Heatr5b Cas9-CKO Strategy

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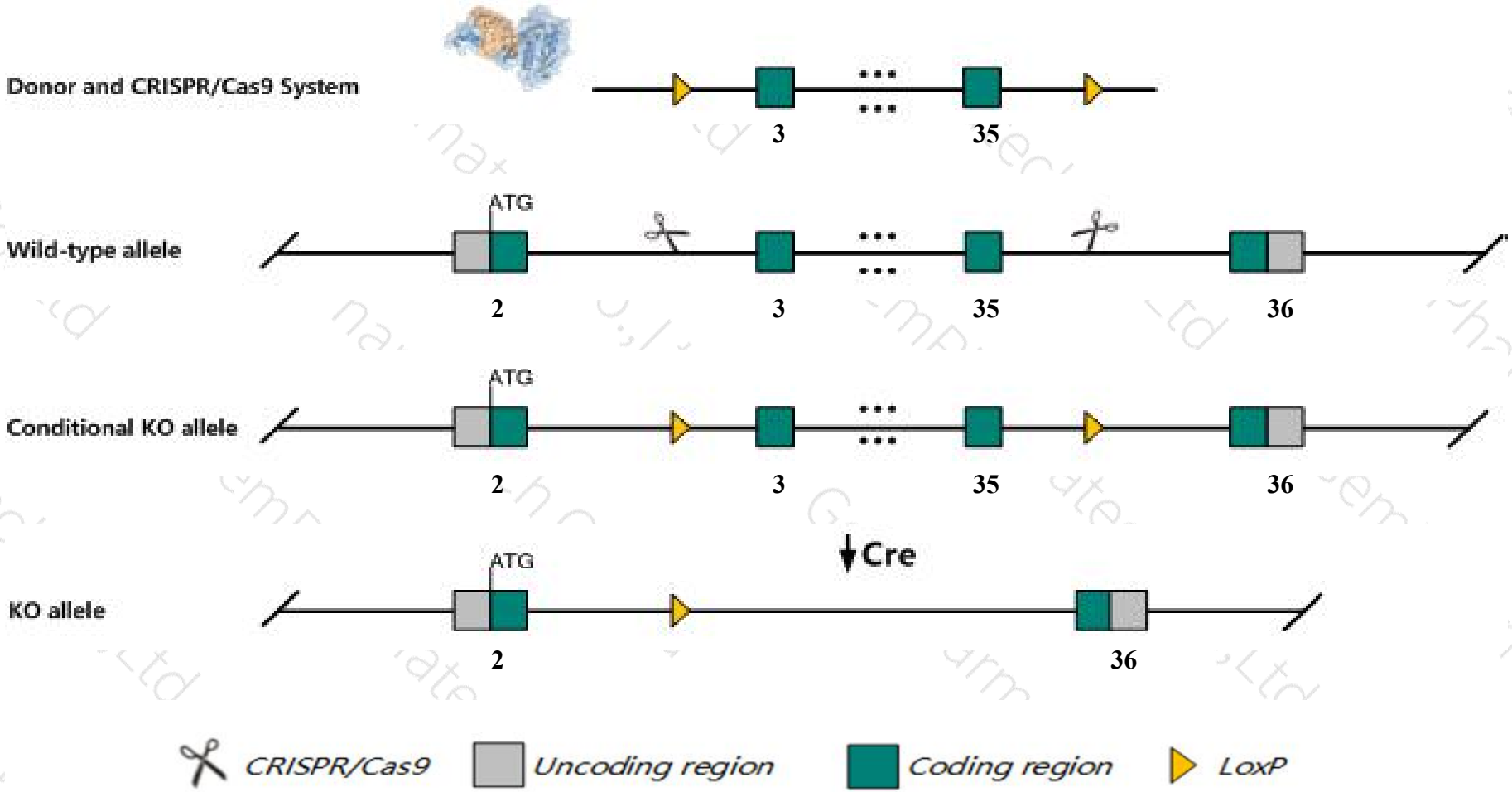
Project Overview

Project Name	***Heatr5b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Heatr5b* gene. The schematic diagram is as follows:



Technical routes

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- The *Heatr5b* gene has 6 transcripts. According to the structure of *Heatr5b* gene, exon3-exon35 of *Heatr5b-201* (ENSMUST00000097281.3) transcript is recommended as the knockout region.The region contains 5782bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Heatr5b* 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

- The flox region is about 3.5 kb away from the 5th end of the *Gpatch11* gene, and its effect is unknown.
- The *Heatr5b* gene is located on the Chr17. 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）

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Heatr5b HEAT repeat containing 5B [Mus musculus (house mouse)]

Gene ID: 320473, updated on 13-Mar-2020

Summary

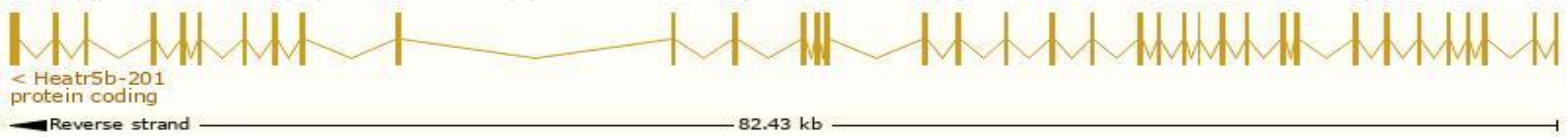
Official Symbol	Heatr5b provided by MGI
Official Full Name	HEAT repeat containing 5B provided byMGI
Primary source	MGI:MGI:2444098
See related	Ensembl:ENSMUSG00000039414
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010013B10Rik, A230048G03Rik, AV071430, D330050P16Rik, mKIAA1414
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 7.6), whole brain E14.5 (RPKM 7.2) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

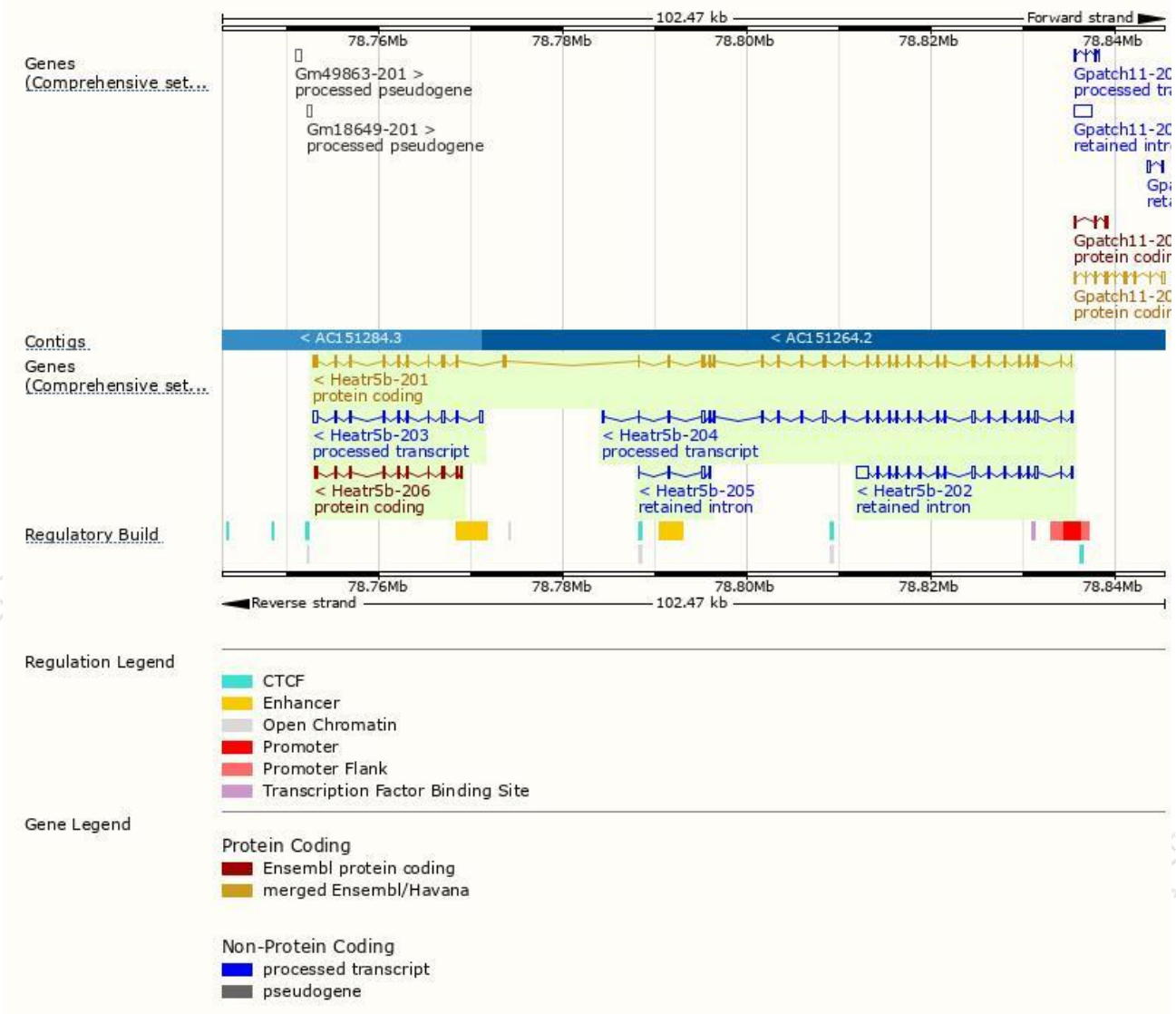
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Heatr5b-201	ENSMUST00000097281.3	6388	2070aa	Protein coding	CCDS37697	Q8C547	TSL:5 GENCODE basic APPRIS P1
Heatr5b-206	ENSMUST00000233850.1	2158	648aa	Protein coding	-	A0A3B2W7H8	GENCODE basic
Heatr5b-204	ENSMUST00000232947.1	4345	No protein	Processed transcript	-	-	
Heatr5b-203	ENSMUST00000232944.1	2255	No protein	Processed transcript	-	-	
Heatr5b-202	ENSMUST00000232917.1	3680	No protein	Retained intron	-	-	
Heatr5b-205	ENSMUST00000233073.1	741	No protein	Retained intron	-	-	

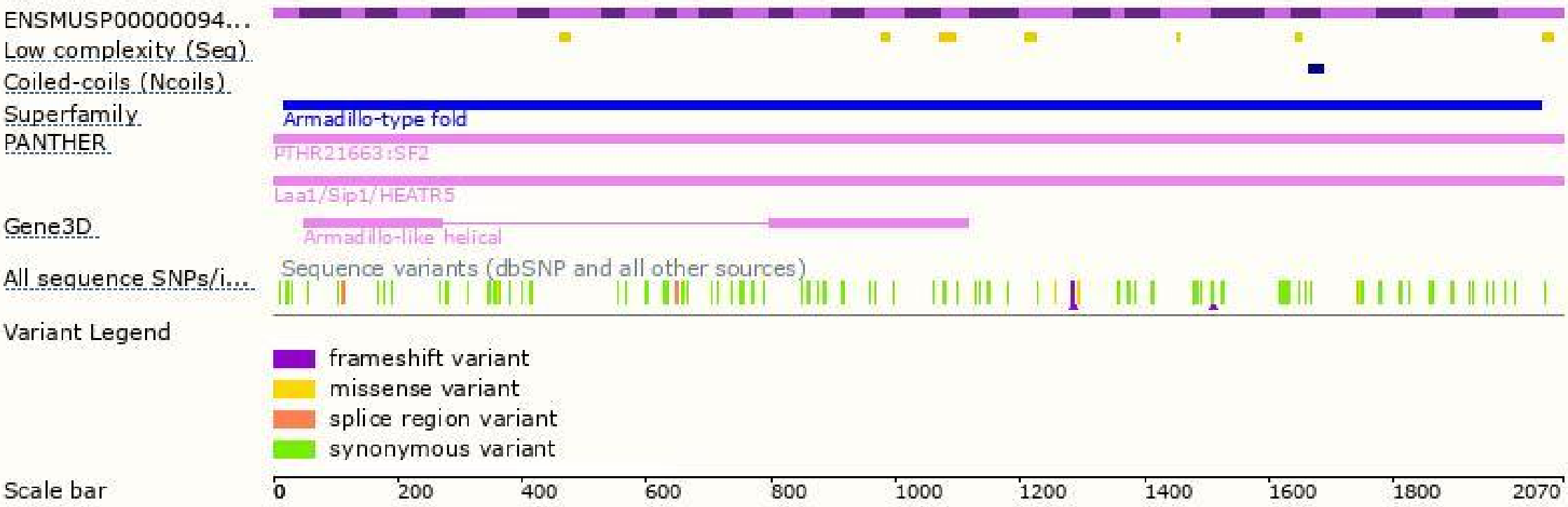
The strategy is based on the design of *Heatr5b-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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