Klb Cas9-CKO Strategy

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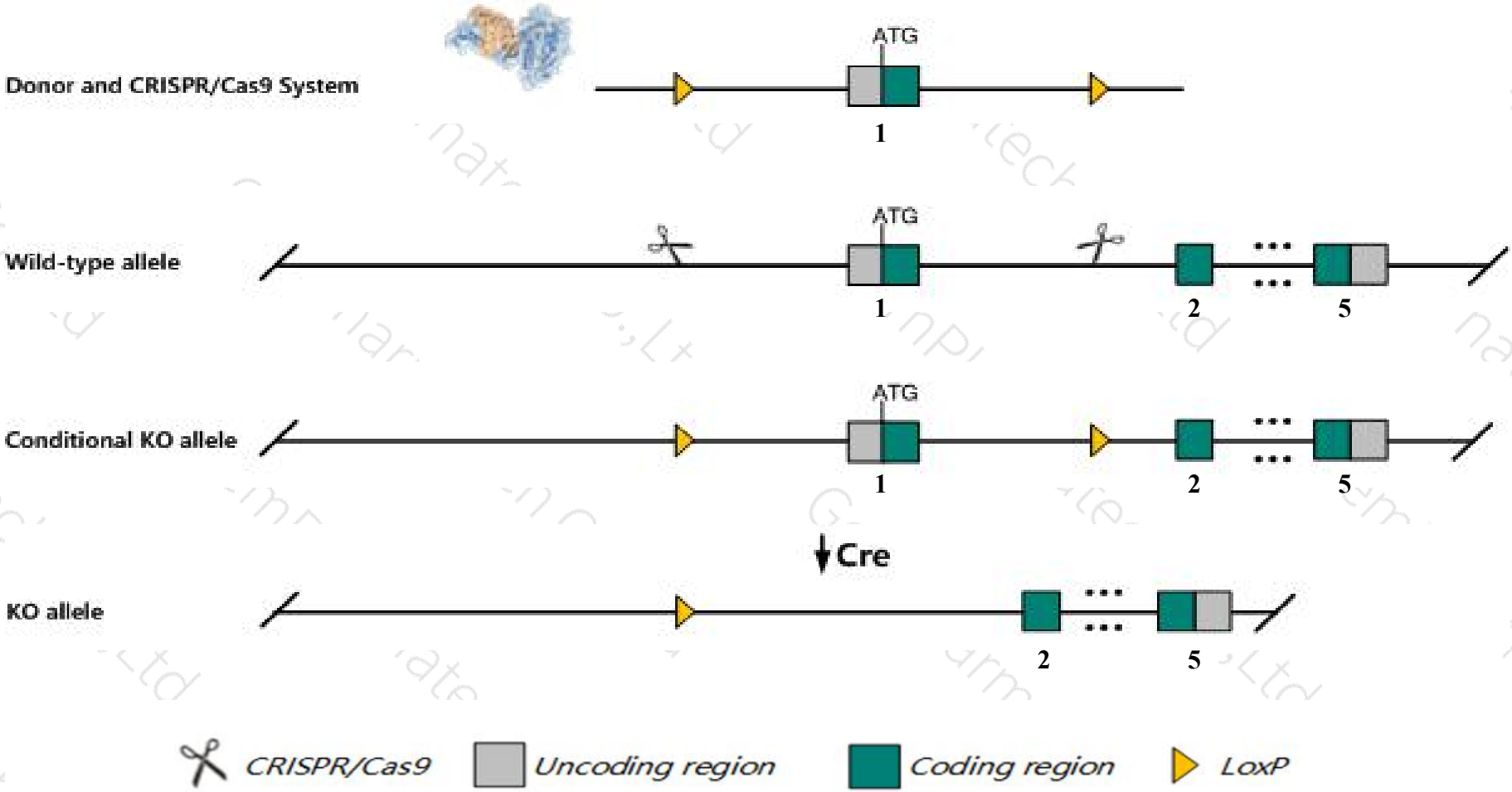
Design Date: 2019-7-18

Project Overview

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

Conditional Knockout strategy

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



Technical routes

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- The *Klb* gene has 3 transcripts. According to the structure of *Klb* gene, exon1 of *Klb-201* (ENSMUST00000031096.10) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Klb* 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,Homozygous null mice display increased bile acid synthesis and excretion, resistance to gallstone formation, and slightly decreased body weight. Mice homozygous for a knock-out allele or a conditional allele activated in adipose tissue exhibit resistanceto FGF21-induced metabolic disruptions.

- The *Klb* gene is located on the Chr5. 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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Klb klotho beta [Mus musculus (house mouse)]

Gene ID: 83379, updated on 19-Feb-2019

Summary

Official Symbol	Klb provided by MGI
Official Full Name	klotho beta provided byMGI
Primary source	MGI:MGI:1932466
See related	Ensembl:ENSMUSG00000029195
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	AV071179, betaKlotho
Expression	Biased expression in subcutaneous fat pad adult (RPKM 12.8), placenta adult (RPKM 10.5) and 10 other tissuesSee more
Orthologs	human all

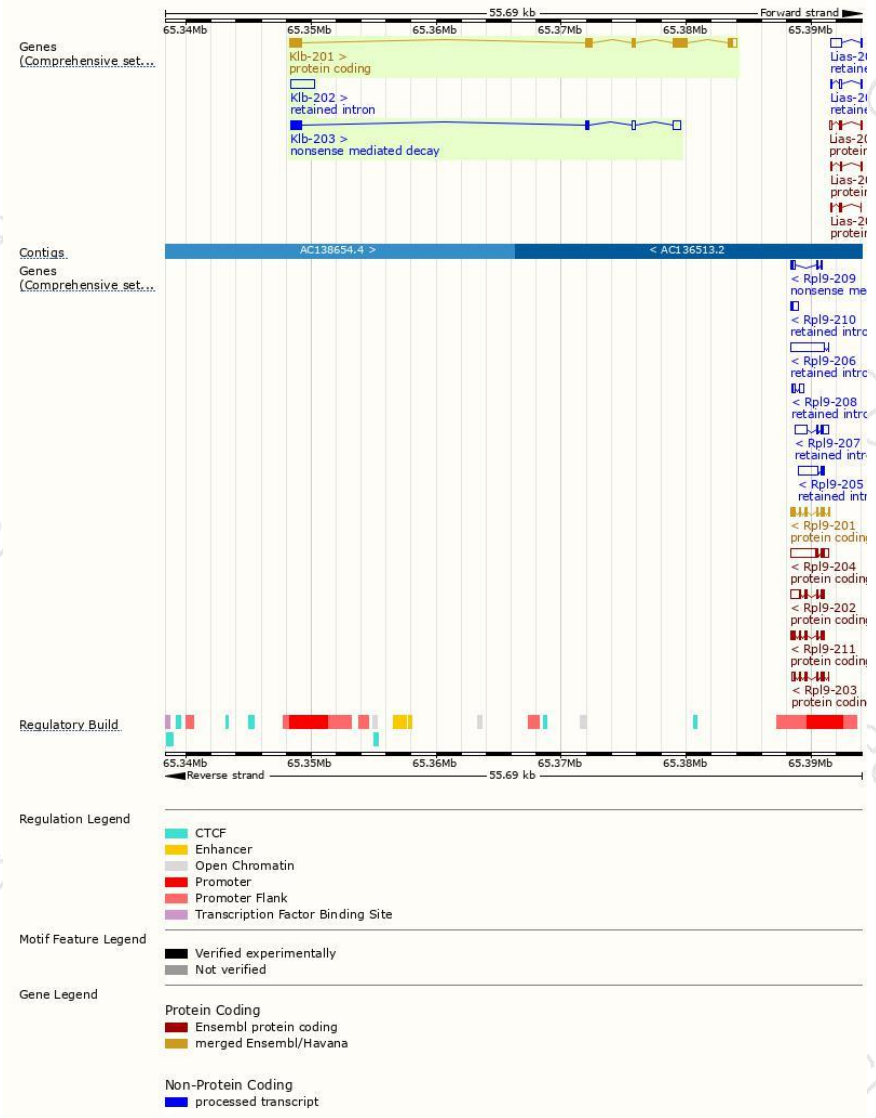
Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

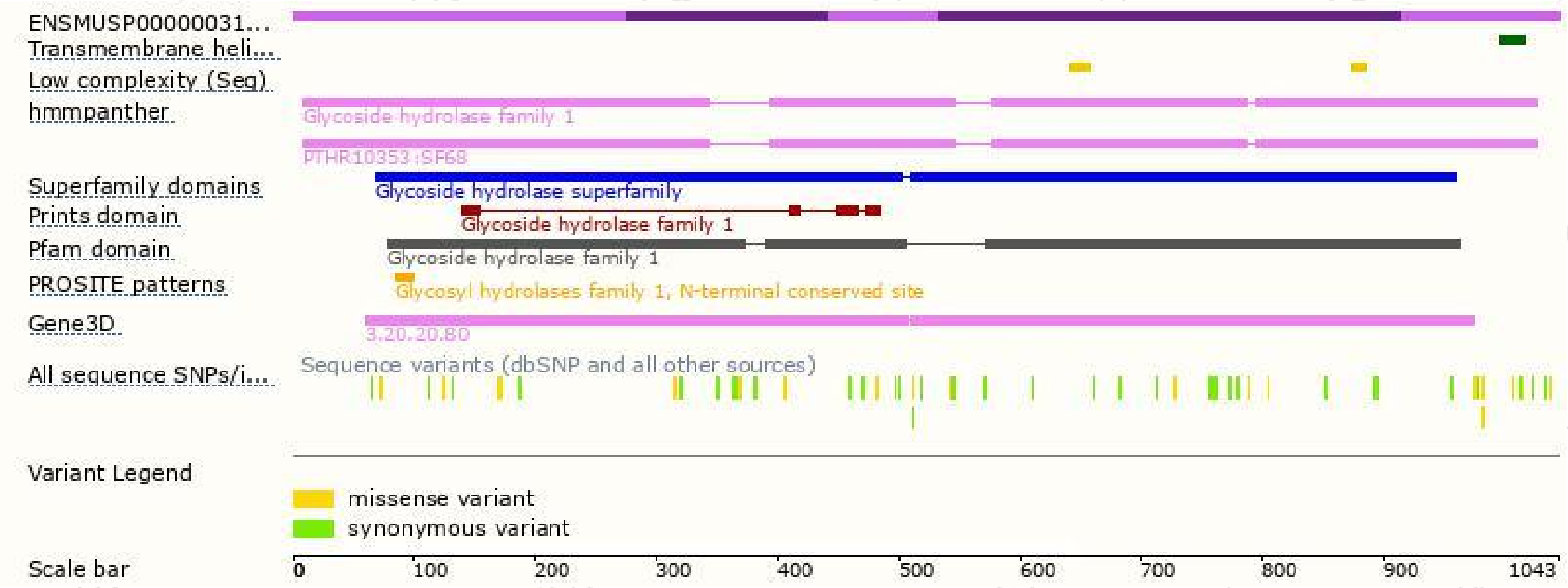
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Klb-201	ENSMUST00000031096.10	3540	1043aa	Protein coding	CCDS19306	Q99N32	TSL:1 GENCODE basic APPRIS P1
Klb-203	ENSMUST00000205084.1	1954	361aa	Nonsense mediated decay	-	S4W6E2	TSL:1
Klb-202	ENSMUST00000203333.1	1839	No protein	Retained intron	-	-	TSL:NA

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

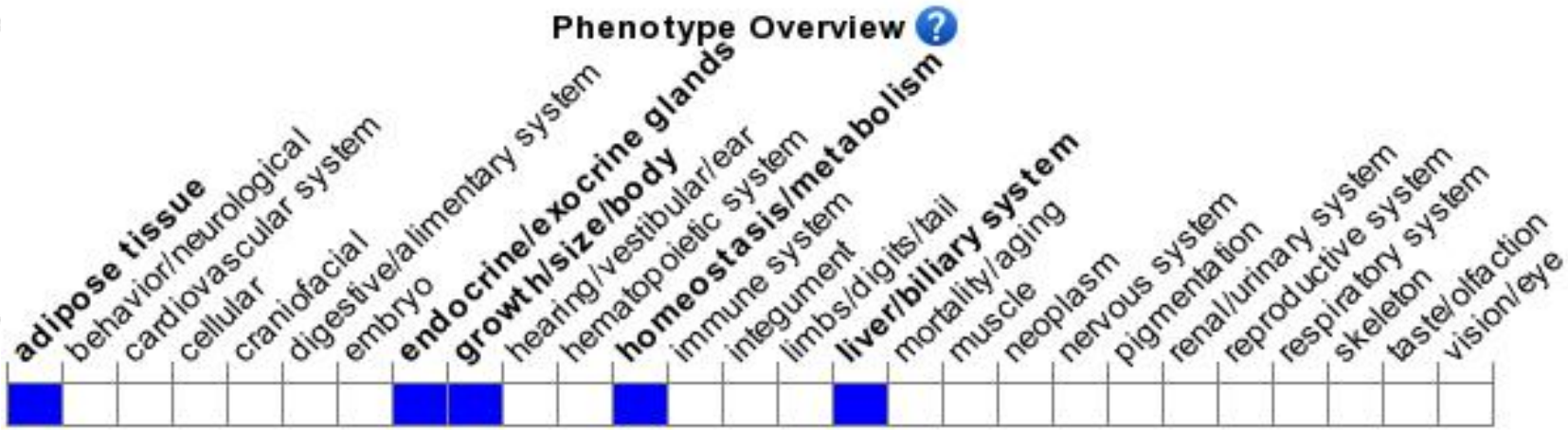
Genomic location distribution



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/).

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

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