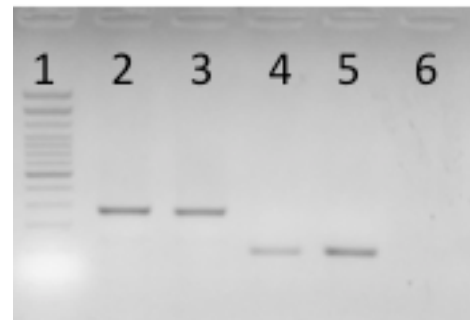


## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	43539
<b>MMRRC Strain Name</b>	B6J.Cg- <i>Cdkn2a</i> <sup>tm4Nesh</sup> /Mmnc
<b>Gene Name(s)</b>	cyclin dependent kinase inhibitor 2A
<b>Breeding Protocol(s)</b>	Backcross and sib-mating
<b>Protocol Date</b>	9/22/2020

### PCR Reaction

	<u><b>1X</b></u>
ddH <sub>2</sub> O	12
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2



#### **Thermal Cycler:**

Step 1: 94C, 5min  
 Step 2: 94C, 30sec  
 Step 3: 60C, 30sec  
 Step 4: 72C, 30sec  
 Step 2 to 4 Cycles: 30  
 Step 5: 72C, 7min

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

**Taq: Apex Taq and Chromataq 5X Buffer**

**Bands: WT: 204bp MUTANT: 352bp**

Primer sequences 5' to 3': Primers are 10uM with respect to each primer  
 p16(43539)F: GGAAGGAAGGAGGGACCCACTG  
 p16(43539)R: AGAGTTCGGGGCGTTGGGC  
 p16(43539)Tom: AAGCCCTCGGGGAAGGACAG