



Multiplex SNaPshot for Age Estimation Using Blood, Saliva and Buccal Swab Samples

Multiplex PCR

Reagents Needed:

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

5 X Primer Mix for Multiplex PCR:

Target ID	Sequence (5'→3')	Conc. (uM)	Amplicon size (bp)
ELOVL2	GGG GYG TAG GGT AAG TGA G	30	187
	CAA CRA ATA AAT ATT CCT AAA ACT CC	30	
FHL2	GGG TTT TGG GAG TAT AGT AGT	1.5	191
	AAA ATA ACC CCC TCC TCC	1.5	
KLF14	AGG TTG TTG TAA TTT AGA AGT TT	1.5	114
	ATA TTT AAC AAC CTC AAA AAT TAT CTT ATC	1.5	
C1orf132	GGG TTA YGT TAT TAA GTT TTG AAG	4	116
	TAA AAC CAA ATT CTA AAA CAT TC	2	
TRIM59	TAT GGT ATY GGT GGT TTG GGG GAG A	1	148
	ATA AAA AAC ACT AC R CTC CAC AAC ATA AC	1	

PCR Mixture:

PCR Component	Vol. (ul)
dH ₂ O	~12.6
10 X Gold ST*R Buffer	2
5 X Primer Mix	4
AmpliTa q Gold (5 U/μL)	0.4 (2 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 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 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:

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)
ELOVL2	(T) ₉ GGG AGG AGA TTT GTA GGT TTA GT	50.0	32
FHL2	(T) ₂₁ GTT TTG GGA GTA TAG TAG TTA T	5.0	43
KLF14	(T) ₂₈ TTA ACA ACC TCA AAA ATT ATC TTA TCT CC	3.0	57
C1orf132	(T) ₄₆ AAA CCA AAA TTT AAA TCT AC	12.0	66
TRIM59	(T) ₅₁ CCT CAA AAA CCR TCR ACC ACC RAC	1.0	75

SBE Reaction Mixture:

Reaction Component	Vol. (ul)
dH ₂ O	~ 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°C for 10 seconds
50°C for 5 seconds
60°C 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 the SNaPshot kit does not support Hot Start PCR.

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

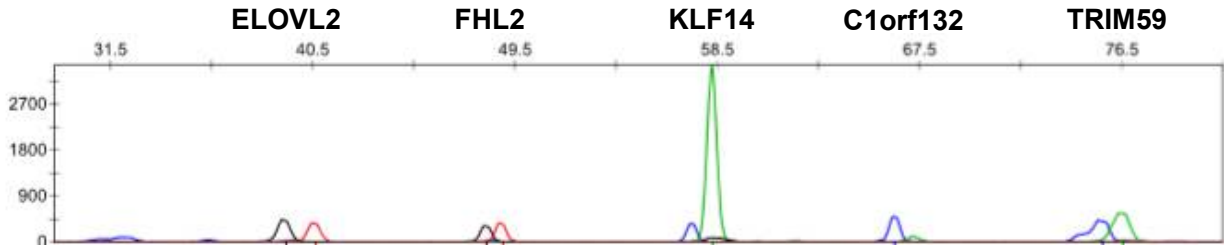
3130 Data Collection Software:

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.


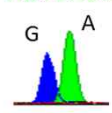
Analysis

Electropherogram



Genes	Chromosomal location (GRCh38)
ELOVL2	chr 6 : 11044628
FHL2	chr 2 : 105399282
KLF14	chr 7 : 130734355
C1orf132	chr 1 : 207823681
TRIM59	chr 3 : 160450189

Age Estimation

Target ID	Methylation*	Coefficients (Blood)	Coefficients (Saliva)	Estimated Age
(Intercept)	(intercept)	8.052	7.527	For BLOOD 8.052
ELOVL2	FORWARD 	55.673	47.078	+ 55.673 × ELOVL2 + 47.141 × FHL2 + 62.870 × KLF14 + (-29.075) × C1orf132 + 23.671 × TRIM59
FHL2	%methyl = $\frac{C \text{ intensity}}{(C+T) \text{ intensity}}$	47.141	42.389	For SALIVA 7.527
KLF14	REVERSE 	62.870	48.479	+ 47.078 × ELOVL2 + 42.389 × FHL2 + 48.479 × KLF14 + (-28.159) × C1orf132 + 41.281 × TRIM59
C1orf132	%methyl = $\frac{G \text{ intensity}}{(G+A) \text{ intensity}}$	-29.075	-28.159	For BUCCAL CELLS -9.277
TRIM59		23.671	41.281	+ 32.589 × ELOVL2 + 49.115 × FHL2 + 41.814 × KLF14 + (-9.228) × C1orf132 + 59.484 × TRIM59

* Methylation value will be in the range of 0 to 1.