

## MMRRC UNC – Genotyping Protocol

|                             |                                                     |
|-----------------------------|-----------------------------------------------------|
| <b>MMRRC Strain ID</b>      | 68224                                               |
| <b>MMRRC Strain Name</b>    | C57BL/6J- <i>Arrb2</i> <sup>tm1.1Mca</sup> /McaMmnc |
| <b>Gene Name(s)</b>         | arrestin, beta 2                                    |
| <b>Breeding Protocol(s)</b> | Backcross and sib-mating                            |
| <b>Protocol Date</b>        | 11/20/2025                                          |

### PCR Reaction

|                        | <b><u>1X</u></b> |
|------------------------|------------------|
| ddH <sub>2</sub> O     | 12.5             |
| 5X Buffer              | 5                |
| 25mM MgCl <sub>2</sub> | 2                |
| 10mM dNTPs             | 0.5              |
| 10uM Primer 1          | 1                |
| 10uM Primer 2          | 1                |
| Taq                    | 1                |
| 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: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: 349bp flox: 507bp

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

Barr(68224)F: GAG TCA CTG TAT GGG TCC CTG

Barr(68224)R: TTG CTG TTC GAT GCT ACA TAA CTC