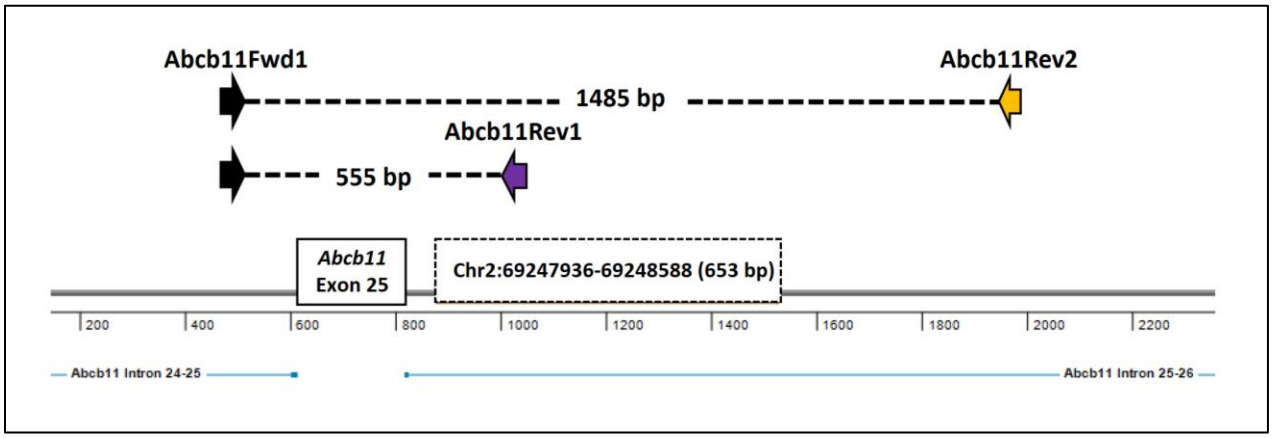
# Vanderbilt Genome Editing Resource

## GENOTYPING PROTOCOL: *Rr327em1Mgn/Vu*

**Investigator:** Mark Magnuson
**Genome edit:** ΔChr2:69247936-69248588 (mm10)
**Common allele name:** *Rr327$^{em1Mgn}$/Vu*




**PCR Primers:**

Abcb11Fwd1: GTCACCCACTGAGTTGCTGTC
Abcb11Rev1: GTCAGCAGGACATCTGGAACTG
Abcb11Rev2: CAGATCTCTAGGACAATGCACTTCC

**Predicted PCR product sizes:**

Homozygous *Rr327$^{em1Mgn}$/Vu* = 832 bp (1485 bp – 653 bp)
Heterozygous *Rr327$^{em1Mgn}$ /Vu* = 832 bp + 555 bp + weak or absent 1485 bp
WT = 555 bp + weak or absent 1485 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 Abcb11Fwd1 | 1.25 µL | 0.5 µM | 65ºC, 10 seconds |
| 10 µM Abcb11Rev1 | 0.625 µL | 0.25 µM | 72ºC, 30 seconds |
| 10 µM Abcb11Rev2 | 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 | |