

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: C57BL/6J-Ambn^{tm1.1Jpsi}/Mmucd MMRRC: 037503-UCD

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

Reagent/Constituent	Volume (µL)
Primer 1. (stock concentration is 20µM)	2.0
Primer 2. (stock concentration is 20µM)	1.0
Primer 3. (stock concentration is 20µM)	1.0
Invitrogen SuperMix	17.0
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	22.000 µL

Comments on protocol:

- We use Invitrogen SuperMix for PCR reaction. The components of the SuperMix include: 22 U/mL complexed recombinant Taq DNA polymerase with Platinum® Taq Antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM MgCl₂, 220 µM dGTP, 220 µM dATP, 220 µM dTTP, 220 µM dCTP, and stabilizers.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input checked="" type="checkbox"/>	94	5:00	1
2. Denaturation	92	0:30	
3. Annealing steps 2-3-4 cycle in sequence	58	0:45	35x
4. Elongation	72	1:30	
5. Amplification	72	7:00	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose:	V:
1. Ambn Common F F DUNELacZF	TGGCTGCTCGTCATTCCATT	Estimated Running:Time: min.	
2. Ambn WT R	ATGTGGCCACTGTGACCATT	Primer Combination	Band
3. Ambn LacZ R	GCTCATCCGCCACATATCCT	1 and 3	400 bp
		2 and 3	841 bp
		1, 2 and 3	400 & 841 bp
			Genotype
			WT
			Null
			+/-

