GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR10455 Amtn iDex**

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	0.5
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_Amtn_comF	TTCCCCAGTCTCTGATTCTGTCTCC
2. CR_Amtn_wtR*	GCACATCCATTTGTAGATTCTTTCCC
3. CR_Amtn_mutR	GCTCACGTTCAGTGAATAGGTGTGC

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	726,1207	wildtype
1 & 3	231	mutant

Allele Description: Exon 3, 4(ENSMUSE00000187697, ENSMUSE00000187693) and flanking splicing regions were constitutively deleted from the Amtn gene ENSMUSG00000029282 using CRISPR Cas9 gene editing technology in mouse zygotes. Note: First 13 coding nucleotides of Exon 3 and SA were not deleted making it an Early stop KO in intron 4. 14 residues then STOP. No SD from either exon is present. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified**)**