

PCR: Unmodulated (amplifluor)

Amplifluor: Yes

Primers

Nucleotide sequence (5' --> 3')

Primer	Sequence
Unmod-1	GAAGGTCGGAGTCAACGGATTCTCCTTCCGGTCATGTT CCT
Unmod-2	GAAGGTGACCAAGTTCATGCTTCTCCTTCCGGTCATGT TCCA
Unmod-R	CAGACAAGGCCATCTTGGACAT

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: Unmod-1			0.5	
Primer: Unmod-2			0.5	
Primer: Unmod-R			7.5	
Amplifluor primer volume				1.0
Water				1.55
Taq Polymerase	Platinum Taq (5U/μl)			0.05

Comments on protocol: DNA Sample (cleaned ear punch in dH2O-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	94.0	240
Denaturation	94.0	10
Annealing	60.0	20
Elongation	72.0	40
Number of cycles (repeat denature, anneal & elongate): 35		
Strand completion (i.e. 72°C, 10 min)	72.0	180
Finish (i.e. 4°C, indefinite)	10.0	Hold

Electrophoresis

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

Image

