

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10953 Top2b EXDEL Stock #: 067441

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_Top2b_comF	TTGTTGTCTTCCTGCTGTGTTGGAC	Estimated Running Time: 90 min.
2. CR_Top2b_wtR*	GCAAGATGTAAGTCAGTGTCAACTGGA	Primer Combination Band (bp) Genotype
3. CR_Top2b_mutR	GCAGGAGCTACAGAAAAGGCTCG	1 & 2, 1 & 3 942,1673 wildtype
		1 & 3 630 mutant

Electrophoresis Protocol:

Allele Description: Exon 5 [ENSMUSE00000120843](#) and flanking splicing regions were constitutively deleted from the Top2b gene [ENSMUSG00000017485.11](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

