Alox5 Cas9-KO Strategy

Designer: Ruirui Zhang
Reviewer: Huimin Su

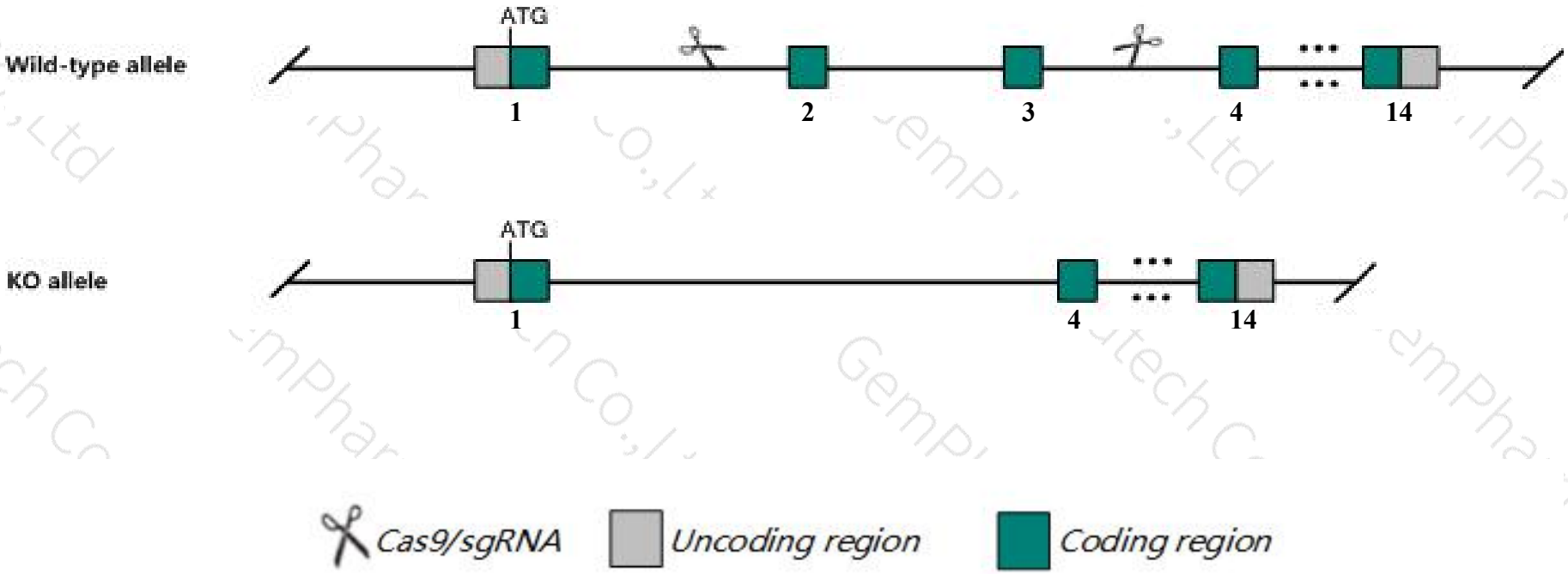
Design Date: 2019-12-16

Project Overview

Project Name	***Alox5***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Alox5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Alox5* gene has 8 transcripts. According to the structure of *Alox5* gene, exon2-exon3 of *Alox5-201* (ENSMUST00000026795.12) transcript is recommended as the knockout region.The region contains 281bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Alox5* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data,nullizygous mice show altered inflammatory responses. One null mutation causes resistance to lethal anaphylaxis, abnormal eicosanoid production and neutrophil recruitment while another leads to increased body fat, bone density, leptin and VLDL cholesterol levels and resistance to autoimmune uveitis.

- The *Alox5* gene is located on the Chr6. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Alox5 arachidonate 5-lipoxygenase [*Mus musculus* (house mouse)]

Gene ID: 11689, updated on 4-Dec-2019

Summary

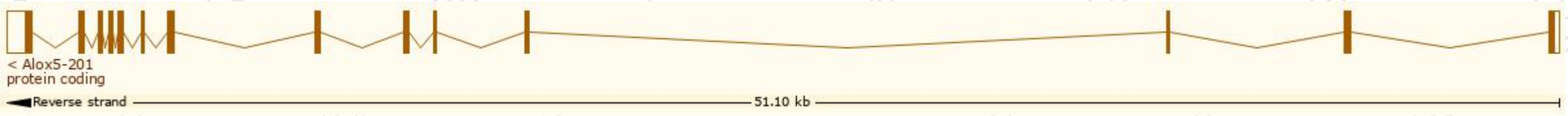
Official Symbol	Alox5 provided by MGI
Official Full Name	arachidonate 5-lipoxygenase provided by MGI
Primary source	MGI:MGI:87999
See related	Ensembl:ENSMUSG00000025701
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5LO; 5LX; 5-LO; 5-LOX; AI850497; F730011J02
Expression	Broad expression in lung adult (RPKM 3.4), heart adult (RPKM 2.7) and 22 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

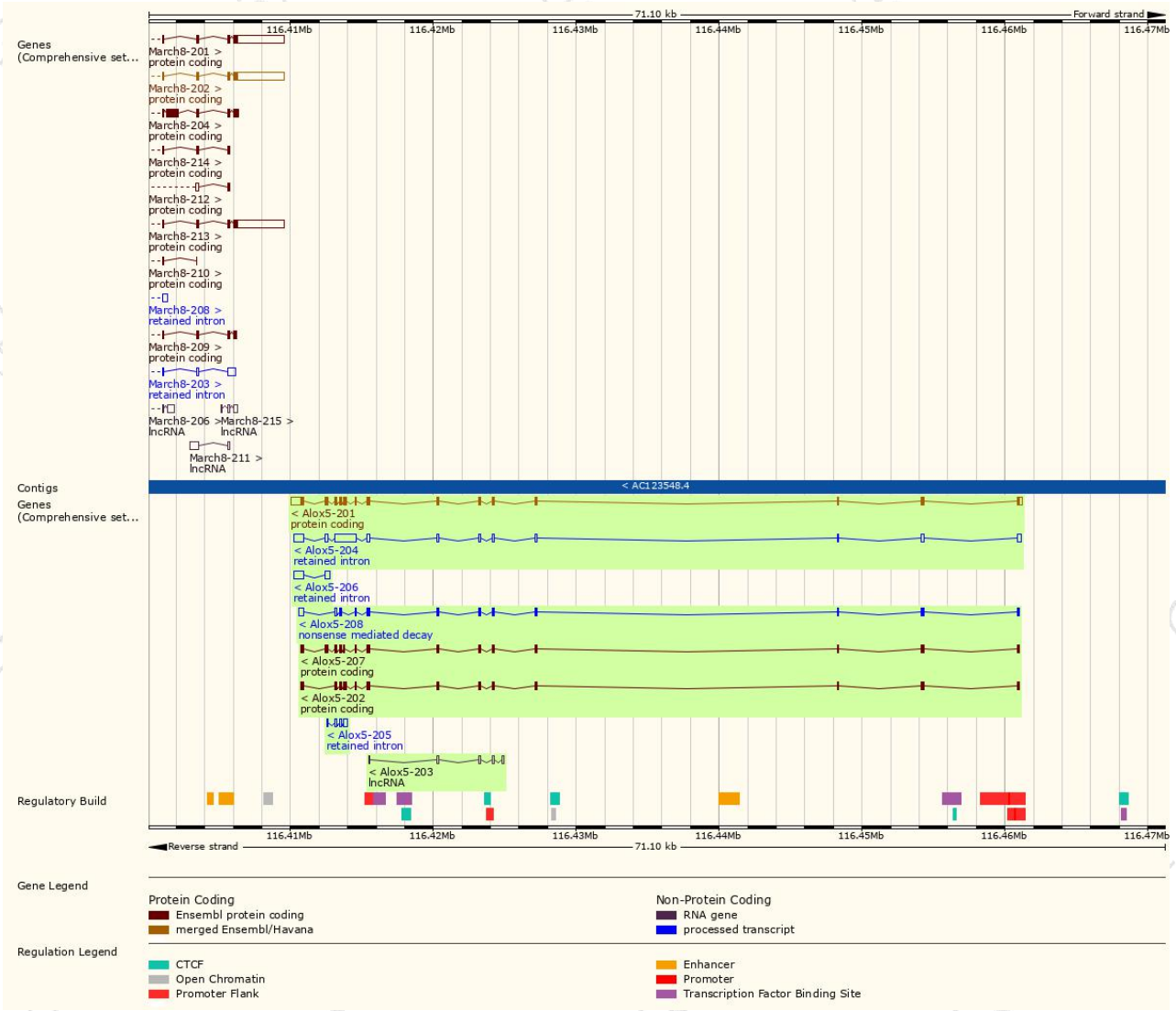
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alox5-201	ENSMUST00000026795.12	2821	674aa	Protein coding	CCDS20452	P48999	TSL:1 GENCODE basic APPRIS P1
Alox5-207	ENSMUST00000170186.1	1929	642aa	Protein coding	-	E9Q6H6	TSL:5 GENCODE basic
Alox5-202	ENSMUST00000164547.7	1854	617aa	Protein coding	-	E9QA93	TSL:5 GENCODE basic
Alox5-208	ENSMUST00000203722.2	1787	434aa	Nonsense mediated decay	-	A0A0N4SW45	TSL:5
Alox5-204	ENSMUST00000167447.7	3576	No protein	Retained intron	-	-	TSL:2
Alox5-206	ENSMUST00000169625.1	956	No protein	Retained intron	-	-	TSL:1
Alox5-205	ENSMUST00000167585.1	546	No protein	Retained intron	-	-	TSL:2
Alox5-203	ENSMUST00000167174.1	659	No protein	lncRNA	-	-	TSL:3

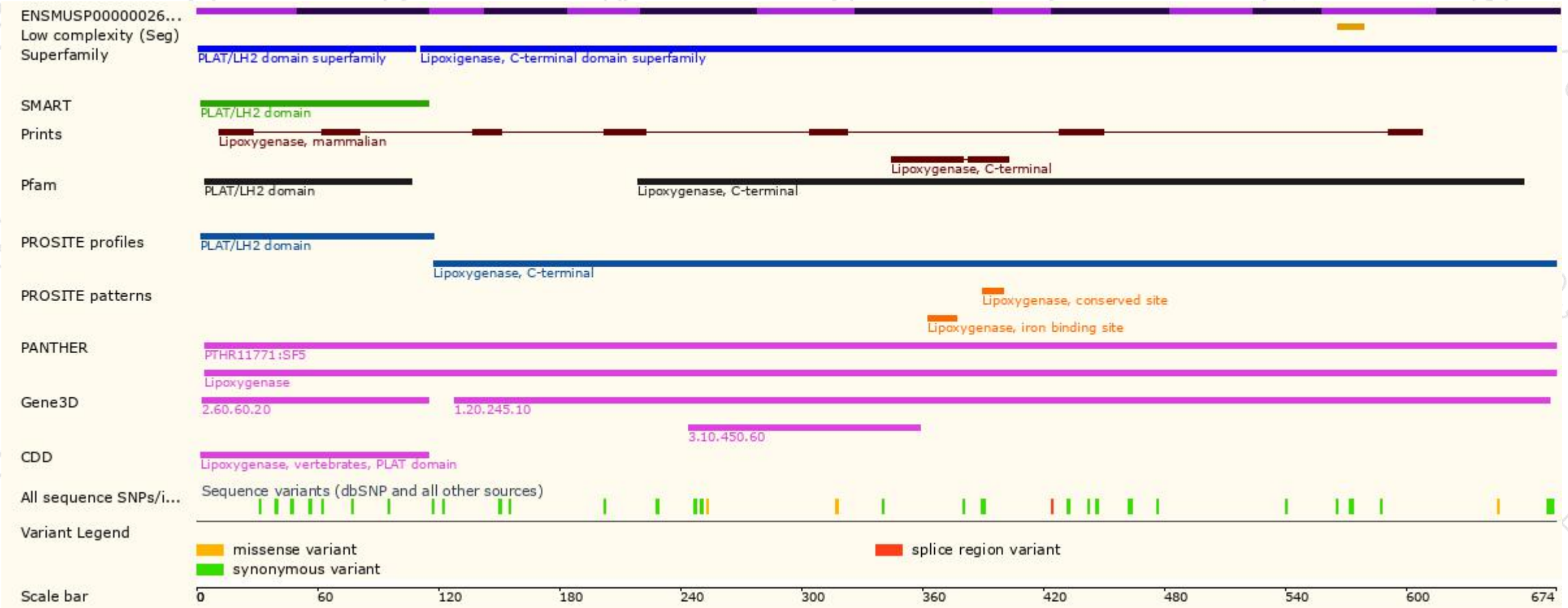
The strategy is based on the design of *Alox5-201* transcript, the transcription is shown below:



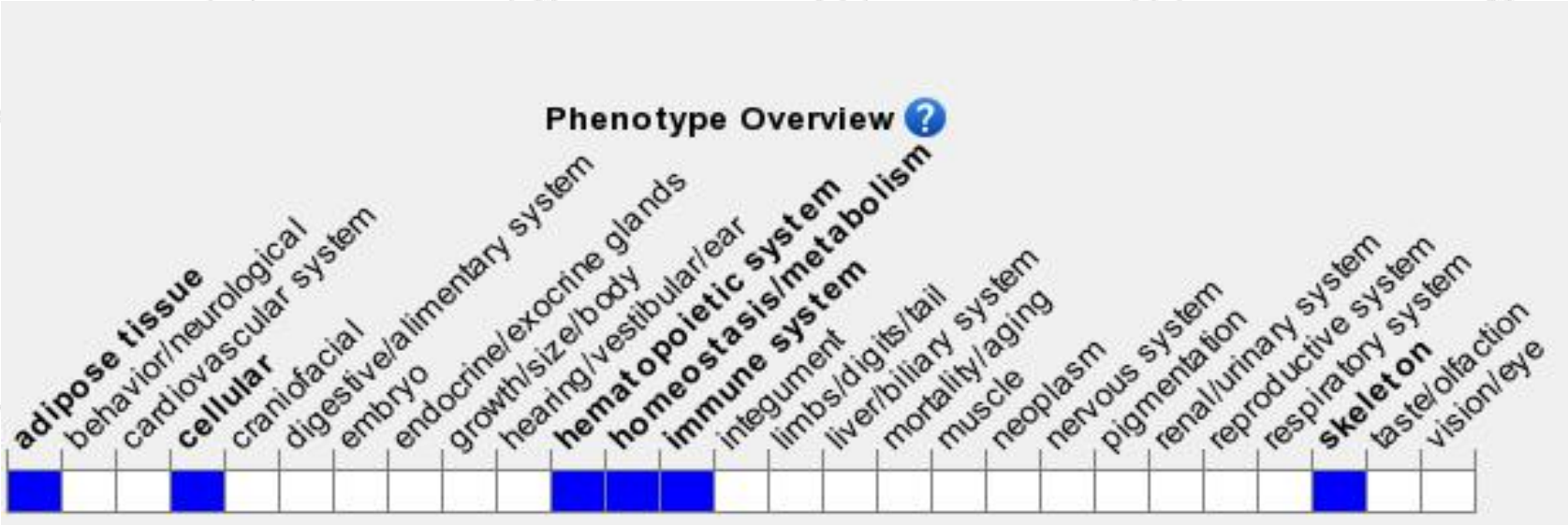
Genomic location distribution



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,nullizygous mice show altered inflammatory responses. One null mutation causes resistance to lethal anaphylaxis, abnormal eicosanoid production and neutrophil recruitment while another leads to increased body fat, bone density, leptin and VLDL cholesterol levels and resistance to autoimmune uveitis.

集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd