Zfp651 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

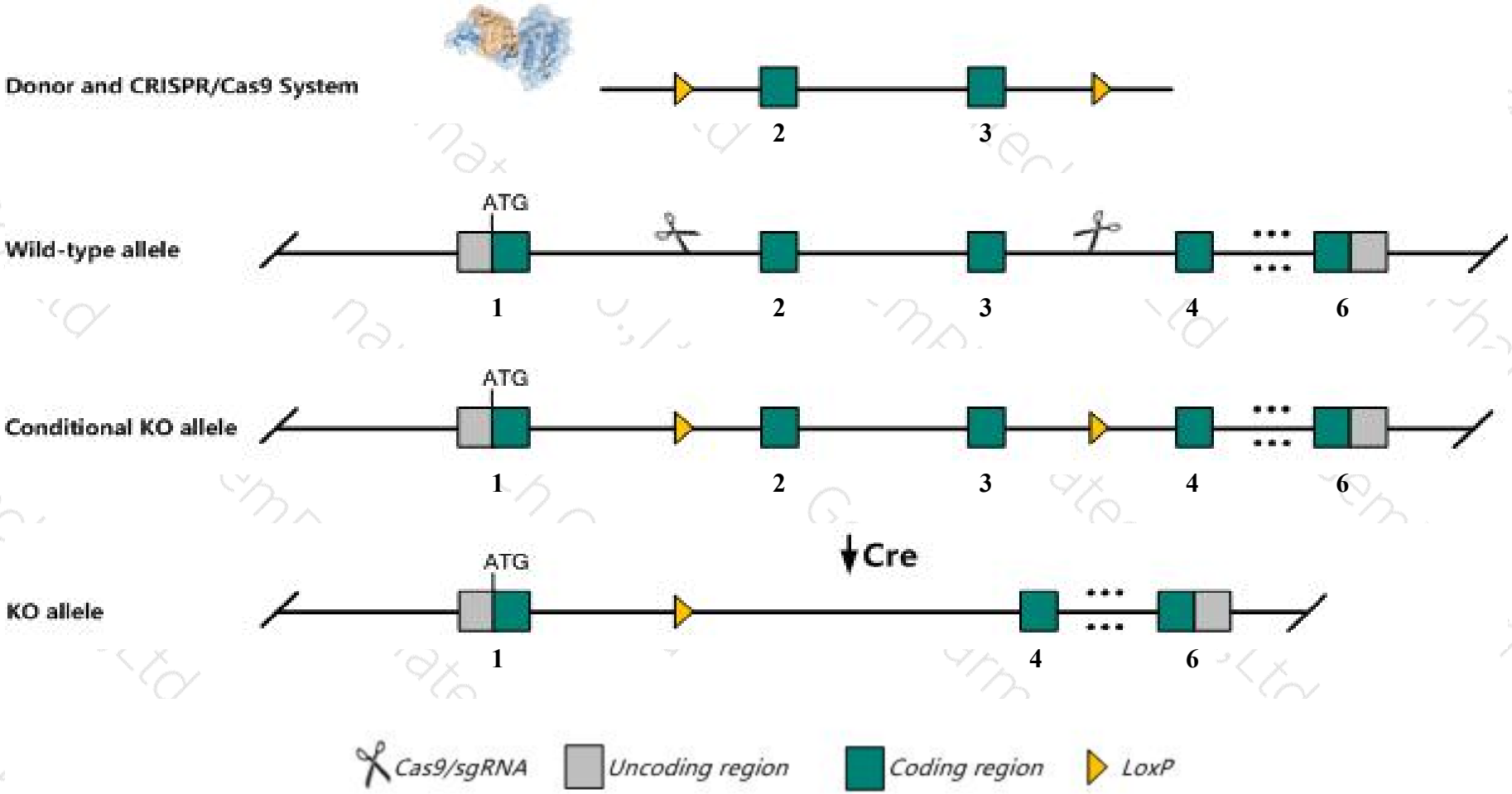
Design Date: 2020-11-10

Project Overview

Project Name	***Zfp651***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Zfp651* gene. The schematic diagram is as follows:



Technical routes

➢ The *Zfp651* gene has 4 transcripts. According to the structure of *Zfp651* gene, exon2-exon3 of *Zfp651-201*(ENSMUST00000093772.3) transcript is recommended as the knockout region.The region contains 1651bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Zfp651* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.

Notice

➢ The *Zfp651* gene is located on the Chr9. 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.

➢ The floxed region is near to the N-terminal of *E530011L22Rik* gene,this strategy may influence the regulatory function of the N-terminal of *E530011L22Rik* gene.

➢ Transcript *Zfp651*-202 may not be affected.

➢ 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）

集萃药康 GemPharmatech

Zfp651 zinc finger protein 651 [Mus musculus (house mouse)]

Gene ID: 270210, updated on 13-Mar-2020

Summary

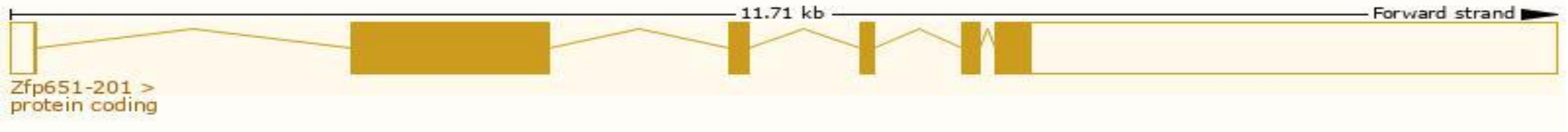
Official Symbol	Zfp651 provided by MGI
Official Full Name	zinc finger protein 651 provided byMGI
Primary source	MGI:MGI:2670992
See related	Ensembl:ENSMUSG00000013419
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4732420M22Rik, BC030045, R74626
Expression	Ubiquitous expression in ovary adult (RPKM 20.0), heart adult (RPKM 16.7) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

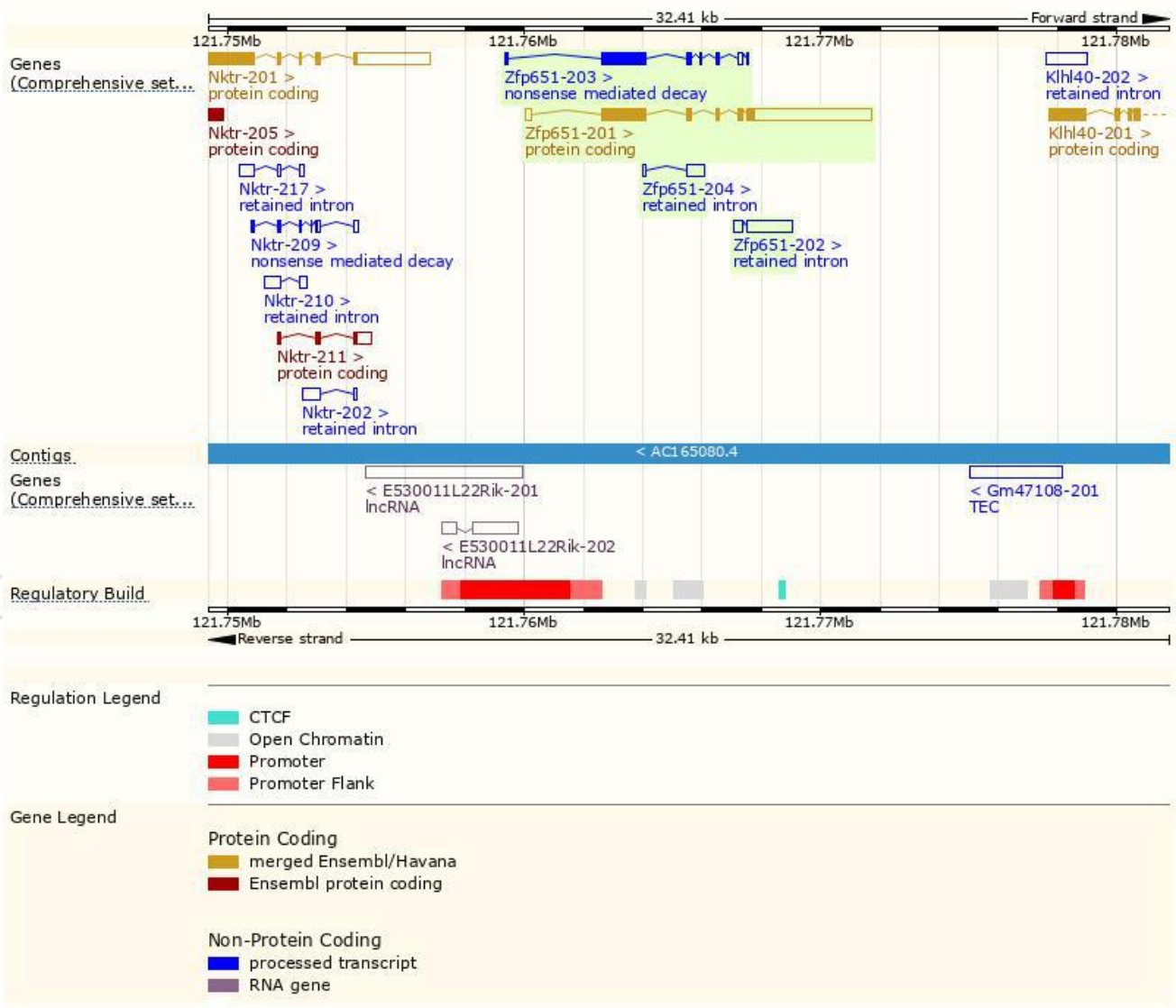
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zfp651-201	ENSMUST00000093772.3	6353	729aa	Protein coding	CCDS52968	E9PZ11	TSL:5 GENCODE basic APPRIS P1
Zfp651-203	ENSMUST00000214732.1	2136	627aa	Nonsense mediated decay	-	A0A1L1STM6	CDS 5' incomplete TSL:5
Zfp651-202	ENSMUST00000213743.1	1862	No protein	Retained intron	-	-	TSL:1
Zfp651-204	ENSMUST00000215081.1	697	No protein	Retained intron	-	-	TSL:2

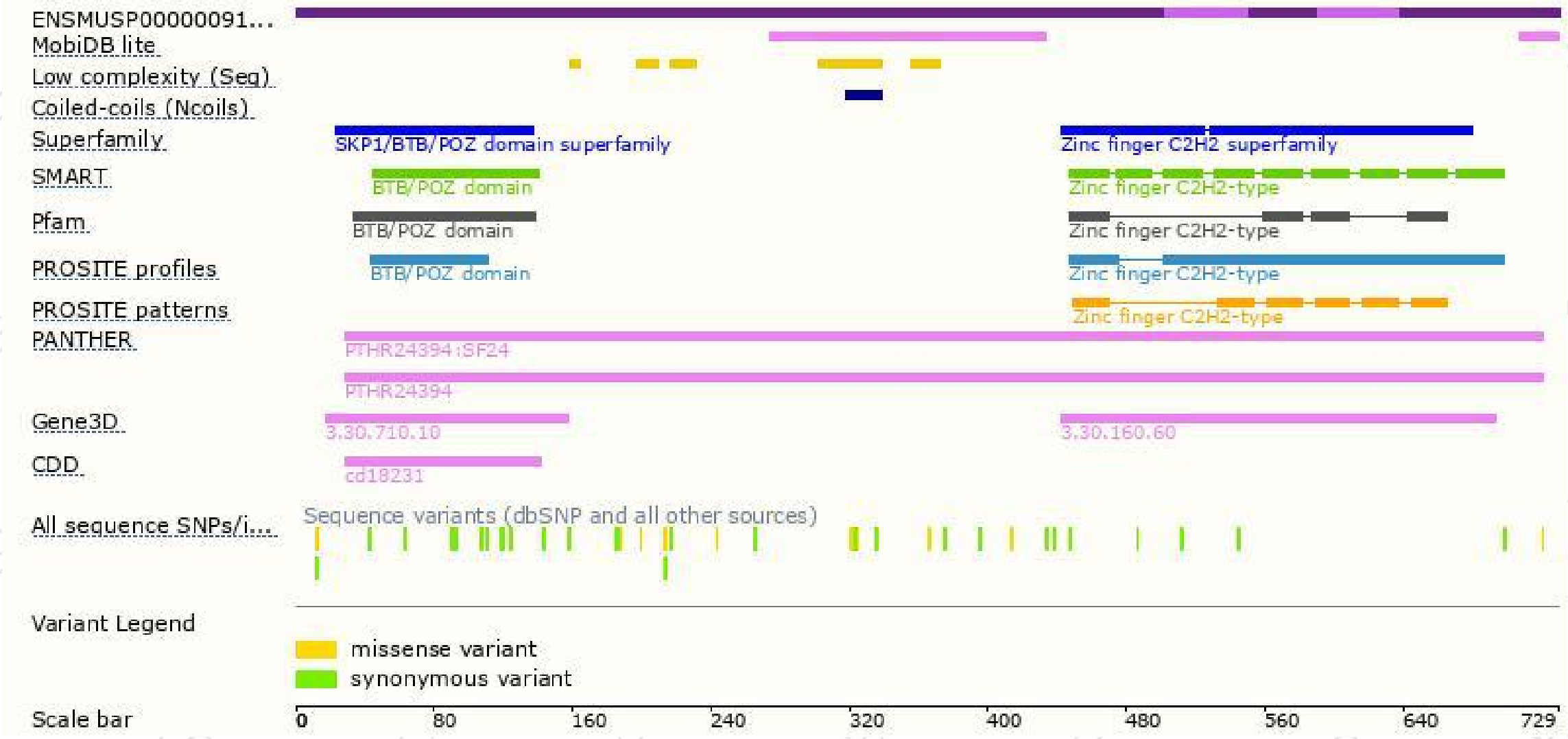
The strategy is based on the design of *Zfp651-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd