

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	43607
<b>MMRRC Strain Name</b>	MRL.129P2- <i>Fcgr1</i> <sup>tm1Sjv</sup> <i>Fas</i> <sup>lpr</sup> /Mmnc
<b>Gene Name(s)</b>	Fc receptor, IgG, high affinity I (Fcgr1)
<b>Breeding Protocol(s)</b>	Random Intra-strain Mating
<b>Protocol Date</b>	12/17/18

### MMRRC #43607 PCR Reaction

#### 2,2 Primer Reaction

##### Thermal Cycler:

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 57°C for 30 sec  
 Step 4: 72°C for 30 sec  
 Step 2 to 4 Cycles: 30  
 Step 5: 72°C for 7 min

Taq: Denville and Chromataq 5X Buffer

**Bands:** MUTANT: 550bp (EC + H)  
 WT: ~1050bp (EC + L2)

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

Fcg(43607)EC: CCTCACACCATAAAGTGACATTT

Fcg(43607)L2: GGAAGTGGGTGAGTGACCTCT

Fcg(43607)H: TCGCCGATAGTGAAACCGAC

Run on 2.0% agarose gel in TAE.

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

1. 100bp Marker (Invitrogen)
2. Homozygous Sample- Wild-type Reaction
3. Homozygous Sample- Wild-type Reaction
4. Homozygous Sample- Wild-type Reaction
5. Homozygous Sample- Wild-type Reaction
6. Homozygous Sample- Wild-type Reaction
7. Homozygous Sample- Wild-type Reaction
8. Homozygous Sample- Wild-type Reaction
9. Wild-type Control- Wild-type Reaction
10. Homozygous Sample- Mutant Reaction
11. Homozygous Sample- Mutant Reaction
12. Homozygous Sample- Mutant Reaction
13. Homozygous Sample- Mutant Reaction
14. Homozygous Sample- Mutant Reaction
15. Homozygous Sample- Mutant Reaction
16. Homozygous Sample- Mutant Reaction
17. Wild-type Control- Mutant Reaction

