

*Apo*f Cas9-CKO Strategy

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Reviewer: Xueting Zhang

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Project Overview

Project Name

Apof

Project type

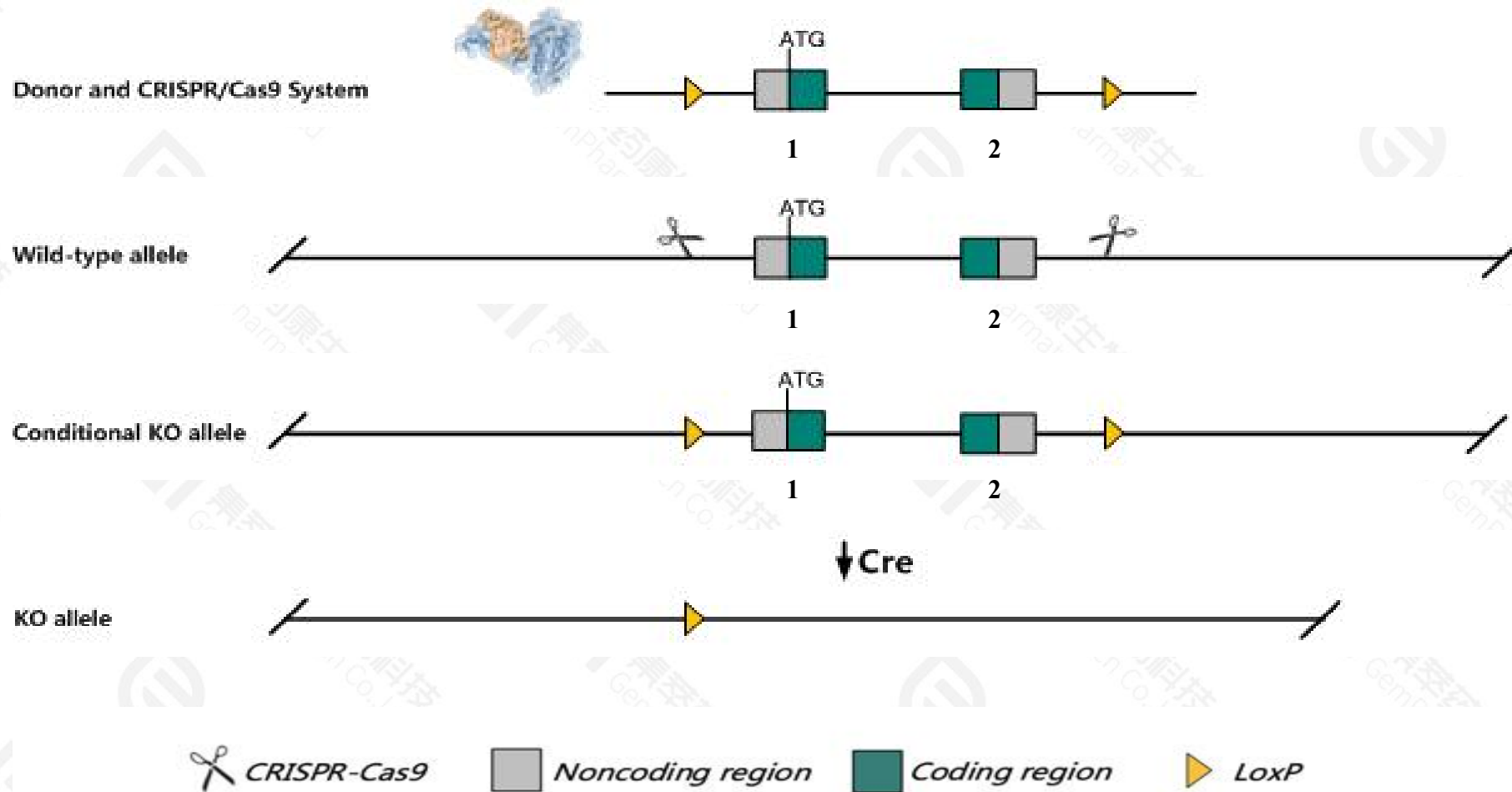
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Apof* gene has 2 transcripts. According to the structure of *Apof* gene, exon1-exon2 of *Apof*-202(ENSMUST00000238970.2) 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 *Apof* 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit impaired ABCG1-mediated cholesterol efflux.
- The KO region contains functional region of the *Stat2* gene. Knockout the region may affect the function of *Stat2* gene.
- This strategy may affect the 3-terminal regulation of the target gene.
- The *ApoE* gene is located on the Chr10. 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)

Apof apolipoprotein F [Mus musculus (house mouse)]

Gene ID: 103161, updated on 24-Apr-2022

Summary



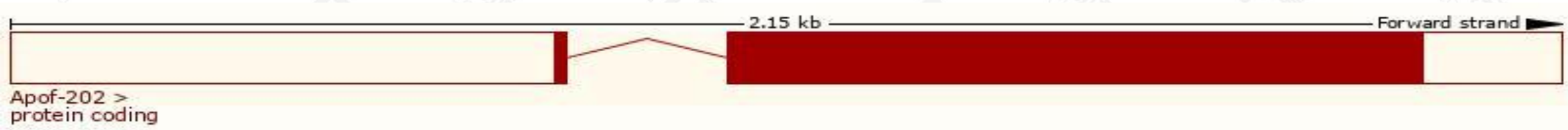
Official Symbol	Apof provided by MGI
Official Full Name	apolipoprotein F provided by MGI
Primary source	MGI:MGI:104539
See related	Ensembl:ENSMUSG00000047631
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI255964
Expression	Biased expression in liver adult (RPKM 507.2) and liver E18 (RPKM 297.1) See more
Orthologs	human all

Transcript information (Ensembl)

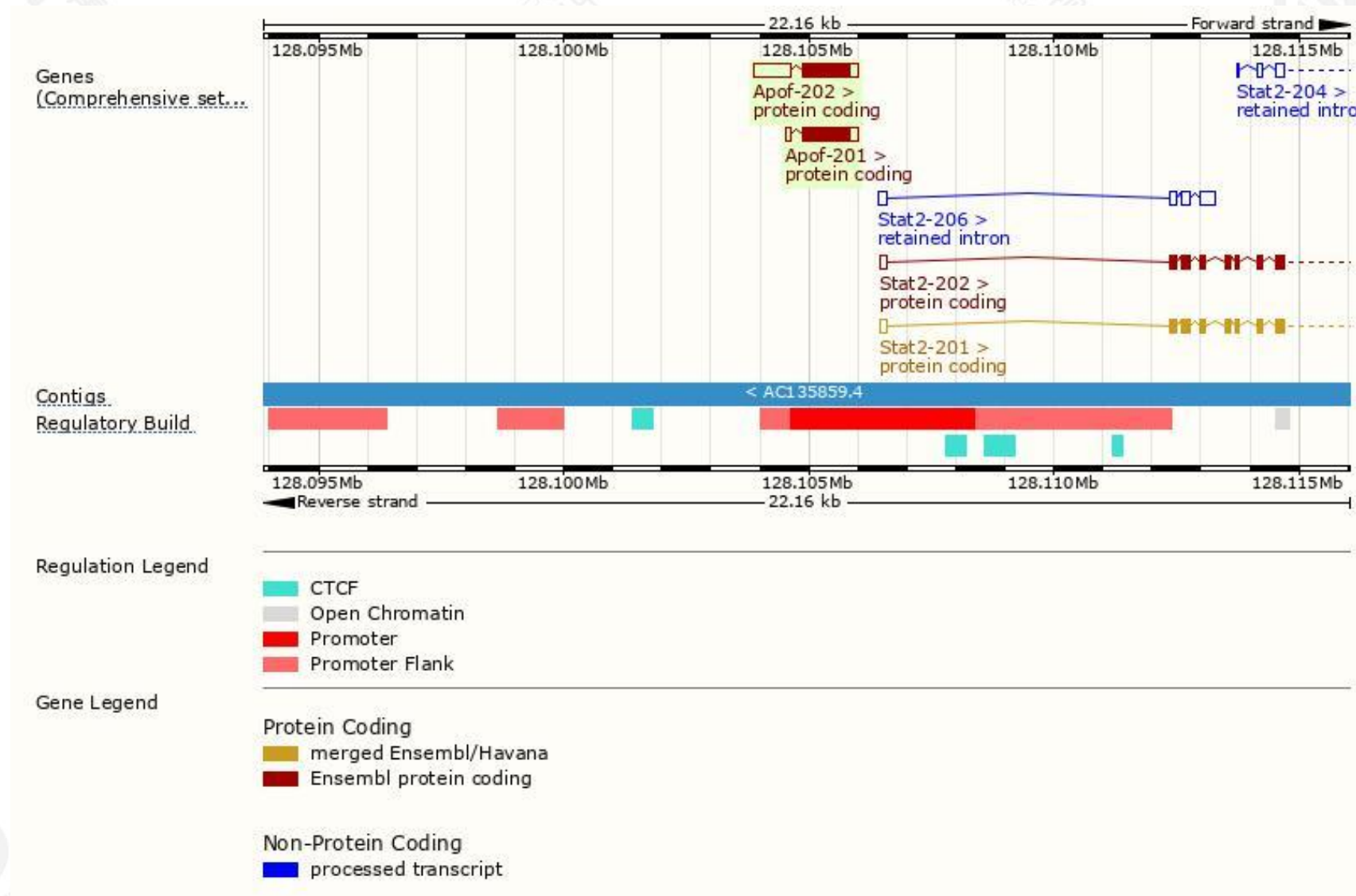
The gene has 2 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags		
ENSMUST00000238970.2	Apof-202	1930	326aa	Protein coding	CCDS24268	A0A0R4J0M4	Ensembl Canonical	GENCODE basic	APPRIS P4
ENSMUST00000050901.5	Apof-201	1258	315aa	Protein coding		Q91V80	GENCODE basic	APPRIS ALT2	TSL:1

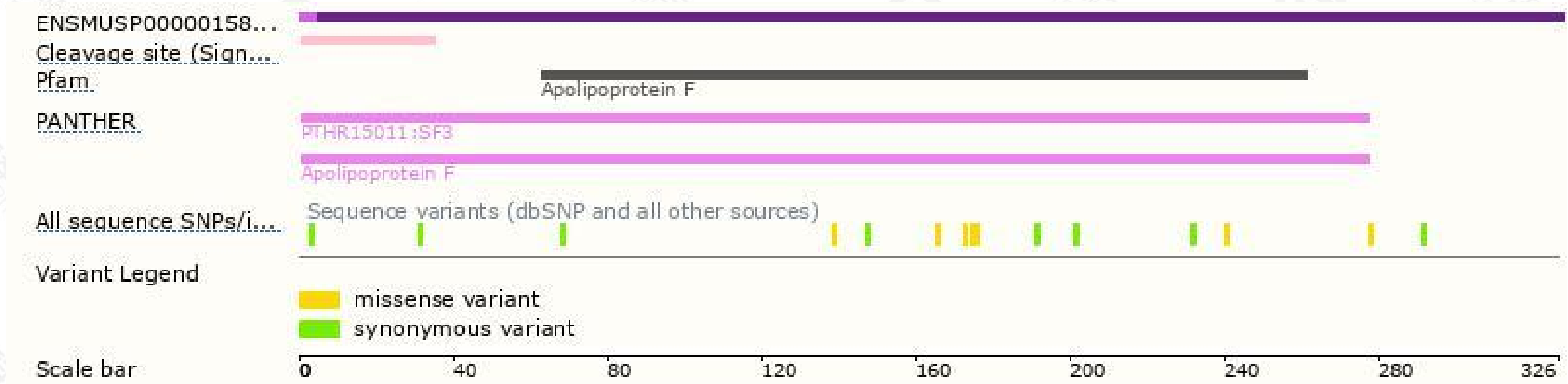
The strategy is based on the design of *Apof-202* transcript,the transcription is shown below:



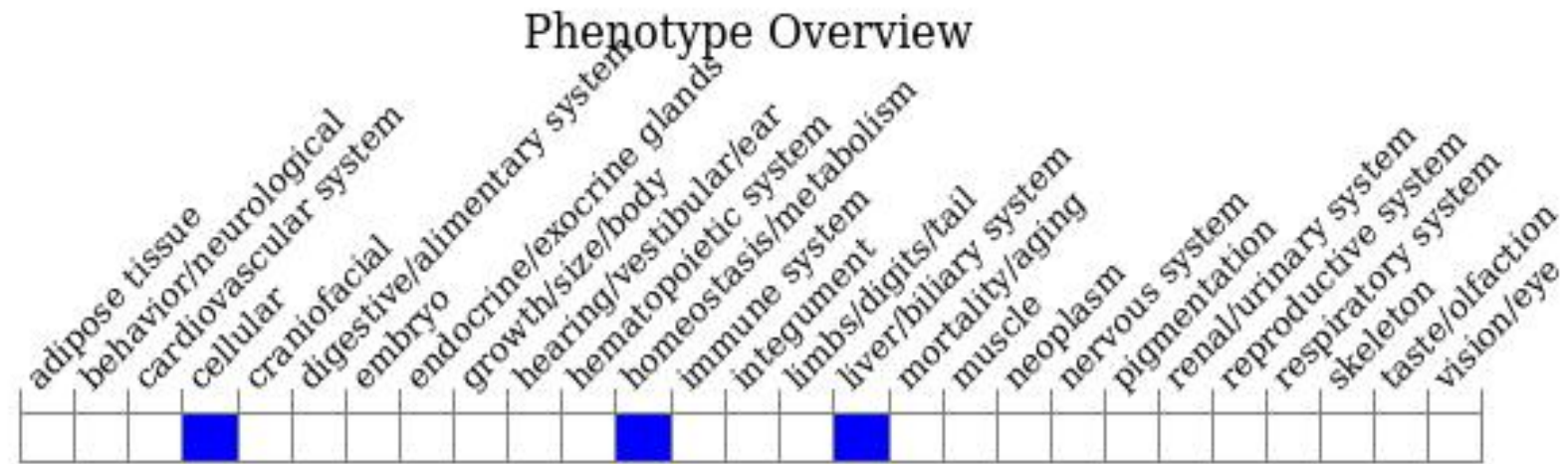
Genomic location distribution



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/>).

According to the existing MGI data, mice homozygous for a knock-out allele exhibit impaired ABCG1-mediated cholesterol efflux.

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