

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11657
MMRRC Strain Name	B6;129S5- <i>Rtn4rl2</i> ^{tm1Lex} /Mmnc
Gene Name(s)	reticulon 4 receptor-like 2
Breeding Protocol(s)	Backcross to B6J
Protocol Date	9/11/2024

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

2,2 Primer Reactions (5+6 for wildtype and 28+Neo for the mutant reaction)

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: 263bp MUTANT: 378bp

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

NIH1305(11657)5: TCCTTGCCACCCAGTACACA

NIH1305(11657)6: CTGAGCTGGCAACGATACAG

NIH1305(11657)28: CCCAGCTACGCAGTACAGT

NIH1305(11657)Neo: GCAGCGCATCGCCTTCTATC