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

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Protocol Name: B6J.Cg-Tg(Tmc1-Tmc1*)1Ajg/Mmucd **MMRRC:** 050609-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless 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
	15

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.
- PCR shown is form 50608 because 50609 DNA was not available.
- Primers 3 through 6 are for the background strain JAX#09146. Primers 5 & 6 are generic LacZ primers.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1x**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**30X**
7. Elongation		72	0:40	

Primers:

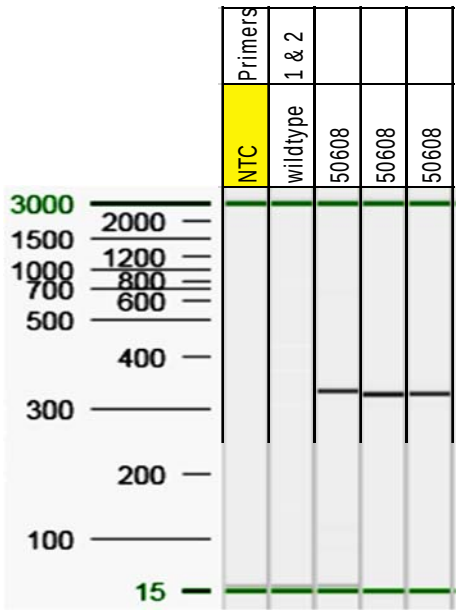
Name	Nucleotide Sequence (5' - 3')
1. 50609-F	GGCCGCTAATACGACTCACTAT
2. 50609-R	AGTAGAGTCCAGGCATGCTAAAAT
3. 09146-wtF	GACAGTTGGTGCTGGGATCT
4. 09146-wtR	AAGTACGAGGCCACTGAGGA
5. 09146-LaczF	CGTGGCCTGATTCATTCC
6. 09146-LaczR	ATCCTCTGCATGGTCAGGTC

Electrophoresis Protocol:

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

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

Primer Combination	**Band** (bp)	Genotype
1 & 2	342	mutant
3 & 4	215	wildtype
5 & 6	315	mutant



PCR protocol developed by MMRRC at University of California, Davis