

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	41164
MMRRC Strain Name	C57BL/6- <i>Nxf1^{em1Ham1}</i> /Mmnc
Gene Name(s)	nuclear RNA export factor 1
Breeding Protocol(s)	Sib-mating
Protocol Date	10/25/2024

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

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: 192bp

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

Nxf(41164)F: ggacagccagggagtctaca

Nxf(41164)R: gacaggagacggcagatgac



After PCR, the products must be sent for Sanger sequencing.

Sequence with the forward primer. The mutations are in bold below.

Wild-type

CATTTTATCTTTCTGCAGGCCAAGGGAG**A**GATTCCAGAA**A**GTAGCGTTTATGAAATGATC

Mutant

CATTTTATCTTTCTGCAGGCCAAGGGAG**G**GATTCCAGAG**G**GTAGCGTTTATGAAATGATC