GENOTYPING BY PCR PROTOCOL

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Protocol Name: C57BL/6NCrl-Plaat3em1(IMPC)Tcp/TcpMmucd **MMRRC:** 066631-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.

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	**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)	

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 66631-koF	TTGATAAGGACTCAAGGACCACAG
2. 66631-koR	AGAGACAGGAAGCTTACATTAGGC
3. 66631-wtF	CTTGATAAGGACTCAAGGACCACAG
4. 66631-wtR	CAGCAGTTCTTTCTTCACTATGGC

Electrophoresis Protocol:

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

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

Primer Combination	**Band** (bp)	Genotype
1 & 2	319, 582	Mutant, wildtype
3 & 4	258	wildtype

Primers 1 and 2 may be sufficient for genotyping