PCR: ICOS NEO

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
pgkNEO for	GATTGAACAAGATGGATTGC
pgkNEO rev	CAGAAGAACTCGTCAAGAAG

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		20.0 mM MgCl2	5.0
dNTPs		2.0 mM	5.0
Primer: **pgkNEO for**		10.0 µM	
Primer: **pgkNEO rev**		10.0 µM	
Water			28.5
Taq Polymerase			0.5
Reagent: 25mM MgCl2			2.0

Comments on protocol: 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	30
Annealing	58.0	30
Elongation	72.0	60
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:	400.0
Estimated running time (min):	10.0

Bands

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