

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6NCrl-Robo1em1(IMPC)Mbp/Mmucd Stock #: 050554

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? <input type="checkbox"/>	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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Robo1_comF	TGTGTGTGTGTGAAGATTTTCATGGTGAC	Estimated Running Time: 90 min.	
2. CR_Robo1_wtR*	GTCCCGTGAGCATACTCAGATAGGA	Primer Combination	Band (bp)
3. CR_Robo1_mutR	GGAAATACATGGAAATGGCCTAAGC	1 & 2, 1 & 3	336,975
		1 & 3	392
			Genotype
			wildtype
			mutant

Allele Description: Exon 3 [ENSMUSE00000318078](#) and flanking splicing regions were constitutively deleted from the Robo1 gene [ENSMUSG00000022883](#) 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)

