



Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: *Sox17CR2m5*

Investigator: Mark Magnuson
 Genome Edit: *Sox17CR2m5*
 Allele Name: *Sox17^{em5Mgn}*

PCR assay to detect the *Sox17CR2m5* allele:

Name	Primer sequence
Sox17CR2Fwd1	CTTGGACTTGTTCTTCAATCTTCC
Sox17CR2m5Rev1	TATGGACGCTTAACCGTTTCGAG

WT = no product, *Sox17CR2m5* = 257 bp

This PCR assay will not differentiate between heterozygous and homozygous mice

PCR assay:

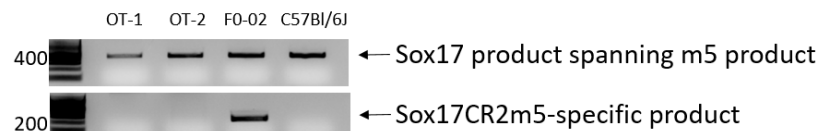
Polymerase	Phusion High-Fidelity DNA Polymerase NEB, catalog #M0530S
Cycling Conditions	Initial denaturation, 98°C, 30 seconds = 1 cycle Denaturation, 98°C, 10 seconds = 35 cycles Annealing, 62°C, 10 seconds Extension, 72°C, 20 seconds Final extension, 72°C, 2 minutes = 1 cycle Hold, 4°C, indefinitely
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

PCR assay to amplify the Sox17CR2m5 region for genotyping by Sanger sequencing and RFLP assay using MseI:

Name	Primer sequence
Sox17CR2Fwd1	CTTGGACTTGTTCTTCAATCTTCC
Sox17CR2Rev2	CCAGAGGAACTCGTAAAGCTG

WT or *Sox17CR2m5* = 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, 20 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



The Sox17CR2m5 mutation introduces a MseI site. If TransnetYX genotyping is no longer desired, RFLP assay may allow assessment of zygosity in this strain. Heterozygous mice have been tested, but not homozygous mice. Note in the image below, the target site was amplified with a forward primer slightly interior to Sox17CR2Fwd1, so the product size is only 341.

