# Multiplex SNaPshot for Body Fluid Identification

## Multiplex PCR

### Reagents Needed:

**4 X Primer Mix**

**AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)**

**Gold ST*R 10 X Buffer (Promega, Madicine, WI)**

### 4 X Primer Mix for Multiplex PCR:

| | Target ID | Sequence (5'→3') | Conc. (uM) | Amplicon size (bp) |
|---|---|---|---|---|
| SE1 | cg17610929 | TTG TTG ATA TGT TTT GAA TTA TTA AG | 2.4 | 174 |
| | | ATA ACT TCC CTT ATC AAC ACC AAC | 2.4 | |
| SE2 | Cg26763284-138d | TGA TTT ATA ATT ATT AGG GAG GGA AAT AG | 0.8 | 105 |
| | | CCT AAA ACA ACC RAT TCC CAA C | 1.6 | |
| BL1 | cg06379435 | TTT ATT GGG GTA TTT TTA TTG GTT AG | 8.0 | 157 |
| | | AAA ATA CAA CTT ACT CCT AAA CAC C | 8.0 | |
| BL3 | cg08792630 | TGT TTT AAG AGG ATG ATA AGG AA | 2.4 | 220 |
| | | CCA CCT CAA TCC AAA CTA ACT ACA | 2.4 | |
| VF1 | cg09765089-231d | TTG GTA GTT TTT GGA TTT TGG AG | 2.4 | 137 |
| | | AAA CRT AAA ACR ACC CRA AC | 19.2 | |
| VF2 | cg26079753-7d | TTT TGT GAG TGT GAG AGA TTT TTA AGA | 1.6 | 176 |
| | | AAA ACC TCC AAA ACA AAA CCT CTA | 1.6 | |
| SA1 | cg09652652-2d | GGG GAT TYG TTT YGT TAG GT | 16.0 | 153 |
| | | CCA TTT CCC CCT TCC TAA AA | 4.0 | |
| MB | cg18069290 | AGG GGY GAA GAG TAG GAA T | 8.0 | 160 |
| | cg09696411 | ATA ATA AAA CAA CCA ATA CAA C | 8.0 | |

### PCR Mixture:

| PCR Component | Vol. (ul) |
|---|---|
| $dH_2O$ | ~11.3 |
| 10 X Gold ST*R Buffer | 2 |
| 4 X Primer Mix | 5 |
| AmpliTaq Gold (5 U/μL) | 0.7 (3.5 U) |
| Bisulfite converted DNA | 1 (~4)* |
| Total | 20 |

### Thermal Cycling:

**95℃ for 11 minutes, then:**

**94℃ for 20 seconds**
**56℃ for 60 seconds**
**72℃ for 30 seconds**
**for 34 cycles, then:**

**72℃ for 7 minutes**
**4℃ soak**

* Please be aware that you should not use too much volume of bisulfite converted DNA because, in our experience, it may cause PCR failure.

# Post-PCR Reaction

## Enzyme Purification of the PCR Product

**Reagents Needed:**

| | |
|---|---|
| PCR product | 5 µL |
| ExoSAP-IT® (USB, Cleveland, OH) | 2 µL |

**Thermal Cycling:**

37℃ for 45 minutes

80℃ for 15 minutes

## Multiplex SNaPshot

**Reagents Needed:**

5 X SBE Primer Mix

5 X Sequencing buffer_BigDye Termination (Applied Biosystems, Foster City, CA)

SNaPshot™ Kit (Applied Biosystems, Foster City, CA)

**5 X SBE Primer Mix:**

| | Target ID | Sequence (5'→3') | Conc. (uM) | Length (nt) |
|---|---|---|---|---|
| SE1 | cg17610929 | $(T)_5$ CCG AAA CCC TCC CCA C | 2.0 | 21 |
| | | $(T)_6$ CCA AAA CCC TCC CCA C | 2.0 | 22 |
| SE2 | Cg26763284-138d | $(T)_6$ CGC GTA ACG ACT ATA AAA CCC TC | 0.15 | 29 |
| | | $(T)_8$ CAC ATA ACA ACT ATA AAA CCC TC | 0.5 | 31 |
| BL1 | cg06379435 | $(T)_{17}$ CCR ATA AAA CCT CAA ACR TAA AAC | 20.0 | 41 |
| BL3 | cg08792630 | $(T)_{21}$ CCR TAA TAA CTT CTA CCT ATA AAT AAA CCC | 3.0 | 51 |
| VF1 | cg09765089-231d | $(T)_{34}$ TCC CCA AAT AAC AAA CRA CRA AAA TC | 4.5 | 60 |
| VF2 | cg26079753-7d | $(T)_{44}$ CRA TCA ACT ACT ATA AAA ACA CC | 4.5 | 67 |
| SA1 | cg09652652-2d | $(T)_{48}$ CCA CGA ATA AAT AAC CAC GAT AAA AC | 7.5 | 74 |
| MB1 | cg18069290 | $(T)_{61}$ GAA CCA ACA ACA AAC RCC AC | 3.0 | 81 |
| MB2 | cg09696411 | $(T)_{69}$ CCT ATC CCR AAA CAA ACR C | 15.0 | 88 |

**SBE Reaction Mixture:**

| Reaction Component | Vol. (ul) |
|---|---|
| $dH_2O$ | ~ 4 |
| 5 X SBE Primer Mix | 2 |
| 5 X Sequencing Buffer | 2 |
| SNaPshot Reaction Mix | 1 |
| Purified PCR Product | > 1 |
| Total | 10 |

**Thermal Cycling:**

96℃ for 10 seconds
50℃ for 5 seconds
60℃ for 30 seconds

for 25 cycles

* Please keep the SNaPshot mixture on ice before putting it into the thermal cycler. Leaving the mixture at ambient temperature may result in a higher background because tha SNaPshot kit does not support Hot Start PCR.

# Post-Single Base Extension

## Enzyme (SAP or CIP) Treatment

**Reagents Needed:**

| | |
|---|---|
| SBE reaction product | 10 µL |
| SAP-Recombinant (USB, Cleveland, OH) | 1 µL |

**Thermal Cycling:**

37℃ for 45 minutes

80℃ for 15 minutes

## Capillary Electrophoresis

**Materials and Reagents Needed:**

Dry heating block, water bath or thermal cycler

3130 capillaries, 33 cm x 50 µm (Applied Biosystems, Foster City, CA)

Performance Optimized Polymer (POP4, Applied Biosystems, Foster City, CA)

Matrix Standard Set DS-02 (dR110, dR6G, dTAMRA™, dROX™, LIZ® Dyes)

(Applied Biosystems, Foster City, CA)

Run Module GS STR POP4 (1 mL) E5

GeneScan™ 120 LIZ™ Size Standard

Hi-Di™ Formamide (Applied Biosystems, Foster City, CA)

**Creating Matrix:**

According to the ABI PRISM®SNaPshot™Multiplex Kit protocol

**Reagents Needed:**

| | |
|---|---|
| GeneScan™ 120 LIZ™ Size Standard | 0.2 µL |
| Hi-Di™ Formamide | 10 µL |
| SNaPshot product | 1~2 µL |

**Thermal Cycling:**

95℃ for 5 minutes

4℃ soak

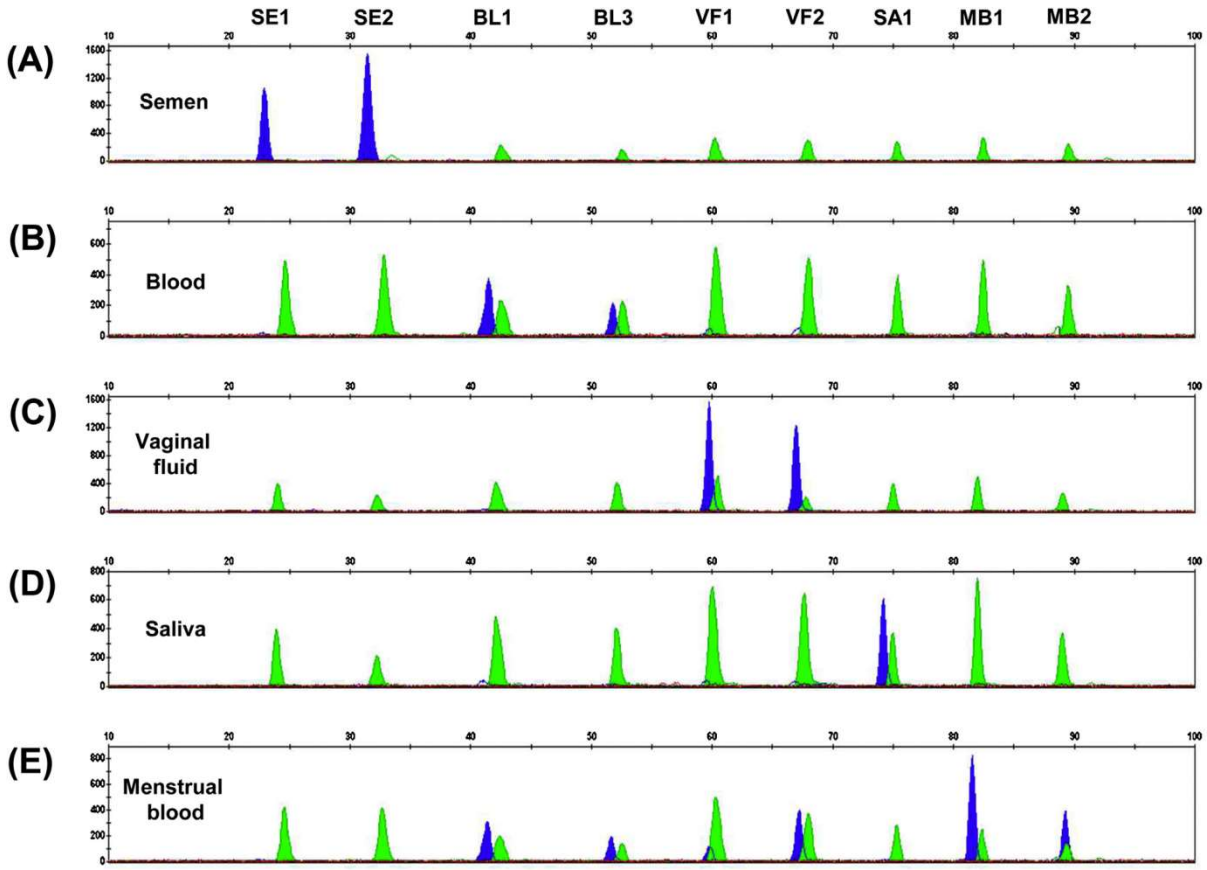
**3130 Data Collection Software:**

Verify that you have chosen GeneScan Run module E5 and the DS-02 GeneScan Matrix Set.

Run prepared samples under the following conditions: injection time of 3 sec, electrophoresis voltage of 15 kV, collection time of 8 min, EP voltage of 15 kV and heat plate temperature of 60℃.

Detect and calculate peak heights with an analytical threshold of 100 rfu.

# Electropherograms




Representative electropherograms of body fluid identification using multiplex methylation SNaPshot; (A) semen, (B) blood, (C) vaginal fluid, (D) saliva, and (E) menstrual blood. SE1, SE2, BL1, BL3, VF1, VF2, SA1, MB1 and MB2 represent cg17610929, cg26763284-138d, cg06379435, cg08792630, cg09765089-231d, cg26079753-7d, cg09652652-2d, cg18069290 and cg09696411, respectively.