

***Hint2* Cas9-CKO Strategy**

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Project Overview

Project Name

Hint2

Project type

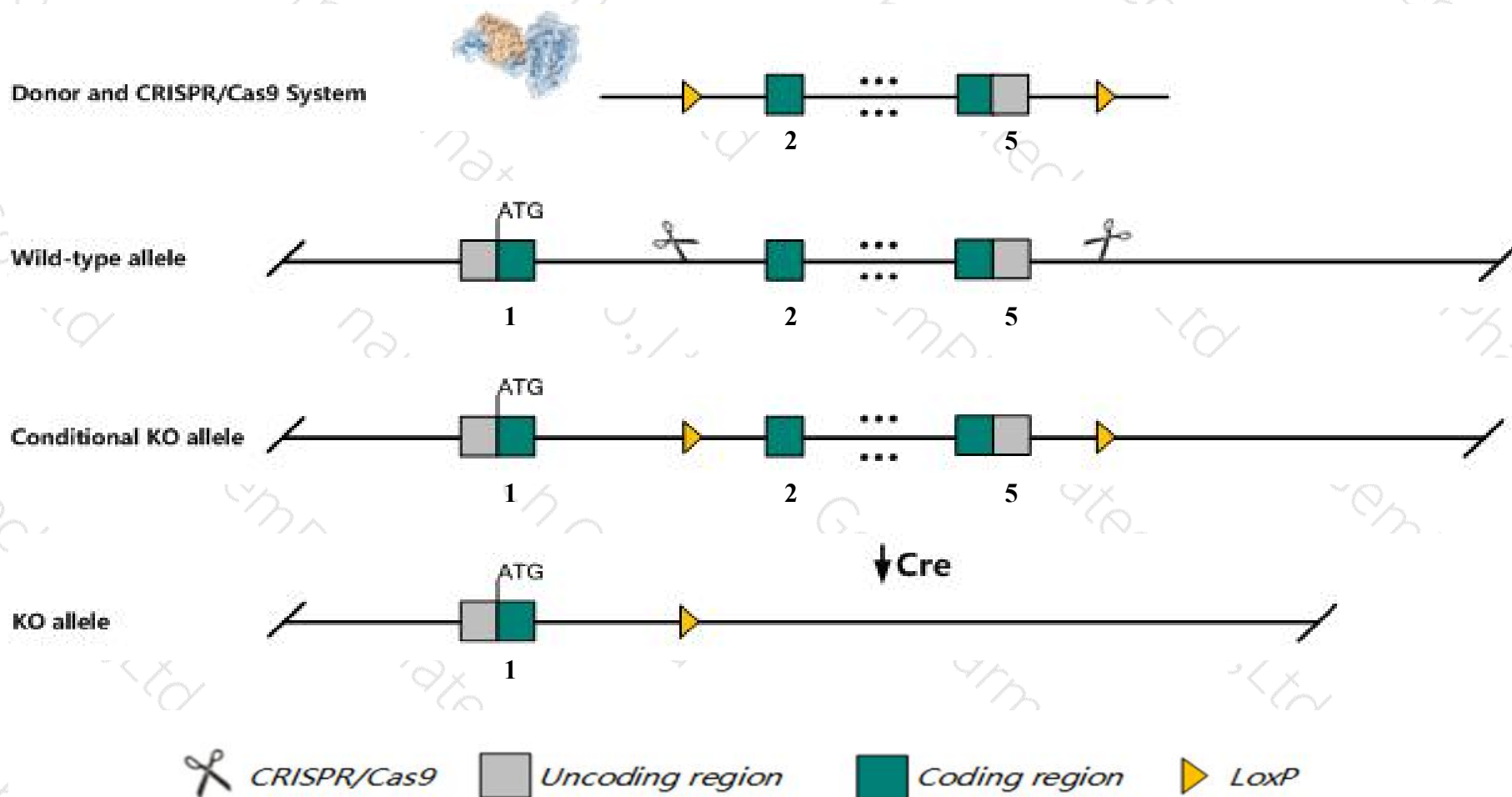
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hint2* gene. The schematic diagram is as follows:



Technical routes

- The *Hint2* gene has 7 transcripts. According to the structure of *Hint2* gene, exon2-exon5 of *Hint2*-201(ENSMUST00000030192.4) 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 *Hint2* 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, mice homozygous for a knock-out allele exhibit alterations in lipid metabolism, glucose homeostasis, and mitochondrial respiration.
- The floxed region is very close to the N-terminal of the *Spag8* gene, this strategy may influence the regulatory function of the N-terminal of *Spag8* gene.
- The *Hint2* gene is located on the Chr4. 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)

Hint2 histidine triad nucleotide binding protein 2 [Mus musculus (house mouse)]

Gene ID: 68917, updated on 13-Mar-2020

Summary



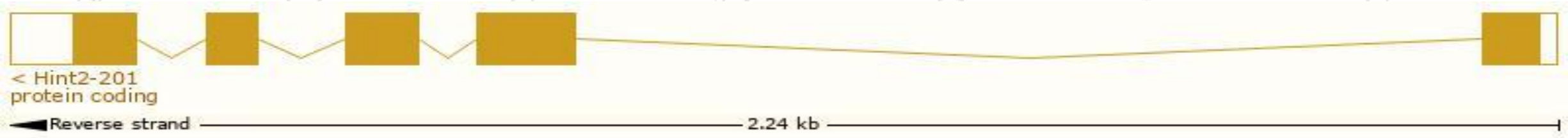
Official Symbol	Hint2 provided by MGI
Official Full Name	histidine triad nucleotide binding protein 2 provided by MGI
Primary source	MGI:MGI:1916167
See related	Ensembl:ENSMUSG00000028470
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1190005L05Rik
Expression	Ubiquitous expression in kidney adult (RPKM 107.6), liver adult (RPKM 61.1) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

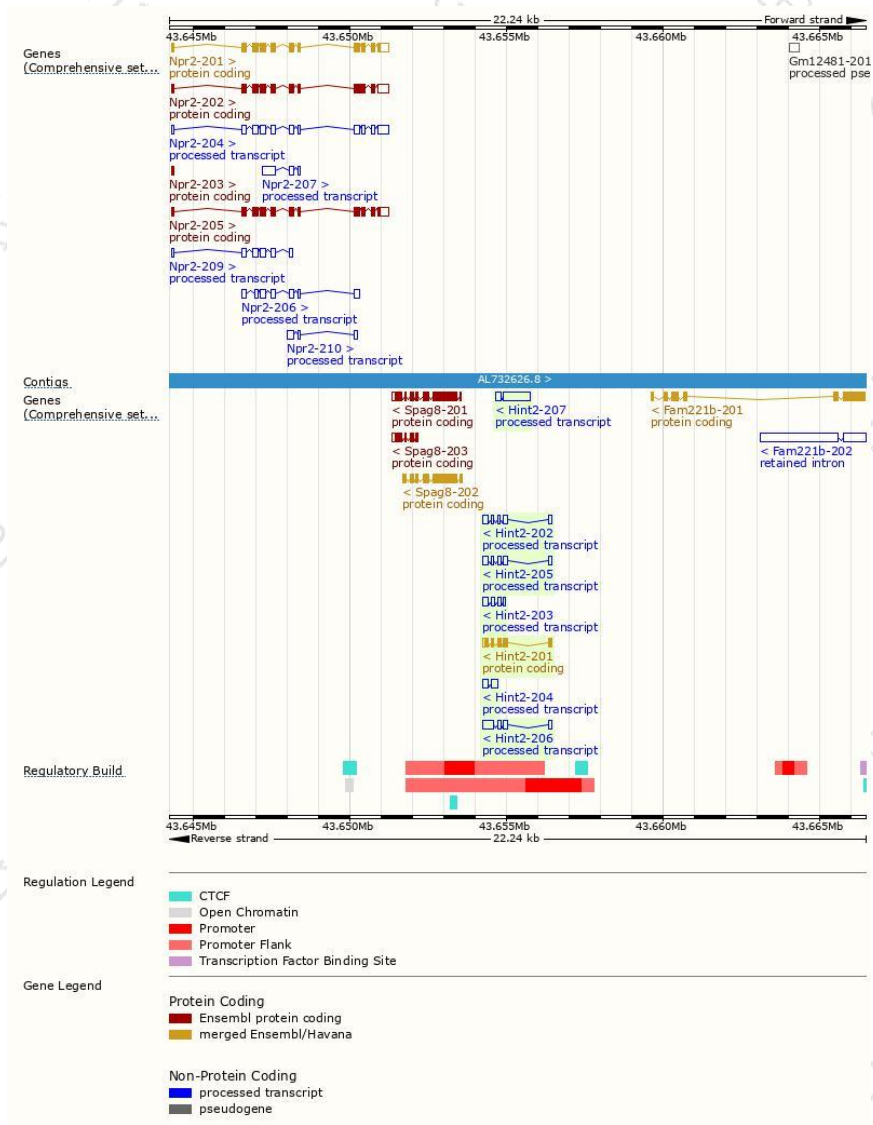
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hint2-201	ENSMUST00000030192.4	610	163aa	Protein coding	CCDS18106	Q5M9J2 Q9D0S9	TSL:1 GENCODE basic APPRIS P1
Hint2-207	ENSMUST00000155985.1	985	No protein	Processed transcript	-	-	TSL:3
Hint2-206	ENSMUST00000151514.1	692	No protein	Processed transcript	-	-	TSL:2
Hint2-205	ENSMUST00000151238.7	600	No protein	Processed transcript	-	-	TSL:5
Hint2-202	ENSMUST00000130593.7	564	No protein	Processed transcript	-	-	TSL:5
Hint2-203	ENSMUST00000134050.7	442	No protein	Processed transcript	-	-	TSL:5
Hint2-204	ENSMUST00000134082.1	398	No protein	Processed transcript	-	-	TSL:2

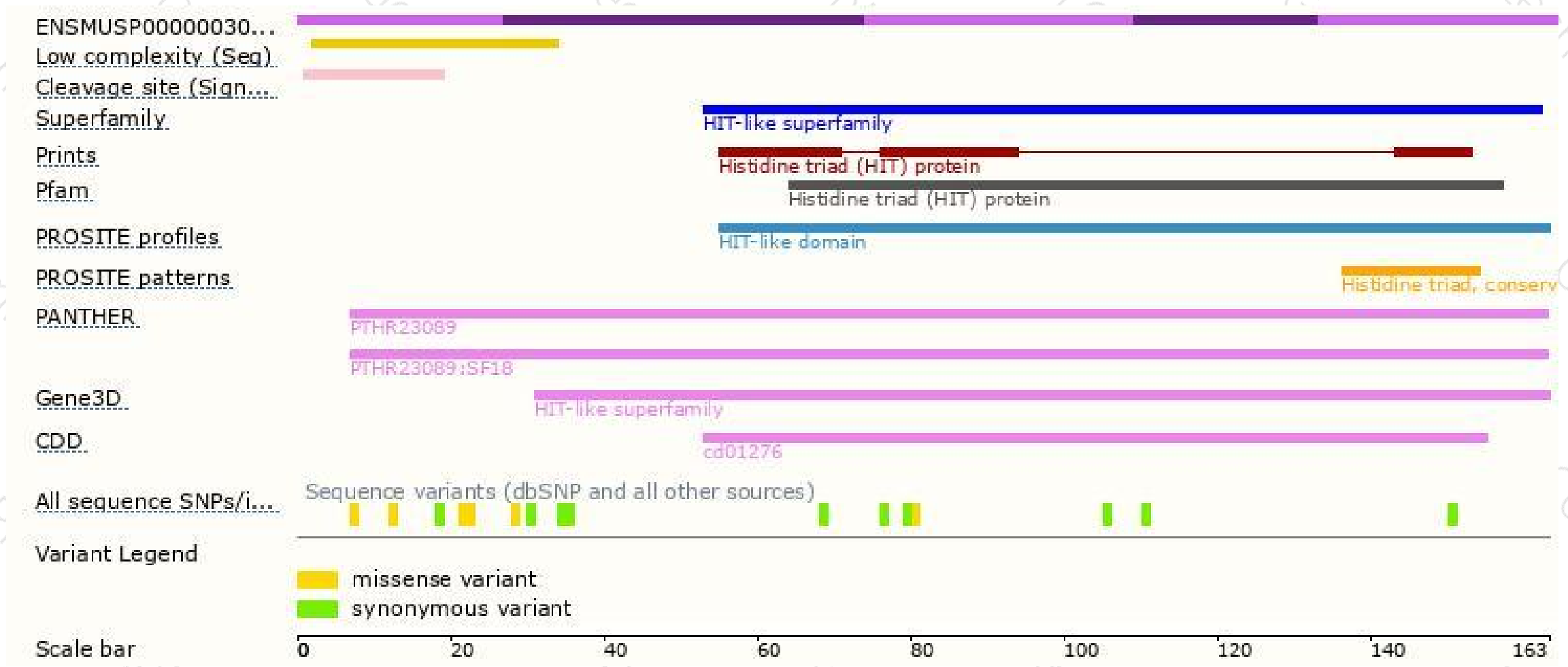
The strategy is based on the design of *Hint2-201* transcript,the transcription is shown below:



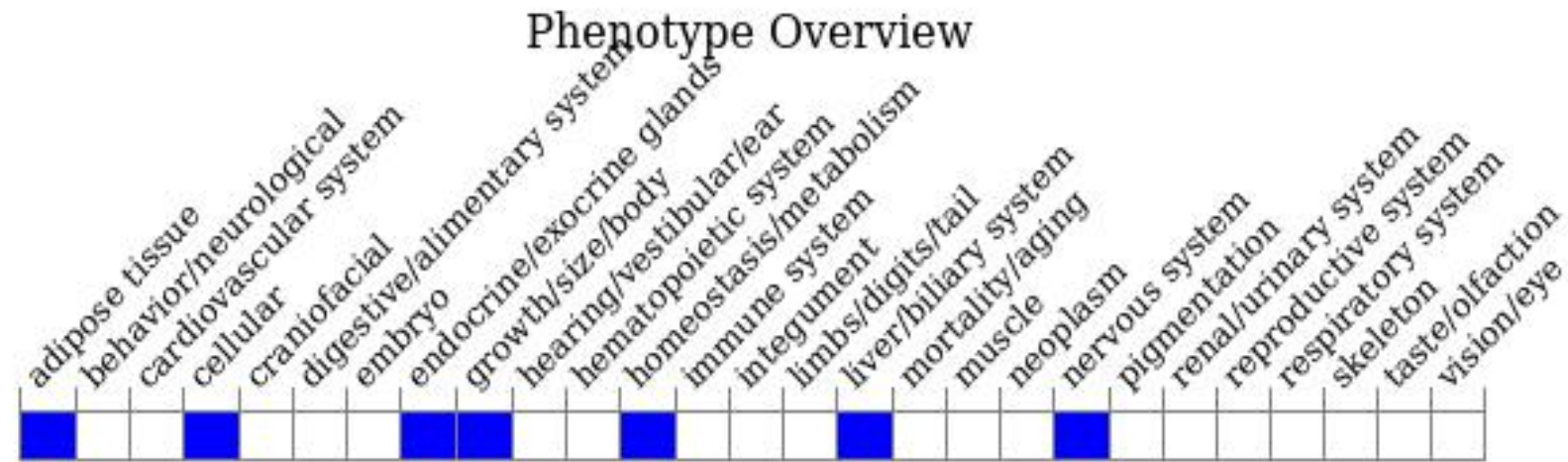
Genomic location distribution



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, mice homozygous for a knock-out allele exhibit alterations in lipid metabolism, glucose homeostasis, and mitochondrial respiration.

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

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