

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: C57BL/6JApb-Ptprc^{loc}/Mmcd (lochy), MMRRC# 031691-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
dH ₂ O	4.6
dNTPs (2.5mM each –amplifluor kit)	0.8
20x amplifluor SNP FAM Primer (amplifluor kit)	0.5
20x amplifluor SNP JOE Primer (amplifluor kit)	0.5
20x lochy specific primer mix (allele 1&2 are 0.5μM, reverse is 7.5μM)	0.5
10x Reaction Mix S Plus	1.0
Platinum Taq (5u/μl)	0.1
DNA Sample (cleaned ear punch in dH ₂ O)	2.0
TOTAL VOLUME OF REACTION:	10μl

Comments on protocol: Perform reactions at room temperature with reagents added in order.

Strategy: program Ampli

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START? [√]	94	4:00	1
2. Denaturation	94	0:10	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:20	35x
4. Elongation	72	0:40	
5. Amplification	72	3:00	1
6. Finish	10	hold	hold

Primers: Nucleotide sequence (5' - 3')

Primer Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1:lochy-1	G	A	A	G	G	T	G	A	C	C	A	A	G	T	T	C	A	T	G	C	T	T	T	G	G	G	T	T	C	C	T	T	G
	A	A	G	C	C	C	T	G	A	T	C	T																					
2:lochy-2	G	A	A	G	G	T	C	G	G	A	G	T	C	A	A	C	G	G	A	T	T	T	G	G	G	T	T	C	C	T	T	G	A
	A	G	C	C	C	T	G	A	T	C	A	A																					
3:lochy-R	A	T	G	A	C	T	G	T	T	C	T	C	T	T	T	G	T	G	T	G	T	T											

