GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: B6.129-Gp2tm1Awl/Mmucd/Mmucd **MMRRC:** 036119-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
Promega GoTaq® Long PCR Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

Strategy:

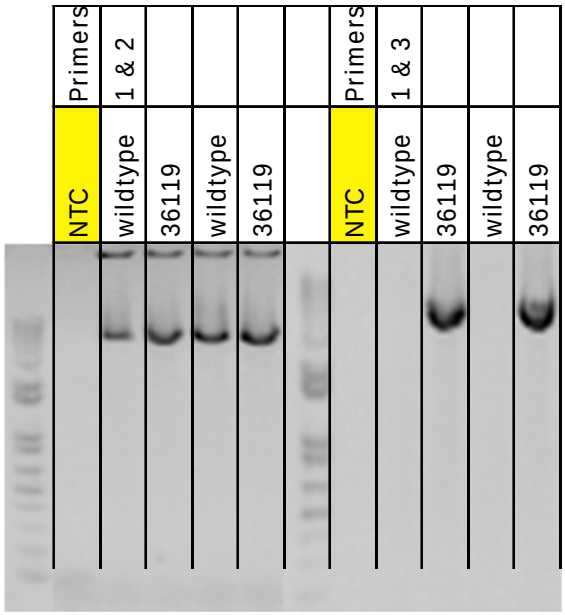
Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	
4. Elongation		68	2:00	
				Go to 2 (9x)
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	
7. Elongation		68	10:00 (+20s/cycle)	
				Go to 5 (24x)

Primers:

Name	Nucleotide Sequence (5' - 3')
1. LR-GP2.KO-comF	GAGATGTAGAAGCAATGGCAGGTGTCG
2. LR-GP2.KO-koR	AAGCCGGTCTTGTCAATCAGGATGATCT
3. LR-GP2.KO-wtR	ATTGTAGATGGAGATACCAGTGTCAGAA

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	4500	mutant
1 & 3	5500	wildtype