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 more than 1/5 PCR volume of bisulfite converted DNA when using Sigma's Imprint ® DNA modification kit because, in our experience, it may cause PCR failure.

Post-PCR Reaction

Enzyme Purification of the PCR Product

Reagents Needed:

PCR product	10 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

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

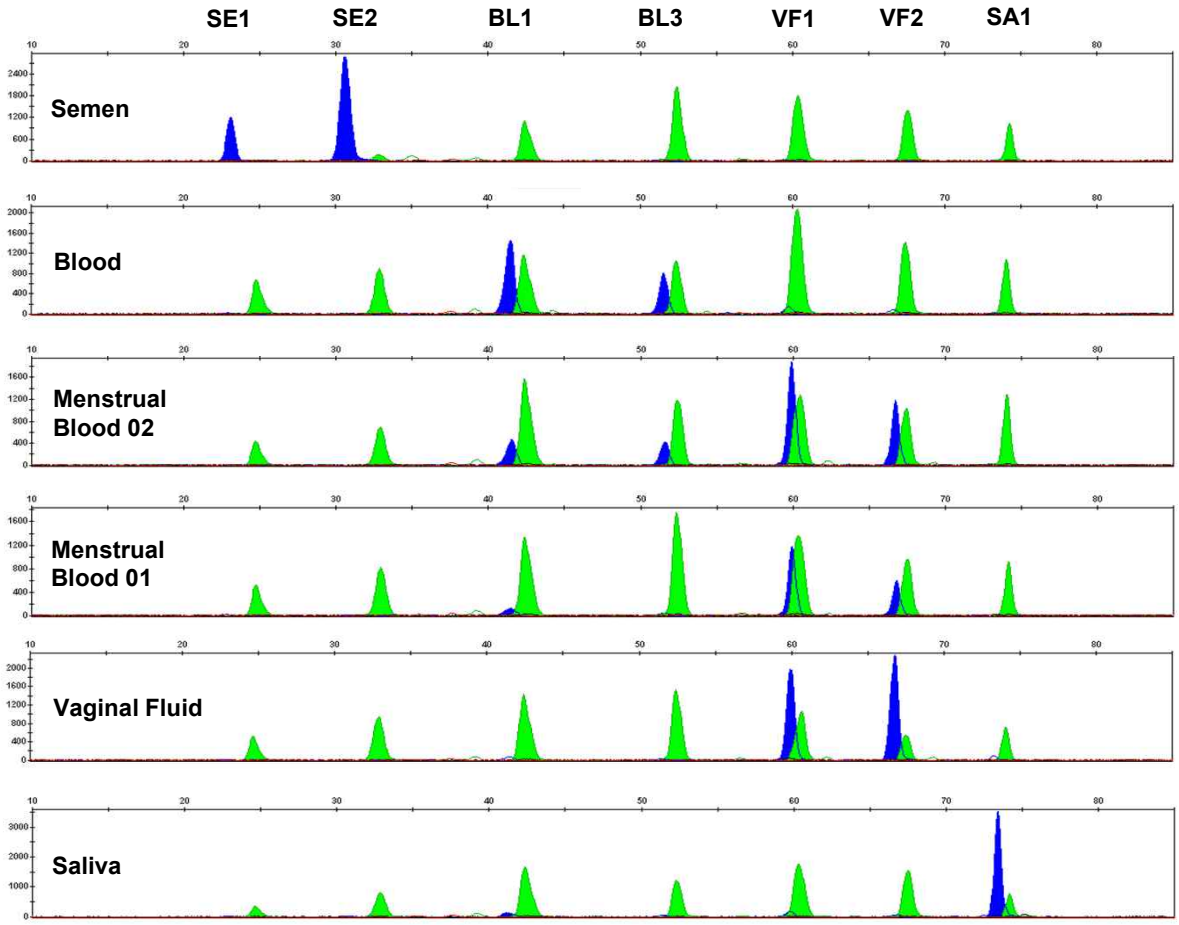
4℃ soak

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℃.

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.