

PCR: Blobby (amplifluor)

Amplifluor: Yes

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Blobby-1	GAAGGTCGGAGTCAACGGATTCTGAGGTATATGCTGAACTCTTCTT
Blobby-2	GAAGGTGACCAAGTTCATGCTACTGAGGTATAGCTGAACTCTTCTA
Blobby-R	CAATGCAAACCTCCAGACGTA

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: Blobby-1			0.5	
Primer: Blobby-2			0.5	
Primer: Blobby-R			7.5	
Amplifluor primer volume				1.0
Water				1.58
Taq Polymerase	Platinum Taq (5U/l)			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	15
Annealing	55.0	20
Elongation	72.0	30
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)	10.0	Hold

Electrophoresis

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

Image

