



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, Madison, 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	GTT TTT TAG GGG AGA AGA GTA GGA AT	8.0	160
	cg09696411	ATA ATA AAA CAA CCA ATA CAA C	8.0	

PCR Mixture:

PCR Component	Vol. (ul)
dH ₂ O	~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°C for 11 minutes, then:
94°C for 20 seconds
56°C for 60 seconds
72°C for 30 seconds
for 34 cycles, then:
72°C for 7 minutes
4°C 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 μ L
ExoSAP-IT® (USB, Cleveland, OH)	2 μ L

Thermal Cycling:

37 °C for 45 minutes
80 °C 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 ₂ O	~ 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 °C for 10 seconds
50 °C for 5 seconds
60 °C for 30 seconds
for 25 cycles

Post-Single Base Extension

Enzyme (SAP or CIP) Treatment

Reagents Needed:

SBE reaction product	10 μ L
SAP-Recombinant (USB, Cleveland, OH)	1 μ L

Thermal Cycling:

37 °C for 45 minutes
80 °C for 15 minutes

Capillary Electrophoresis

Materials and Reagents Needed:

Dry heating block, water bath or thermal cycler
3130 capillaries, 33 cm x 50 μ m (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 μ L
Hi-Di™ Formamide	10 μ L
SNaPshot product	1~2 μ L

Thermal Cycling:

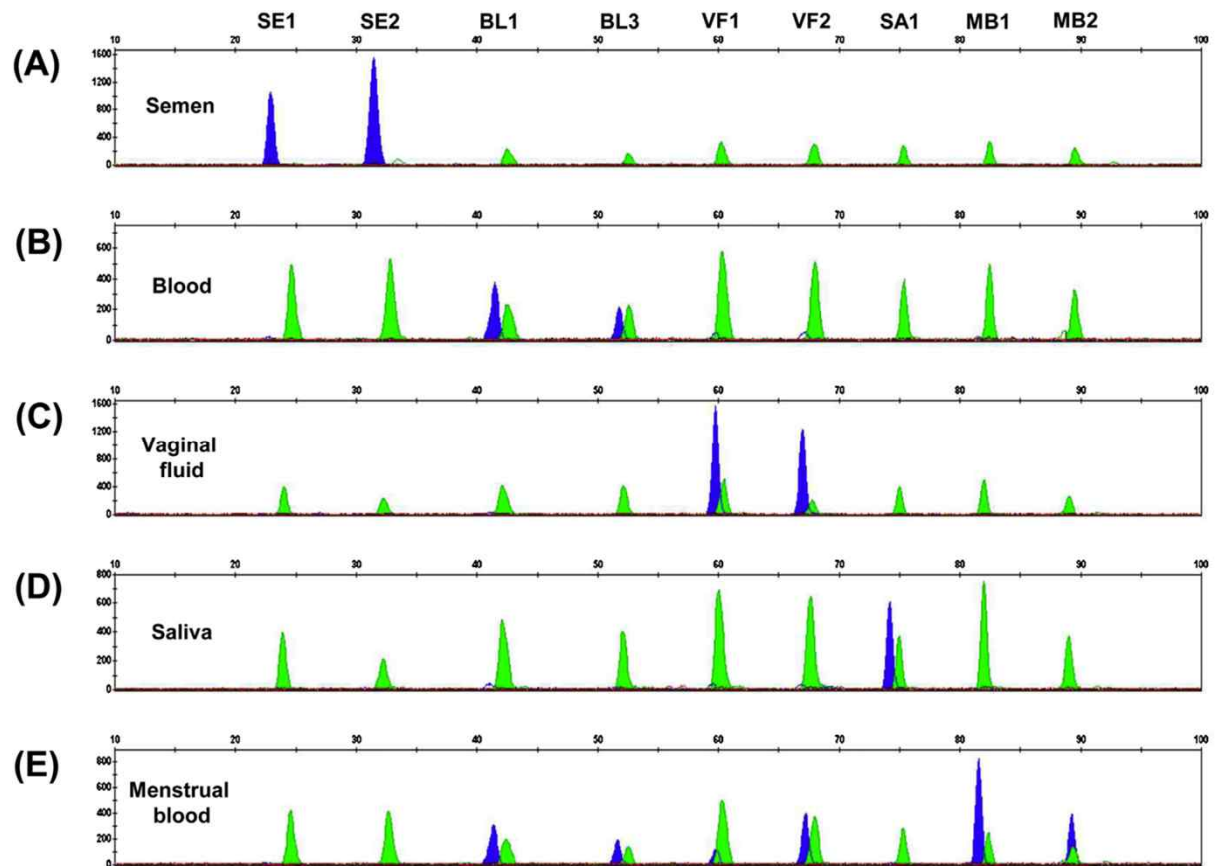
95 °C for 5 minutes
4 °C soak

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 °C.

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.