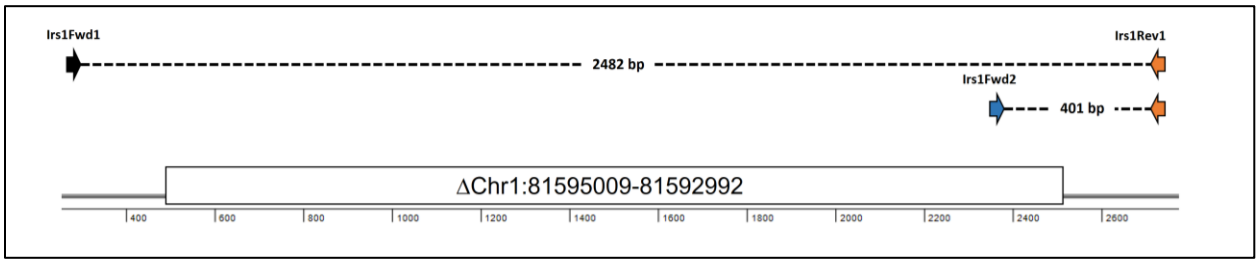
Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: *Rr*^{413em1Mgn}*/Vu*

Investigator: Mark Magnuson
Genome edit: ΔChr1:81595009-81592992 (mm10)
Common allele name: *Rr413*^{em1Mgn}*/Vu*



PCR Primers:

Irs1Fwd1: ACCTGCTTTCTGCTCTCCTCTC
Irs1Rev1: AGCTCTGCCCAAATGTTAAGGAG
Irs1CRFwd2: CCACCTCCACTACCACCTATATG

Predicted PCR product sizes:

Homozygous *Rr413*^{em1Mgn}*/Vu* =	464 bp (= 2482 bp – 2018 bp)
Heterozygous *Rr413*^{em1Mgn}*/Vu* =	464 bp + 401 bp + weak or absent 2482 bp
WT =	401 bp + weak or absent 2482 bp

Component	25 ul reaction	Final concentration	PCR program
5X Phusion Reaction Buffer (NEB #M0530S)	5.0 µL	1X	98ºC, 30 seconds
10 mM dNTPs	0.5 µL	200 µM	98ºC, 10 seconds
10 µM Irs1Rev1	1.25 µL	0.5 µM	66ºC, 10 seconds
10 µM Irs1Fwd1	0.625 µL	0.25 µM	72ºC, 30 seconds
10 µM Irs1CRFwd2	0.625 µL	0.25 µM	Go to 2, 38 X
Phusion DNA Polymerase (NEB #M0530S)	0.25 µL	0.02 U/µl	72ºC, 2 minutes
Nuclease-free water	16.25 µL		4ºC, ∞
Genomic DNA	0.5 µL	Less than 1 µg