

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	43504
<b>MMRRC Strain Name</b>	B6J.B6N- <i>Tmem35a</i> <sup>tm1(KOMP)Vlcg</sup> /Mmnc
<b>Gene Name(s)</b>	transmembrane protein 35A (Tmem35a)
<b>Breeding Protocol(s)</b>	Intra-strain breeding
<b>Protocol Date</b>	3/23/18

### MMRRC #43504 PCR Reaction

**Note: Strain is X-Linked**

**Thermal Cycler:**

Step 1: 94°C, 5min  
 Step 2: 94°C, 30sec  
 Step 3: 57°C, 30sec  
 Step 4: 72°C, 30sec  
 Step 2 to 4 Cycles: 30  
 Step 5: 72°C, 7min

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

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

**Bands:** WT: 543bp (F+R)  
 MUTANT: ~450bp (F+MR)

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

Tmem(43504)F      GGA AGT TGC CTT CTC ACT TCA GG  
 Tmem(43504)R      TTC CGA GCT GCA AAG TTG TTA GG  
 Tmem(43504)MR    GCT GGC TTG GTC TGT CTG TCC TA

1. 100bp Marker (Invitrogen)
2. Homozygous Sample
3. Wild-type Sample
4. Heterozygous Sample
5. Homozygous Sample
6. Homozygous Sample
7. Homozygous Sample
8. Wild-type Control

