

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: C57BL/6JApb-Jak3^{will}/Mmcd (*Willow*), MMRRC # 031694-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	38.20
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: JAK3-F	1.0
Primer 2 (stock concentration is 10μM) Name: JAK3-RWT	2.0
Primer 3 (stock concentration is 10μM) Name: JAK3-RM	1.0
Taq Polymerase	0.05
DNA Sample	2.0
TOTAL VOLUME OF REACTION:	50μl

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
1:JAK3-F	G	A	G	G	C	A	G	A	G	A	C	T	T	A	C	C	A	C														
2:JAK3-RWT	C	C	C	C	C	A	C	A	T	G	C	C	C	G	C	C	C	A	C	T	C	A	C	C	T	G	G	C				
3:JAK3-RM	C	A	T	C	C	A	C	C	C	A	C	C	C	A	C	G	T	T	C	C	T	T	T	C	C	C	A	C	A	T		

Electrophoresis Protocol:

% Agarose: 3 V : 400

Estimated Running Time (min): 20

Nur	Band (bp)	genotype
1		Wt/Wt
2		Wil/Wil