GENOTYPING PROTOCOL

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Protocol Name: CR1526 Erlec1 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_ Erlec1-comF	cagccagtagacgcttaaaatggc
2. CR_ Erlec1-wtR	CAGAAGATTCTTAGCCAACATATTCCC
3. CR_ Erlec1-mutR	CCTCATACGTGGTGTTTATACACCC

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2,1 & 3	700, 1121	wildtype
1 & 3	386	mutant

Allele Description: Exons 4-5 (ENSMUSE00000254768, ENSMUSE00000254763) and flanking splicing regions were constitutively deleted from the Erlec1 gene (ENSMUSG00000020311) using CRISPR Cas9 editing technology in C57BL6/NCrl zygotes and subsequently backcrossed in C57BL6/NCrl.