

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	211
<b>MMRRC Strain Name</b>	B6.129S7- <i>Chrn2</i> <sup>tm1Mdb</sup> /Mmnc
<b>Gene Name(s)</b>	Cholinergic receptor, nicotinic, beta polypeptide 2 ( <i>Chrn2</i> )
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6J
<b>Protocol Date</b>	9/7/13

### MMRRC #211 PCR Reaction

	<b>1X</b>
ddH <sub>2</sub> O	12
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 94<sup>0</sup>C for 5 min  
 Step 2: 94<sup>0</sup>C for 45 sec  
 Step 3: 60<sup>0</sup>C for 60 sec  
 Step 4: 72<sup>0</sup>C for 60 sec  
 Step 5: 35x from Step 2 to 4  
 Step 6: 72<sup>0</sup>C for 7 min

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

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

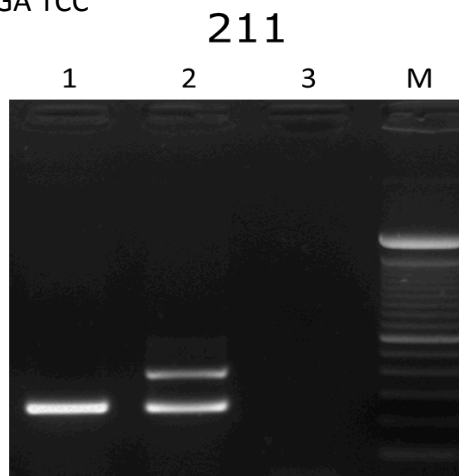
Beta2KO (211): GAG ACT AGT GAG ACG TGC TAC TTC CAT TTG

Beta2WF (211): CTC TGA CTG TAA AGG CAG TGG TTG CTA TAG

Beta2WR (211): TAG CTA TTG ACG ACG TCT TTA AGA TCC

**Bands expected:** MUTANT ~400bp  
 WT 250bp

Run on 2.0% agarose gel in TAE.



Lane 1: WT; Lane 2: Het; Lane 3: H<sub>2</sub>O;  
 M: 100 bp DNA ladder (Invitrogen)