Il5 Cas9-CKO *Strategy*

Designer: **Jinling Wang**

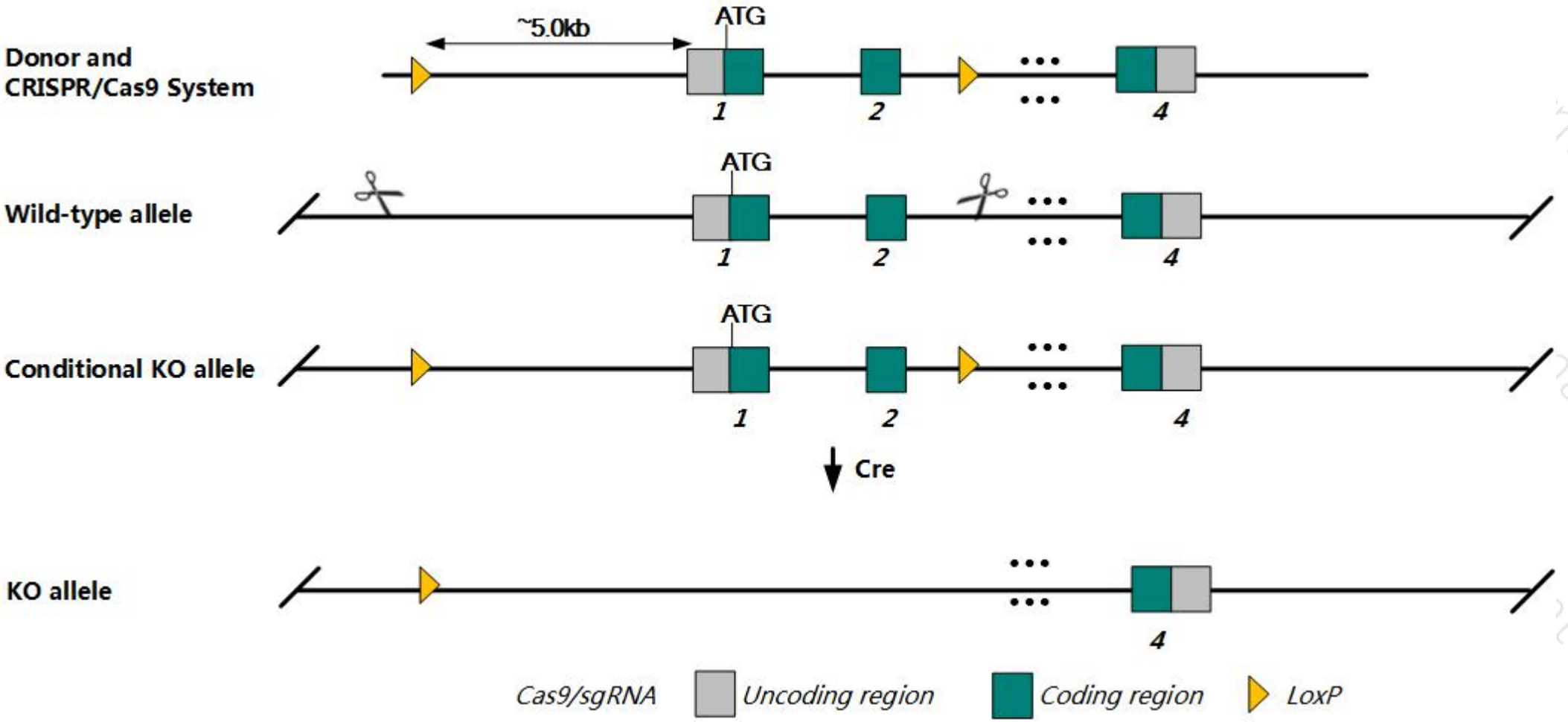
Design Date: **2019-8-8**

Project Overview

Project Name	***Il5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Il5* gene. The schematic diagram is as follows:



Technical routes

- The *Il5* gene has 1 transcripts. According to the structure of *Il5* gene, the predicted promoter region and exon1-2 of *Il5*-201 (ENSMUST00000048605.2) transcript is recommended as the knockout region.The region contains the predicted promoter sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Il5* 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.

Notice

- According to the existing MGI data: Homozygotes for a targeted null mutation exhibit loss of normal airway hyperreactivity resulting from aeroallergen challenge, reduced numbers of CD5+ B cells in the peritoneal cavity at 2 weeks, and some altered responses to schistosomiasis infection.
- The *Il5* gene is located on the Chr11. 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）

集萃药康 GemPharmatech

Il5 interleukin 5 [*Mus musculus* (house mouse)]

Gene ID: 16191, updated on 8-Dec-2018

Summary

Official Symbol	Il5 provided by MGI
Official Full Name	interleukin 5 provided by MGI
Primary source	MGI:MGI:96557
See related	Ensembl:ENSMUSG00000036117
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	Il-5
Expression	Low expression observed in reference dataset See more
Orthologs	human all

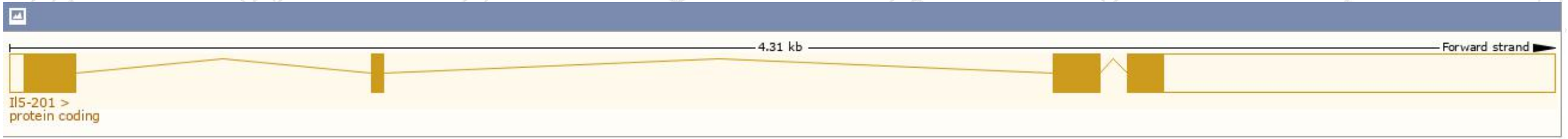
Transcript information （Ensembl

The gene has 1 transcripts, and all transcripts are shown below ：

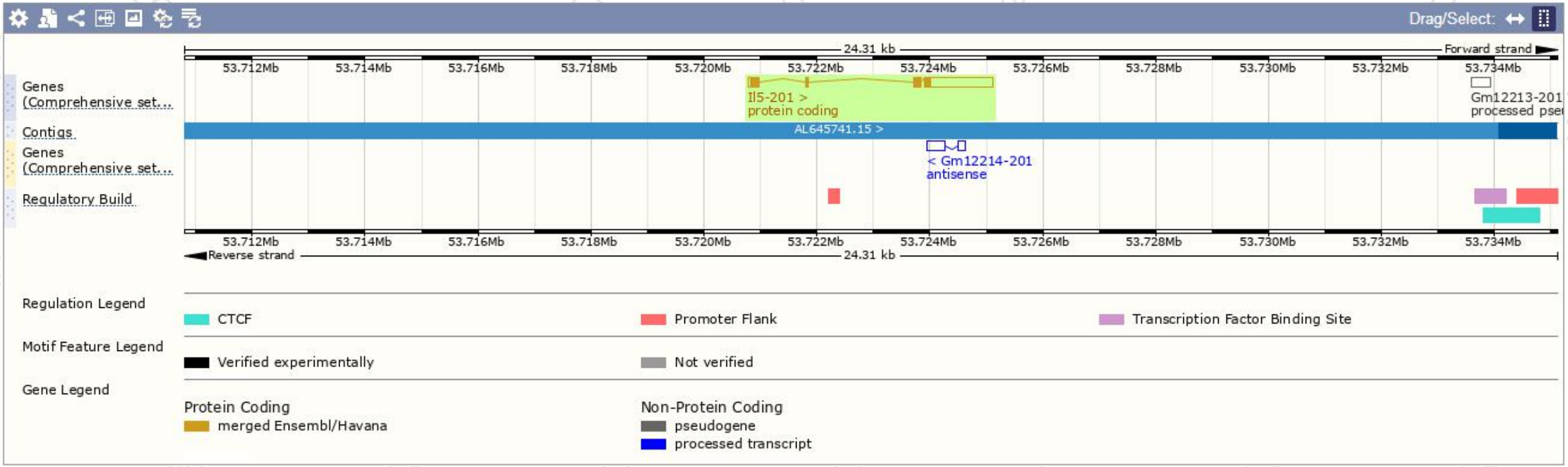
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Il5-201	ENSMUST00000048605.2	1537	133aa	Protein coding	CCDS24685	P04401 Q5SV01	NM_010558 NP_034688	TSL:1 GENCODE basic APPRIS P1

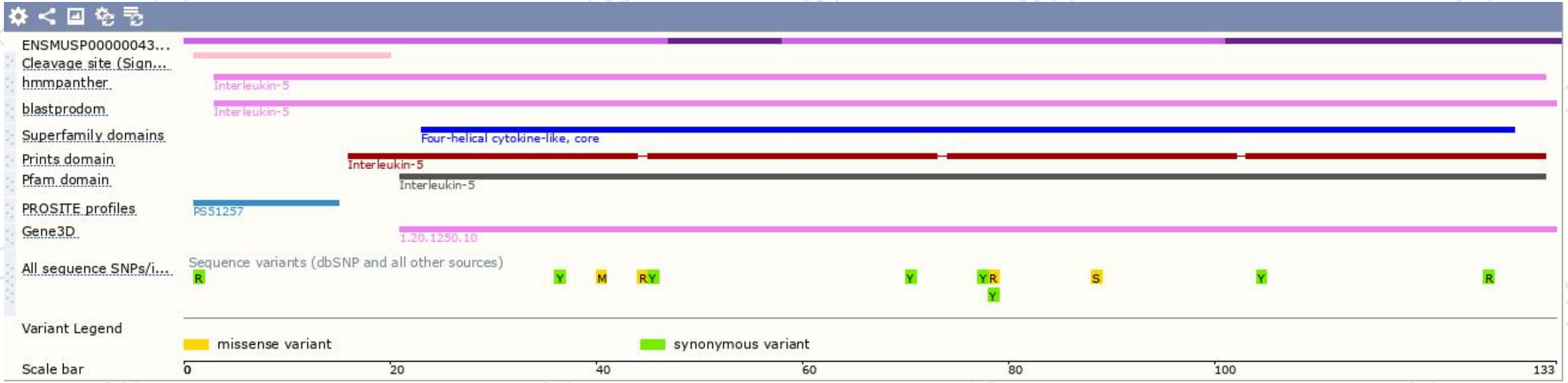
The strategy is based on the design of *Il5-201* transcript,The transcription is shown below



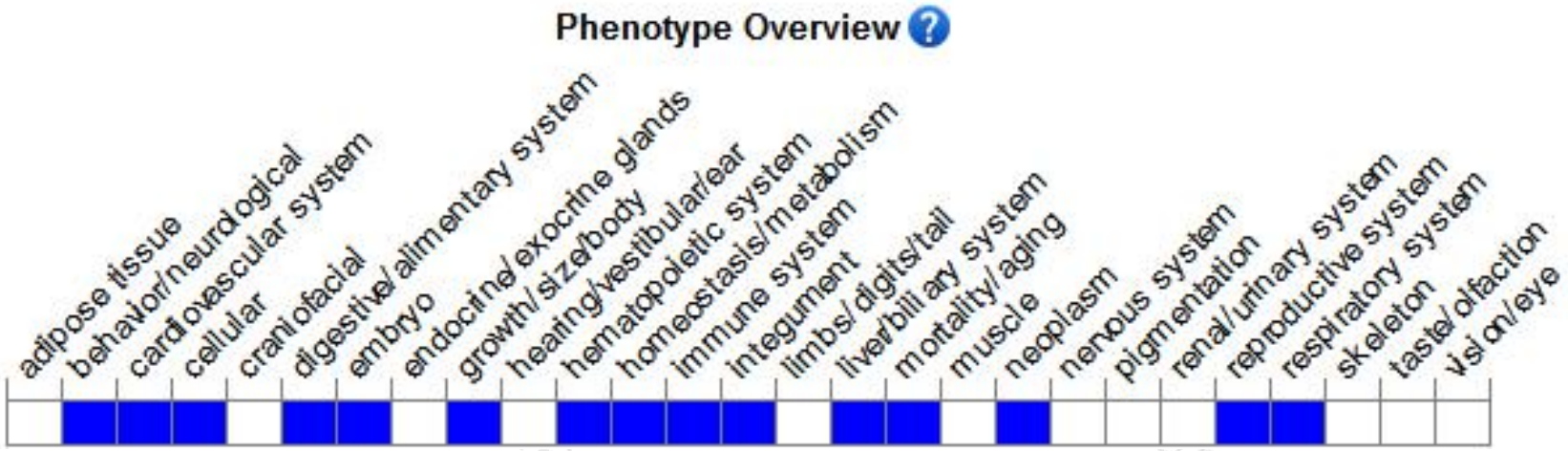
Genomic location distribution



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/) .

Homozygotes for a targeted null mutation exhibit loss of normal airway hyperreactivity resulting from aeroallergen challenge, reduced numbers of CD5+ B cells in the peritoneal cavity at 2 weeks, and some altered responses to schistosomiasis infection.

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

集萃药康生物科技
GemPharmatech Co.,Ltd