

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/6-Cd4^{Thth}/Mmcd (*Thoth*), MMRRC # 031683-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
ddH ₂ O	1.58
dNTPs (2.5mM each –amplifluor kit)	0.4
20x amplifluor SNP FAM Primer (amplifluor kit)	0.25
20x amplifluor SNP JOE Primer (amplifluor kit)	0.25
20x 9B6 64 primer mix (allele 1&2 are 0.5μM, reverse is 7.5μM)	1.0
10x Reaction Mix S Plus	0.5
Platinum Taq (5u/μl)	0.05
DNA Sample (cleaned ear punch in dH ₂ O)	1.0
TOTAL VOLUME OF REACTION:	5μl

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 [✓]	95	4:00	1
2. Denaturation	95	0:10	
3. Annealing	60	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: 9B6 64-1	G	A	A	G	G	T	C	G	G	A	G	T	C	A	A	C	G	G	A	T	T	A	G	G	C	G	A	A	C	C	T	C	C
	T	G	C	A	A	A	T	C	T	A																							
2: 9B6 64-2	G	A	A	G	G	T	G	A	C	C	A	A	G	T	T	C	A	T	G	C	T	G	C	G	A	A	C	C	T	C	C	T	G
	C	A	A	A	T	C	G																										
3: 9B6 64-R	G	G	G	C	A	C	C	T	C	A	A	C	T	T	C	T	T	T	T	G	T	A											

