GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: C57BL/6N-*Gt(ROSA)26Sor*$^{tm1(FLP1)Dym}$/Mmcd, (FLPeR) **MMRRC #** 034413-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	10.775
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Primer 3 (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25.000 µL**

Comments on protocol:

- *Protocol may work with other DNA extraction methods.*
- *Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.*
- *Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.*

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	**1**
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**40x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 34413-comF	AAAGTCGCTCTGAGTTGTTAT
2. 34413-mutR	GCGAAGAGTTTGTCCCTCAACC
3. 34413-wtR	GGAGCGGGAGAAATGGATATG

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90

Estimated Running Time: 90 **min.**

Primer Combination	Band	Genotype
1 and 2	300 bp	Mutant
1 and 3	603 bp	Wild-type

NTC= 34413comF/mutR/wtR