

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	65283
MMRRC Strain Name	C57BL/6J- <i>Gpr85^{em1(Gpr85*,cre)Blr}</i> /Mmnc
Gene Name(s)	G protein-coupled receptor 85; cre recombinase
Breeding Protocol(s)	Backcross
Protocol Date	8/22/2024

PCR Reaction

FLAG 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: 56C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: 240bp MUTANT: ~275bp

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

FLAG(65283)F1: AAATCAAACGGAAGGCAAGA

FLAG(65283)R: CCCAGGGAAGTCAGTTTCAG

PCR Reaction

Cre 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: 56C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: No Band Cre: ~250bp

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

CreF: GGACATGTTTCAGGGATCGCCAGGC

CreR: CGACGATGAAGCATGTTTAGCTG