GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: **CR11423 Ankrd46 EXDEL**

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_Ankrd46_comF	GCACCTCAGATAGAGTGTTCTGC
2. CR_Ankrd46_wtR*	CTCACGCTTGGAATACATTTCCT
3. CR_Ankrd46_mutR	GCACTTGTGTATGATAGACCCCC

Electrophoresis Protocol:

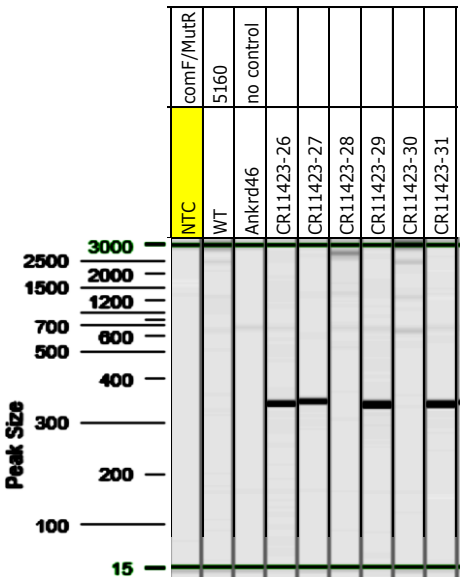
Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	412, 3125	wildtype
1 & 3	335	mutant

Allele Description: Exon 3-4(ENSMUSE00000408435, ENSMUSE00000382796) and flanking splicing regions were constitutively deleted from the Ankrd46 Gene ENSMUSG00000048307 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 2790bp deletion from Chr15: 36,483,815 – 36,486,604 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)



PCR protocol developed by MMRRC at University of California, Davis