

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

Protocol:

NAME OF PCR: Zmpste24 0033-UCD

Reagent/ Constituent	Volume (uL)
DNA Sample	15.0
10x Buffer (w/ 15 mM MgCl ₂)	5.5
dNTPs (stock concentration is 2mM)	5.5
Primer 1 (stock concentration is 20 uM) Name: Z-UP	2.75
Primer 2 (stock concentration is 20 uM) Name: Z-DN	2.75
Primer 3 (stock concentration is 20 uM) Name:	-
Water	22
Taq Polymerase	1.5
Other ?	
TOTAL VOLUME OF REACTION:	50 ul

Comments on protocol (e.g., different concentration of MgCl₂, etc): _____

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [X]	95	5 min.	1
2. Denaturation	95	1 min	\
3. Annealing } steps 2-3-4 will cycle in sequence	60	2 min.	> 40
4. Elongation	72	3 min.	/
5. Amplification (i.e., 72°C, 10 min)	72	10 min.	1
6. Finish (i.e., 4°C, indefinite)	4	N/A	N/A

Primers:

Primer Name	Nucleotide sequence (5' - 3')																																														
	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: Z-up	G	T	C	T	G	G	T	T	G	T	T	T	G	A	T	T	A	G	A	T	G	G	G	T	C																						
2: Z-dn	G	C	T	A	C	A	T	A	G	T	G	A	A	C	A	C	C	A	G	G	C	C	A																								

Electrophoresis Protocol:

% Agarose: 2.0% mV : 100

Estimated Running Time (min): 60

Number	Band (kB)	genotype
1	300 bp	+/+
2	220 bp	-/-
3	220/300 bp	+/-

PASTE GEL PICTURE HERE, CLEARLY
 INDICATING LADDER, WATER CONTROL, DNA
 CONTROL, & DIAGNOSTIC SAMPLES