Awat2 Cas9-CKO Strategy

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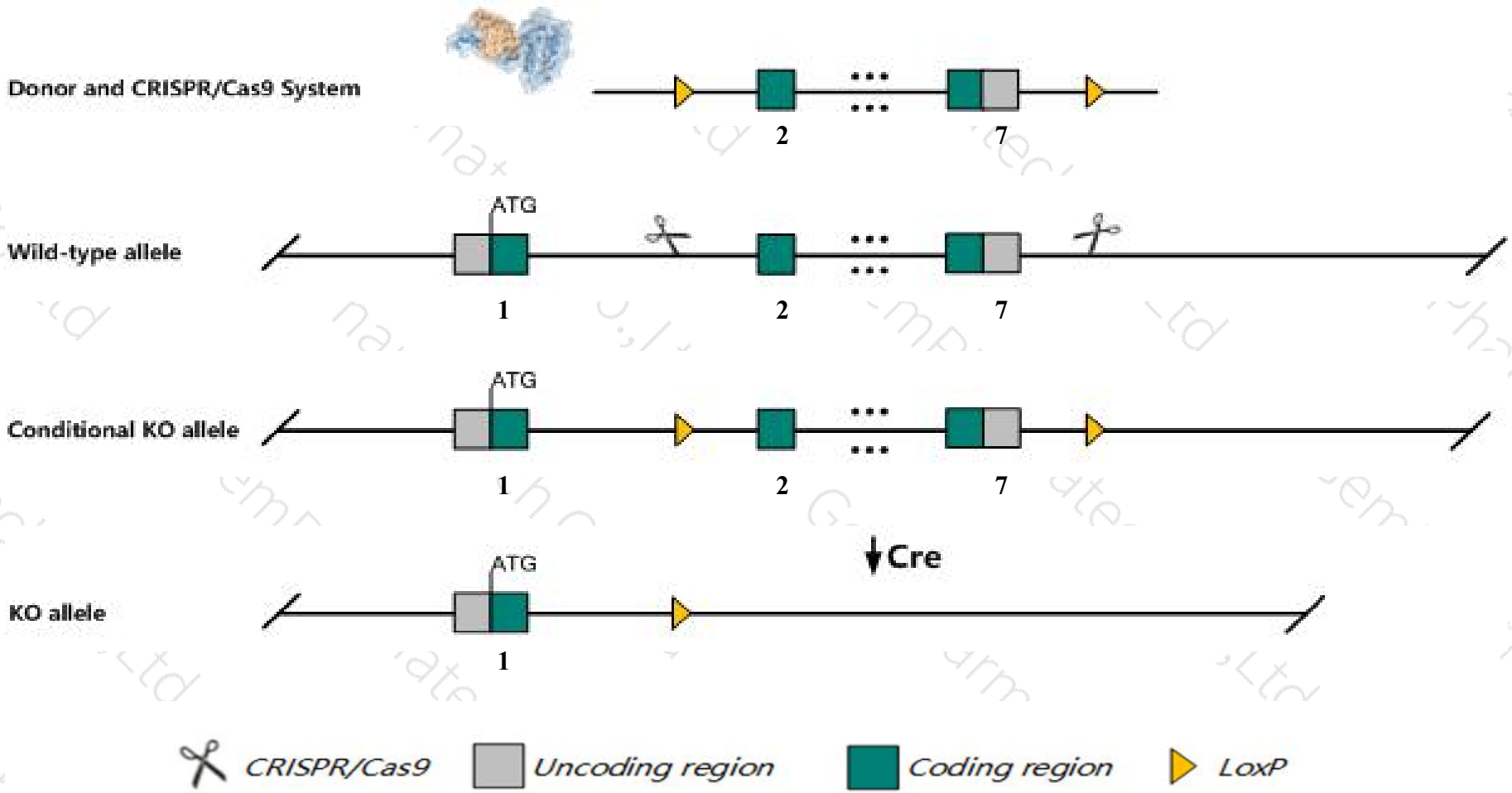
Design Date:2020-4-7

Project Overview

Project Name	***Awat2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Awat2* gene. The schematic diagram is as follows:



Technical routes

- The *Awat2* gene has 2 transcripts. According to the structure of *Awat2* gene, exon2-exon7 of *Awat2-201* (ENSMUST00000033567.14) transcript is recommended as the knockout region.The region contains 917bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Awat2* 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

➢ The *Awat2* gene is located on the ChrX. 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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Awat2 acyl-CoA wax alcohol acyltransferase 2 [Mus musculus (house mouse)]

Gene ID: 245532, updated on 13-Mar-2020

Summary

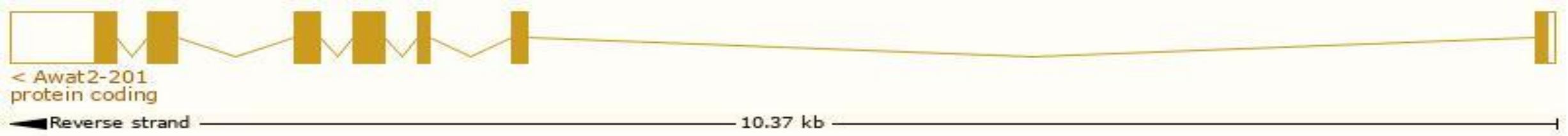
Official Symbol	Awat2 provided by MGI
Official Full Name	acyl-CoA wax alcohol acyltransferase 2 provided by MGI
Primary source	MGI:MGI:3045345
See related	Ensembl:ENSMUSG00000031220
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9430062J17Rik, ARAT, Dgat2l4, WS
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

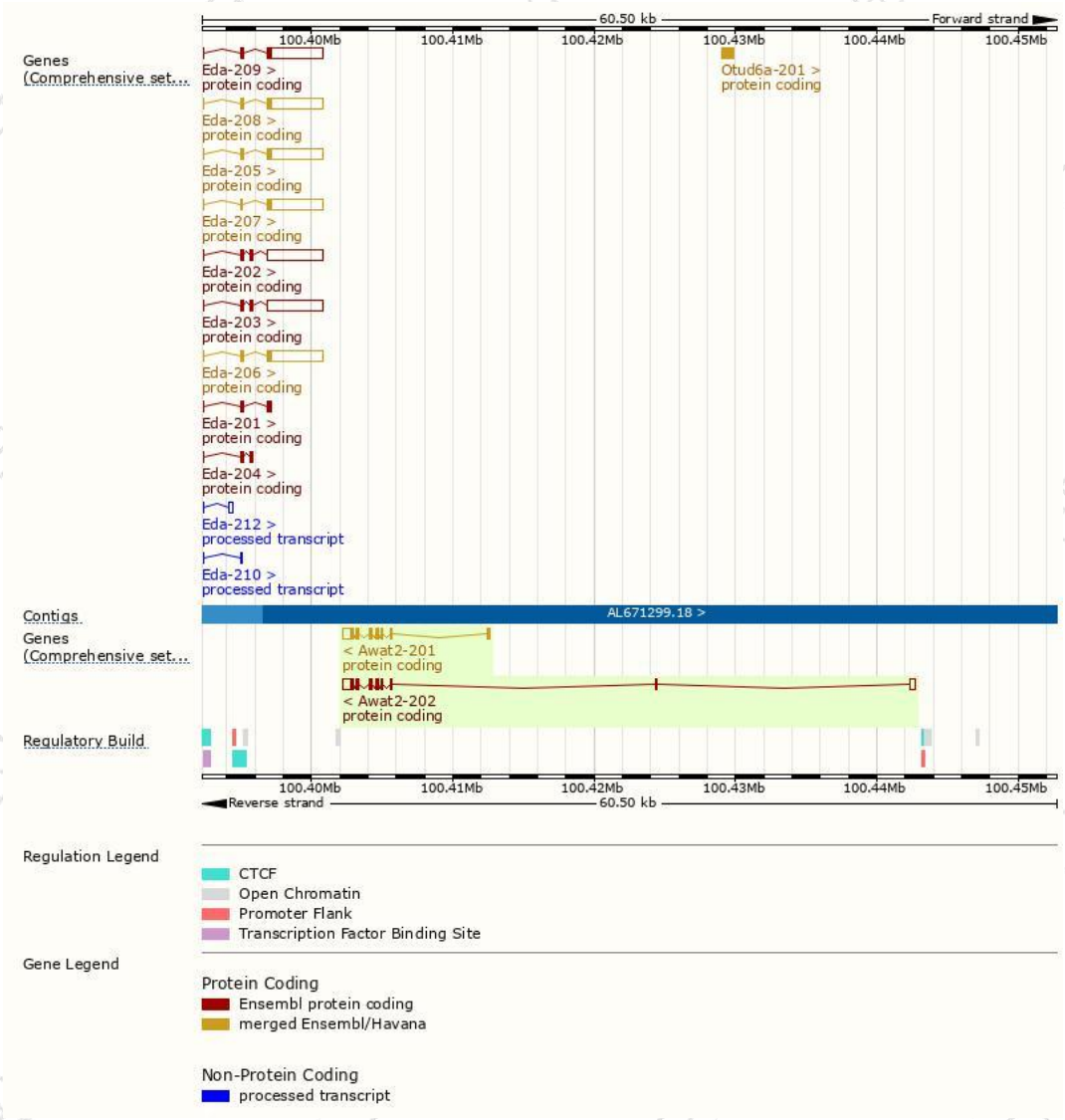
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Awat2-201	ENSMUST00000033567.14	1619	333aa	Protein coding	CCDS30300	A2ADU0 Q6E1M8	TSL:1 GENCODE basic APPRIS P1
Awat2-202	ENSMUST00000147103.1	1984	282aa	Protein coding	CCDS72412	Q6E1M8	TSL:1 GENCODE basic

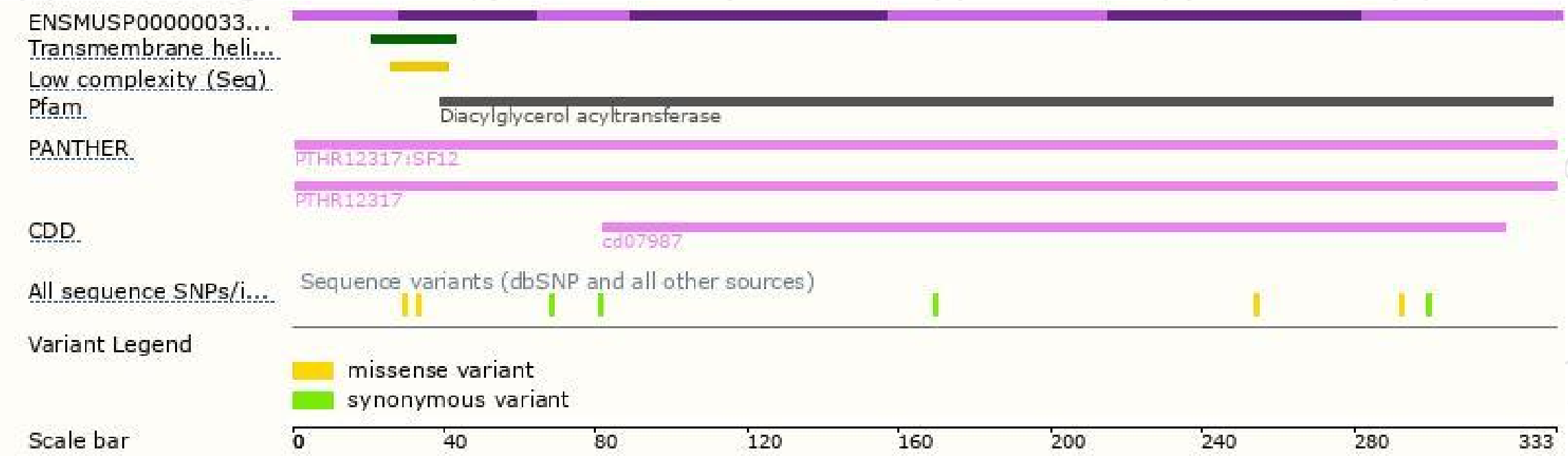
The strategy is based on the design of *Awat2-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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