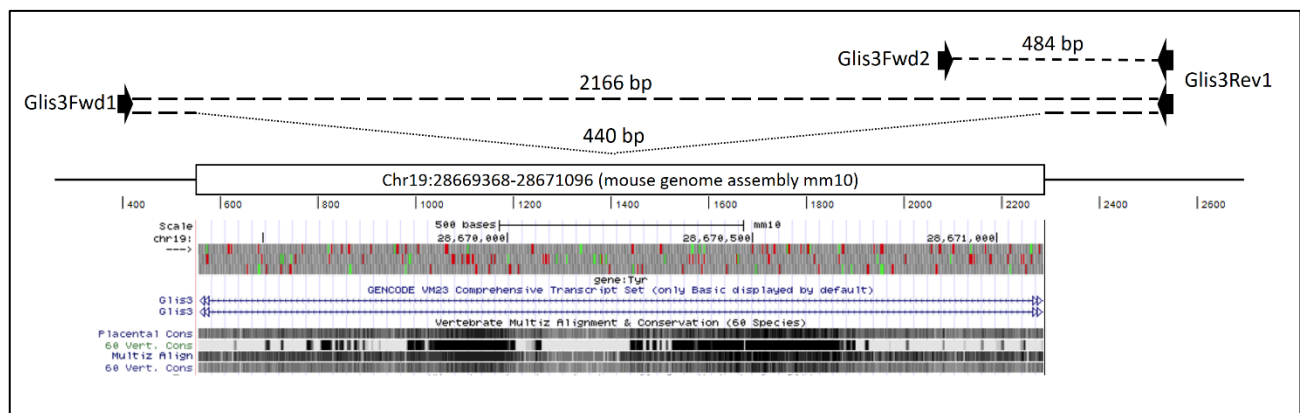# Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: $Rr^{Chr19Glis3\Delta1729Mgn}/Vu$

Investigator: Mark Magnuson
Genome edit: Chr19:28669368-28671096 (mm10)
Common allele name: $Rr^{Chr19Glis3\Delta1729Mgn}/Vu$

PCR Primers:

Glis3Fwd1: GCACCATATACCTGACCGATG
Glis3Fwd2: CTAAGCTGTAGCGCAGAAACTC
Glis3Rev1: CGTCTTGGCCATACTTATACCTG

Predicted PCR product sizes:

Homozygous = $Rr^{Chr19Glis3\Delta1729Mgn}/Vu$ = 440 bp
Heterozygous = $Rr^{Chr19Glis3\Delta1729Mgn}/Vu$ = 440 bp and 484 bp
WT = 484 bp

The following PCR program does not amplify the full length WT product of 2166 bp, but extension times can be adjusted if desired.

Component	25 ul reaction	Final concentration	PCR program
5X Phusion Reaction Buffer (NEB #M0530)	5.0 µL	1X	98°C, 30 seconds
10 mM dNTPs	0.5 µL	200 µM	98°C, 10 seconds
10 µM Glis3_Rev1	1.25 µL	0.5 µM	63°C, 10 seconds
10 µM Glis3_Fwd1	0.625 µL	0.25 µM	72°C, 30 seconds
10 µM Glis3_Fwd2	0.625 µL	0.25 µM	Go to 2, 35 X
Phusion DNA Polymerase (NEB #M0530)	0.25 µL	0.02 U/µl	72°C, 2 minutes
Nuclease-free water	16.25 µL		4°C, ∞
Genomic DNA	0.5 µL	Less than 1 µg	

HET HET HET HOMO WT

600
500
400

WT (484 bp)
$Rr^{Chr19Glis3\Delta1729}$ (440 bp)