

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11256 Grk3 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 (°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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90
1. CR_Grk3_comF	ACTGAGAAGATGCCTCCAGAAGGTC	Estimated Running Time: 90 min.
2. CR_Grk3_wtR**	CGACAGTTCCAGATGAAGGAGGA	Primer Combination Band (bp) Genotype
3. CR_Grk3_mutR	CCTCTCATGTGATTCTTGGAATGC	1 & 2, 1 & 3 1124, 1665* wildtype
		1 & 3 719 mutant

Allele Description: Exon 2 (ENSMUSE00001001538) and flanking splicing regions were constitutively deleted from the Grk3 gene ENSMUSG00000042249 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*May not see larger wildtype band

**wtR primer untested (ePCR verified)

