

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11745 2900092C05Rik 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? <input type="checkbox"/>	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_2900092C05Rik_comF	CCACACAACACTACTAACAGCAACC	Estimated Running Time: 90 min.	
2. CR_2900092C05Rik_wtR*	GTTAGAGACATCCCACTTAGAGATGA	Primer Combination	Band (bp)
3. CR_2900092C05Rik_mutR	CCTTCTCTGGTATGACCAACAATCC	1 & 2, 1 & 3	385, 2231
		1 & 3	1085
			wildtype
			mutant

Allele Description: Exon 4 ENSMUSE00000198271 and flanking splicing regions were constitutively deleted from the 2900092C05Rik Gene ENSMUSG00000030385 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1146bp deletion from Chr7: 12284116 -12285261 GRCm39 was sequence verified. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)

