

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36515
<b>MMRRC Strain Name</b>	STOCK Tg(Ednrb-icre)1Lby/Mmnc
<b>Gene Name(s)</b>	Promotor: endothelin receptor type B (Ednrb) Transgene: codon-improved cre recombinase sequence [icre (Bacteriophage P1)]; Transgene: Tg(Ednrb-icre)1Lby
<b>Breeding Protocol(s)</b>	Backcross to CD-1 (Charles River)
<b>Protocol Date</b>	2/26/13

### MMRRC # 36515 PCR 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 45 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

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

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

**Bands:** MUTANT: 522bp

WT: No Band

**Primer sequences 5' to 3':**

E<sub>Cre</sub>1: GACAGGCAGGCCTTCTCTGAA

E<sub>Cre</sub>2: CTTCTCCACACCAGCTGTGGA

Run on 2% agarose gel in TAE.