Tfpt Cas9-CKO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

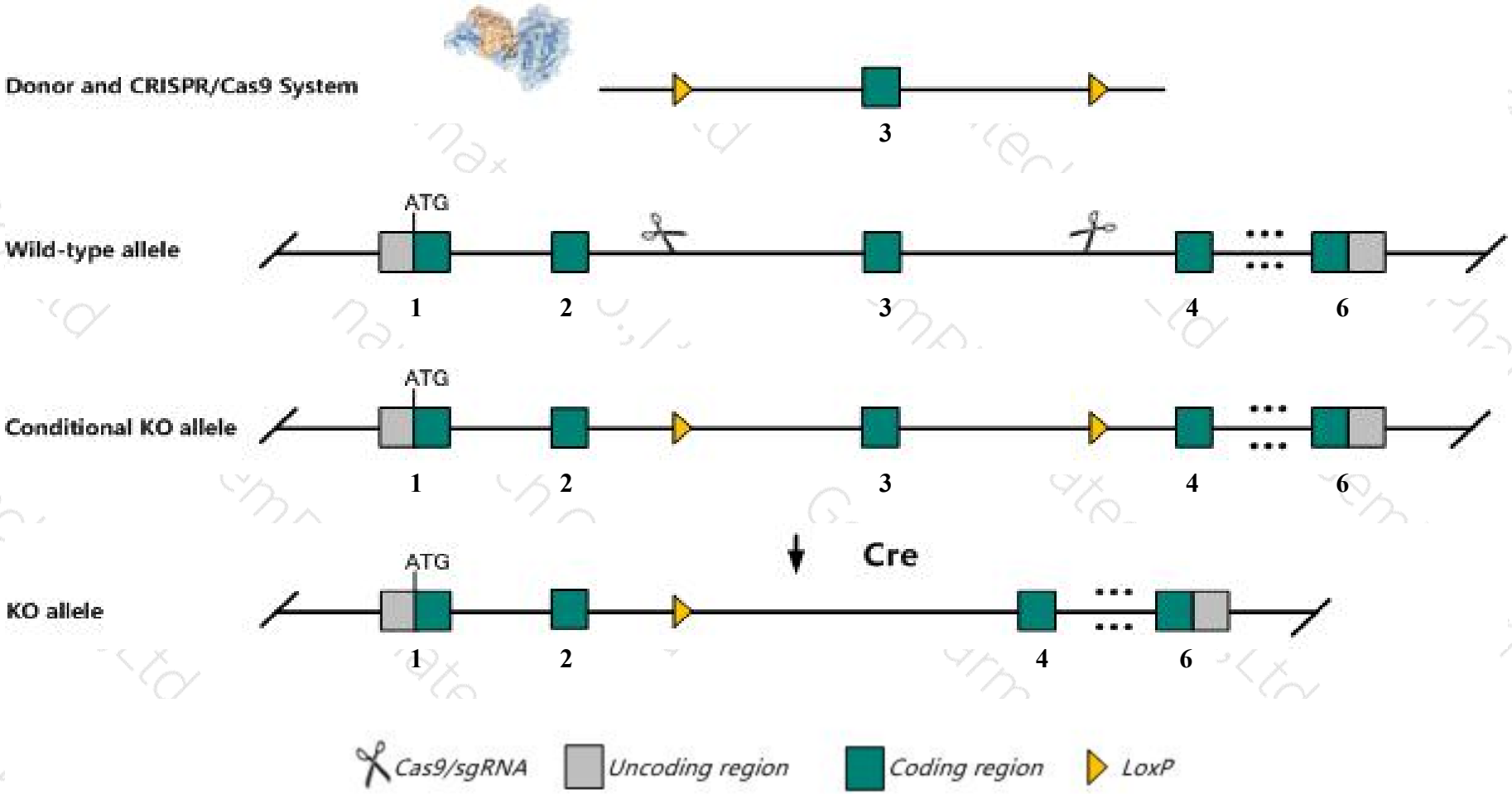
Design Date: 2020-8-24

Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Tfpt* gene has 7 transcripts. According to the structure of *Tfpt* gene, exon3 of *Tfpt-202*(ENSMUST00000108641.9) transcript is recommended as the knockout region.The region contains 71bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tfpt* 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 *Tfpt* 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 had no effect on *Tfpt*-206.

➢ 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

Tfpt TCF3 (E2A) fusion partner [Mus musculus (house mouse)]

Gene ID: 69714, updated on 13-Mar-2020

Summary

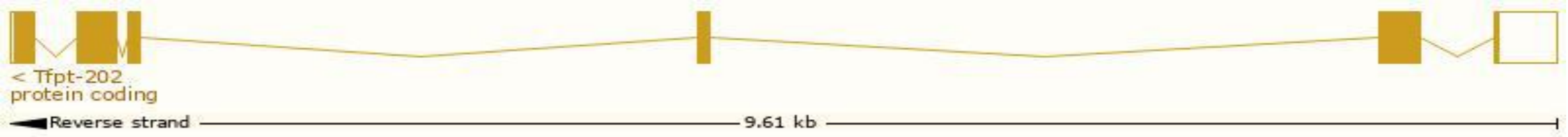
Official Symbol	Tfpt provided by MGI
Official Full Name	TCF3 (E2A) fusion partner provided byMGI
Primary source	MGI:MGI:1916964
See related	Ensembl:ENSMUSG00000006335
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2400004F01Rik, AI450389, Amida, FB1
Expression	Ubiquitous expression in CNS E11.5 (RPKM 12.3), CNS E14 (RPKM 9.8) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

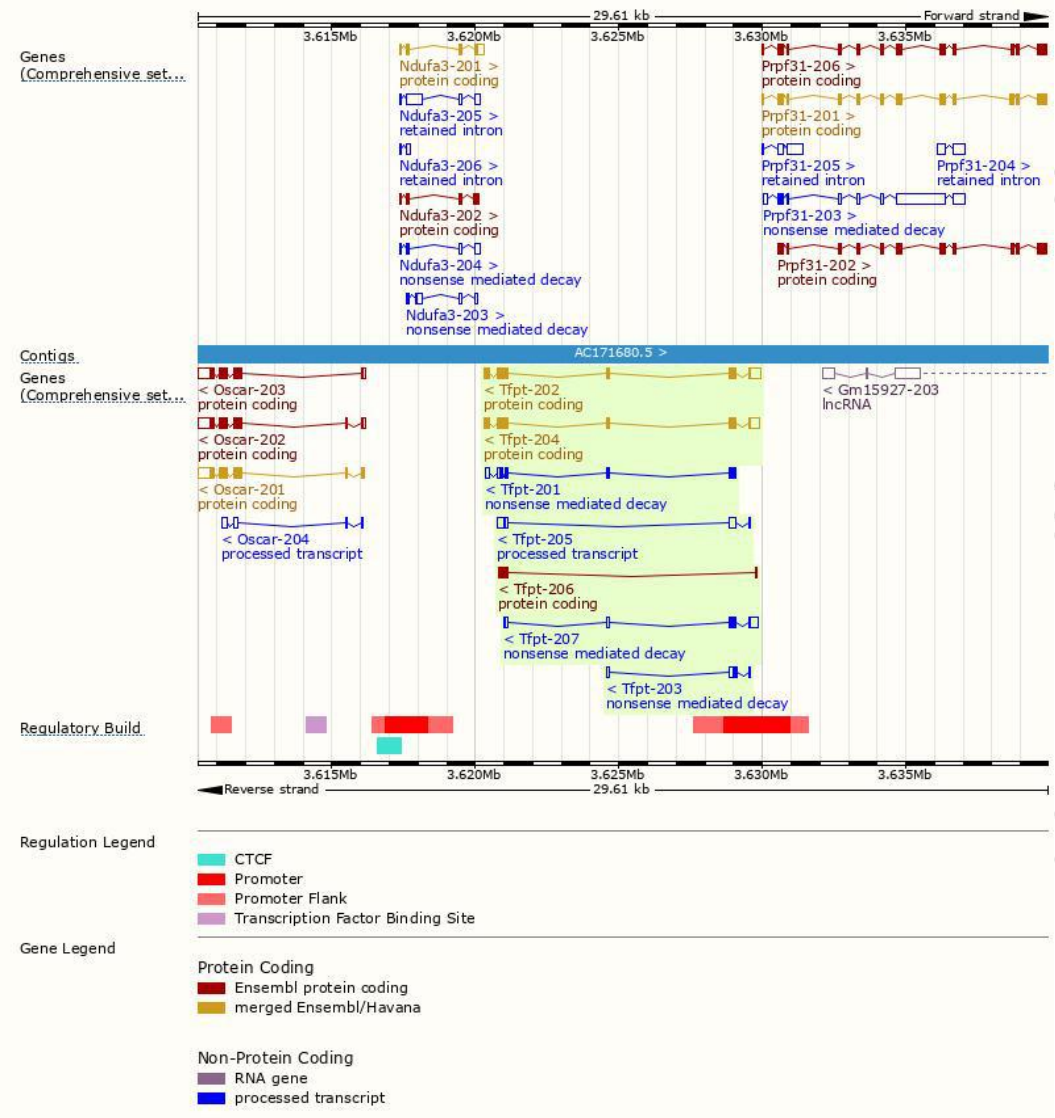
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tfpt-202	ENSMUST00000108641.9	1166	259aa	Protein coding	CCDS71873	Q3U1J1	TSL:1 GENCODE basic APPRIS ALT2
Tfpt-204	ENSMUST00000155592.7	1091	249aa	Protein coding	CCDS20720	Q3U1J1	TSL:1 GENCODE basic APPRIS P3
Tfpt-206	ENSMUST00000205596.1	315	84aa	Protein coding	-	A0A0U1RPD5	CDS 3' incomplete TSL:5
Tfpt-207	ENSMUST00000206370.1	722	97aa	Nonsense mediated decay	-	A0A0U1RQ56	TSL:5
Tfpt-201	ENSMUST00000058880.8	710	156aa	Nonsense mediated decay	-	F8WIZ9	CDS 5' incomplete TSL:5
Tfpt-203	ENSMUST00000153143.1	389	45aa	Nonsense mediated decay	-	D6RHM4	TSL:3
Tfpt-205	ENSMUST00000156194.2	562	No protein	Processed transcript	-	-	TSL:3

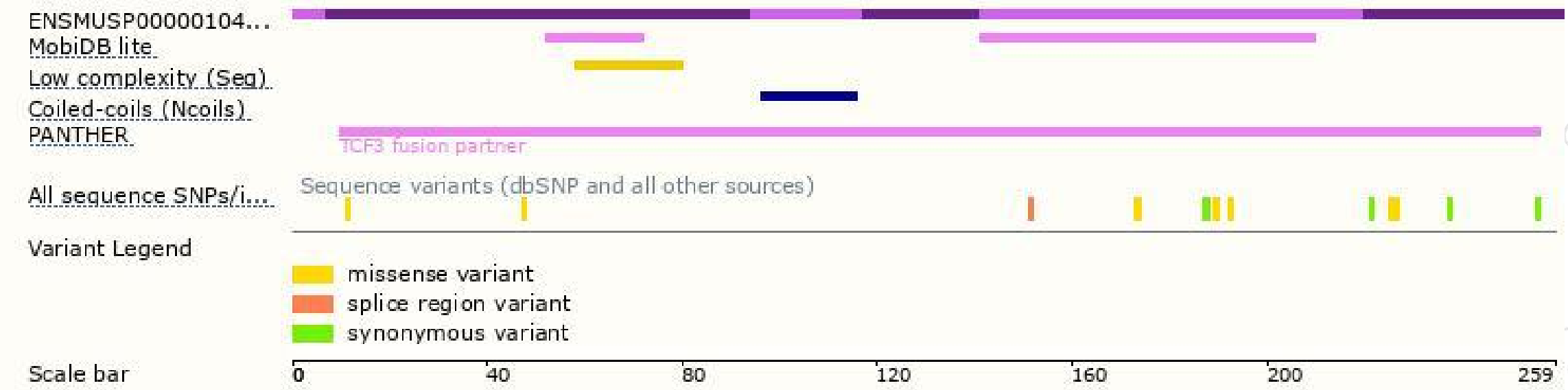
The strategy is based on the design of *Tfpt-202* transcript,the transcription is shown below:



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