

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11647
MMRRC Strain Name	B6;129S5- <i>Galnt10</i> ^{Gt(neo)⁷⁸Lex} /Mmnc
Gene Name(s)	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (<i>Galnt10</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	12/6/12

MMRRC# 11647 PCR Reaction

2, 2 primer reactions: (P3+P5) for wild-type and (LTR+P3) for mutant reaction

Thermal Cycler:

94°C for 5 min
94°C for 15 sec
65°C for 30 sec, decrease 1 degree/cycle
72°C for 40 sec
steps 2-4, 10x
94°C for 15 sec
5°C for 30 sec
72°C for 40 sec
steps 5-7, 30x
72°C for 5 min

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
20 uM Primer F/R	2.0
Taq	0.5
ddH ₂ O	13.5
25mM MgCl ₂	2.0

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

Bands: MUTANT: 541bp
WT: 541bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

LTR (11647): AAATGGCGTTACTTAAGCTAGCTTGC

P3 (11647): AGGAAGACCACCAGCTCTAGGCCAAC

P5 (11647): AATTGGCATGAAGCCAGGAGGAGG

Run on 2.0% agarose gel in TAE.