

Orai1 Cas9-CKO Strategy

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Project Overview

Project Name

Orai1

Project type

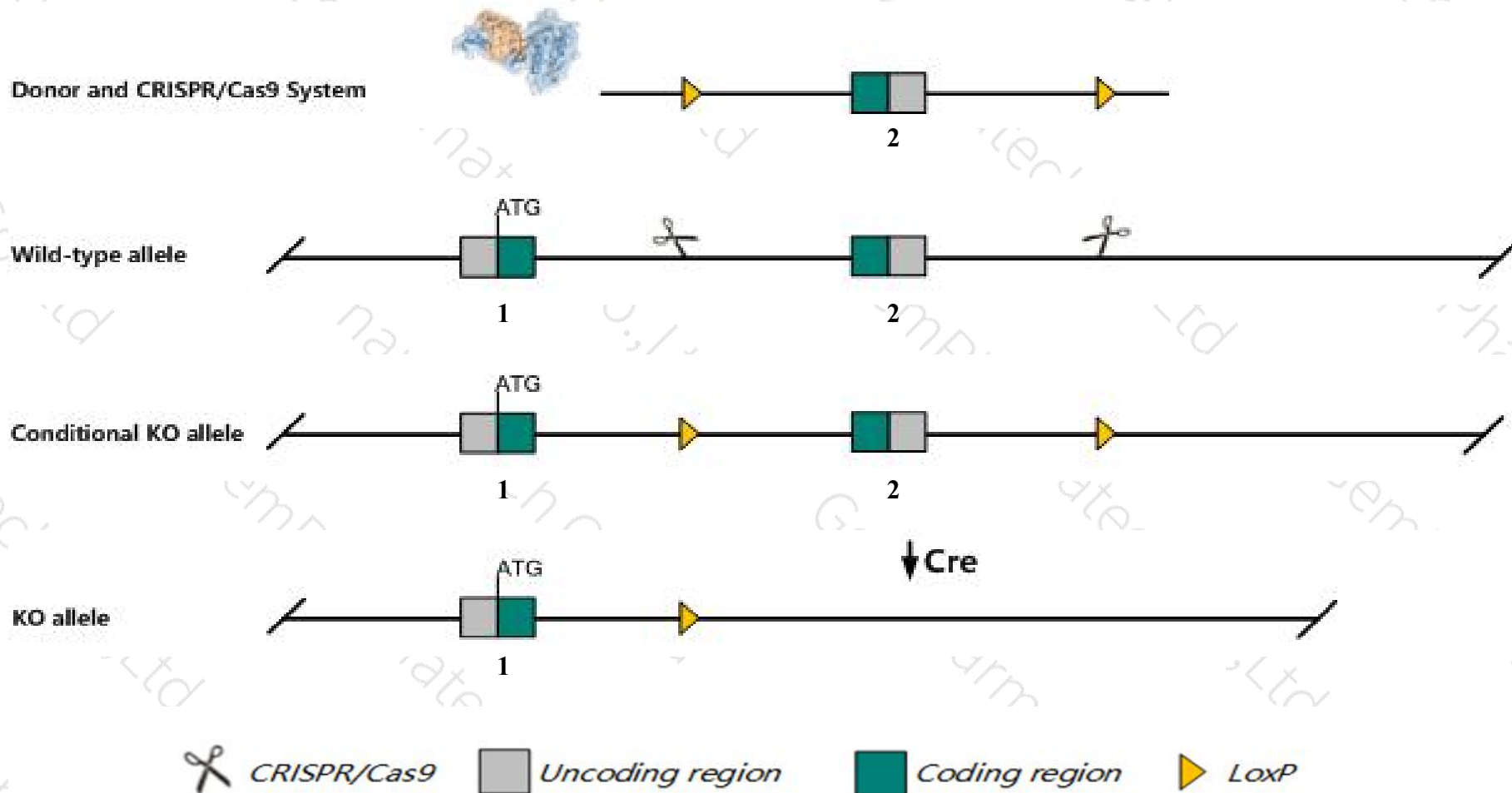
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Orai1* gene. The schematic diagram is as follows:



Technical routes

- The *Orail* gene has 3 transcripts. According to the structure of *Orail* gene, exon2 of *Orail*-202 (ENSMUST00000121652.7) transcript is recommended as the knockout region. The region contains 606bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Orail* 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 null allele exhibit small body size and defective mast cell degranulation, cytokine secretion and passive anaphalaxis.
- The *Orail* gene is located on the Chr5. 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)

Orai1 ORAI calcium release-activated calcium modulator 1 [Mus musculus (house mouse)]

Gene ID: 109305, updated on 13-Mar-2020

Summary



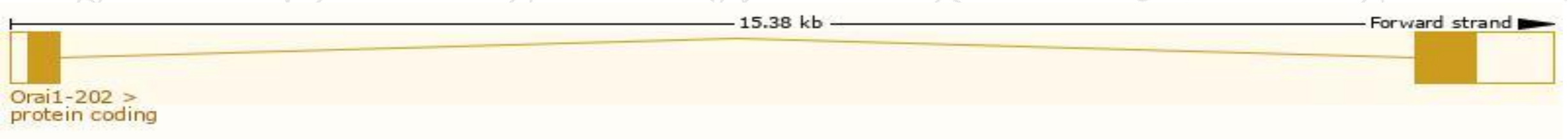
Official Symbol	Orai1 provided by MGI
Official Full Name	ORAI calcium release-activated calcium modulator 1 provided by MGI
Primary source	MGI:MGI:1925542
See related	Ensembl:ENSMUSG00000049686
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	D730049H07Rik, Tmem142a, orai-1
Expression	Ubiquitous expression in duodenum adult (RPKM 54.2), lung adult (RPKM 35.6) and 26 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

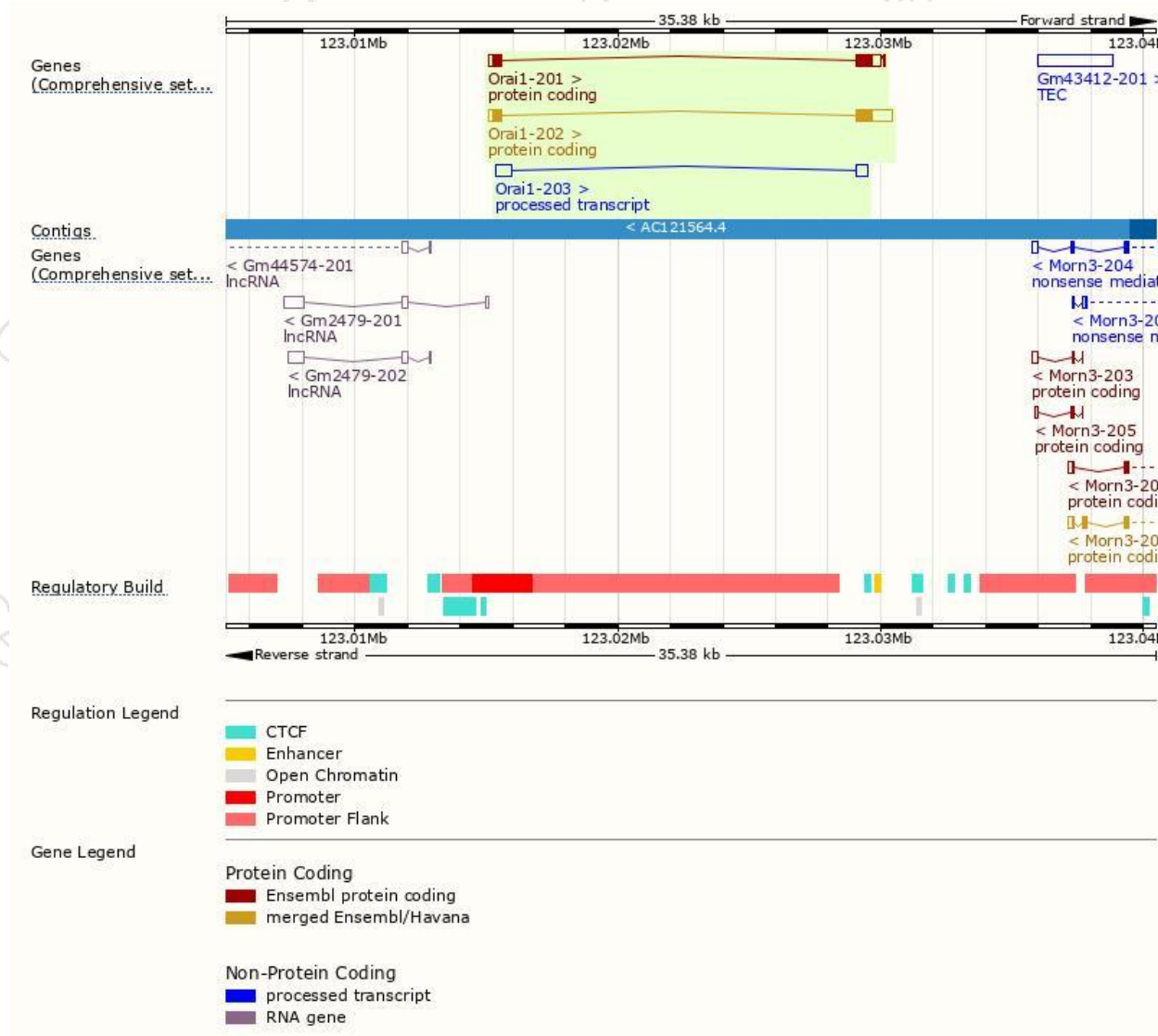
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Orai1-202	ENSMUST00000121652.7	1875	304aa	Protein coding	CCDS39261	A3KCG1 Q8BWG9	TSL:1 GENCODE basic APPRIS P1
Orai1-201	ENSMUST00000051016.4	1482	304aa	Protein coding	CCDS39261	A3KCG1 Q8BWG9	TSL:1 GENCODE basic APPRIS P1
Orai1-203	ENSMUST00000152213.2	1065	No protein	Processed transcript	-	-	TSL:3

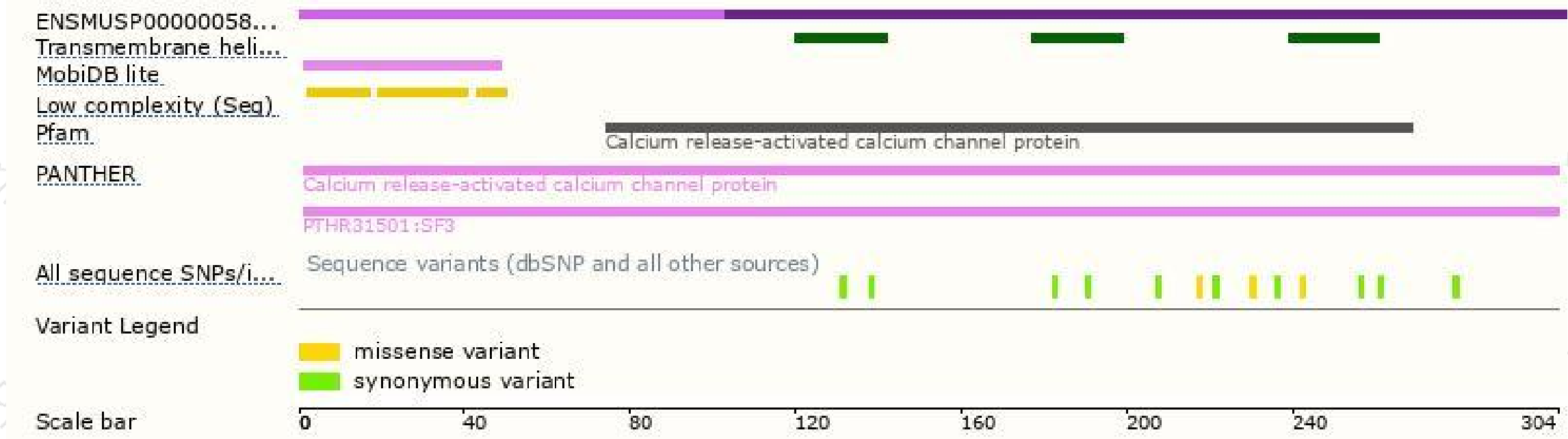
The strategy is based on the design of *Orai1-202* transcript,the transcription is shown below:



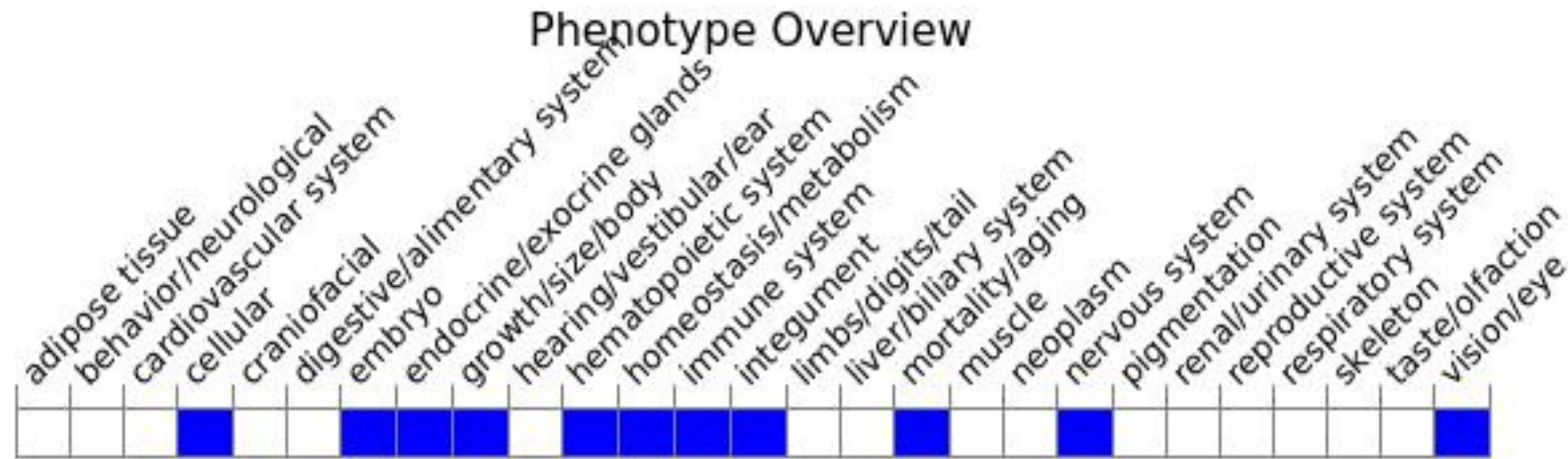
Genomic location distribution



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 null allele exhibit small body size and defective mast cell degranulation, cytokine secretion and passive anaphylaxis.

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

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