Why do forensic DNA labs care about the use of reference materials?

PCR-based DNA Profiling Standard

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<td><strong>would like your input into the desired number of components &amp; loci certified</strong></td>
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**History of SRM Work and Certificates**

- **1995** – released SRM 2391 with certified values for D1580, DQA1+PM, and 4 monoplex STR loci
- **1996** – addition of 13 core STR loci to SRM
- **1998** – released SRM 2391 certificate with 17 STRs (13 core loci + FFFL)
- **2000** – renewal (SRM 2391a) due to Quality Assurance Standard requirement; now includes 21 STRs (Penta D, Penta E, D19S433, D2S1338 added)
- **2003** – renewal (SRM 2391b) due to Quality Assurance Standard required; includes 22 STRs (SE33 added)
- **2007** – miniFiler kit released (with different primer sets and D16 dropout seen in Component 8), new miniSTR assays developed at NIST, and new commercial kits with new loci on the horizon
- **2008** – SRM 2391b certificate revised with additional 26 miniSTR loci including D25S441, D10S1248, and D22S1045
- **2010** – due to limited supply of current sample components, new DNA sources will be needed for SRM 2391c

Certified values for the NIST reference materials have evolved as the technology for DNA testing has improved...

**GAS Standard 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.

**Genomic materials in SRM 2391 were originally selected to “light-up” all the types on a PM+DQA1 (PolyMarker and DQa1) reverse dot blot strip to confirm that all probes were working properly.** Cell lines 9947A and 9948 were added due to work by Ron Fourney’s RCMP lab.

**Some STR Typing Measurement Issues**

- **STR genotypes are generated using PCR amplification and electrophoretic sizing that involves an internal size standard with each sample.**
- The forensic DNA community almost exclusively uses STR typing kits to obtain results (there are different kits available that examine the same common markers). PCR amplification is expected to generate consistent genotypes as long as primer positions are not changed between kits. Primer changes can result in allele dropout due to primer site mutations.
- Occasionally new commercial kits are created with additional loci. General STR repeat nomenclature rules have been established but do have some subjectivity in them permitting possible differences in how STR alleles are named.

**Examples:**
- Certain human use approval for 10 mL whole blood. Obtain blood and appropriate stain cards / stain-media following protocol, prepare 500 stains (20 µL/stain), dry and store for at least a week. Analyze at least 5 randomly selected samples Evaluate results: are they all qualitatively identical? Now analyze at least two samples in parallel with SRM 2395 Maintain records that the SRM data obtained was correct as well as the data from your stain. Package and store stains appropriately (dry and cold!)

**Make Your Own (MYO) Traceable Material**

Prepare a "lot" of DNA samples: stain, swab, cell pellet, extract, etc.

Assure that the MYO samples are:
- Homogenous
- Stable
- Reproducible

Analyze the appropriate SRM and MYO “in parallel”

Confirm that your results for the SRM are correct (agree with certificate) and your results for the MYO are consistent (agree with your prior results).

Maintain the records of the now traceable MYO and the SRM analysis. You may use the MYO as frequently as you desire in your Laboratory System instead of the SRM. Keep a record of the use of the MYO and results.

**If at any time there is a discrepancy with the results obtained for the MYO, a new lot must be made!!!**

Remember:

There must always be a direct comparison to the SRM. The “Lot” is Traceable not the source of the material.

For more information on NIST SRMs, see:

- http://www.nist.gov/srm

Poster available for download from STRBase: http://www.csti.nist.gov/biotech/strbase/pub_pres/Promega2009poster_SRM2391c.pdf