Multiplex SNaPshot for Body Fluid Identification

Multiplex PCR

Reagents Needed:

5 X Primer Mix
AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10 X Buffer (Promega, Madicine, WI)

5 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	3.0	174
		ATA ACT TCC CTT ATC AAC ACC AAC	3.0	
SE2	cg26763284-138d	TGA TTT ATA ATT ATT AGG GAG GGA AAT AG	1.0	105
		CCT AAA ACA ACC RAT TCC CAA C	2.0	
BL1	cg06379435	TTT ATT GGG GTA TTT TTA TTG GTT AG	10.0	157
		AAA ATA CAA CTT ACT CCT AAA CAC C	10.0	
BL3	cg08792630	TGT TTT AAG AGG ATG ATA AGG AA	3.0	220
		CCA CCT CAA TCC AAA CTA ACT ACA	3.0	
VF1	cg09765089-231d	TTG GTA GTT TTT GGA TTT TGG AG	3.0	137
		AAA CRT AAA ACR ACC CRA AC	24.0	
VF2	cg26079753-7d	TTT TGT GAG TGT GAG AGA TTT TTA AGA	2.0	176
		AAA ACC TCC AAA ACA AAA CCT CTA	2.0	
SA1	cg09652652-2d	GGG GAT TYG TTT YGT TAG GT	16.0	153
		CCA TTT CCC CCT TCC TAA AA	4.0	

PCR Mixture:

PCR Component	Vol. (ul)
dH_2O	~12.4
10 X Gold ST*R Buffer	2
5 X Primer Mix	4
AmpliTaq Gold (5 U/µL)	0.6 (3 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:

10 X SBE Primer Mix

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

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

10 X SBE Primer Mix:

	Target ID	Sequence (5'→3')	Conc. (uM)	Length (nt)
SE1	cg17610929	$(T)_5$ CCG AAA CCC TCC CCA C	4.0	21
		$(T)_6$ CCA AAA CCC TCC CCA C	4.0	22
SE2	cg26763284-138d	$(T)_6$ CGC GTA ACG ACT ATA AAA CCC TC	0.3	29
		$(T)_8$ CAC ATA ACA ACT ATA AAA CCC TC	1.0	31
BL1	cg06379435	$(T)_{17}$ CCR ATA AAA CCT CAA ACR TAA AAC	40.0	41
BL3	cg08792630	$(T)_{21}$ CCR TAA TAA CTT CTA CCT ATA AAT AAA CCC	6.0	51
VF1	cg09765089-231d	$(T)_{34}$ TCC CCA AAT AAC AAA CRA CRA AAA TC	9.0	60
VF2	cg26079753-7d	$(T)_{44}$ CRA TCA ACT ACT ATA AAA ACA CC	9.0	67
SA1	cg09652652-2d	$(T)_{48}$ CCA CGA ATA AAT AAC CAC GAT AAA AC	15.0	74

SBE Reaction Mixture:

Reaction Component	Vol. (ul)
dH_2O	~ 5
10 X SBE Primer Mix	1
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

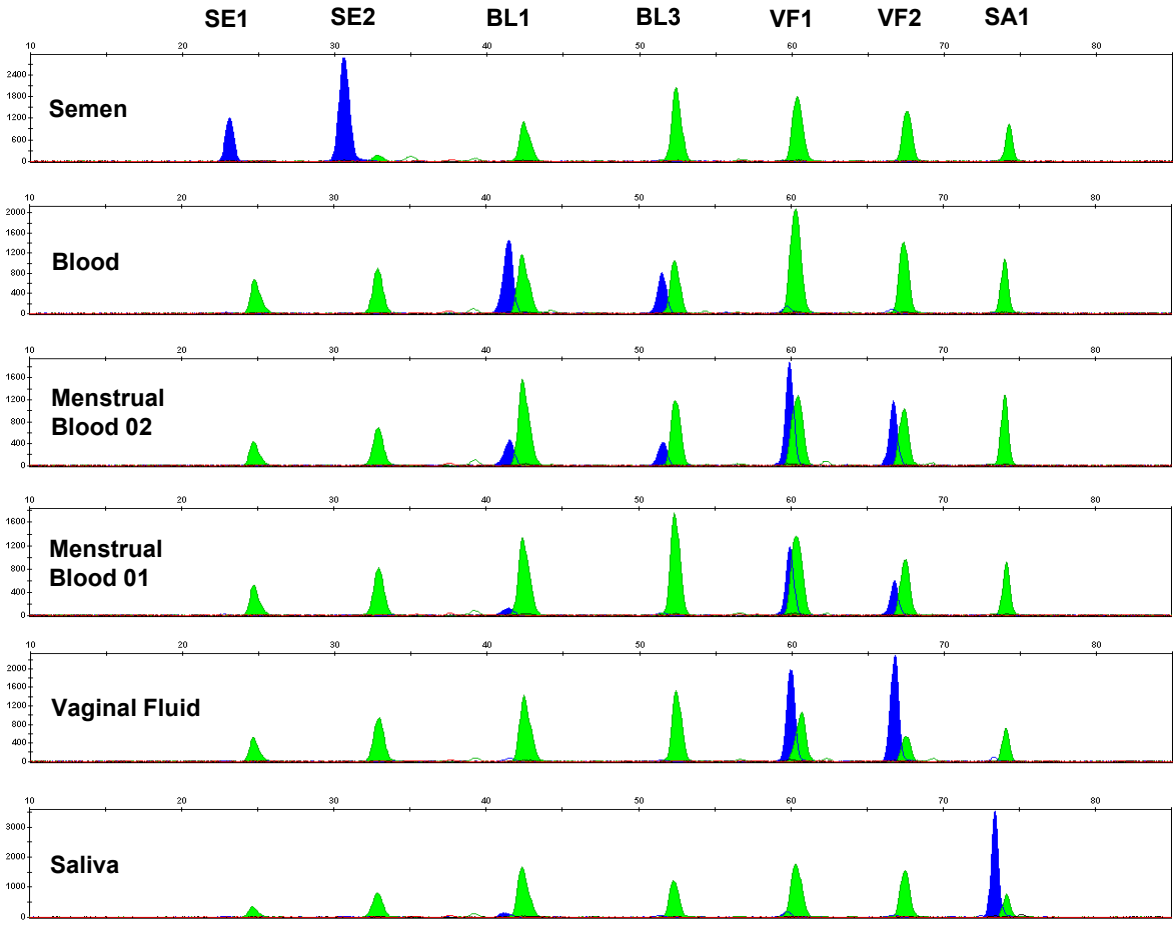
3130 Data Collection Software:

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. SE1, SE2, BL1, BL3, VF1, VF2, and SA1 represent cg17610929, cg26763284-138d, cg06379435, cg08792630, cg09765089-231d, cg26079753-7d, and cg09652652-2d, respectively. Because all SBE primers were designed to be in the reverse direction, a blue peak represents the nucleotide G as a methylation signal and a green peak represents the nucleotide A as an unmethylation signal.