

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	67237
<b>MMRRC Strain Name</b>	C57BL/6NTac-Adora2a <sup>em1(IMPC)<sup>H</sup></sup> /Mmnc
<b>Gene Name(s)</b>	adenosine A2a receptor
<b>Breeding Protocol(s)</b>	Intra-strain mating
<b>Protocol Date</b>	2/6/2026

### PCR Reaction

	<b>1X</b>
ddH <sub>2</sub> O	13.5
5X Buffer	5.0
10mM dNTPs	0.5
10uM Primer 1	1.25
10uM Primer 2	1.25
10uM Primer 3	1.25
Taq	0.25
DNA	2

#### **Thermal Cycler:**

Step 1: 98C, 30sec

Step 2: 98C, 10sec

Step 3: 58C, 10sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 5min

Taq: **Invitrogen Platinum SuperFi DNA Polymerase (12351-010)**

Bands: WT: 520bp MUTANT: 414bp

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

Adora(67237)KOF: GATGGGGAAAGGCCAGAGAG

Adora(67237)WTF: ACTGGCTTCTGCGCTGCCTG

Adora(67237)ComR: GCCAAGTCACTTCCCCTCTC