

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR1171 Agbl4 exdel

Protocol: GoTaq® Long PCR Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® Long PCR Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.6
Primer 2. (stock concentration is 20µM) wtR	1.2
Primer 3. (stock concentration is 20µM) mutR	0.3
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 (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Agbl4-comF	TGACAAAACAGCATGGGCAAGG	Estimated Running Time: 90 min.	
2. CR_Agbl4-wtR	GAAGCACTCGGTCAAAGGCTCG	Primer Combination	Band (bp)
3. CR_Agbl4-mutR	CAATCAGCATACCAGCAGTCAACC	1 & 2, 1 & 3	593, 1441
		1 & 3	481
			Genotype
			wildtype
			mutant

Allele Description: Exon 5 [ENSMUSE00000631513](#) and flanking splicing regions were constitutively deleted from the Agbl4 gene [ENSMUST00000106592.7](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

