GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **B6.129S3-*Slc6a1*^{tm1Lst}/Mmcd** **MMRRC #** **000426-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	10.775
10x Buffer without $MgCl_2$ (AB)	2.5
$MgCl_2$ (AB) (stock concentration is 25mM)	1.7
Betaine (Sigma) (stock concentration is 5M) *Optional*	6.5
dNTPs (Invitrogen) (stock concentration is 10mM)	0.5
DMSO (Sigma)	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 (AB Amplitaq)	0.2
DNA (50-200 ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65^{o} C decreasing in temperature by 1.0 ^{o} C; next 30 cycles anneal at 55^{o} 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 (^{o}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 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: mGAT1 17830-799	GCTAAGGGGCCTCTACGGAAGCCTCCAGAGGC
2: mGAT1 17399-430	GACATTTGGCTTACTAGTGAGGAAACAAGAGC
3: GFP37 995-64	CCATCTAATTCAACAAGAATTGGGACAACTCC

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90 **Estimated Running Time:** 90 **min.**

Primer Combination	Expected Bands	Genotype
1 and 2	431 bp	WT +/+
2 and 3	335 bp	KO -/-