

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: 129-Ago2^{Gt(XE344)Byg}Mmucd MMRRC: 037123-UCD

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

Reagent/Constituent	Volume (μL)
Water	6.5
2X Buffer KAPPA2G fest genotyping Mix	10.0
Primer Mix (Triplet at 10μM each)	2.0
Lysed DNA (HOT SHOT lysis protocol see below)	1.5
TOTAL VOLUME OF REACTION:	20.000 μL

Comments on protocol:

HOT-SHOT lysis protocol (biopsies)

- Add 100 ul of HOT-SHOT lysis buffer (0.2mM EDTA pH8, 25mM NaOH) to the earpunches
- Place at +95C for 30min
- Place at +4C until use it

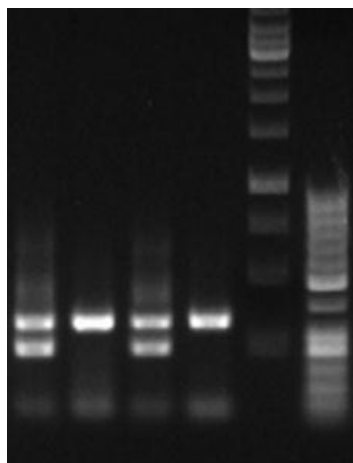
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	3:00	1
2. Denaturation	95	0:15	
3. Annealing steps 2-3-4 cycle in sequence	57	0:15	40x
4. Elongation	72	0:10	
5. Amplification			1
6. Finish	12	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 2%	5 μL
1. 2C2Up-1	GAGACCCACCCGCTCATGTC	Estimated Running:Time: 15 min.	
2. 2C2Up-2	ATATAGGCTCCTGCTGCAACTATG	Primer Combination	Band
3. 86R	CACTCCAACCTCCGCAAATC	1 & 2	336 bp
		1 & 3	250 bp
			Genotype
			WT
			MUTANT



Lanes: 1 & 3 are Heterozygotes mice
Lanes: 2 & 4 are Wild-type mice