PCR: Fluoro

Amplifluor: Yes

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Fluoro-1	GAAGGTGACCAAGTTCATGCTTTGGGAGTGTCTGTGGACTCT
Fluoro-2	GAAGGTCGGAGTCAACGGATTCTGGGAGTGTCTGTGGACTG
Fluoro-R	TACCTCAAGACCCCTGCAA

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	20x Primer mix concentration (µM)	Volume (µL)
DNA Sample				1.0
10x Buffer	10x Reaction Mix S Plus			0.5
dNTPs		2.5 mM		0.4
20x amplifluor SNP FAM Primer				
20x amplifluor SNP JOE Primer				
Primer: **Fluoro-1**			1.0	
Primer: **Fluoro-2**			0.5	
Primer: **Fluoro-R**			7.5	
Amplifluor primer volume				1.0
Water				1.55
Taq Polymerase	Platinum Taq (5U/l)			0.05

Comments on protocol: DNA Sample (cleaned ear punch in dH20-see cleanup protocol).

Reactions to be done at room temperature with reagents added in order.

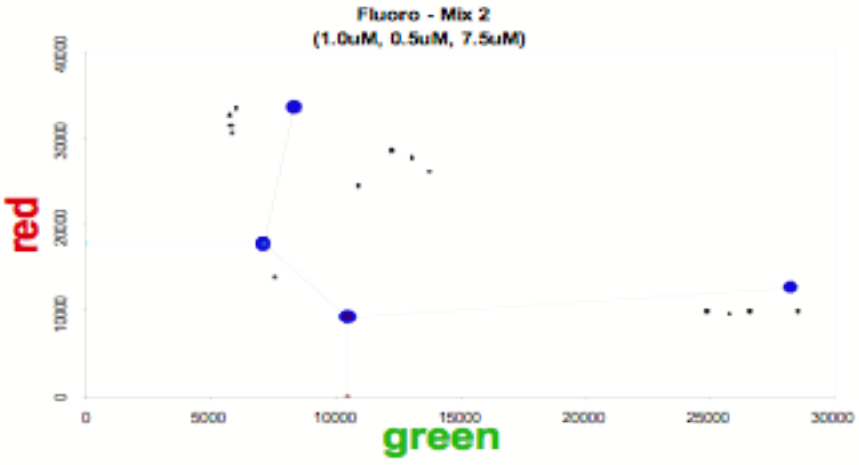
Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **Yes**		
Initiation/Melting	95.0	240
Denaturation	95.0	10
Annealing	60.0	20
Elongation	72.0	40
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	180
Finish (i.e. 4°C, indefinite)	25.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	
Volt:	
Estimated running time (min):	

Image


Fluoro - Mix 2
(1.0uM, 0.5uM, 7.5uM)
red
green
0
5000
10000
15000
20000
25000
30000
0
10000
20000
30000
40000