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Copy of poster available:
http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2006_Kline.pdf

National Institute of Standards and Technology (NIST) has produced several PCR-based DNA Profiling Standard Reference Materials (SRMs) for the forensic community. Primary uses of these materials are validation and calibration of currently used methods for quality assurance purposes [1]. As advances in methodologies occur and new loci become accepted for use in the human identity testing community, there becomes a need to update the certified values for current SRM materials to keep pace. To that end we are updating the Certificates of Analysis for the SRM 2391b PCR-based DNA Profiling Standard and the SRM 2395 Human Y-Chromosome DNA Profiling Standard. These certificate updates involve typing the SRM components with the additional loci and confirming the certified repeat numbers through DNA sequencing. We intend to sequence all STR alleles where no commercial kit is available with previously calibrated allelic ladders. **In this presentation, we detail our progress towards obtaining certified values for new autosomal and Y-chromosome STR loci.** Updated information on SRM components will be made available on the NIST STRBase website [2] as well as revised SRM Certificates.

SRM 2391b certificate updates include genetic typing with mini-short tandem repeat (miniSTR) loci, which produce smaller sized polymerase chain reaction (PCR) products used for analyzing degraded DNA [3]. At present 26 miniSTR loci have been selected for inclusion on the updated certificate: D1GATA113E02, D1S1627, D1S1677, D2S441, D2S1776, D3S3053, D3S4529, D4S2364, D4S2408, D5S2500, D6S474, D6S1017, D8S1115, D9S1122, D9S2157, D10S1248, D10S1435, D11S4463, D12A2A63A05, D14S1434, D17S974, D17S1301, D18S853, D20S482, D20S1082, D22S1045 [4,5].

SRM 2395 currently contains certified values for 22 different Y-STR loci confirmed through DNA sequencing and 5 loci that have been typed but not sequenced. Since SRM 2395 became available in July 2003, Applied Biosystems released the Yfiler kit containing 17 Y-STRs, including DYS635 [6]. We have sequenced the DYS635 locus with the 5 male components of SRM 2395 to enable coverage of the DYS635 locus [7]. When it is completed, the updated certificate will include DYS635 certified values as well as approximately 20 new Y-STR loci chosen from a screen of more than 60 additional markers (beyond the 17 Yfiler loci) based on their informational value and availability from genetic genealogy companies [8]. The loci include DYS449, DYS481, DYS505, DYS522, DYS532, DYS534, DYS570, DYS576, DYS607, DYS715, and DYS717.

Progress Towards Certificate Updates

SRM 2391b

https://srmors.nist.gov/view_detail.cfm?srm=2391B

The 12 components of SRM 2391b have certified values for all 22 STR loci present in the commercial autosomal STR kits Profiler Plus, COfiler, SGM Plus, Identifier, SEfiler, PowerPlex 16, PowerPlex ES, and FFL. In addition, certified values are available for D1S80, amelogenin, and DQA1 + PM. **In an effort to keep current with new developments in the field, we are adding to this information with new autosomal STR assays and loci.**

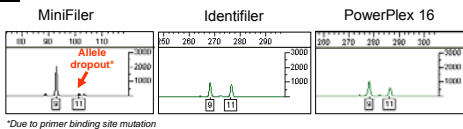
We recently tested the new miniSTR kit AmpFISTR® MiniFiler™ across the 10 extracted DNA samples present in SRM 2391b (in a beta-test format kindly provided by Applied Biosystems). We found all components to be concordant with previous certified values for the 8 STRs present in MiniFiler **with the exception of an allele dropout at D16S539 in component Genomic 8 (see below).**

New miniSTR loci that are being developed at NIST and adopted by other laboratories are being carefully genotyped and sequenced to enable world-wide calibration for these loci. Genotyping results across 26 new non-CODIS (NC) miniSTR loci (see Table to the right) include the recently recommended European core loci, D10S1248, D2S441, and D22S1045 [10], which have been sequenced.

New MiniFiler Kit Tested with SRM 2391b

All allele calls with MiniFiler for CSF1PO, D7S820, D13S317, D18S51, D21S11, FGA, and D16S539 (with the exception noted to the right) match previously certified values.

**D16S539
SRM 2391b
Genomic 8**



See Poster #44 for more information on the miniSTR loci

Components of SRM 2391b

Locus	Genomic 1	Genomic 2	Genomic 3	Genomic 4	Genomic 5	Genomic 6	Genomic 7	Genomic 8	Genomic 9947A	Genomic 9948
D1GATA113	11,11	12,13	11,11	13,13	11,12	11,12	10,12	10,12	11,12	7,12
D1S1627	10,14	13,14	13,14	11,12	14,15	11,13	11,14	13,14	13,14	11,13
D1S1677 (NC02)	12,13	14,16	14,17	14,15	13,14	13,14	12,13	14,16	13,14	13,14
D2S441 (NC02)	11,14	11,14	10,14	12,14	11,14	10,11	11,14	11,13	10,14	11,12
D2S1776	11,12	11,11	8,10	11,12	12,13	11,12	11,12	11,12	10,10	10,12
D3S3053	9,12	10,11	9,11	11,11	11,11	9,9	11,11	9,9	9,11	9,11
D3S4529	14,15	13,16	14,16	15,16	13,15	15,17	14,16	14,14	13,13	12,12
D4S2364 (NC02)	9,9	9,10	9,10	9,10	9,10	9,9	9,9	9,9	9,10	9,10
D4S2408	10,10	9,9	8,9	9,10	10,11	9,9	8,11	11,11	9,10	10,10
D5S2500	17,18	17,24	17,18	17,18	14,15	14,18	14,20	14,18	14,23	14,17
D6S474	15,17	14,17	14,15	14,16	15,18	14,17	15,17	17,17	14,18	17,17
D6S1017	10,10	10,12	10,12	7,10	8,9	10,10	7,12	10,12	9,10	8,8
D6S1115	16,16	16,16	16,17	9,17	9,15	9,16	9,18	15,16	9,18	15,17
D6S1122	11,12	12,13	12,12	12,12	11,13	11,12	11,12	13,13	12,13	12,15
D6S2157	8,13	9,11	11,13	11,11	7,14	11,13	12,15	11,11	7,13	7,11
D10S1248 (NC01)	14,16	13,15	13,16	12,12	14,15	14,15	13,14	11,15	13,15	12,15
D10S1435	13,13	11,14	13,14	12,12	11,12	12,12	12,12	11,13	10,11	12,13
D11S4463	14,14	13,14	14,15	11,12	14,16	16,17	14,15	14,17	12,13	12,14
D12A2A63	14,17	13,17	12,15	16,18	13,15	14,18	16,17	14,15	13,13	13,18
D14S1434 (NC01)	13,14	11,13	14,15	10,11	13,14	13,14	10,14	13,13	11,13	13,14
D17S974	9,11	9,10	9,9	7,9	11,12	9,9	11,11	8,9	7,10	10,11
D17S1301	11,11	11,12	11,12	12,13	11,11	11,11	11,12	12,12	12,12	11,12
D18S853	11,14	11,11	11,11	11,13	10,15	11,14	14,14	12,13	11,14	11,11
D20S482	14,14	14,16	15,15	14,15	14,15	14,14	14,14	15,16	14,15	13,14
D20S1082	11,15	14,15	11,11	14,15	11,14	11,15	14,15	11,15	11,14	11,15
D22S1045 (NC01)	14,15	11,16	15,16	17,18	11,14	11,15	11,15	16,17	11,14	16,18

Values in bold font have been sequenced

SRM 2395

https://srmors.nist.gov/view_detail.cfm?srm=2395

The five male components of SRM 2395 have certified values for the commercial STR kits PowerPlex Y and Yfiler (including DYS635 with sequence information noted below).

Future SRM 2395 Certificate of Analysis updates will include information on Y-STR nomenclature issues such as Y-GATA-H4 [11,12]. An important value with use of SRM components is that issues with potential nomenclature can be resolved fairly easily because a common set of standard samples may be run in any laboratory to verify and calibrate their results.

Many of the genetic genealogy companies utilize SRM 2395 to verify their Y-STR results. However, with this dynamic field, new loci are constantly being added (see [8] for a listing of current loci used by each company). Thus, we are in the process of testing new loci and sequencing alleles present in the SRM 2395 male components as part of adding value to the current DNA samples (see Table to the right).

DYS635 (GATA-C4) Sequence Information on SRM 2395 Components

Component	Type	DNA Sequence of STR Repeat Region
A	23	(TCTA) ₈ (TGTA) ₂ (TCTA) ₂ (TGTA) ₂ (TCTA) ₂ (TGTA) ₂ (TCTA) ₃
B	21	(TCTA) ₄ (TGTA) ₂ (TCTA) ₂ (TGTA) ₂ (TCTA) ₁₁
C	23	(TCTA) ₄ (TGTA) ₂ (TCTA) ₂ (TGTA) ₂ (TCTA) ₁₃
D	21	(TCTA) ₄ (TGTA) ₂ (TCTA) ₂ (TGTA) ₂ (TCTA) ₁₁
E	21	(TCTA) ₆ (TGTA) ₂ (TCTA) ₂ (TGTA) ₂ (TCTA) ₁₁

See Poster #2 for more information on the Y-STR loci

SRM 2395 Male Components	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385 a/b	DYS438	DYS439
A	14	13	29	25	11	13	13	12-15	12	12
B	14	13	28	23	11	11	12	14-17	9	12
C	16	14	32	21	12	11	13	17-20	11	11
D	15	12	28	22	10	11	14	14-15	11	11
E	17	14	31	24	10	12	14	13-15	10	11

SRM 2395 Male Components	DYS388	DYS437	DYS447	DYS448	DYS456	DYS458	DYS460	DYS461	DYS462	DYS635	H4
A	12	15	24	19	15	16	11	12	11	23	12
B	15	14	25	21	15	15	10	13	11	21	12
C	12	14	25	21	15	17	9	13	12	23	12
D	12	16	23	21	15	16	11	11	13	21	12
E	13	14	26	20	15	16	11	12	12	21	11

Yfiler loci in blue; not all Y-STR loci under investigation are listed

SRM 2395 Male Components	DYS449	DYS481	DYS522	DYS532	DYS534	DYS570	DYS576	DYS607	DYS715	DYS717
A	28	22	10	15	15	17	18	15	14	16
B	32	23	11	11	15	18	16	15	11	16
C	30	28	10	12	15	18	17	15	12	16
D	28	23	12	15	14	17	18	13	13	19
E	27	28	12	9	14	18	17	14	12	13

Establishing Traceability to NIST SRMs

Traceability requires the establishment of an unbroken chain of comparisons to stated references (see <http://ts.nist.gov/traceability/>). In the case of DNA testing with autosomal STR markers, the reference materials would be SRM 2391b. Just as chain of custody is used to convey integrity of connections between the final result obtained on a DNA sample and the evidence collected, materials deemed traceable to NIST-created materials must have a record associated with them.

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Acknowledgments and Disclaimer

Special thanks to Jan Redman for assistance in preparation and maintenance of SRM materials and Applied Biosystems for the MiniFiler kit beta-test materials.

These projects were funded by the National Institute of Justice through interagency agreement 2003-IJ-R-029 to the NIST Office of Law Enforcement Standards. Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.



For more information, see STRBase:

<http://www.cstl.nist.gov/biotech/strbase/srm2395.htm>