

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: C57BL/6JApb-*Trp53*^{Bbl}/Mmcd (*B-blast*), MMRRC # 031688-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
ddH ₂ O	4.65
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 B_blast specific primer mix (allele 1&2 are 0.5μM, reverse is 7.5μM)	2.0
10x Reaction Mix S Plus	1.0
Platinum Taq (5u/μl)	0.05
DNA Sample (ear punch)	0.5
TOTAL VOLUME OF REACTION:	10μl

Comments on protocol: Perform reactions at room temperature with reagents added in order.
 Final MgCl₂ conc. is 2.5mM.

Strategy: program Amp2

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? [√]	95	4:00	1
2. Denaturation	95	0:10	
3. Annealing } steps 2-3-4 will cycle in sequence	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:Bblast-1	G	A	A	G	G	T	C	G	G	A	G	T	C	A	A	C	G	G	A	T	T	C	A	A	A	T	T	T	C	C	T	T	C
	C	C	G	G	T																												
2:Bblast-2	G	A	A	G	G	T	G	A	C	C	A	A	G	T	T	C	A	T	G	C	T	T	A	C	A	A	A	T	T	T	C	C	T
	T	C	C	A	C	C	C	G	G	A																							
3:Bblast-R	G	G	C	T	T	C	T	G	A	C	T	T	A	T	T	C	T	T	G	C	T	C	T	T									

