Apoa1 Cas9-CKO Strategy

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Project Overview

Project Name	***Apoa1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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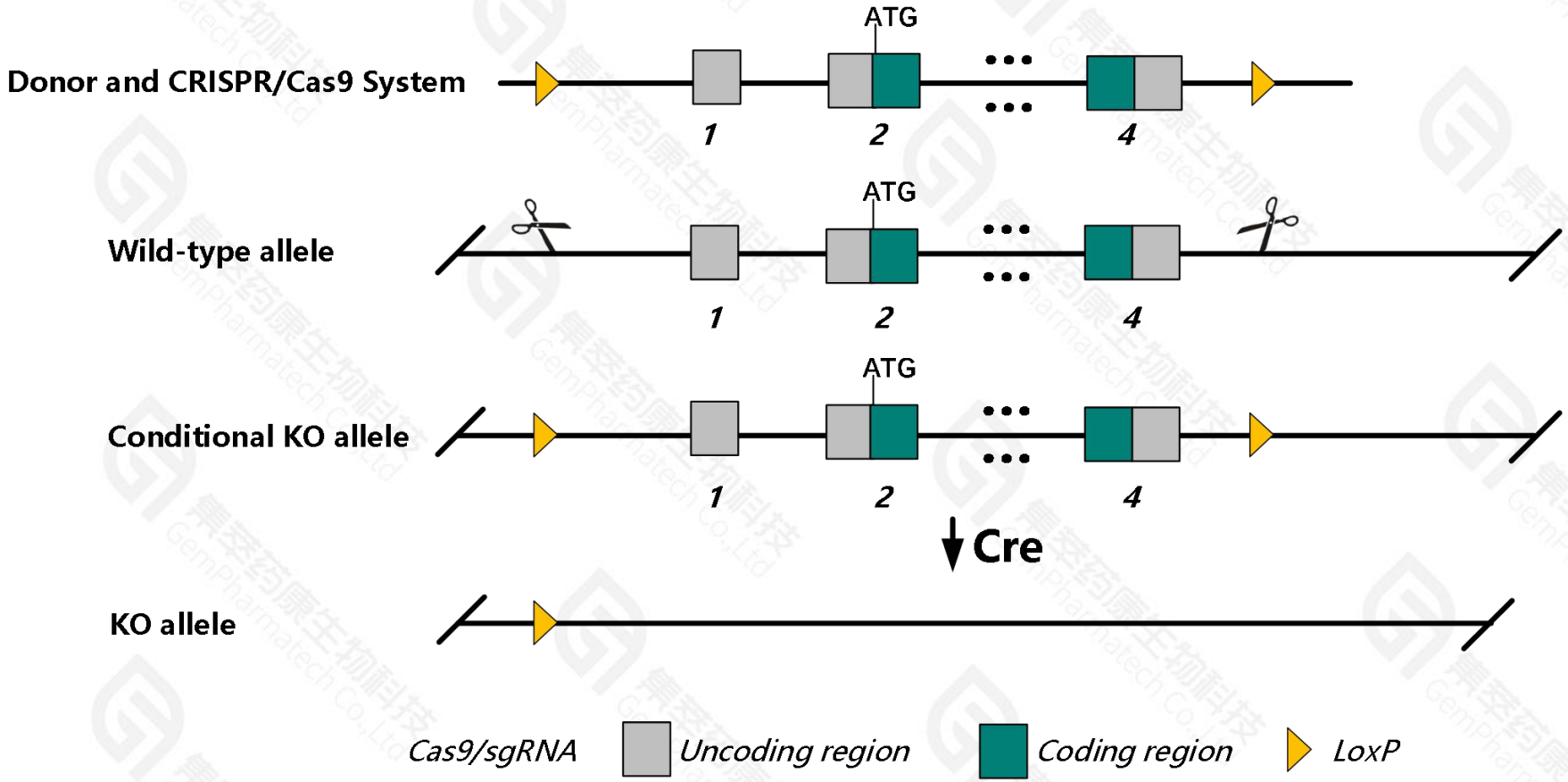
This model will use CRISPR/Cas9 technology to edit the *Apoa1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Apoa1* gene has 2 transcripts. According to the structure of *Apoa1* gene, exon1-exon4 of *Apoa1-201*(ENSMUST00000034588.9) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Apoa1* 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,homozygotes for targeted null mutations exhibit reduced high density lipoprotein (HDL), non-HDL cholesterol, and cholesterol ester levels, increased plasma triglyceride and free cholesterol levels, and impaired corticosteroid synthesis.

➢ The floxed region is near to the C-terminal of *Sik3* gene,this strategy may influence the regulatory function of the C-terminal of *Sik3* gene.

➢ The *Apoa1* gene is located on the Chr9. 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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Apoa1 apolipoprotein A-I [Mus musculus (house mouse)]

Gene ID: 11806, updated on 13-Mar-2020

Summary

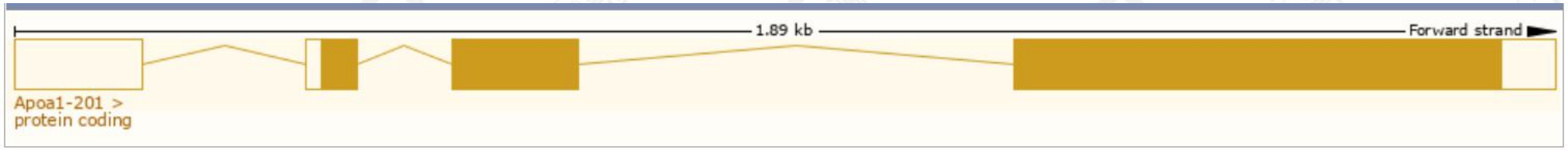
Official Symbol	Apoa1 provided by MGI
Official Full Name	apolipoprotein A-I provided byMGI
Primary source	MGI:MGI:88049
See related	Ensembl:ENSMUSG00000032083
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Alp-1, Apoa-1, Brp-14, Ltw-1, Lvtw-1, Sep-1, Sep-2, Sep2, apo-AI, apoA-I
Summary	This gene encodes a preproprotein that is proteolytically cleaved to yield a signal peptide and a proproptein that is subsequently processed to generate the active mature peptide. The encoded protein is the major protein component of plasma high density lipoprotein (HDL). This protein facilitates the removal of cholesterol and other fats from tissues by transporting them to the liver for excretion. This protein is a cofactor for lecithin cholesterolacyltransferase, an enzyme that catalyzes the conversion of free cholesterol to cholesteryl esters. Mutations in this gene in humans causes familial HDL deficiency, Tangier disease and familial visceral amyloidosis. Similar clinical features are exhibited by mice with mutations in this gene. This gene is clustered with three other apolipoprotein genes on chromosome 9. [provided by RefSeq, Dec 2013]
Expression	Biased expression in liver E18 (RPKM 4031.2), duodenum adult (RPKM 3636.0) and 7 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

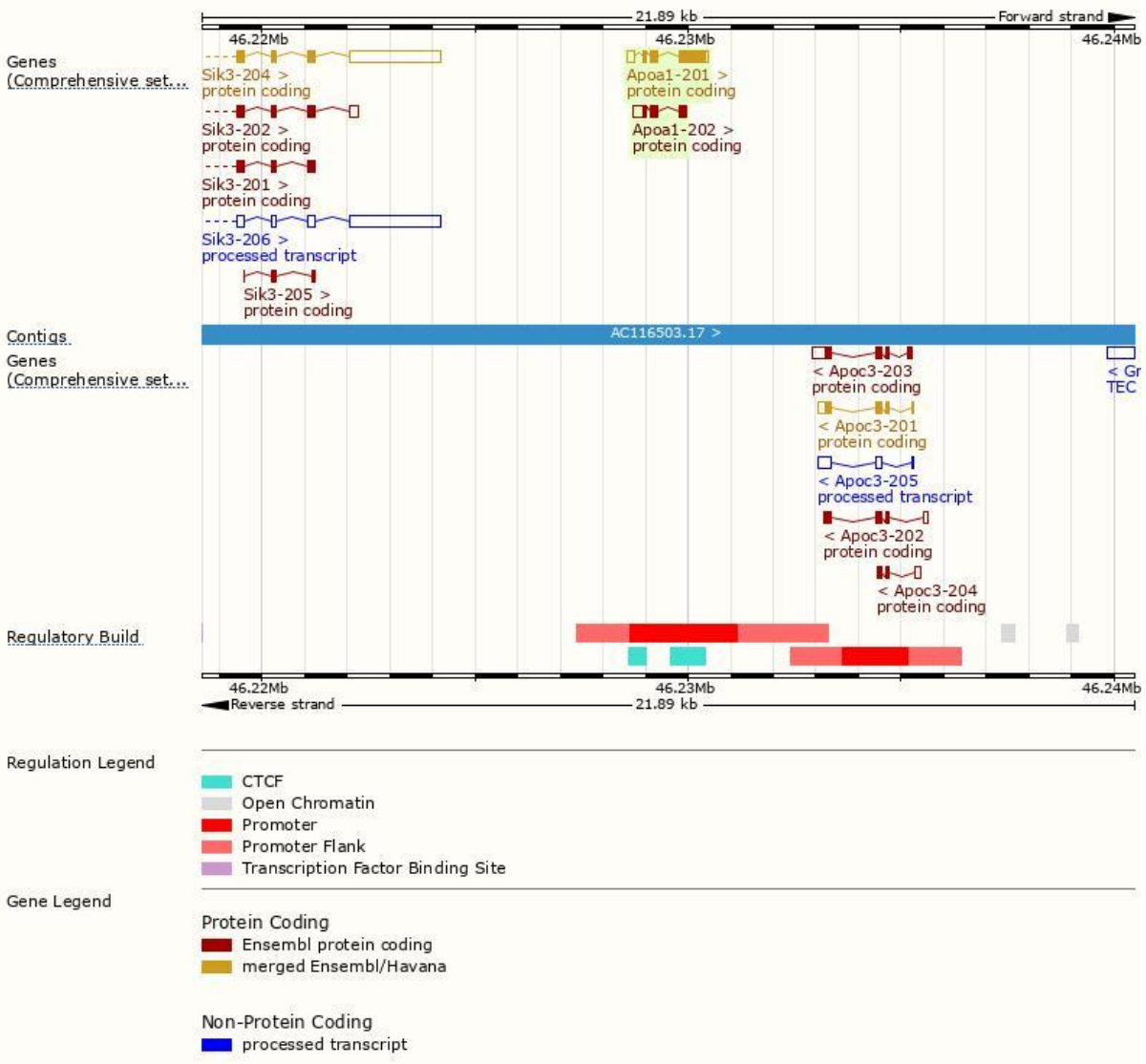
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Apoa1-201	ENSMUST00000034588.8	1035	264aa	Protein coding	CCDS40610	Q00623	TSL:1 GENCODE basic APPRIS P1
Apoa1-202	ENSMUST00000132155.1	602	122aa	Protein coding	-	A0A1L1STX7	CDS 3' incomplete TSL:1

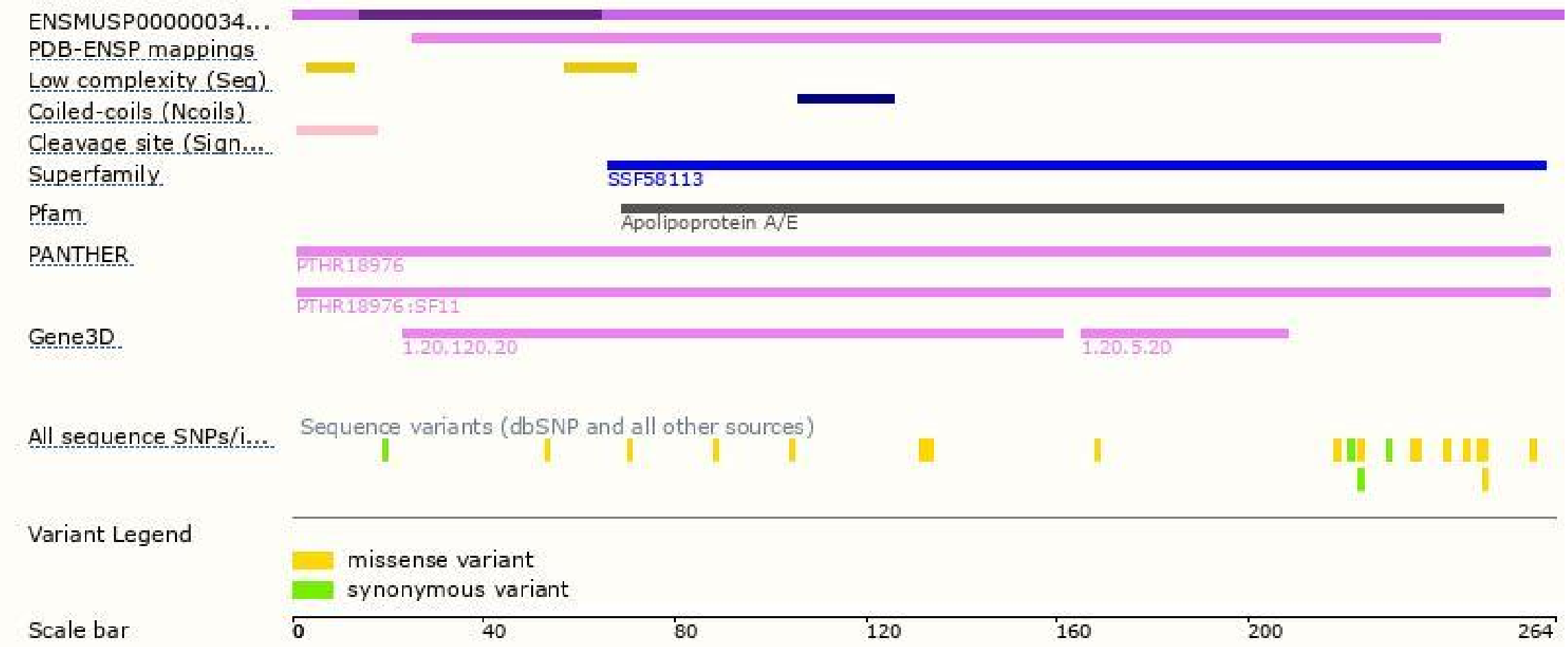
The strategy is based on the design of *Apoa1-201* transcript,the transcription is shown below:



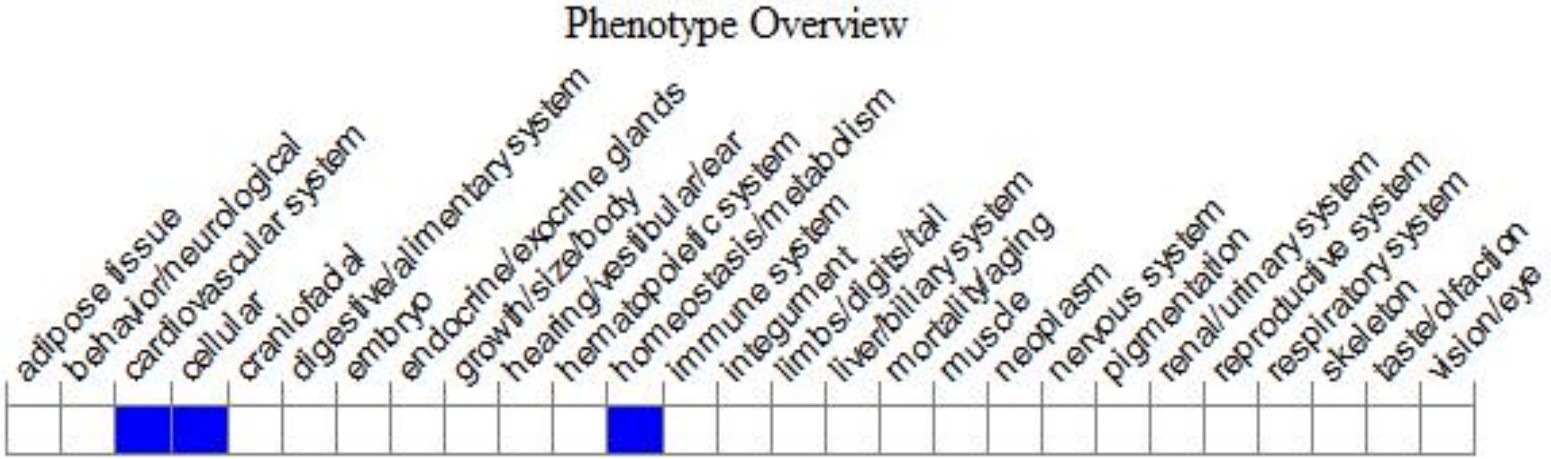
Genomic location distribution



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/).

According to the existing MGI data,homozygotes for targeted null mutations exhibit reduced high density lipoprotein (HDL), non-HDL cholesterol, and cholesterol ester levels, increased plasma triglyceride and free cholesterol levels, and impaired corticosteroid synthesis.

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