

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11659
MMRRC Strain Name	B6;129S5- <i>Adcy10</i> ^{tm1Lex} /Mmnc
Gene Name(s)	adenylate cyclase 10 (<i>Adcy10</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	3/3/15

MMRRC# 11659 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

2,2 Primer Reactions (P28 + P33) for wild-type and (Neo + P71) for mutant reaction

Thermal Cycler:

94°C for 30 sec
 55°C for 30 sec
 72°C for 45 sec
 35x

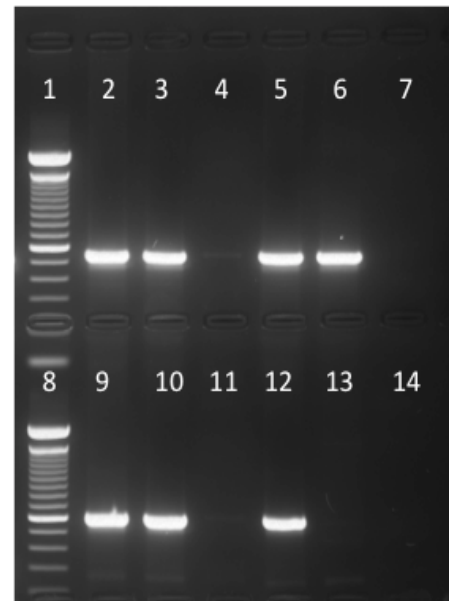
Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: 597bp
 WT: 500bp

Primer sequences 5' to 3':

P28 - (11659): GTCTGGCCACACACTAAGG
 P33 - (11659): CTCCAGCTCCGATGAAGG
 Neo(11659): GCAGCGCATCGCCTTCTATC
 P71- (11659): CTGGGCTGTCCTCAAGCTC

Run on 2.0% agarose gel in TAE.



1. 100bp Marker
2. Wild-type Reaction Heterozygous Sample
3. Wild-type Reaction Heterozygous Sample
4. Wild-type Reaction Heterozygous Sample
5. Wild-type Reaction Heterozygous Sample
6. Wild-type Reaction Wild-type Control
7. Wild-type Reaction Negative Control
8. 100bp Marker
9. Mutant Reaction Heterozygous Sample
10. Mutant Reaction Heterozygous Sample
11. Mutant Reaction Heterozygous Sample
12. Mutant Reaction Heterozygous Sample
13. Mutant Reaction Wild-type Control
14. Mutant Reaction Negative Control