GENOTYPING PROTOCOL

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Protocol Name: **CR1465 Sh3rf2 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_ Sh3rf2-comF	gtgtgagggagctaagttctgagtcc
2. CR Sh3rf2-wtR*	AAGAGACGGGTTGGCTCTGAGC
3. CR Sh3rf2-mutR	CAGGTTGGACATGCCTAGTAGTCC

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	502, 836	wildtype
1 & 3	~430	mutant

Allele Description: Exon 5 (ENSMUSE00000484601) and flanking splicing regions were constitutively deleted from the Sh3rf2 gene ENSMUSG00000057719 using CRISPR Cas9 editing technology in C57BL6/NCrl zygotes and subsequently backcrossed in C57BL6/NCrl.

*wtR not used below (ePCR verified)