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

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 dH2O 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), and 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 ºC using the 3100 POP™-6 sieving polymer (Applied Biosystems, P/N 4316355), 1X Genetic Analyzer Buffer with EDTA (P/N 4028242), and a 36 cm array (P/N 4315931).

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

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>Control DNA 007 Genotype</th>
<th>Control DNA 9347A Genotype</th>
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<tbody>
<tr>
<td>TH01</td>
<td>7, 9, 3</td>
<td>8, 9, 3</td>
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<tr>
<td>AmelO</td>
<td>X, Y</td>
<td>X, X</td>
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<tr>
<td>FGA</td>
<td>24, 26</td>
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<tr>
<td>D18S51</td>
<td>12, 16</td>
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<td>D16S539</td>
<td>9, 10</td>
<td>11, 12</td>
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<tr>
<td>D2S1338</td>
<td>20, 23</td>
<td>19, 23</td>
</tr>
</tbody>
</table>

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

Dye Blob ~78bp; Artifact Peak ~85 bp