

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

Protocol Name: CR1154 Cpne7 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_Cpne7-comF	AGAAAAGCAAGCGTGAGTCTTGTGC	Estimated Running Time: 90 min.	
2. CR_Cpne7-wtR	ATGGCCGTGTCATCTTCTTCTGG	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Cpne7-mutR	CCCTGAAGGGTCTGTACCCAAGG	1 & 2, 1 & 3	620,5458
		1 & 3	394
			wildtype
			mutant

**Allele Description:** Exon 3 through 10 ([ENSMUSE00001282544](#), [ENSMUSE00001205792](#), [ENSMUSE00001285604](#), [ENSMUSE00001301756](#), [ENSMUSE00000293662](#), [ENSMUSE00000293084](#), [ENSMUSE00001249165](#), [ENSMUSE00001295928](#)) and flanking splicing regions were constitutively deleted from the Cpne7 gene [ENSMUST00000037900.8](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

