

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: **CR1393 1700025G04Rik EXDEL**

Protocol: **GoTaq® G2 Colorless Master Mix(Promega)**

Reagent/Constituent	Volume (μL)
Water	3.9
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20μM) comF	0.6
Primer 2. (stock concentration is 20μM) wtR	1.2
Primer 3. (stock concentration is 20μM) mutR	0.3
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 (°C)	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 (↓1°C/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')	Agarose: 1.5%	V: 90
1. CR_1700025G04Rik-comF	cgtccagctgaagtatgctgctg	Estimated Running Time: 90 min.	
2. CR_1700025G04Rik-wtR	CTTCAGCTCTACCAGGTTTCTTGG	Primer Combination	Band (bp)
3. CR_1700025G04Rik-mutR	GAACGTGAAGAATGGGCTCTTTGG	1 & 2, 1 & 3	514,957
		1 & 3	325
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 3 [ENSMUSE00000258187](#) and flanking splicing regions were constitutively deleted from the 1700025G04Rik gene [ENSMUST00000044581.13](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

