GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR1516 Dcaf6 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_ Dcaf6-comF	aggcagttttcagagtttgaggacag
2. CR Dcaf6-wtR	CACCCACAAGGCGCCTTTCC
3. CR Dcaf6-mutR	GCACTTGGTATAACAGCACACCGAG

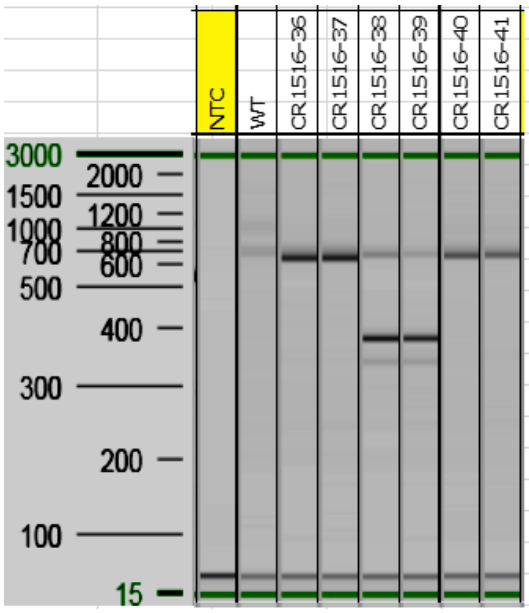
Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	718, 1710	wildtype
1 & 3	416	mutant

Allele Description: Exon 7 (ENSMUSE00000229487) and flanking splicing regions were constitutively deleted from the Dcaf6 gene ENSMUSG00000026571 using CRISPR Cas9 editing technology in C57BL6/NCrl zygotes and subsequently backcrossed in C57BL6/NCrl.



PCR protocol developed by MMRRC at University of California, Davis