

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	11666
<b>MMRRC Strain Name</b>	B6;129S5-Slc12a6 <sup>Gt(OST458419)Lex</sup> /Mmnc
<b>Gene Name(s)</b>	solute carrier family 12, member 6 (Slc12a6)
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6J
<b>Protocol Date</b>	9/6/11

### MMRRC# 11666 PCR Reaction

**Note: Run 2,2 primer reactions  
(1503-5' + 1503-3' for wild-type) and (1503-5' +  
LTR-rev for mutant)**

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 15 sec  
 Step 3: 65°C for 30 sec, Decrease 1°C/cycle  
 Step 4: 72°C for 40 Sec  
 Step 5: go to step 2, 10 Cycles,  
 Step 6: 94°C for 15 sec  
 Step 7: 55°C for 30 sec  
 Step 8: 72°C for 40 sec  
 Step 9: go to step 6 to 8, 30 Cycles  
 Step 10: 72°C for 7 min

	<b><u>1X</u></b>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer F/R	1/1
Taq	0.5
ddH <sub>2</sub> O	13.0
25mM MgCl <sub>2</sub>	2.0

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

**Bands:** MUTANT: 177bp  
 WT: 145bp

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

LTR-rev (11666): ATAAACCCTCTTGCAGTTGCATC  
 1503-5' (11666): CATGTGCTTTCACAAGCATGTGACC  
 1503-3' (11666): TCCTAATCAGTGAGCACCAAGG

Run on 2.0% agarose gel in TAE.