GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

Protocol: **NAME OF PCR: C57BL/6J-*Inpp5d*^{m1Btlr}/Mmcd (*styx*), MMRRC #030820-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol: *Styx* genotyping is performed by amplifying the region containing the mutation using PCR, followed by *BstE* II restriction enzyme digestion. The reverse PCR primer introduces a *BstE* II site (5'-GGTNACC-3') into the wild type PCR product, while the *styx* mutation would alter this introduced site. The lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs).

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?.CHECK HERE [x]	94	10	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0.50	32
3. Annealing	58	0.50	32
4. Elongation	72	0.50	32
5. Amplification (i.e., 72°C, 10 min)	72	10	**1**
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Styx(F)	ATTCCGACTTTTTGAAGACGGGCTCCAG
2: Styx (R)	ACCCCTCTCTCAGGGCTCTCGGTT
3:	
4:	

Restriction Digest:

10µl PCR reaction
17µl ddH_2O
3µl NEB Buffer 3
0.5µl *BstE* II
Incubate 2 hours – overnight at **60°C**

Electrophoresis Protocol:

% Agarose: __3____ V : _____
Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.108	*styx*
(i.e. 3&4)		
(i.e. 1&2&3)		

Products after digest:

wt - 82 bp, 26 bp.

***stix* - 108 bp.**

Second primer introduces a *BstE* II site. Digestion of the PCR product with *BstE* II will give two bands for the WT and a single band for the mutant.

The following sequence of 108 nucleotides (from Genbank genomic region **NC_000067** for linear DNA sequence of *Inpp5d*) is amplified:

49323 attccgac tttttgaaga cgggctccag caacctccct cacctgaaga agctgatgtc

49381 actgctctgc aaggagctcc atgggtaacg gagagccctg agagaggggt

The primer binding sites are underlined; the introduced *BstE* II site is highlighted in gray; the nucleotide that will be changed by PCR is shown in purple text. This site is destroyed by the *styx* mutation with the mutated T shown in red text.