

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	71776
MMRRC Strain Name	C57BL/6J- <i>Nfe2l2</i> ^{em1Mmaj} /Mmnc
Gene Name(s)	nuclear factor, erythroid derived 2, like 2
Breeding Protocol(s)	Backcross
Protocol Date	1/16/2025

PCR Reaction

	<u>1X</u>
ddH ₂ O	11.5
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	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: 290bp MUTANT: 242bp

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

Nfe(71776)F: GATGCCTTCTTCTTGCCTGTAG

Nfe(71776)R: AAAGGGACAGGATAAGTATGACA

Nfe(71776)R2: TCCACACGGGTAGTTCACTACA

Run on 2% agarose gel