Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: *Sox17CR2m3*

Investigator: Mark Magnuson
Genome Edit: *Sox17CR2m3*
Allele Name: *Sox17*^{em4Mgn}

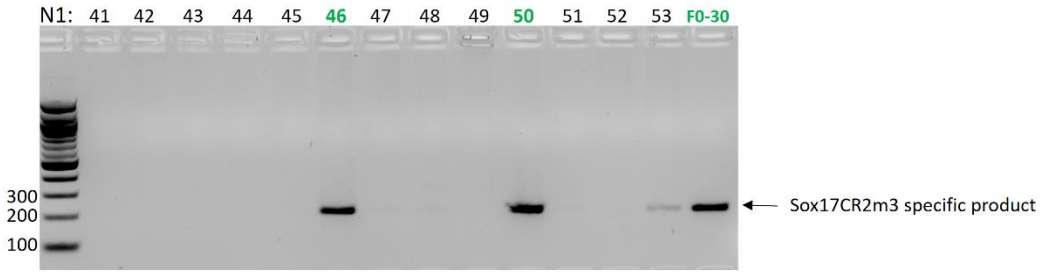
PCR assay to detect the Sox17CR2m3 allele:

Name	Primer sequence
Sox17CR2m3Fwd	GACCACTGGCGTTTATTTTGC
Sox17CR2Rev2	CCAGAGGAACTCGTAAAGCTG

WT = no product, *Sox17CR2m3* = 209 bp
This PCR assay will not differentiate between heterozygous and homozygous mice

PCR assay:

Polymerase	EconoTaq PLUS GREEN 2x Master Mix Lucigen Catalog #3033-1
Annealing temperature	55°C
Cycling Conditions	Initial denaturation, 94°C, 2 min = 1 cycle Denaturation, 94°C, 30 seconds = 35 cycles Annealing, 55°C, 30 seconds Extension, 72°C, 20 seconds Final extension, 72°C, 5 minutes = 1 cycle Hold, 4°C, indefinitely



PCR assay to amplify the Sox17CR2m3 region for genotyping by Sanger sequencing:

Name	Primer sequence
Sox17CR2Fwd1	CTTGGACTTGTTCTTCAATCTTCC
Sox17CR2Rev2	CCAGAGGAACTCGTAAAGCTG

WT or *Sox17CR2m3* = 394 bp

Polymerase	Phusion High-Fidelity DNA Polymerase NEB, catalog #M0530S
Cycling Conditions	Initial denaturation, 98°C, 30 seconds = 1 cycle Denaturation, 98°C, 10 seconds = 35 cycles Annealing, 62°C, 10 seconds Extension, 72°C, 30 seconds Final extension, 72°C, 2 minutes = 1 cycle Hold, 4°C, indefinitely
Reaction components (company protocol recommendations)	1x Phusion GC buffer 200 uM dNTPs 0.5 uM Sox17CR2Fwd1 primer 0.5 uM Sox17CR2Rev2 primer 3% DMSO 1 unit per 50 ul PCR Phusion DNA polymerase Variable template DNA input Nuclease-free water to volume

Example results: