

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	365
<b>MMRRC Strain Name</b>	B6;SJL-Tg(DBH-PTPN11)33Bmas/Mmnc
<b>Gene Name(s)</b>	Dopamine beta-hydroxylase (DBH Human), Protein tyrosine phosphatase, non-receptor type 11 (PTPN11 Chicken)
<b>Breeding Protocol(s)</b>	Intra-strain Mating
<b>Protocol Date</b>	12/2/13

### MMRRC #365 PCR Reaction

**Note:** In order to distinguish from strain #393, this genotyping requires *Pst*I restriction digestion.

	<b>1X</b>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25 mM MgCl <sub>2</sub>	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, 5 min  
 Step 2: 94 °C for 30 s  
 Step 3: 58 °C for 30 s  
 Step 4: 72 °C for 45 s  
 Step 5: 34 x 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.

SHP2-F (365): CTGGCCTGATGAATATTCCTAAAGGA

SHP2-R (365): TTGAACCATTCTGACCGTTGTGA

**Bands expected:** Tg+, 409 bp; WT, no band

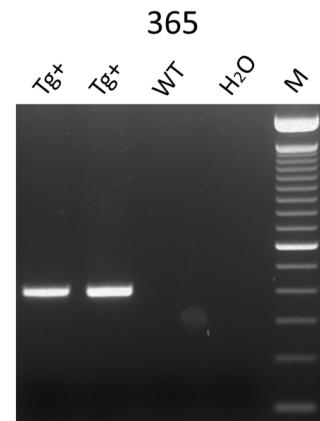
***Pst*I restriction digestion mix for each PCR sample**

9.6 μl H<sub>2</sub>O  
 4.0 μl NEB Buffer 3, 10X  
 0.4 μl BSA, 100X  
 1.0 μl *Pst*I

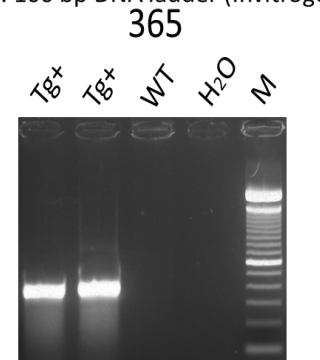
**Add 15 μl Mix to each 25ul PCR product:** incubate at 37 °C for 3 hours - overnight.  
 Then 80°C for 20 minutes

**Expected Results:** Tg+, 409 bp only

Run on a 2% agarose gel.



M: 100 bp DNA ladder (Invitrogen)



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