Als2cl Cas9-CKO Strategy

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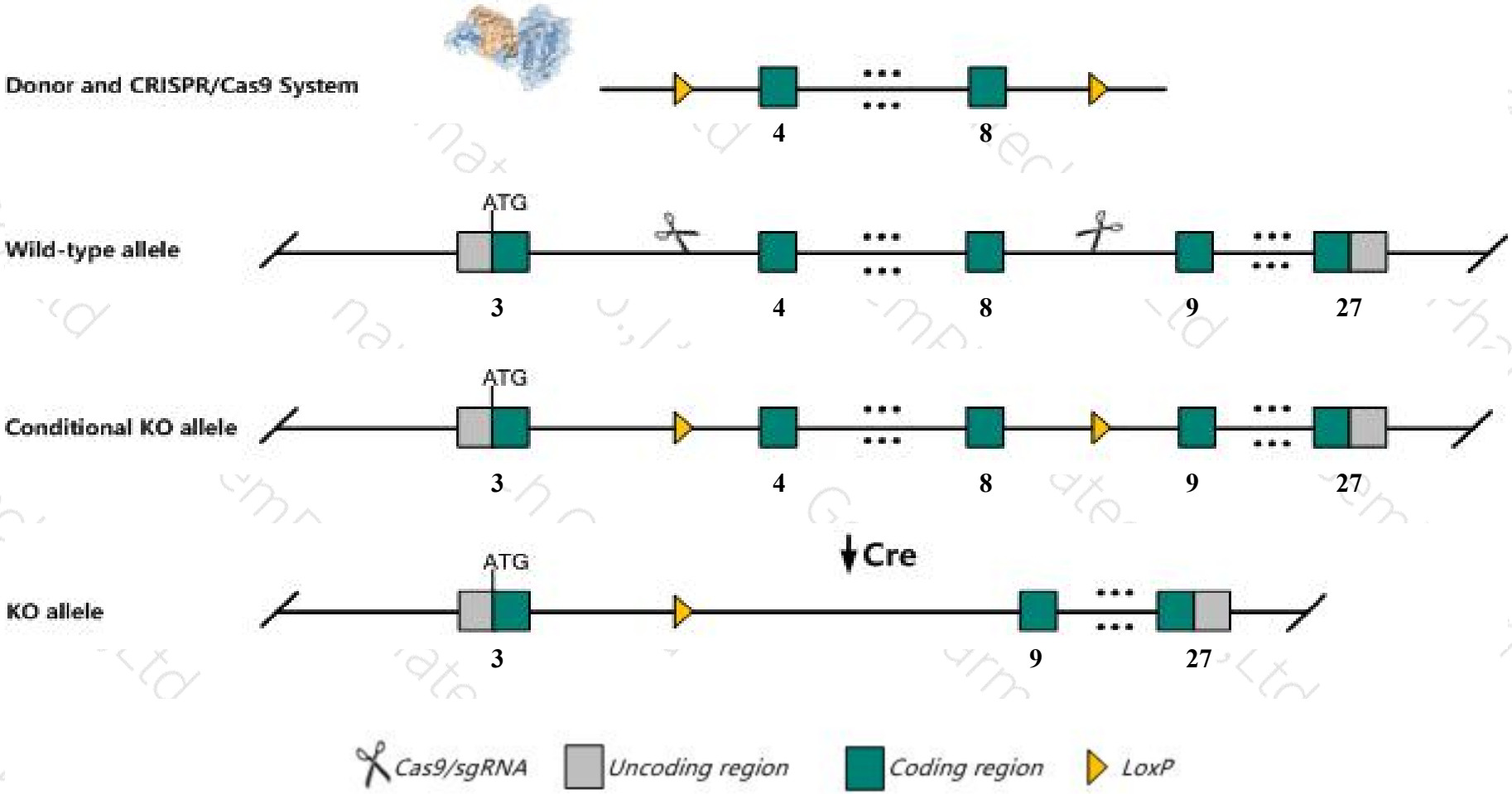
Project Overview

Project Name	***Als2cl***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Als2cl* gene. The schematic diagram is as follows:



Technical routes

➢ The *Als2cl* gene has 8 transcripts. According to the structure of *Als2cl* gene, exon4-exon8 of *Als2cl-206*(ENSMUST00000155014.1) transcript is recommended as the knockout region.The region contains 683bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Als2cl* 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

➢ The *Als2cl* 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）

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Als2cl ALS2 C-terminal like [Mus musculus (house mouse)]

Gene ID: 235633, updated on 20-Mar-2020

Summary

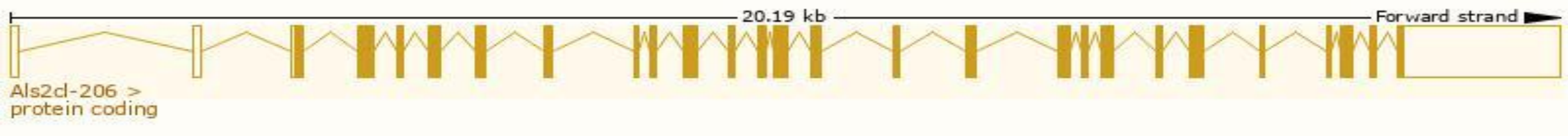
Official Symbol	Als2cl provided by MGI
Official Full Name	ALS2 C-terminal like provided byMGI
Primary source	MGI:MGI:2447532
See related	Ensembl:ENSMUSG00000044037
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	79H19D, 79H19E, BC022645, D930044G19Rik, mRn.49018
Expression	Broad expression in lung adult (RPKM 21.1), thymus adult (RPKM 17.2) and 19 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

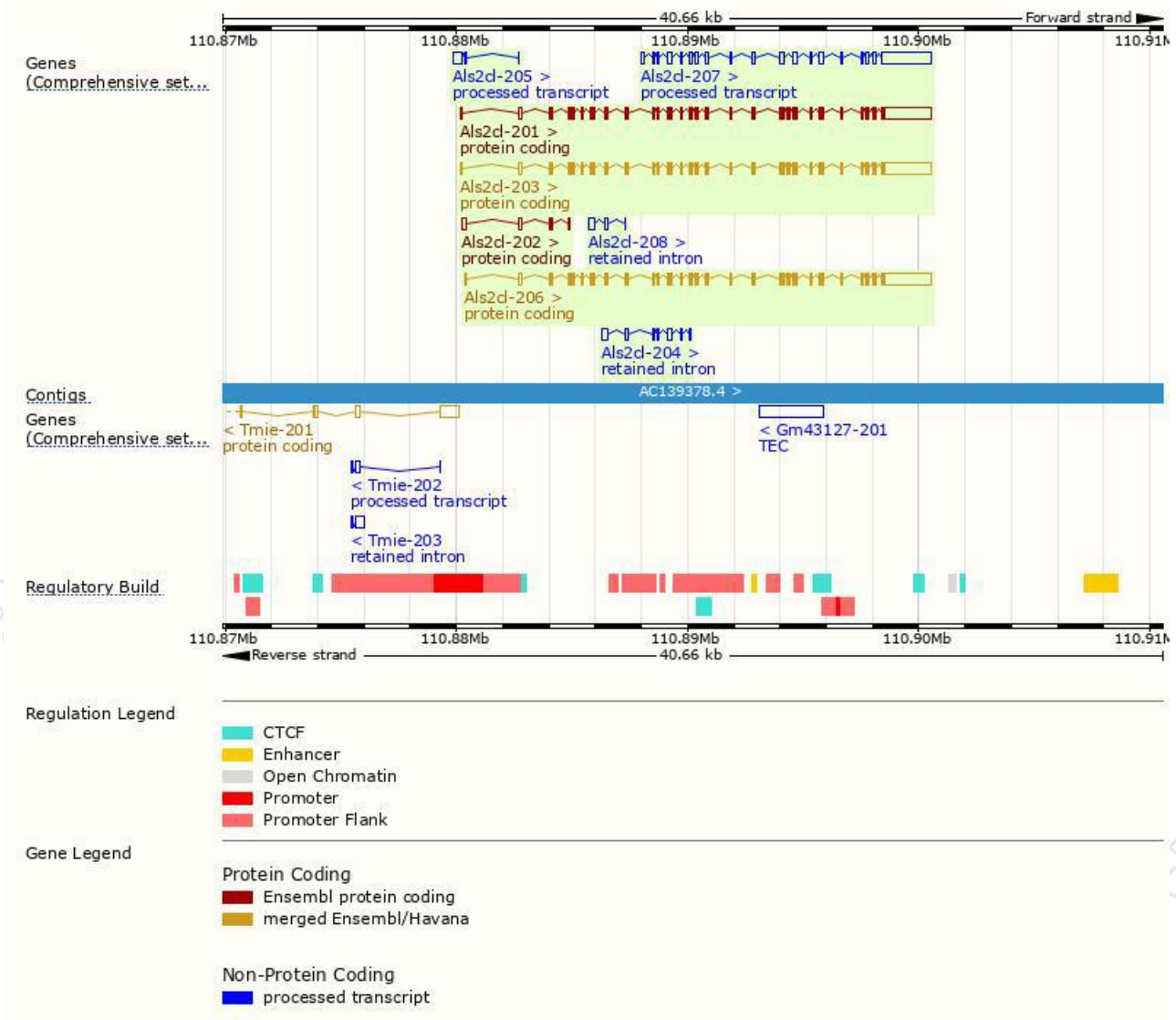
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Als2cl-206	ENSMUST00000155014.1	5150	952aa	Protein coding	CCDS23579	A0A0R4J1X4	TSL:1 GENCODE basic APPRIS P1
Als2cl-201	ENSMUST00000084926.8	5079	952aa	Protein coding	CCDS23579	A0A0R4J1X4	TSL:1 GENCODE basic APPRIS P1
Als2cl-203	ENSMUST00000130386.7	5056	952aa	Protein coding	CCDS23579	A0A0R4J1X4	TSL:1 GENCODE basic APPRIS P1
Als2cl-202	ENSMUST00000123389.7	478	39aa	Protein coding	-	A0A0G2JEP6	CDS 3' incomplete TSL:5
Als2cl-207	ENSMUST00000156673.1	4122	No protein	Processed transcript	-	-	TSL:1
Als2cl-205	ENSMUST00000143416.1	485	No protein	Processed transcript	-	-	TSL:5
Als2cl-204	ENSMUST00000141845.7	824	No protein	Retained intron	-	-	TSL:5
Als2cl-208	ENSMUST00000200613.1	364	No protein	Retained intron	-	-	TSL:5

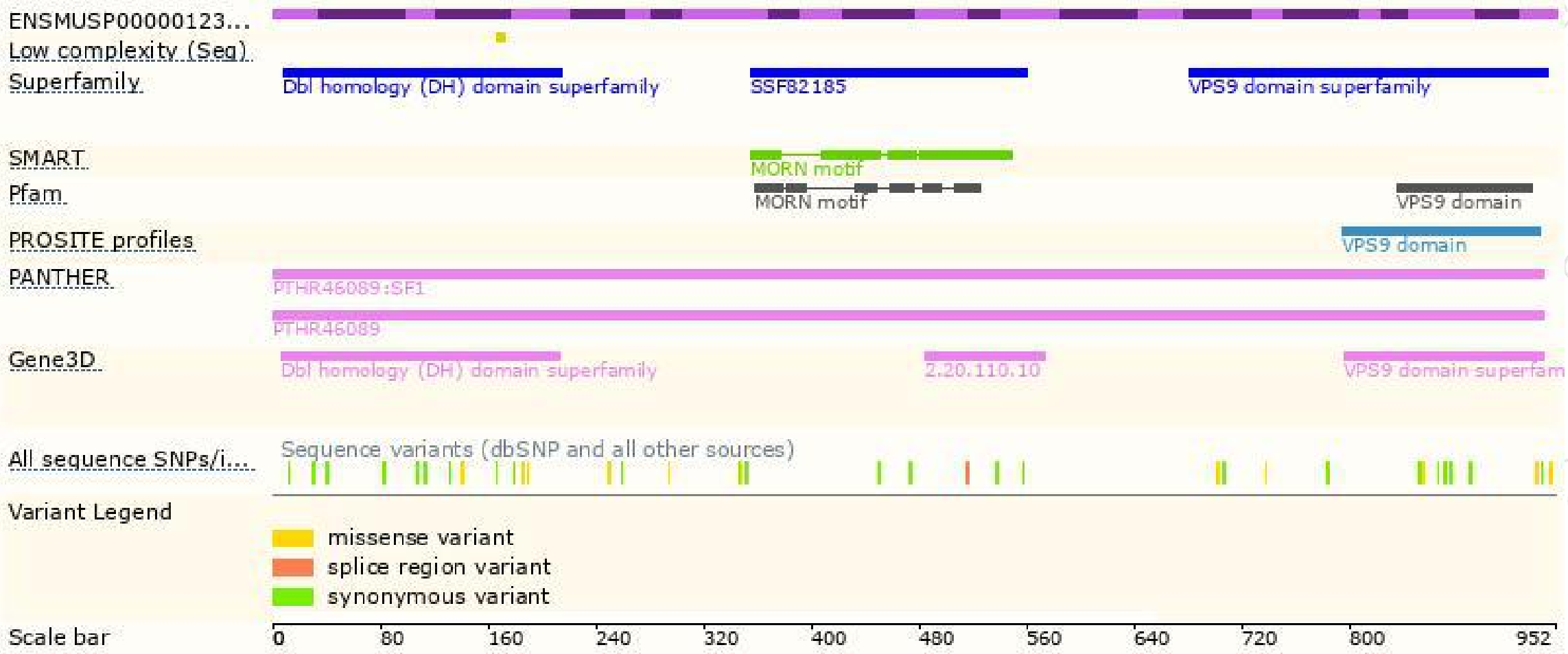
The strategy is based on the design of *Als2cl-206* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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