

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36520
MMRRC Strain Name	B6;129S- <i>Ttll5</i> ^{tm1N} /Mmnc
Gene Name(s)	tubulin tyrosine ligase-like family, member 5 (Ttll5)
Breeding Protocol(s)	Sib-Mating
Protocol Date	2/26/13

MMRRC # 36520 PCR Reaction

Thermal Cycler:

Step 1: 94° for 5 min

Step 2: 94°C for 30 sec

Step 3: 56°C for 30 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

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

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

Bands: MUTANT: 310bp

WT: 245bp

Primer sequences 5' to 3':

DL1(36519-20): 5'- TTG ACT CCC AGA GTA CTG AGA GAG- 3'

Lox1(36519-20): 5'- AAC CAC CAG TTC CTG TGT ACA TG- 3'

Run on 2% agarose gel in TAE.