexor HY System (Promega). Component A of SRM 2372: Human DNA Quantitation Standard was used to calibrate each assay. The range of the qPCR results are displayed in Table 1.

**Table 1. Description of Components in SRM 2391c**

| Component | Description | Quantity [a] |
|---|---|---|
| A | 50 µL of anonymous female genomic DNA | 1.4 – 1.9 ng DNA/µL |
| B | 50 µL of anonymous male genomic DNA | 1.3 – 1.5 ng DNA/µL |
| C | 50 µL of anonymous male genomic DNA | 1.3 – 2.0 ng DNA/µL |
| D | 50 µL of mixed-source (Components A and C) | 1.4 – 2.0 ng DNA/µL |
| E | Two 6 mm punches of CRL-1486 cells spotted on 903 paper | 7.5 x$10^4$ cells per punch |
| F | Two 6 mm punches of HTB-157 cells, spotted on FTA paper | 7.5 x$10^4$ cells per punch |

[a] DNA concentrations and cell counts are nominal values and are **not** intended for use as quantitative standards.

The STR genotypes for this SRM result from analyses performed at NIST; Palm Beach Sheriff's Office (West Palm Beach, FL); Bode Technology Group (Lorton, VA); Promega Corp. (Madison, WI); and Life Technologies (Foster City, CA).

**All results are concordant across all kits and all laboratories.**

In order to avoid any potential null alleles, the SRM 2391c components were tested with 22 different genotyping kits found in Table 2.

**There were no discordant results observed in the tested loci with these materials.**

**Table 2. STR Genotyping kits and primer mixes used at NIST to certify SRM 2391c**

| Kit Provider | | | Primer Mixes |
|---|---|---|---|
| *Life Technologies* | *Promega* | *Qiagen* | *NIST* |
| Identifiler | Powerplex 16 | ESSplex | 26plex [3] |
| Identifiler Plus | Powerplex 16 HS | IDplex | miniSTRs [4,5] |
| NGM | Powerplex ESX 17 | | |
| NGM SElect | Powerplex ESI 17 | | |
| COfiler | Powerplex ES | | |
| Profiler | Powerplex S5 | | |
| Profiler Plus | Powerplex Y | | |
| Profiler Plus ID | FFFL | | |
| SGM Plus | | | |
| SEfiler | | | |
| MiniFiler | | | |
| Yfiler | | | |


**References:**

**[1]** FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (2011); http://www.fbi.gov/about-us/lab/codis/qas-standards-for-forensic-dna-testing-laboratories-effective-9-1-2011

**[2]** Sambrook, J. and Russell D.W. (2001) Molecular Cloning a Laboratory Manual, Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.

**[3]** Hill, C.R., Butler, J.M., Vallone, P.M. (2009) A 26plex autosomal STR assay to aid human identity testing. *J. Forensic Sci.* 54(5): 1008-1015.

**[4]** Hill, C.R., Kline, M.C., Coble, M.D., Butler, J.M. (2008) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *J. Forensic Sci.* 53(1):73-80.

**[5]** Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* 50: 43-53.

**[6]** Perlin MW and Sinelnikov A. (2009) An information gap in DNA evidence interpretation. *PLoS One* 4(12):e8327; http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0008327

**[7]** JCGM 100:2008. Guide to the Expression of Uncertainty in Measurement (GUM). BIPM, Sèvres, France; http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf.

**[8]** Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results;* NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC (1994); http://physics.nist.gov/Pubs/guidelines/TN1297/tn1297s.pdf.


**Table 3. Certified Genotypes of 41 STR Loci and Amelogenin**

| | Component | | | | | |
|---|---|---|---|---|---|---|
| Locus | A | B | C | D | E | F |
| D1S1656 | 17.3,17.3 | 11,14 | 11,15 | 11,15,17.3 | 11,16.3 | 17.3,17.3 |
| D2S1338 | 18,23 | 17,17 | 19,19 | 18,19,23 | 19,20 | 17,17 |
| D2S441 | 10,10 | 10,14 | 10,10 | 10 | 10,10 | 14,14 |
| D3S1358 | 15,16 | 15,19 | 16,18 | 15,16,18 | 14,15 | 16,17 |
| D5S818 | 11,12 | 12,13 | 10,11 | 10,11,12 | 11,13 | 11,13 |
| D7S820 | 11,11 | 10,10 | 10,12 | 10,11,12 | 8,10 | 8,12 |
| D8S1179 | 13,14 | 10,13 | 10,17 | 10,13,14,17 | 11,13 | 10,13 |
| D8S1115 | 15,16 | 15,17 | 9,9 | 9,15,16 | 9,16 | 9,17 |
| D10S1248 | 15,16 | 13,13 | 12,16 | 12,15,16 | 14,14 | 14,15 |
| D12S391 | 18.3,22 | 19,24 | 19,23 | 18.3,19,22,23 | 17,22 | 18,19 |
| D13S317 | 8,8 | 9,12 | 11,11 | 8,11 | 8,12 | 8,11 |
| D16S539 | 10,11 | 10,13 | 10,10 | 10,11 | 11,12 | 9,11 |
| D18S51 | 12,15 | 13,16 | 16,19 | 12,15,16,19 | 14,17 | 17,22 |
| D19S433 | 13,14 | 16,16.2 | 13.2,15.2 | 13,13.2,14,15.2 | 14,14 | 13,14 |
| D21S11 | 28,32.2 | 32,32.2 | 29,30 | 28,29,30,32.2 | 29,30 | 29,32.2 |
| D22S1045 | 15,15 | 15,17 | 16,16 | 15,16 | 16,17 | 11,15 |
| CSF1PO | 10,10 | 10,11 | 10,12 | 10,12 | 10,11 | 10,11 |
| FGA | 21,23 | 20,23 | 24,26 | 21,23,24,26 | 20,23 | 21,25 |
| Penta D | 9,13 | 8,12 | 10,11 | 9,10,11,13 | 14,14 | 9,10 |
| Penta E | 5,10 | 7,15 | 12,13 | 5,10,12,13 | 13,19 | 11,15 |
| SE33 | 16,18 | 17,18 | 28.2,31.2 | 16,18,28.2,31.2 | 22,30.2 | 12,21 |
| TH01 | 8,9.3 | 6,9.3 | 6,8 | 6,8,9.3 | 6,9.3 | 7,9.3 |
| TPOX | 8,8 | 8,11 | 11,11 | 8,11 | 8,11 | 8,8 |
| vWA | 18,19 | 17,18 | 16,18 | 16,18,19 | 17,18 | 16,18 |
| Amelogenin | X,X | X,Y | X,Y | X,Y | X,X | X,Y |
| DYS19 | | 14 | 15 | 15 | | 17 |
| DYS385a | | 13 | 13 | 13 | | 12 |
| DYS385b | | 17 | 15 | 15 | | 16 |
| DYS389I | | 13 | 12 | 12 | | 13 |
| DYS389II | | 31 | 27 | 27 | | 30 |
| DYS390 | | 23 | 24 | 24 | | 24 |
| DYS391 | | 10 | 11 | 11 | | 12 |
| DYS392 | | 11 | 13 | 13 | | 11 |
| DYS393 | | 12 | 13 | 13 | | 13 |
| DYS437 | | 14 | 16 | 16 | | 15 |
| DYS438 | | 10 | 11 | 11 | | 10 |
| DYS439 | | 11 | 12 | 12 | | 11 |
| DYS448 | | 20 | 19 | 19 | | 20 |
| DYS456 | | 15 | 15 | 15 | | 15 |
| DYS458 | | 17.2 | 17 | 17 | | 18 |
| DYS635 | | 20 | 21 | 21 | | 21 |
| Y GATA H4 | | 11 | 11 | 11 | | 11 |

**Table 4. Genotypes of 26 Reference STR Loci and 1 Information STR Locus**

| | Component | | | | | |
|---|---|---|---|---|---|---|
| Locus | A | B | C | D | E | F |
| D1GATA113 | 12,12 | 12,12 | 7,12 | 7,12 | 12,12 | 7,13 |
| D1S1627 | 13,14 | 11,14 | 14,14 | 13,14 | 13,14 | 13,14 |
| D1S1677 | 13,15 | 12,13 | 14,15 | 13,14,15 | 14,16 | 15,15 |
| D2S1776 | 12,12 | 9,12 | 12,13 | 12,13 | 9,11 | 11,11 |
| D3S3053 | 9,11 | 11,12 | 9,11 | 9,11 | 9,11 | 11,11 |
| D3S4529 | 14,16 | 13,14 | 13,15 | 13,14,15,16 | 13,16 | 12,15 |
| D4S2364 | 9,10 | 8,9 | 9,9 | 9,10 | 9,10 | 10,10 |
| D4S2408 | 8,9 | 9,10 | 8,8 | 8,9 | 8,8 | 8,11 |
| D5S2500 | 18,18 | 17,17 | 14,14 | 14,18 | 17,17 | 17,17 |
| D6S1017 | 8,10 | 8,10 | 8,10 | 8,10 | 10,13 | 12,12 |
| D6S474 | 16,18 | 14,15 | 14,15 | 14,15,16,18 | 14,16 | 14,18 |
| D9S1122 | 11,12 | 11,13 | 10,10 | 10,11,12 | 11,11 | 12,13 |
| D9S2157 | 7,11 | 12,15 | 13,15 | 7,11,13,15 | 11,11 | 9,11 |
| D10S1435 | 11,14 | 12,14 | 11,12 | 11,12,14 | 12,13 | 12,13 |
| D11S4463 | 13,14 | 13,14 | 13,14 | 13,14 | 14,15 | 14,17 |
| D12ATA63 | 13,15 | 15,17 | 12,12 | 12,13,15 | 12,17 | 12,15 |
| D14S1434 | 10,14 | 10,14 | 13,14 | 10,13,14 | 10,14 | 13,14 |
| D17S1301 | 11,13 | 10,10 | 12,12 | 11,12,13 | 11,14 | 12,12 |
| D17S974 | 10,11 | 9,11 | 9,11 | 9,10,11 | 9,10 | 10,10 |
| D18S853 | 11,13 | 11,14 | 11,15 | 11,13,15 | 11,14 | 11,12 |
| D20S1082 | 11,14 | 11,15 | 11,15 | 11,14,15 | 11,15 | 11,15 |
| D20S482 | 14,15 | 13,14 | 13,15 | 13,14,15 | 15,15 | 14,15 |
| F13A01 | 4,5 | 3.2,7 | 5,6 | 4,5,6 | 5,7 | 5,6 |
| F13B | 8,9 | 9,10 | 10,10 | 8,9,10 | 9,10 | 8,10 |
| FESFPS | 12,12 | 11,11 | 11,13 | 11,12,13 | 11,12 | 10,11 |
| LPL | 10,11 | 10,10 | 10,12 | 10,11,12 | 10,11 | 10,12 |
| Penta C | 11,12 | 12,13 | 5,9 | 5,9,11,12 | 12,13 | 12,12 |

The SRM delivers Reference Values for loci assigned from repeat counts based on electrophoretic base pair (bp) size differences between non-sequenced alleles compared to sequenced alleles.

NIST Information Value is data that may be of interest and use to the SRM user, but insufficient information is available to access the confidence of the assignment (Penta C)

## Component D: The Mixture

Component D is a mixture prepared by gravimetrically combining three parts of Component A with one part of Component C. The preparative composition of this mixture was verified using peak height ratios from the multiplex assays used to assign genotypes and results from the TrueAllele electropherogram deconvolution software package [6].

The certified ratio for Component D, the mass of Component A relative to that of Component C, is **3.1 ± 0.1 Component A / Component C.** The uncertainty in the value, calculated according to the method described in the *Guide to the Expression of Uncertainty in Measurement* (GUM) [7, 8], is expressed as an expanded uncertainty, *U*. The expanded uncertainty is calculated as $U = k \times u_c$, where $u_c$ is the combined uncertainty and the coverage factor $k$=2.6 corresponds to approximately 95 % confidence.

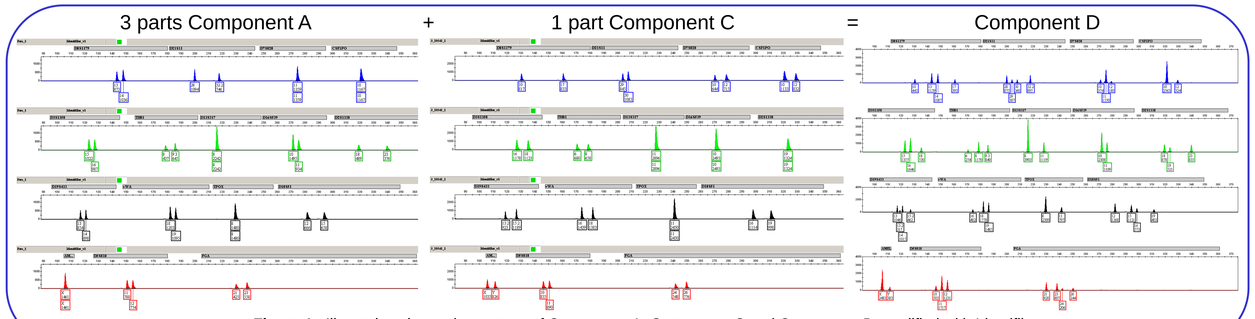



**Figure 1**. Illustrative electropherograms of Component A, Component C and Component D amplified with Identifiler

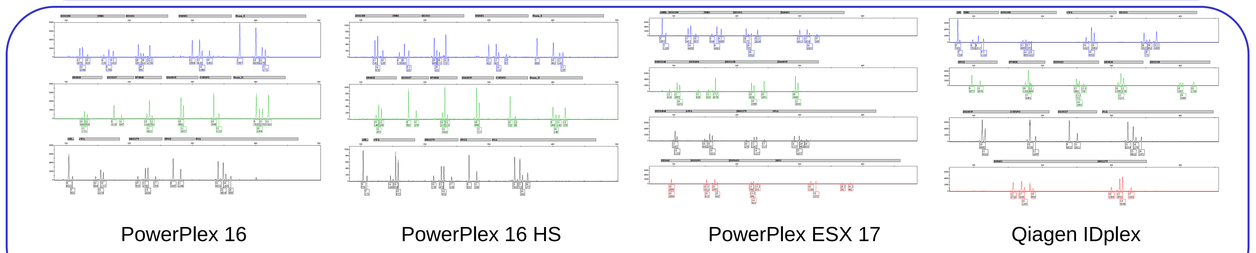



**Figure 2**. Illustrative electropherograms of Component D amplified with Promega: PP16, PP16 HS, PP ESX 17 and Qiagen IDplex

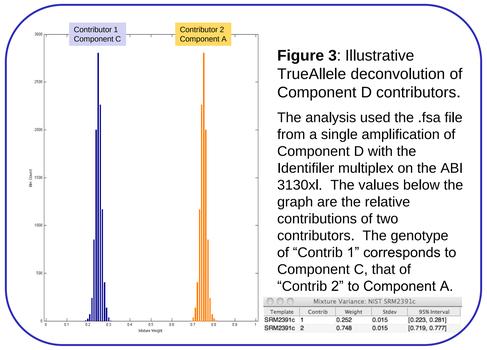



**Figure 3**: Illustrative TrueAllele deconvolution of Component D contributors.

The analysis used the .fsa file from a single amplification of Component D with the Identifiler multiplex on the ABI 3130xl. The values below the graph are the relative contributions of two contributors. The genotype of "Contrib 1" corresponds to Component C, that of "Contrib 2" to Component A.

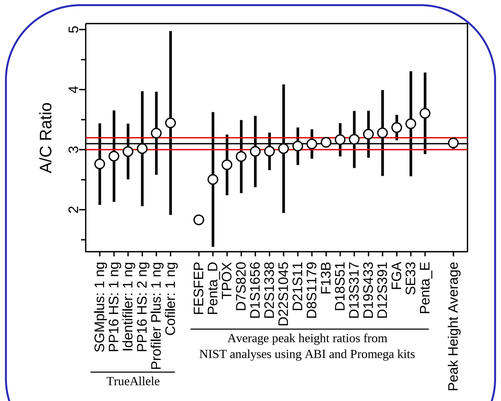



**Figure 4**: Summary of mass ratio evidence for Component D

**Table 5. Results for TrueAllele Deconvolution of Component D Profiles**

| | | TrueAllele | | Weight / (1-Weight) | | |
|---|---|---|---|---|---|---|
| Multiplex | Input ng | Weight | Stdev | Ratio | u | $U_{95}$ |
| COfiler | 1 | 0.775 | 0.036 | 3.44 | 0.79 | 1.53 |
| Identifiler | 1 | 0.748 | 0.015 | 2.97 | 0.24 | 0.47 |
| PP16 HS | 1 | 0.743 | 0.025 | 2.89 | 0.39 | 0.76 |
| PP16 HS | 2 | 0.751 | 0.029 | 3.02 | 0.49 | 0.96 |
| Profiler Plus | 1 | 0.766 | 0.019 | 3.27 | 0.36 | 0.69 |
| SGM Plus | 1 | 0.734 | 0.024 | 2.76 | 0.35 | 0.68 |

**Figure 4 is the summary of mass ratio evidence for Component D.**

The dots represent the mean estimate for each of the lines of evidence, with bars spanning the full 95 % level of confidence interval on the mean. Bars are not shown for the two loci characterized with only one multiplex. The "Peak Height Average" is the mean of the **140** discrete peak height ratios provided by the Life Technologies and Promega STR kits listed in Table 2 for 17 loci with unshared alleles for Components A and C. The red horizontal lines bound the 3.1 ± 0.1 95 % level of confidence interval about the preparative value.

**Figure 5 shows the electropherograms of the D21S11 locus and peak height ratios of Components A and C.**

This data is for 16 different amplification kits. It displays the intrinsic variability that can be expected from different primer sets and primer ratios in multiplexed kits. While some of these kits are using the same "primer mixes", the reaction buffers and/or amplification conditions have changed. These changes may affect the amplification efficiencies and therefore the peak height ratios.

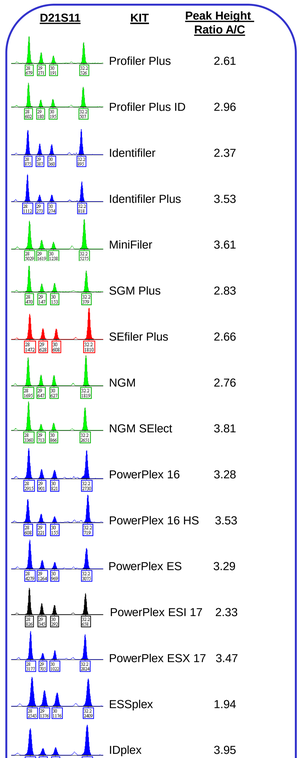



**Figure 5**: D21S11 for Component D

*Poster available for download from STRBase http://www.cstl.nist.gov/biotech/strbase/pub_pres/KlineISHI2011poster.pdf*