GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR1206 Atxn7l2 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 (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_Atxn7l2-comF	GAGGAAGGAGCTTGAAGCAGCAG
2. CR_Atxn7l2-wtR	CTCCAGACTGACAATGTCCTAAGAGG
3. CR_Atxn7l2-mutR	TGGAAGACTGGAAGCCCAAAGC

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	504,1903	wildtype
1 & 3	356	mutant

Allele Description: Exon 4 through 6 (ENSMUSE00000662145, ENSMUSE00001281910, ENSMUSE00000402522) and flanking splicing regions were constitutively deleted from the Atxn7l2 gene ENSMUST00000102633.8 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.