GENOTYPING PROTOCOL

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Protocol Name: **CR1520 Dusp12 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_ Dusp12-comF	caggaggtagtgataagaagtgcgg
2. CR_ Dusp12-wtR*	CTTTTTCAAAGGTAAGCTGGTCGG
3. CR_ Dusp12 -mutR	AGCGAAGAGTTCCTGAGGTAAATTCC

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2,1 & 3	620, 1377	wildtype
1 & 3	450	mutant

Allele Description: Exons 2-3 (ENSMUSE00001301586, ENSMUSE00001312249) and flanking splicing regions were constitutively deleted from the Dusp12 gene ENSMUSG00000026659 using CRISPR Cas9 editing technology in C57BL6/NCrl zygotes and subsequently backcrossed in C57BL6/NCrl.

wtR not included in reaction image below.