

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10961 Chsy1 EXDEL Stock #: 067396

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_Chys1_comF	GGCATAGCATAGAAGACGACTCTGC	Estimated Running Time: 90 min.
2. CR_Chys1_wtR	CGTACTTGTCAGGTAGTGATCGTGC	Primer Combination Band (bp) Genotype
3. CR_Chys1_mutR	AGGTCATCTTGACCAGACGAAAGTG	1 & 2, 1 & 3 955,1695 wildtype
		1 & 3 480 mutant

Electrophoresis Protocol:

Allele Description: Exon 2 [ENSMUSE00000263913](#) and flanking splicing regions were constitutively deleted from the Chsy1 gene [ENSMUSG00000032640.10](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

