

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10035 Lrrtm1 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_Lrrtm1_comF	CCGTGGCTGTGAAC TTTGTATGC	Estimated Running Time: 90 min.	
2. CR_Lrrtm1_wtR	GTGAGCTGCATTAACCCTGTGAAC T	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Lrrtm1_mutR	CACTCTCAGATT CCTGAATATGGACAGA	1 & 2, 1 & 3	509, 2109
		1 & 3	459
			wildtype
			mutant

**Allele Description:** Exon 2 [ENSMUSE00000495593](#) was constitutively deleted from the 5'UTR through the 3' UTR from Lrrtm1 gene [ENSMUSG00000060780](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

