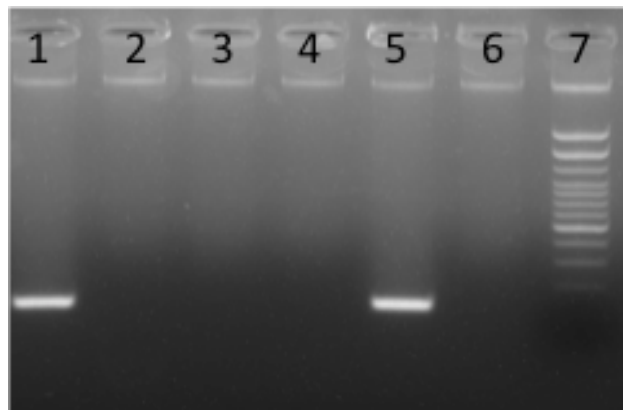


MMRRC UNC – Genotyping Protocol

| | |
|-----------------------------|---|
| MMRRC Strain ID | 37106 |
| MMRRC Strain Name | C57BL/6-Tg(H2-K-Cebpa)1Ipp/Mmnc |
| Gene Name(s) | CCAAT/enhancer binding protein (C/EBP), alpha |
| Breeding Protocol(s) | Backcross |
| Protocol Date | 5/14/2024 |

PCR Reaction

| | <u>1X</u> |
|------------------------|-----------|
| ddH ₂ O | 12.5 |
| 5X Buffer | 5 |
| 25mM MgCl ₂ | 2 |
| 10mM dNTPs | 0.5 |
| 10uM Primer 1 | 1 |
| 10uM Primer 2 | 1 |
| Taq | 1.0 |
| DNA | 2 |



Thermal Cycler:

Step 1: 94C, 5min
 Step 2: 94C, 45sec
 Step 3: 58C, 45sec
 Step 4: 72C, 60sec
 Step 2 to 4 Cycles: 35
 Step 5: 72C, 7min

1. Mutant Sample
2. Wild-type Sample
3. Wild-type Sample
4. Wild-type Control
5. Positive Control
6. Negative Control
7. 100bp Ladder (Invitrogen)

Taq: Apex Taq from Genesee Scientific (42-401)

Bands: WT: No Band MUTANT: ~250bp

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

Cebpa(37106)For: CCATCTCCACAGTTTCACTTCTGC
 Cebpa(37106)Rev: CCGCGGCTCCGCCTCGTAGAAGTCG