Vcan Cas9-KO Strategy

Designer: Shilei Zhu

Reviewer: Linyan Wu

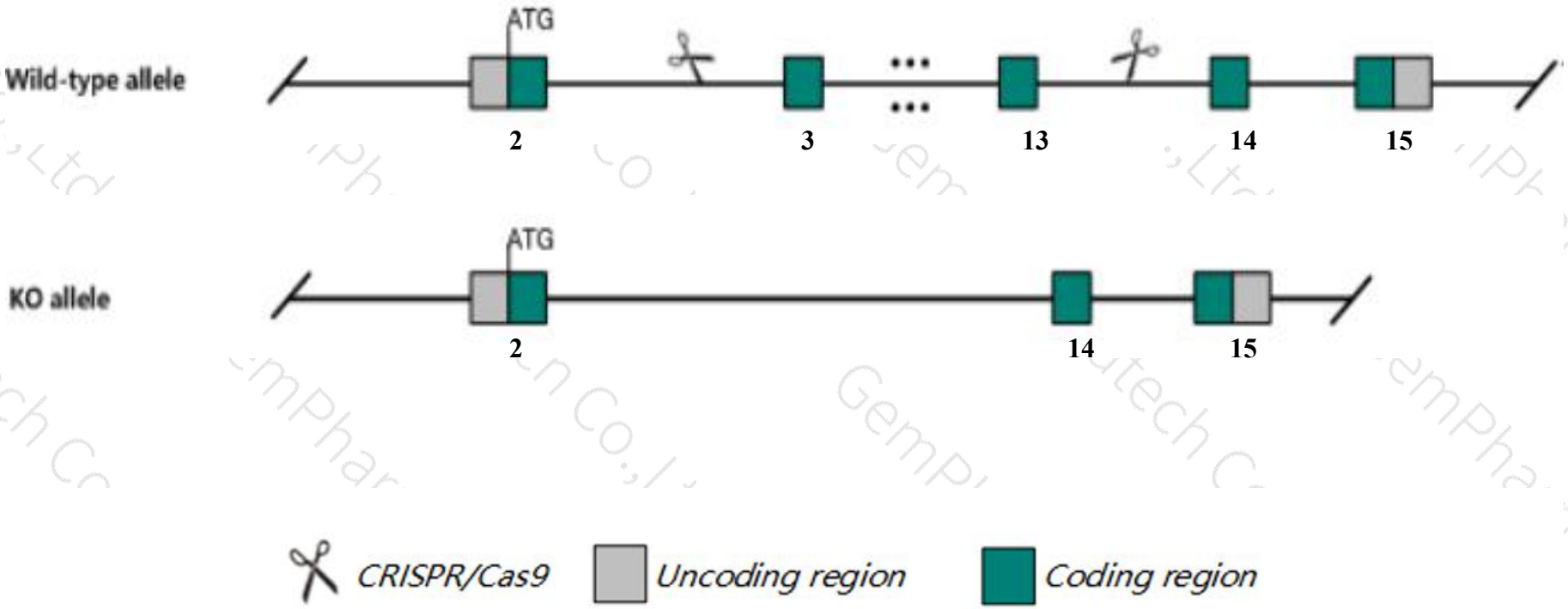
Design Date: 2020-9-23

Project Overview

Project Name	***Vcan***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

➢ The *Vcan* gene has 9 transcripts. According to the structure of *Vcan* gene, exon3-exon13 of *Vcan-203*(ENSMUST00000109546.8) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

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

Notice

➢ According to the existing MGI data,homozygotes for an insertional mutation exhibit anterior-posterior segmental defects of the heart, lack endocardial cushions of the conus and atrioventricular region, and die and around embryonic day 10.5.

➢ The *Vcan* gene is located on the Chr13. 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.

➢The deleted area contains GM16318-201.

➢The KO region deletes most of the coding sequence, but does not result in frameshift.

Gene information（NCBI）

集萃药康 GemPharmatech

Vcan versican [Mus musculus (house mouse)]

Gene ID: 13003, updated on 13-Mar-2020

Summary

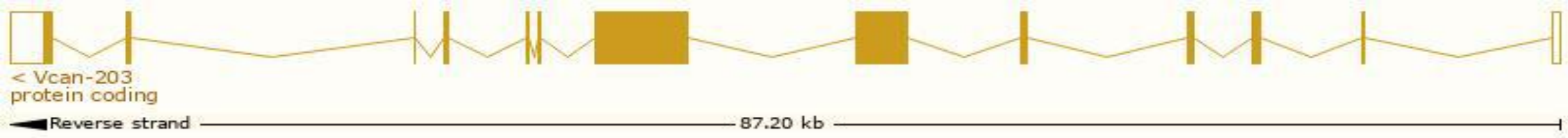
Official Symbol	Vcan provided by MGI
Official Full Name	versican provided byMGI
Primary source	MGI:MGI:102889
See related	Ensembl:ENSMUSG00000021614
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5430420N07Rik, 9430051N09, Cspg2, DPEAAE, NG2, PG-M, PG-M(V0), PG-M(V1), hdf
Expression	Biased expression in limb E14.5 (RPKM 25.2), CNS E11.5 (RPKM 14.1) and 9 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

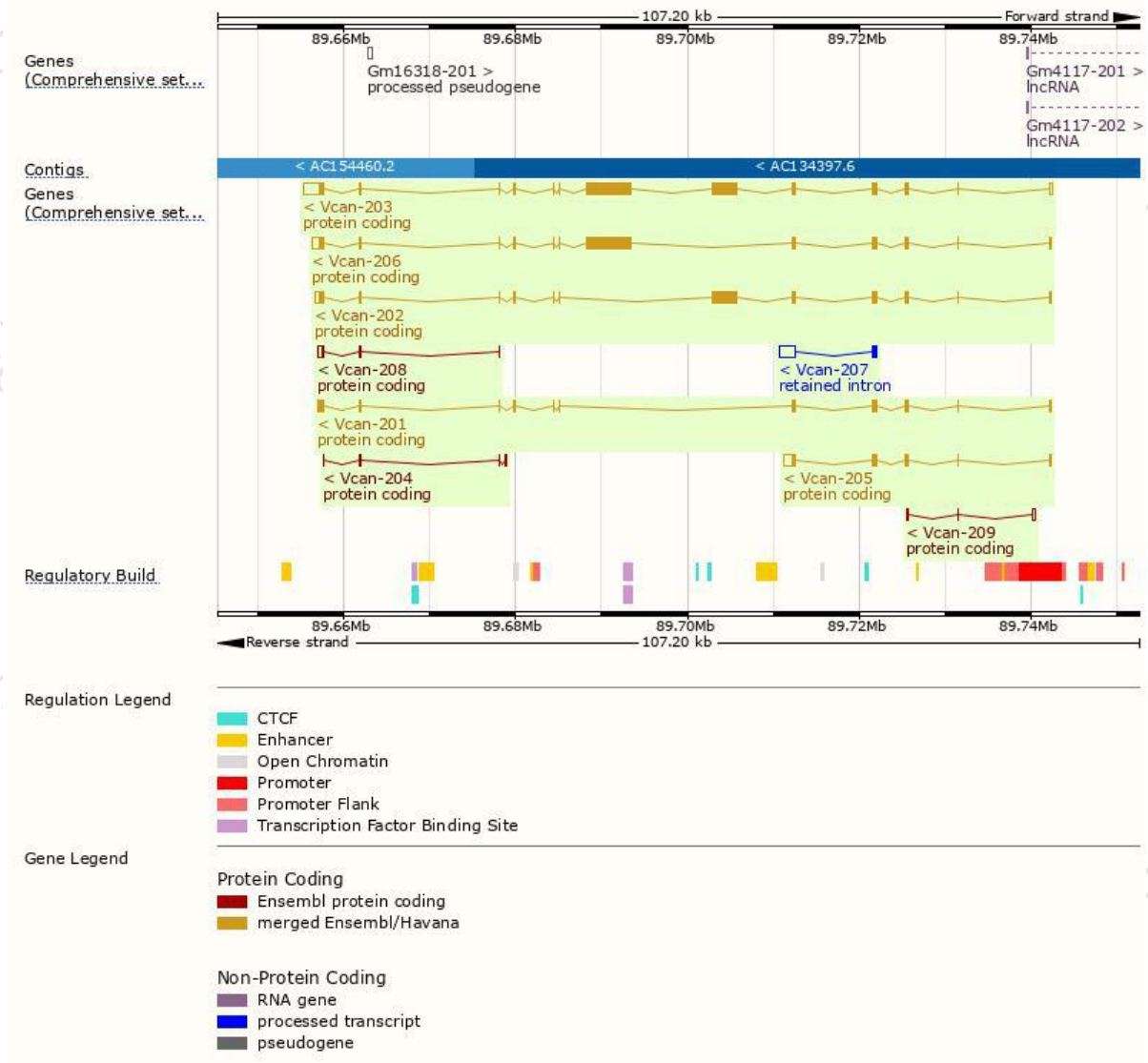
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vcan-203	ENSMUST00000109546.8	12427	3354aa	Protein coding	CCDS36740	E9PYH0	TSL:1 GENCODE basic
Vcan-206	ENSMUST00000159910.7	8332	2394aa	Protein coding	CCDS49323	G3XA35	TSL:5 GENCODE basic
Vcan-202	ENSMUST00000109544.8	5702	1615aa	Protein coding	CCDS49324	E9QMK2	TSL:5 GENCODE basic
Vcan-201	ENSMUST00000109543.8	2321	655aa	Protein coding	CCDS49322	E9QMK3	TSL:1 GENCODE basic APPRIS P1
Vcan-205	ENSMUST00000159337.7	2273	368aa	Protein coding	CCDS49321	Q8BS97	TSL:1 GENCODE basic
Vcan-208	ENSMUST00000160740.7	861	141aa	Protein coding	-	F7B603	CDS 5' incomplete TSL:2
Vcan-209	ENSMUST00000162715.1	615	100aa	Protein coding	-	E0CZC0	CDS 3' incomplete TSL:3
Vcan-204	ENSMUST00000159285.1	294	98aa	Protein coding	-	F7B6F7	CDS 5' and 3' incomplete TSL:5
Vcan-207	ENSMUST00000160029.1	2144	No protein	Retained intron	-	-	TSL:1

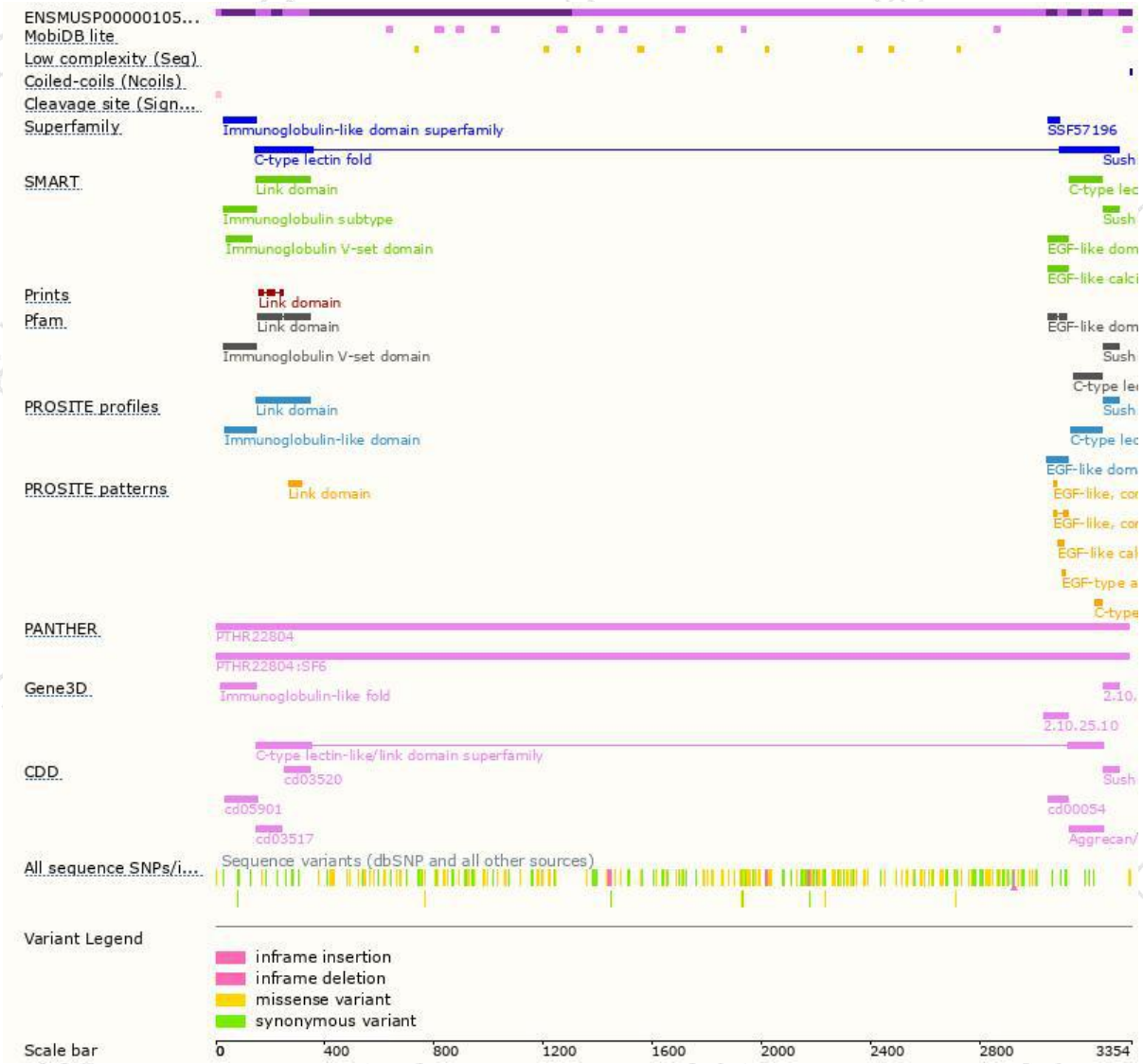
The strategy is based on the design of *Vcan-203* transcript,the transcription is shown below:



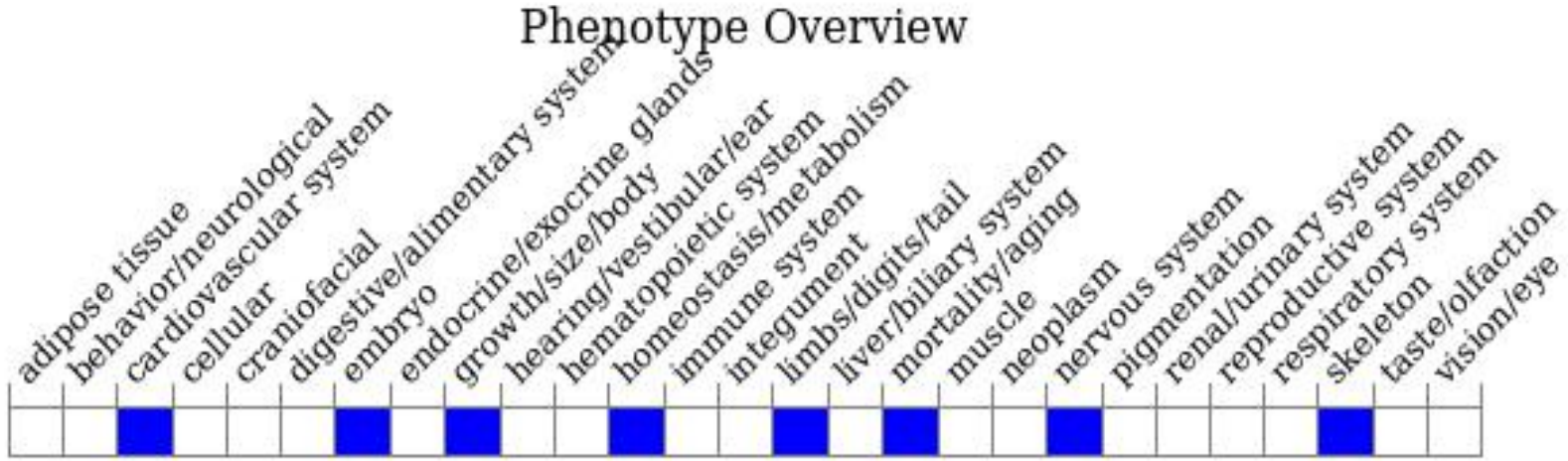
Genomic location distribution



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,homozygotes for an insertional mutation exhibit anterior-posterior segmental defects of the heart, lack endocardial cushions of the conus and atrioventricular region, and die and around embryonic day 10.5.

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd