

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	421
MMRRC Strain Name	B6.129S7- <i>Chrna5</i> ^{tm1Mdb} /Mmnc
Gene Name(s)	Cholinergic receptor, nicotinic, alpha polypeptide 5 (<i>Chrna5</i>)
Breeding Protocol(s)	Sib-mating (homo x homo)
Protocol Date	9/30/13

MMRRC# 421 PCR Reaction

	1X
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
20 uM Primer F/R	1.0
Taq	1.0
ddH ₂ O	13.5
25mM MgCl ₂	2.0

NOTE: Run two 2-primer reactions: (A5F & A5R;) and (Neo1 & Neo2). **Combine to run.**

Thermal Cycler:

94°C for 60 sec
 61°C for 60 sec
 72°C for 60 sec
 35x

Taq: **Apex and Chromataq 10X Buffer**

Bands: MUTANT: 290bp
 WT: 380bp

Primers are 10uM with respect to each primer (20uM total).

Primer sequences 5' to 3':

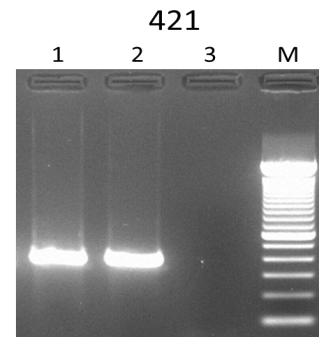
A5 WTForward: GTG AAA GAG AAC GAC GTC CGC

A5 WTRreverse: GCC TCA GCC CCT GAA TGG TAG

Neo1: CTT TTT GTC AAG ACC GAC CTG TCC G

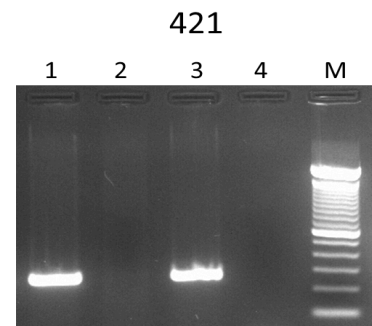
Neo2: CTC GAT GCG ATG TTT CGC TTG GTG

Run on 2.0% agarose gel in TAE



Primers: A5F + A5R

Lane 1: WT; Lane 2: Het; Lane 3: H₂O
 M: 100 bp DNA ladder (Invitrogen)



Primers: Neo1 & Neo2

Lane 1: Tg+; Lane 2: WT; Lane 3: H₂O
 M: 100 bp DNA ladder (Invitrogen)