

Nfkb2 Cas9-CKO Strategy

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Design Date: 2019-08-08

Project Overview

Project Name

Nfkb2

Project type

Cas9-CKO

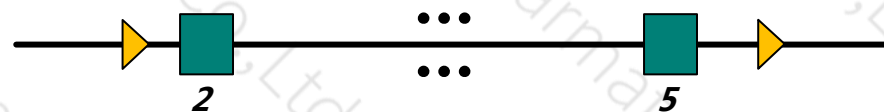
Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nfkb2* gene. The schematic diagram is as follows:

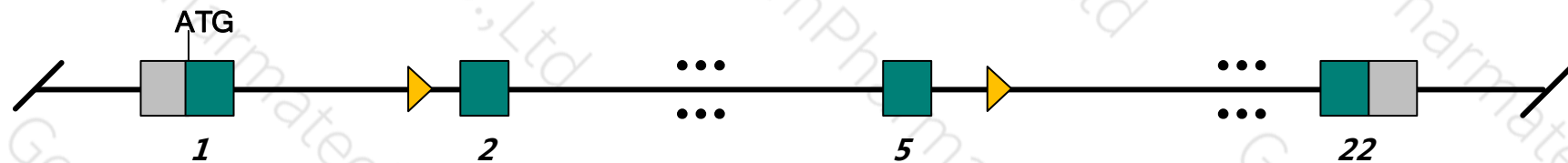
Donor and CRISPR/Cas9 System



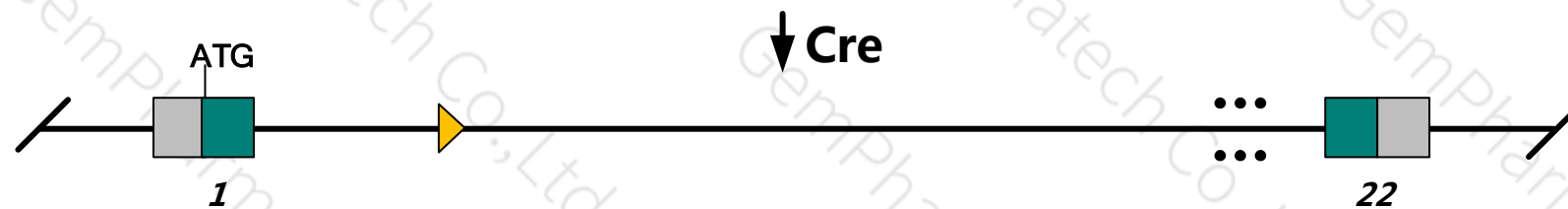
Wild-type allele



Conditional KO allele



KO allele



Cas9/sgRNA



Uncoding region



Coding region



LoxP

Technical routes

- The *Nfkb2* gene has 8 transcripts. According to the structure of *Nfkb2* gene, exon2-exon5 of *Nfkb2-202* (ENSMUST00000111881.3) transcript is recommended as the knockout region. The region contains 374bp of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nfkb2* 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.

- According to the existing MGI data, Homozygotes for targeted null mutations exhibit gastric hyperplasia, enlarged lymph nodes, enhanced cytokine production by activated T cells, absence of Peyer's patches, increased susceptibility to *Leishmania major*, and early postnatal mortality.
- Intron 1-2 (258 bp) & Intron 5-6 (270 bp) are small, and insertion of loxp at both ends may affect normal splicing of the gene.
- The *Nfkb2* gene is located on the Chr19. 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)

Nfkb2 nuclear factor of kappa light polypeptide gene enhancer in B cells 2, p49/p100 [*Mus musculus* (house mouse)]

Gene ID: 18034, updated on 30-Jul-2019

Summary

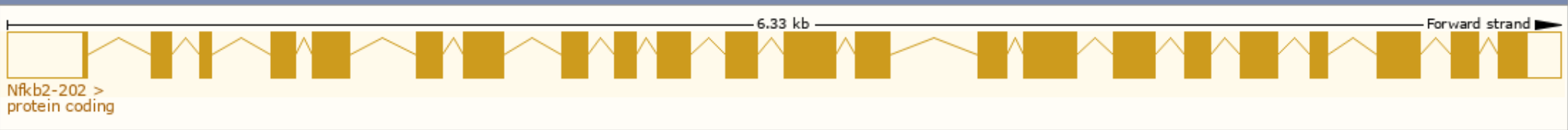
Official Symbol	Nfkb2 provided by MGI
Official Full Name	nuclear factor of kappa light polypeptide gene enhancer in B cells 2, p49/p100 provided by MGI
Primary source	MGI:MGI:1099800
See related	Ensembl:ENSMUSG00000025225
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	lyt; p49; p52; p50B; p49/p100; NF-kappaB2
Expression	Broad expression in spleen adult (RPKM 67.0), mammary gland adult (RPKM 33.7) and 15 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

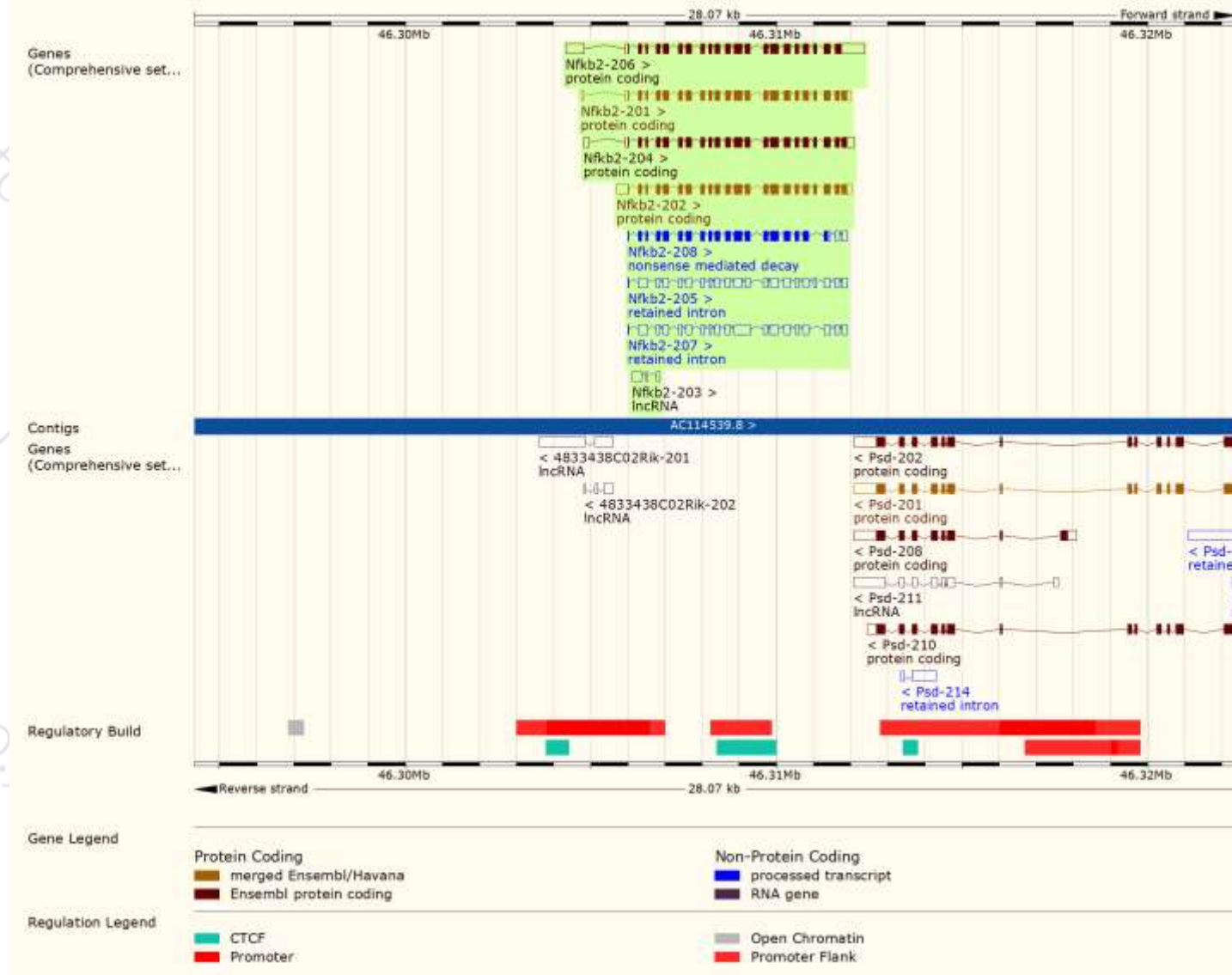
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfkb2-202	ENSMUST00000111881.3	3141	899aa	Protein coding	CCDS29874	Q3UG25 Q9WTK5	TSL:1 GENCODE basic APPRIS P2
Nfkb2-204	ENSMUST00000236591.1	3132	899aa	Protein coding	CCDS29874	Q3UG25	GENCODE basic APPRIS P2
Nfkb2-201	ENSMUST00000073116.12	2979	899aa	Protein coding	CCDS29874	Q3UG25 Q9WTK5	TSL:1 GENCODE basic APPRIS P2
Nfkb2-206	ENSMUST00000237330.1	3846	878aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Nfkb2-208	ENSMUST00000237791.1	2636	776aa	Nonsense mediated decay	-	-	-
Nfkb2-207	ENSMUST00000237761.1	2837	No protein	Retained intron	-	-	-
Nfkb2-205	ENSMUST00000236820.1	2827	No protein	Retained intron	-	-	-
Nfkb2-203	ENSMUST00000235868.1	384	No protein	lncRNA	-	-	-

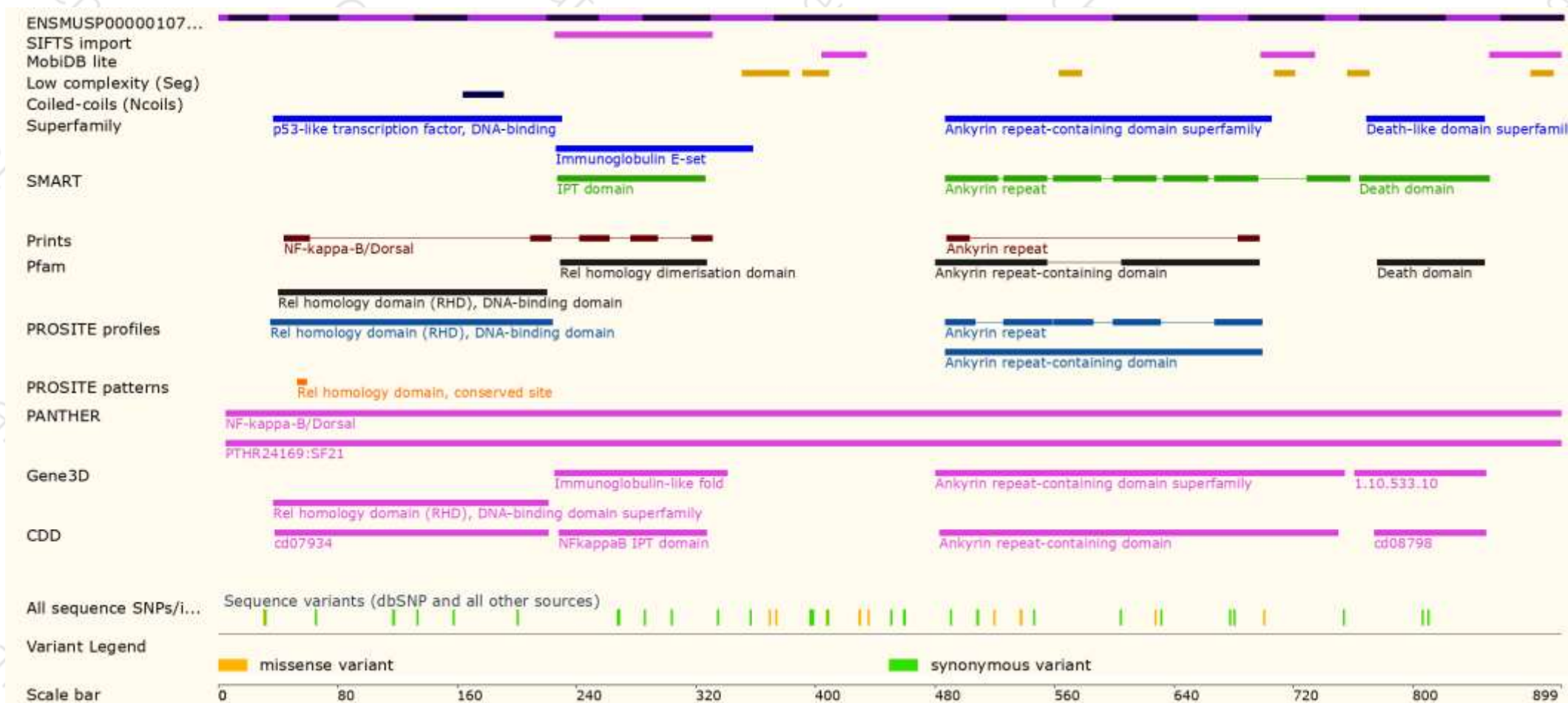
The strategy is based on the design of *Nfkb2-202* transcript,The transcription is shown below



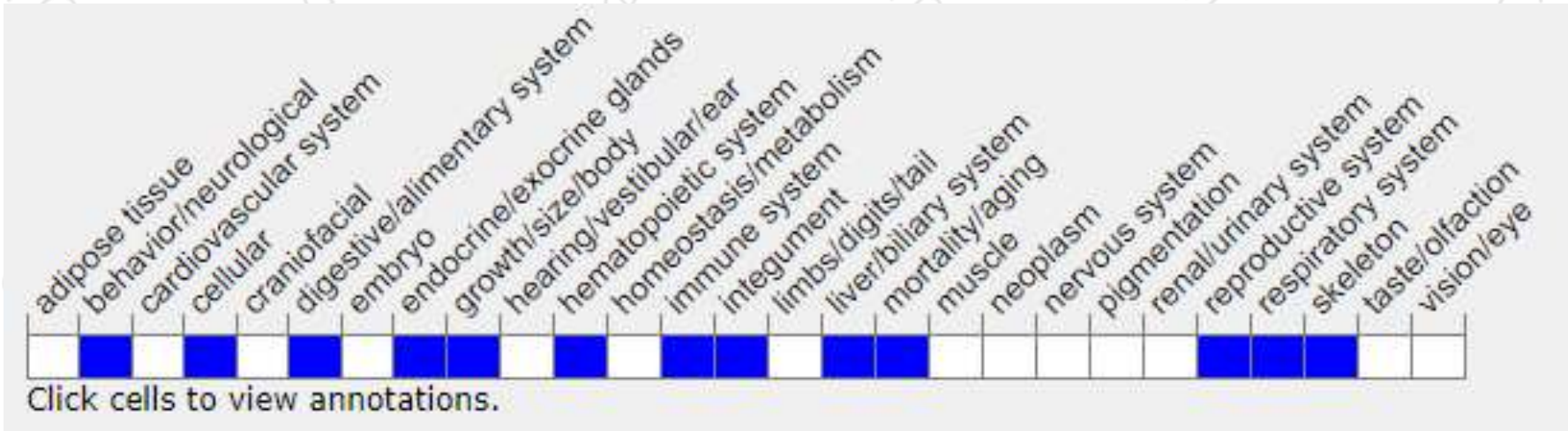
Genomic location distribution



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

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