Hba-x Cas9-CKO Strategy

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Project Overview

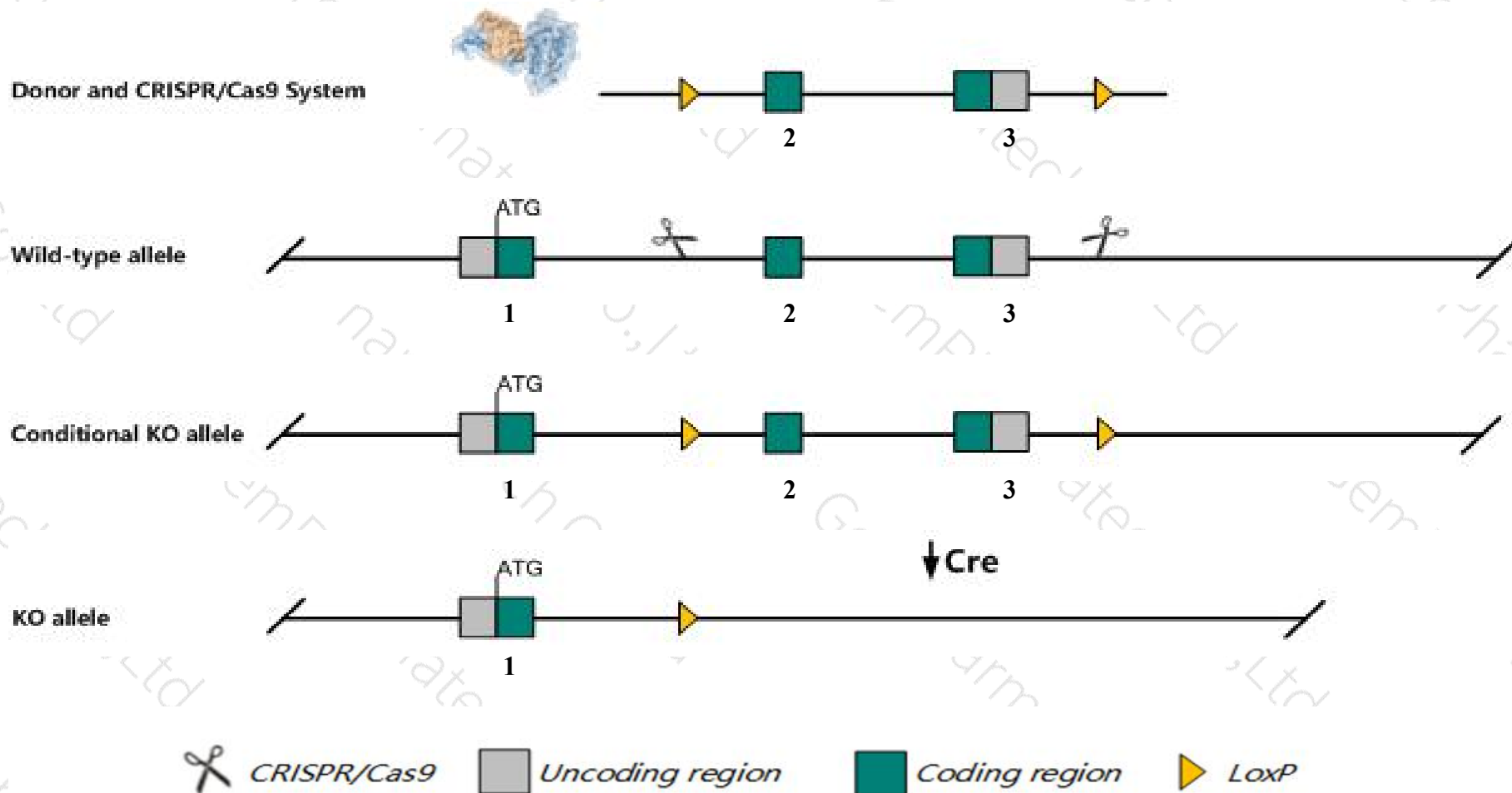|	
Project Name	*Hba-x*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Technical routes

➤ The *Hba-x* gene has 2 transcripts. According to the structure of *Hba-x* gene, exon2-exon3 of *Hba-x-201*(ENSMUST00000020531.8) transcript is recommended as the knockout region.The region contains 334bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Hba-x* 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele show background sensitive prenatal lethality, variation in postnatal size and pallor, reduced litter size, and a thalassemia-like picture with anisocytosis, poikilocytosis, polychromasia, reduced mean corpuscular volume and hemoglobin, and increased platelet count.

➢ The *Hba-x* gene is located on the Chr11. 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）

Hba-x hemoglobin X, alpha-like embryonic chain in Hba complex [Mus musculus (house mouse)]

Gene ID: 15126, updated on 13-Mar-2020

▲ Summary

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Official Symbol	Hba-x provided by MGI
Official Full Name	hemoglobin X, alpha-like embryonic chain in Hba complex provided by MGI
Primary source	MGI:MGI:96019
See related	Ensembl:ENSMUSG00000055609
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	AI450015, Hbz
Expression	Biased expression in placenta adult (RPKM 2628.2) and CNS E11.5 (RPKM 744.9)See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hba-x-201	ENSMUST00000020531.8	801	142aa	Protein coding	CCDS24522	P06467 Q78PA4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Hba-x-202	ENSMUST00000145569.1	579	156aa	Protein coding	-	A7M7S6	CDS 5' incomplete TSL:3

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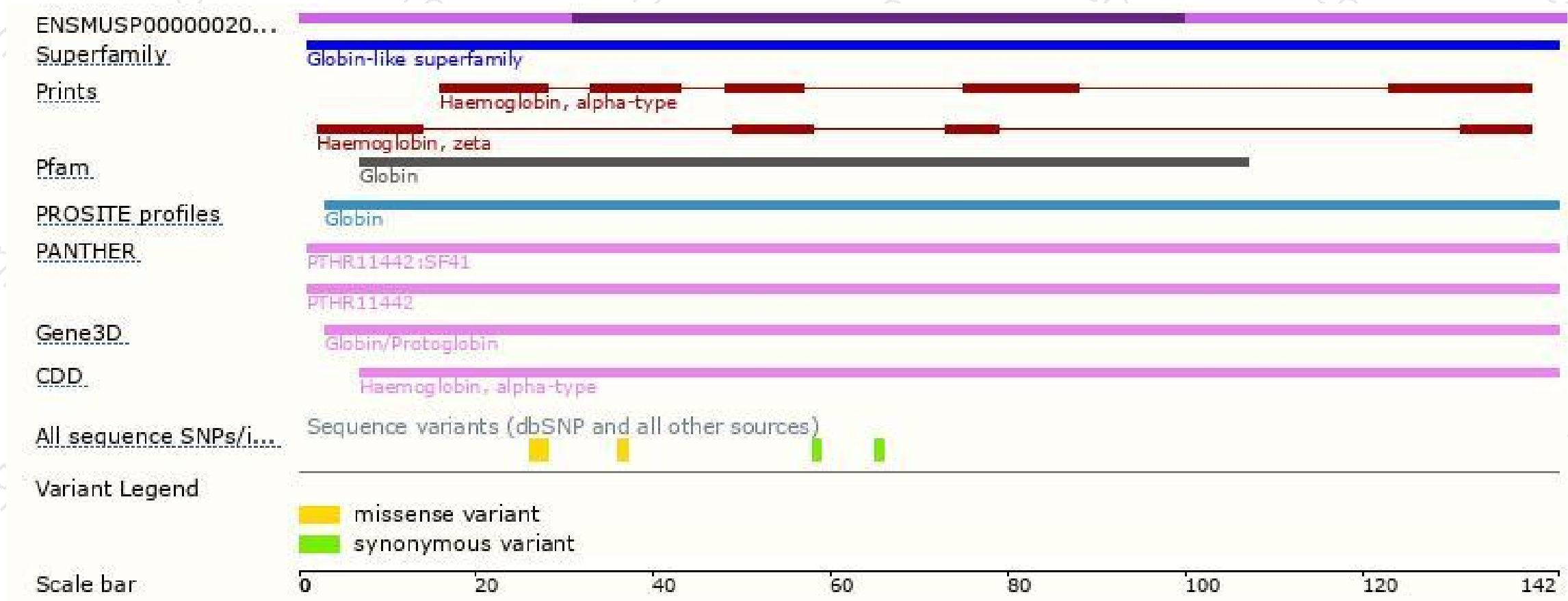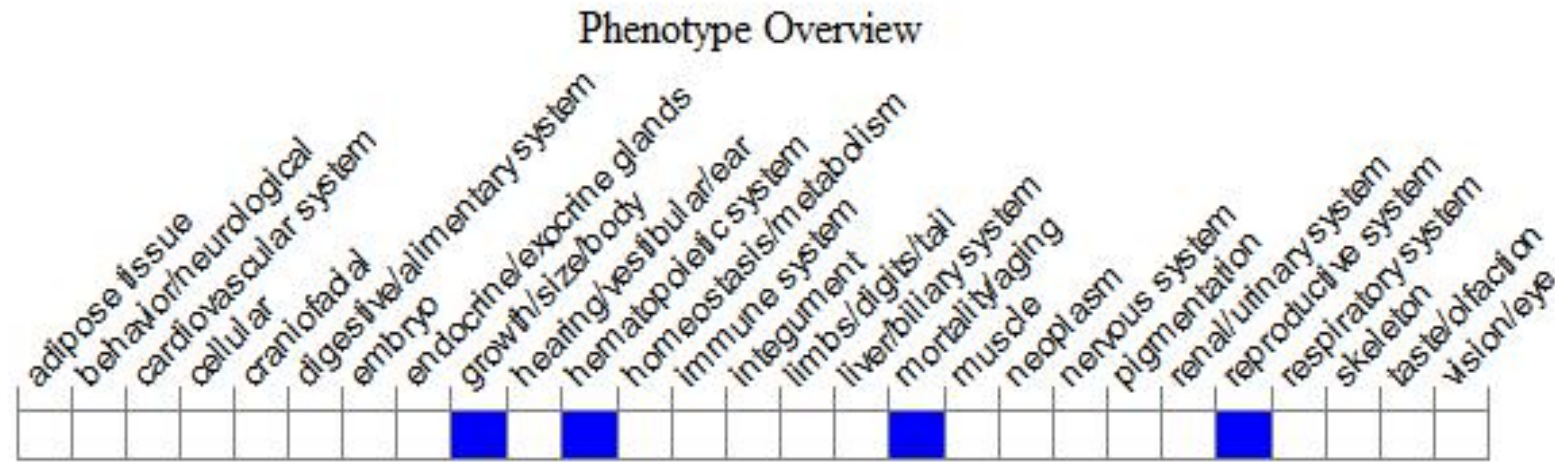

1.72 kb · Forward strand

Hba-x-201 >
protein coding
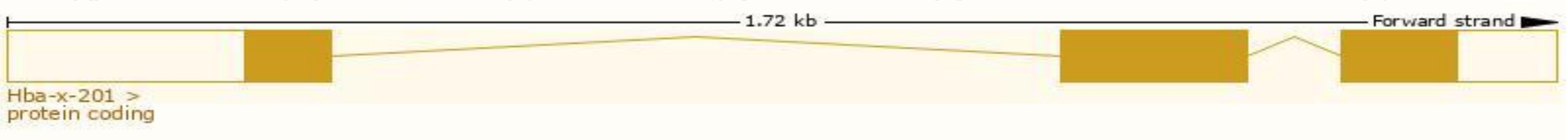
Genomic location distribution
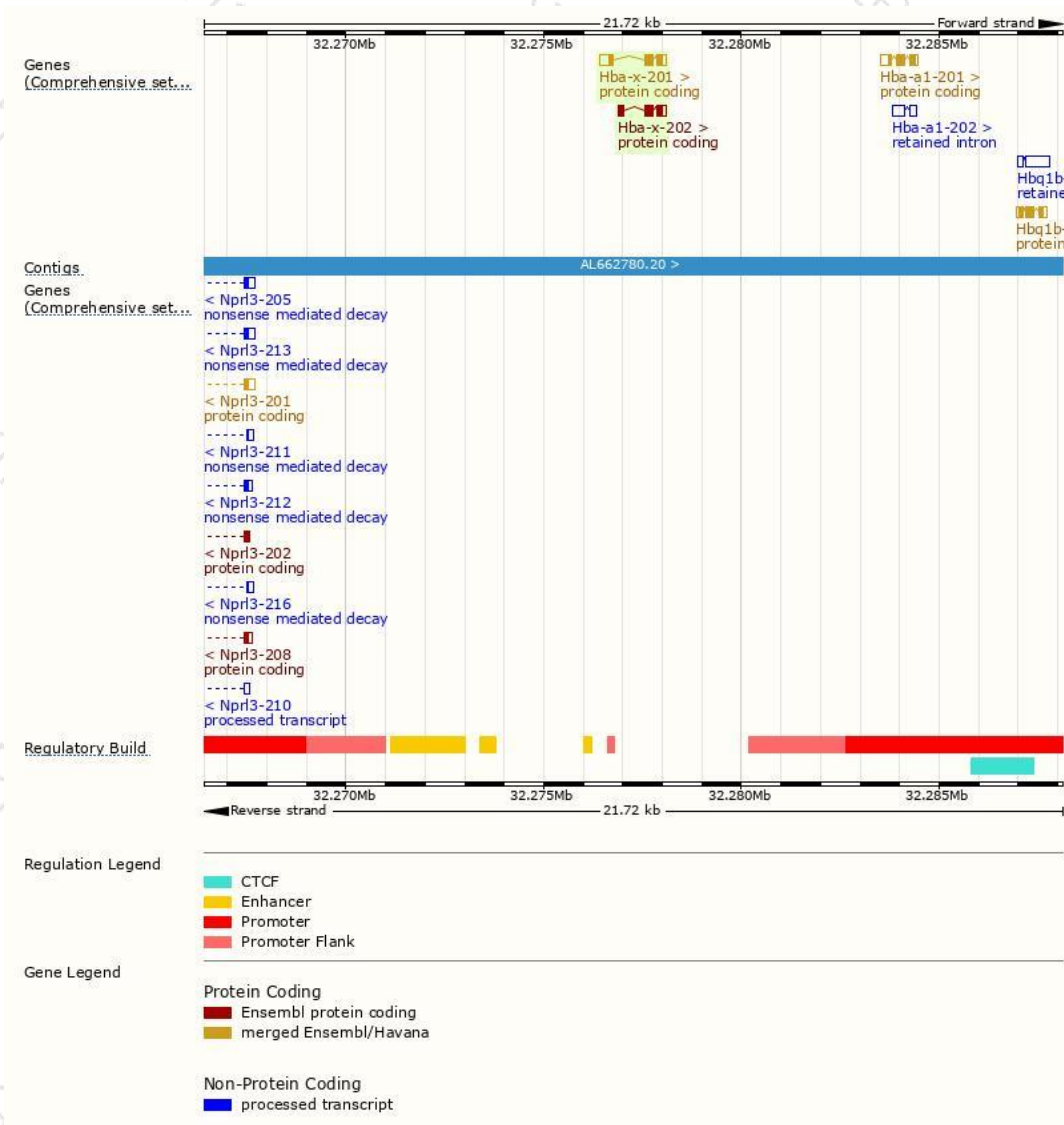
Protein domain

Mouse phenotype description(MGI)

Phenotype Overview

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

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