GENOTYPING PROTOCOL

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Protocol Name: CR1509 Ube2f 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_ Ube2f-comF	cctaagccctgagccatctagc
2. CR Ube2f-wtR*	CCACCATGTTGTAGGCATCGG
3. CR Ube2f-mutR	ATCATTGGTCCCTGGAATTTCGG

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2,1 & 3	538, 839	wildtype
1 & 3	400	mutant

Allele Description: Exon 6 (ENSMUSE00000903028) and flanking splicing regions were constitutively deleted from the Ube2f gene ENSMUSG00000034343 using CRISPR Cas9 editing technology in C57BL6/NCrl zygotes and subsequently backcrossed in C57BL6/NCrl.

*wtR not used below (ePCR verified)