PCR: CD28 Hotstart

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
CD28EX2	CTGCTTGTGGTAGATAGCAACGA
CD28IN2	CCTGAGTCCTGATCTGTCAGACT
HSVTK	ATTCGGCAATGACAAGACGTTGG

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: **CD28EX2**		6.0 µM	
Primer: **CD28IN2**		6.0 µM	
Primer: **HSVTK**		6.0 µM	
Water			1.4
Taq Polymerase			
Reagent: Q Solution			0.2

Comments on protocol: DNA Sample (ear punch)

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	95.0	900
Denaturation	95.0	30
Annealing	64.0	30
Elongation	72.0	60
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:	Ethidium Bromide: 2% Agarose. Gel Red: 1.5% Agarose
% Agarose:	
Volt:	350.0
Estimated running time (min):	30.0

Bands

Number	Band (bp)	Genotype
1.	600	WT
2.	750	MUTANT