

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: CR11500 Rps9 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:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Rps9_comF	GCCAGAAGCTGGGTTTGTCTG	Estimated Running Time: 90 min.	
2. CR_Rps9_wtR*	GCTCATACACACCTTCAAACAGAC	Primer Combination	Band (bp)
3. CR_Rps9_mutR	CCAGTCACTTCAGACAGGTGCA	1 & 2, 1 & 3	420, 1972
		1 & 3	404
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 3-4 (ENSMUSE00000134589, ENSMUSE00000134587) and flanking splicing regions were constitutively deleted from the Rps9 Gene ENSMUSG00000006333 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1568bp deletion from Chr 7: 3707543 - 3709110 GRCm39 was screened by PCR analysis. 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)

