Ctla4 Cas9-KO Strategy

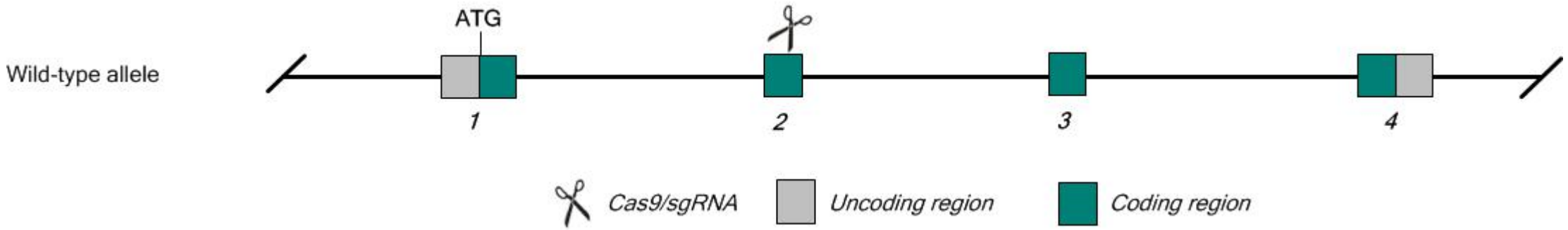
Designer: Qiong Zhou

Project Overview

Project Name	***Ctla4***
Project type	**Cas9-KO**
Strain background	**BALB/cJGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ctla4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ctla4* gene has 3 transcripts. According to the structure of *Ctla4* gene, partial exon2 of *Ctla4-201* (ENSMUST00000027164.8) transcript is recommended as the knockout region.The region contains key coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ctla4* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of BALB/cJGpt 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 BALB/cJGpt mice.

Notice

➢ According to the existing MGI data,Mice homozygous for a knock-out allele exhibit lethality at 3 to 4 weeks of age, decreased T cell numbers, abnormal T cell physiology, inflammation in mutliple organs, abnormal thymus morphology, and lymph node hypoplasia.

➢ The *Ctla4* gene is located on the Chr1. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Ctla4 cytotoxic T-lymphocyte-associated protein 4 [Mus musculus (house mouse)]

Gene ID: 12477, updated on 9-Apr-2019

Summary

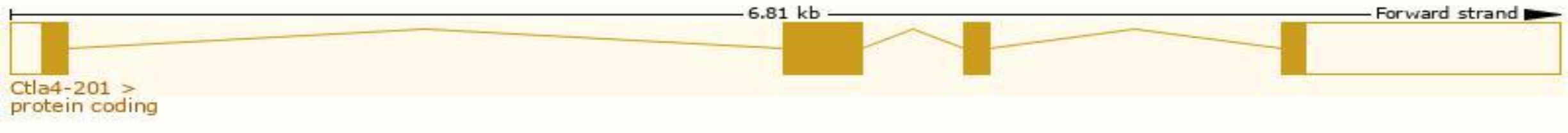
Official Symbol	Ctla4 provided by MGI
Official Full Name	cytotoxic T-lymphocyte-associated protein 4 provided byMGI
Primary source	MGI:MGI:88556
See related	Ensembl:ENSMUSG00000026011
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cd152, Ctla-4, Ly-56
Summary	This gene is a member of the immunoglobulin superfamily, and encodes a protein that functions as a negative regulator of T-cell responses. Alternatively spliced transcript variants encoding different isoforms have been described for this gene. [provided by RefSeq, Aug 2013]
Expression	Biased expression in thymus adult (RPKM 1.4), spleen adult (RPKM 0.8) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

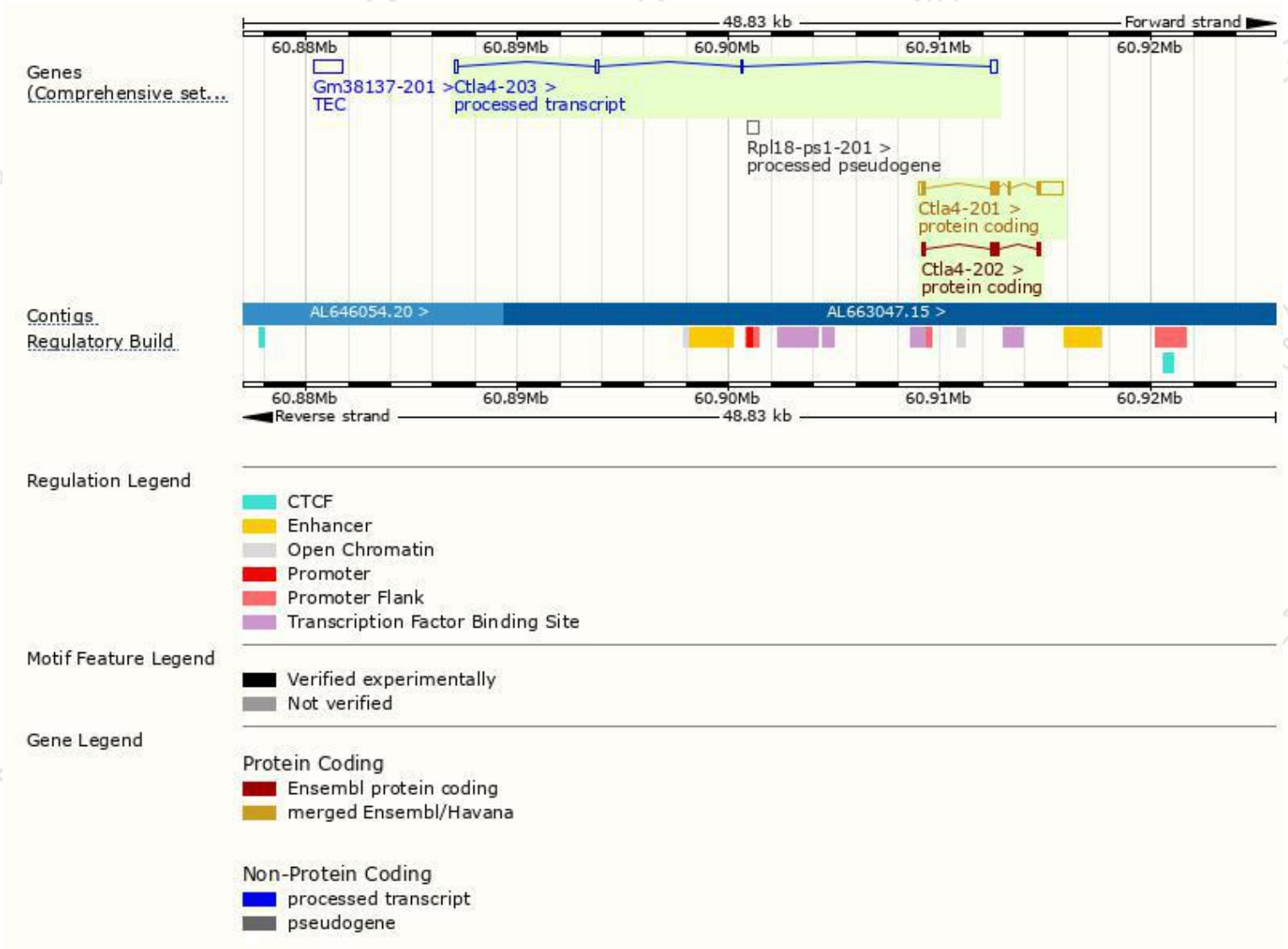
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctla4-201	ENSMUST00000027164.8	1933	223aa	Protein coding	CCDS14993	Q6GTR6	TSL:1 GENCODE basic APPRIS P1
Ctla4-202	ENSMUST00000097720.3	614	174aa	Protein coding	CCDS69893	Q5SSM0	TSL:5 GENCODE basic
Ctla4-203	ENSMUST00000124816.1	691	No protein	Processed transcript	-	-	TSL:3

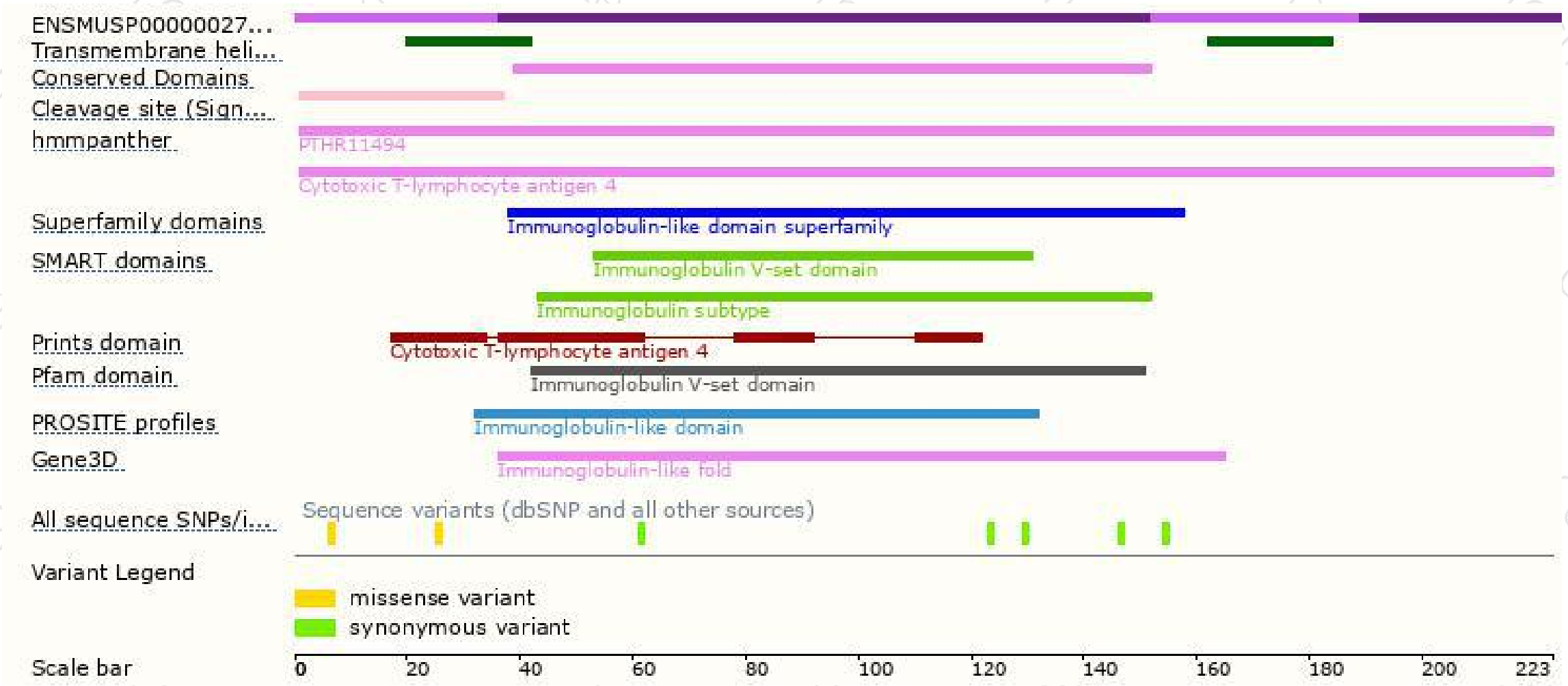
The strategy is based on the design of *Ctla4-201* transcript,The transcription is shown below



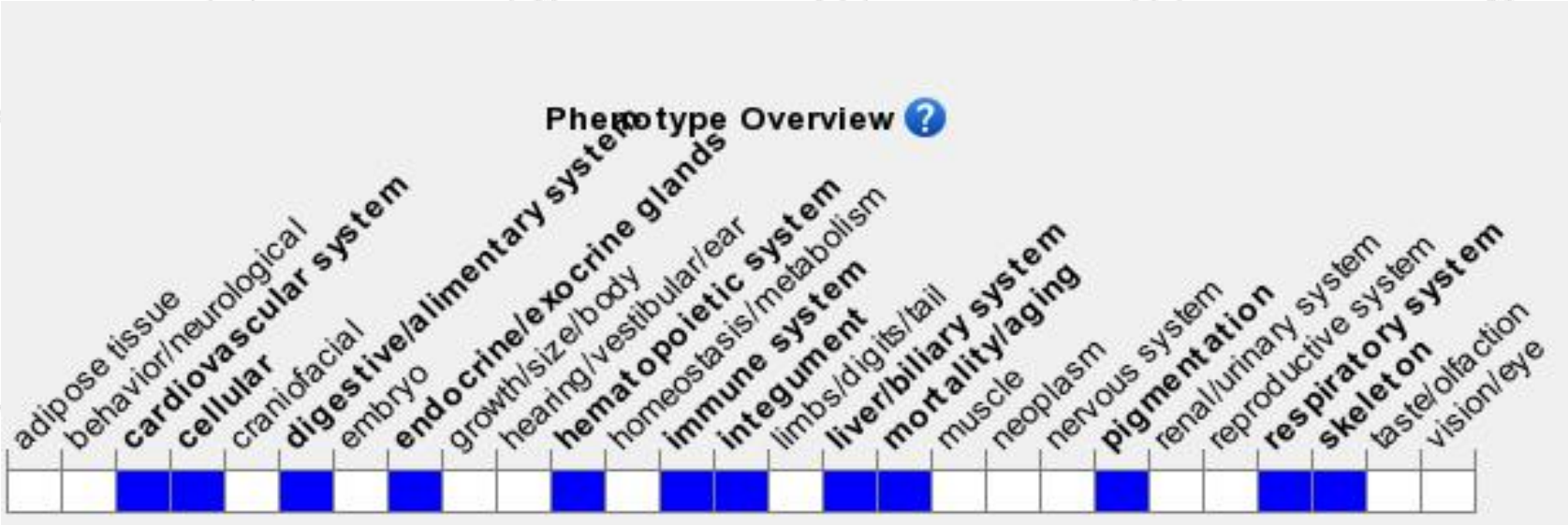
Genomic location distribution



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,Mice homozygous for a knock-out allele exhibit lethality at 3 to 4 weeks of age, decreased T cell numbers, abnormal T cell physiology, inflammation in mutliple organs, abnormal thymus morphology, and lymph node hypoplasia.

集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd