PCR: ICOS KO and Neo

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
ICOS Fwd	GGTGTCCATCAAGAATCCAATG
ICOS Rev	CCTTCTTAACTACTATTGGTACCTTG
Neo Fwd	GATTGAACAAGATGGATTGC
Neo Rev	CAGAAGAACTCGTCAAGAAG

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			4.0
10x Buffer		20.0 mM MgCl2	5.0
dNTPs		2.0 mM	5.0
Primer: **ICOS Fwd**		10.0 µM	
Primer: **ICOS Rev**		10.0 µM	
Primer: **Neo Fwd**		10.0 µM	
Primer: **Neo Rev**		10.0 µM	
Water			31.5
Taq Polymerase			0.5

Comments on protocol: DNA Sample (gen DNA 1:20)

May need optimizing as original used different polymerase

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 animal is het.

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	98.0	30
Denaturation	98.0	10
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)		

Electrophoresis

Running buffer:	
% Agarose:	1.5
Volt:	
Estimated running time (min):	

Bands

Number	Band (bp)	Genotype
1.	1050	ICOS
2.	800	NEO