

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36560
MMRRC Strain Name	C(Cg)- <i>Efnb2</i> ^{tm1And} /Mmnc
Gene Name(s)	ephrin B2 (Efnb2)
Breeding Protocol(s)	Backcross to Balb/cJ
Protocol Date	2/26/13

MMRRC # 36560 PCR Reaction

2,2 Primer Reaction: (RAP a + RAP b) for wild-type and (LacZF + LacZR) for mutant reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 58°C for 30 sec

Step 4: 72°C for 60 sec

Step 2 to 4 Cycles: 33

Step 5: 72°C for 7 min

Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: MUTANT: ~180bp

WT: 592bp

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM WT Primer	1
10uM Mut Primer	1
Taq	0.5
DNA	2

Primer sequences 5' to 3':

LacZ F: CTGTCGTCGTCCTCCCTCAAC

LacZ R: GGCCTTCCTGTAGCCAGCTT

RAP a: AGGACTGGGTGGCTTCCAACCTCCAGACAC

RAP b: AGCTTCTCATTGCTGCGCGCCAGGTTTCAGG

Run on 2% agarose gel in TAE.