Btk Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

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Project Overview

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

Conditional Knockout strategy

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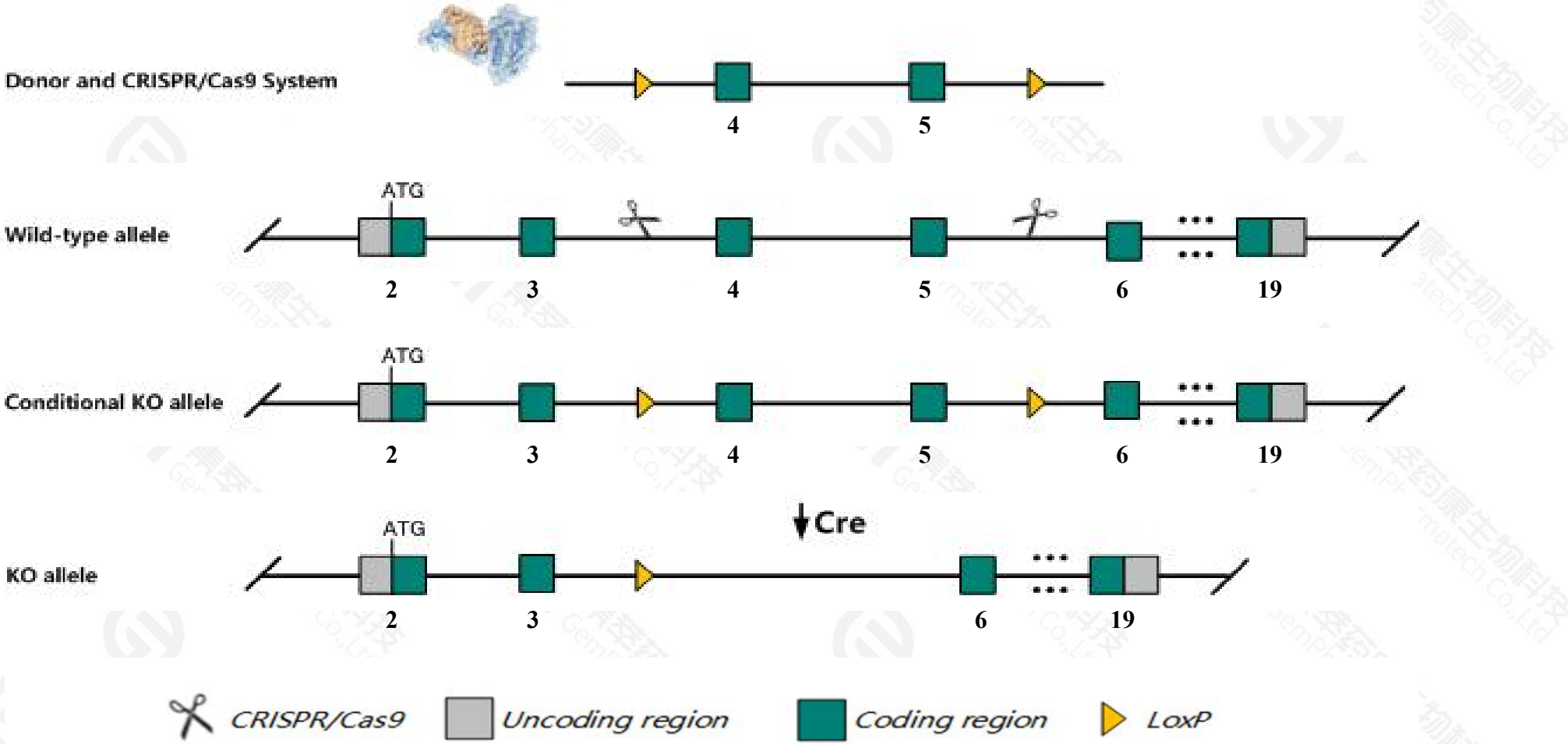
This model will use CRISPR/Cas9 technology to edit the *Btk* gene. The schematic diagram is as follows:



Technical routes

➢ The *Btk* gene has 5 transcripts. According to the structure of *Btk* gene, exon4-exon5 of *Btk-201*(ENSMUST00000033617.13) transcript is recommended as the knockout region.The region contains 151bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Btk* 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,mutants have immune defects including reduced B cell numbers, serum immunoglobulin deficiencies, and defective responses to B cell activators and thymus-independent antigens. B-1 B cells are absent in these mice.

➢ Transcript *Btk*-203 will be not affected.

➢ The *Btk* gene is located on the ChrX. 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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Btk Bruton agammaglobulinemia tyrosine kinase [Mus musculus (house mouse)]

Gene ID: 12229, updated on 13-Mar-2020

Summary

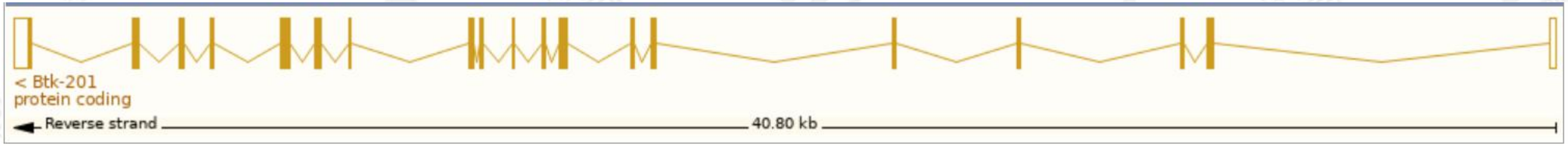
Official Symbol	Btk provided by MGI
Official Full Name	Bruton agammaglobulinemia tyrosine kinase provided byMGI
Primary source	MGI:MGI:88216
See related	Ensembl:ENSMUSG00000031264
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI528679, xid
Expression	Biased expression in liver E14 (RPKM 7.3), liver E14.5 (RPKM 5.9) and 11 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

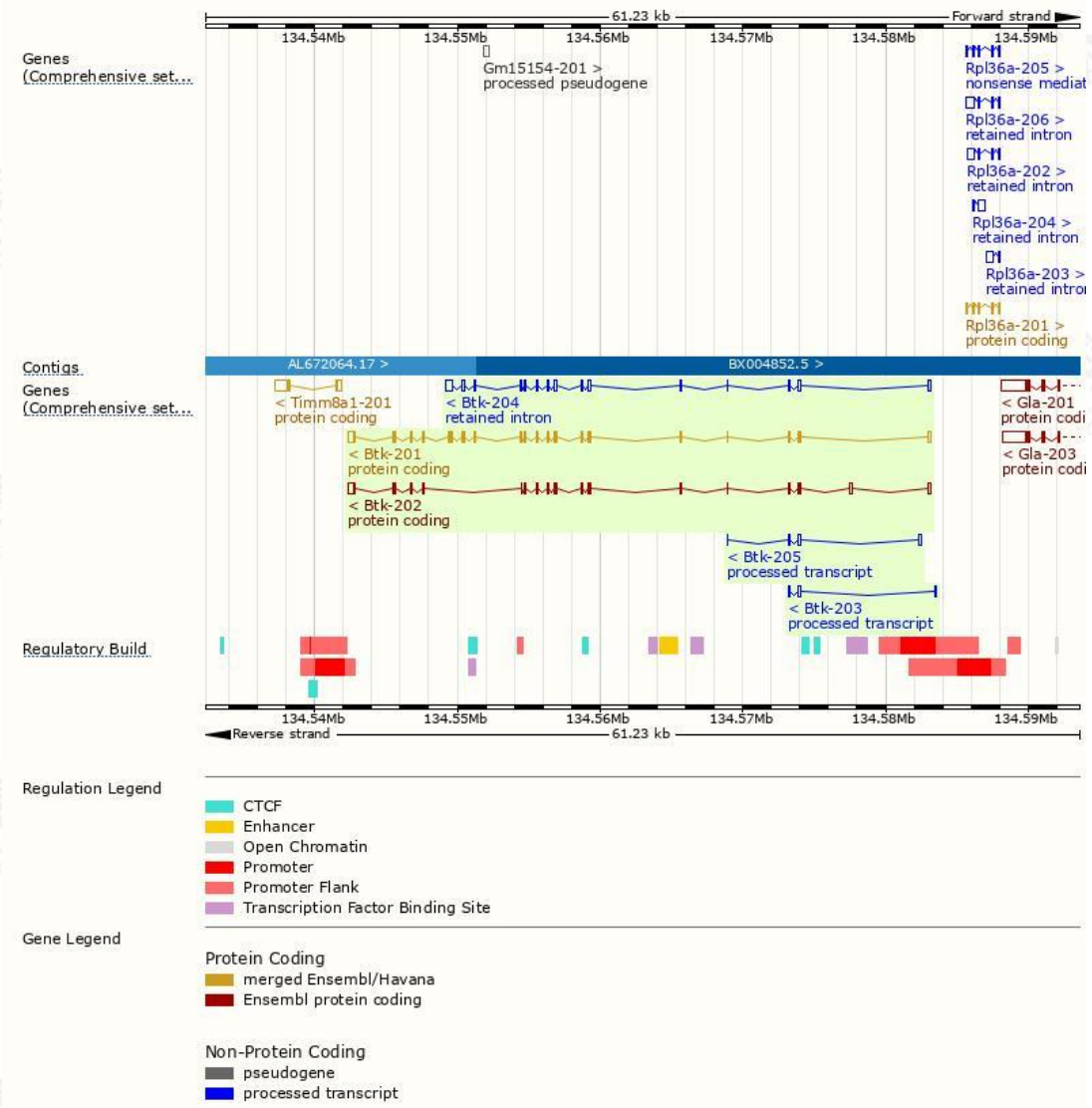
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Btk-201	ENSMUST00000033617.12	2540	659aa	Protein coding	CCDS30396	P35991	TSL:1 GENCODE basic APPRIS P1
Btk-202	ENSMUST00000113213.1	2121	483aa	Protein coding	-	A2BDW0	TSL:5 GENCODE basic
Btk-205	ENSMUST00000150245.7	481	No protein	Processed transcript	-	-	TSL:2
Btk-203	ENSMUST00000128333.1	388	No protein	Processed transcript	-	-	TSL:3
Btk-204	ENSMUST00000132664.7	1920	No protein	Retained intron	-	-	TSL:5

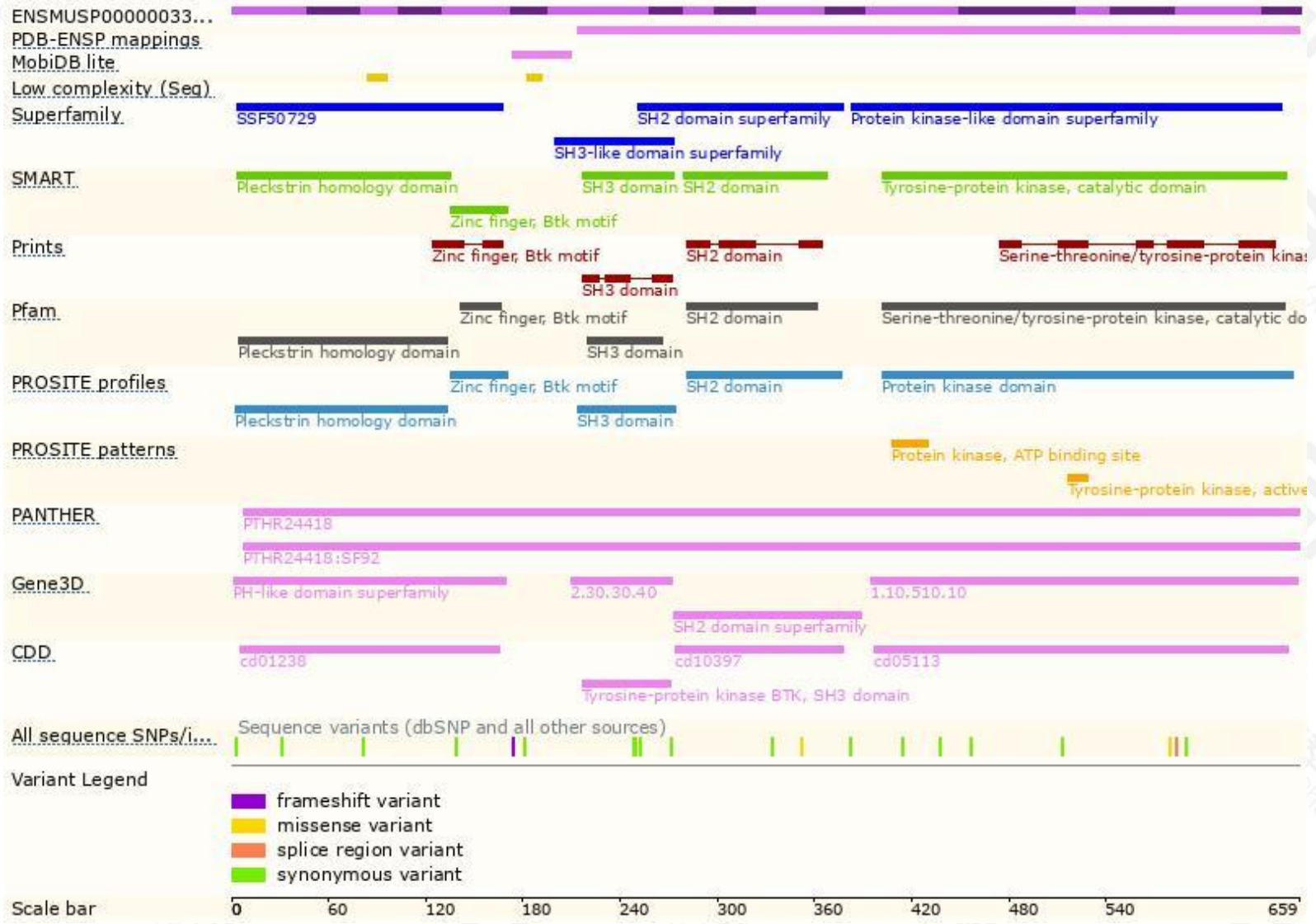
The strategy is based on the design of *Btk-201* transcript,the transcription is shown below:



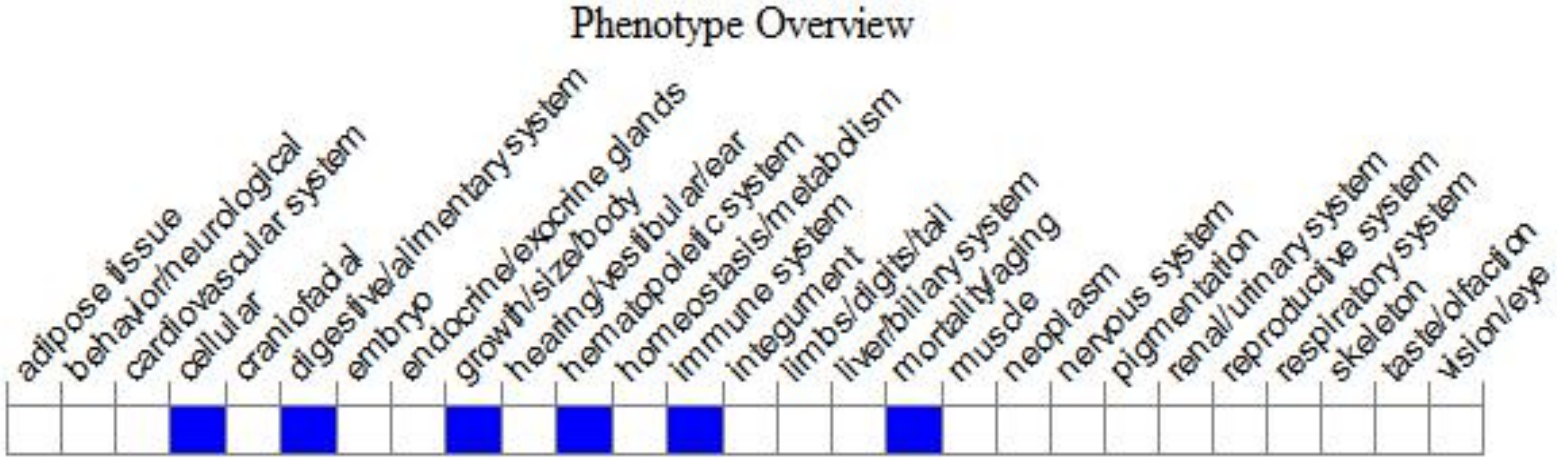
Genomic location distribution



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,mutants have immune defects including reduced B cell numbers, serum immunoglobulin deficiencies, and defective responses to B cell activators and thymus-independent antigens. B-1 B cells are absent in these mice.

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If you have any questions, you are welcome to inquire.
Tel: 400-9660890

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