GENOTYPING PROTOCOL

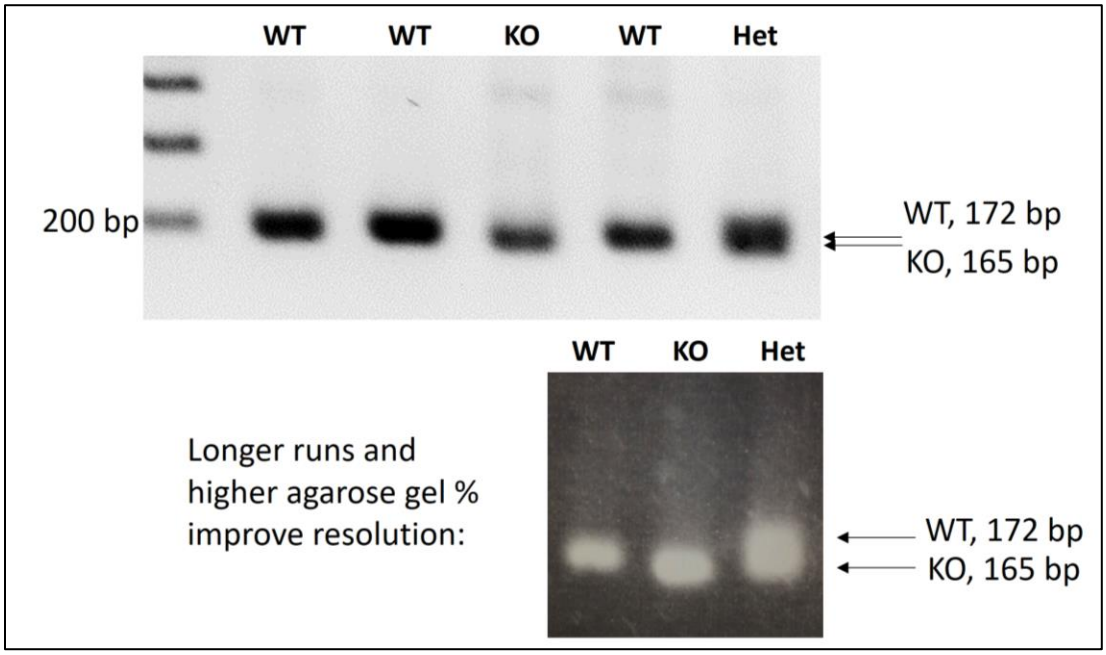
Investigator: Mark Magnuson
Genome Edit: Zfp92 KO
Allele name: *Zfp92*^{em1Mgn}

Primers:
KD2-Fwd: AATTCCCGACCACATAACTG
KD-2 Rev: GCAAGAAAGTTCCAAAGCAGAGTC

Predicted PCR Product: WT = 172 bp, KO = 165 bp

PCR protocol:

20 µL PCR reaction		PCR program
5x Phusion HF buffer	= 4 µL	1. 98ºC, 30 sec
10 mM dNTPs	= 0.4 µL	2. 98ºC, 10 sec
10 µM KD2-F2:	= 1.0 µL	3. 61ºC, 15 sec
10 µM Zfp92 7bpRev:	= 1.0 µL	4. 72ºC, 20 sec
DMSO	= 0.6 µL	5. Go to 2, 40 X
Phusion Polymerase	= 0.2 µL	6. 72ºC, 7 min
Water	= 11.8 µL	7. 4ºC, ∞
Genomic DNA (about 50 ng)	= 1.0 µL	



Knockout Allele Confirmation PCR:
When WT and KO bands cannot be clearly distinguished this assay will amplify only the KO allele.

Primers:

KD2-F2:	CATGCTGCTTGCCTGAGTTTCC
Zfp92 7bp Rev:	GATGGCTGTAGTTCTCACTTGC

Predicted PCR Product: WT = none, KO = 250 bp

PCR protocol:

20 µL PCR reaction		PCR program
5x Phusion HF buffer	= 4 µL	1. 98ºC, 2 min
10 mM dNTPs	= 0.4 µL	2. 98ºC, 10 sec
10 µM KD2-F2:	= 1.0 µL	3. 67ºC, 15 sec
10 µM Zfp92 7bpRev:	= 1.0 µL	4. 72ºC, 15 sec
DMSO	= 0.6 µL	5. Go to 2, 40 X
Phusion Polymerase	= 0.2 µL	6. 72ºC, 7 min
Water	= 11.8 µL	7. 4ºC, ∞
Genomic DNA (about 50 ng)	= 1.0 µL	