

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	240
<b>MMRRC Strain Name</b>	Pdyn-EGFP
<b>Gene Name(s)</b>	Prodynorphin (Pdyn)
<b>Breeding Protocol(s)</b>	Outcross to Swiss Webster(Taconic)
<b>Protocol Date</b>	3/24/17

### MMRRC #240 PCR Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 57°C for 30 sec

Step 4: 72°C for 30 sec

Step 2 to 4 Cycles: 30

Step 5: 72°C for 7 min

	<b>1X</b>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

Taq: **Denville Taq and Chromataq 5X Buffer**

**Bands:** MUTANT: ~600bp

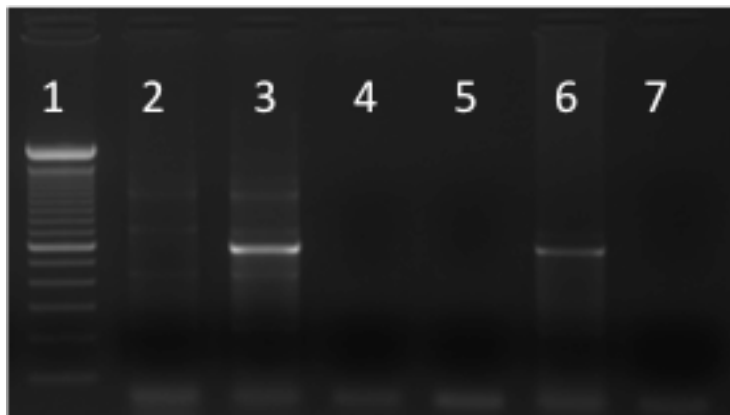
WT: No Band

**Primer sequences 5' to 3':**

Pdyn(240)5Abox: GCGCGCCCTACGCAGAAACCCATTTTCCTATGC

Pdyn(240)EGFPR2: TAGCGGCTGAAGCACTGCA

Run on 2.0% agarose gel in TAE.



1. 100bp Marker (Invitrogen)
2. Wild-type Sample
3. Transgenic Sample
4. Wild-type Sample
5. Wild-type Control
6. Positive Control
7. Negative Control