

Tlr8 Cas9-CKO Strategy

Designer:

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Project Overview

Project Name

Tlr8

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

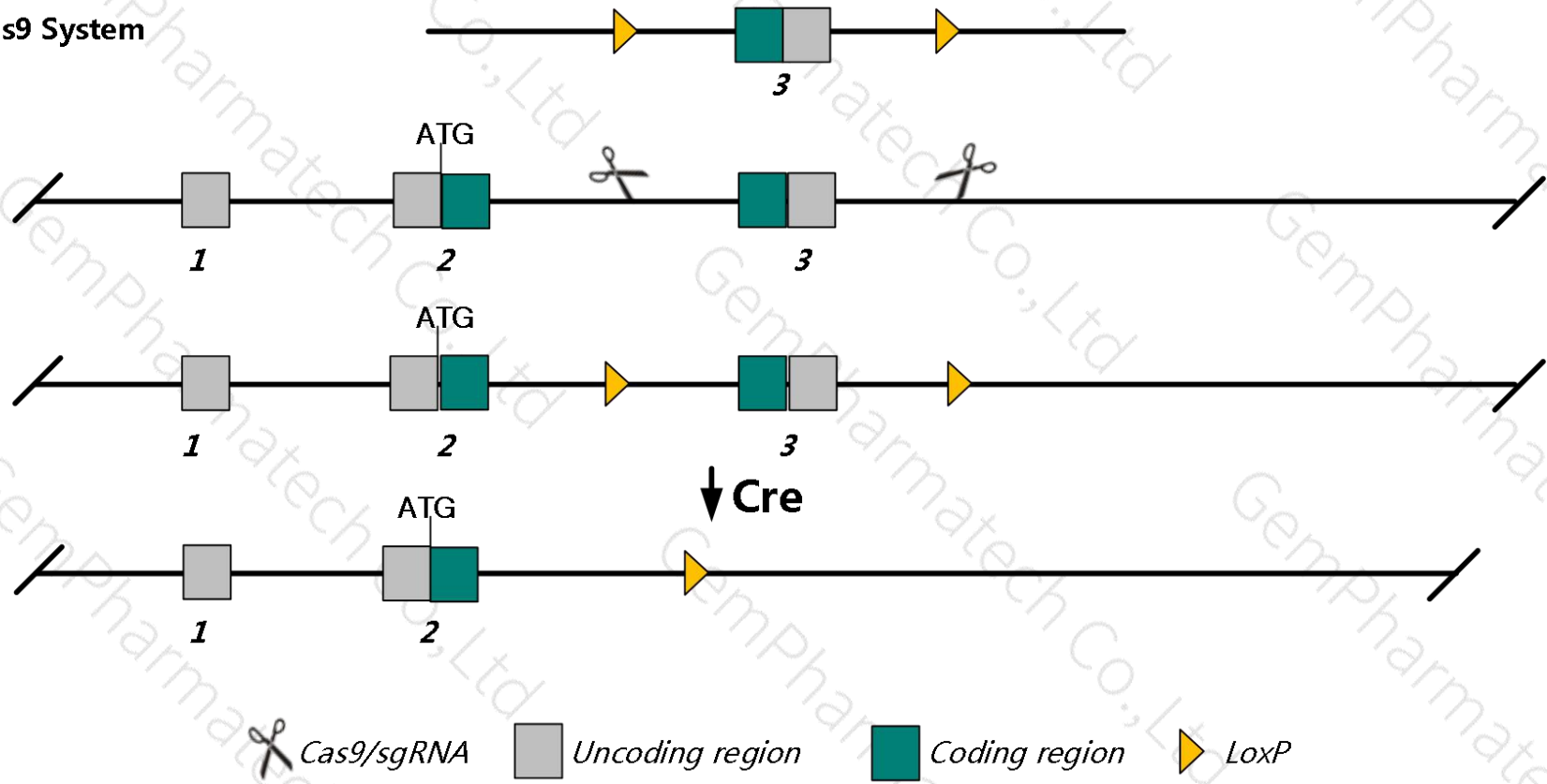
This model will use CRISPR/Cas9 technology to edit the *Tlr8* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Tlr8* gene has 5 transcripts. According to the structure of *Tlr8* gene, exon 3 of *Tlr8*-202 (ENSMUST00000112170.1) transcript is recommended as the knockout region. The region contains the most coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tlr8* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 or cell types.

- According to the existing MGI data : Mice homozygous for a knock-out allele exhibit increased anti-nuclear antigen antibodies, altered immunoglobulin levels, decreased marginal zone, B-1a, and B-1b cells, splenomegaly, and glomerulonephritis.
- The *Tlr8* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Tlr8 toll-like receptor 8 [*Mus musculus* (house mouse)]

Gene ID: 170744, updated on 9-Sep-2018

Summary

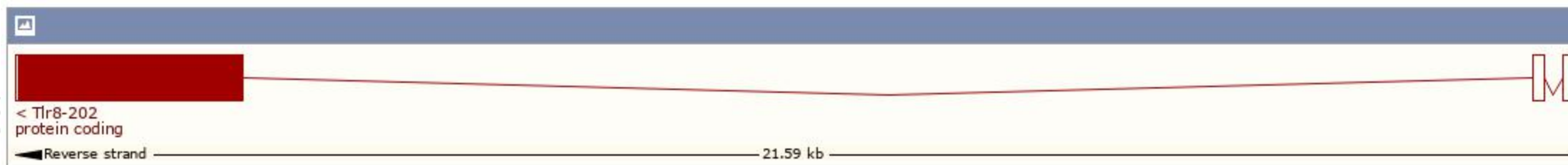
Official Symbol	Tlr8 provided by MGI
Official Full Name	toll-like receptor 8 provided by MGI
Primary source	MGI:MGI:2176887
See related	Ensembl:ENSMUSG00000040522 Vega:OTTMUSG00000019575
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information (Ensembl)

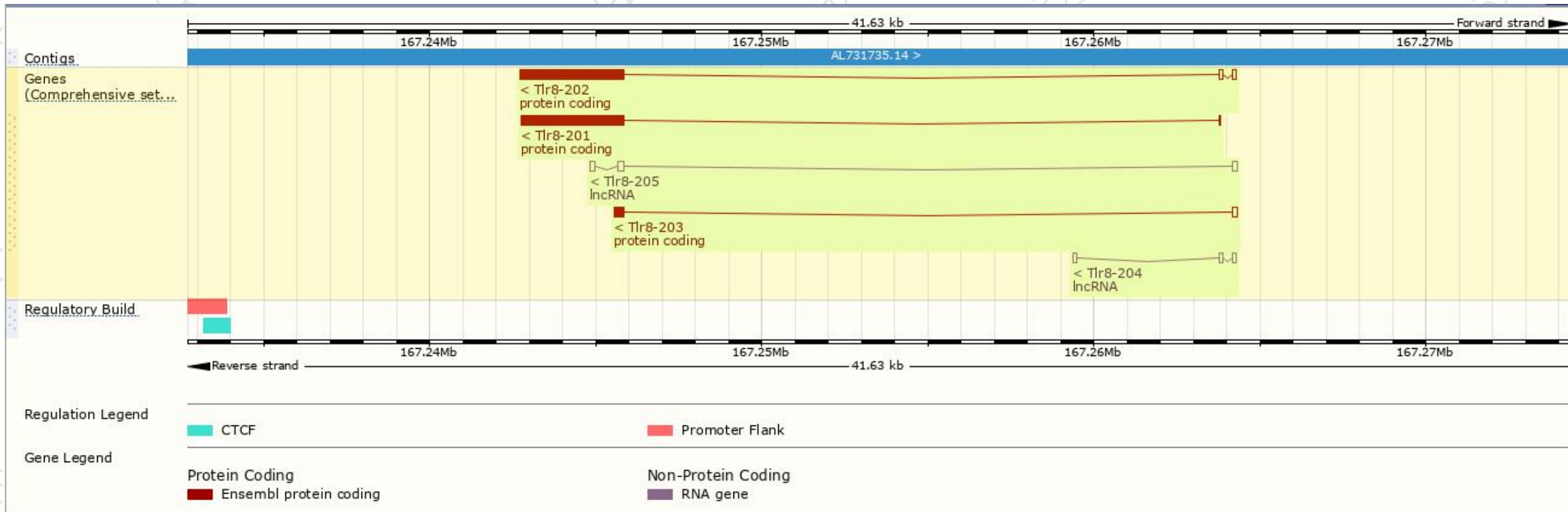
The gene has 5 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Tlr8-202	ENSMUST00000112170.1	3431	1032aa	Protein coding	CCDS41209	P58682	NM_133212 NP_573475	TSL:2 GENCODE basic APPRIS P1
Tlr8-201	ENSMUST00000049023.2	3158	1032aa	Protein coding	CCDS41209	P58682	-	TSL:1 GENCODE basic APPRIS P1
Tlr8-203	ENSMUST00000133722.1	485	100aa	Protein coding	-	A2AHI8	NM_001313760 NP_001300689	CDS 3' incomplete TSL:2
Tlr8-205	ENSMUST00000148370.1	491	No protein	Processed transcript	-	-	-	TSL:2
Tlr8-204	ENSMUST00000138311.1	387	No protein	Processed transcript	-	-	-	TSL:2

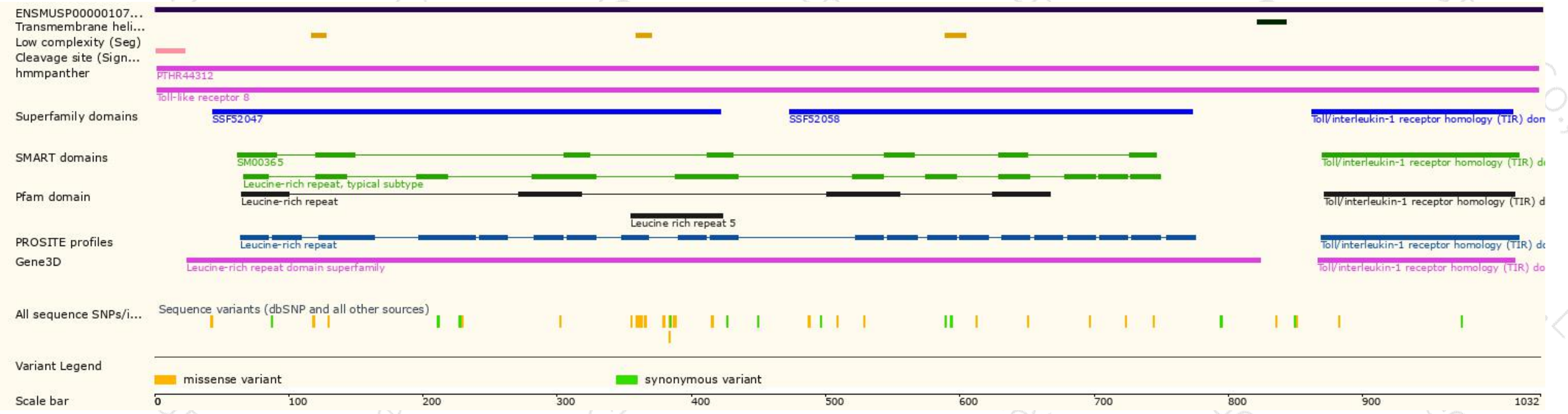
The strategy is based on the design of *Tlr8-202* transcript, The transcription is shown below



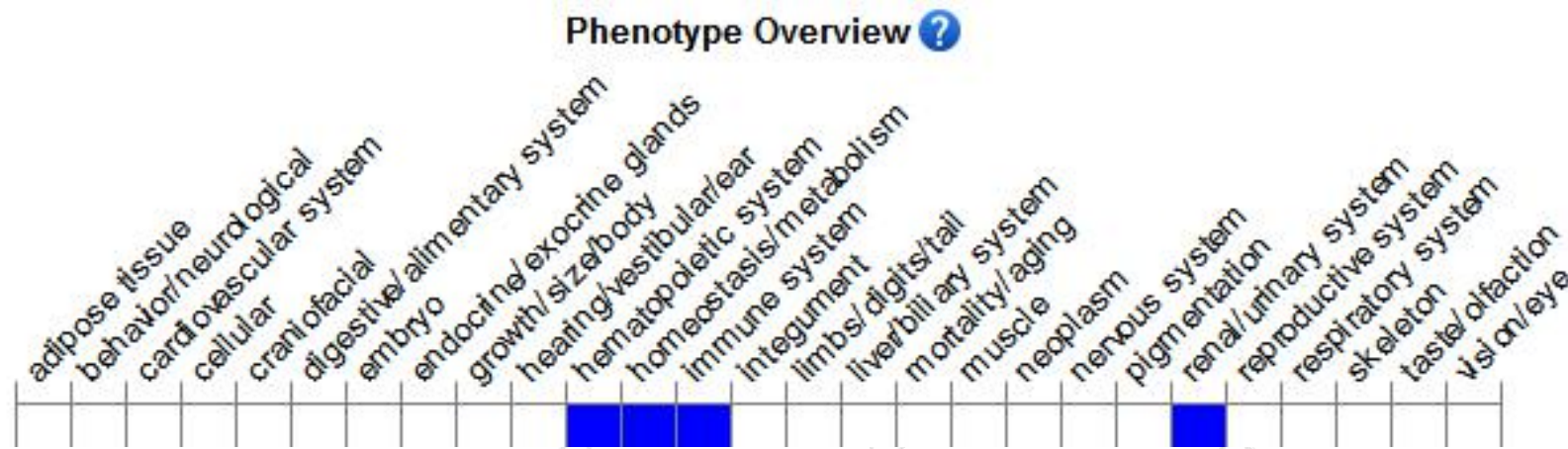
Genomic location distribution



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/>).

Mice homozygous for a knock-out allele exhibit increased anti-nuclear antigen antibodies, altered immunoglobulin levels, decreased marginal zone, B-1a, and B-1b cells, splenomegaly, and glomerulonephritis