GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: STOCK *Mecp2*$^{tm1.1Jae}$/Mmucd **MMRRC #** 000011-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	13.1
10x Buffer (with $MgCl_2$)	2.5
$MgCl_2$: (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20µM) Nsi-5	0.5
Primer 2 (stock concentration is 20µM) Nsi-3	0.5
Taq Polymerase	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**20µL**

Comments on protocol:

Use GibCO Taq polymerase; the 10x buffer is supplied w/o $MgCl_2$; we supplement with 2mM $MgCl_2$, ie. 2µl for a 50µl reaction. The two PCR primers are over 2kb apart in the wild-type allele (no product will be generated due to the long distance); in the mutant allele, the 2kb or so sequence is deleted and the PCR reaction generates a product of about 300bp.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	95	2:00	**1**
2. Denaturation	} steps 2-3-4 will cycle in sequence	95	0:45	} **30x**
3. Annealing		55	0:45	
4. Elongation		72	1:00	
5. Amplification		72	5:00	**1**
6. Finish		4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Nsi-5	CAC CAC AGA AGT ACT ATG ATC
2: Nsi-3	ATG CTG ACA AGC TTT CTT CTA

Electrophoresis Protocol:

% Agarose: 1.5 **V:** _____

Estimated Running Time (min): _____

Expected Band	Genotype
250 bp	1 lox KO
No band	WT +/+