Apob Cas9-CKO Strategy

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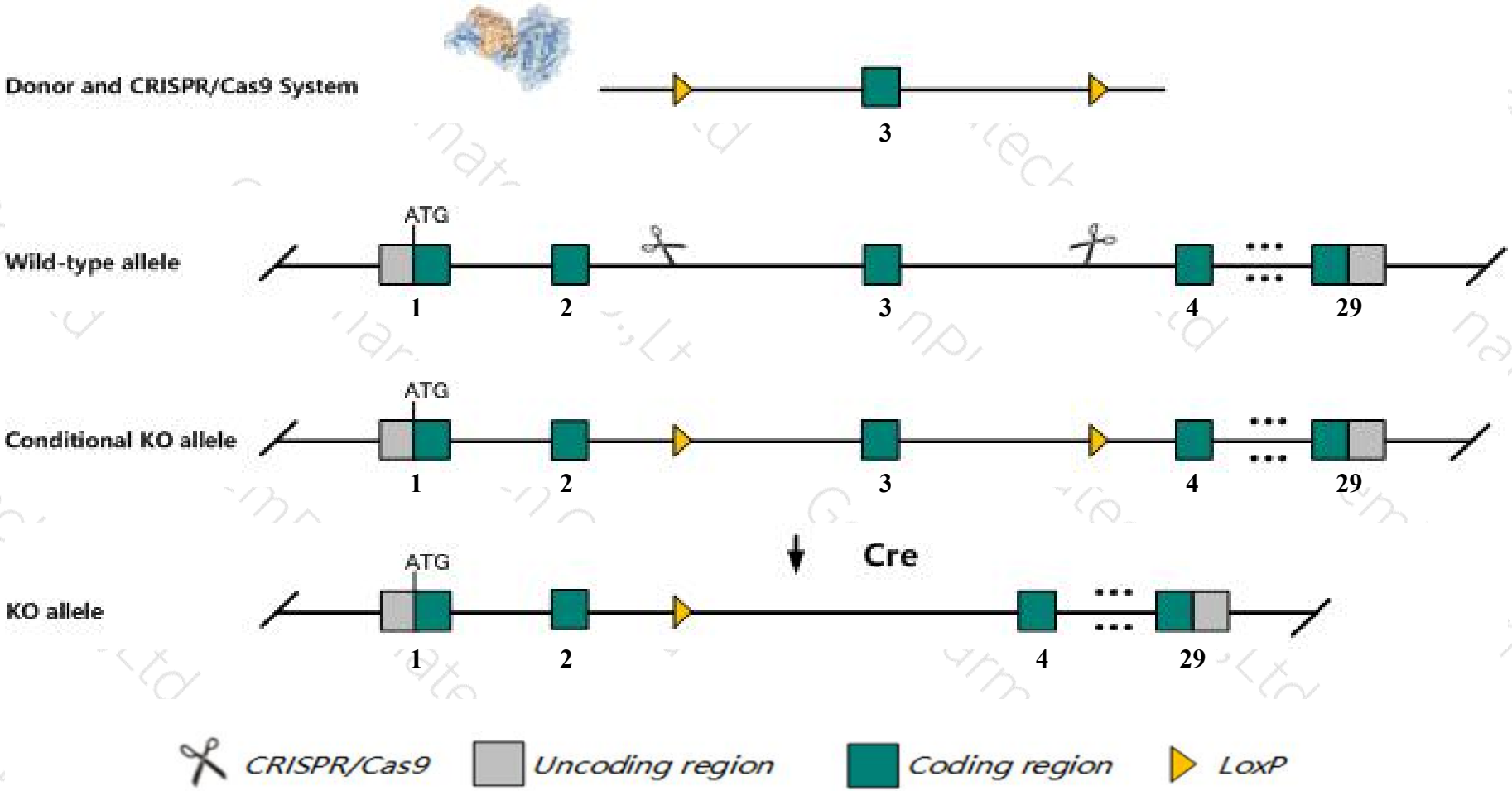
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Apob* gene has 4 transcripts. According to the structure of *Apob* gene, exon3 of *Apob-202* (ENSMUST00000037811.12) transcript is recommended as the knockout region.The region contains 116bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Apob* 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 mutants usually die by midgestation and longer survivors exhibit exencephaly. Heterozygotes show reduced plasma cholesterol and apolipoprotein levels. Single isoform B100 and B48 null mutants are viable.

➢ The *Apob* gene is located on the Chr12. 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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Apob apolipoprotein B [Mus musculus (house mouse)]

Gene ID: 238055, updated on 5-Mar-2019

Summary

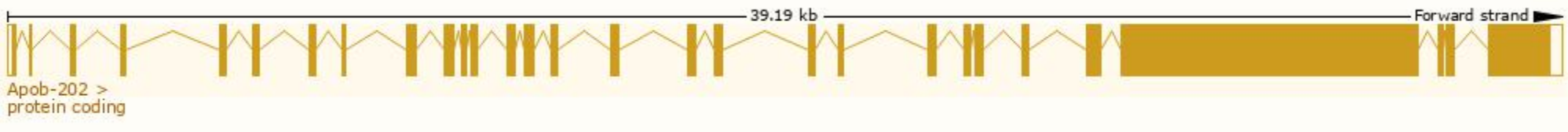
Official Symbol	Apob provided by MGI
Official Full Name	apolipoprotein B provided byMGI
Primary source	MGI:MGI:88052
See related	Ensembl:ENSMUSG00000020609
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI315052, Apo B-100, apob-100, apob-48
Summary	This gene product is the main apolipoprotein of chylomicrons and low density lipoproteins. It occurs in plasma as two main isoforms, apoB-48 and apoB-100. Unlike the apoB-48 and apoB-100 structural equivalents in human, which are synthesized exclusively in the gut and liver, respectively, the mouse apoB-48 isoform is also found in mouse liver. The intestinal and the hepatic forms of apoB are encoded by a single gene from a single, very long mRNA. The two isoforms share a common N-terminal sequence. The shorter apoB-48 protein is produced after RNA editing of the apoB-100 transcript at residue 2179 (CAA->UAA), resulting in the creation of a stop codon, and early translation termination. [provided by RefSeq, Jul 2008]
Expression	Biased expression in placenta adult (RPKM 182.2), liver adult (RPKM 82.4) and 5 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

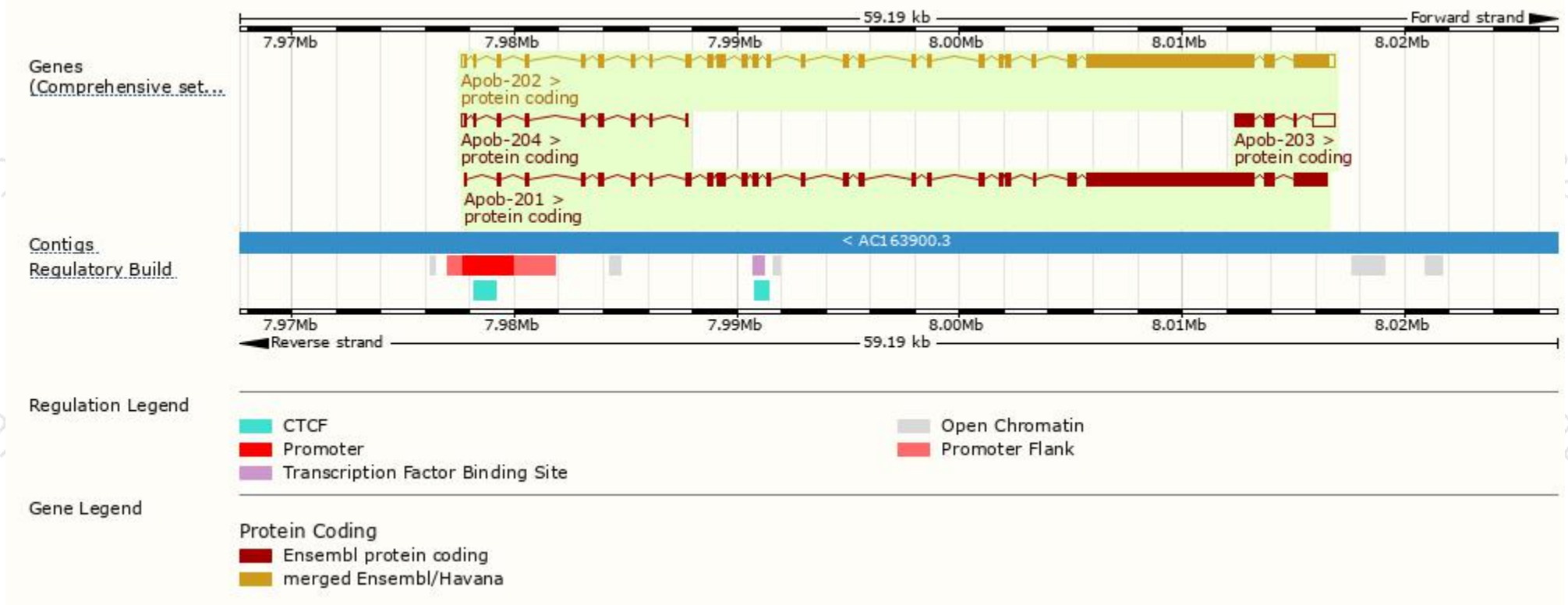
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Apob-202	ENSMUST00000037811.12	13934	4505aa	Protein coding	CCDS49022	E9Q414	TSL:1 GENCODE basic APPRIS P1
Apob-201	ENSMUST00000037520.13	13369	4456aa	Protein coding	-	E9Q1Y3	CDS 3' incomplete TSL:5
Apob-203	ENSMUST00000171239.1	2174	411aa	Protein coding	-	F7A3M3	CDS 5' incomplete TSL:1
Apob-204	ENSMUST00000171271.7	1118	329aa	Protein coding	-	E9Q4G4	CDS 3' incomplete TSL:1

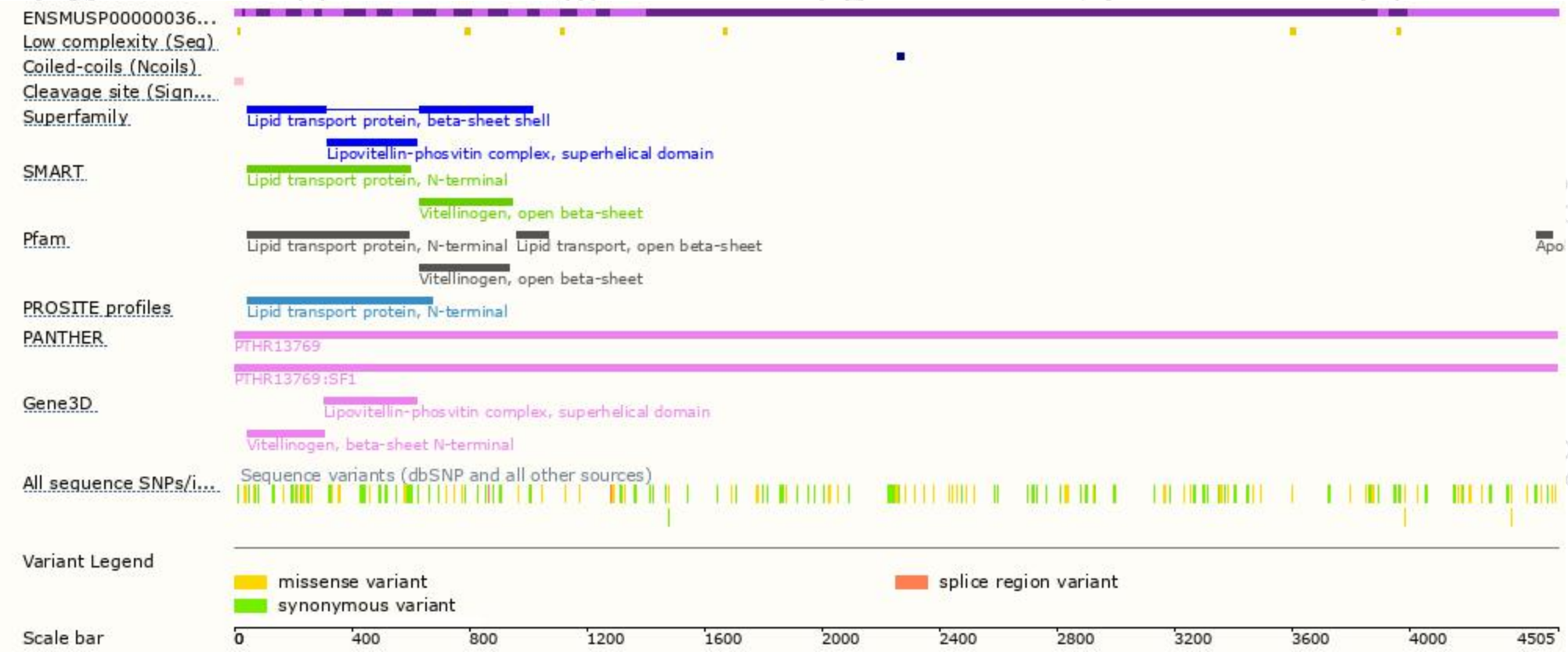
The strategy is based on the design of *Apob-202* transcript,The transcription is shown below



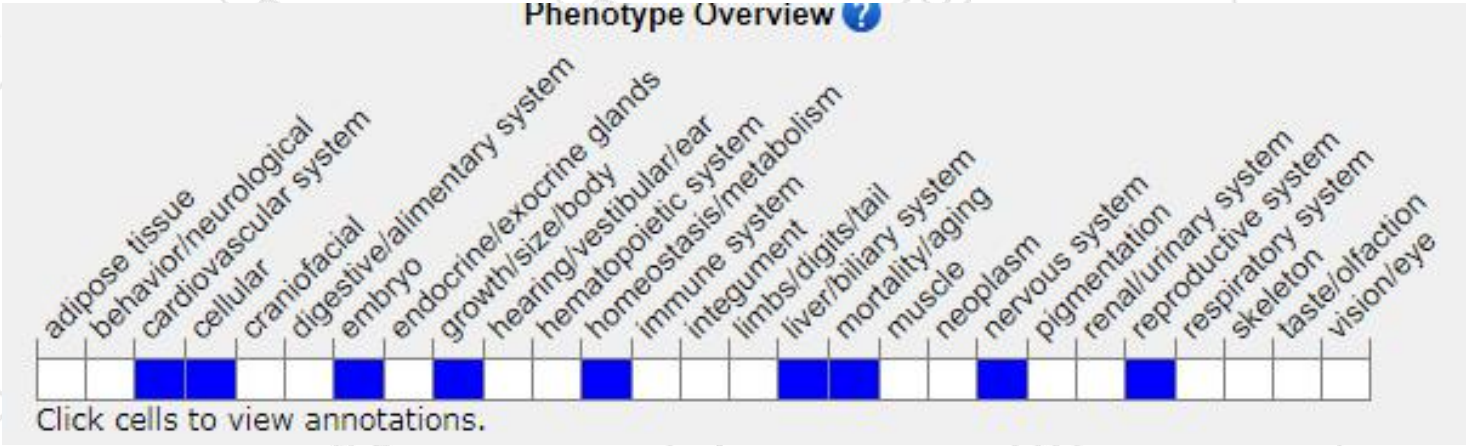
Genomic location distribution



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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