Crlf3 Cas9-KO Strategy

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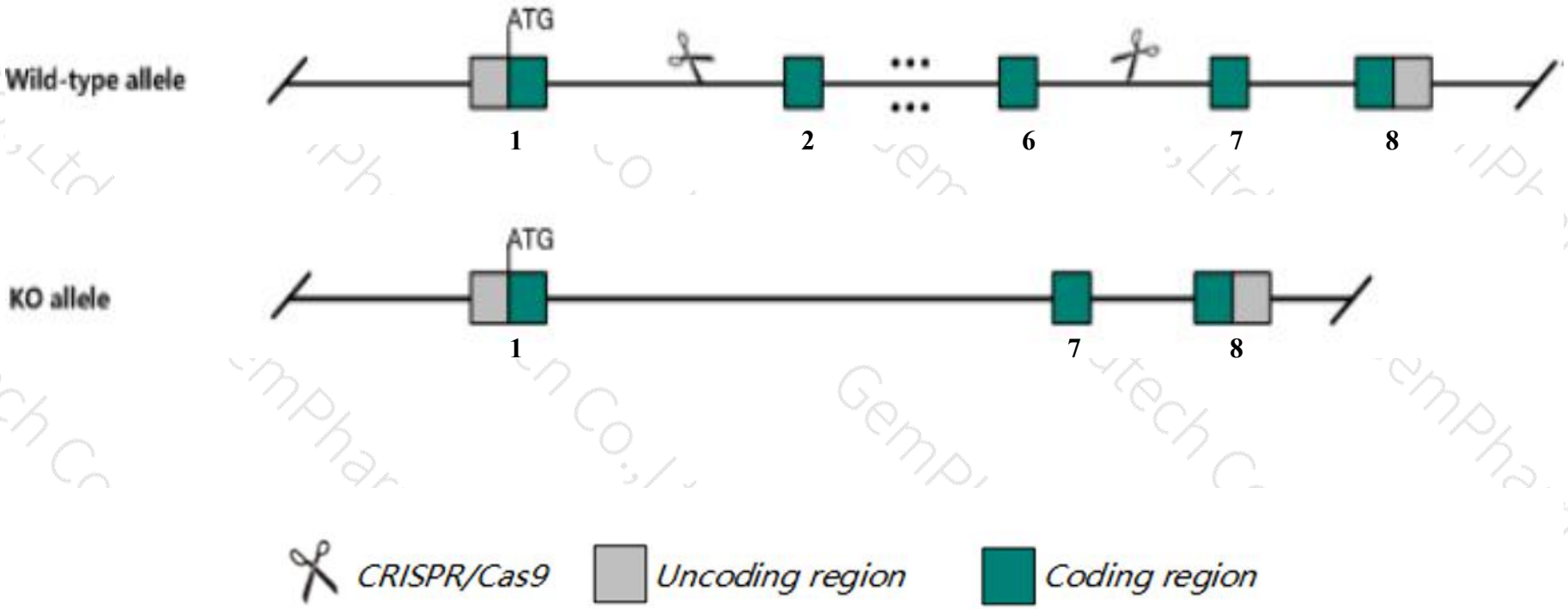
Project Overview

Project Name	***Crlf3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Crlf3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Crlf3* gene has 8 transcripts. According to the structure of *Crlf3* gene, exon2-exon6 of *Crlf3-201* (ENSMUST00000061283.14) transcript is recommended as the knockout region.The region contains 830bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Crlf3* gene. The brief process is as follows: CRISPR/Cas9 system

Notice

➢ According to the existing MGI data,Mice homozygous for a knock-out allele exhibit decreased lean body mass, decreased platelet cell number and increased circulating fructosamine level.

➢ The *Crlf3* 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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Crlf3 cytokine receptor-like factor 3 [Mus musculus (house mouse)]

Gene ID: 54394, updated on 13-Mar-2020

Summary

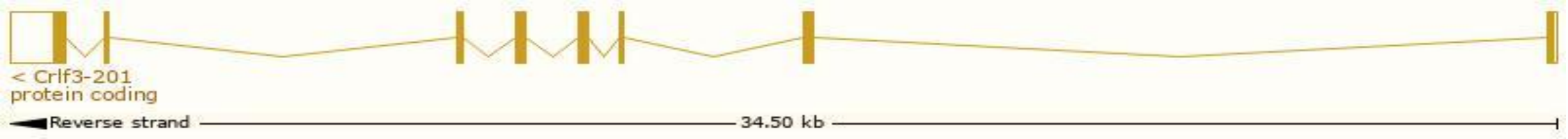
Official Symbol	Crlf3 provided by MGI
Official Full Name	cytokine receptor-like factor 3 provided by MGI
Primary source	MGI:MGI:1860086
See related	Ensembl:ENSMUSG00000017561
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BB164954, Creme9, Cytor4
Summary	This gene encodes a cytokine receptor-like factor that contains a fibronectin type 3 domain. The encoded protein may act as a negative regulator of the cell cycle. There is a pseudogene for this gene on chromosome 1. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Mar 2013]
Expression	Broad expression in liver E14 (RPKM 9.6), liver E14.5 (RPKM 8.9) and 25 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

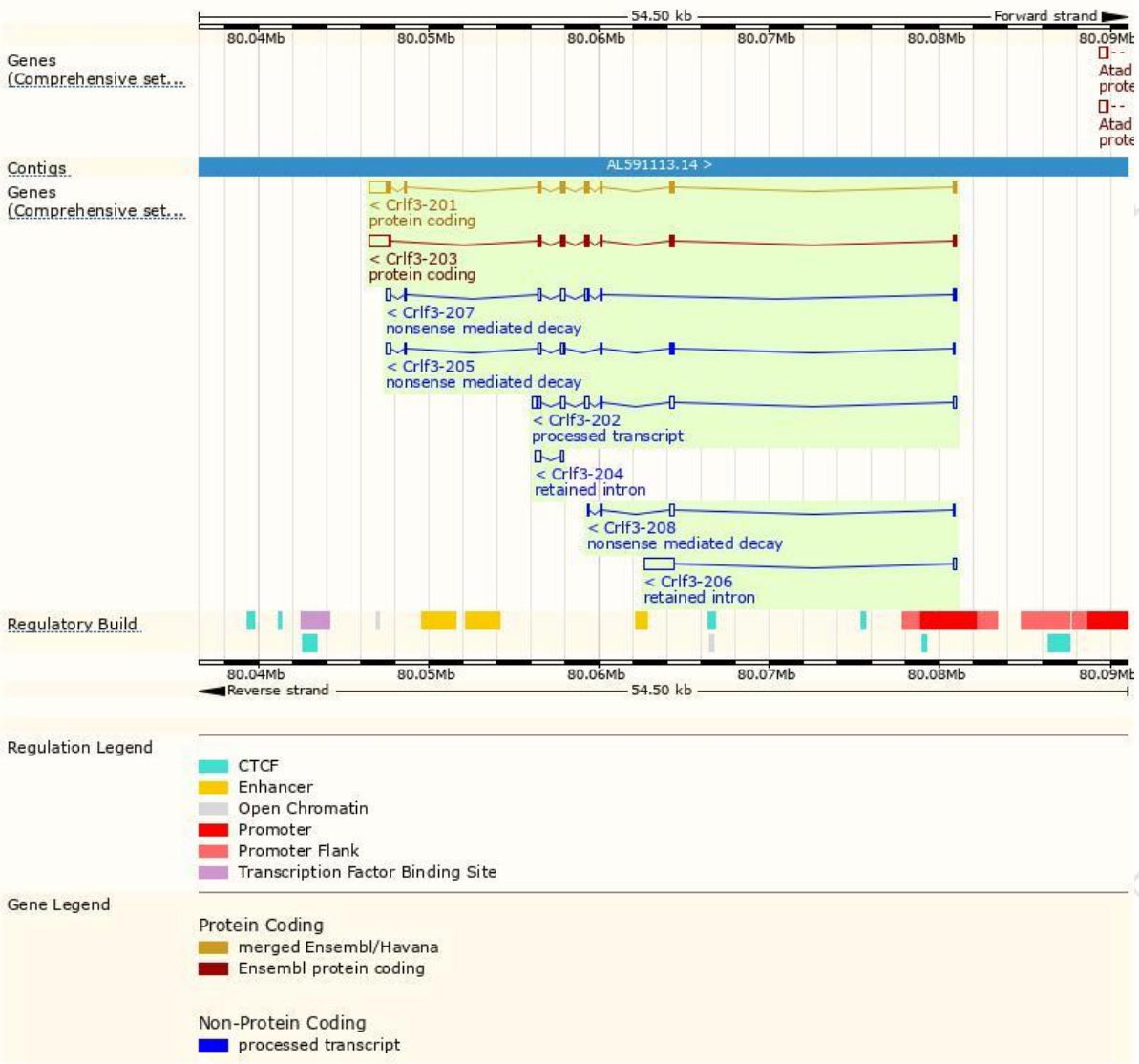
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Crlf3-201	ENSMUST00000061283.14	2396	442aa	Protein coding	CCDS25126	Q9Z2L7	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Crlf3-203	ENSMUST00000103233.9	2230	344aa	Protein coding	CCDS70260	Q9Z2L7	TSL:1 GENCODE basic
Crlf3-205	ENSMUST00000177825.7	1151	168aa	Nonsense mediated decay	-	J3QMV5	TSL:5
Crlf3-207	ENSMUST00000178893.7	1138	79aa	Nonsense mediated decay	-	J3QPM4	TSL:5
Crlf3-208	ENSMUST00000179855.1	436	25aa	Nonsense mediated decay	-	J3QP70	CDS 5' incomplete TSL:3
Crlf3-202	ENSMUST00000092858.10	1186	No protein	Processed transcript	-	-	TSL:1
Crlf3-206	ENSMUST00000178280.1	1871	No protein	Retained intron	-	-	TSL:2
Crlf3-204	ENSMUST00000147164.1	476	No protein	Retained intron	-	-	TSL:1

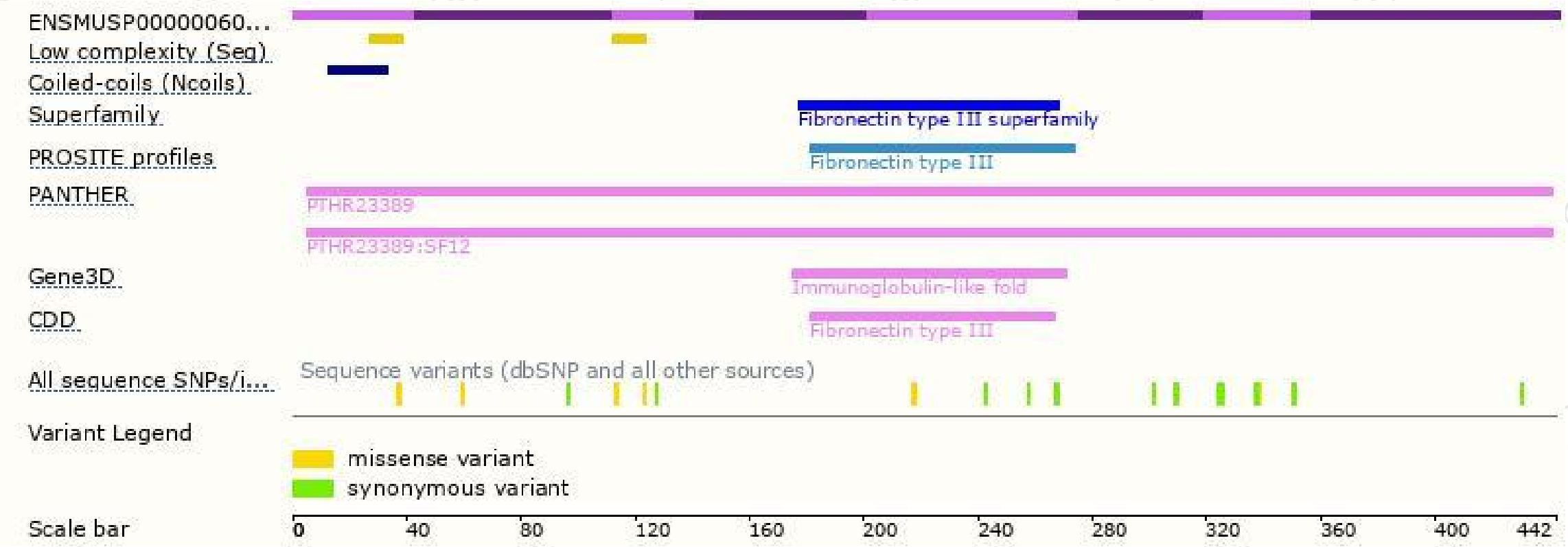
The strategy is based on the design of *Crlf3-201* transcript,The transcription is shown below



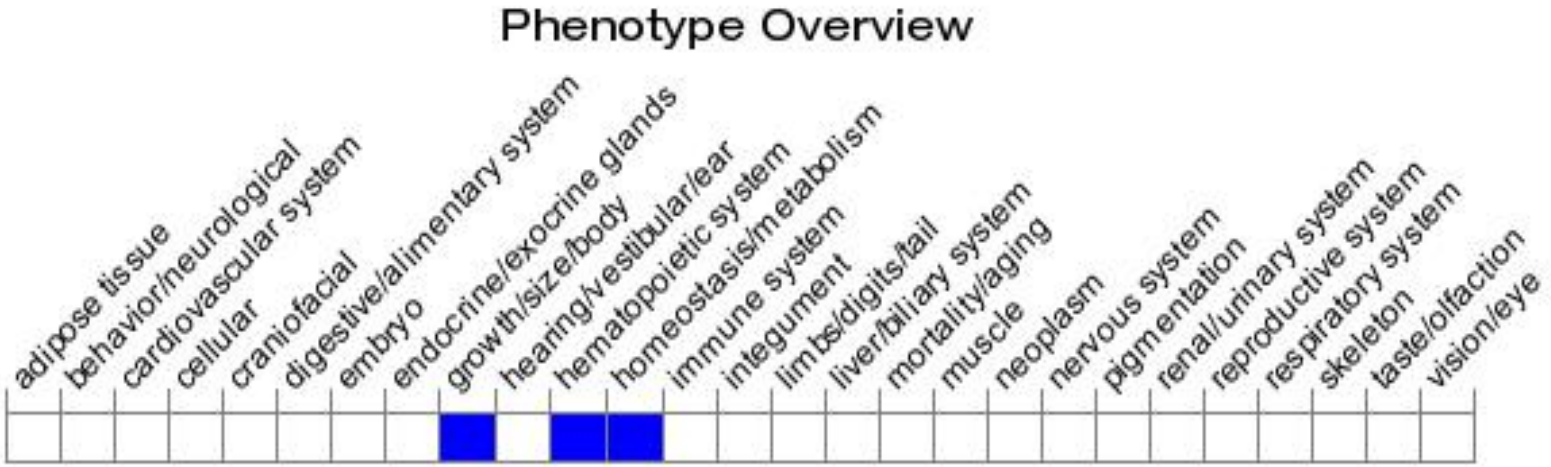
Genomic location distribution



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,Mice homozygous for a knock-out allele exhibit decreased lean body mass, decreased platelet cell number and increased circulating fructosamine level.

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

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