

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1159 Mark4 exdel

Protocol: GoTaq® Long PCR Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® Long PCR Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.6
Primer 2. (stock concentration is 20µM) wtR	1.2
Primer 3. (stock concentration is 20µM) mutR	0.3
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
<b>TOTAL VOLUME OF REACTION:</b>	<b>15.00 µL</b>

### Comments on protocol:

- Protocol may work with other DNA extraction methods.

### Strategy:

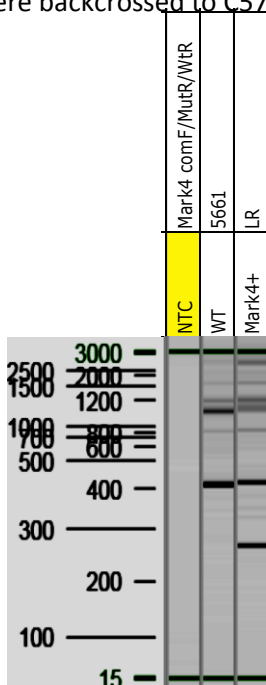
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	2:00	<b>1x</b>
2. Denaturation	94	0:10	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	65 (↓1°C/cycle)	0:30	<b>10x</b>
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>	55	0:30	<b>25x</b>
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_Mark4-comF	GCATGTGAACCTGTGTGAAAGGAG	Estimated Running Time: 90 min.	
2. CR_Mark4-wtR	TCAATGATCTTAATAGCGACCTGTGG	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Mark4-mutR	CCCTGATACTGATCCCAGCTCCAG	1 & 2, 1 & 3	415,941
		1 & 3	272
			wildtype
			mutant

**Allele Description:** Exon 3, 4([ENSMUSE00000198453](#) ,[ENSMUSE00000198460](#)) and flanking splicing regions were constitutively deleted from the Mark4 gene [ENSMUST00000085715.6](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.



PCR protocol developed by MMRRC at University of California, Davis