

Alkbh8 Cas9-CKO Strategy

Designer: Xueting Zhang
reviewer: Yanhua Shen
Date: 2020-02-26

Project Overview

Project Name

Alkbh8

Project type

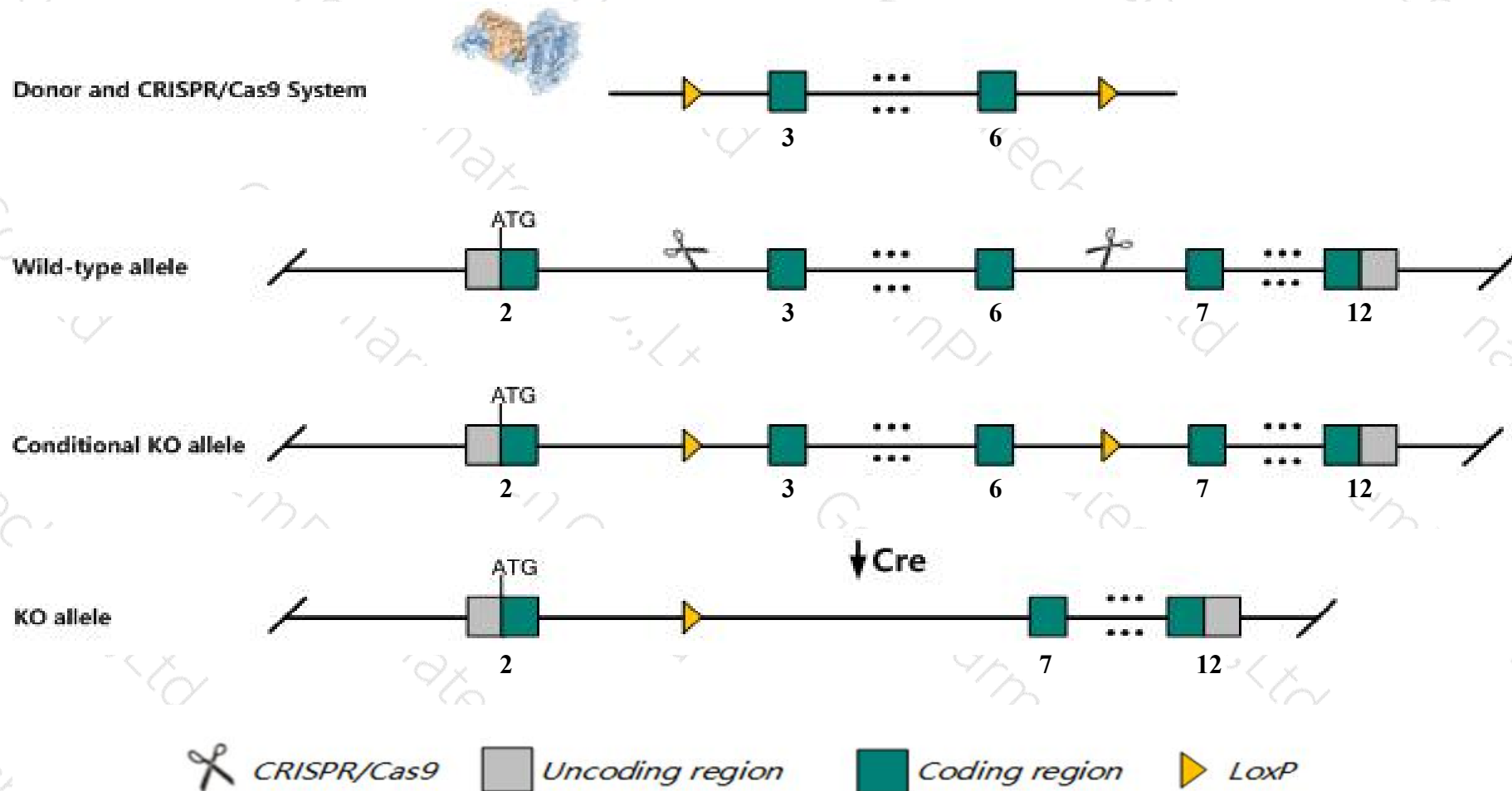
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Alkbh8* gene. The schematic diagram is as follows:



Technical routes

- The *Alkbh8* gene has 10 transcripts. According to the structure of *Alkbh8* gene, exon3-exon6 of *Alkbh8-204* (ENSMUST00000211933.1) transcript is recommended as the knockout region. The region contains 571bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Alkbh8* 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, Homozygous mutants show no obvious phenotype at 20 months of age.
- Transcript *Alkbh8*-210 may not be affected.
- The floxed region is near to the N-terminal of *Gm24918* gene, this strategy may influence the regulatory function of the N-terminal of *Gm24918* gene.
- The *Alkbh8* 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)

Alkbh8 alkB homolog 8, tRNA methyltransferase [*Mus musculus* (house mouse)]

Gene ID: 67667, updated on 10-Oct-2019

Summary

- Official Symbol

Alkbh8 provided by MGI
- Official Full Name

alkB homolog 8, tRNA methyltransferase provided by MGI
- Primary source

MGI:MGI:1914917
- See related

Ensembl:ENSMUSG00000025899
- Gene type

protein coding
- RefSeq status

PROVISIONAL
- Organism

Mus musculus
- Lineage

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as

Abh8; 4930562C03Rik; 8030431D03Rik; 9430088N01Rik
- Expression

Broad expression in CNS E18 (RPKM 3.8), whole brain E14.5 (RPKM 3.5) and 23 other tissues [See more](#)
- Orthologs

[human](#) [all](#)

Genomic context

Location: 9; 9 A1

See Alkbh8 in [Genome Data Viewer](#)

Exon count: 14

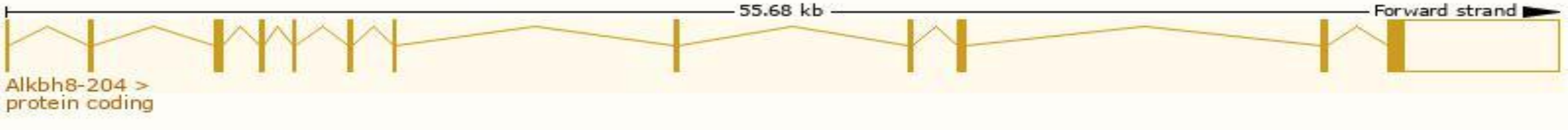
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (3335231..3391154)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (3335231..3385847)

Transcript information (Ensembl)

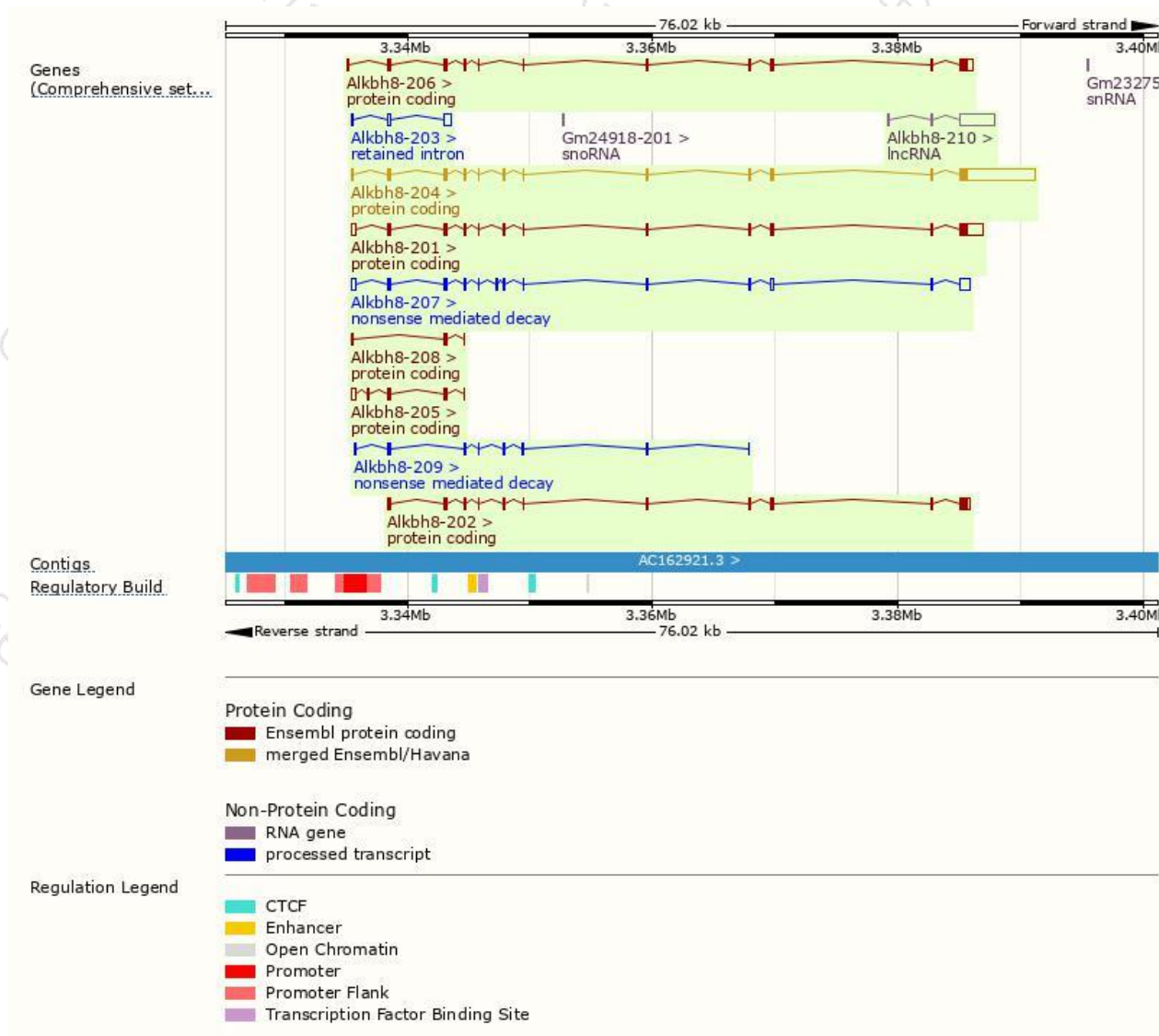
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alkbh8-204	ENSMUST00000211933.1	7674	664aa	Protein coding	CCDS22792	Q80Y20	TSL:1 GENCODE basic APPRIS P2
Alkbh8-201	ENSMUST00000053407.12	3603	664aa	Protein coding	CCDS22792	Q80Y20	TSL:1 GENCODE basic APPRIS P2
Alkbh8-202	ENSMUST00000165105.1	2256	664aa	Protein coding	CCDS22792	Q80Y20	TSL:5 GENCODE basic APPRIS P2
Alkbh8-206	ENSMUST00000212294.1	2584	629aa	Protein coding	-	Q80Y20	TSL:1 GENCODE basic APPRIS ALT2
Alkbh8-205	ENSMUST00000212154.1	761	142aa	Protein coding	-	A0A1D5RLN3	CDS 3' incomplete TSL:3
Alkbh8-208	ENSMUST00000212666.1	369	57aa	Protein coding	-	A0A1D5RLQ8	CDS 3' incomplete TSL:2
Alkbh8-207	ENSMUST00000212358.1	2687	199aa	Nonsense mediated decay	-	A0A1D5RL93	TSL:1
Alkbh8-209	ENSMUST00000212817.1	750	60aa	Nonsense mediated decay	-	A0A1D5RLF6	TSL:5
Alkbh8-203	ENSMUST00000211884.1	852	No protein	Retained intron	-	-	TSL:1
Alkbh8-210	ENSMUST00000212955.1	3076	No protein	lncRNA	-	-	TSL:1

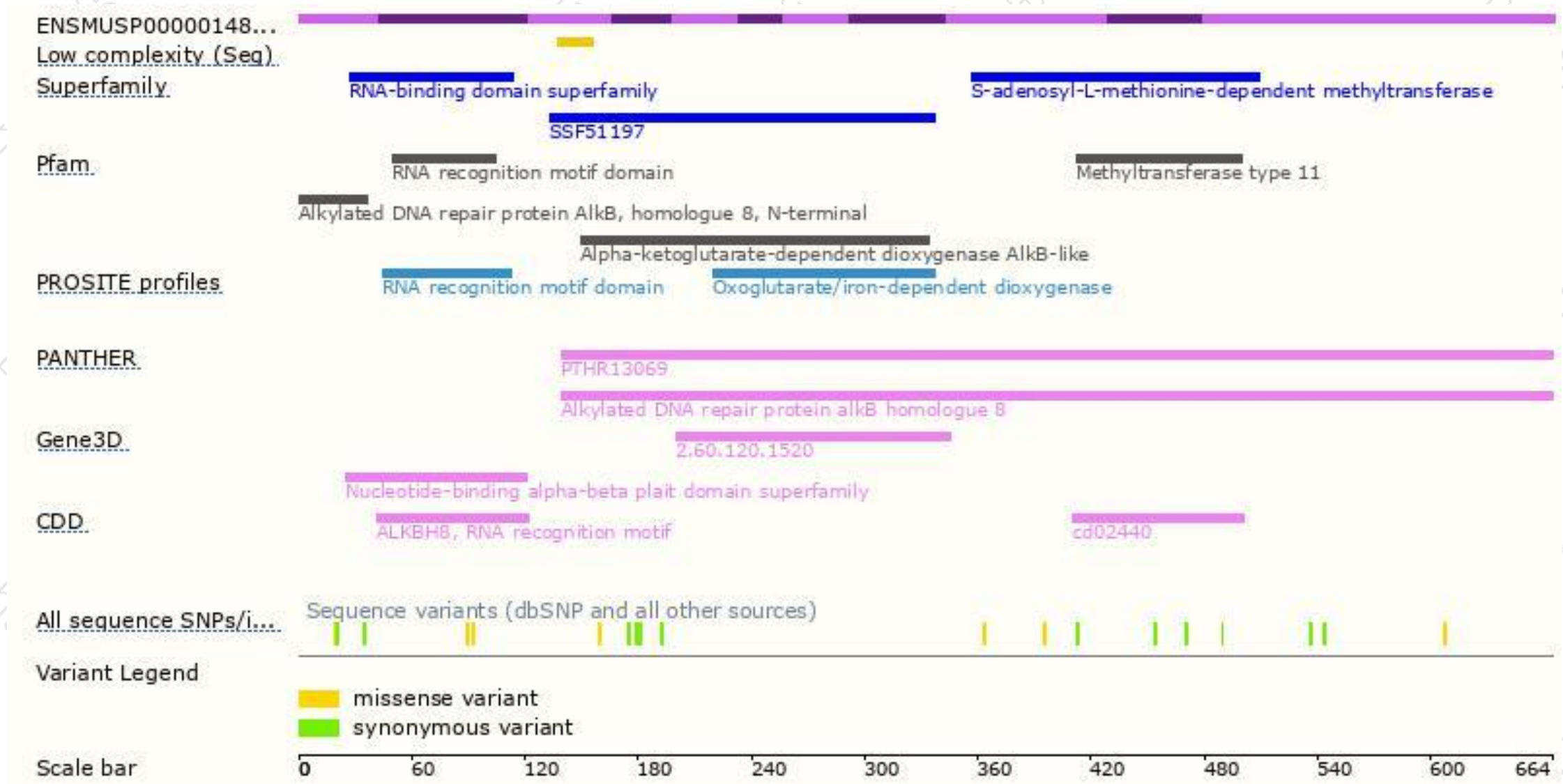
The strategy is based on the design of *Alkbh8-204* transcript,The transcription is shown below



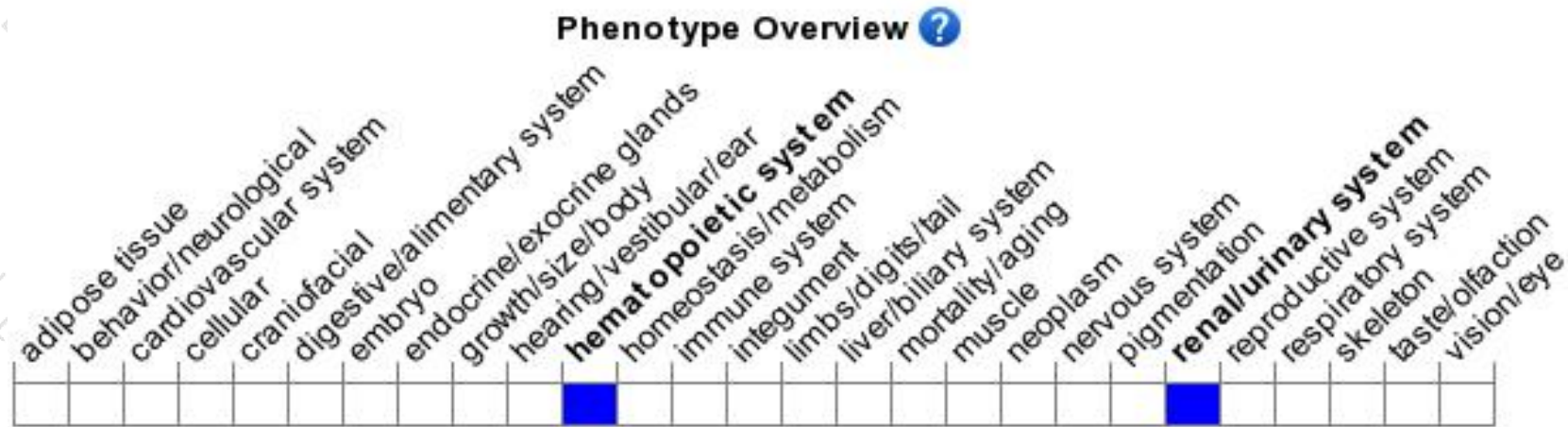
Genomic location distribution



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, Homozygous mutants show no obvious phenotype at 20 months of age.

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

Tel: 400-9660890

