

Ascl2 Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruiuri Zhang

Design Date: 2020-6-23

Project Overview

Project Name

Ascl2

Project type

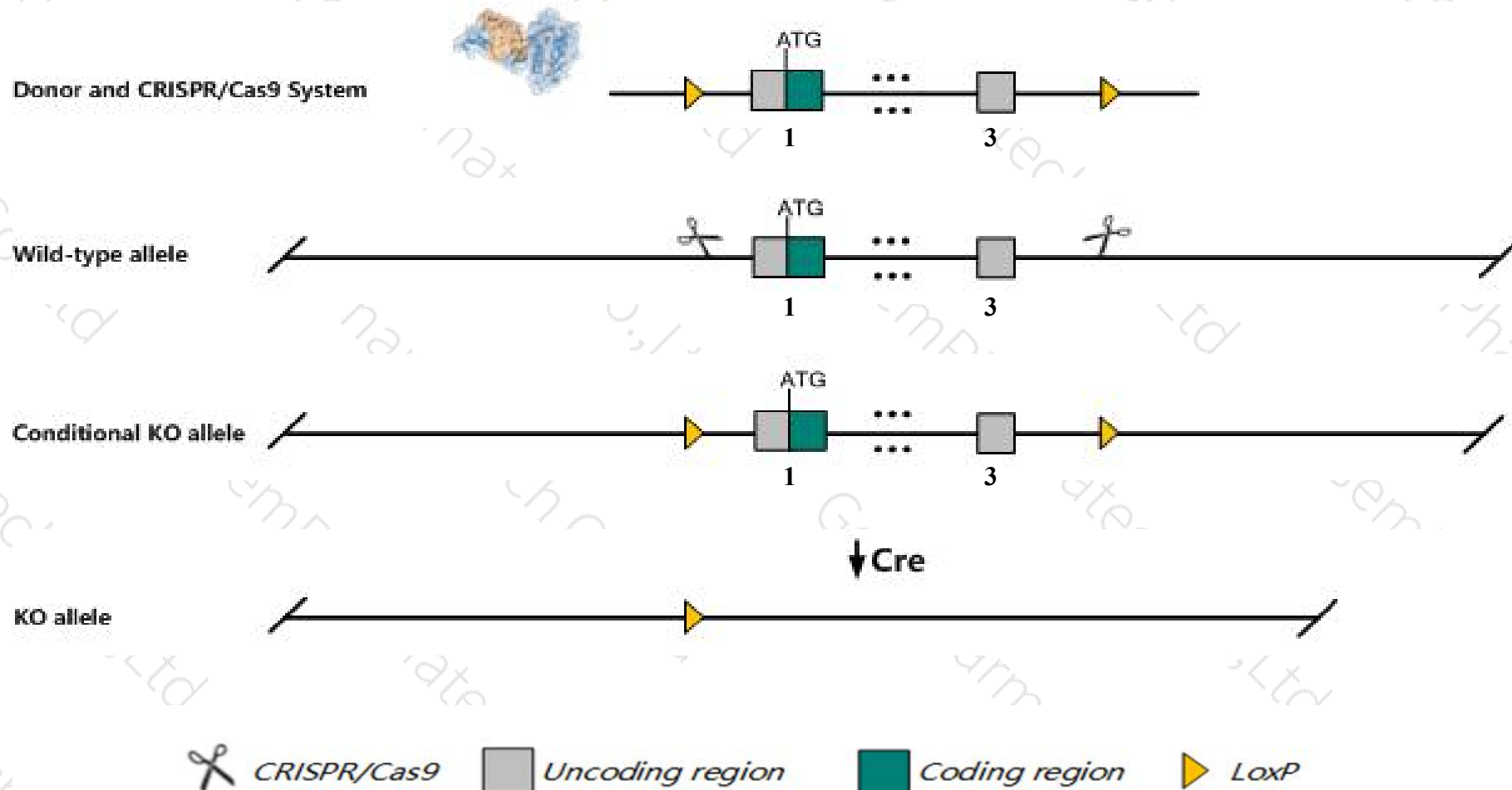
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ascl2* gene. The schematic diagram is as follows:



- The *Ascl2* gene has 2 transcripts. According to the structure of *Ascl2* gene, exon1-exon3 of *Ascl2*-201(ENSMUST00000009392.5) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ascl2* 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, mice homozygous for a knock-out allele or heterozygous for a maternally inherited allele exhibit embryonic lethality during organogenesis associated with abnormal embryogenesis.
- The *Ascl2* gene is located on the Chr7. 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)

Ascl2 achaete-scute family bHLH transcription factor 2 [*Mus musculus* (house mouse)]

Gene ID: 17173, updated on 31-Mar-2020

Summary



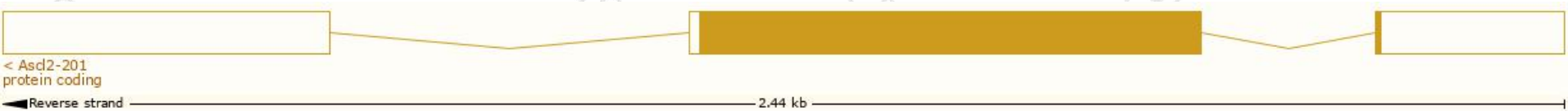
Official Symbol	Ascl2 provided by MGI
Official Full Name	achaete-scute family bHLH transcription factor 2 provided by MGI
Primary source	MGI:MGI:96920
See related	Ensembl:ENSMUSG00000009248
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mash2; bHLHa45; 2410083I15Rik
Expression	Broad expression in placenta adult (RPKM 4.7), duodenum adult (RPKM 3.4) and 22 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

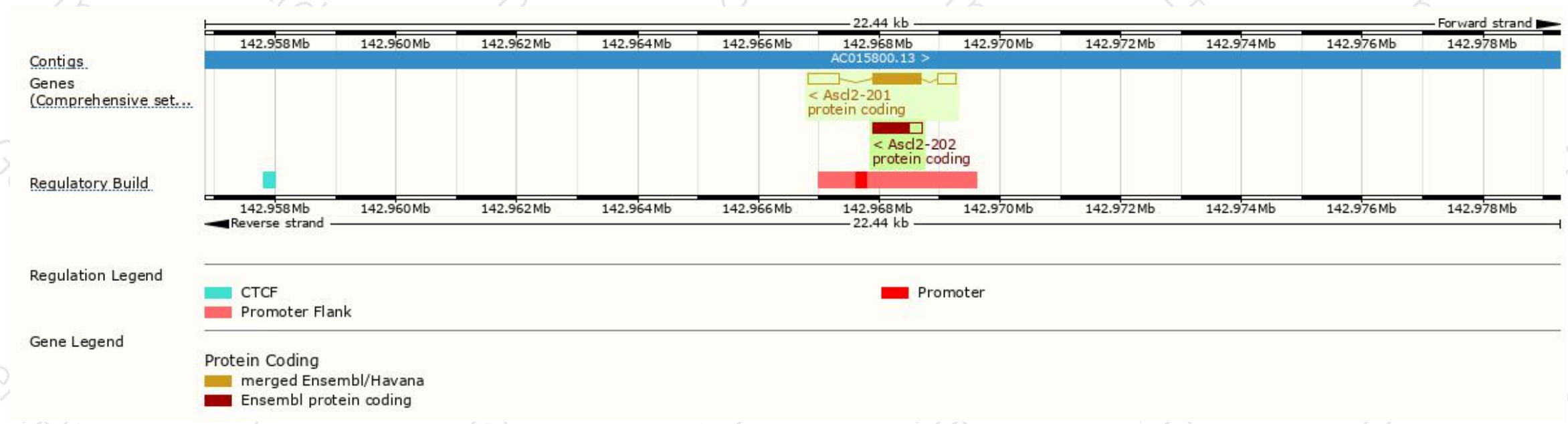
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ascl2-201	ENSMUST00000009392.5	1605	263aa	Protein coding	CCDS40194	Q35885 Q3TJR9	TSL:1 GENCODE basic APPRIS P2
Ascl2-202	ENSMUST00000121862.2	814	195aa	Protein coding	-	D3Z7Q6	TSL:NA GENCODE basic APPRIS ALT2

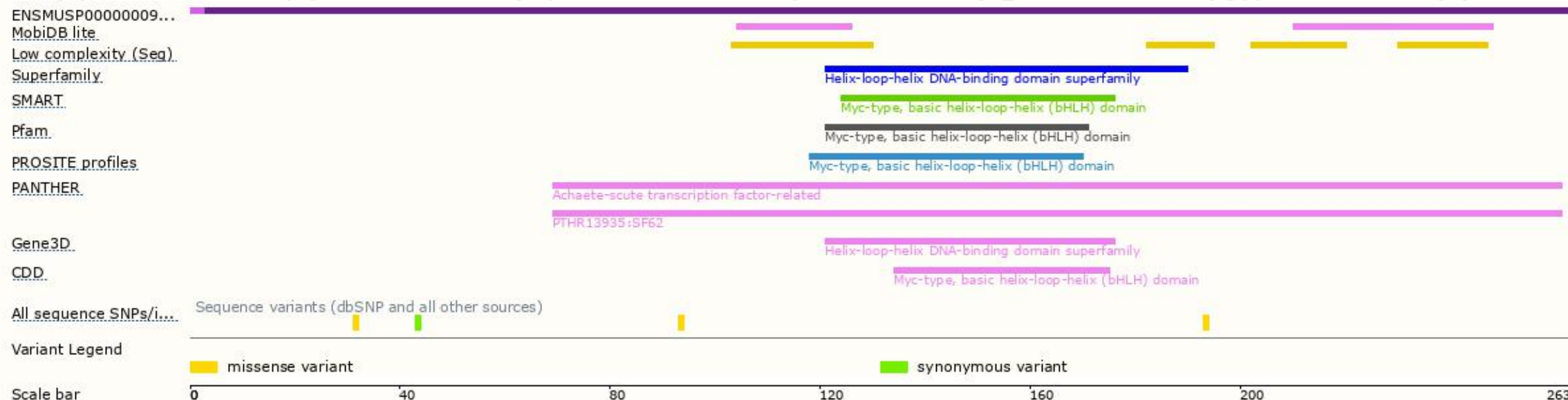
The strategy is based on the design of *Ascl2-201* transcript,the transcription is shown below:



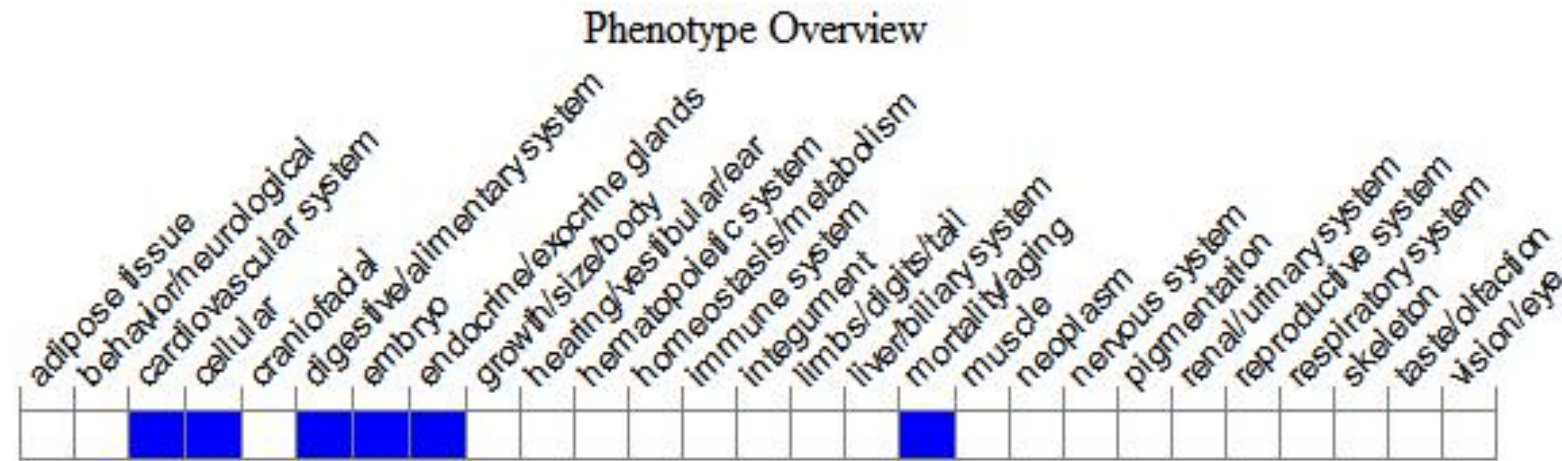
Genomic location distribution



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, mice homozygous for a knock-out allele or heterozygous for a maternally inherited allele exhibit embryonic lethality during organogenesis associated with abnormal embryogenesis.

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

Tel: 400-9660890

