

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36482
<b>MMRRC Strain Name</b>	B6;129S5-Peli1tm1.1Lex/Mmnc
<b>Gene Name(s)</b>	pellino 1 (Peli1)
<b>Breeding Protocol(s)</b>	Random Intra-strain Mating Backcross to C57BL/6J
<b>Protocol Date</b>	6/27/13

### MMRRC #36482 PCR Reaction

**Thermal Cycler:**

Step1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 55°C for 30 sec  
 Step 4: 72°C for 30 sec  
 Step 5: Step 2 to 4 Cycles: 32  
 Step 6: 72°C for 7 min

Taq: **Apex and chromataq Buffer**

	<b><u>1X</u></b>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

Primers are 10uM with respect to each primer

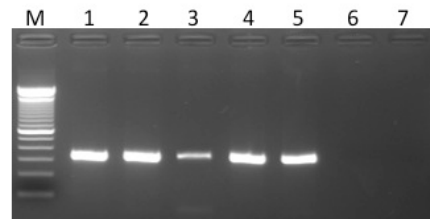
**Primer sequences 5' to 3':**

Neo3A: GCAGCGCATCGCCTTCTATCGCC  
 P8: GAGAACTCAGACAGCCTTAGAG  
 P2: CCTGACCAGTGAACAGAGATTTG  
 P4: GATGGCATAAATACGGTCAC

**Bands:** P2 + P4, WT: 276 bp  
 Neo3A + P8, MUTANT: 336 bp

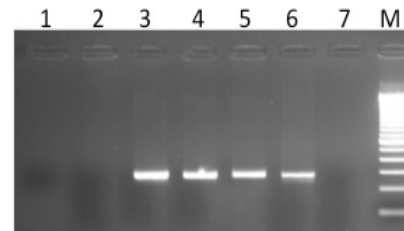
Run on 2% agarose gel in TAE.

#### 36482 (Neo3A + P8)



Lane 1-2, 4-5: Hom; Lane 3: Het; Lane 6: WT; Lane 7: ddH<sub>2</sub>O;  
 M: 100 bp DNA ladder Invitrogen

#### 36482 (P2 + P4)



Lane 1 and 2: Hom; Lane 3 and 6: WT; Lane 4 and 5: Het;  
 Lane 7: ddH<sub>2</sub>O; M: 100 bp DNA ladder Invitrogen