GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: C57BL/6JApb-*Lig4*^{tiny}/Mmcd (*Tiny*), MMRRC # 031685-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
dH_20	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 Tiny specific primer mix (allele primers 0.5µM, reverse primer 7.5µM)	0.5
10x Reaction Mix S Plus	1.0
Platinum Taq (5u/µl)	0.1
DNA Sample (cleaned ear punch in dH_20)	2.0
TOTAL VOLUME OF REACTION:	**10µl**

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

Strategy: program Amp2

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	60	0:20	**30x**
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:Tiny-1	G A A G G T C G G A G T C A A C G G A T T A C A A A G A T G G C G
	C G C T G T A
2:Tiny-2	G A A G G T G A C C A A G T T C A T G C T C A A A G A T G G C G C
	G C T G T G
3:Tiny-R	A A A T G G G G T G A G A G A G C C T T