

Ctf1 Cas9-CKO Strategy

Designer:

Bingxuan Li

Reviewer:

Ruirui Zhang

Design Date:

2020-3-25

Project Overview

Project Name

Ctf1

Project type

Cas9-CKO

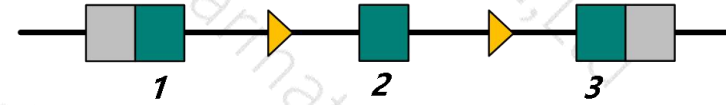
Strain background

C57BL/6JGpt

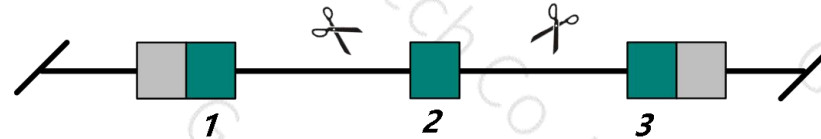
Conditional Knockout strategy

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

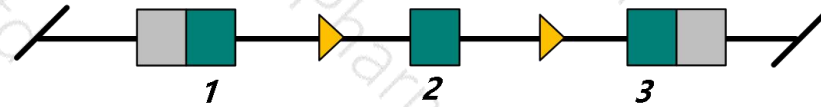
Donor and CRISPR/Cas9 System



Wild-type allele

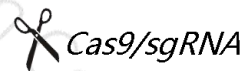
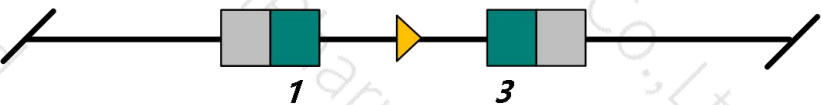


Floxed allele



Cre

Cre recombination



Cas9/sgRNA



Uncoding region



Coding region



loxP

- The *Ctfl* gene has 5 transcripts. According to the structure of *Ctfl* gene, the exon2 of *Ctfl*-201 (ENSMUST00000047393.6) transcript is recommended as the knockout region. The region contains 119bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ctfl* 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 and cell types.

- According to the existing MGI data, mice homozygous for a knock-out allele show a significant reduction in grip strength and increased motoneuron cell death in the spinal cord and brainstem nuclei between embryonic day 14 and the first postnatal week.
- The *Ctfl* gene is located on the Chr7. 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)

Ctf1 cardiotrophin 1 [*Mus musculus* (house mouse)]

Gene ID: 13019, updated on 12-Aug-2019

Summary

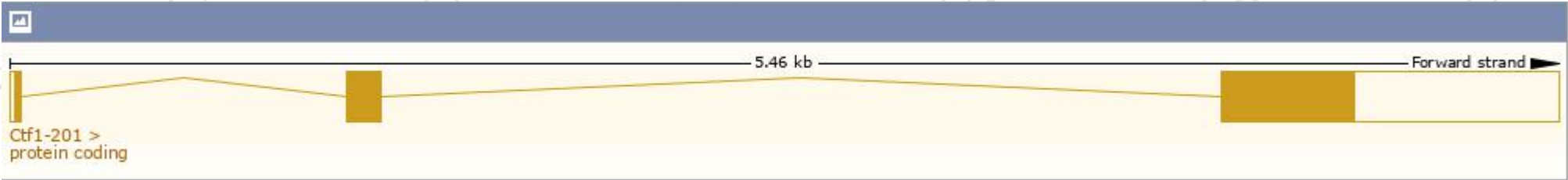
Official Symbol	Ctf1 provided by MGI
Official Full Name	cardiotrophin 1 provided by MGI
Primary source	MGI:MGI:105115
See related	Ensembl:ENSMUSG000000042340
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CT-1
Expression	Ubiquitous expression in limb E14.5 (RPKM 16.6), ovary adult (RPKM 10.7) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

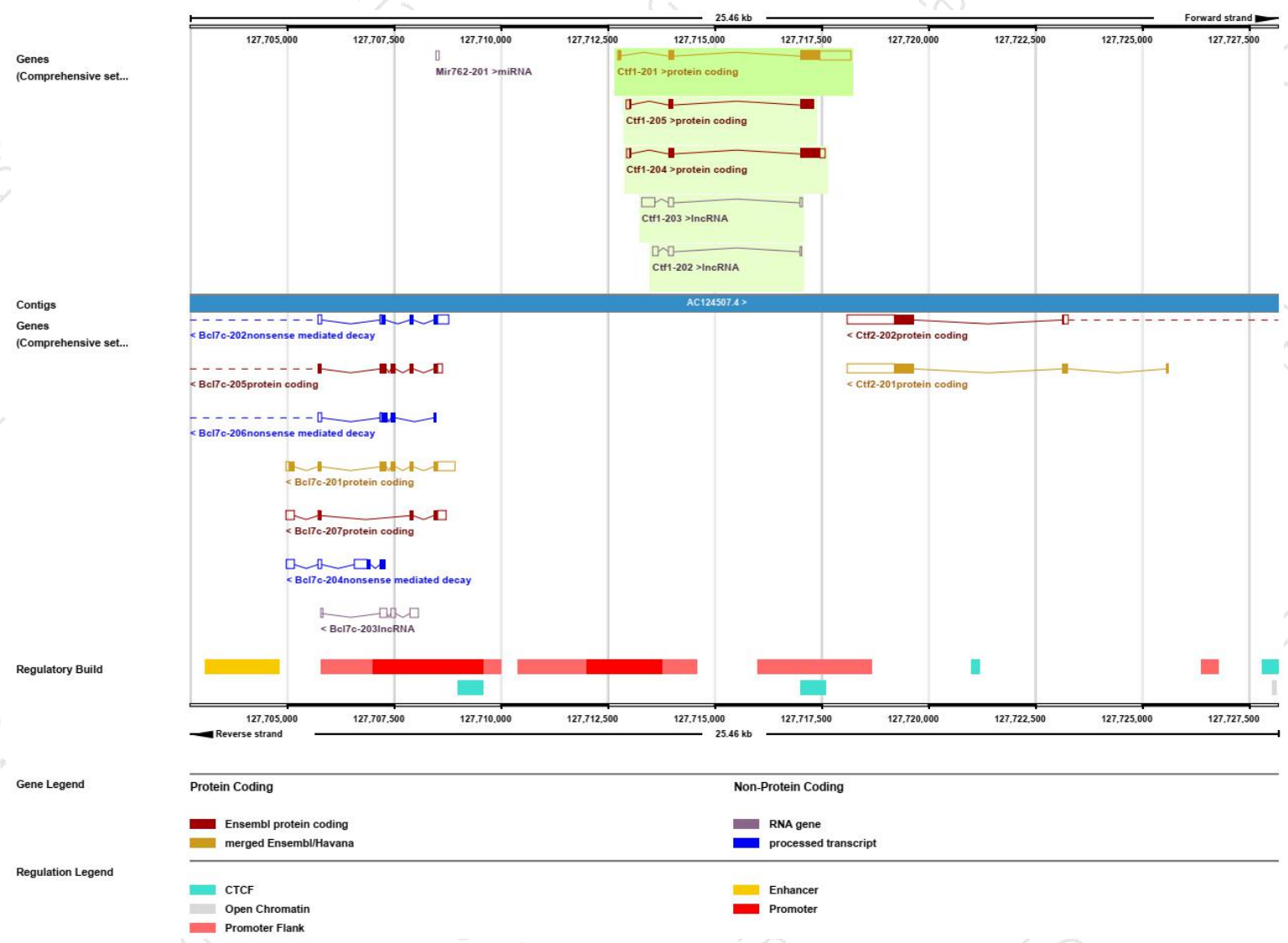
The gene has 5 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden)					Filter		
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctf1-201	ENSMUST00000047393.6	1352	203aa	Protein coding	CCDS21875	Q541U3 Q60753	TSL:1 GENCODE basic APPRIS P1
Ctf1-204	ENSMUST00000206506.1	784	196aa	Protein coding	CCDS85423	A0A0U1RNJ2	TSL:2 GENCODE basic
Ctf1-205	ENSMUST00000206997.1	528	149aa	Protein coding	-	A0A0U1RQ09	CDS 3' incomplete TSL:3
Ctf1-203	ENSMUST00000206073.1	450	No protein	lncRNA	-	-	TSL:5
Ctf1-202	ENSMUST00000205655.1	285	No protein	lncRNA	-	-	TSL:5

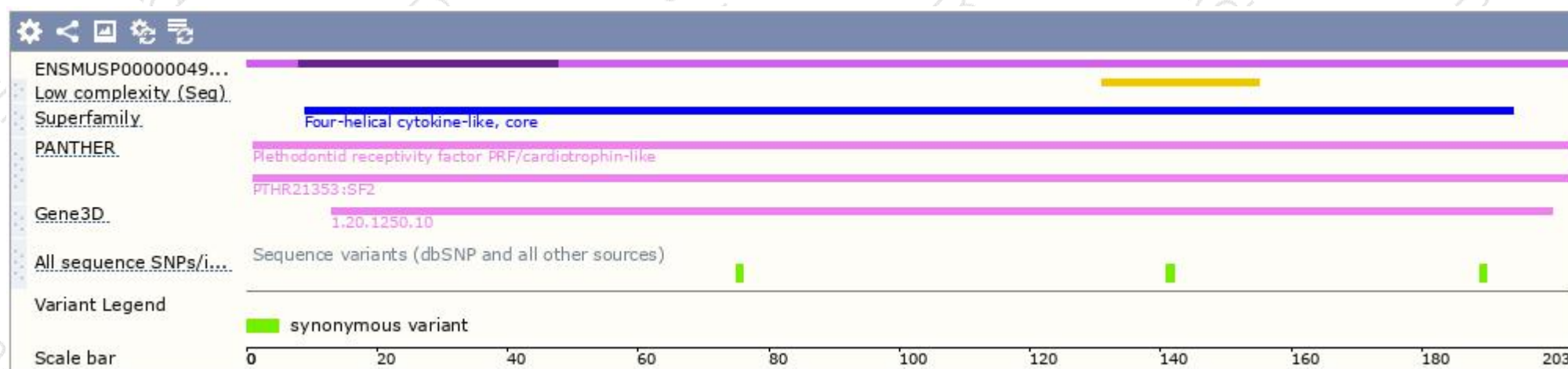
The strategy is based on the design of *Ctf1-201* transcript, The transcription is shown below



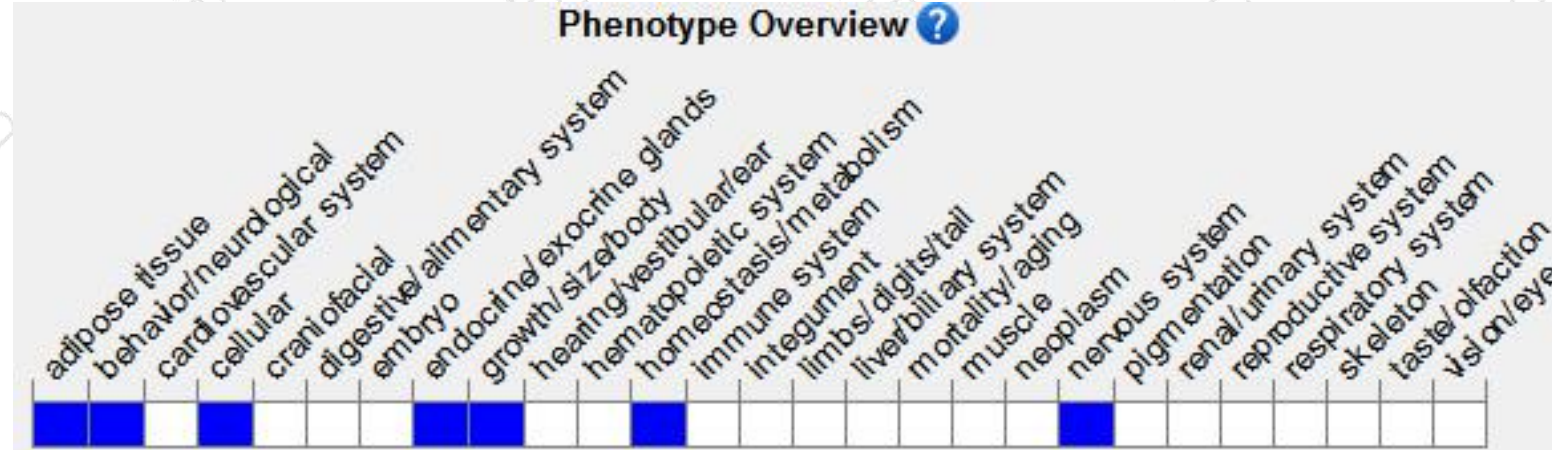
Genomic location distribution



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/>) .

Mice homozygous for a knock-out allele show a significant reduction in grip strength and increased motoneuron cell death in the spinal cord and brainstem nuclei between embryonic day 14 and the first postnatal week.

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



集萃药康生物科技
GemPharmatech Co.,Ltd

