

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

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Protocol:

NAME OF PCR C57BL/6J-Tg(pUR288)1Vij #30330-UCD

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM MgCl ₂	1.7
5M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer 1: Z1924 (20 µM)	0.5
Primer 2: Z2046 (20 µM)	0.5
Taq Polymerase-5 Units/µl	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25 µl

Comments on protocol: Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; final 30 cycles anneal at 55° C. *Concentration of MgCl₂ may be adjusted to reduce non-specific banding.

Cycling:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	5:00	1
2. Denaturation	94	0:20	\
3. Annealing	65 to 55	0:30	> x 40
4. Elongation			
5. Final Extension	72	5:00	1
6. Finish	25	Hold	--

Primers:

Primer Name	Nucleotide sequence (5'- 3')
1 Z1924	GTCTCGTTGCTGCATAAACC
2 Z2046	TCGTCTGCTCATCCATGACC

Electrophoresis Protocol:

% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

Primer Combinations	Band size (bp)	genotype
1 & 2	500	Mutant

