GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: C57BL/6NCrl-Bcorem1(IMPC)Mbp/Mmucd Stock #: 67172

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_Bcor_wtF	TGCGGCGGAAACACGGT
2. CR_Bcor_kiF	GTCGGCCTCTGCAACACG
3. CR_Bcor_IVQC-R	CTGTGGCGTGGTCCCTTTCACT
	Do two separate PCRs for the two primer combinations.

Electrophoresis Protocol:

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

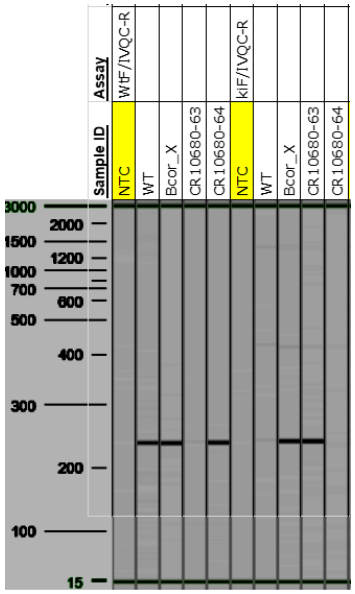
Estimated Running **90** min.

Primer Combination	Band (bp)	Genotype
1 & 3	240	wt
2 & 3	243	mutant

Allele Description: Six base pairs "GGCGGA" of the 5'UTR were constitutively deleted using optimized CRISPR Cas9 KI technology utilizing Ribonucleoprotein (RNP) in the presence of a synthetic single strand DNA repair template harboring the desired KI. Zygotes were electroporated and subsequent progeny were screened for the presence of the correctly targeted allele via homology directed repair (HDR). The KI contains a 6 bp deletion, which ablates positions 1-16 of protospacer and will protect the HDR derived locus from re-cutting by Cas9. Key progeny were sequence confirmed.

cgactccctcctccccacttcgccccctttcccggcccgcatgcggtcggcctctgcaacacggtccaactccttgaaagtaaacttgccgcgagctgccag

ggcggaa>a



WT = #64; Mutant (6 bp deletion) = #63