

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	420
MMRRC Strain Name	B6.129S7- <i>Chrna7</i> ^{tm2Bay} /Mmnc
Gene Name(s)	Cholinergic receptor, nicotinic, alpha polypeptide 7 (<i>Chrna7</i>)
Breeding Protocol(s)	Sib-mate (homozygous lethal)
Protocol Date	9/17/12

MMRRC #420 PCR Reaction

	1X
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

Thermal Cycler:

94°C for 30 sec

60°C for 30 sec

72°C for 45 sec

35x

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

Bands: MUTANT: ~220bp after restriction digest
WT: ~440bp

Primer sequences 5' to 3':

P1 (420): cc tgg tcc tgc tgt gtt aaa ctg ctt c

P2 (420): ctg ctg gga aat cct agg cac act tga g

Reaction requires a restriction digest! Add 15 ul mix to each 25ul PCR rxn:

9.6ul HOH
4.0ul NEB Buffer 1, 10X
0.4ul BSA 100X
1.0ul **SacI**

Run on 2.0% agarose gel in TAE.