

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1724 Nnat Gendel

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
<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-Nnat-comF	GAATTTGTAGGCTTGGGTGTGTCG	Estimated Running Time: 90 min.	
2. CR-Nnat-wtR*	GGTACACAGGGATTGCGGCAAT	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR-Nnat-mutR	CGATCTTCATGGTAGGATCTTGTGC	1 & 2, 1 & 3	586,2183
		1 & 3	659
			wildtype
			mutant

**Allele Description:** Exon 1-3([ENSMUSE00000552733](#), [ENSMUSE00001309983](#), [ENSMUSE00000680629](#)) were constitutively deleted from the Nnat gene [ENSMUST00000109526.1](#) 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)

