PCR: ICOS/NEO - Hotstart

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
ICOS F	CCAAAATGAGCTGCTGTCAA
ICOS R	ACTTCTGCAGCCTGTCCATT
Neo F	TGAATGAACTGCAGGACGAGG
Neo R	AAGGTGAGATGACAGGAGATC

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: **ICOS F**		10.0 µM	
Primer: **ICOS R**		10.0 µM	
Primer: **Neo F**		10.0 µM	
Primer: **Neo R**		10.0 µM	
Water			3.2
Taq Polymerase			

Comments on protocol: Note that this is a hotstart PCR reaction.

Also note difference in dNTP concentration.

Results: if no ICOS band and Neo present then genotype is hom KO.

If no Neo band then genotype is WT. If both ICOS and Neo bands appear then genotype is het.

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **Yes**		
Initiation/Melting	95.0	900
Denaturation	95.0	20
Annealing	58.0	20
Elongation	72.0	30
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	60
Finish (i.e. 4°C, indefinite)	10.0	Hold

Electrophoresis

Running buffer:	Ethidium Bromide: 400V. Gel Red: 300V.
% Agarose:	2.0
Volt:	
Estimated running time (min):	10.0

Bands

Number	Band (bp)	Genotype
1.	242	ICOS
2.	180	NEO