Nfrkb Cas9-CKO Strategy

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Project Overview

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

Conditional Knockout strategy

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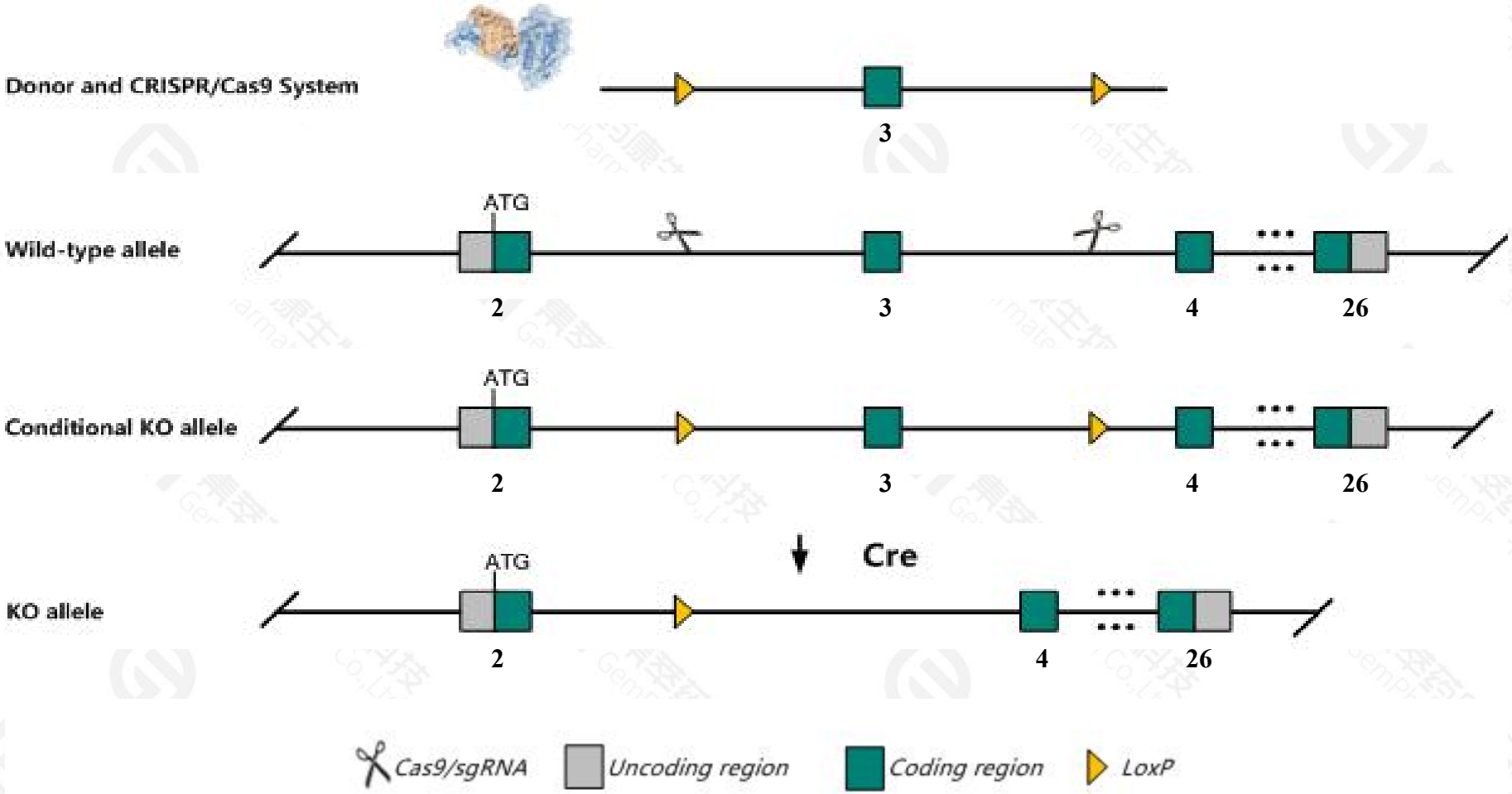
This model will use CRISPR/Cas9 technology to edit the *Nfrkb* gene. The schematic diagram is as follows:



Technical routes

➢ The *Nfrkb* gene has 10 transcripts. According to the structure of *Nfrkb* gene, exon3 of *Nfrkb-201*(ENSMUST00000086167.12) transcript is recommended as the knockout region.The region contains 202bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Nfrkb* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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 *Nfrkb* gene is located on the Chr9. 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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Nfrkb nuclear factor related to kappa B binding protein [Mus musculus (house mouse)]

Gene ID: 235134, updated on 3-Jan-2021

Summary

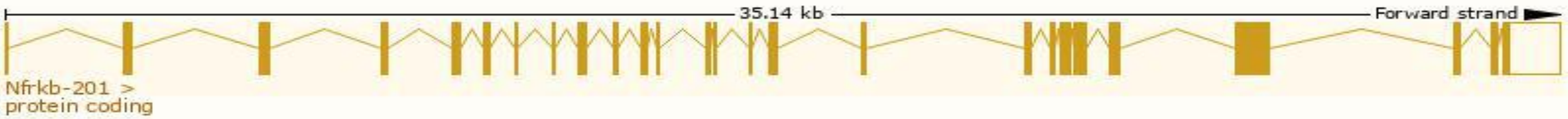
Official Symbol	Nfrkb provided by MGI
Official Full Name	nuclear factor related to kappa B binding protein provided by MGI
Primary source	MGI:MGI:2442410
See related	Ensembl:ENSMUSG00000042185
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A530090G11Rik
Expression	Ubiquitous expression in thymus adult (RPKM 21.3), spleen adult (RPKM 11.1) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

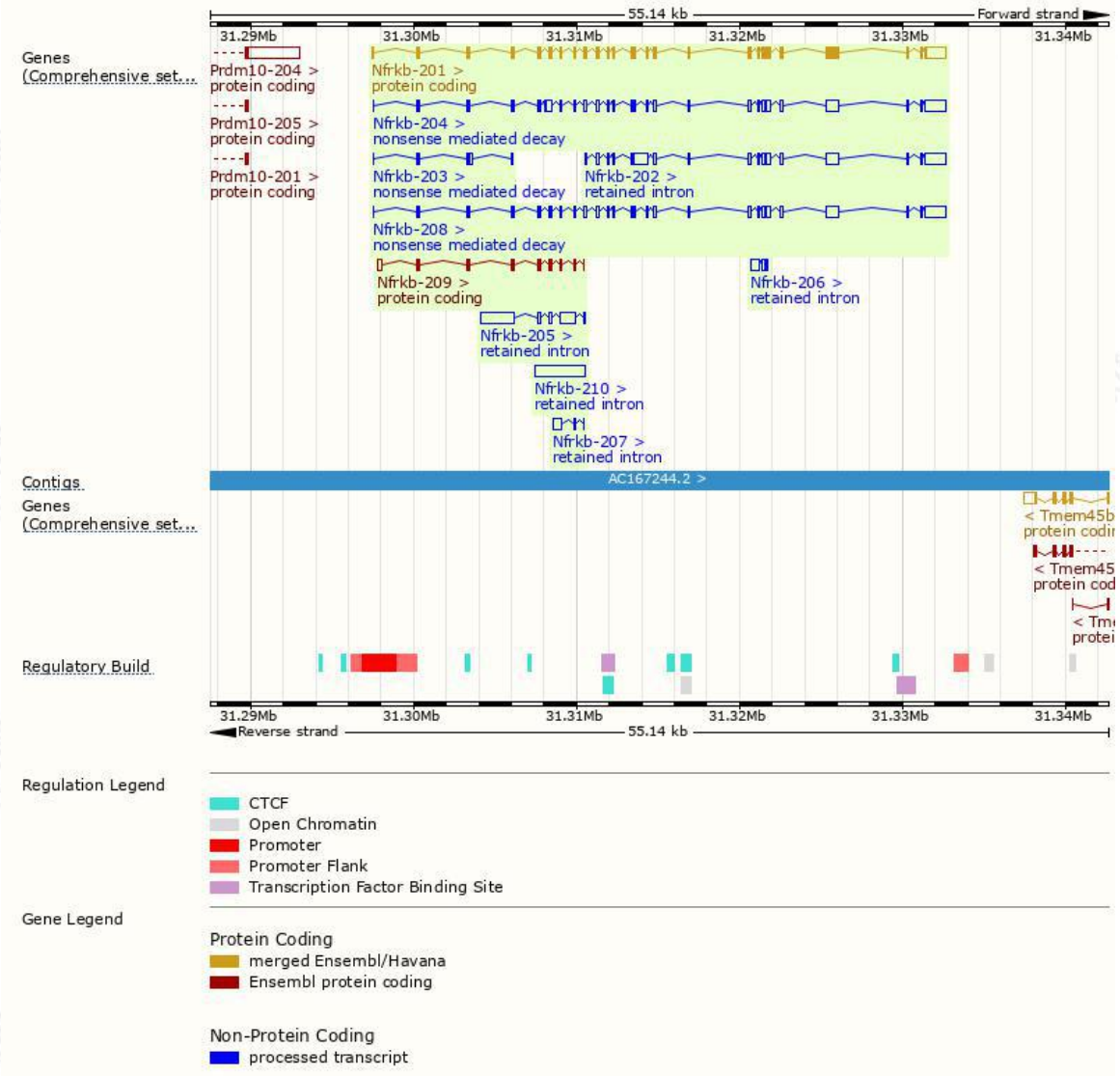
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfrkb-201	ENSMUST00000086167.12	5161	1296aa	Protein coding	CCDS22949		TSL:1 , GENCODE basic , APPRIS P1 ,
Nfrkb-209	ENSMUST00000215211.2	1338	324aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Nfrkb-204	ENSMUST00000132329.8	5398	215aa	Nonsense mediated decay	-		TSL:1 ,
Nfrkb-208	ENSMUST00000152593.8	5208	258aa	Nonsense mediated decay	-		TSL:1 ,
Nfrkb-203	ENSMUST00000131540.8	645	114aa	Nonsense mediated decay	-		TSL:3 ,
Nfrkb-202	ENSMUST00000128375.8	4808	No protein	Retained intron	-		TSL:1 ,
Nfrkb-205	ENSMUST00000134528.2	3295	No protein	Retained intron	-		TSL:1 ,
Nfrkb-210	ENSMUST00000215544.2	3048	No protein	Retained intron	-		TSL:NA ,
Nfrkb-206	ENSMUST00000143558.2	819	No protein	Retained intron	-		TSL:2 ,
Nfrkb-207	ENSMUST00000150179.2	669	No protein	Retained intron	-		TSL:5 ,

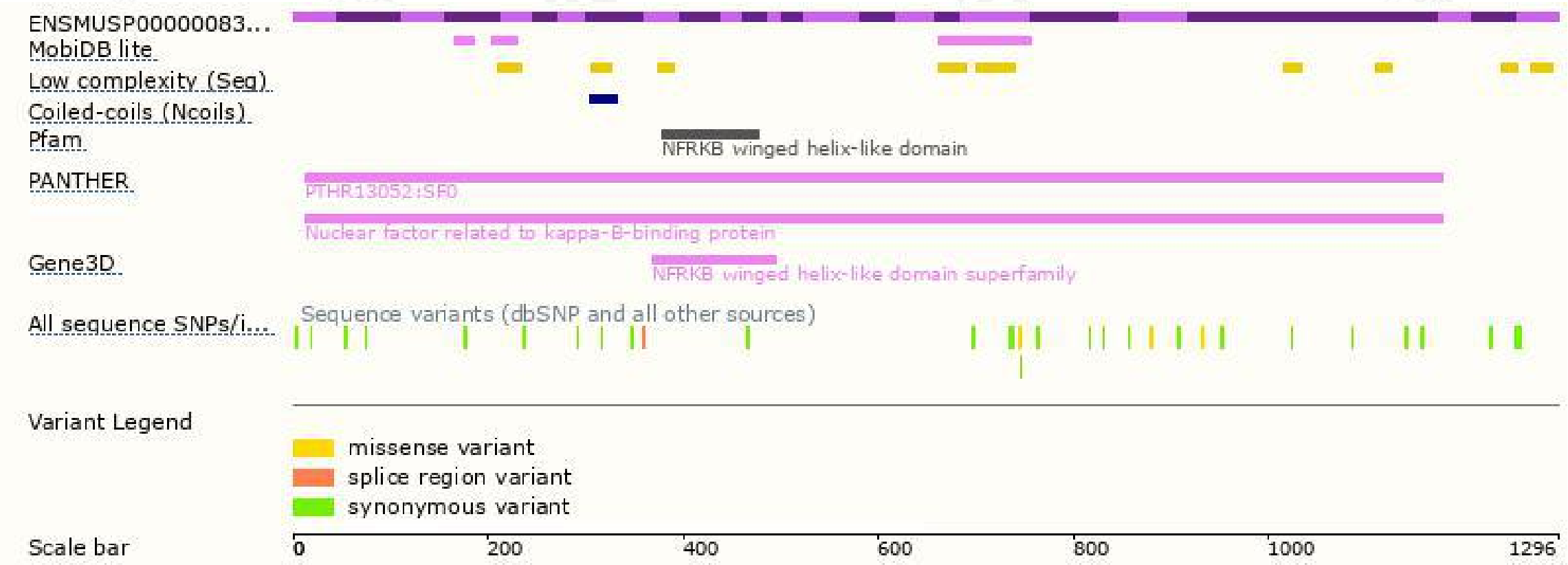
The strategy is based on the design of *Nfrkb-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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