

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36519
<b>MMRRC Strain Name</b>	B6.Cg- <i>Ttll5</i> <sup>tm1.1N</sup> /Mmnc
<b>Gene Name(s)</b>	tubulin tyrosine ligase-like family, member 5 (Ttll5)
<b>Breeding Protocol(s)</b>	Sib-mating (heterozygous x homozygous) Backcross to C57BL/6
<b>Protocol Date</b>	2/26/13

### MMRRC # 36519 PCR Reaction

**2,2 Primer Reaction:** (DL1 + Lox1) for wild-type and (DL1 + SAR2) for mutant reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 56°C for 30 sec

Step 4: 72°C for 30 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

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

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

**Bands:** MUTANT: 250bp

WT: 246bp

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

DL1(36519): 5'-TTG ACT CCC AGA GTA CTG AGA GAG-3'

Lox1(36519): 5'-AAC CAC CAG TTC CTG TGT ACA TG-3'

SAR2(36519): 5'-TGG CAA CAG CAA CAC AAC T-3'

Run on 2% agarose gel in TAE.