Aldob Cas9-CKO Strategy

Designer: Yang Zeng
Reviewer: Yanhua Shen

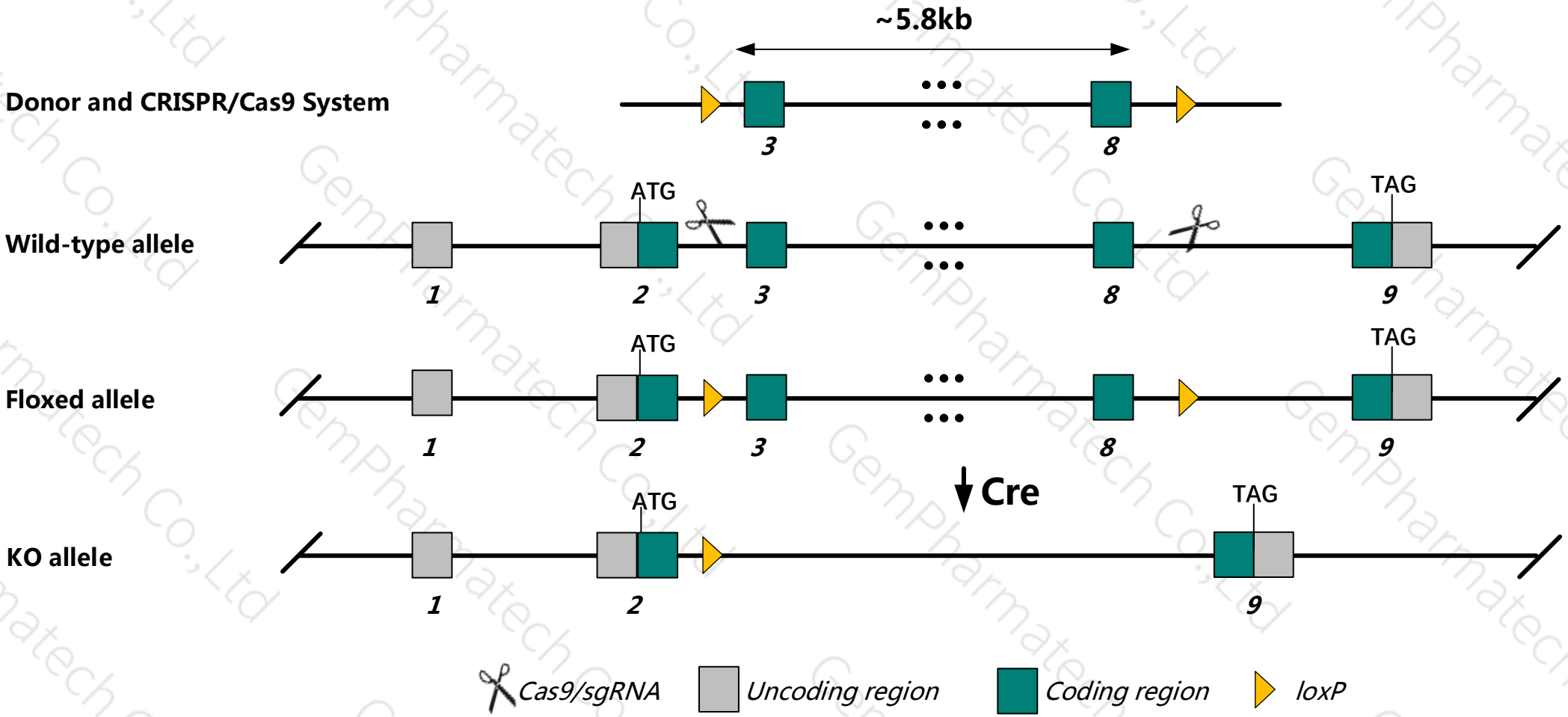
Design Date: 2019-10-31

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Aldob* gene has 3 transcripts. According to the structure of *Aldob* gene, exon3-exon8 of *Aldob-201* (ENSMUST00000029987.9) transcript is recommended as the knockout region.The region contains 887bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Aldob* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,Following exposure to a 40% fructose diet, mice homozygous for a null allele exhibit failure to thrive, liver pathology and dysfunction, and a high mortality rate.

- The *Aldob* gene is located on the Chr4. 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）

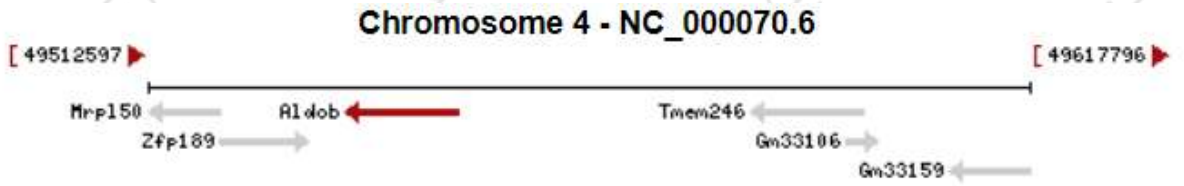
集萃药康 GemPharmatech

Aldob aldolase B, fructose-bisphosphate [*Mus musculus* (house mouse)]

Gene ID: 230163, updated on 12-Aug-2019

Summary

Official Symbol	Aldob provided by MGI
Official Full Name	aldolase B, fructose-bisphosphate provided by MGI
Primary source	MGI:MGI:87995
See related	Ensembl:ENSMUSG00000028307
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	Aldo2; Aldo-2; BC016435
Summary	This gene encodes a subunit of the homotetrameric enzyme aldolase B, an isozyme of the class I fructose 1,6-bisphosphate aldolase enzyme. This enzyme catalyzes the conversion of fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Homozygous knockout mice for this gene exhibit liver damage and death following fructose ingestion. A pseudogene of this gene has been identified in the genome. [provided by RefSeq, Aug 2015]
Expression	Biased expression in kidney adult (RPKM 1344.8), liver adult (RPKM 757.5) and 5 other tissues See more
Orthologs	human all

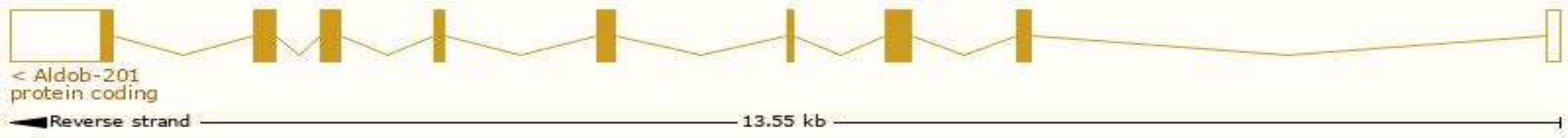


Transcript information（Ensembl）

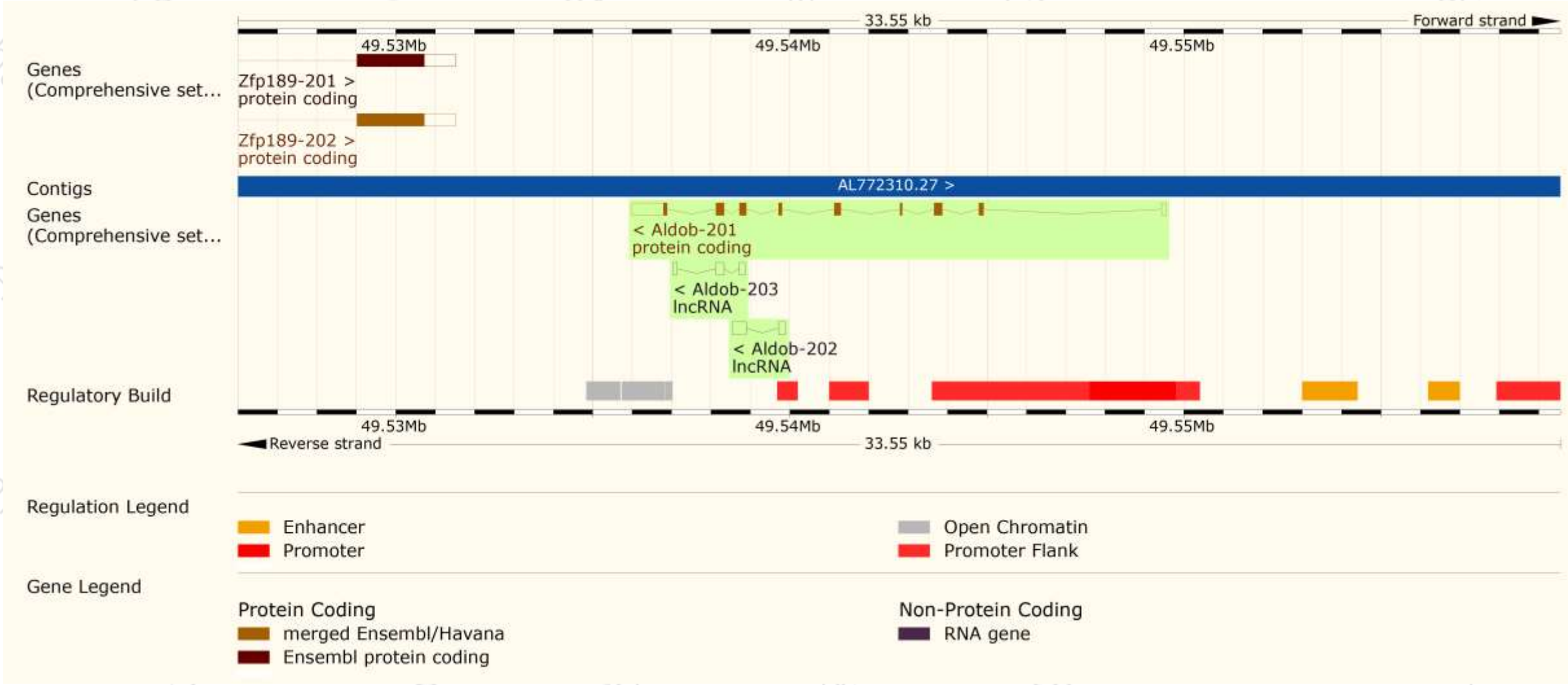
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aldob-201	ENSMUST00000029987.9	2016	364aa	Protein coding	CCDS18176	Q3UER1 Q91Y97	TSL:1 GENCODE basic APPRIS P1
Aldob-202	ENSMUST00000144372.1	530	No protein	lncRNA	-	-	TSL:2
Aldob-203	ENSMUST00000148415.1	454	No protein	lncRNA	-	-	TSL:3

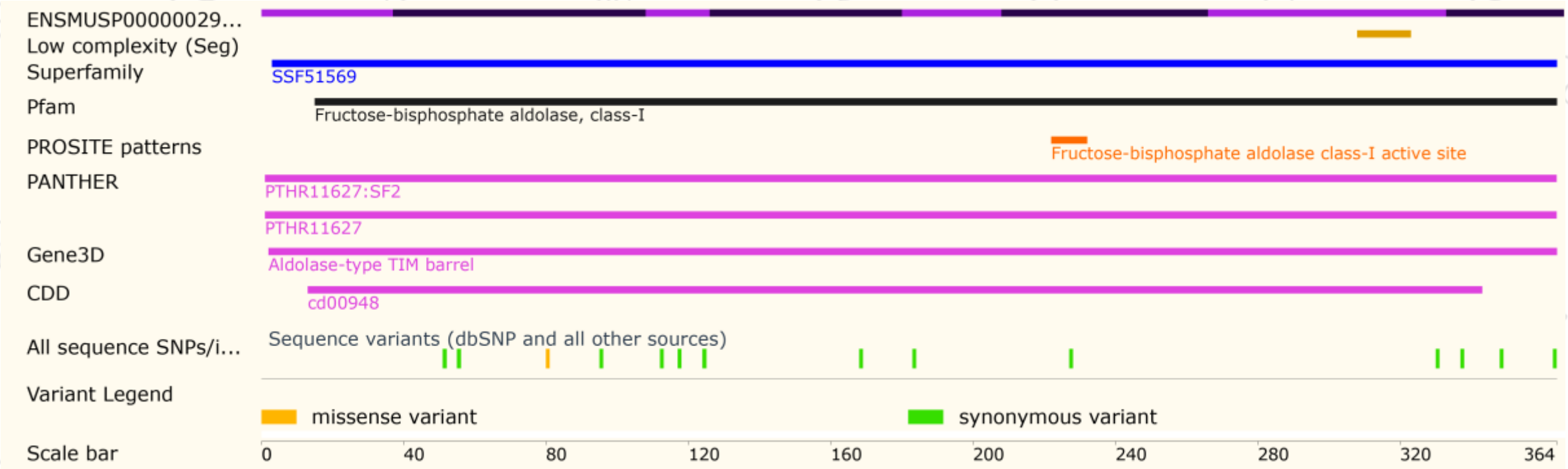
The strategy is based on the design of *Aldob-201* transcript,The transcription is shown below



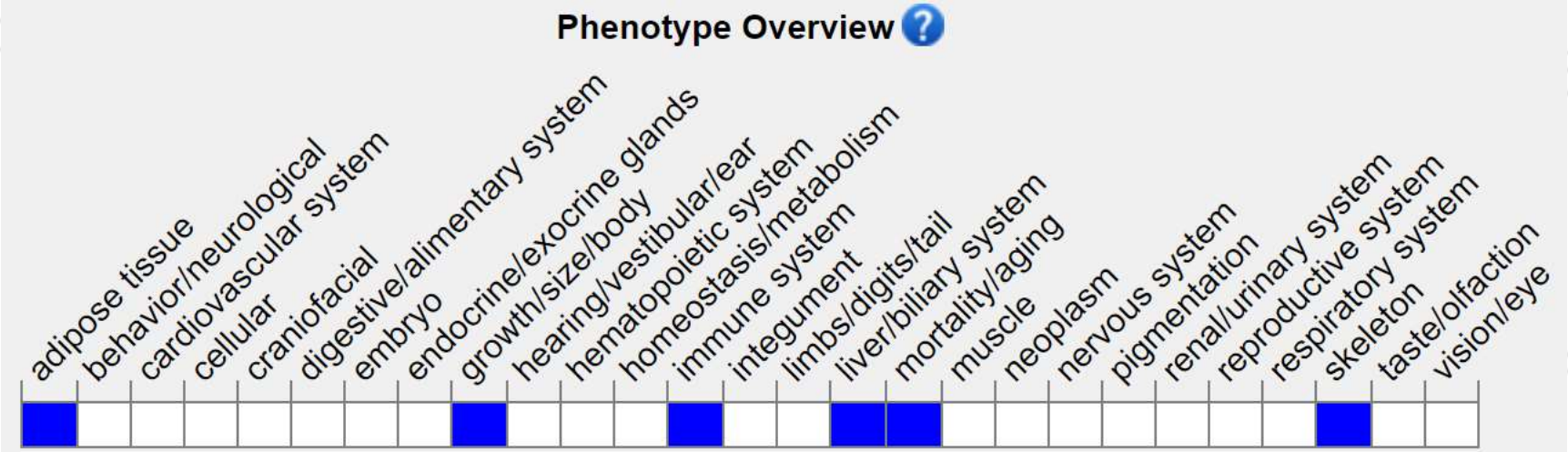
Genomic location distribution



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,Following exposure to a 40% fructose diet, mice homozygous for a null allele exhibit failure to thrive, liver pathology and dysfunction, and a high mortality rate.

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