Blnk Cas9-CKO Strategy

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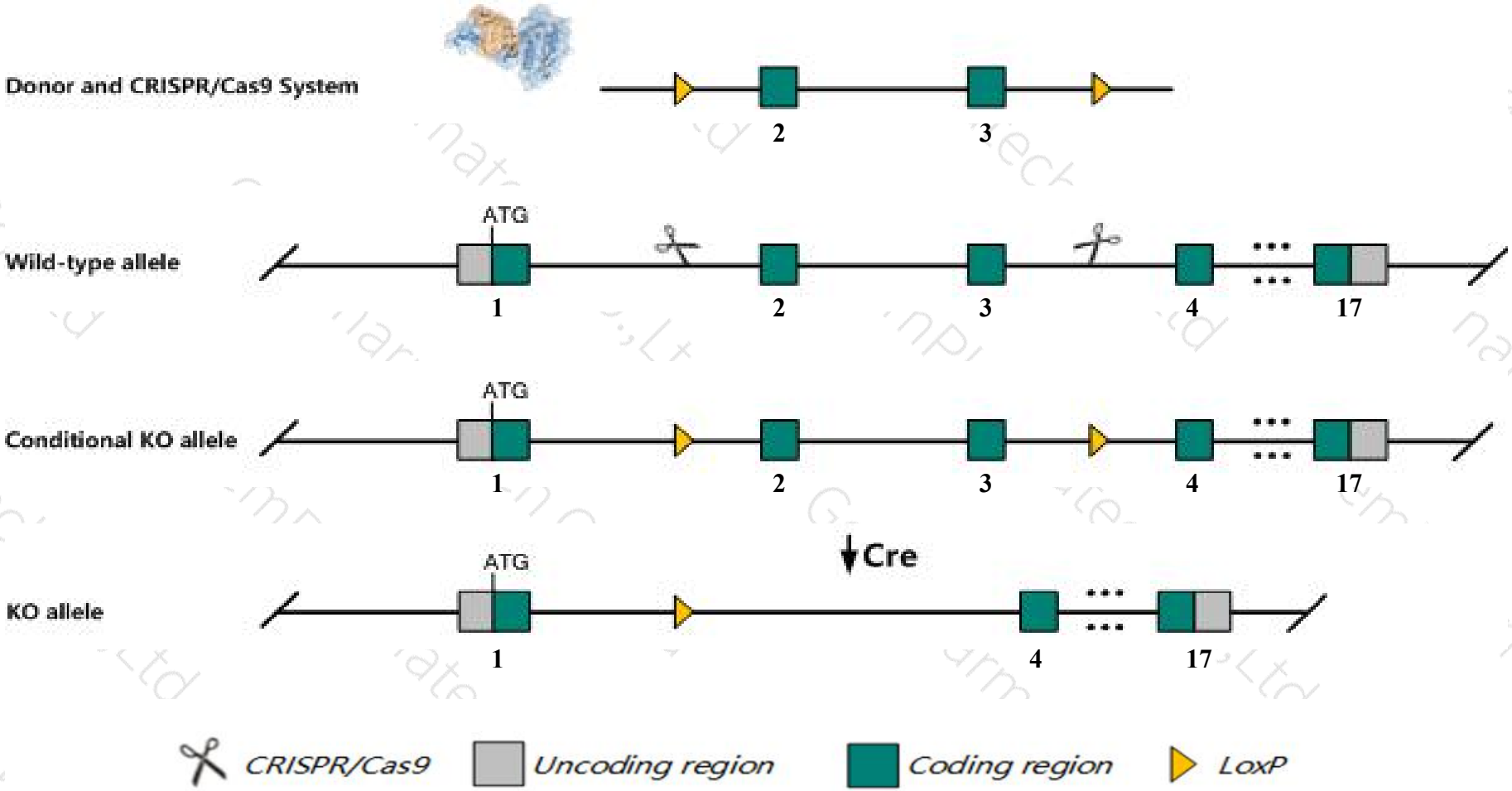
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Blnk* gene has 3 transcripts. According to the structure of *Blnk* gene, exon2-exon3 of *Blnk-201* (ENSMUST00000054769.6) transcript is recommended as the knockout region.The region contains 116bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Blnk* 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,Homozygotes for targeted null mutations exhibit a partial block in pre-B cell development, a lack of B1 B cells, reduced numbers of mature B cells, lower IgM and IgG3 serum levels, poor IgM immune responses, and a high incidence of pre-B cell lymphoma.

➢ The *Blnk* 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）

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Blnk B cell linker [*Mus musculus* (house mouse)]

Gene ID: 17060, updated on 10-Oct-2019

Summary

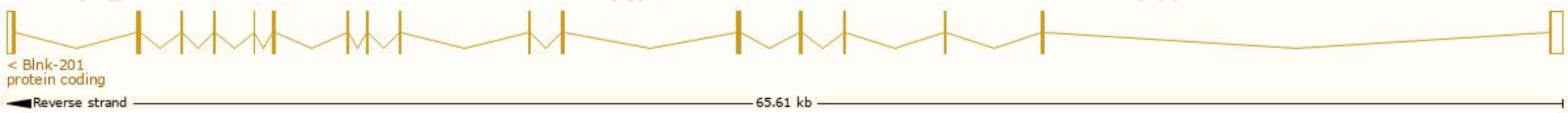
Official Symbol	Blnk provided by MGI
Official Full Name	B cell linker provided by MGI
Primary source	MGI:MGI:96878
See related	Ensembl:ENSMUSG00000061132
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bca; BASH; Ly57; Ly-57; Lyw-57; SLP-65
Expression	Biased expression in spleen adult (RPKM 24.5), colon adult (RPKM 14.5) and 14 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

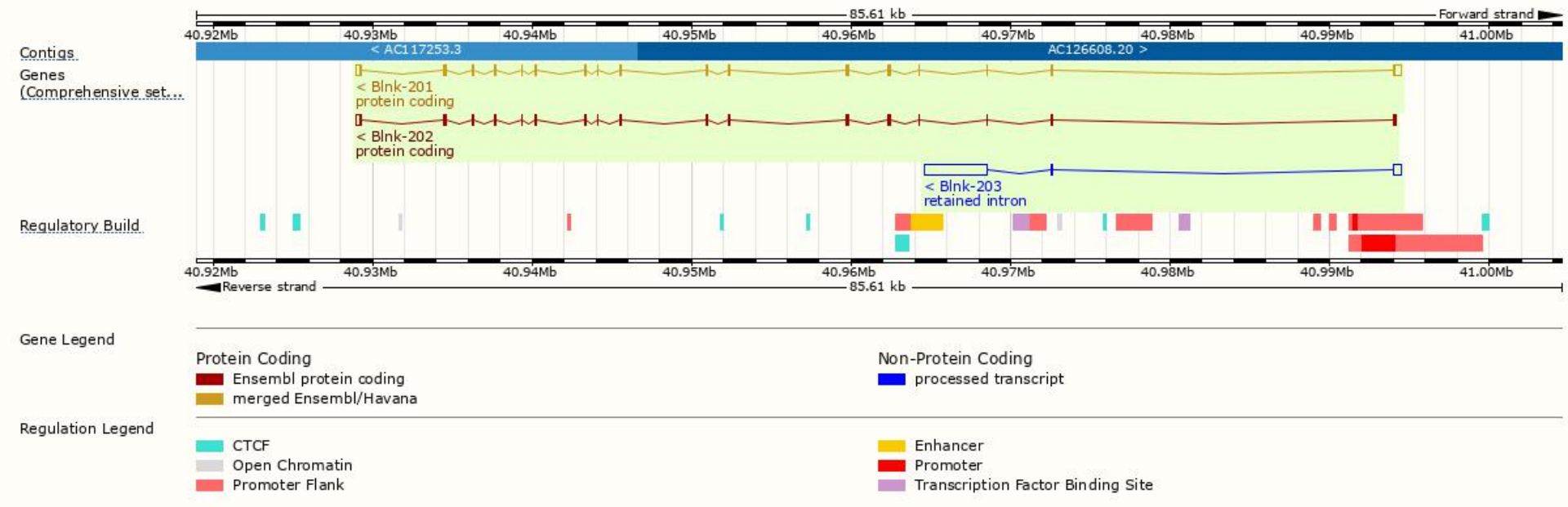
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Blnk-201	ENSMUST00000054769.6	2097	457aa	Protein coding	CCDS37983	Q9QUN3	TSL:1 GENCODE basic APPRIS P1
Blnk-202	ENSMUST00000117695.7	1701	454aa	Protein coding	-	D3YWR2	TSL:1 GENCODE basic
Blnk-203	ENSMUST00000134568.1	4534	No protein	Retained intron	-	-	TSL:1

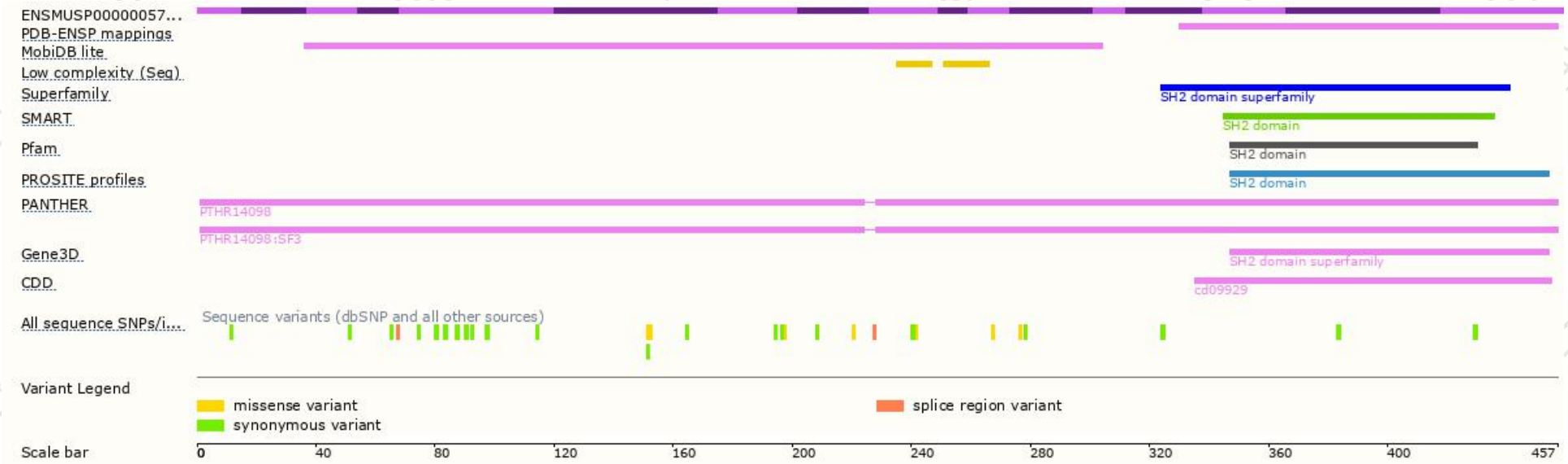
The strategy is based on the design of *Blnk-201* transcript,The transcription is shown below



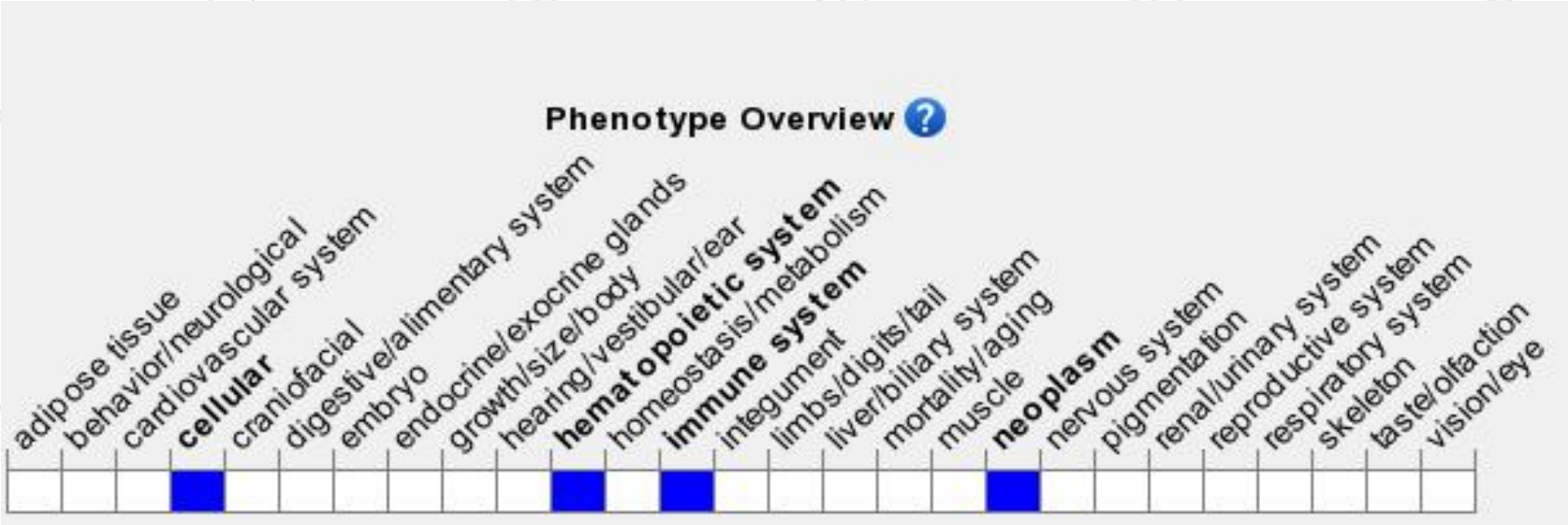
Genomic location distribution



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/).

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

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