Fank1 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

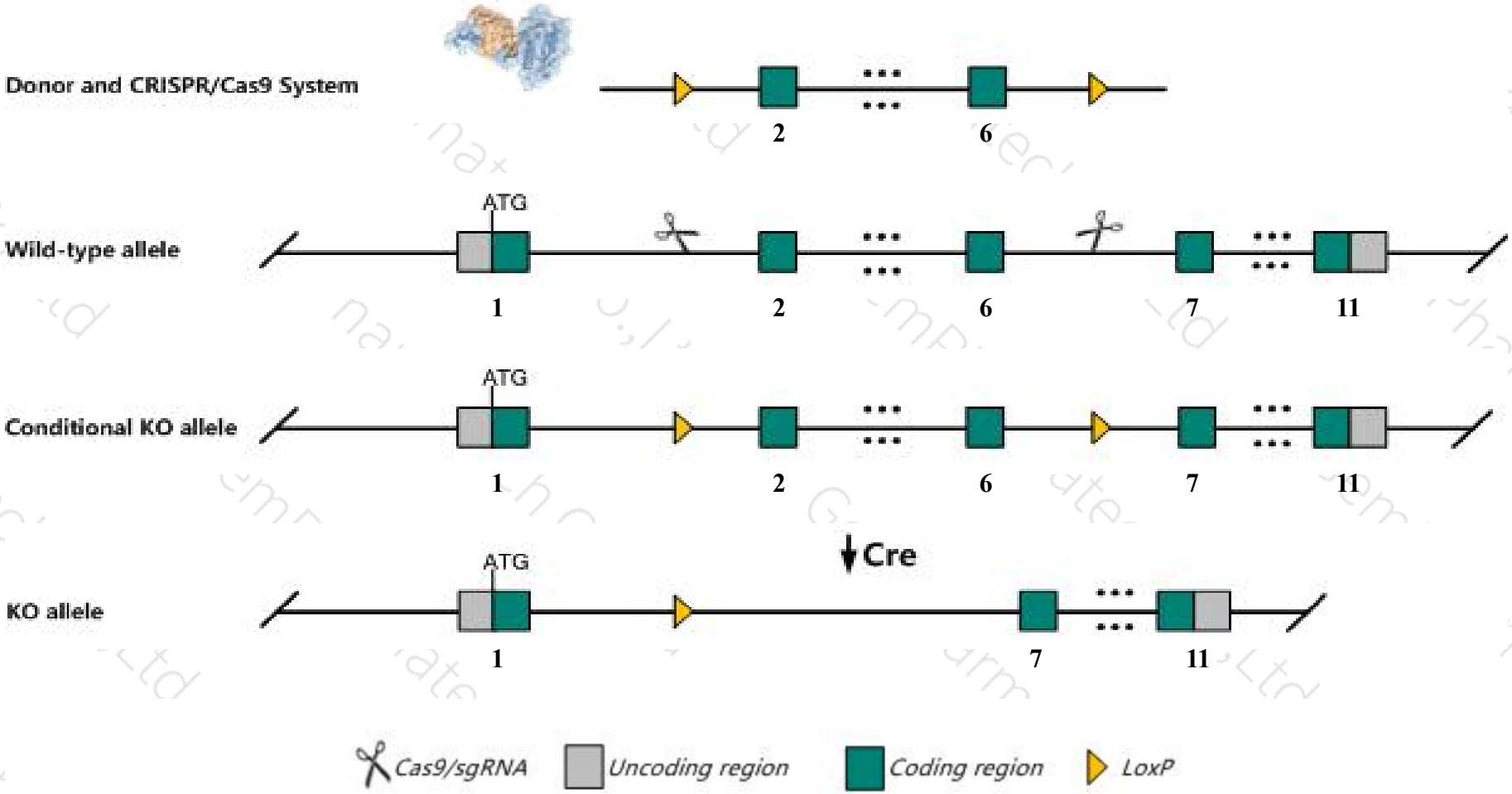
Design Date: 2020-8-6

Project Overview

Project Name	***Fank1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Fank1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Fank1* gene has 5 transcripts. According to the structure of *Fank1* gene, exon2-exon6 of *Fank1-201*(ENSMUST00000065359.11) transcript is recommended as the knockout region.The region contains 526bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Fank1* 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 are viable and fertile; males show normal spermatogenesis with no detectable alterations in sperm morphology, count and motility or number of apoptotic cells in testes.

➢ The effect on transcript *Fank1*-203&205 is unknown.

➢ The *Fank1* 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）

集萃药康 GemPharmatech

Fank1 fibronectin type 3 and ankyrin repeat domains 1 [Mus musculus (house mouse)]

Gene ID: 66930, updated on 13-Mar-2020

Summary

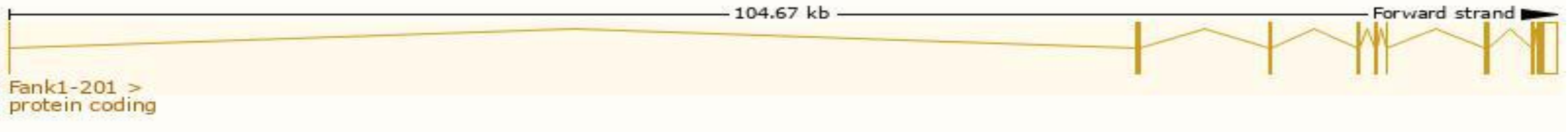
Official Symbol	Fank1 provided by MGI
Official Full Name	fibronectin type 3 and ankyrin repeat domains 1 provided byMGI
Primary source	MGI:MGI:1914180
See related	Ensembl:ENSMUSG00000053111
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700007B22Rik, AI850911, FANK1S
Expression	Biased expression in testis adult (RPKM 86.5) and CNS E18 (RPKM 2.7)See more
Orthologs	human all

Transcript information（Ensembl）

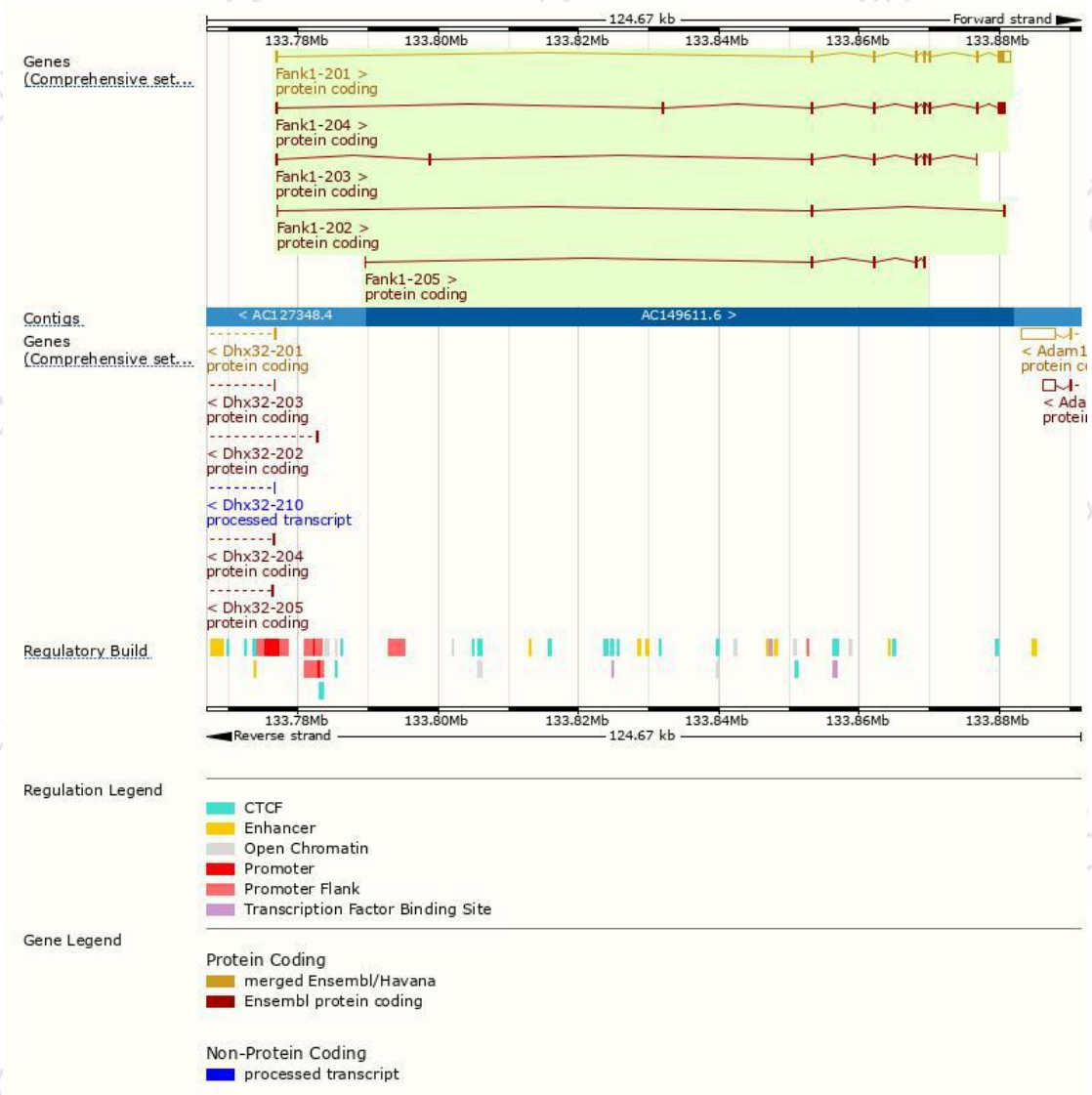
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fank1-201	ENSMUST00000065359.11	2004	344aa	Protein coding	CCDS21937	Q9DAM9	TSL:1 GENCODE basic APPRIS P1
Fank1-204	ENSMUST00000209511.1	1392	343aa	Protein coding	-	A0A1B0GSJ7	TSL:5 GENCODE basic
Fank1-203	ENSMUST00000151031.1	824	152aa	Protein coding	-	D3Z7V1	CDS 3' incomplete TSL:3
Fank1-205	ENSMUST00000211077.1	515	96aa	Protein coding	-	A0A1B0GRK5	CDS 3' incomplete TSL:3
Fank1-202	ENSMUST00000121560.1	475	126aa	Protein coding	-	D3YW22	TSL:5 GENCODE basic

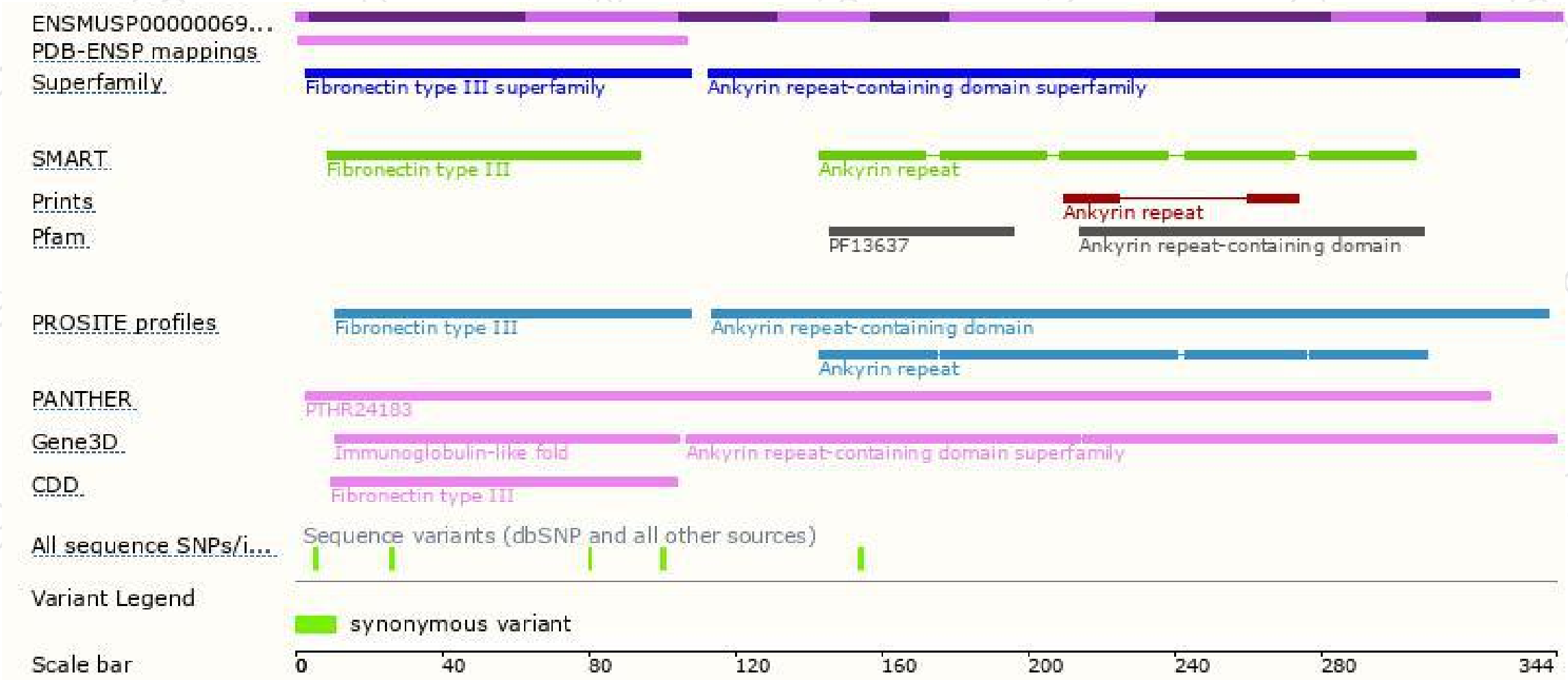
The strategy is based on the design of *Fank1-201* transcript,the transcription is shown below:



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