Protocol for Using the miniSTR System "miniSGM" on the ABI 3100 Instrument

Reagents Included:

- MiniSGM Primer mix, 100 reactions per tube + 10% overfill (**1 blue topped tube**). Approximately 550 µL, 1 µM conc.
- Allelic ladders, 20 µL (**1 red topped tube**)

Materials Needed:

- PCR mix from any STR kit (e.g. SGM Plus PCR mix)
- TaqGold DNA polymerase (5 U/µL)
- Capillary Array
- POP-6 or POP-4 polymer
- Matrix standards for ABI 3100
- Genetic Analyzer Buffer
- GeneScan and Genotyper software programs

Primer Sequences (Butler et al. (2003), *JFS*, 48(5): 1054-1064)*

Locus		MiniSGM Primer Sequences (5'-3')	Distance 3'end from STR repeat
TH01	F	6FAM-CCTGTTCCTCCCTTATTTCCC	0
	R	GTTTCTTGGGAACACAGACTCCATGGTG	1
AMELO	F	6FAM-CCCTGGGCTCTGTAAAGAATAGTG	X = 126 bp
	R	ACACAGGCTTGAGGCCAAC	Y = 132 bp
FGA	F	6FAM-AAATAAAATTAGGCATATTTACAAGC	3
	R	GCTGAGTGATTTGTCTGTAATTG	23
D18S51	F	VIC-TGAGTGACAAATTGAGACCTT	5
	R	GTCTTACAATAACAGTTGCTACTATT	33
D16S539	F	NED-ATACAGACAGACAGACAGGTG	0
	R	GCATGTATCTATCATCCATCTCT	16
D2S1338	F	PET-TGGAAACAGAAATGGCTTGG	3
	R	GATTGCAGGAGGGAAGGAAG	3

*PDF available at http://www.cstl.nist.gov/biotech/strbase/pub_pres/Butler2003d.pdf

Note: The reverse primer for TH01 has a 7-nucleotide tail (e.g., GTTTCTTT) added at the 5'-end to promote non-template addition.

Comments, suggestions, issues, notes for user…

- In our hands, this assay is sensitive to ~20 pg DNA at 32 cycles of PCR.

- Information for detection of PCR products using an ABI 310 can be found in the Butler et al. (2003) reference above, or can be sent to you via e-mail (see contact info to the right).

- Be aware that at very low template concentrations (e.g. <50pg) a PCR artifact can often be seen as either a 9.3 or 10 allele for TH01 (~86bp). A dye blob is also observed at around 79bp, however, this generally falls outside of the bin for allele 7.

- The allelic ladders included show very weak signal for the larger FGA and D18S51 alleles.

PCR Conditions:

Preparation of Master Mix:

___ (# reactions) x **10.5 µL PCR mix (from an ABI kit)** = _____

___ (# reactions) x **0.4 µL Taq Gold** = _____

___ (# reactions) x **5.5 µL primer mix (blue-topped tube)** = _____
16.4 µL – includes overfill for pipetting

Preparation of Individual PCR Reactions:

15 µL master mix *(from above)*
10 µL DNA template (or dI H_2O to bring up the volume)

Thermal Cycling

Thermal cycling was performed with the GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e., ramp speeds of 1 °C/s):

95 °C for 10 minutes
32 cycles: 94 °C for 1 minute
55 °C for 1 minute
72 °C for 1 minute

60 °C for 45 minutes
25 °C forever

Detection of PCR Products

We have used the ABI 3100 with POP6 polymer, capillaries, and buffer used for STR typing with commercial kits. The MiniSGM assay uses 6FAM (blue), VIC (green), NED (yellow), and PET (red) dyes.

ABI 3100

Prior to running any samples with the MiniSGM STR system on the ABI 3100, a 5 dye matrix needs to be established under the "**G5 filter**" with the dyes 6FAM (blue), VIC (green), NED (yellow), PET (red), and LIZ (orange) using **matrix standard set DS-33** (P/N 4318159). Samples are typically prepared with **15 µL Hi-Di™ formamide** (Applied Biosystems, P/N 4311320), **0.35 µL GS500 LIZ** (P/N 4322682), and with **1 µL PCR product**.

The samples may be run using the default module **GeneScan36_POP4DefaultModule**, which performs an electrokinetic injection onto the 16-capillary array for 10 s at 3,000 volts. The STR alleles are then separated at 15,000 volts for approximately 30 minutes with a run temperature of 60 °C using the 3100 POP™-6 sieving polymer (Applied Biosystems, P/N 4316355), 1X Genetic Analyzer Buffer with EDTA (P/N 402824), and a 36 cm array (P/N 4315931).

Contact Information for Technical Details:

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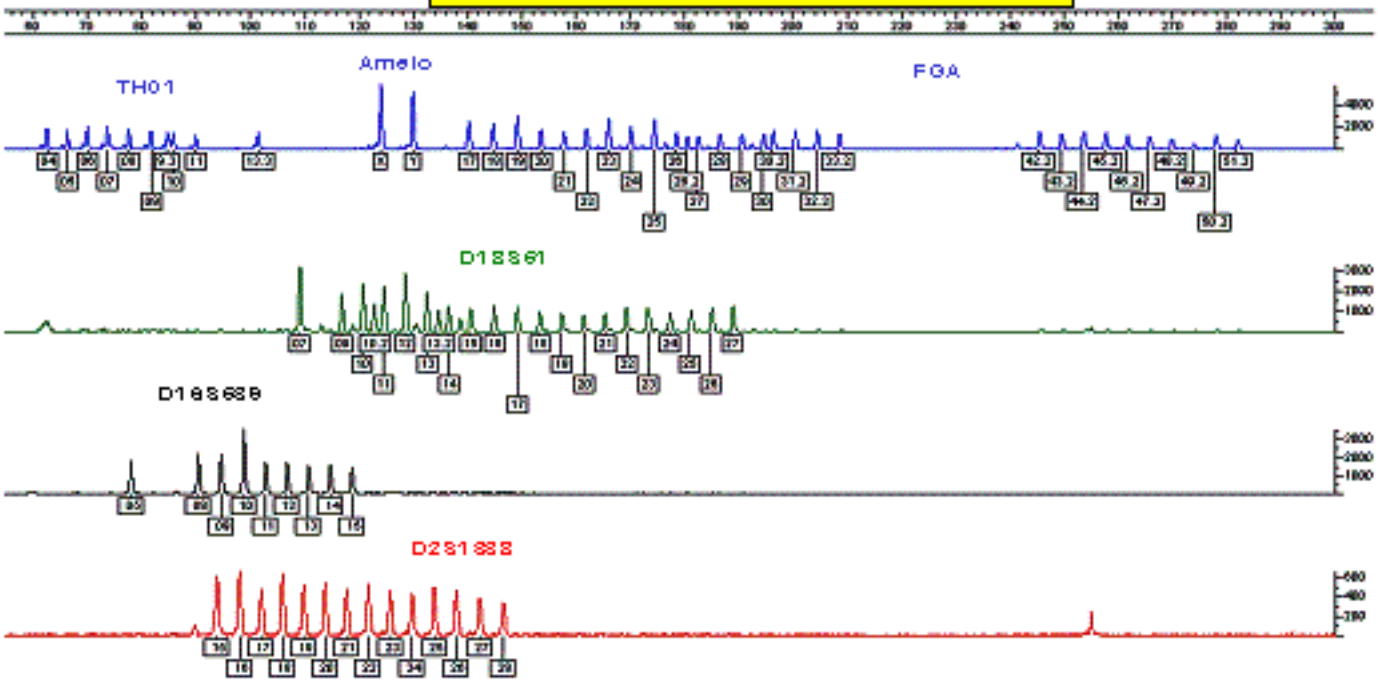
Allelic Ladders with miniSGM markers



Expected Control Results

STR Locus	Control DNA 007 Genotype	Control DNA 9947A Genotype
TH01	7, 9.3	8, 9.3
Amelo	X, Y	X, X
FGA	24, 26	23, 24
D18S51	12, 15	15, 19
D16S539	9, 10	11, 12
D2S1338	20, 23	19, 23

Macro Information:

A downloadable research macro using fixed bins will be posted for you on the STRBase website:
http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

Also posted is a document with allele size ranges for each bin (for those wanting to build their own macro).

Negative control from sensitivity series using the suggested conditions - 32 cycles, 2U Taq