GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Protocol: 415

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	5
dNTPs (stock concentration is 2mM)	3
Primer **1** (stock concentration is 20 uM) Name: **Nsi-5**	0.5
Primer **2** (stock concentration is 20 uM) Name: **Nsi-3**	0.5
Primer **3** (stock concentration is 20 uM) Name: ---------	-
Water	39.5
Taq Polymerase	0.5
Other ?	
TOTAL VOLUME OF REACTION:	**50 ul**

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

We use GibCO Taq polymerase; the 10x buffer is supplied w/o $MgCl_2$; we supplement with 2mM $MgCl_2$, ie. 2ul for a 50 ul reaction.

Strategy:

Steps	Temp (^oC)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	2 min	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	45 sec	
3. Annealing	55	45 sec	**30**
4. Elongation	72	1 min	
5. Amplification (i.e., 72^{o}C, 10 min)	72	5 min	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

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: Nsi-5	**C**	**A**	**C**	**C**	**A**	**C**	**A**	**G**	**A**	**A**	**G**	**T**	**A**	**C**	**T**	**A**	**T**	**G**	**A**	**T**	**C**												
2: Nsi-3	**A**	**T**	**G**	**C**	**T**	**G**	**A**	**C**	**A**	**A**	**G**	**C**	**T**	**T**	**T**	**C**	**T**	**T**	**C**	**T**	**A**												
3:																																	

Electrophoresis Protocol:

% Agarose: 1.5 **mV :** _____

Estimated Running Time (min): _____

Number	Band (kB)	genotype
1	0.25	1 lox
2	No band	Wild type
3	--------	---------