F10 Cas9-CKO Strategy

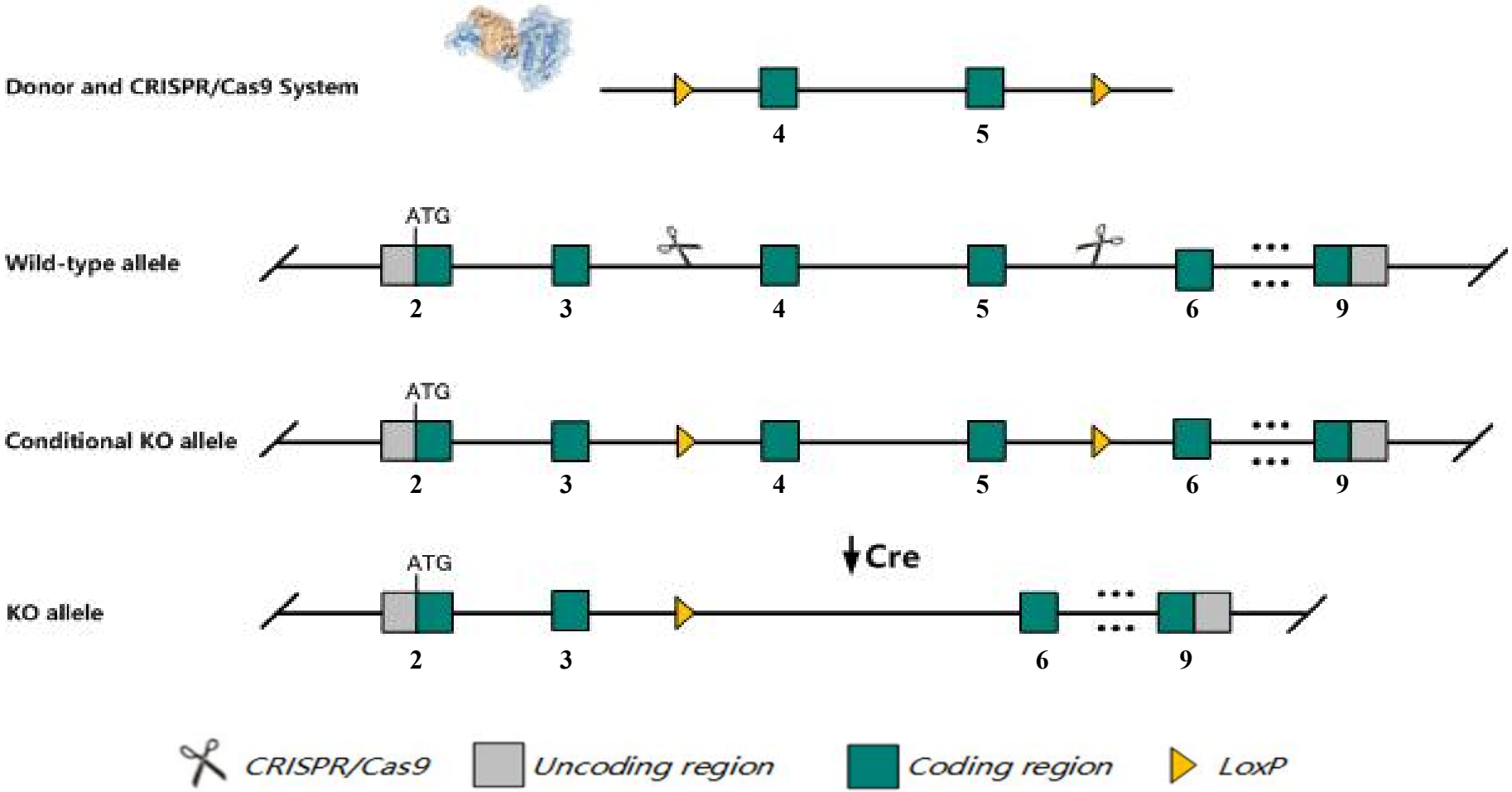
Designer:

Project Overview

Project Name	***F10***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *F10* gene. The schematic diagram is as follows:



Technical routes

The *F10* gene has 5 transcripts. According to the structure of *F10* gene, exon4-exon5 of *F10-202* (ENSMUST00000063820.11) transcript is recommended as the knockout region.The region contains 139bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *F10* 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.

Notice

According to the existing MGI data,Most homozygous mice die from fatal bleeding events at embryonic and neonatal stages, with the remaining homozygous mice dying before weaning stages.

The *F10* gene is located on the Chr8. 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

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F10 coagulation factor X [Mus musculus (house mouse)]

Gene ID: 14058, updated on 31-Jan-2019

Summary

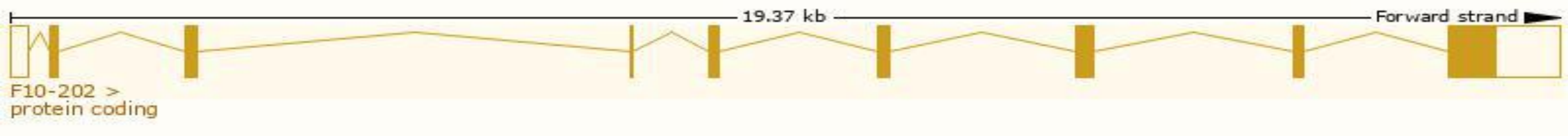
Official Symbol	F10 provided by MGI
Official Full Name	coagulation factor X provided by MGI
Primary source	MGI:MGI:103107
See related	Ensembl:ENSMUSG00000031444
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cf10, fX
Summary	This gene encodes factor X, a component of both the intrinsic and extrinsic blood coagulation pathways. The encoded protein is a zymogen that undergoes further processing in a vitamin K-dependent manner to generate mature factor X, a heterodimer comprised of disulfide-linked heavy and light chains. The mature factor X is proteolytically activated either by factor IXa (intrinsic pathway) or factor VIIa (extrinsic pathway) to form factor Xa serine endopeptidase. Activated factor Xa catalyzes the conversion of prothrombin to thrombin. A complete lack of the encoded protein is fatal to mice. A severe deficiency of the encoded protein in mice causes age-dependent iron deposition and cardiac fibrosis. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Aug 2015]
Expression	Biased expression in liver E18 (RPKM 103.5), liver adult (RPKM 83.2) and 3 other tissues See more
Orthologs	human all

Transcript information Ensembl

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

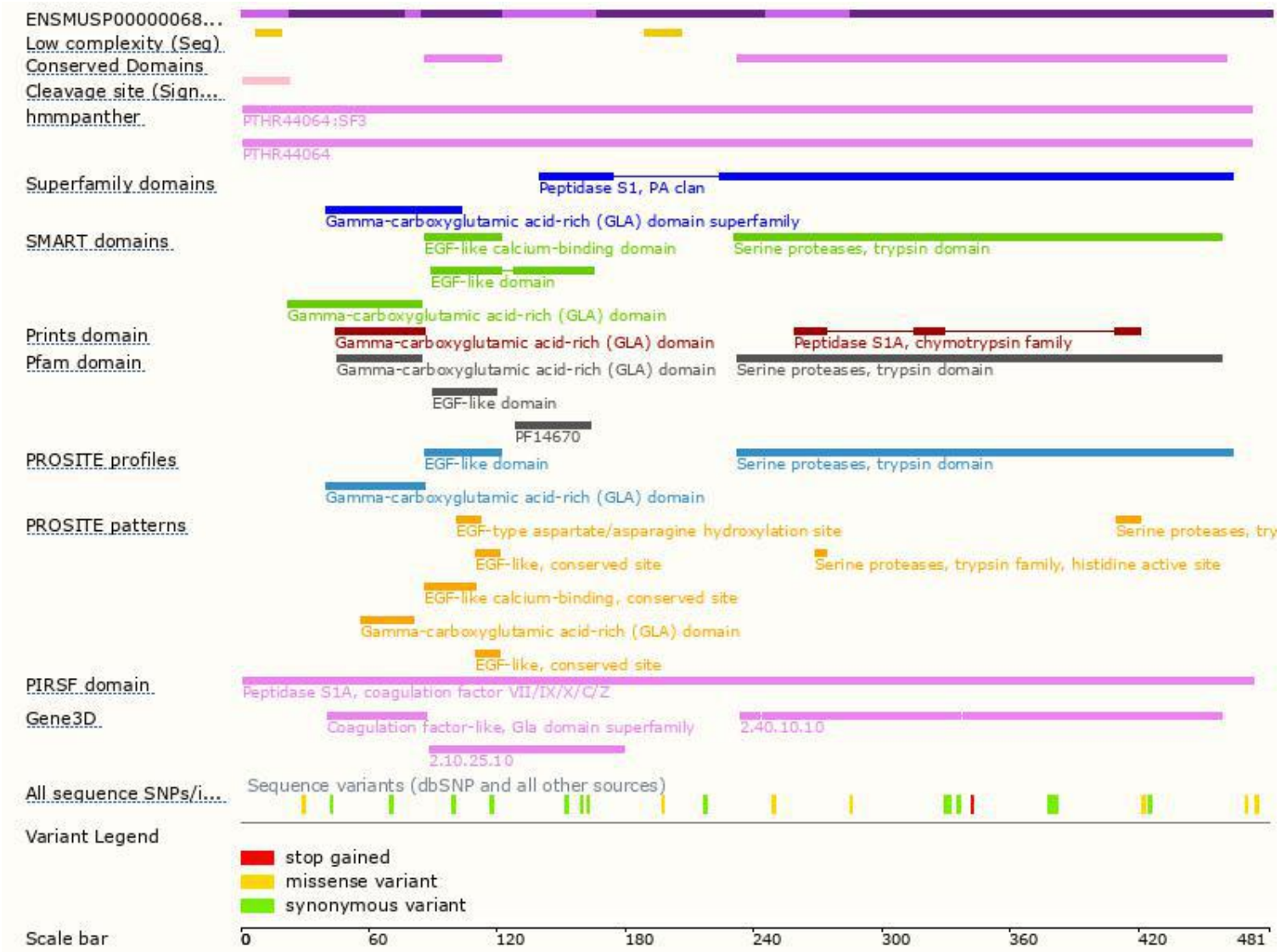
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
F10-202	ENSMUST00000063820.11	2503	481aa	Protein coding	CCDS40226	O88947 Q3TBR2	TSL:1 GENCODE basic APPRIS P3
F10-201	ENSMUST00000033821.10	1903	493aa	Protein coding	CCDS57606	Q3U3V1	TSL:1 GENCODE basic APPRIS ALT2
F10-205	ENSMUST00000152034.1	1449	319aa	Protein coding	-	D3Z521	TSL:5 GENCODE basic
F10-204	ENSMUST00000128418.7	1395	321aa	Protein coding	-	D3Z215	TSL:5 GENCODE basic
F10-203	ENSMUST00000123768.7	609	119aa	Protein coding	-	D3Z7R3	CDS 3' incomplete TSL:2

The strategy is based on the design of *F10-202* transcript,The transcription is shown below

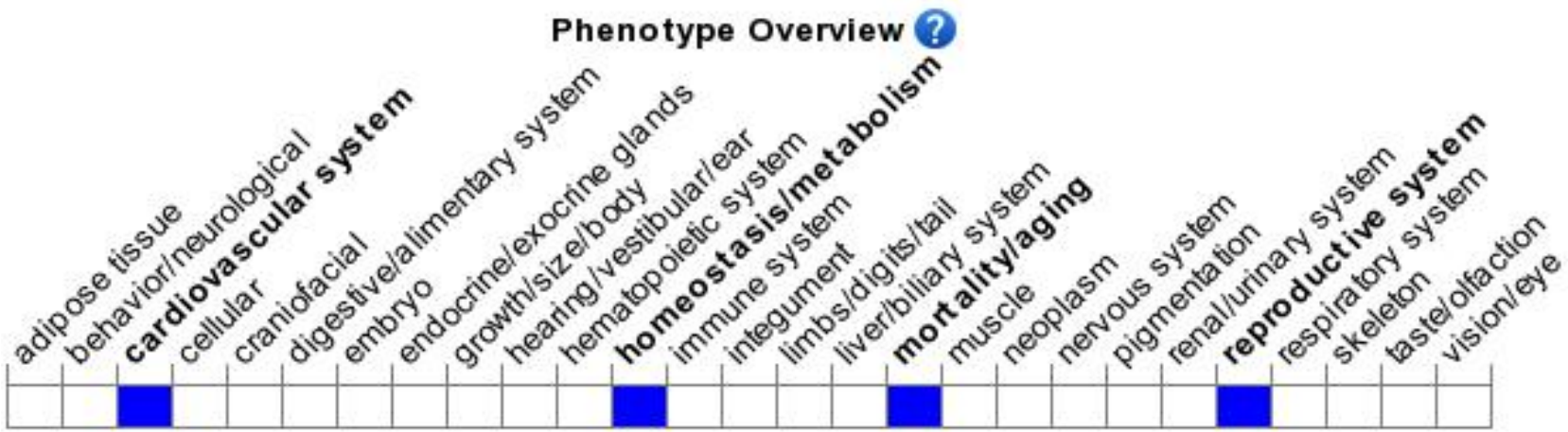


Genomic location distribution

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/).

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集萃药康
GemPharmatech

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd