



# GENOTYPING PROTOCOL

**Investigator:** Mark Magnuson

**Genome Edit:** Zfp800 KO

**Allele name:** *Zfp800<sup>em1Mgn</sup>*

## Primers:

Zfp800Fwd: ATGGGTTTGTGCTTCTCTGTCC

Zfp800Rev: GTGAAGGAGAGCAAAGACACTGG

**Predicted PCR Product:** WT = 188 bp, KO = 172 bp

## Validated PCR protocol:

### 20 $\mu$ L PCR reaction

5x Phusion HF buffer	= 4.0 $\mu$ L
10 mM dNTPs	= 0.4 $\mu$ L
10 $\mu$ M Zfp800Fwd:	= 1.0 $\mu$ L
10 $\mu$ M Zfp800Rev:	= 1.0 $\mu$ L
Phusion Polymerase	= 0.2 $\mu$ L
Nuclease-free water	= 12.4 $\mu$ L
Genomic DNA (about 50 ng)	= 1.0 $\mu$ L

### PCR program

1. 98°C, 2 min
2. 98°C, 10 sec
3. 69°C, 15 sec
4. 72°C, 15 sec
5. Go to 2, 35X
6. 72°C, 7 min
7. 4°C, $\infty$

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

