

Hfe Cas9-CKO Strategy

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Project Overview

Project Name

Hfe

Project type

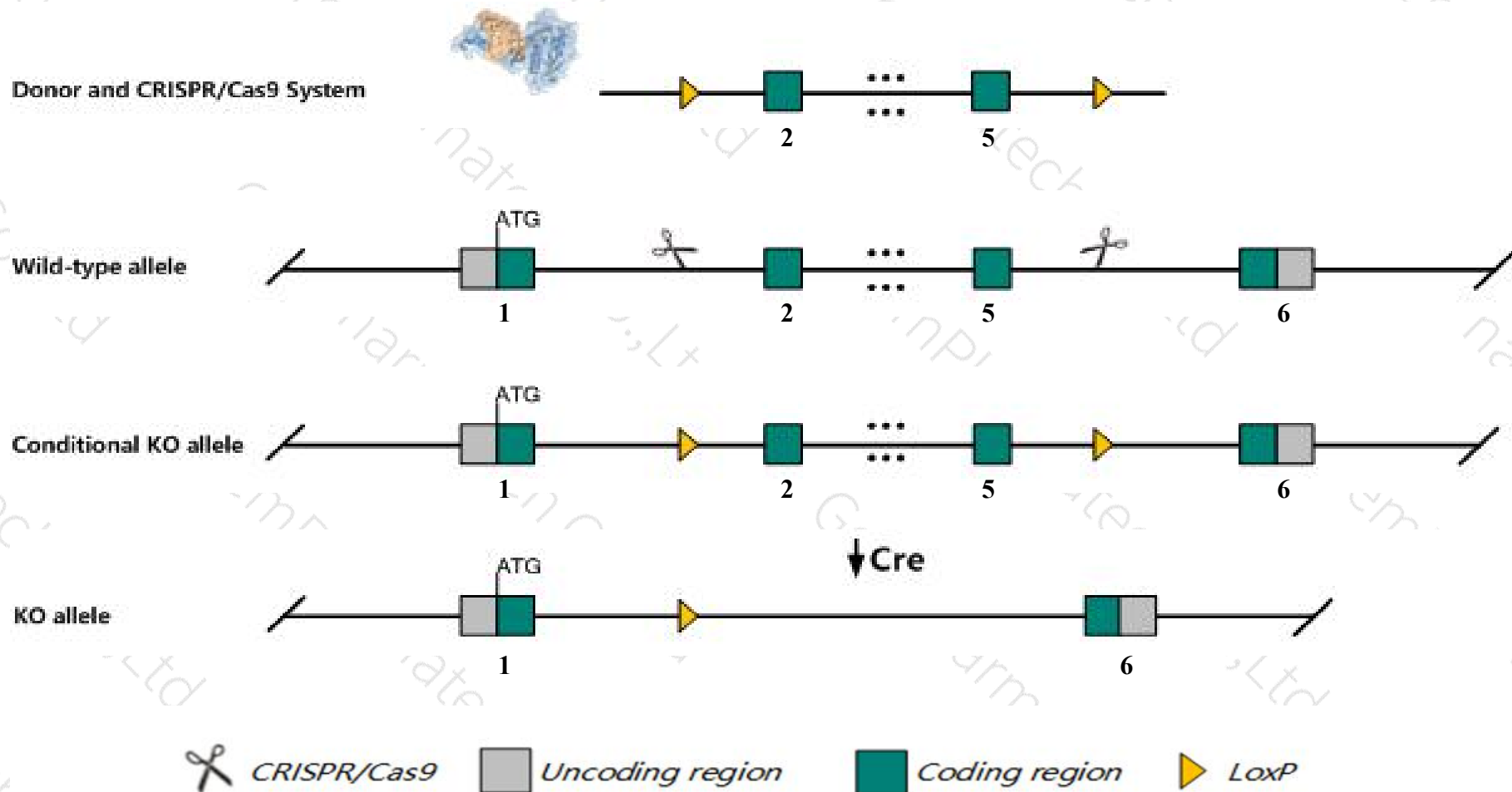
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hfe* gene. The schematic diagram is as follows:



- The *Hfe* gene has 4 transcripts. According to the structure of *Hfe* gene, exon2-exon5 of *Hfe*-202 (ENSMUST00000091706.13) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Hfe* gene. The brief process is as follows: CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- According to the existing MGI data, Mutation of this gene affects iron metabolism. Homozygotes for targeted null mutations exhibit increased intestinal iron absorption and an elevated hepatic iron load but reduced duodenal iron stores. Heterozygotes also accumulate more iron than normal.
- The KO region deletes most of the coding sequence, but does not result in frameshift.
- The *Hfe* gene is located on the Chr13. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Hfe homeostatic iron regulator [*Mus musculus* (house mouse)]

Gene ID: 15216, updated on 22-Oct-2019

Summary

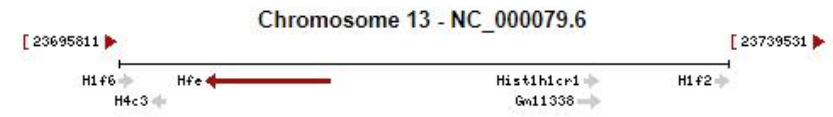
- Official Symbol** Hfe provided by [MGI](#)
- Official Full Name** homeostatic iron regulator provided by [MGI](#)
- Primary source** [MGI:MGI:109191](#)
- See related** [Ensembl:ENSMUSG000000006611](#)
- Gene type** protein coding
- RefSeq status** VALIDATED
- Organism** [Mus musculus](#)
- Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as** MR2
- Expression** Broad expression in subcutaneous fat pad adult (RPKM 24.7), liver adult (RPKM 10.9) and 19 other tissues [See more](#)
- Orthologs** [human](#) [all](#)

Genomic context

Location: 13 A3.1; 13 9.88 cM [See Hfe in Genome Data Viewer](#)

Exon count: 8

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	13	NC_000079.6 (23702034..23710854, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	13	NC_000079.5 (23795710..23802680, complement)

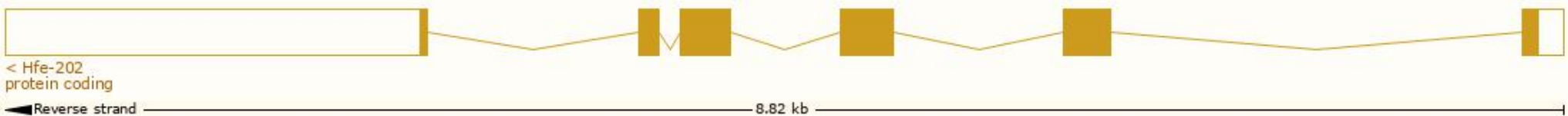


Transcript information (Ensembl)

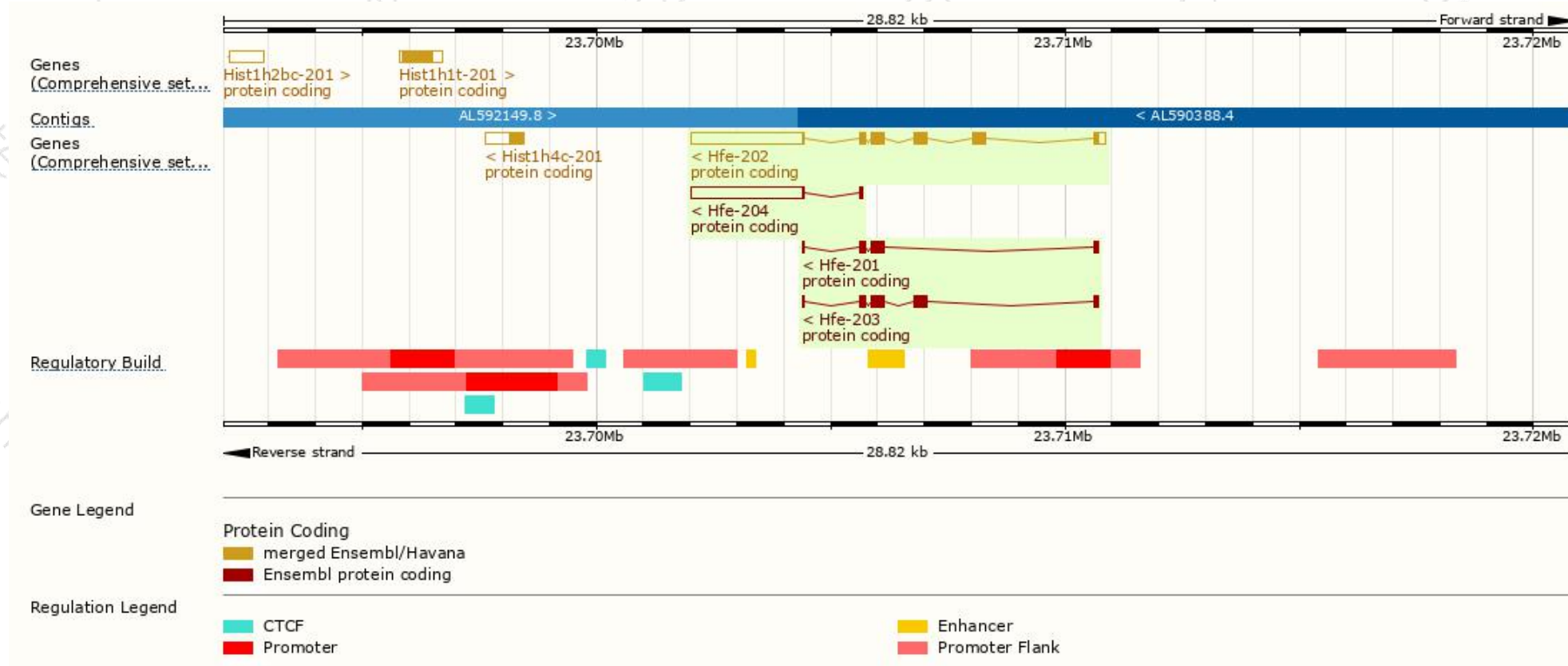
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hfe-202	ENSMUST00000091706.13	3572	359aa	Protein coding	CCDS26360	P70387	TSL:1 GENCODE basic APPRIS P1
Hfe-203	ENSMUST00000091707.12	816	271aa	Protein coding	CCDS84012	Q5SZ87	TSL:5 GENCODE basic
Hfe-204	ENSMUST00000151243.1	2458	35aa	Protein coding	-	F7CW53	CDS 5' incomplete TSL:1
Hfe-201	ENSMUST00000006787.7	516	171aa	Protein coding	-	Q5SZ91	TSL:5 GENCODE basic

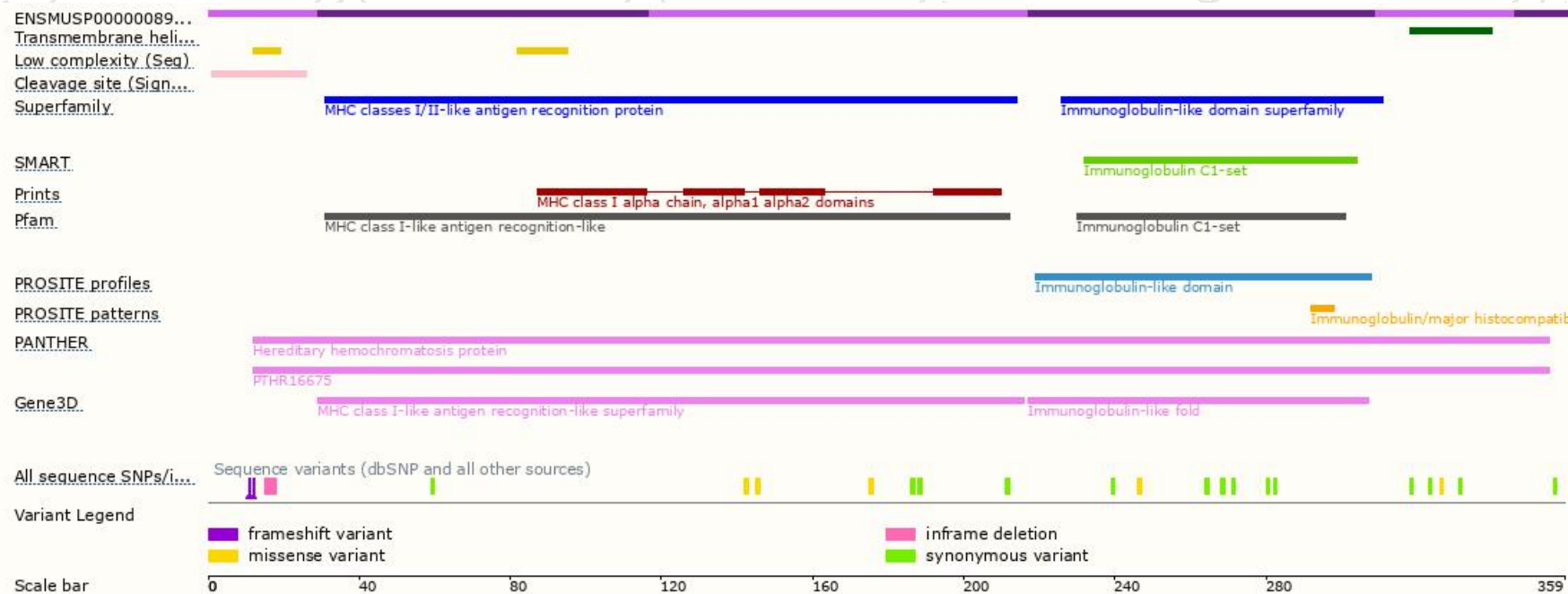
The strategy is based on the design of *Hfe-202* transcript,The transcription is shown below



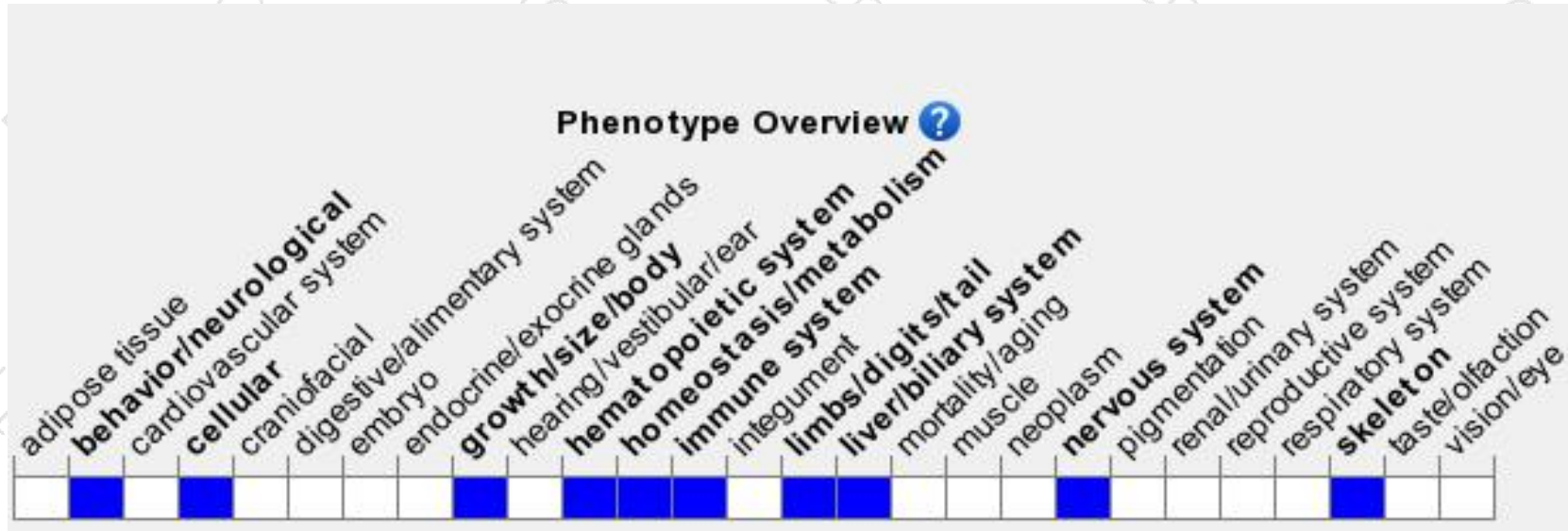
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, Mutation of this gene affects iron metabolism. Homozygotes for targeted null mutations exhibit increased intestinal iron absorption and an elevated hepatic iron load but reduced duodenal iron stores. Heterozygotes also accumulate more iron than normal.

If you have any questions, you are welcome to inquire.

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