GENOTYPING PROTOCOL

Investigator: Mark Magnuson
Genome Edit: Zfp329 KO
Allele name: *Zfp329*^{em1Mgn}

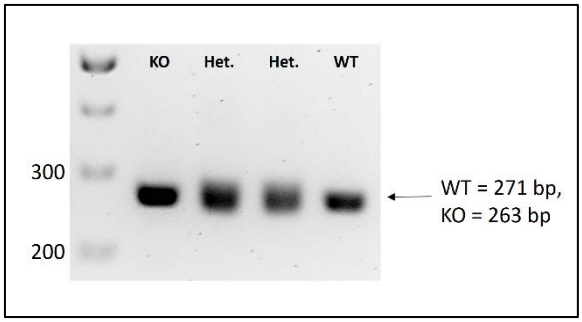
Primers:
Zfp329Fwd: ATGGAGGGATTTACAAGAGAGG
Zfp329Rev: GGCAACTATGTAAGGTTTGGTC

Predicted PCR Product: WT = 271 bp, KO = 263 bp

Validated PCR protocol:

20 µL PCR reaction		PCR program
5x Phusion HF buffer	= 4.0 µL	1. 98ºC, 2 min
10 mM dNTPs	= 0.4 µL	2. 98ºC, 10 sec
10 µM Zfp329Fwd:	= 1.0 µL	3. 64ºC, 15 sec
10 µM Zfp329Rev:	= 1.0 µL	4. 72ºC, 20 sec
Phusion Polymerase	= 0.2 µL	5. Go to 2, 35X
Nuclease-free water	= 12.4 µL	6. 72ºC, 7 min
Genomic DNA (about 50 ng)	= 1.0 µL	7. 4ºC, ∞

Run on agarose gel ≥ 2.5% (improved resolution with longer runs and higher agarose gel percentage)



Note that differentiating between the WT and KO is challenging on an agarose gel for this assay. Higher percentage agarose gels may allow for differentiation, but untested.

It is recommended to either sequence validate the samples or spike in a known WT PCR product at a 50:50 ratio in all the non-heterozygous samples, heat to 95°C for 10 minutes, then allow to cool to room temperature. Run the randomly annealed PCR product into a 10% TBE gel and identify KO samples by presence of a heterozygous doublet.