Fancl Cas9-KO Strategy

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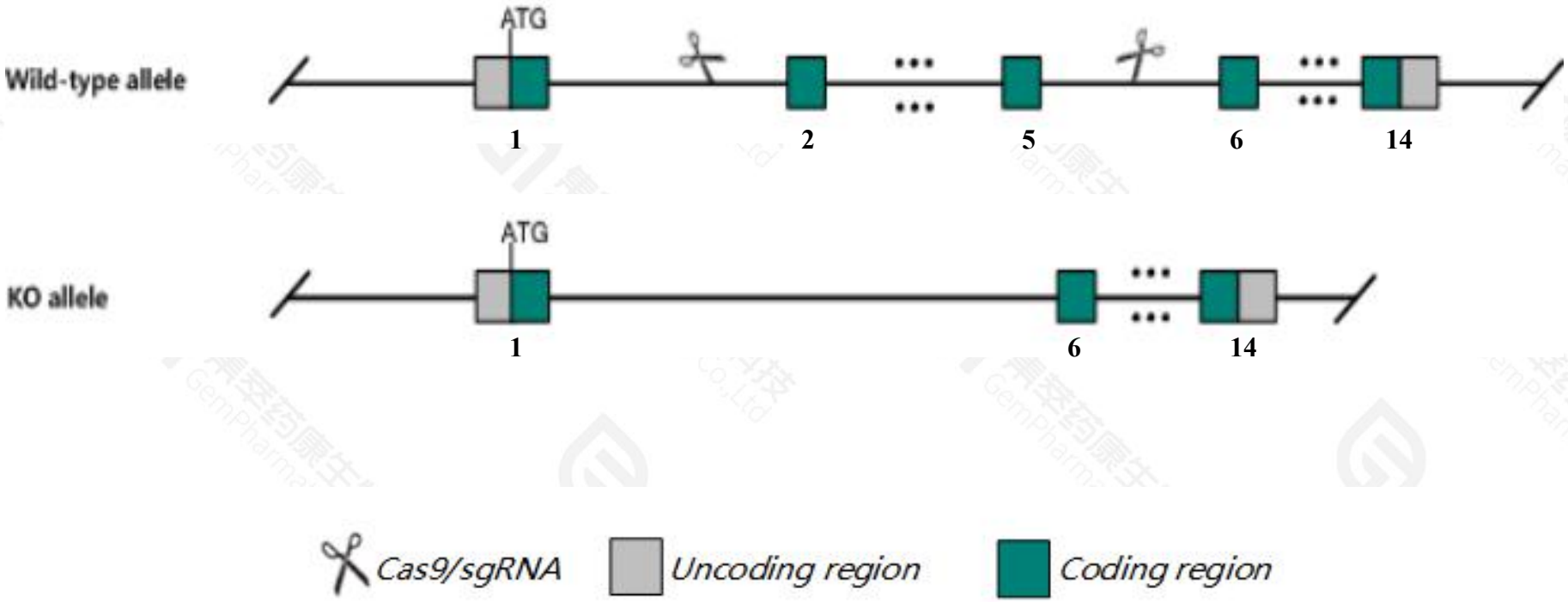
Design Date: 2021-5-24

Project Overview

Project Name	***Fancl***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

➢ The *Fancl* gene has 5 transcripts. According to the structure of *Fancl* gene, exon2-exon5 of *Fancl-201*(ENSMUST00000004120.9) transcript is recommended as the knockout region.The region contains 278bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Fancl* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,homozygosity for mutations that inactivate the allele results in male and female infertility due to a defects in primordial germ cell proliferation. Homozygosity is embryonic lethal on some backgrounds.

➢ Transcript *Fancl-203* may not be affected.

➢ The *Fancl* gene is located on the Chr11. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Fancl Fanconi anemia, complementation group L [Mus musculus (house mouse)]

Gene ID: 67030, updated on 17-Nov-2020

Summary

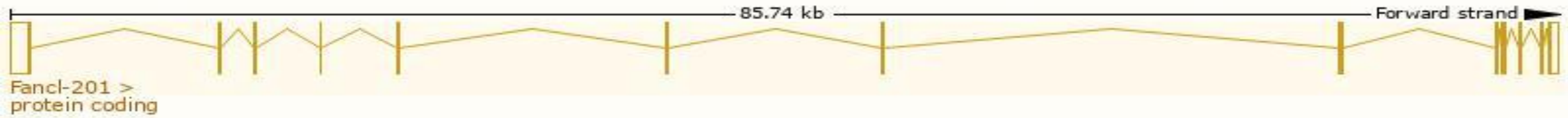
Official Symbol	Fancl provided by MGI
Official Full Name	Fanconi anemia, complementation group L provided byMGI
Primary source	MGI:MGI:1914280
See related	Ensembl:ENSMUSG00000004018
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010322C19Rik, AW554273, B230118H11Rik, P, Phf, Phf9, Pog, gcd
Summary	This gene encodes the complementation group L subunit of the multimeric Fanconi anemia (FA) nuclear complex composed of proteins encoded by over ten Fanconi anemia complementation (FANC) group genes. The FA complex is necessary for protection against DNA damage. This gene product, an E3 ubiquitin ligase, catalyzes and is required for the monoubiquitination of the protein encoded by the Fanconi anemia, complementation group D2 gene, a critical step in the FA pathway (PMID: 12973351, 21229326). In mouse, mutations of this E3 ubiquitin ligase gene can lead to infertility in adult males and females, and a deletion of this gene can cause embryonic lethality in some genetic backgrounds. A pseudogene of this gene has been identified on chromosome 1. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Mar 2013]
Expression	Ubiquitous expression in liver E14 (RPKM 7.9), CNS E11.5 (RPKM 7.5) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

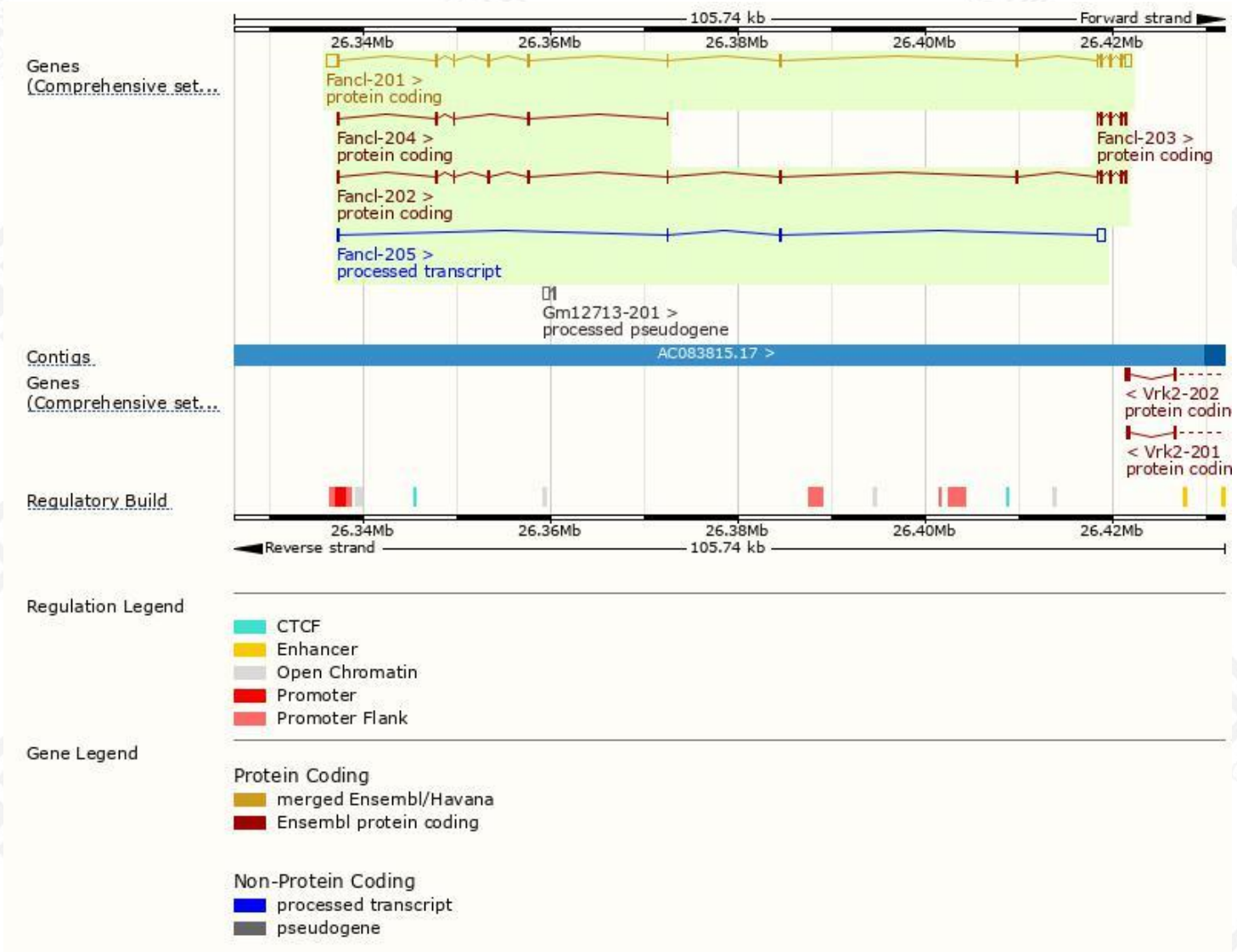
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fancl-201	ENSMUST00000004120.9	2724	375aa	Protein coding	CCDS24485		TSL:1 , GENCODE basic , APPRIS P1 ,
Fancl-202	ENSMUST00000109509.8	1187	370aa	Protein coding	CCDS70152		TSL:1 , GENCODE basic ,
Fancl-203	ENSMUST00000134445.2	577	145aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Fancl-204	ENSMUST00000136830.2	383	123aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Fancl-205	ENSMUST00000143471.2	1065	No protein	Processed transcript	-		TSL:1 ,

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

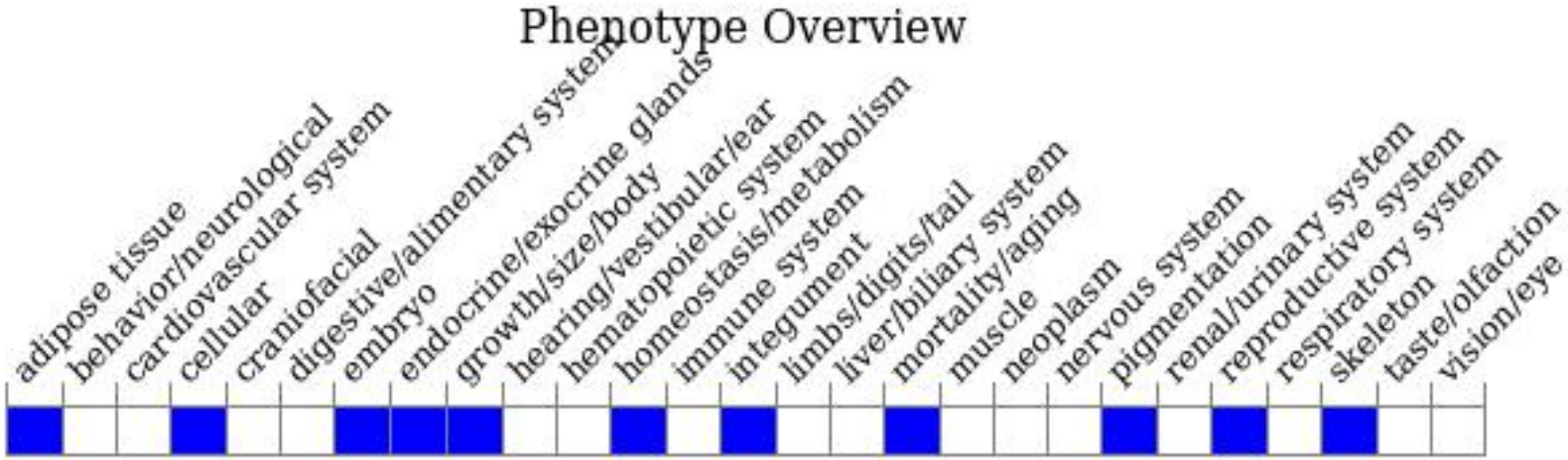


Genomic location distribution



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,homozygosity for mutations that inactivate the allele results in male and female infertility due to a defects in primordial germ cell proliferation. Homozygosity is embryonic lethal on some backgrounds.

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

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