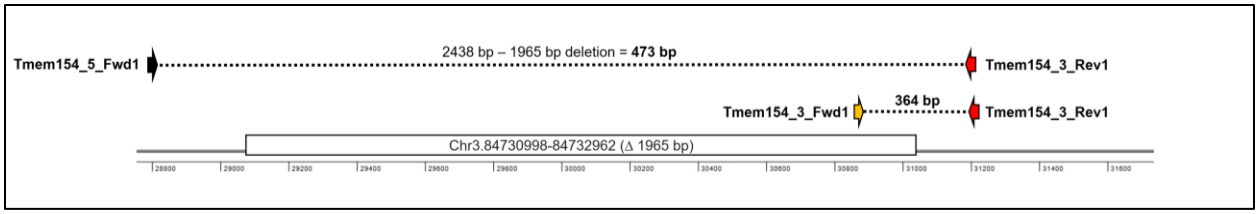
Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: *Rr414^{em1Mgn}/Vu*

Investigator: Mark Magnuson
Genome edit: Chr3:Δ84730998-84732962 (mm10)
Common allele name: *Rr414^{em1Mgn}*



PCR Primers:

Tmem154_5_Fwd1: GCTTCACCTCTGACTTCAGGAGT
Tmem154_3_Fwd1: AGGCTTGGCAGGTGTTTATGG
Tmem154_3_Rev1: ACCTGACTGCTGCTTACTCTGTCG

Predicted PCR product sizes:

Homozygous = *Rr414*^{em1Mgn} = 473 bp
Heterozygous = *Rr414*^{em1Mgn} = 473 bp and 364 bp
WT = 364 bp

Component	25 ul reaction	Final concentration	PCR program
5X Phusion Reaction Buffer (NEB #M0530)	5.0 µL	1X	98ºC, 30 seconds
10 mM dNTPs	0.5 µL	200 µM	98ºC, 10 seconds
10 µM Tmem154_5_Rev1	1.25 µL	0.5 µM	66ºC, 10 seconds
10 µM Tmem154_5_Fwd1	0.625 µL	0.25 µM	72ºC, 30 seconds
10 µM Tmem154_3_Rev1	0.625 µL	0.25 µM	Go to 2, 35 X
Phusion DNA Polymerase (NEB #M0530)	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