Fancb Cas9-CKO Strategy

Designer:Xiaojing Li

Reviewer: JiaYu

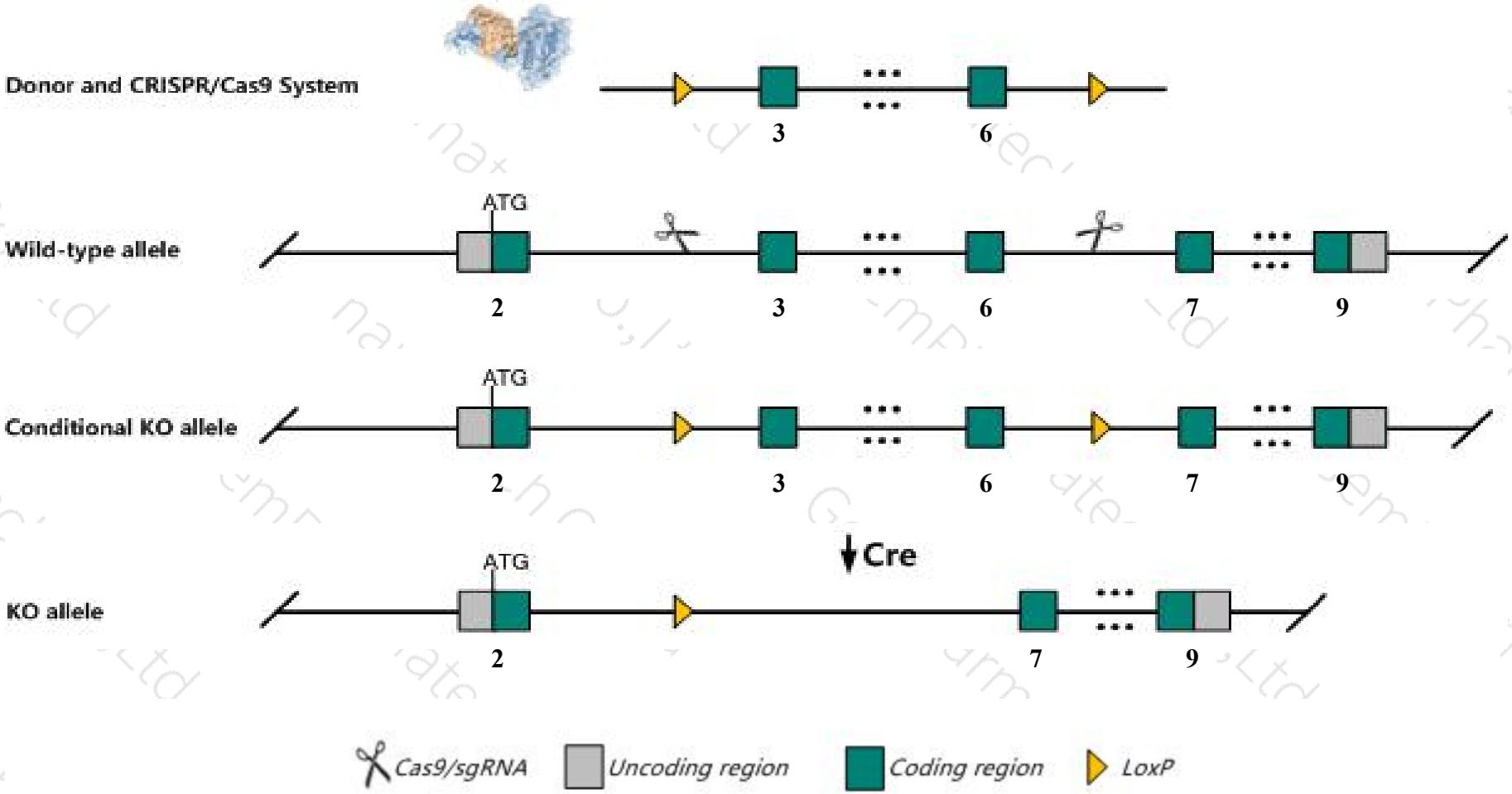
Design Date: 2020-7-29

Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Fancb* gene has 2 transcripts. According to the structure of *Fancb* gene, exon3-exon6 of *Fancb-202*(ENSMUST00000167446.7) transcript is recommended as the knockout region.The region contains 554bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Fancb* 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

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit male infertility with oligozoospermia, reduced primordial germ cells and defects in the maintenance of undifferentiated spermatogonia.

➢ The *Fancb* gene is located on the ChrX. 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）

集萃药康 GemPharmatech

Fancb Fanconi anemia, complementation group B [Mus musculus (house mouse)]

Gene ID: 237211, updated on 13-Mar-2020

Summary

Official Symbol	Fancb provided by MGI
Official Full Name	Fanconi anemia, complementation group B provided byMGI
Primary source	MGI:MGI:2448558
See related	Ensembl:ENSMUSG00000047757
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	BC022692
Expression	Broad expression in liver E14 (RPKM 2.2), CNS E11.5 (RPKM 2.0) and 20 other tissuesSee more
Orthologs	human all

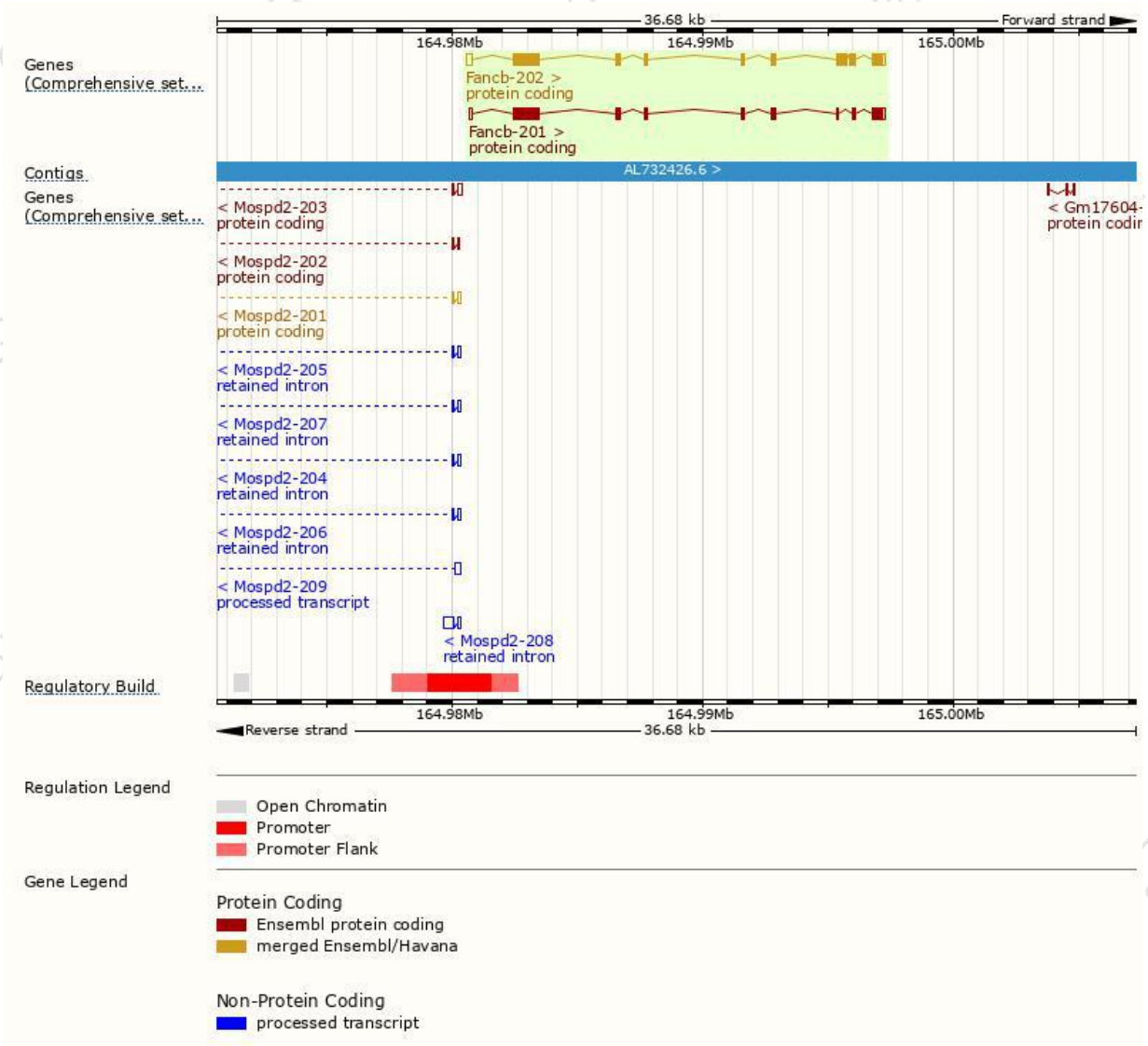
Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

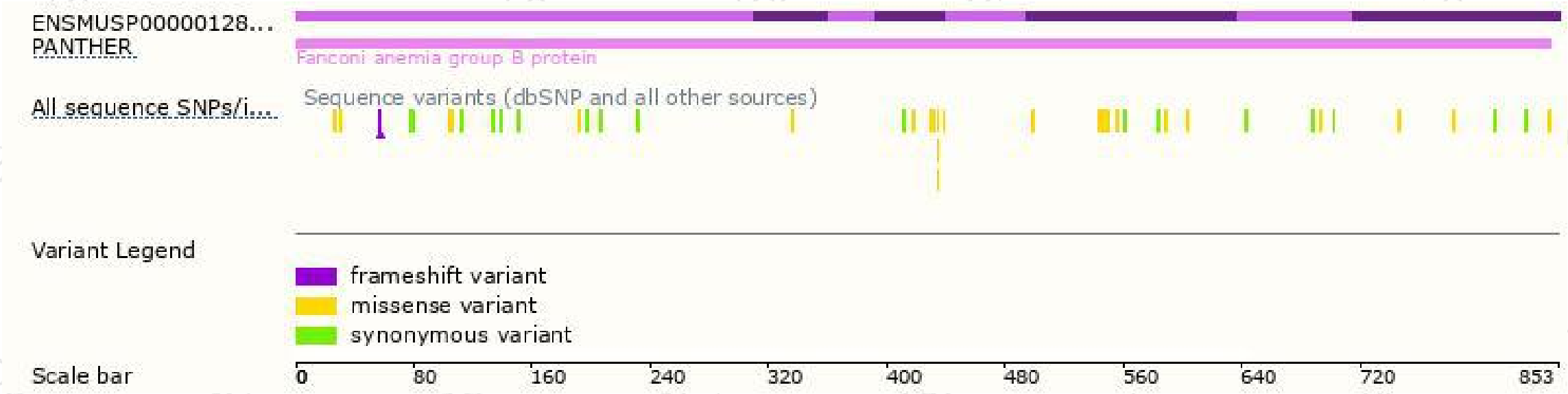
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fancb-202	ENSMUST00000167446.7	2953	853aa	Protein coding	CCDS53242	Q3TEX6	TSL:1 GENCODE basic APPRIS P2
Fancb-201	ENSMUST00000057150.7	2395	706aa	Protein coding	-	B1AW75	TSL:5 GENCODE basic APPRIS ALT2

The strategy is based on the design of *Fancb-202* transcript,the transcription is shown below:

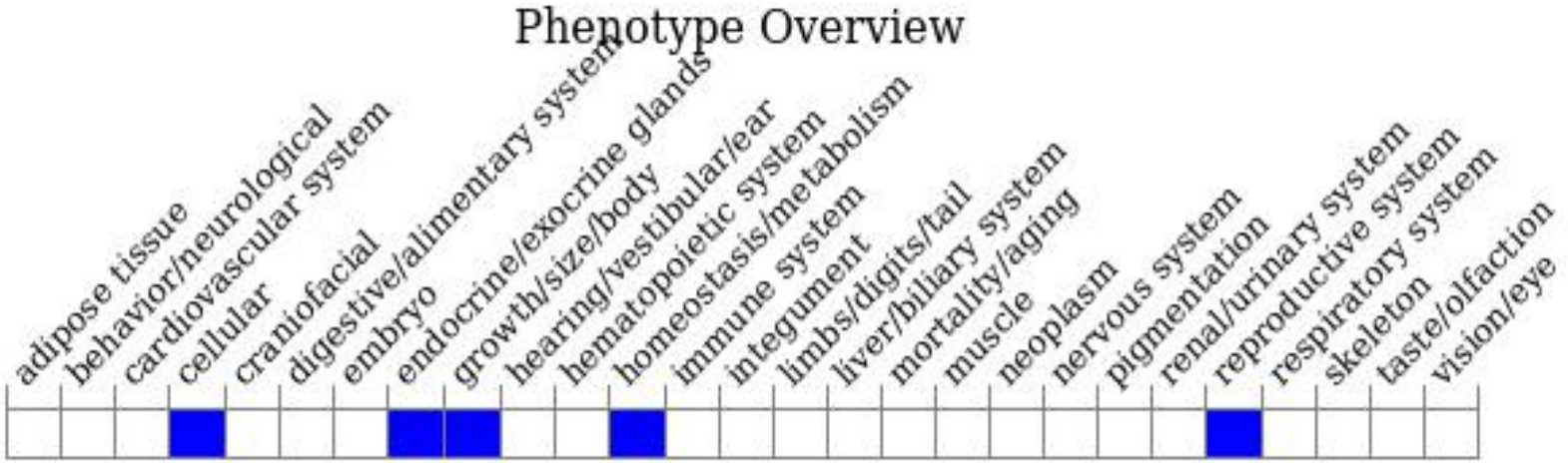
Genomic location distribution



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 knock-out allele exhibit male infertility with oligozoospermia, reduced primordial germ cells and defects in the maintenance of undifferentiated spermatogonia.

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