

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10764 Efcab5 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 (°C)	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 (↓1°C/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')	Agarose: 1.5% V: 90
1. CR Efcab5 comF	ACGTGTGAAAGGACAAGTAAGGAGC	Estimated Running Time: 90 min.
2. CR Efcab5 wtR	AGCTGGTCCAGTTGAGTTCTGATGG	Primer Combination Band (bp) Genotype
3. CR Efcab5 mutR	CCATGATGCTGGCATTCAAGTG	1 & 2, 1 & 3 693, 1392 wildtype
		1 & 3 357 mutant

Electrophoresis Protocol:

Allele Description: Exon 4 ENSMUSE00000342832 and flanking splicing regions were constitutively deleted from the Efcab5 gene [ENSMUST00000108400.7](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

