

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10738 Eef1d 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. Eef1d-cond-IV5F	AGCTGGAGGTGAGTTTGGAGGTG	Estimated Running Time: 90 min.
2. Eef1d-cond-IV5R	CACTCCTGCTGCTGGTGTGG	Primer Combination Band (bp) Genotype
3. Eef1d-cond-IV3R	CTCACTCCAGCCAAGGATTTCTGG	1 & 2, 1 & 3 531, 3450 wildtype
		1 & 3 521 mutant

Electrophoresis Protocol:

Allele Description: Exons 2 ENSMUSE00000898037 and 3 ENSMUSE00001250574 and flanking splicing regions were constitutively deleted from the Eef1d gene [ENSMUST00000109975.9](#) 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)

