

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

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: 204-CreER-IRES-GFP **MMRRC:** 037536-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 proteinase 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 Ladder
- Lane 2 = Tail DNA from positive 204CT2IG mouse
- Lane 3 = Tail DNA from positive 204CT2IG mouse
- Lane 4 = Tail DNA from negative (wild-Type) mouse

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.0%	V: 120
1. 692.F	GAA CTC AGG TTG CCA GAT CC	Estimated Running Time: 30 min.	
2. CreER.R	AGTGCTGCCTCTGACCTCAT	Primer Combination	Band (bp)
		1 and 2	426
		1 and 2	0
			Genotype
			KO or MT/-
			WT +/-

