Wls Cas9-CKO Strategy

Designer: Jinling Wang

Reviewer: Longyun Hu

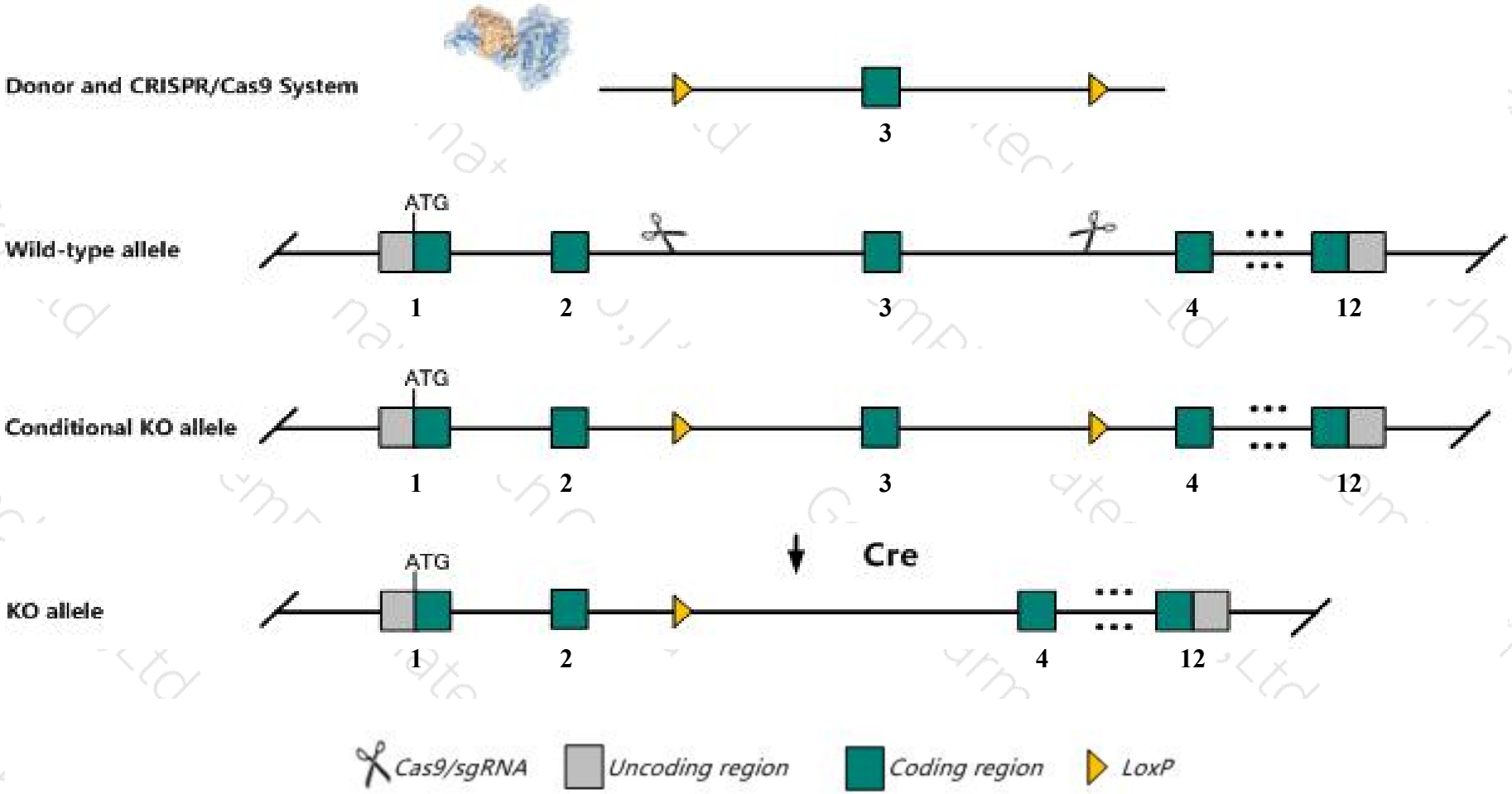
Design Date: 2022-3-2

Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Wls* gene has 7 transcripts. According to the structure of *Wls* gene, exon3 of *Wls-201*(ENSMUST00000068952.9) transcript is recommended as the knockout region.The region contains 125bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Wls* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,mice homozygous for null alleles exhibit impaired body axis and triploblastic development dying prior to E10.5. Mice homozygous for a floxed allele activated in keratinocytes exhibit a psoriasiform dermatitis-like phenotype.

➢ The *Wls* gene is located on the Chr3. 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）

集萃药康 GemPharmatech

Wls wntless WNT ligand secretion mediator [Mus musculus (house mouse)]

Gene ID: 68151, updated on 5-Mar-2019

Summary

Official Symbol	Wls provided by MGI
Official Full Name	wntless WNT ligand secretion mediator provided by MGI
Primary source	MGI:MGI:1915401
See related	Ensembl:ENSMUSG00000028173
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	5031439A09Rik, AI173978, AI987742, EVI, Gpr177
Expression	Broad expression in bladder adult (RPKM 41.9), limb E14.5 (RPKM 30.9) and 21 other tissues See more
Orthologs	human all

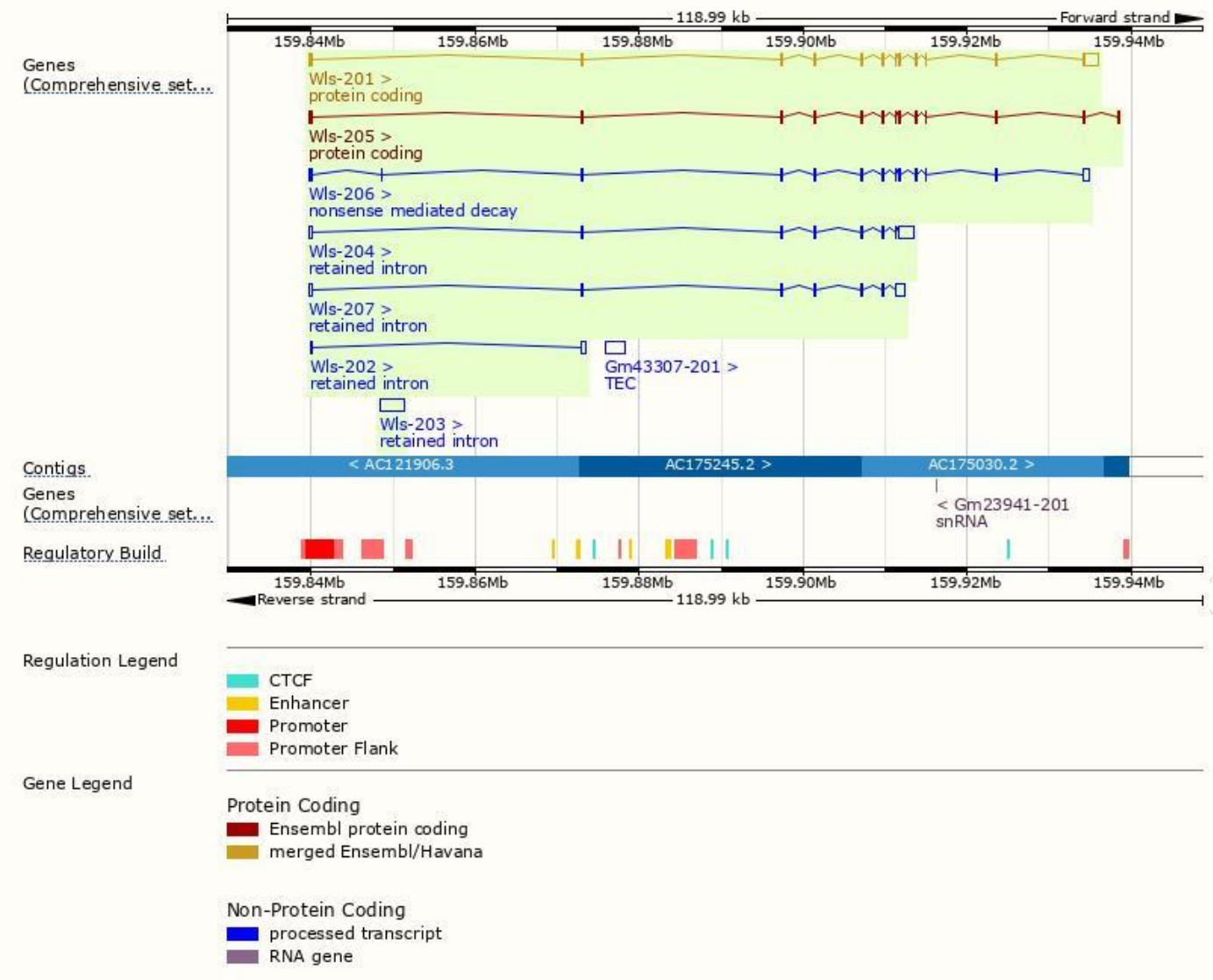
Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

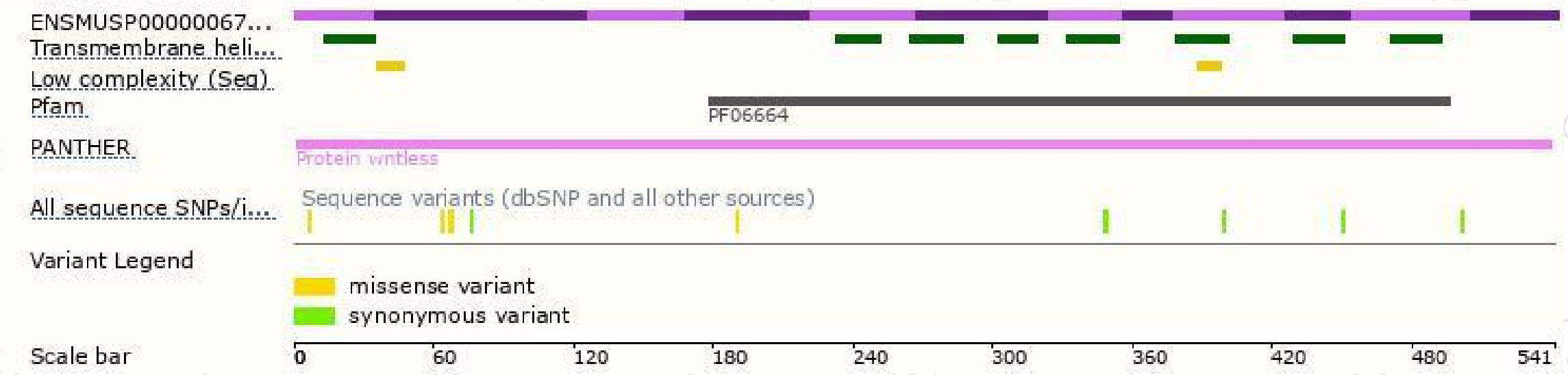
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wls-201	ENSMUST00000068952.9	3559	541aa	Protein coding	CCDS38684	Q6DID7	TSL:1 GENCODE basic APPRIS P1
Wls-205	ENSMUST00000198878.1	2050	541aa	Protein coding	CCDS38684	Q6DID7	TSL:1 GENCODE basic APPRIS P1
Wls-206	ENSMUST00000200191.1	2453	37aa	Nonsense mediated decay	-	A0A0G2JEH4	TSL:1
Wls-204	ENSMUST00000197328.4	2949	No protein	Retained intron	-	-	TSL:1
Wls-203	ENSMUST00000196782.1	2824	No protein	Retained intron	-	-	TSL:NA
Wls-207	ENSMUST00000200571.4	2338	No protein	Retained intron	-	-	TSL:1
Wls-202	ENSMUST00000196276.1	634	No protein	Retained intron	-	-	TSL:1

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

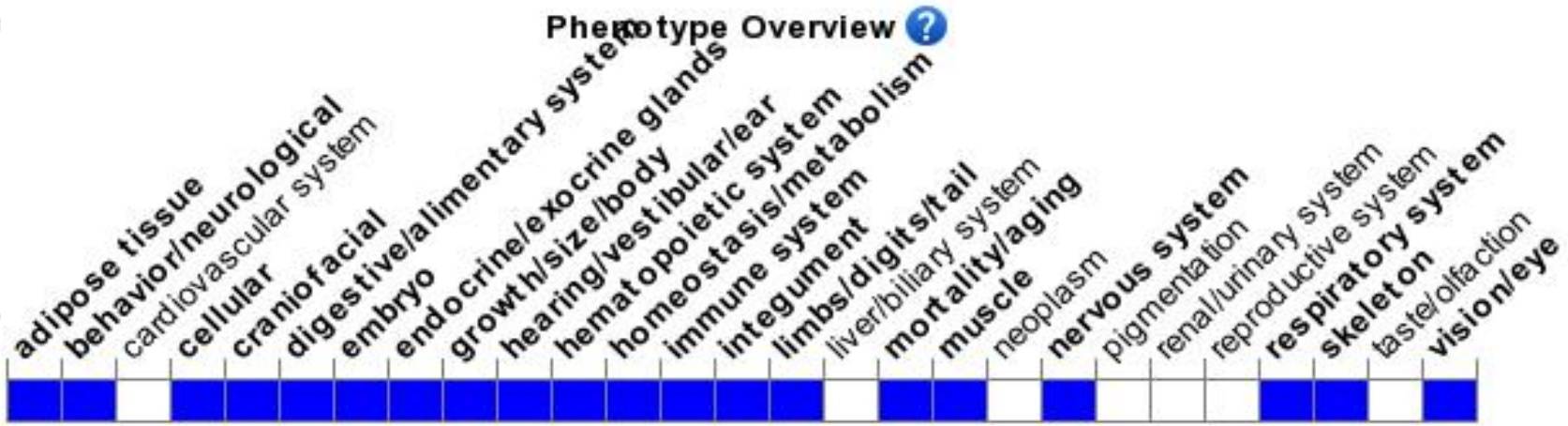
Genomic location distribution



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 null alleles exhibit impaired body axis and triploblastic development dying prior to E10.5. Mice homozygous for a floxed allele activated in keratinocytes exhibit a psoriasiform dermatitis-like phenotype.

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