

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: STOCK Tg(Ckm-GCGR)1Qwg/Mmucd MMRRRC: 036506-UCD

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

Reagent/Constituent	Volume (μL)
Water	
10x Buffer (contains / without 15mM MgCl ₂)	
dNTPs (stock concentration is mM)	
Primer 1 (stock concentration is 100μM)	
Primer 2 (stock concentration is 100μM)	
Taq Polymerase	
Additives / Other (if applicable):	
DNA sample extracted <input type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	
TOTAL VOLUME OF REACTION:	μL

Comments on protocol:

We use SIGMA REExtract-N-Amp™ Tissue PCR Kit XNAT which includes Extraction solution (24ml), Tissue Preparation solution (3ml), Neutralization solution B (24ml), and REExtract-N-Amp PCR Reaction Mix (1.2ml, including buffer, salts, dNTPs, Tag polymerase, REDTaq dye, and JumpStart Taq antibody).

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	3:00	1
2. Denaturation	94	1:00	
3. Annealing } steps 2-3-4 will cycle in sequence	55	1:00	35
4. Elongation	72	2:00	
5. Amplification	72	10:00	1
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Forward	5'-GCAAAGTGCTATGGGAGGAG
2: Reverse	5'-TCAATGGTGATGGTGATGATG

Electrophoresis Protocol:

Agarose: 1% V: 50 Estimated Running Time: 45 min

Primer Combination	Band	Genotype
Pimer1+2	369 bp	MCK ^{Gcgr}

