

GENOTYPING BY PCR PROTOCOL

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530-754-MMRRC

Protocol Name: C57BL/6N-Chrnb2tm1.1Geno/Mmucd **MMRRC:** 066430-UCD

Protocol:

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	
15	

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.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	68 (+20s/cycle)	2:00	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 66430_B2_GT_F1	CAGGCACACTATTCTTCCG	Estimated Running Time: 90 min.		
2. 66430_A4XFPM_R	CTGTTGTAGTTGTACTCCAGCTTG	Primer Combination	Band (bp)	Genotype
3. 66430_A2_GT_R1	GCAATGAAGCGTACACCGTCC	1 & 2	478	mutant
		1 & 3	202	wildtype

