

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: C57BL/6JApb-Jak3^{b^{fy}}/Mmcd (*Buffy*), MMRRC# 031689-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	37.7
10x Buffer (20mM MgCl ₂)	5.0
MgCl ₂ (stock concentration is 25mM)	1.25
dNTPs (stock concentration is 10mM)	1.0
Primer 1 (stock concentration is 10μM) Name: Buf-F	1.0
Primer 2 (stock concentration is 10μM) Name: Buf-RWT	1.0
Primer 3 (stock concentration is 10μM) Name: Buf-RM	1.0
Taq Polymerase	0.05
DNA Sample	2.0
TOTAL VOLUME OF REACTION:	50 μl

Comments on protocol:

Strategy: 62C Short

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START? []	94	2:00	1
2. Denaturation	94	0:20	
3. Annealing } steps 2-3-4 will cycle in sequence	62	0:20	35x
4. Elongation	72	0:30	
5. Amplification	72	10:00	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:Buf-F	G	C	G	A	C	T	C	C	A	G	G	C	A	T	T	A	T	T	T	C													
2:Buf-RWT	C	T	G	T	C	A	T	A	G	T	C	C	T	G	C	G	G	G	C	T	G	C	G	G	C	A	G	A					
3:Buf-RM	A	C	G	C	A	G	G	C	G	G	T	A	A	G	A	A	G	A	A	A	G	G	A	G	T	C	A	T	A	G	T	C	C
	T	G	C	G	G	G	C	T	G	C	G	G	T	G	G	G																	

Electrophoresis Protocol:

% Agarose: 3 mV : 400

Estimated Running Time (min): 20

Number	Band (bp)	Genotype
1	271	Wt/Wt
2	292	Buf/Buf