



GENOTYPING PROTOCOL

Investigator: Mark Magnuson

Genome Edit: Zfhx4 KO

Allele name: *Zfhx4^{em1Mgn}*

Primers:

Zfhx4Fwd-1: CGATGGTTGTGTTAGTGATGG

Zfhx4Rev-1: GGATCAGGTCCTATGAGGTTTG

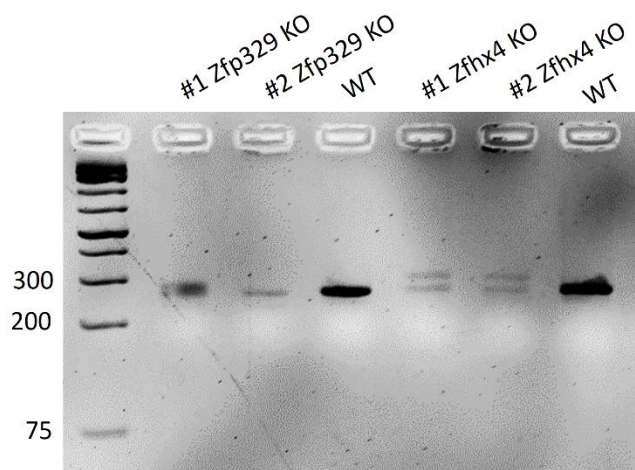
Predicted PCR Product: WT = 279 bp, KO = 272 bp

Validated PCR protocol:

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5 X Q5 Reaction Buffer	= 10 µL	1. 98°C, 30 seconds
10 mM dNTPs	= 1.0 µL	2. 98°C, 10 seconds
10 µM Zfhx4Fwd-1	= 2.5 µL	3. 71°C, 10 seconds
10 µM Zfhx4Rev-1	= 2.5 µL	4. 72°C, 20 seconds
Q5 High-Fidelity DNA Polymerase	= 0.5 µL	5. Go to 2, 35 X
Nuclease-free water	= 32.5 µL	6. 72°C, 2 minutes
+ 1 ul gDNA (about 50 ng/ul)		7. 4°C, ∞

Run on agarose gel ≥ 3.0% (improved resolution with longer runs and higher agarose gel percentage)



Both males used for cryopreservation are confirmed heterozygous, however, the allele appears to be an insertion—not a deletion. This line has been sequence confirmed multiple times.