

Orai2 Cas9-CKO Strategy

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Project Overview

Project Name

Orai2

Project type

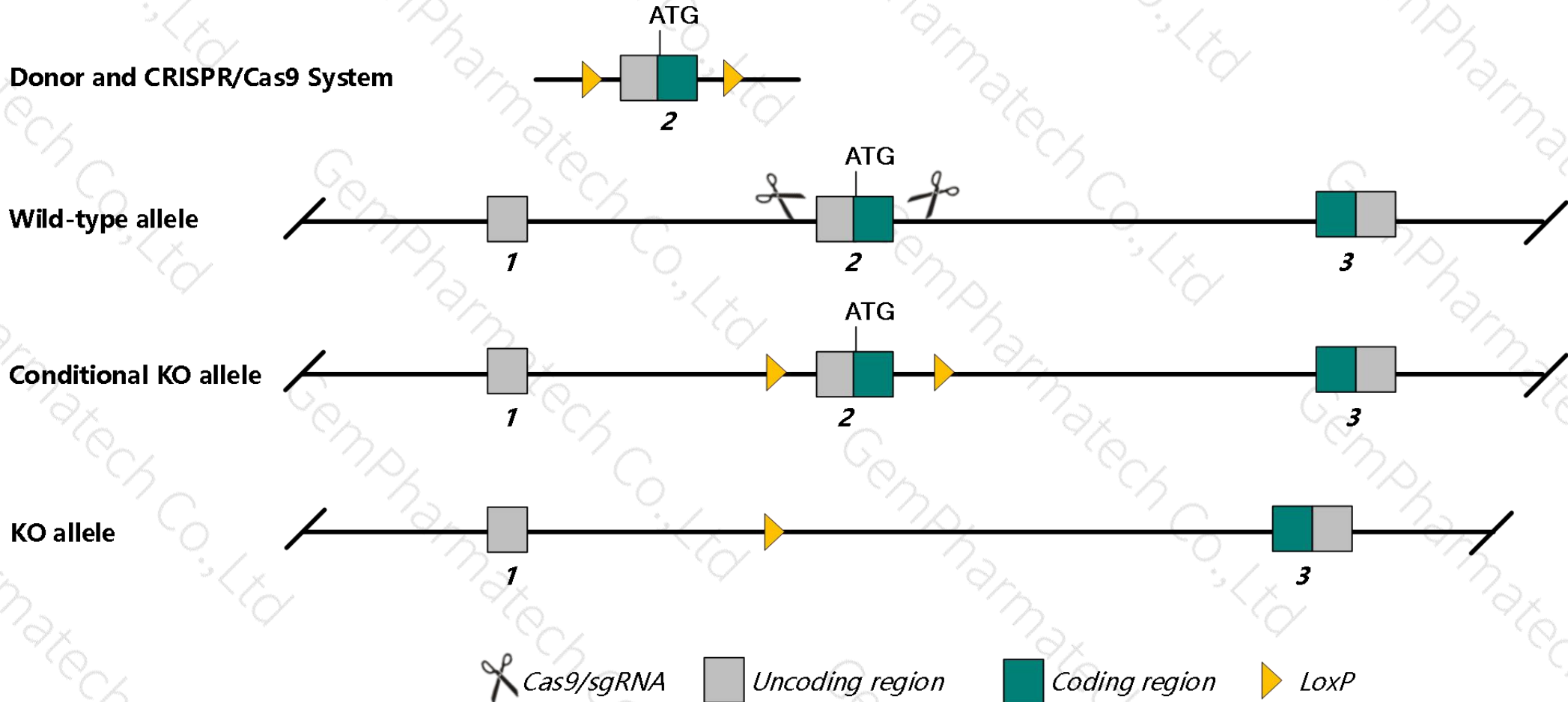
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Orai2* gene. The schematic diagram is as follows:



Technical routes

- The *Orai2* gene has 4 transcripts. According to the structure of *Orai2* gene, exon2 of *Orai2-201* (ENSMUST00000041048.5) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Orai2* 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 KO mice show increased SOCE (store-operated Ca^{2+} entry) through CRAC (Ca^{2+} release-activated Ca^{2+}) channels in T cells and higher CRAC currents.
- The *Orai2* 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)

Orai2 ORAI calcium release-activated calcium modulator 2 [*Mus musculus* (house mouse)]

Gene ID: 269717, updated on 17-Sep-2019

Summary

Official Symbol Orai2 provided by MGI
Official Full Name ORAI calcium release-activated calcium modulator 2 provided by MGI
Primary source MGI:MGI:2443195
See related Ensembl:ENSMUSG00000039747
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 Tmem142b; A730041O15Rik
Expression Broad expression in thymus adult (RPKM 40.1), spleen adult (RPKM 26.5) and 20 other tissues [See more](#)
Orthologs [human](#) [all](#)

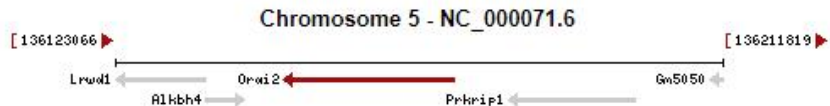
Genomic context

Location: 5; 5 G2

See Orai2 in [Genome Data Viewer](#)

Exon count: 5

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	5	NC_000071.6 (136147456..136172564, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	5	NC_000071.5 (136623329..136646526, complement)

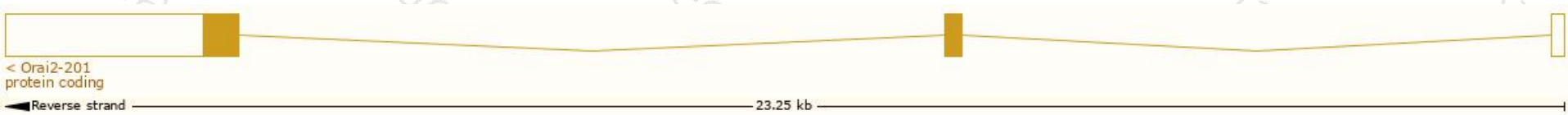


Transcript information (Ensembl)

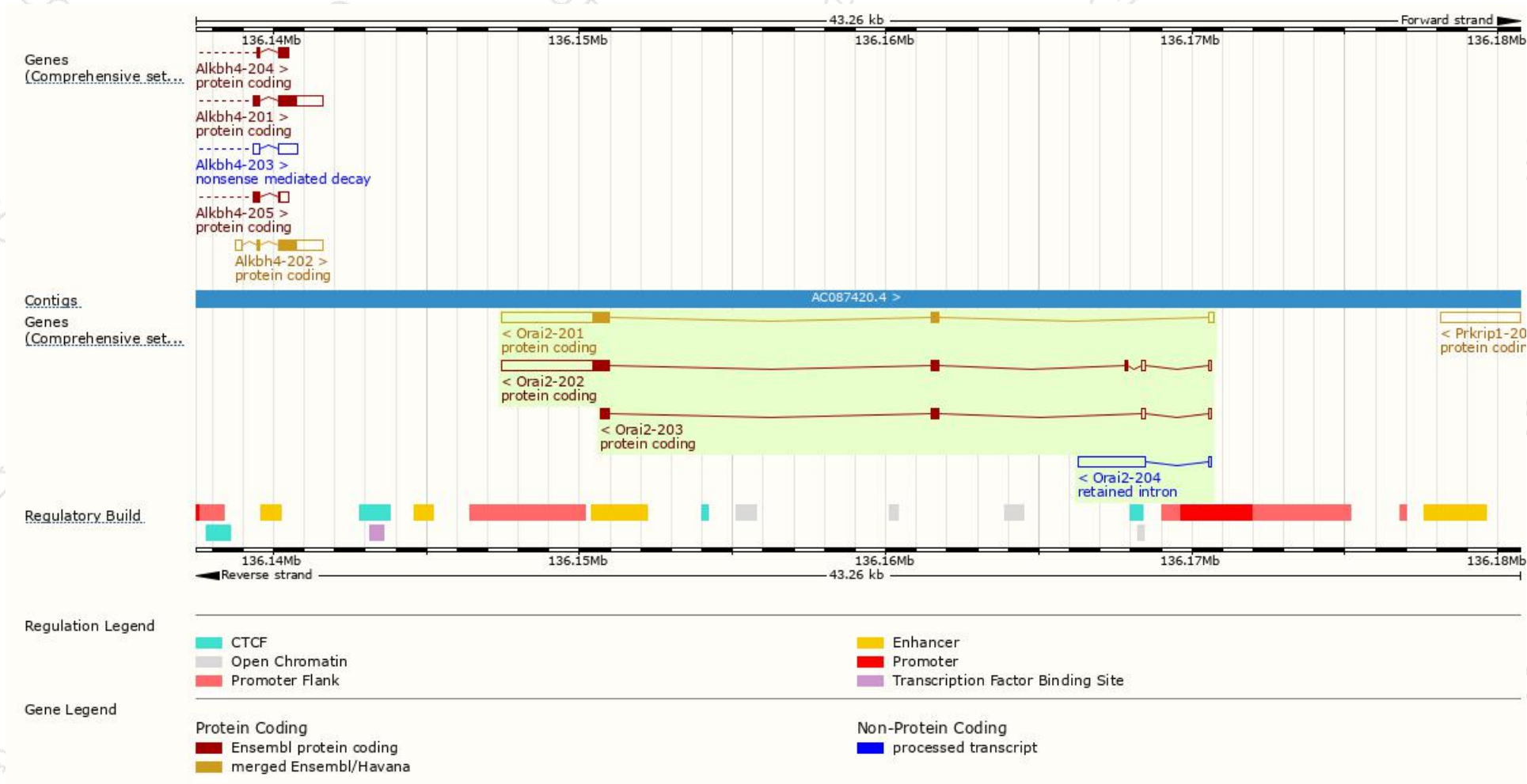
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Orai2-201	ENSMUST00000041048.5	3908	250aa	Protein coding	CCDS39326	Q14BR6 Q8BH10	TSL:1 GENCODE basic APPRIS P1
Orai2-202	ENSMUST00000196454.4	3990	264aa	Protein coding	-	A5CVE1	TSL:5 GENCODE basic
Orai2-203	ENSMUST00000197052.1	693	158aa	Protein coding	-	A0A0G2JFL5	CDS 3' incomplete TSL:3
Orai2-204	ENSMUST00000198268.1	2265	No protein	Retained intron	-	-	TSL:1

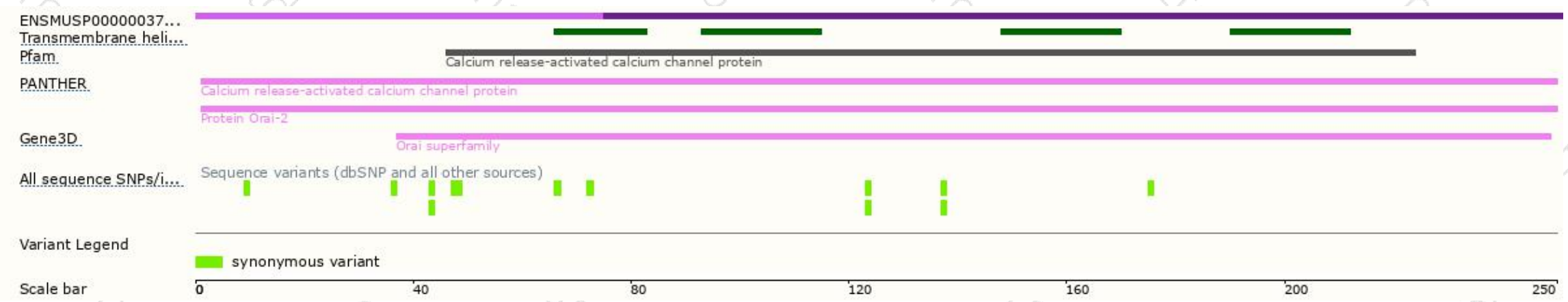
The strategy is based on the design of *Orai2-201* transcript, the transcription is shown below:



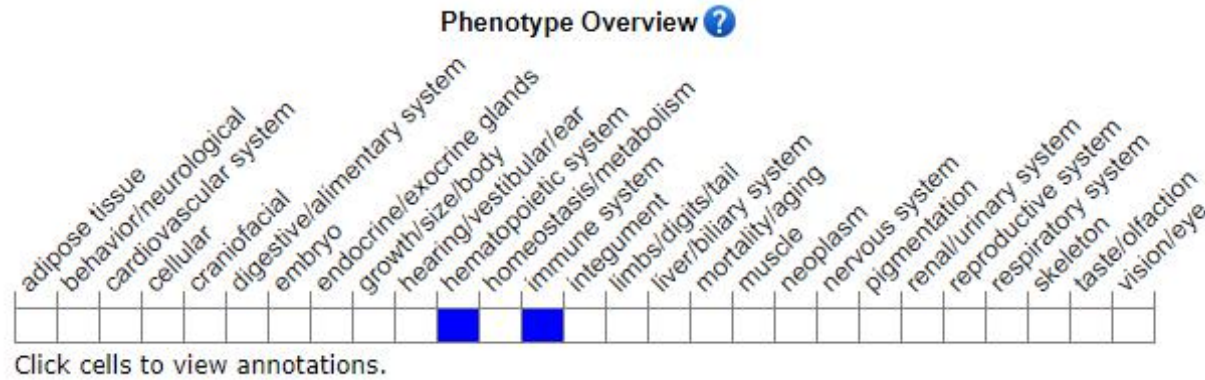
Genomic location distribution



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, homozygous KO mice show increased SOCE (store-operated Ca^{2+} entry) through CRAC (Ca^{2+} release-activated Ca^{2+}) channels in T cells and higher CRAC currents.

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

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