GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Protocol:

NAME OF PCR: **ATOX 1** **0019-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	1.0
10x Buffer	5.0
dNTPs (stock concentration is 10mM)	4.0
Primer **1** (stock concentration is 10 uM) Name: Atox A8	5.0
Primer **2** (stock concentration is 10 uM) Name: Atox E5	5.0
Primer **3** (stock concentration is 10 uM) Name: AF4	5.0
Water	15.0
Taq Polymerase	1.0
Other ? 25mM MgCl2	6.0
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): ____25 mM MgCl2_____

If DNA is not very pure +/- will give .7 kb, 1.3 kb, and 1.5 kb. Can scale PCR rxn down to 20 ul and use 1.0 ul DNA

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [**X**]	96	2 min.	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	96	20 sec.	\
3. Annealing	68	1 min.	> **34**
4. Elongation	72	4 min.	/
5. Amplification (i.e., 72^{o}C, 10 min)	72	10 min.	**1**
6. Finish (i.e., 4^{o}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: Atox A8	**G**	**C**	**C**	**C**	**T**	**G**	**G**	**G**	**T**	**T**	**T**	**A**	**C**	**T**	**T**	**C**	**C**	**C**	**A**	**T**	**A**	**C**	**T**	**G**	**A**	**G**	**A**	**C**	**A**	**G**	**T**	**A**	**C**
2: Atox E5	**C**	**C**	**T**	**A**	**G**	**T**	**A**	**C**	**T**	**G**	**A**	**G**	**A**	**T**	**G**	**G**	**C**	**A**	**G**	**A**	**G**	**G**	**C**	**C**	**T**	**G**	**C**	**A**	**G**				
3: AF4	**C**	**G**	**A**	**C**	**T**	**G**	**G**	**C**	**C**	**G**	**G**	**C**	**T**	**G**	**G**	**G**	**T**	**G**	**T**	**G**	**T**	**G**	**G**	**C**	**G**	**G**	**A**						

Electrophoresis Protocol:

% Agarose: **1.3%** **mV :** **120**

Estimated Running Time (min): **30**

Number	Band (kB)	genotype
1	0.7 kp	+/+
2	0.7+1.3 kb	+/-
3	1.3 kb	-/-

Notes: On Gel Pic,
Lane 1 = Ladder
Lane 2 = Water
Lane 3 = WT (+/+)
Lane 4 = Atox1 Control +/-