

PCR: Lochy (amplifluor)

Amplifluor: Yes

Primers

Nucleotide sequence (5' --> 3')

Primer	Sequence
Lochy-1	GAAGGTGACCAAGTTCATGCTTTGGGTTCCCTGAAGCCCTGATCT
Lochy-2	GAAGGTCGGAGTCAACGGATTTGGGTTCCCTGAAGCCC TGATCAA
Lochy-R	ATGACTGTTCTCTTTGTGTGTT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	20x Primer mix concentration (μM)	Volume (μL)
DNA Sample				2.0
10x Buffer	10x Reaction Mix S Plus			1.0
dNTPs		2.5 mM		0.8
20x amplifluor SNP FAM Primer				
20x amplifluor SNP JOE Primer				
Primer: Lochy-1			0.5	
Primer: Lochy-2			0.5	
Primer: Lochy-R			7.5	
Amplifluor primer volume				0.5
Water				4.6
Taq Polymerase	Platinum Taq (5U/μl)			0.1

Comments on protocol: DNA Sample (cleaned ear punch in dH2O-see cleanup protocol).

The reaction needs to be done at room temperature with reagents added in order (dh2O, dNTPs, SNP FAM, SNP JOE, Lochy, 10x reaction mix, Taq, DNA sample).

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: Yes		
Initiation/Melting	94.0	240
Denaturation	94.0	10
Annealing	55.0	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

