

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	68119
MMRRC Strain Name	C57BL/6J- <i>Tardbp</i> ^{em1Toco} /Mmnc
Gene Name(s)	TAR DNA binding protein
Breeding Protocol(s)	Sib-mating
Protocol Date	5/30/2023

PCR Reaction

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

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 55C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: 550bp

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

TDP(68119)F: CCTTGAGCTAACTTGATTACCTTTATGAGC

TDP(68119)R: TGCTTGCATGGAATGAATTCGG

Requires a Restriction Enzyme Digestion

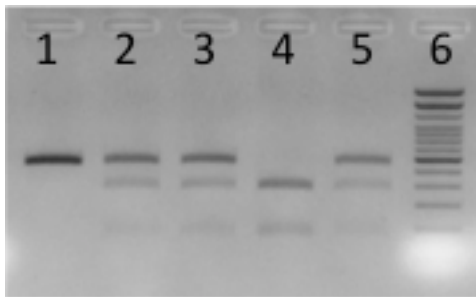
	<u>1X (ul)</u>
Water	10
NEB Cutsmart Buffer	4
Hinfl	<u>1</u>
	15 total

Add 15ul to each PCR sample

Digest at 37C overnight and then run on an agarose gel

Expected Bands post restriction enzyme digestion

WT: 550bp
Mutant: 378bp and 172bp



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