Clcf1 Cas9-CKO *Strategy*

Designer: **Huan Wang**

Design Date: **2019-7-24**

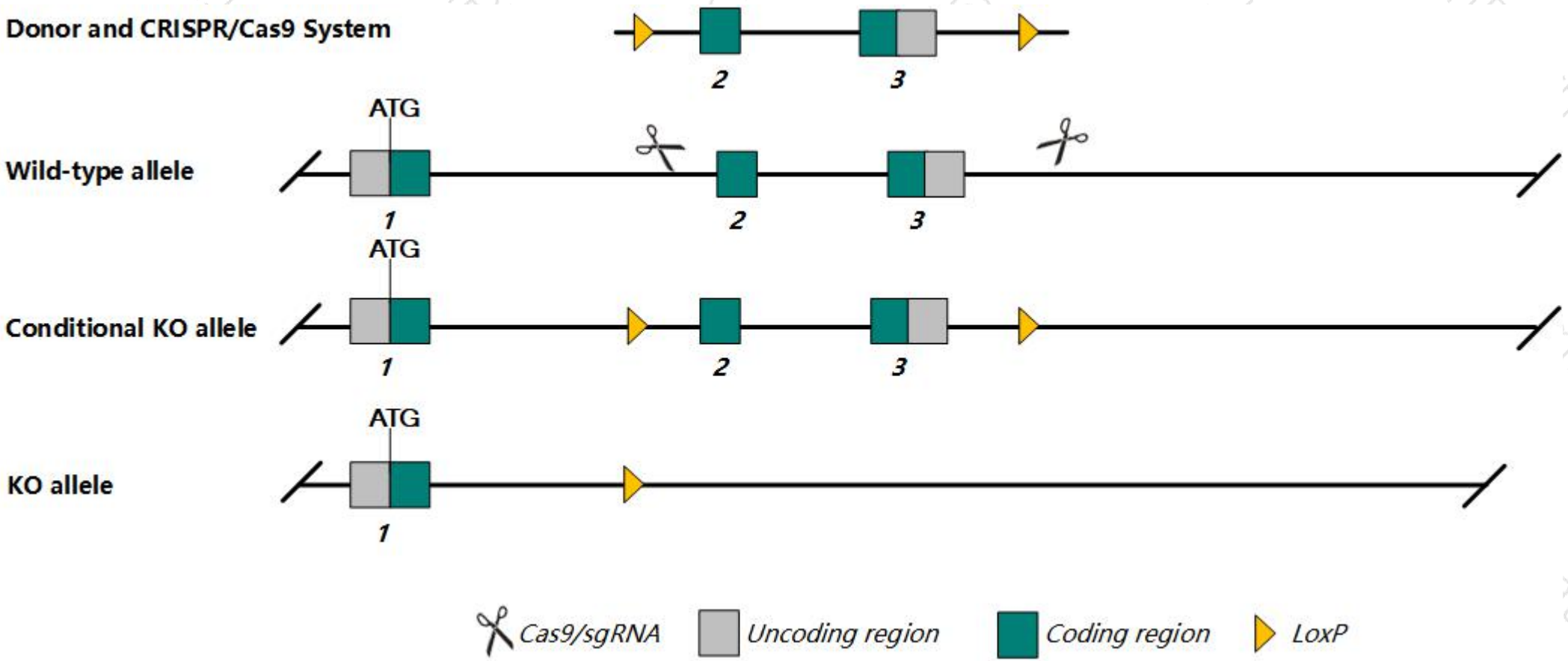
Project Overview

Project Name	***Clcf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

集萃药康 GemPharmatech

This model will use CRISPR/Cas9 technology to edit the *Clcf1* gene. The schematic diagram is as follows:



Technical routes

- The *Clcf1* gene has * transcript.According to the structure of *Clcf1* gene, exon2-3 of *Clcf1*-201 transcript is recommended as the knockout region.The region contains most of coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Clcf1* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 or cell types.

Notice

- The *Clcf1* gene is located on the Chr*. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Clcf1 cardiotrophin-like cytokine factor 1 [*Mus musculus* (house mouse)]

Gene ID: 56708, updated on 2-Oct-2018

Summary

Official Symbol	Clcf1 provided by MGI
Official Full Name	cardiotrophin-like cytokine factor 1 provided by MGI
Primary source	MGI:MGI:1930088
See related	Ensembl:ENSMUSG00000040663 Vega:OTTMUSG00000028319
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	CLC; Bsf3; BSF-3; NNT-1
Expression	Broad expression in spleen adult (RPKM 32.4), mammary gland adult (RPKM 16.1) and 15 other tissues See more
Orthologs	human all

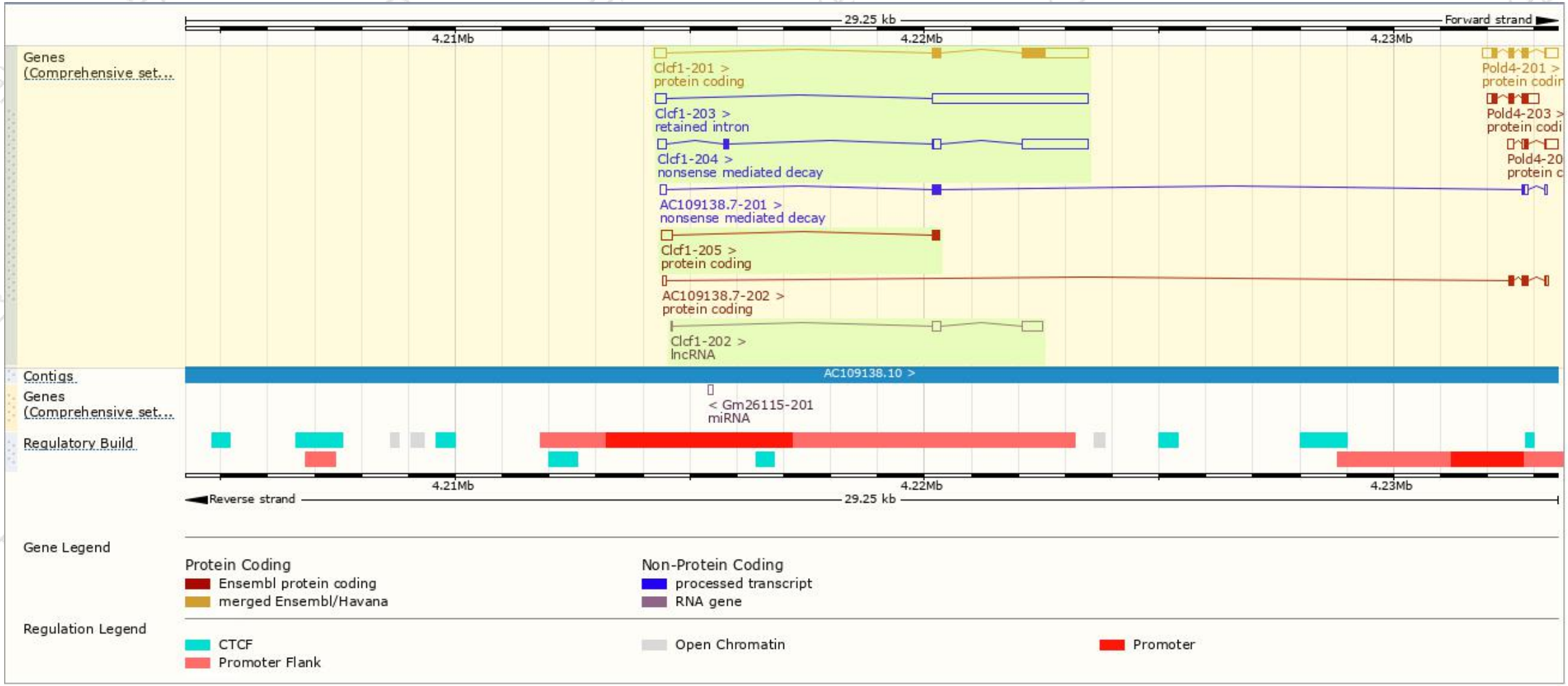
Transcript information（Ensembl）

The gene has * transcripts, and all transcripts are shown below:

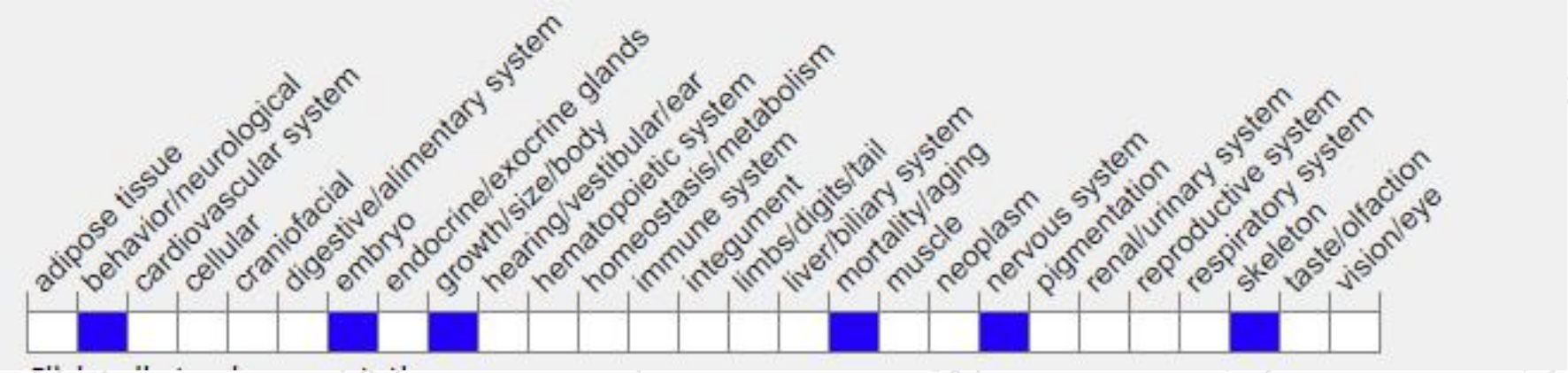
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Clcf1-201	ENSMUST00000046506.6	1847	225aa	Protein coding	CCDS29423	Q9QZM3	NM_001310038 NM_001310039 NM_019952 NP_001296967 NP_001296968 NP_064336	TSL:1 GENCODE basic APPRIS P1
Clcf1-202	ENSMUST00000126457.1	646	No protein	Processed transcript	-	-	-	TSL:3
Clcf1-203	ENSMUST00000132305.1	3550	No protein	Retained intron	-	-	-	TSL:2
Clcf1-204	ENSMUST00000138090.1	1878	44aa	Nonsense mediated decay	-	D6RIL9	-	TSL:1

The strategy is based on the design of *Clcf1*-201 transcript,The transcription is shown below

Genomic location distribution



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

Mice homozygous for a knock-out allele exhibit postnatal lethality associated with a failure to suckle and decreased facial and spinal motor neurons.

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

集萃药康生物科技
GemPharmatech Co.,Ltd