

GENOTYPING PROTOCOL

UC Davis Mouse Biology Program

Protocol Name: C57BL/6J-Grin2bem1Zuks/Mmucd MMRRRC: 050686

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

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 OF REACTION:	15.00 µL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	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 (↓ 1°C/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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	:	90
1. 50686-F	TCGGCCAAATCTAGGAGGGA	Estimated Running	90	min.
2. 50686-R	ATCGGCCCTTGCTTTCAGG	Primers	Band (bp)	Genotype
		1 & 2	706	Wildtype/mutant

sequence with 50686-F	
AGCGAGATgCtGTCAGTGG	mutant
AGCGAGATTCGGTCAGTGG	wildtype

	band sizes	
Alternative	mutant	wildtype
Digest with either HinfI or TfiI	598	386
	108	212
		108

