

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	43537
MMRRC Strain Name	B6.Cg- <i>Cdkn2a</i> ^{tm3.1Nesh Tyr^{c-2j}} /Mmnc
Gene Name(s)	cyclin-dependent kinase inhibitor 2A (Cdkn2a)
Breeding Protocol(s)	Sib-mating
Protocol Date	3/14/19

MMRRC #43537 PCR Reaction

2,2 Primer Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 60°C for 30 sec

Step 4: 72°C for 30 sec

Step 2 to 4 Cycles: 30

Step 5: 72°C for 7 min

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

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

Bands: MUTANT: 434bp (F + Luc)
 WT: 777bp (F + R)

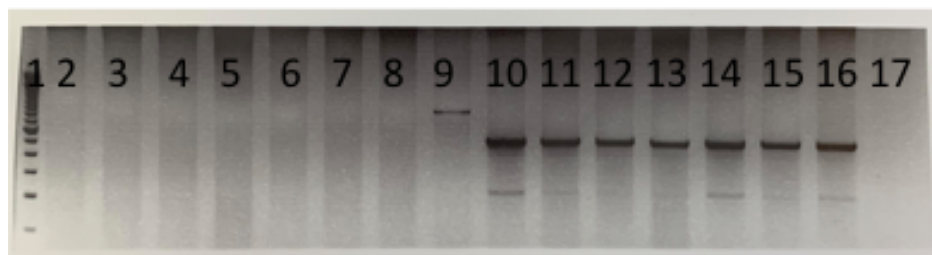
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

p16(43537)F GAAGGAAGGAGGGACCCACTGGTCAC

p16(43537)R TCGCACAACCACCCAACCTGTCTAGA

p16(43537)Luc CACGGTAGGCTGCGAAATG

Run on 2.0% agarose gel in TAE.



Lane 1. 100bp Ladder (Invitrogen)
 Lanes 2-8: Wild-type Reaction for Homozygous Samples
 Lane 9: Wild-type Reaction for Wild-type Control
 Lanes 10-16: Mutant Reaction for Homozygous Samples
 Lane 17: Mutant Reaction for Wild-type Control

Note: A non-specific band is present in the mutant reaction at ~200bp.