

PCR: Perforin(prf-/-)

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
JT272	CCACTCCACCTTGACTTCAAAAAGGCG
JT98	TGGGCAGCAGTCCTGGTTGGTGACCTT
01Neo2A	CGGAGAACCTGCGTGCAATC

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer	2x Mix Qiagen multiplex PCR Kit		5.0
dNTPs			
Primer: JT272		10.0 µM	
Primer: JT98		10.0 µM	
Primer: 01Neo2A		10.0 µM	
Water			3.0
Taq Polymerase			

Comments on protocol: DNA Sample (ear punch)

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: Yes		
Initiation/Melting	95.0	900
Denaturation	95.0	20
Annealing	60.0	30
Elongation	72.0	60
Number of cycles (repeat denature, anneal & elgonate): 35		
Strand completion (i.e. 72°C, 10 min)	72.0	240
Finish (i.e. 4°C, indefinite)	20.0	Hold

Electrophoresis

Running buffer:	1.5% sodium borate
% Agarose:	2.0
Volt:	350.0
Estimated running time (min):	20.0

Bands

Number	Band (bp)	Genotype
1.	975	Perforin KO/KO
2.	600	wt/wt