

Gale Cas9-CKO Strategy

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Project Overview

Project Name

Gale

Project type

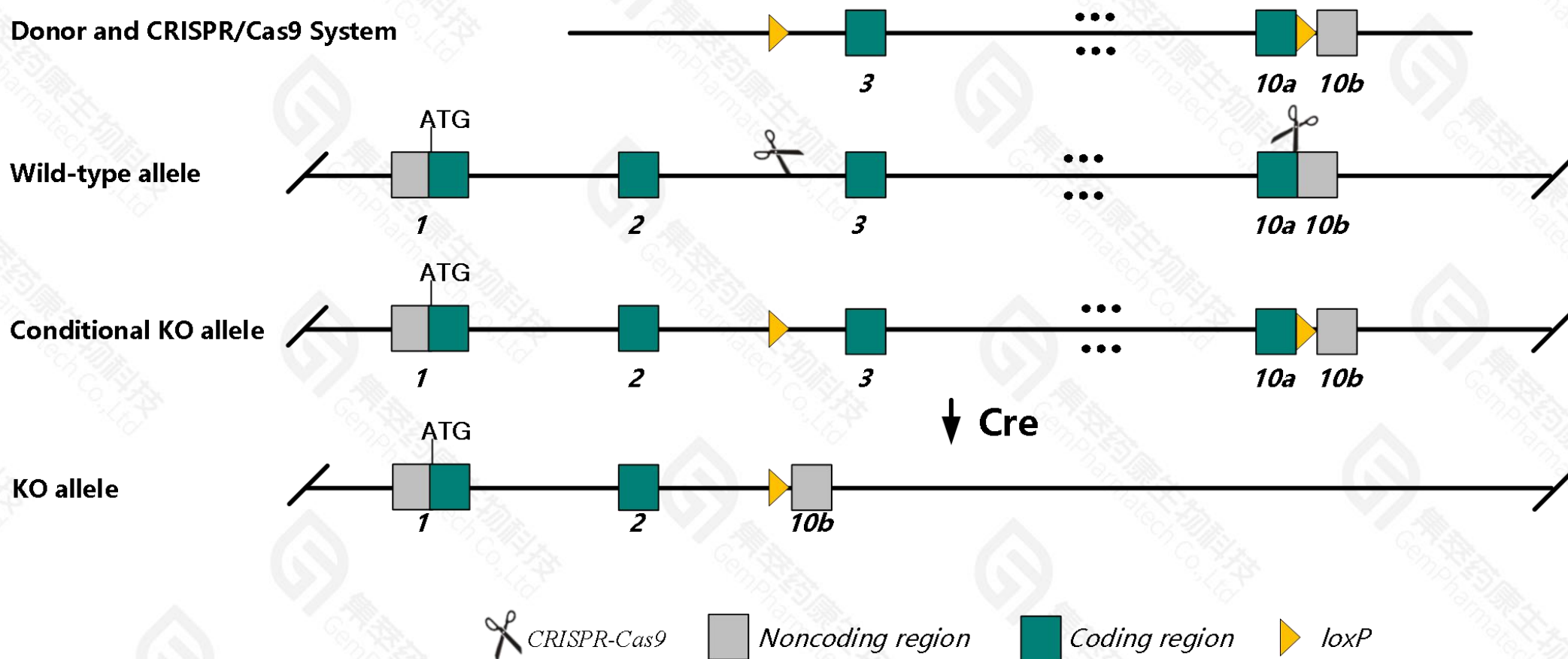
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Gale* gene has 7 transcripts. According to the structure of *Gale* gene, from exon3 to coding region of exon10 of *Gale*-201(ENSMUST00000102540.2) transcript is recommended as the knockout region. The region contains most 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 *Gale* 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.

- The intron3 is only 321bp and the second Loxp is inserted in front of the 3'UTR, which may affect mRNA splicing.
- The insertion site is only about 360bp from 3' end of *Lypla2* gene, and the insertion of Loxp may affect its expression.
- Deletion of exons from exon 3 to coding region of exon 10 does not cause *Gale* gene frame-shift mutation, but most of the coding region is deleted.
- The *Gale* gene is located on the Chr4. 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)

Gale galactose-4-epimerase, UDP [Mus musculus (house mouse)]

Gene ID: 74246, updated on 17-Feb-2021

Summary



Official Symbol Gale provided by [MGI](#)

Official Full Name galactose-4-epimerase, UDP provided by [MGI](#)

Primary source [MGI:MGI:1921496](#)

See related [Ensembl:ENSMUSG00000028671](#)

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 2310002A12Rik, AI323962

Expression Broad expression in colon adult (RPKM 100.7), large intestine adult (RPKM 78.5) and 22 other tissues [See more](#)

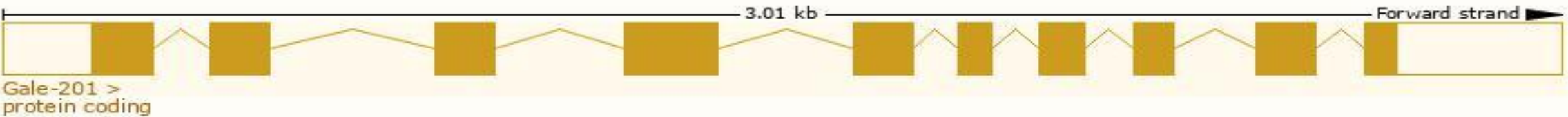
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

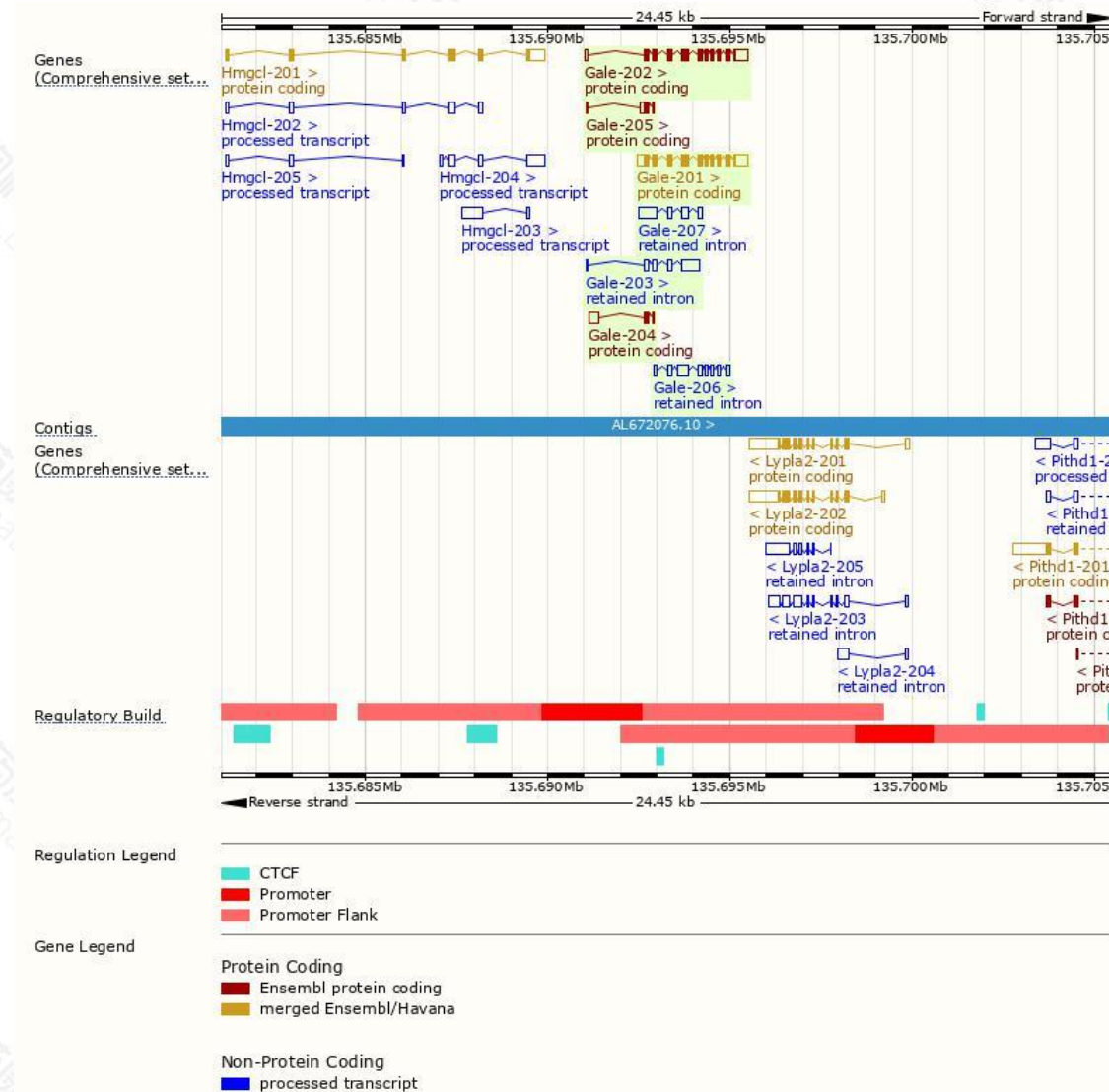
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gale-201	ENSMUST00000102540.2	1537	347aa	Protein coding	CCDS18796		TSL:1 , GENCODE basic , APPRIS P1 ,
Gale-202	ENSMUST00000102541.10	1430	347aa	Protein coding	CCDS18796		TSL:1 , GENCODE basic , APPRIS P1 ,
Gale-204	ENSMUST00000143304.2	457	57aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Gale-205	ENSMUST00000149636.2	327	60aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Gale-206	ENSMUST00000150430.2	930	No protein	Retained intron	-		TSL:5 ,
Gale-207	ENSMUST00000153542.8	892	No protein	Retained intron	-		TSL:5 ,
Gale-203	ENSMUST00000128929.8	885	No protein	Retained intron	-		TSL:2 ,

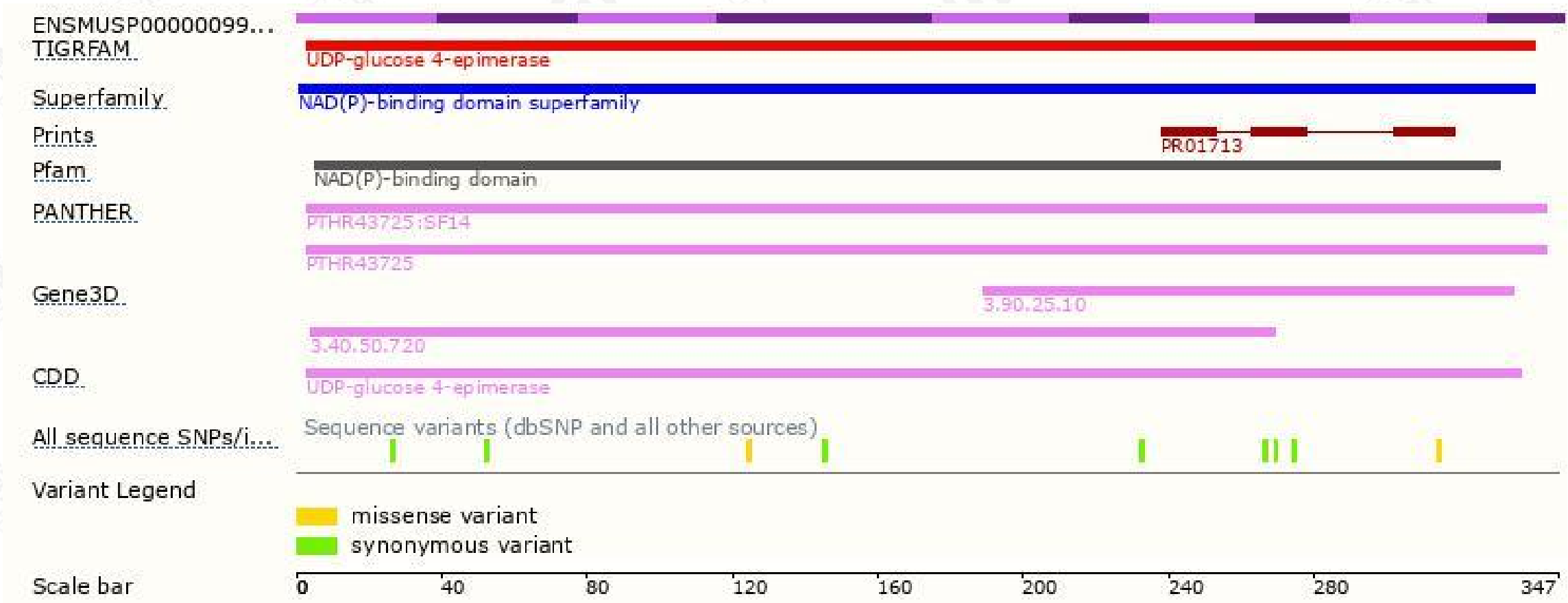
The strategy is based on the design of *Gale-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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