



GENOTYPING PROTOCOL

Investigator: Mark Magnuson

Genome Edit: Jazf1 KO

Allele name: *Jazf1^{em1Mgn}*

Primers:

Jazf170-D: GCTCTCGATGTAGCACCATGACAG (spans 5'UTR and Exon 2)

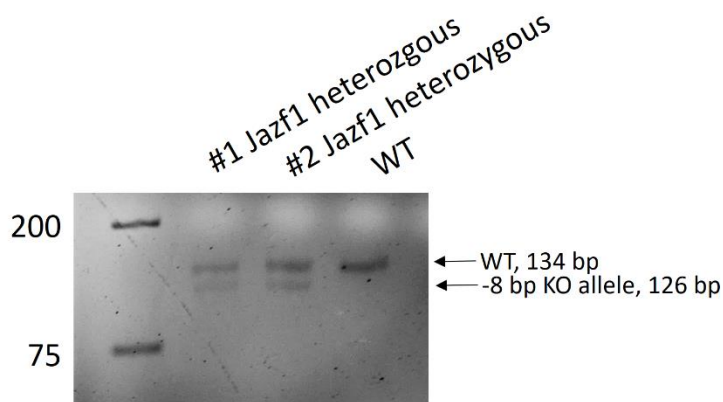
Jazf170-R: CACCGATGTGGTTGTCCTCGATG (nts in red are in intron 1-2, rest is in Exon 2)

Predicted PCR Product: WT = 134 bp, KO = 126 bp

Validated PCR protocol:

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 Jazf170-D	= 2.5 µL	3. 71°C, 10 seconds
10 µM Jazf170-R	= 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)



Note that these were the two males used for sperm cryopreservation. Both are heterozygous.