

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	394
MMRRC Strain Name	B6.129- <i>Inpp5d</i> ^{tm1Rkh} /Mmnc
Gene Name(s)	Inositol polyphosphate-5-phosphatase D (Inpp5d)
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	10/21/13

MMRRC #394 PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94 °C for 5 min
 Step 2: 94 °C for 30 sec
 Step 3: 60 °C for 30 sec
 Step 4: 72 °C for 45 sec
 Step 5: 35x from step 2 to step 4
 Step 6: 72 °C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

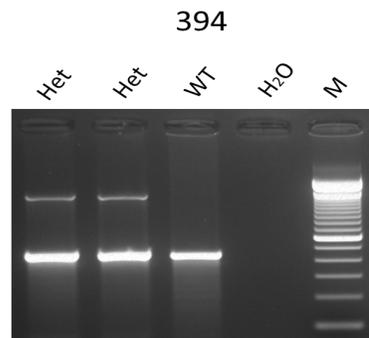
SHIP A (394): TCT GTG CAG CTC AGT TTC CTC T

SHIP B (394): CGT CCC ACC ATC CTA TGA CAT AA

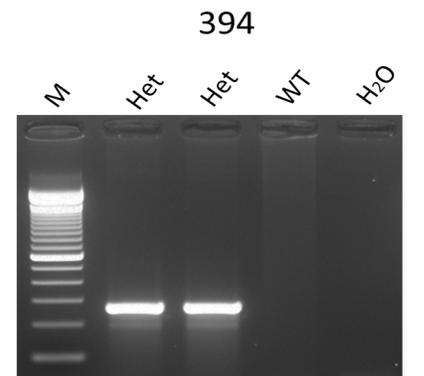
TK promoter (394): CTG CAT CTG CGT GTT CGA ATT

Bands expected: WT: primers SHIP A + SHIP B: 448 bp
 Mut: primers SHIP B + TK promoter: 265 bp

Run on 2% agarose gel in TAE.



Primers: SHIP A + SHIP B
 M: 100 bp DNA ladder (Invitrogen)



Primers: SHIP B + TK promoter
 M: 100 bp DNA ladder (Invitrogen)