

GENOTYPING BY PCR PROTOCOL

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Protocol Name: C57BL/6J-Tg(Cb1n2-cre/ERT2)NIDA167Htz/Mmucd **MMRRC: 043816-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM) comF	0.45
Primer 2. (stock concentration is 20μM) wtR	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	

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')	Argar 1.5%	V: 90
1. 43816-Cb1n2-5F	AATTCCTCTAGATGTCTTGGGC	Estimated	90 min.
2. cre-100at	GCGAACCTCATCACTCGTTGCATCG	Primer	Band (bp)
3. R6kr-3end	CAGGTTGAACTGCTGATCAACAGATC	1 & 2	480
4. 43816-Cb1n2-3R	AGGCTGGAGCCAAAGTCGGCTGGC	3 & 4	490
			Genotype
			mutant
			mutant

