GENOTYPING BY PCR PROTOCOL FORM

Mouse Biology Program: UC DAVIS

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Protocol Name: **CR1074 Il18r1 exdel**

Protocol: ***GoTaq® Long PCR Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	5.7
GoTaq® Long PCR Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.12
Primer 2. (stock concentration is 20µM)	0.12
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		68	2:00	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**25x**
7. Elongation		68	2:00 (↑20sec/cycle)	
8. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. CR-il18r1-xdF	GACTGAGATGAACATGTGTACCCAGG
2. CR-il18r1-xdR	CAGGTGCCATATCATCTGAAGTCC

Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2	1089	wildtype
1 & 2	259	mutant

Allele Description: Exon 3 ENSMUSE00000309702 and flanking splicing regions were constitutively deleted from the Il18r1 gene ENSMUST00000108044.3 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.