GENOTYPING PROTOCOL

UC Davis Mouse Biology Program

Protocol Name: **B6.129X1-Slc17a7tm1Edw/Mmucd** **MMRRC: 032097**

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	5.15
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
Primer 3. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

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	
8. Finish		4	∞	n/a

Primers:

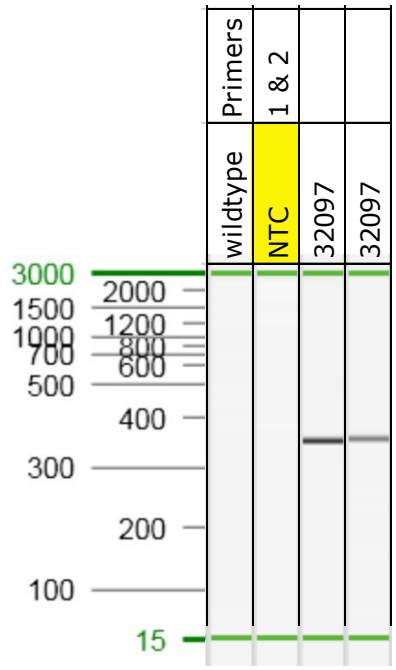
Name	Nucleotide Sequence (5' - 3')
1. 32097-koF	GACTCGGATCTGCATCTGCT
2. 32097-koR	GGGGAACTTCCTGACTAGGG
3. 32097-wtF	CCAAGCAAGGTTAAGCCTAG
4. 32097-wtR	GGTGAATTTGGAAAAGAGC

Electrophoresis Protocol:

Agarose: **1.5%** : **90**

Estimated Running **90** min.

Primers	Band (bp)	Genotype
1 & 2	~380	Mutant
3 & 4	649	Wildtype



PCR protocol developed by MMRRC at University of California, Davis

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6.129-*Slc17a7*^{tm1Edw}/Mmcd MMRRC# 032097-UCD

DNA Extraction Method: NaOH _____ Proteinase K _ _X___ Other_____

Protocol:

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.4
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	0.5
Primer **4** (stock concentration is 20 uM)	0.5
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): _____

Strategy:

Steps	Temp (^{o}C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	4:00	**1**
2. Denaturation	95	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	57	0:30	**30x**
4. Elongation	72	0:45	
5. Amplification (i.e., 72^{o}C, 10 min)	72	5:00	**1**
6. Finish (i.e., 4^{o}C, indefinite)		n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: KO5'	GACTCGGATCTGCATCTGCT
2: KO3'	GGGGAACTTCCTGACTAGGG
3: WT5'	CCAAGCAAGGTTAAGCCTAG
4: WT3'	GGTGAATTTGGAAAAGAGC

Electrophoresis Protocol:

% Agarose: 1 **V : 100**

Estimated Running Time (min): 30

Primer combination	Band (kB)	Genotype
(i.e. 1&2)	0.35	KO allele
(i.e. 3&4)	0.65	WT allele
(i.e. 1&2&3)		