GENOTYPING PROTOCOL

UC Davis Mouse Biology Program

Protocol Name: B6J;129S7-Gt(ROSA)26Sortm4Rray/Mmucd **MMRRC: 043519**

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:

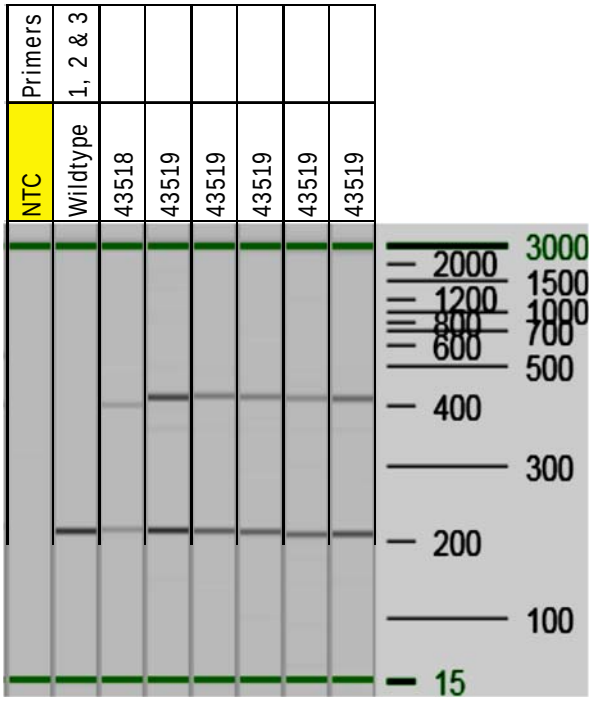
Name	Nucleotide Sequence (5' - 3')
1. 43519-comF	GCACTTGCTCTCCCAAAGTC
2. 43519-mutR	GGGCGTACTTGGCATATGAT
3. 43519-wtR	CTTTAAGCCTGCCCAGAAGA

Electrophoresis Protocol:

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

Estimated Running **90** min.

Primers	Band (bp)	Genotype
1 & 2	493	Mutant
1 & 3	252	Wildtype



PCR protocol developed by MMRRC at University of California, Davis