Mki67 Cas9-CKO Strategy

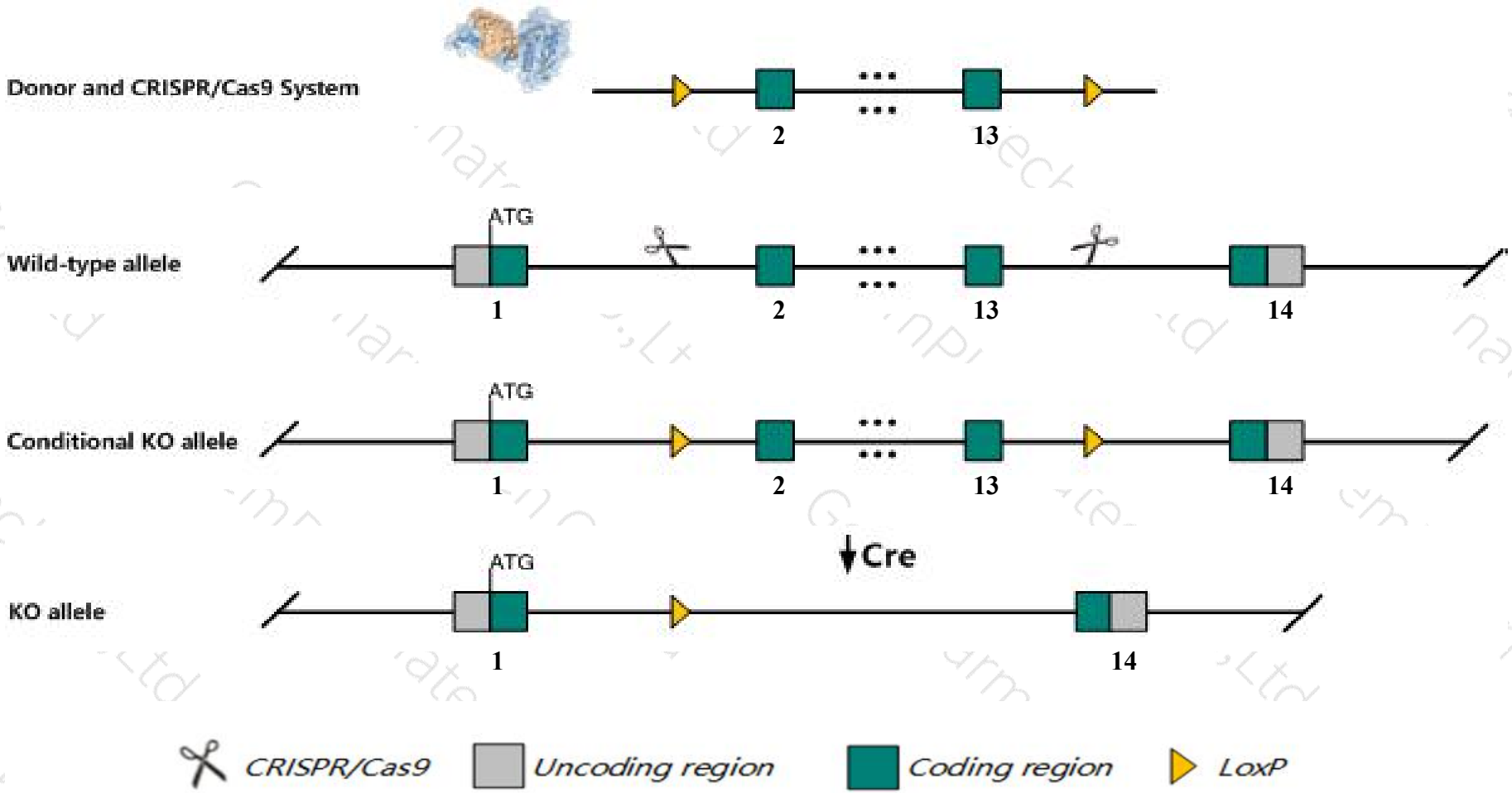
Designer:Xueting Zhang
Reviewer:Yanhua Shen
Date:2019-10-20

Project Overview

Project Name	***Mki67***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mki67* gene. The schematic diagram is as follows:



Technical routes

- The *Mki67* gene has 2 transcripts. According to the structure of *Mki67* gene, exon2-exon13 of *Mki67-201* (ENSMUST00000033310.8) transcript is recommended as the knockout region.The region contains 9397bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mki67* gene. The brief process is as follows:CRISPR/Cas9 system 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

➢ According to the existing MGI data,Mice carrying a reporter allele show expression in actively dividing cells.

➢*Gm45240* gene is located inside of *Mki67* gene,*Gm45240* gene will be deleted together in this strategy.

➢The effect on transcript *Mki67*-202 is unknown.

➢ The *Mki67* gene is located on the Chr7. 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

Mki67 antigen identified by monoclonal antibody Ki 67 [*Mus musculus* (house mouse)]

Gene ID: 17345, updated on 15-Oct-2019

Summary

Official Symbol	Mki67 provided by MGI
Official Full Name	antigen identified by monoclonal antibody Ki 67 provided by MGI
Primary source	MGI:MGI:106035
See related	Ensembl:ENSMUSG00000031004
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	Ki67; Ki-67; D630048A14Rik
Expression	Biased expression in liver E14 (RPKM 21.8), liver E14.5 (RPKM 14.9) and 8 other tissues See more
Orthologs	human all

Genomic context

Location: 7; 7 F3

See Mki67 in Genome Data Viewer

Exon count: 14

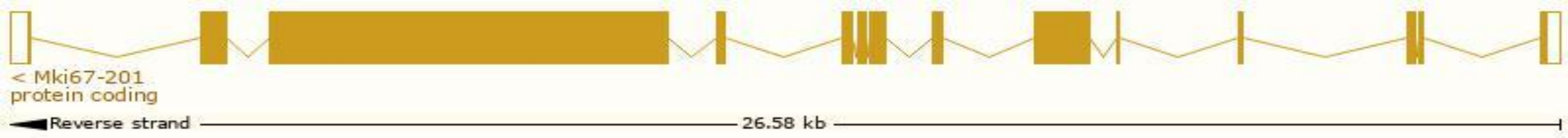
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (135689784..135716450, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (142881471..142908062, complement)

Transcript information（Ensembl）

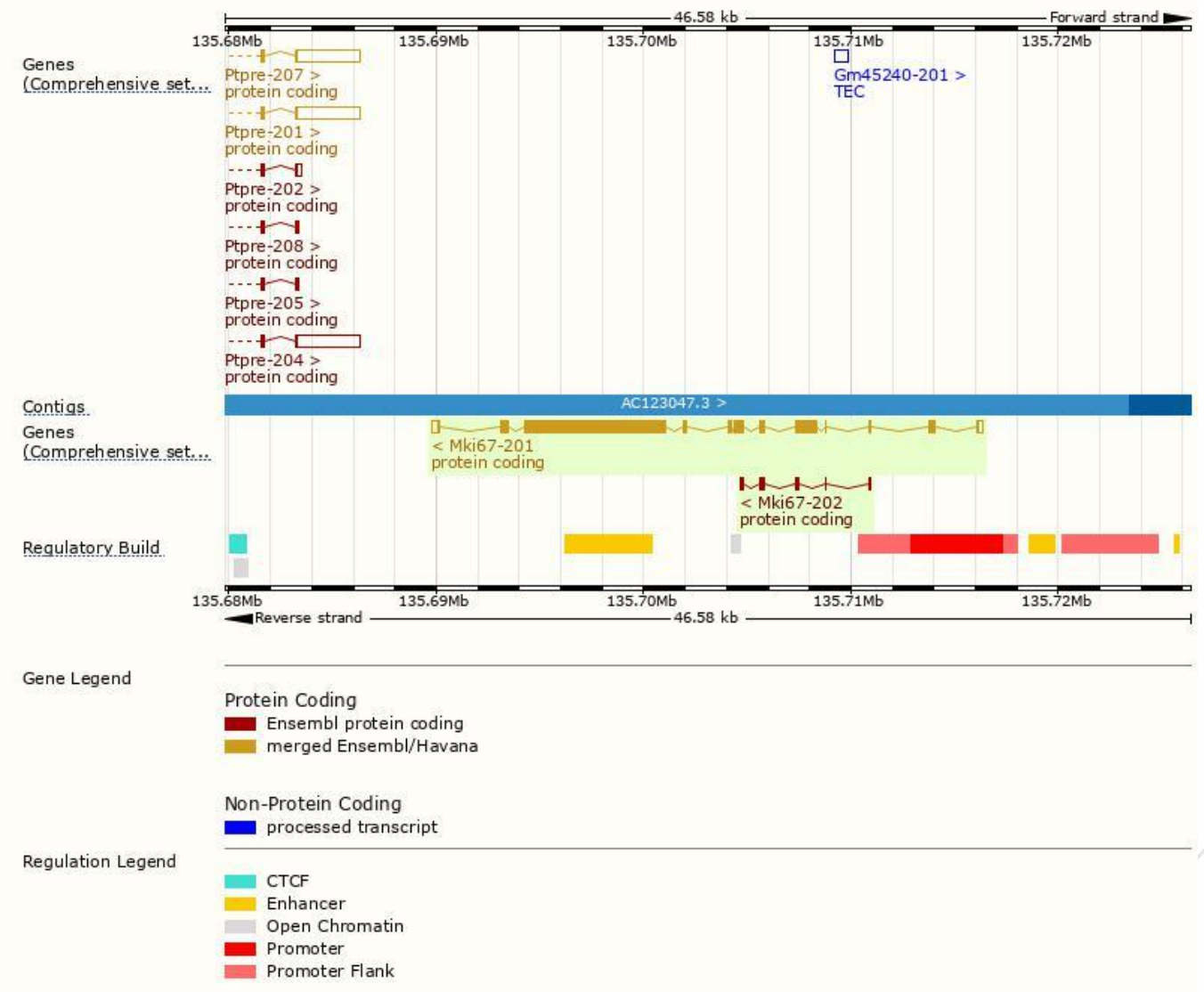
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mki67-201	ENSMUST00000033310.8	10061	3177aa	Protein coding	CCDS52421	E9PVX6	TSL:5 GENCODE basic APPRIS P1
Mki67-202	ENSMUST00000211238.1	575	192aa	Protein coding	-	A0A1B0GQV9	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3

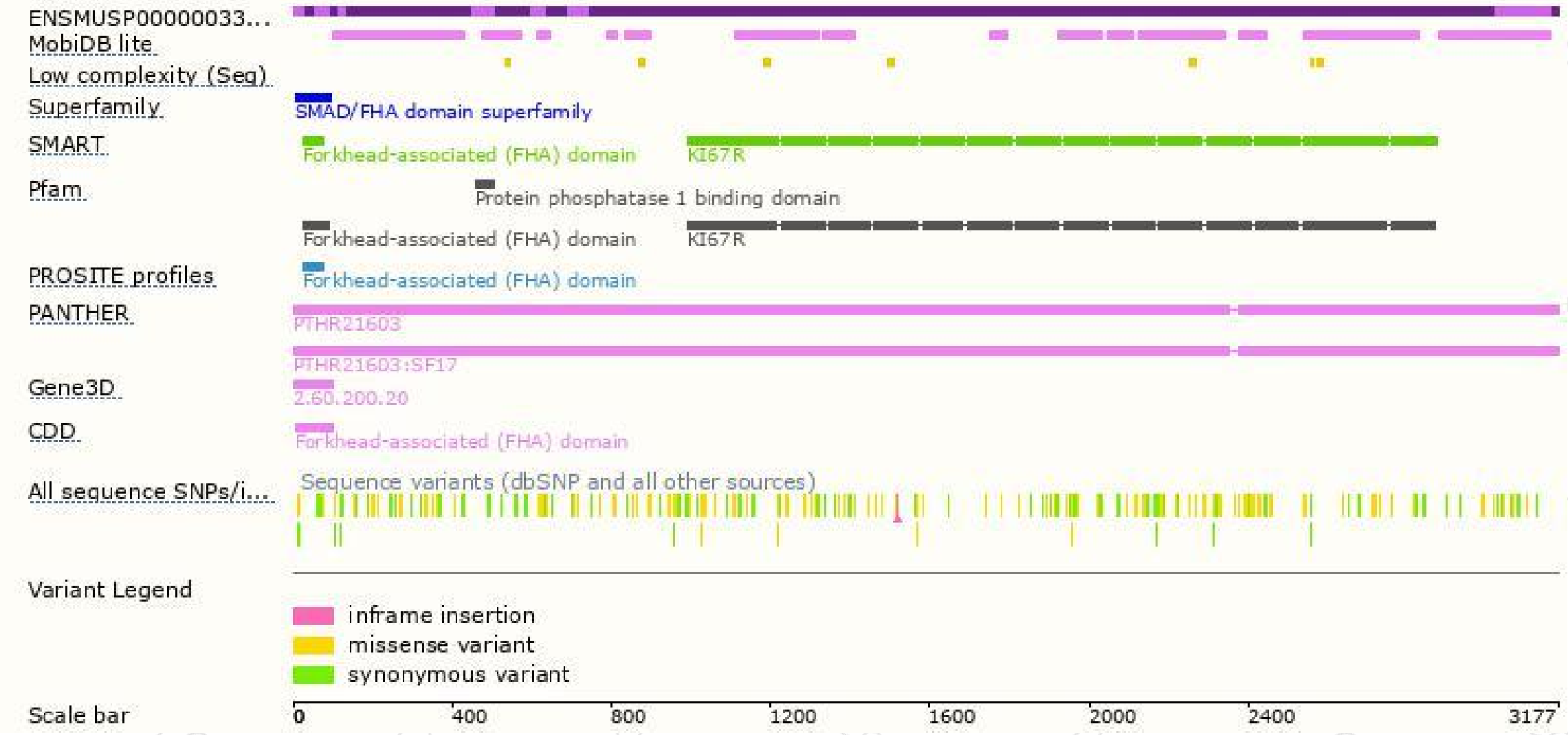
The strategy is based on the design of *Mki67-201* transcript,The transcription is shown below



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