

GENOTYPING BY PCR PROTOCOL

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Protocol Name: B6J.FVB-Tg(Chrna6*^{L280S})2Lst/Mmucd **MMRRC: 050701-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	4.7
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
Primer 4. (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	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	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 50701-α6-F	CTCCGTTCTGTCAAGCTTG	Estimated Running Time: 90 min.		
2. 50701-α6-R	ACGAGTGCTCTGAATTCTCTG	Primer	Band (bp)	Genotype
3. 50701-α4-F	CTGCTGCTCATCACCGAGATC	3 & 4	332	wildtype
4. 50701-α4-R	CAGATGTCACCCAAGATGCCG	1, 2, 3 & 4	~200, 332	Tg

