

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	66961
MMRRC Strain Name	B6J;B6N- <i>Trp73^{tm1a(KOMP)Wtsi}</i> /WjlMmnc
Gene Name(s)	transformation related protein 73
Breeding Protocol(s)	Backcross and sib-mating
Protocol Date	9/19/2025

PCR Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1
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: WT: ~260bp MUTANT: ~290bp

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

Trp73(66961)F: GACCGACATTGTTAAGCGCTG

Trp73(66961)R: TCAGCCTCTGGACTGTAGT