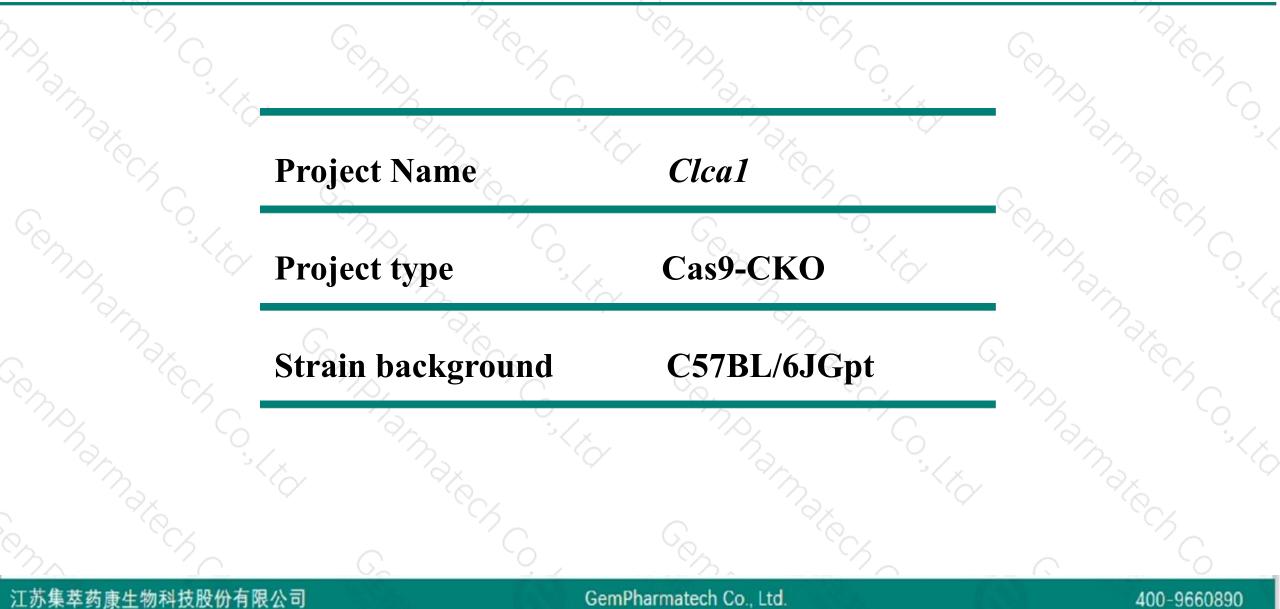


Clca1 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Yang Zeng Xueting Zhang 2019-10-17

Project Overview

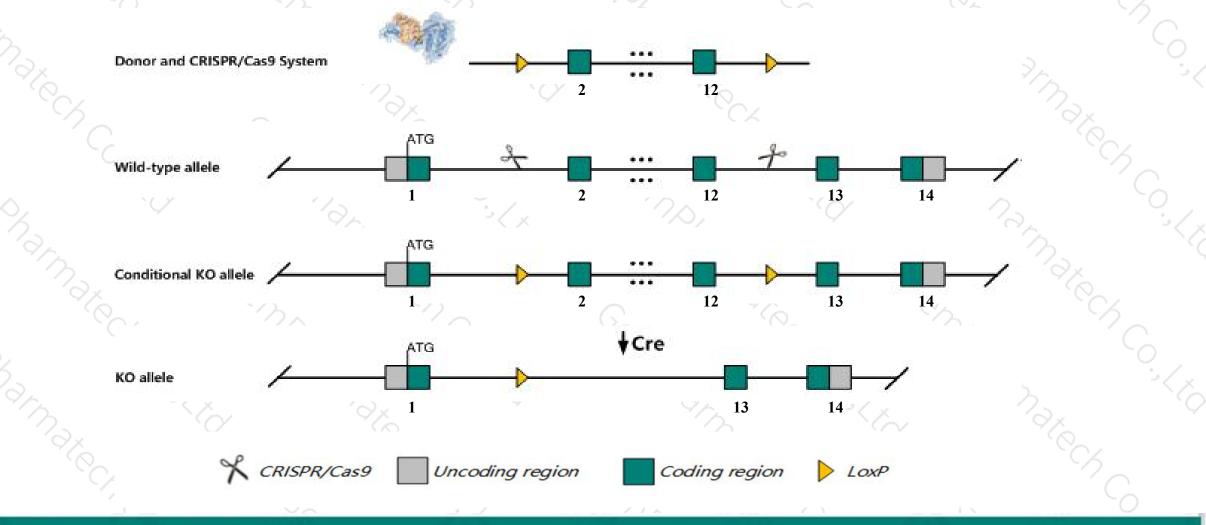




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Clca1 gene. The schematic diagram is as follows:



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The Clca1 gene has 3 transcripts. According to the structure of Clca1 gene, exon2-exon12 of Clca1-201 (ENSMUST00000029919.6) transcript is recommended as the knockout region. The region contains 1954bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Clca1* 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 an exacerbated mucin response.
- The Clca1 gene is located on the Chr3. 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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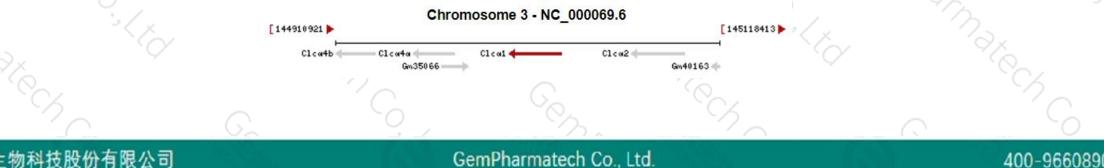
Clca1 chloride channel accessory 1 [Mus musculus (house mouse)]

Gene ID: 23844, updated on 12-Aug-2019

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Summary

Official Symbol Clca1 provided by MGI chloride channel accessory 1 provided by MGI Official Full Name Primary source MGI:MGI:1346342 Ensembl:ENSMUSG0000028255 See related Gene type protein coding RefSeg status VALIDATED Mus musculus Organism Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as gob5; Clca2; Clca3; gob-5 Biased expression in colon adult (RPKM 313.3), large intestine adult (RPKM 280.7) and 3 other tissues See more Expression Orthologs human all



Transcript information (Ensembl)



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

Name 🖕	Transcript ID	bp 🎄	Protein 🖕	Translation ID	Biotype 💧	CCDS 🍦	UniProt 🖕	Flags
Clca1-201	ENSMUST0000029919.6	3655	<u>913aa</u>	ENSMUSP0000029919.5	Protein coding	<u>CCDS17889</u> &	<u>Q9D7Z6</u> @	TSL:1 GENCODE basic APPRIS P1
Clca1-203	ENSMUST00000198832.1	629	No protein	(-	IncRNA	-	-	TSL:3
Clca1-202	ENSMUST00000195901.1	261	No protein	(. .)	IncRNA	-	-	TSL:3

28.96 kb

The strategy is based on the design of Clca1-201 transcript, The transcription is shown below

< Clca1-201 protein coding

Reverse strand

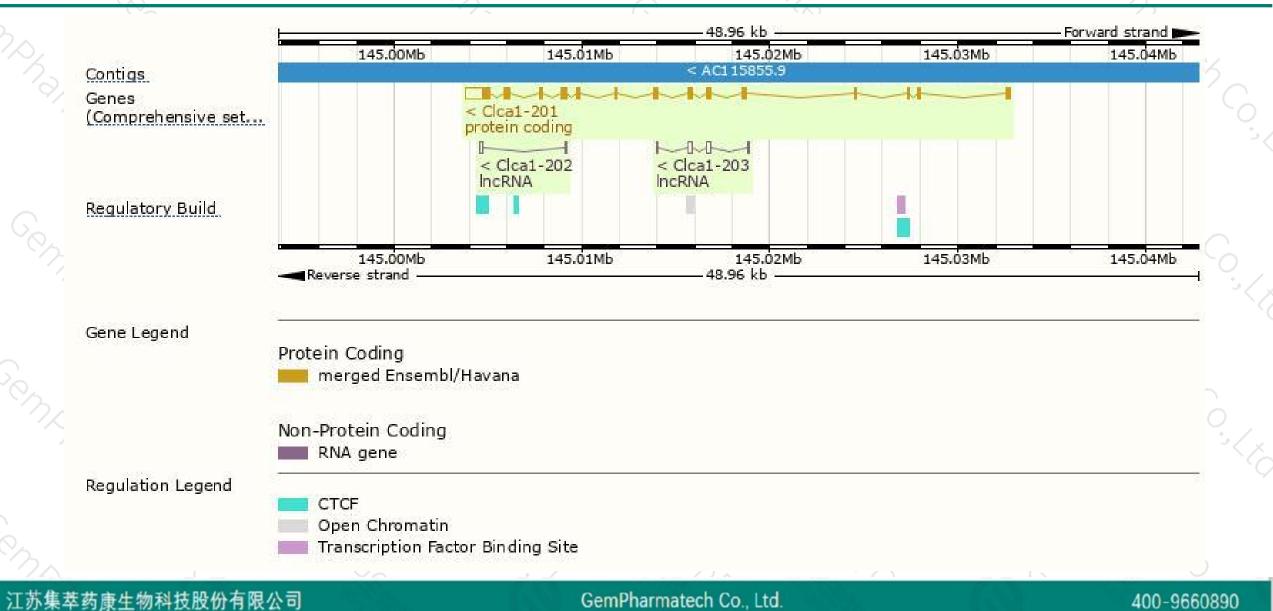
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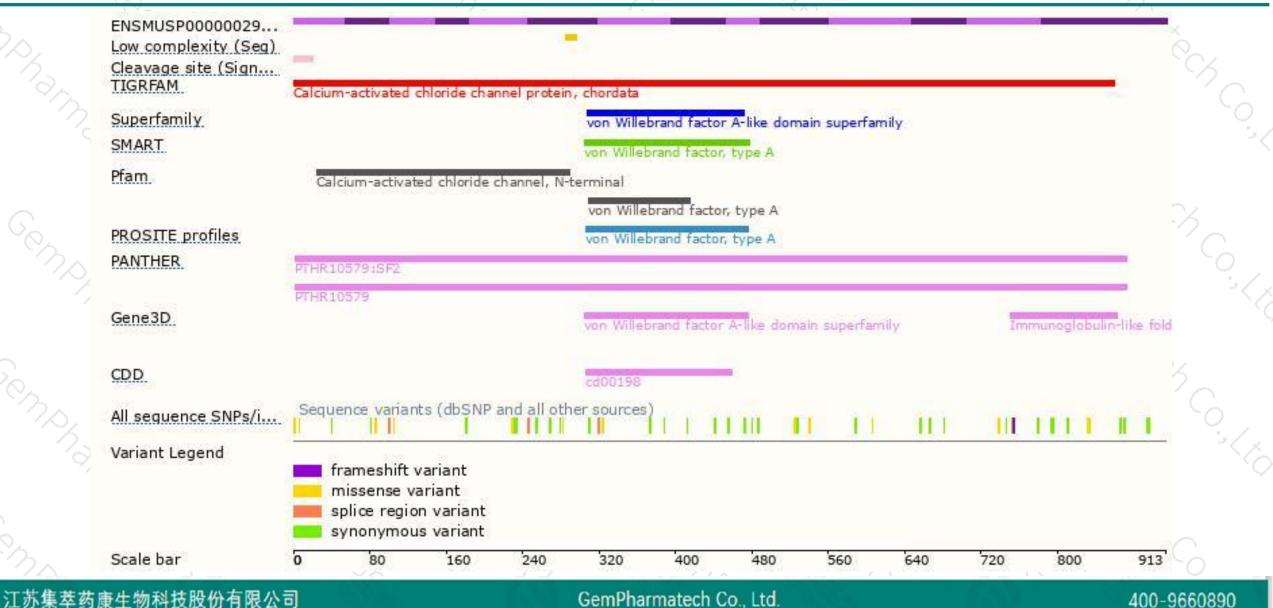
Genomic location distribution



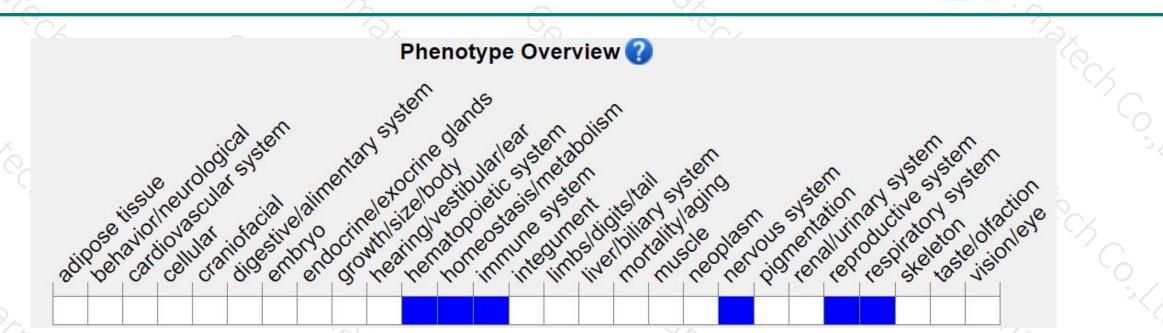


Protein domain





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 an exacerbated mucin response.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



