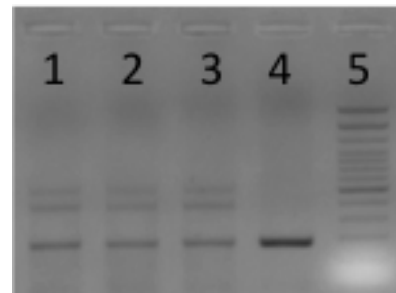


MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11606
MMRRC Strain Name	B6;129P2-B3gnt3 ^{tm1Dgen} /Mmnc
Gene Name(s)	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	6/11/21

PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2



1. Heterozygous Sample
 2. Heterozygous Sample
 3. Heterozygous Sample
 4. Wild-type Control
 5. 100bp Ladder (Invitrogen)
- Note: Non-specific band for mutant samples at ~600bp.

Thermal Cycler:

Step 1: 94C, 5min
 Step 2: 94C, 45sec
 Step 3: 60C, 45sec
 Step 4: 72C, 60sec
 Step 2 to 4 Cycles: 30
 Step 5: 72C, 7min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 288bp MUTANT: 505bp
 Note: Mutant mice have a non-specific band at ~600bp.

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

NIH1793(11606)1 GTGGGGCACCTGATCCAGAACGTGG
 NIH1793(11606)2 TGACACACGTGGGCTAGGGGGGAA
 NIH1793(11606)N GGGCCAGCTCATTCTCCCACTCAT