

***Heatr3* Cas9-CKO Strategy**

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Design Date: 2020-11-3

Project Overview

Project Name

Heatr3

Project type

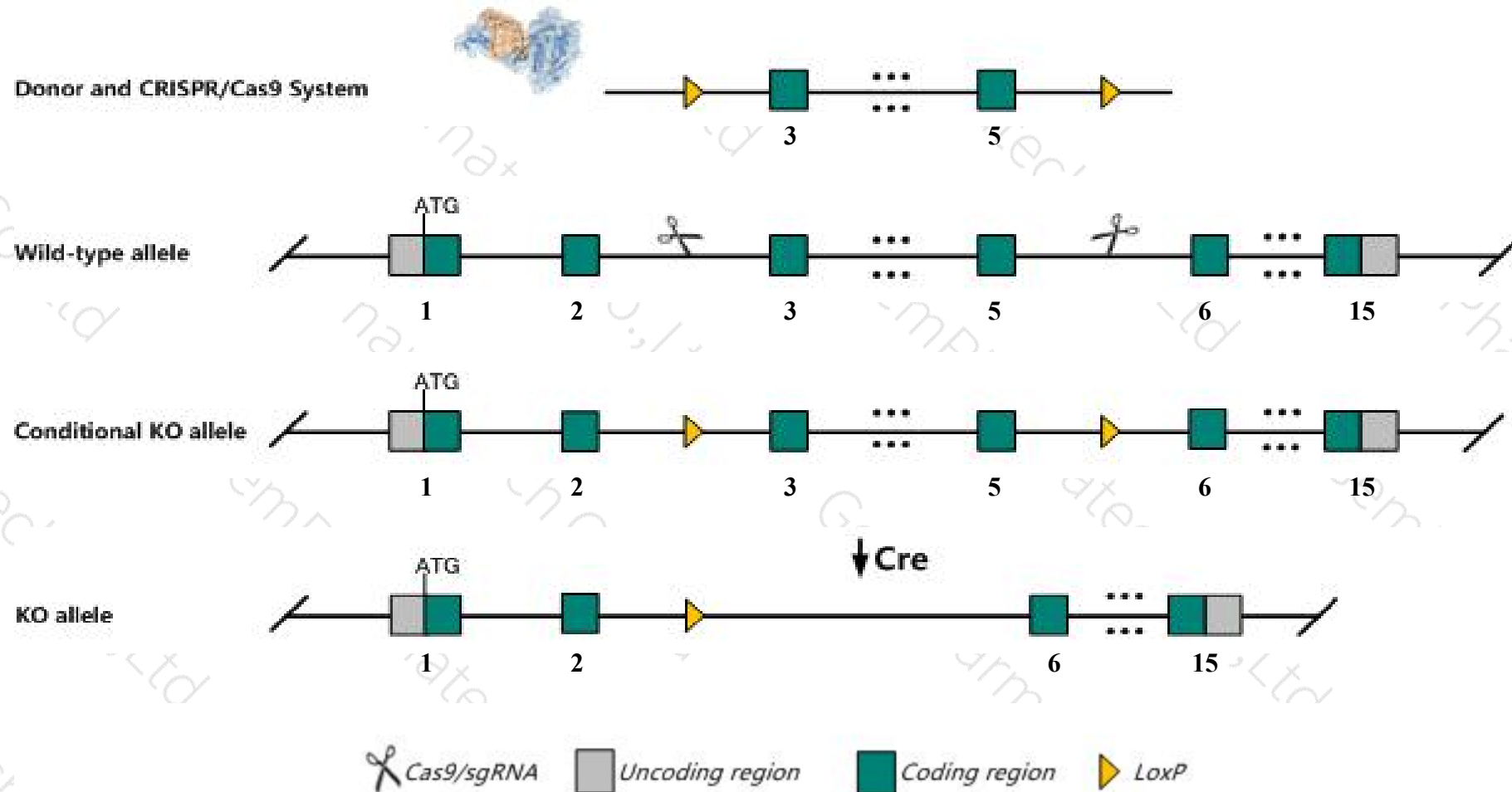
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Heatr3* gene. The schematic diagram is as follows:



Technical routes

- The *Heatr3* gene has 5 transcripts. According to the structure of *Heatr3* gene, exon3-exon5 of *Heatr3*-201(ENSMUST00000034079.13) transcript is recommended as the knockout region. The region contains 308bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Heatr3* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was 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.

- The *Heatr3* gene is located on the Chr8. 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.
- The N-terminal of *Cfap161* gene will remain several amino acids ,it may remain the partial function of *Cfap161* gene.
- Transcript *Heatr3*-203&205&205 may not be affected.
- The floxed region is near to the N-terminal of *Gm9988* gene and the C-terminal of *Mir7071* gene. this strategy may influence the regulatory function of the N-terminal of *Gm9988* gene and the C-terminal of *Mir7071* gene.
- 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)

Heatr3 HEAT repeat containing 3 [Mus musculus (house mouse)]

Gene ID: 234549, updated on 13-Mar-2020

Summary



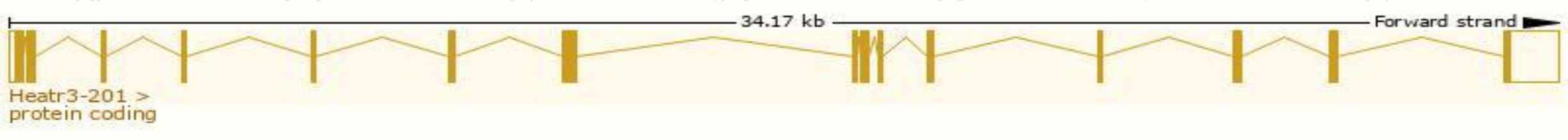
Official Symbol	Heatr3 provided by MGI
Official Full Name	HEAT repeat containing 3 provided by MGI
Primary source	MGI:MGI:2444491
See related	Ensembl:ENSMUSG00000031657
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C030036P15Rik
Expression	Ubiquitous expression in CNS E11.5 (RPKM 13.1), limb E14.5 (RPKM 13.0) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

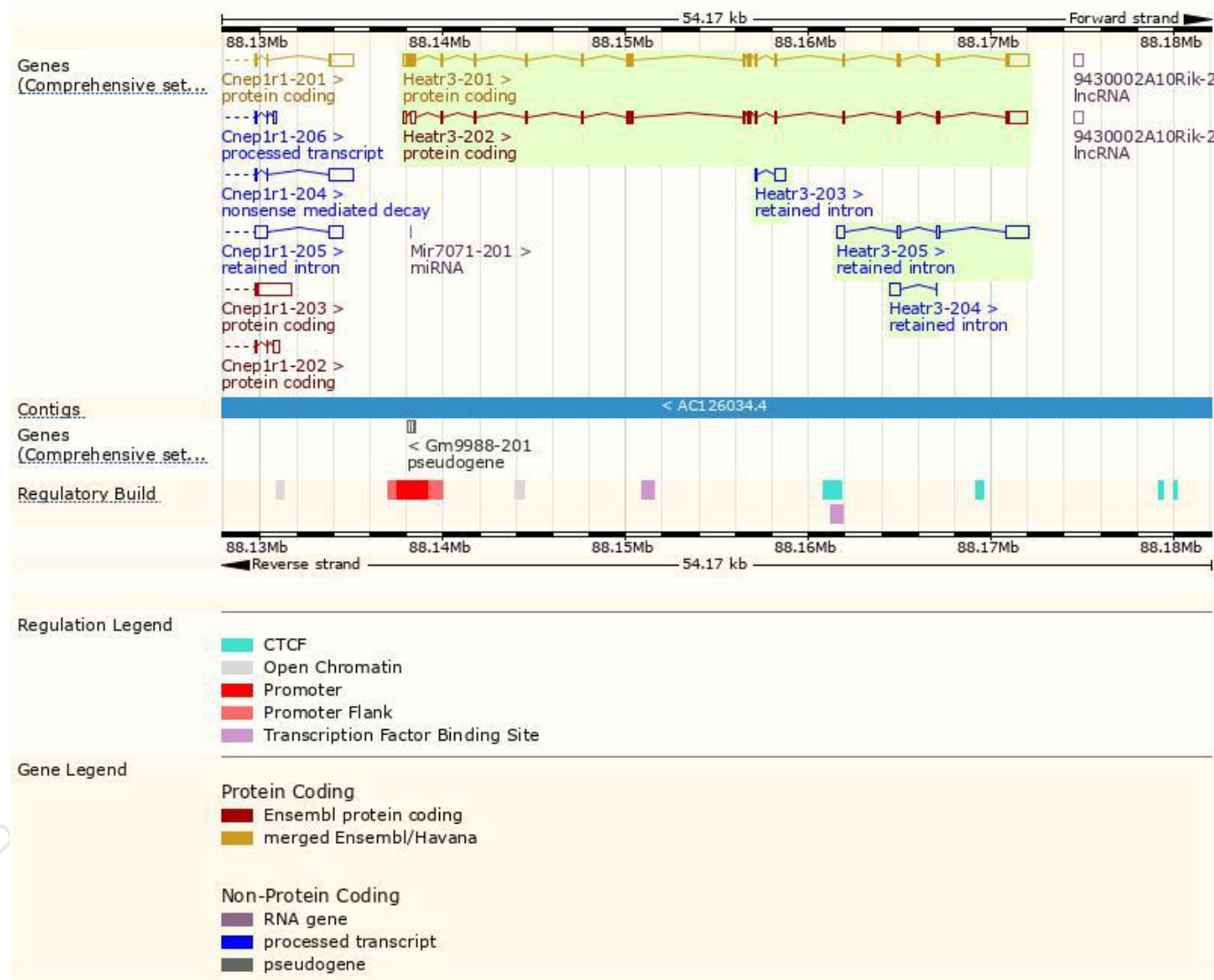
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Heatr3-201	ENSMUST00000034079.13	3251	679aa	Protein coding	CCDS22508	Q8BQM4	TSL:1 GENCODE basic APPRIS P1
Heatr3-202	ENSMUST00000121949.1	3086	562aa	Protein coding	-	E9PWH6	TSL:1 GENCODE basic
Heatr3-205	ENSMUST00000146946.1	1939	No protein	Retained intron	-	-	TSL:1
Heatr3-203	ENSMUST00000131682.1	688	No protein	Retained intron	-	-	TSL:3
Heatr3-204	ENSMUST00000137452.1	600	No protein	Retained intron	-	-	TSL:3

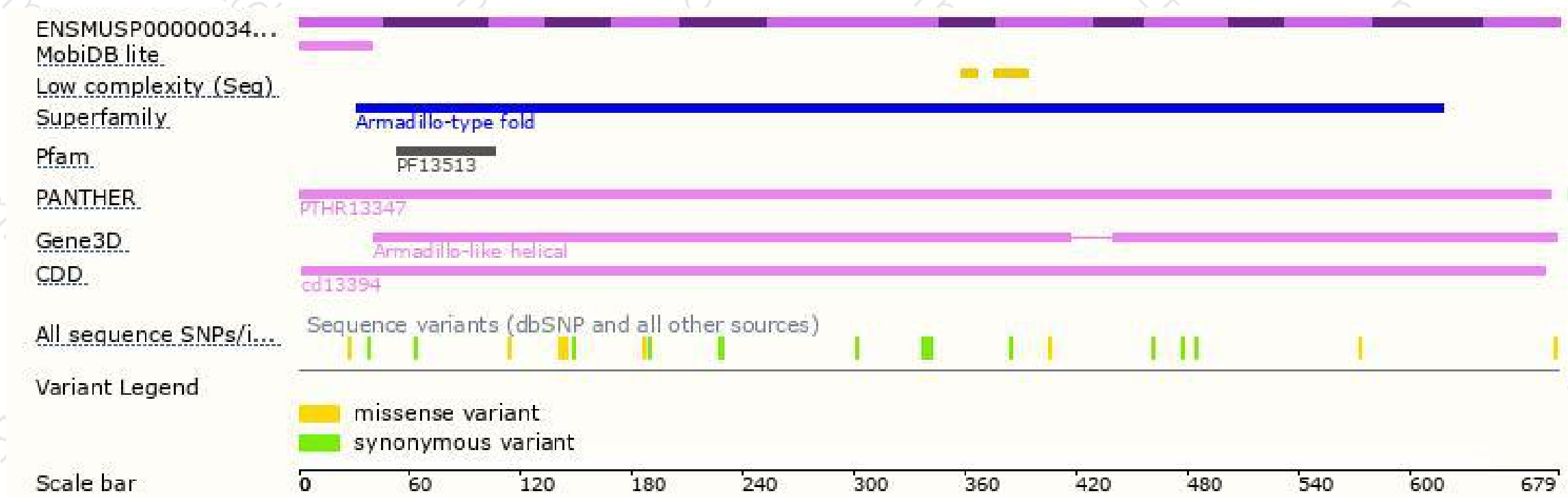
The strategy is based on the design of *Heatr3-201* transcript,the transcription is shown below:



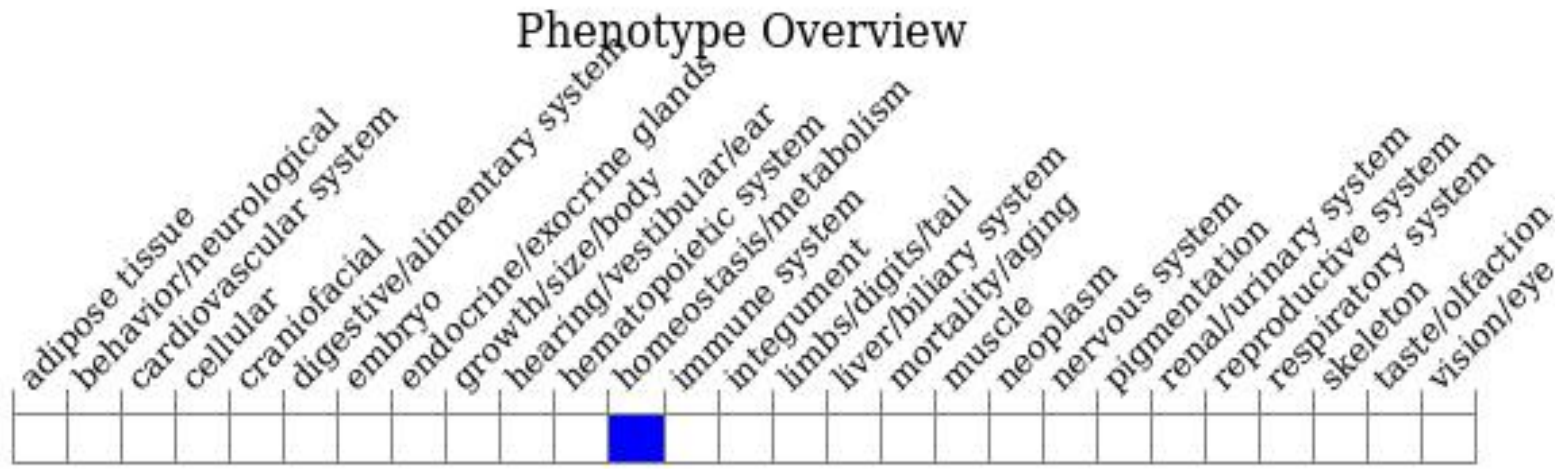
Genomic location distribution



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/>).

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

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