

***Chd4* Cas9-CKO Strategy**

Designer:

Huan Wang

Design Date:

2019-7-24

Project Overview

Project Name

Chd4

Project type

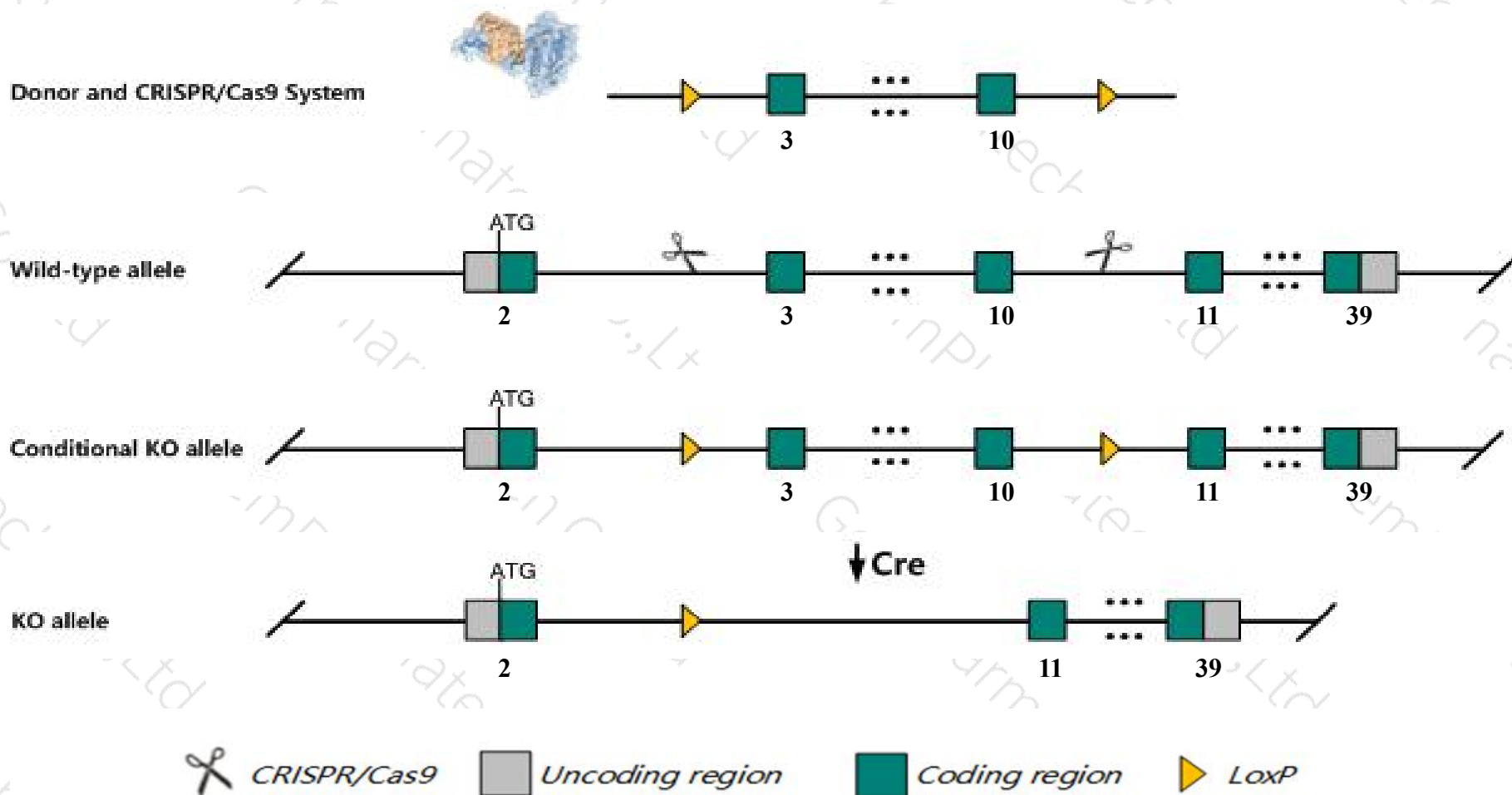
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Chd4* gene. The schematic diagram is as follows:



Technical routes

- The *Chd4* gene has 8 transcripts. According to the structure of *Chd4* gene, exon3-exon10 of *Chd4-201* (ENSMUST00000056889.14) transcript is recommended as the knockout region. The region contains 1361bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Chd4* 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 gene trapped allele exhibit embryonic lethality between E3.5 and E4.5, absent blastocoele failure of trophectoderm function and increased apoptosis in blastocysts.
- The *Chd4* gene is located on the Chr6. 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)

Chd4 chromodomain helicase DNA binding protein 4 [Mus musculus (house mouse)]

Gene ID: 107932, updated on 19-Mar-2019

Summary



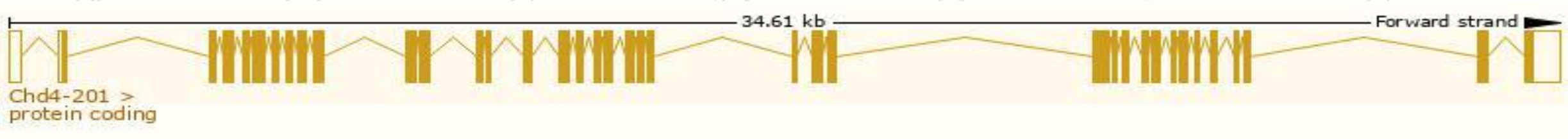
Official Symbol	Chd4 provided by MGI
Official Full Name	chromodomain helicase DNA binding protein 4 provided by MGI
Primary source	MGI:MGI:1344380
See related	Ensembl:ENSMUSG00000063870
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9530019N15Rik, AA617397, BC005710, D6Ert380e, Mi-2beta, mKIAA4075
Expression	Ubiquitous expression in CNS E11.5 (RPKM 47.4), CNS E14 (RPKM 29.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

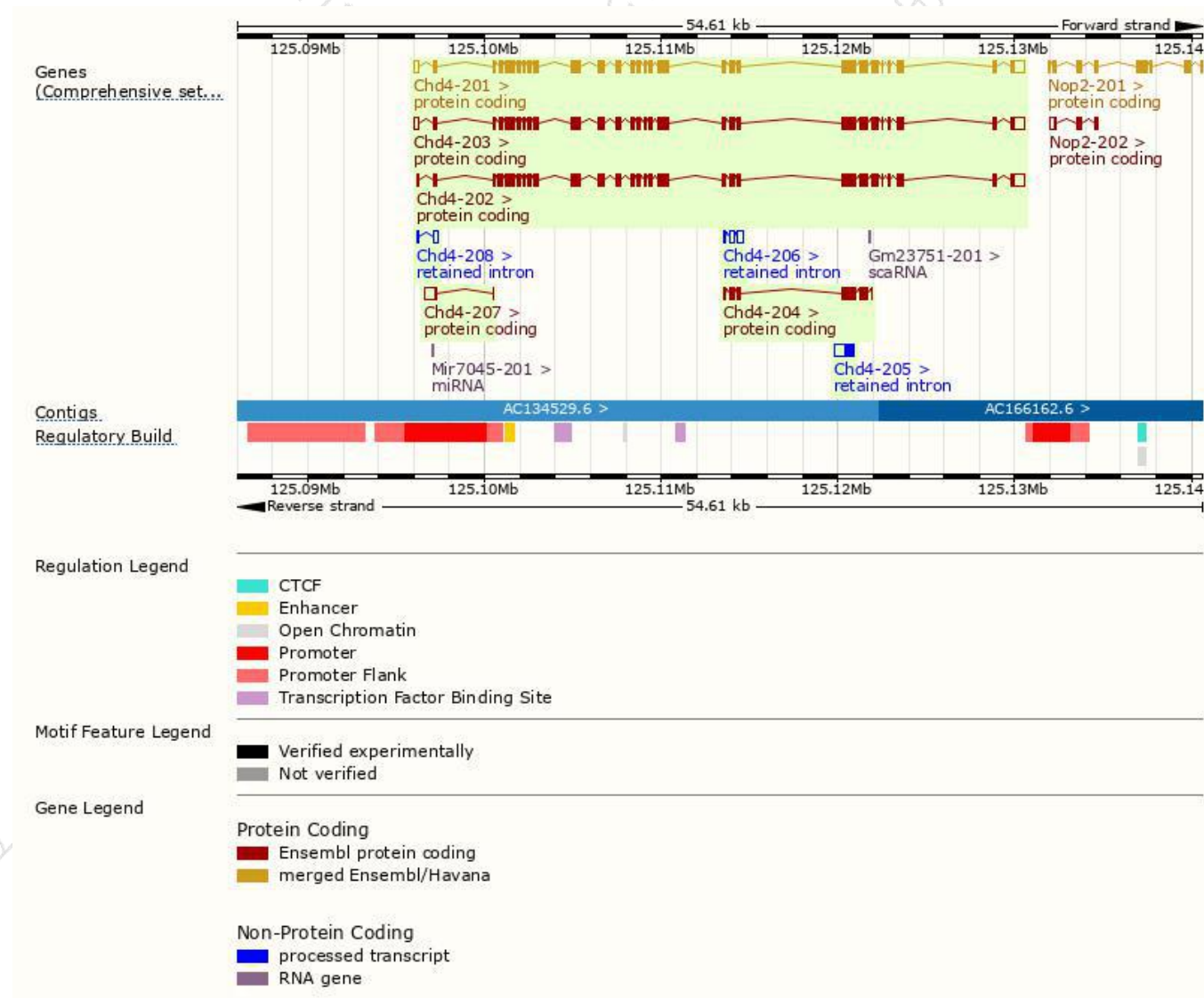
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chd4-201	ENSMUST00000056889.14	6697	1915aa	Protein coding	CCDS20543	Q6PDQ2	TSL:1 GENCODE basic APPRIS P2
Chd4-203	ENSMUST00000112392.7	6652	1902aa	Protein coding	-	E9QAS4	TSL:5 GENCODE basic APPRIS ALT2
Chd4-202	ENSMUST00000112390.7	6537	1922aa	Protein coding	-	E9QAS5	TSL:5 GENCODE basic APPRIS ALT2
Chd4-204	ENSMUST00000124317.1	1332	444aa	Protein coding	-	F6WR45	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Chd4-207	ENSMUST00000155261.1	750	51aa	Protein coding	-	G5E8V7	CDS 3' incomplete TSL:1
Chd4-205	ENSMUST00000132794.1	821	No protein	Retained intron	-	-	TSL:3
Chd4-206	ENSMUST00000138968.1	618	No protein	Retained intron	-	-	TSL:2
Chd4-208	ENSMUST00000155932.1	369	No protein	Retained intron	-	-	TSL:2

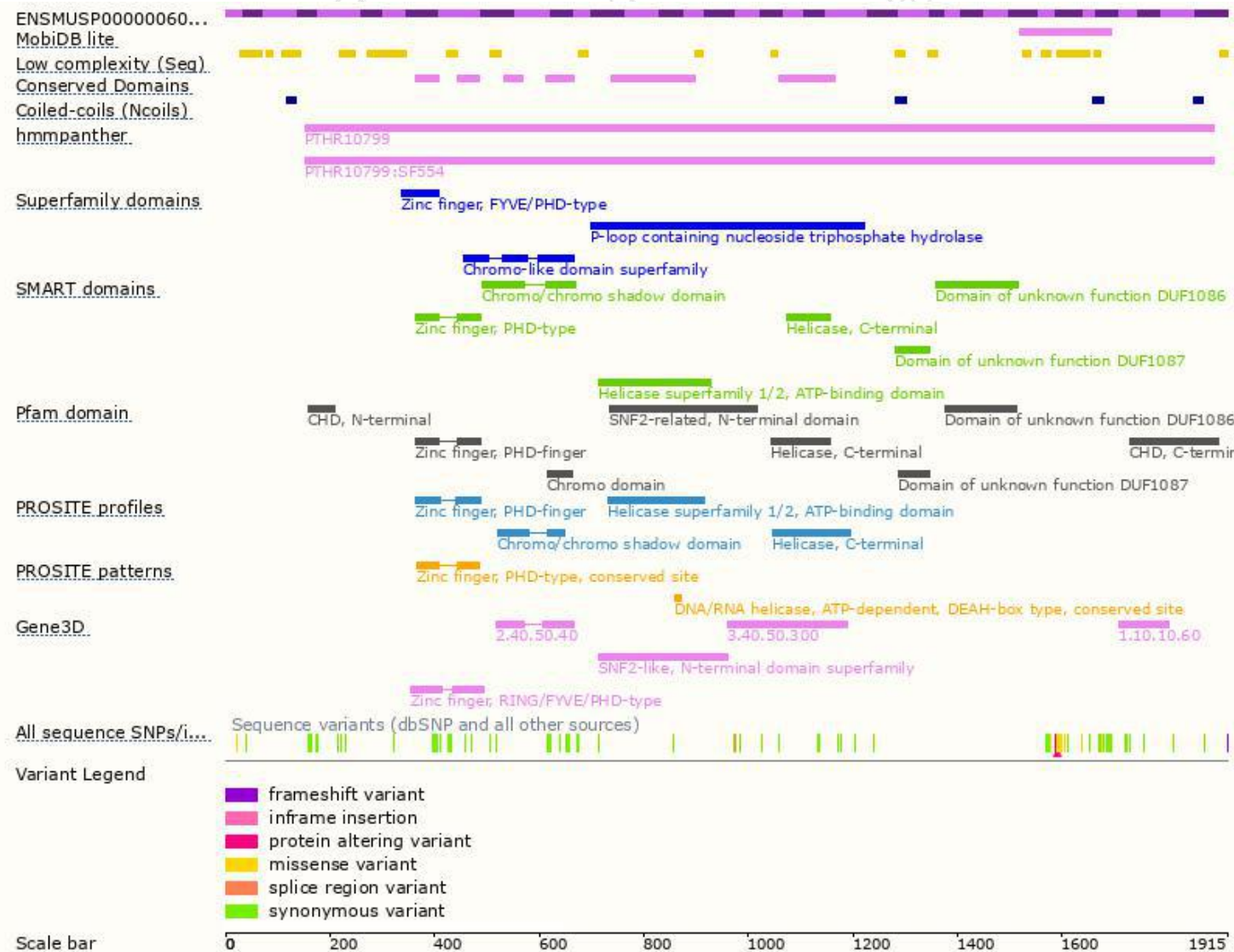
The strategy is based on the design of *Chd4-201* transcript,The transcription is shown below



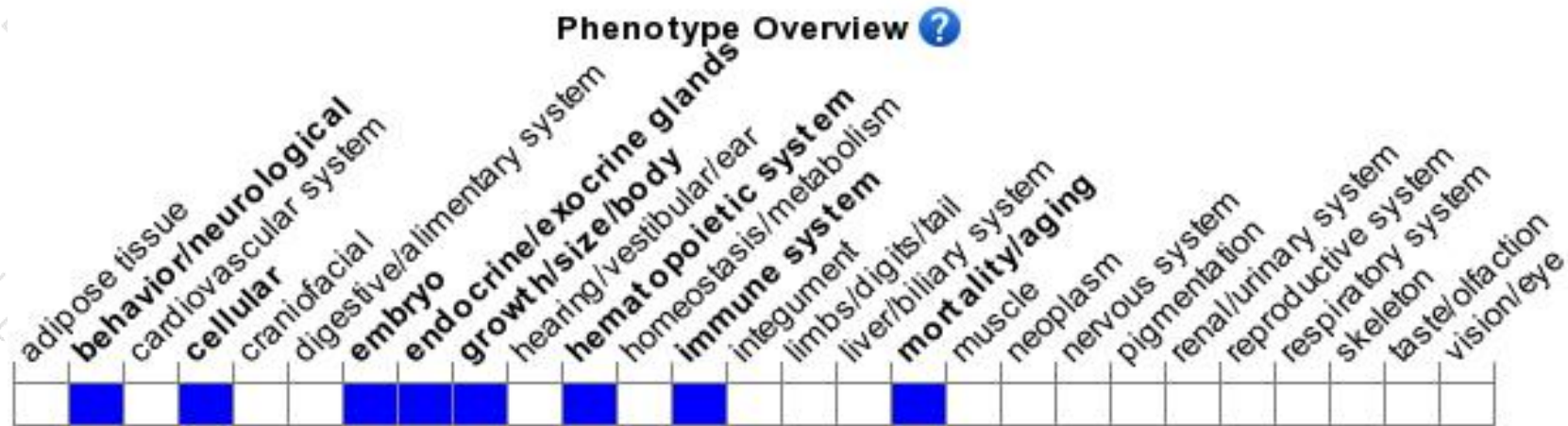
Genomic location distribution



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, Mice homozygous for a gene trapped allele exhibit embryonic lethality between E3.5 and E4.5, absent blastocoele failure of trophoctoderm function and increased apoptosis in blastocysts.

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

Tel: 400-9660890

