

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11684 Srrd EXDEL Stock # 75999

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_Srrd_comF	CTTTGTCCCTAAACATTGCCTCTGG	Estimated Running Time: 90 min.	
2. CR_Srrd_wtR*	TCCCTTCTCTTTTGTGCCATCTTAGC	Primer Combination	Band (bp)
3. CR_Srrd_mutR	GTAGACGCTGCCTTTCATTTAACAACC	1 & 2, 1 & 3	458, 1053
		1 & 3	385
			Genotype
			wildtype
			mutant

Allele Description: Exon 2 ENSMUSE00001278226 and flanking splicing regions were constitutively deleted from the Srrd Gene ENSMUSG00000029346 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 668bp deletion from Chr 5:112488669 - 112489336 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)

