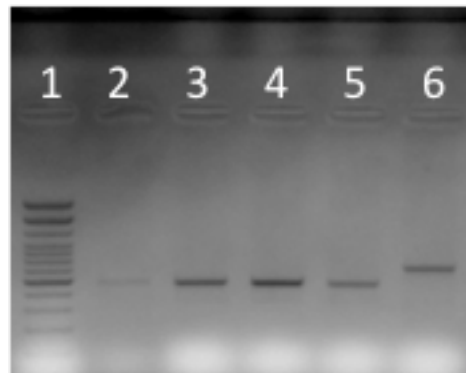


MMRRC UNC – Genotyping Protocol

| | |
|-----------------------------|---|
| MMRRC Strain ID | 68218 |
| MMRRC Strain Name | C57BL/6J- <i>Cmklr1^{em1Shai}</i> /Mmnc |
| Gene Name(s) | chemerin chemokine-like receptor 1 |
| Breeding Protocol(s) | Backcross and sib-mating |
| Protocol Date | 12/5/2023 |

PCR Reaction

| | <u>1X</u> |
|------------------------|------------------|
| ddH ₂ O | 11.5 |
| 5X Buffer | 5.0 |
| 25mM MgCl ₂ | 2 |
| 10mM dNTPs | 0.5 |
| 10uM Primer 1 | 1 |
| 10uM Primer 2 | 1 |
| 10uM Primer 3 | 1 |
| Taq | 1 |
| DNA | 2 |



Thermal Cycler:

Step 1: 94C, 5min
 Step 2: 94C, 45sec
 Step 3: 55C, 45sec
 Step 4: 72C, 60sec
 Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

Bands: WT: 725bp MUTANT: 580bp

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

| | |
|----------------|-------------------------|
| Chem(68218)5F1 | GGAGCAGGAAACAGAATAGGAC |
| Chem(68218)3F1 | ATCACCTTCTCCTCTGCTGG |
| Chem(68218)3R1 | GGTTTGACTGTCATGTTGCCATA |

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