

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	65440
MMRRC Strain Name	C.129S4(Cg)- <i>Igh-7^{tm1Led}</i> /Mmnc
Gene Name(s)	immunoglobulin heavy chain 7 (heavy chain of IgE)
Breeding Protocol(s)	Backcross followed by sib-mating
Protocol Date	9/4/2020

PCR Reaction

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

2,2 Primer Reactions (F+R for wild-type reaction and F+NR for mutant reaction)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 58C, 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

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

Bands: WT: ~330bp MUTANT: ~650bp

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

Igh(65440)F: TCTGAATCCCCAGGCC

Igh(65440)R: TTGGATAAAGAGGCCAAACTTGC

Igh(65440)NR: TCGTGCTTACGGTATCGCC



Wild-type Reaction

1. 100bp Ladder (Invitrogen)
2. Homozygous Sample
3. Homozygous Sample
4. Wild-type Control

Mutant Reaction

5. 100bp Ladder (Invitrogen)
6. Homozygous Sample
7. Homozygous Sample
8. Wild-type Control