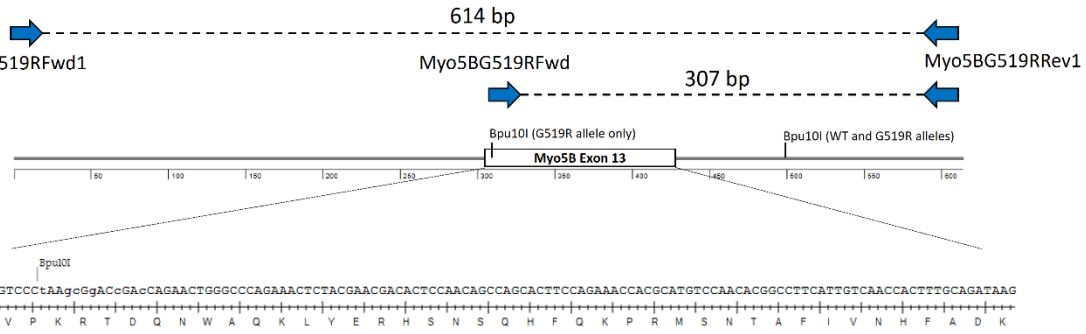
Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: *Myo5B G519R*

Investigator: James Goldenring
Genome Edit: *Myo5B*^{G519R}
Allele name: *Myo5B*^{em1Jrgo}

Overview of genotyping strategy:



Bpu10I cleavage products for 614 bp Myo5BG519RFwd1-Rev1 PCR assay:

WT = 499 and 115 bp
Myo5B G519R = 310, 189, 115 bp

Assay for detection of *Myo5B G519R* allele:

This assay will not differentiate between heterozygous and homozygous animals. Nucleotides in red are specific to the designed Myo5B G519R edited allele.

PCR Primers: Myo5BG519RFwd: CCTAAGCGGACCGACCAGAAC
Myo5BG519RRev1: GTGCCTCGTCTTCTCAACAGTCTG

PCR product = 307 bp

Components	50 ul reaction	Final concentration	PCR program*
5x Phusion Reaction Buffer (NEB #M0530S)	10.0 µL	1X	98ºC, 30 seconds
10 mM dNTPs	1.0 µL	200 µM	98ºC, 10 seconds
10 µM Primer forward	2.5 µL	0.5 µM	67ºC, 10 seconds
10 µM Primer reverse	2.5 µL	0.5 µM	72ºC, 30 seconds
Phusion DNA Polymerase (NEB #M0530S)	0.5 µL	0.02 U/µl	Go to 2, 35 X
Nuclease-free water	32.5 µL		72ºC, 2 minutes
Genomic DNA	1.0 µL	Less than 1 µg	4ºC, ∞

Assay for detection of *Myo5B G519R* allele:

This PCR assay amplifies both WT and Myo5B G519R alleles. Digestion with Bpu10I can be used to detect heterozygous versus homozygous Myo5B G519R animals.

PCR Primers: Myo5BG519RFwd1: GAGCGTCTTCAGACAACTCCACA
Myo5BG519RRev1: GTGCCTCGTCTTCTCAACAGTCTG

PCR product = 614 bp

Components	50 ul reaction	Final concentration	PCR program*
5x Phusion Reaction Buffer (NEB #M0530S)	10.0 µL	1X	98ºC, 30 seconds
10 mM dNTPs	1.0 µL	200 µM	98ºC, 10 seconds
10 µM Primer forward	2.5 µL	0.5 µM	67ºC, 10 seconds
10 µM Primer reverse	2.5 µL	0.5 µM	72ºC, 30 seconds
Phusion DNA Polymerase (NEB #M0530S)	0.5 µL	0.02 U/µl	Go to 2, 35 X
Nuclease-free water	32.5 µL		72ºC, 2 minutes
Genomic DNA	1.0 µL	Less than 1 µg	4ºC, ∞

If you use Phusion HF polymerase you can add 0.5 ul Bpu10I enzyme directly to 10 µl PCR product and incubate for 1 hour at 37°C. Use of other polymerases may require purification of the PCR product prior to digestion using the Bpu10I buffer provided by NEB. Run digested PCR samples on a 2% or greater agarose gel to separate digest products. Optimization may be required to reliably assess zygosity.