

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	69807
MMRRC Strain Name	STOCK <i>Nckap1^{Gt(XE068)Byg}/EanMmnc</i>
Gene Name(s)	NCK-associated protein 1
Breeding Protocol(s)	Intra-strain (homo embryonic lethal)
Protocol Date	5/21/24

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 (CF+WR for Wild-type and CF+MR for Mutant)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 60C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq

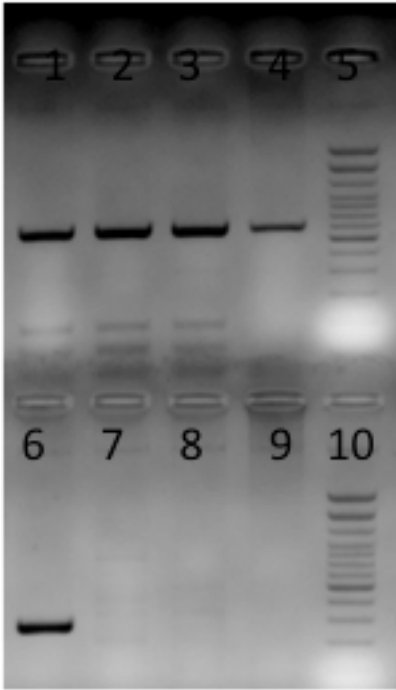
Bands: WT: 710bp MUTANT: 325bp

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

Nap(69807)CF CTGTCTGCCTGCCTCTGCTTTTTG

Nap(69807)WR CCCACCCCCAGGGTTTGAGTAGAG

Nap(69807)MR CCACCCTGGGGTTCGTGTCCTAC



Wild-type Reaction

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

Mutant Reaction

6. Heterozygous Sample
7. Wild-type Sample
8. Wild-type Sample
9. Wild-type Control
10. 100bp Marker (Invitrogen)