

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: Bt.129(Cg)-Atox1^{Gt(IRESBetageo)3Pgr}/Mmucd MMRRC # 000019-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	16.65
10x Buffer (contains 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) Atox A8	1.0
Primer 2 (stock concentration is 10μM) Atox E5	0.4
Primer 3 (stock concentration is 10μM) AF4	1.0
Taq Polymerase	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

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

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3:00	1
2. Denaturation	94	0:20	} 34
3. Annealing } steps 2-3-4 will cycle in sequence	68	1:00	
4. Elongation	72	4:00	
5. Amplification	72	10:00	1
6. Finish	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Atox A8	GCC CTG GGT TTA CTT CCC ATA CTG AGA CAG TAC
2: Atox E5	CCT AGT ACT GAG ATG GCA GAG GCC TGC AG
3: AF4	CGA CTG GCC GGC TGG GTG TGT GGC GGA

Electrophoresis Protocol:

% Agarose: 2 mV: 80120

Estimated Running Time (min): 90

Expected Bands	Genotype
700 bp	WT +/+
700 / 1300 bp	Het +/-
1300bp	KO -/-