GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11656 Efcab10 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_Efcab10_comF	GTCAGACACAAACATTCTGCCTCG
2. CR_Efcab10_wtR2*	GAAGGGTAGTGGTGCTCTATATGC
3. CR_Efcab10_mutR	CTCTTTAACCCTATCATCACTCCTCTCC

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	1046, 1476	wildtype
1 & 3	1035	mutant

Allele Description: Exon 3 ENSMUSE00000106612 and flanking splicing regions were constitutively deleted from the Efcab10 Gene ENSMUSG00000020562 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 441bp deletion from Chr 12: 33448449 - 33448889 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.

Note: 6bp intronic deletion (Chr 12: 33447827 - 33447832) upstream of Exon 3 has also been detected in these mice.

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

*Run in Simplex