Polr2k Cas9-CKO Strategy

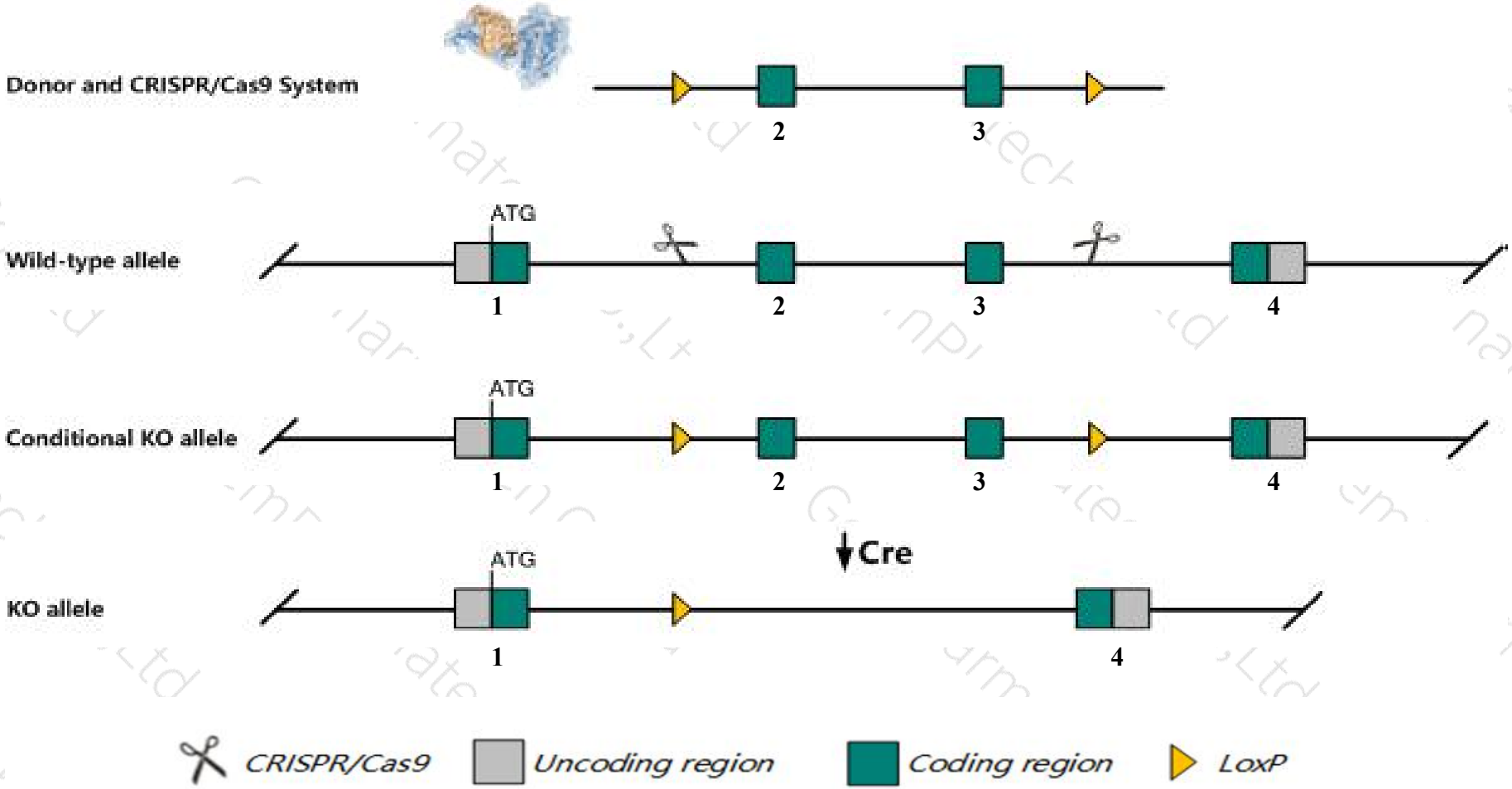
Designer:Xueting Zhang
Reviewer:Yanhua Shen
Date:2019-12-02

Project Overview

Project Name	***Polr2k***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Polr2k* gene. The schematic diagram is as follows:



Technical routes

- The *Polr2k* gene has 2 transcripts. According to the structure of *Polr2k* gene, exon2-exon3 of *Polr2k-201* (ENSMUST00000057177.6) transcript is recommended as the knockout region.The region contains 163bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Polr2k* 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 and cell types.

Notice

➢ The floxed region is near to the N-terminal of *Spag1* gene,this strategy may influence the regulatory function of the N-terminal of *Spag1* gene.

➢ The *Polr2k* gene is located on the Chr15. 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

Polr2k polymerase (RNA) II (DNA directed) polypeptide K [*Mus musculus* (house mouse)]

Gene ID: 17749, updated on 12-Aug-2019

Summary

Official Symbol	Polr2k provided by MGI
Official Full Name	polymerase (RNA) II (DNA directed) polypeptide K provided by MGI
Primary source	MGI:MGI:102725
See related	Ensembl:ENSMUSG00000045996
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	MafY; Mt1a; RPB12; RPABC4; RPB7.0; RPB10alpha; ABC10-alpha
Expression	Broad expression in CNS E11.5 (RPKM 21.0), liver E14 (RPKM 19.6) and 17 other tissues See more
Orthologs	human all

Genomic context

Location: 15; 15 B3.1

See Polr2k in Genome Data Viewer

Exon count: 4

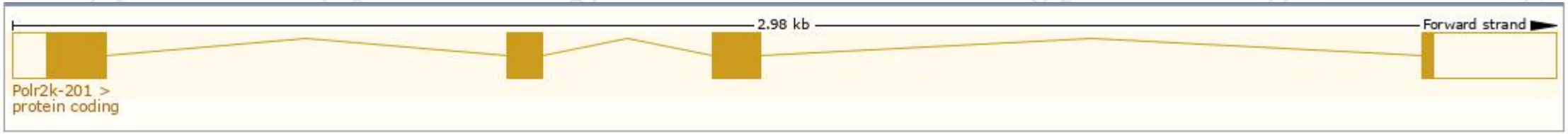
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	15	NC_000081.6 (36174010..36177012)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	15	NC_000081.5 (36103772..36106767)

Transcript information（Ensembl）

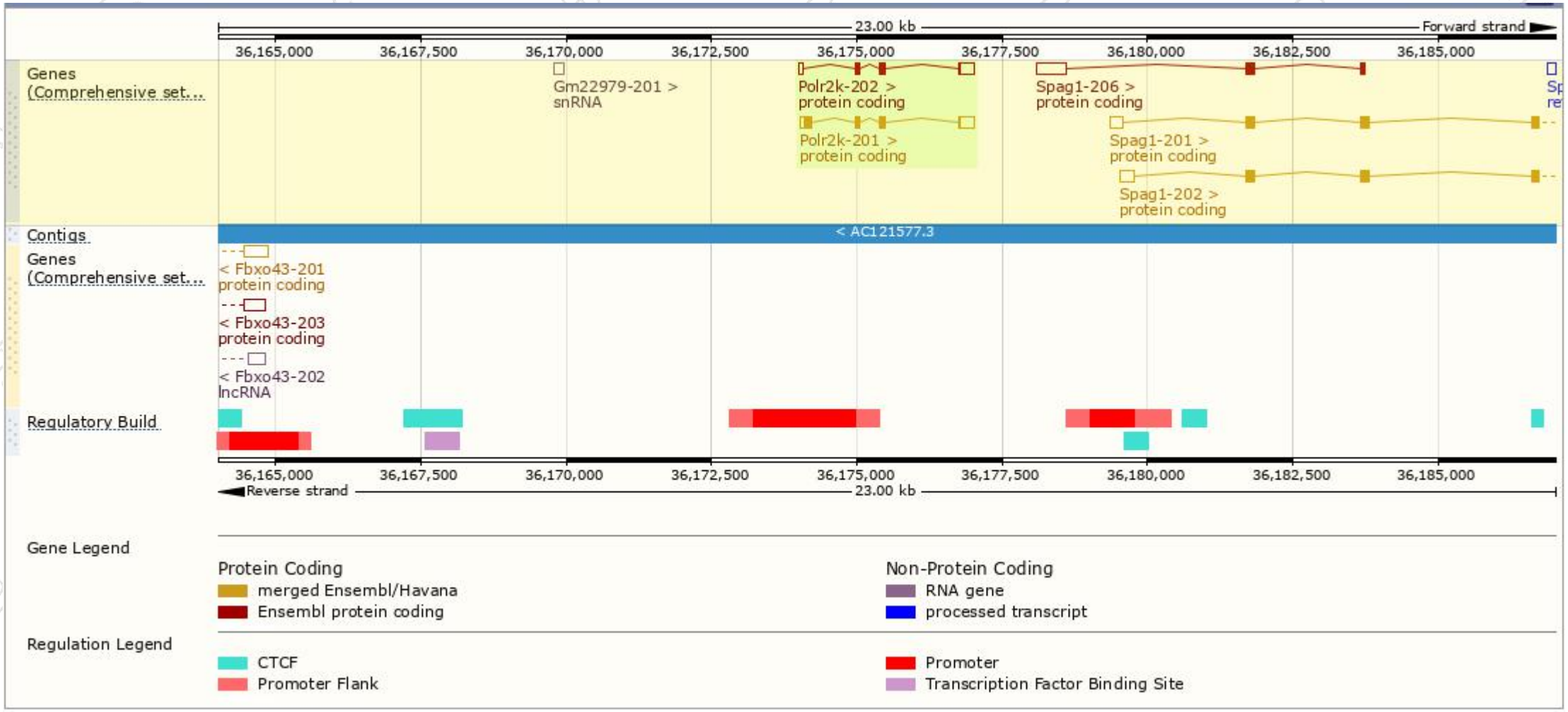
The gene has 2 transcripts,and all the transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Polr2k-201	ENSMUST00000057177.6	601	99aa	Protein coding	CCDS27426	Q8BFX0	TSL:1 GENCODE basic
Polr2k-202	ENSMUST00000180159.7	489	58aa	Protein coding	CCDS56980	Q545V5 Q63871	TSL:1 GENCODE basic APPRIS P1

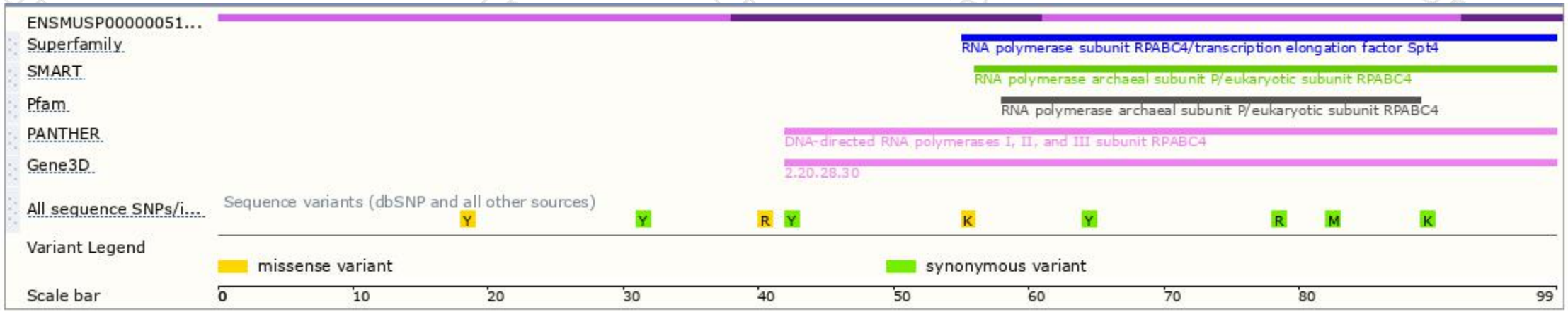
The strategy is based on the design of *Polr2k-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd