GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: B6J-Tg(Fcer1g-cre)NIDA168Htz/Mmucd **MMRRC:** 043798-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 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

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	

Primers:

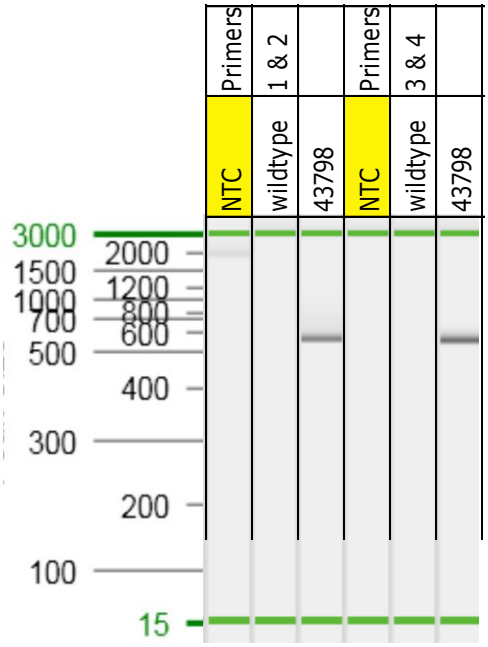
Name	Nucleotide Sequence (5' - 3')
1. 43798-Fcer1g-F	AGTCTTACTCTTTCCACTTCTC
2. 43798-CRE-100at	GCGAACCTCATCACTCGTTGCATCG
3. 43798-R6kr-3end	CAGGTTGAACTGCTGATCAACAGATC
4. 43798-Fcer1g-R	TAACGCTCCCTGAAGCCTTCCCCTTA

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	~575	mutant
3 & 4	~575	wildtype



PCR protocol developed by MMRRC at University of California, Davis