GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR1230 Crisp2 Exdel

Protocol:

Reagent/Constituent	Volume (µL)
Water	9.975
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
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
Taq Polymerase 5Units/µL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25.000 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	**1**
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1ºC/cycle)	0:30	**40x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. CR_Crisp2-comF	GCCATATTGCACCAGTGTATTGGG
2. CR_Crisp2-wtR	CTGCCAGTAGGGTTAACTGATCTCCTC
3. CR_Crisp2-mutR	CATTGTCATAATGGTAGGAAGTGTGG

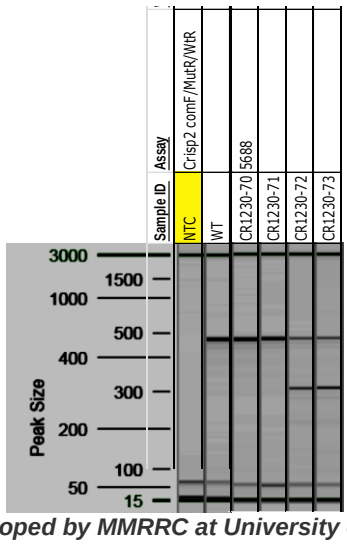
Electrophoresis Protocol:

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

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2, 1 & 3	487,1942*	wildtype
1 & 3	312	mutant

Allele Description: Exon 5, 6(ENSMUSE00000136438 , ENSMUSE00000136444)and flanking splicing regions were constitutively deleted from the Crisp2 gene ENSMUST00000024724.13 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.



***May not see larger wildtype band