GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: B6;129S-*Zmpste24*^{tm1Sgy}/Mmucd **MMRRC #** 000033-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	18.05
10x Buffer (without 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 2mM)	0.5
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Taq Polymerase	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Strategy:

Steps	Temp (^oC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	5:00	**1**
2. Denaturation	95	1:00	
3. Annealing } steps 2-3-4 will cycle in sequence	60	2:00	} **30x**
4. Elongation	72	3:00	
5. Amplification	72	10:00	**1**
6. Finish	4	∞	n/a

Primers:

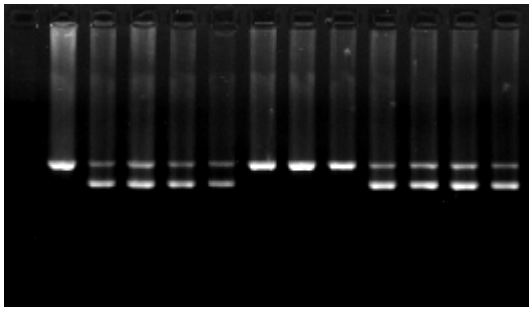
Primer Name	Nucleotide Sequence (5' - 3')
1: Z-up	GTC TGG TTG TTT GAT TAG ATG GGT C
2: Z-dn	GCT ACA TAG TGA ACA CCA GGC CA

Electrophoresis Protocol:

% Agarose: 2 **mV:** 100

Estimated Running Time (min): 60

Expected Bands	Genotype
300 bp	WT +/+
220 / 300 bp	Het +/-
220bp	KO -/-



Sample Gel From Left to Right:
1 Kb+ Ladder
Controls: H_2O, B6, *ZmpSte24* +/-
ZmpSte24 samples 302 - 311