GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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NAME OF PCR: 841-CreER-IRES-GFP **MMRRC:** 037576-UCD

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
2x Buffer GoTaq Green Master Mix (Promega)	6.0
Primer Mix (1.25 µM of each primer in water)	5.0
DNA From Tail Digest (2mm tail digested with protinase K and diluted to a final volume of 200 ul)	1.0
TOTAL VOLUME OF REACTION:	**12.0 µL**

Comments on protocol:

- Lane 1 = All Star 1kb DNA Ladder
- Lane 2 = Tail DNA from positive 841CT2IG male mouse
- Lane 3 = Negative control for PCR – No DNA
- Lane 4 = All Star 100 bp Ladder

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	1:00	
3. Annealing	steps 2-3-4 cycle in sequence	58	1:00	**34x**
4. Elongation		72	1:00	
5. Amplification		72	10:00	**1**
6. Finish		16	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 841.F	AGA TTA GGG ACC AGG AGA CTG A
2. CreER.R	AGTGCTGCCTCTGACCTCAT

Electrophoresis Protocol:

Argarose: **1.0%** V: **120**

Estimated Running:Time: **30** min.

Primer Combination	**Band** (bp)	**Genotype**
1 and 2	475	KO or MT/-
1 and 2	0	WT +/+