

Vcan Cas9-KO Strategy

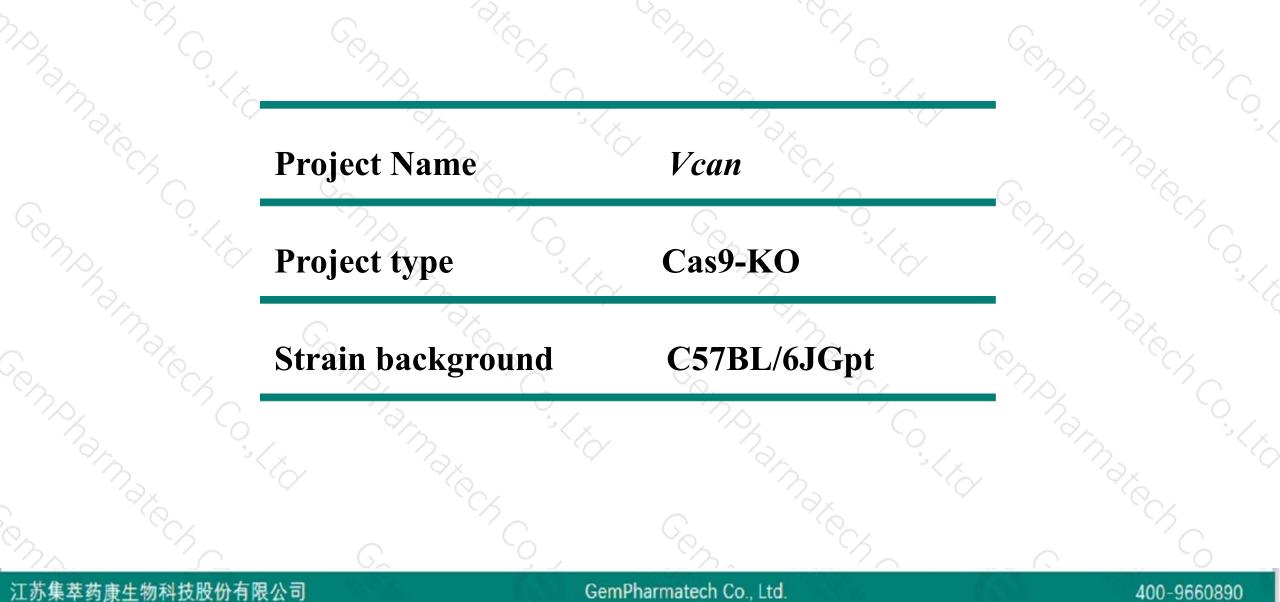
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Reviewer: Linyan Wu

Design Date: 2020-9-23

Project Overview

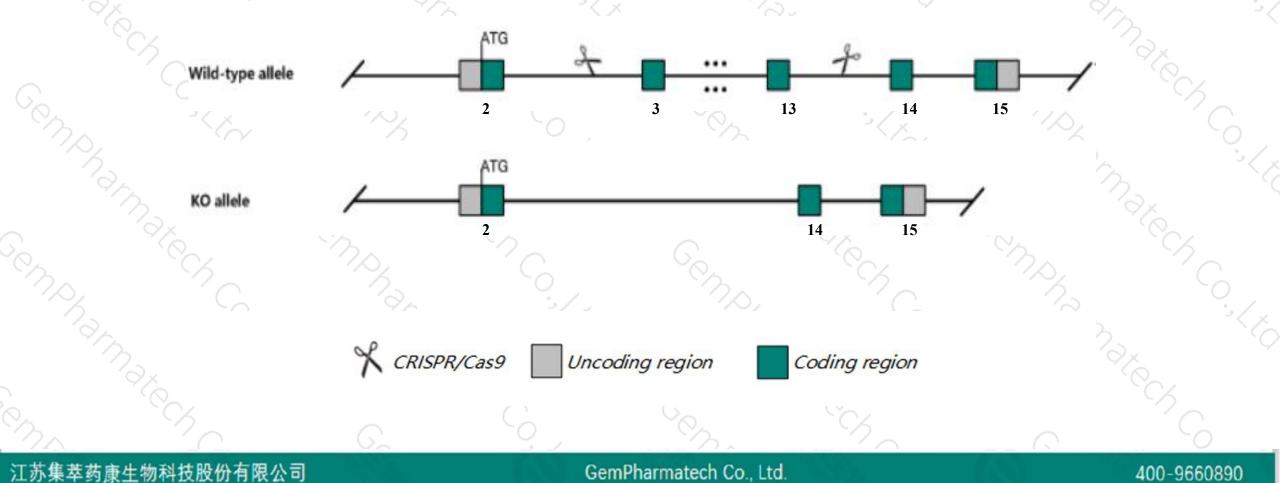




Knockout strategy



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





> 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.



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)



< ?

Vcan versican [Mus musculus (house mouse)]

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

Summary

Official SymbolVcan provided by MGIOfficial Full Nameversican provided byMGIPrimary sourceMGI:MGI:102889See relatedEnsembl:ENSMUSG0000021614Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
Myomorpha; Muroidea; Murinae; Mus; MusAlso known as5430420N07Rik, 9430051N09, Csp92, DPEAAE, NG2, PG-M(V0), PG-M(V1), hdfExpressionBiased expression in limb E14.5 (RPKM 25.2), CNS E11.5 (RPKM 14.1) and 9 other tissues
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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	<u>3354aa</u>	Protein coding	CCDS36740	E9PYH0	TSL:1 GENCODE basic	
Vcan-206	ENSMUST00000159910.7	8332	<u>2394aa</u>	Protein coding	CCDS49323	<u>G3XA35</u>	TSL:5 GENCODE basic	
Vcan-202	ENSMUST00000109544.8	5702	<u>1615aa</u>	Protein coding	CCDS49324	E9QMK2	TSL:5 GENCODE basic	
Vcan-201	ENSMUST00000109543.8	2321	<u>655aa</u>	Protein coding	CCDS49322	E9QMK3	TSL:1 GENCODE basic APPRIS P1	
Vcan-205	ENSMUST00000159337.7	2273	<u>368aa</u>	Protein coding	CCDS49321	<u>Q8BS97</u>	TSL:1 GENCODE basic	
Vcan-208	ENSMUST00000160740.7	861	<u>141aa</u>	Protein coding	673 J	<u>F7B603</u>	CDS 5' incomplete TSL:2	
Vcan-209	ENSMUST00000162715.1	615	<u>100aa</u>	Protein coding	-	E0CZC0	CDS 3' incomplete TSL:3	
Vcan-204	ENSMUST00000159285.1	294	<u>98aa</u>	Protein coding	(22)	<u>F7B6F7</u>	CDS 5' and 3' incomplete TSL:5	
Vcan-207	ENSMUST00000160029.1	2144	No protein	Retained intron	670	-	TSL:1	

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

< Vcan-203 protein coding

Reverse strand

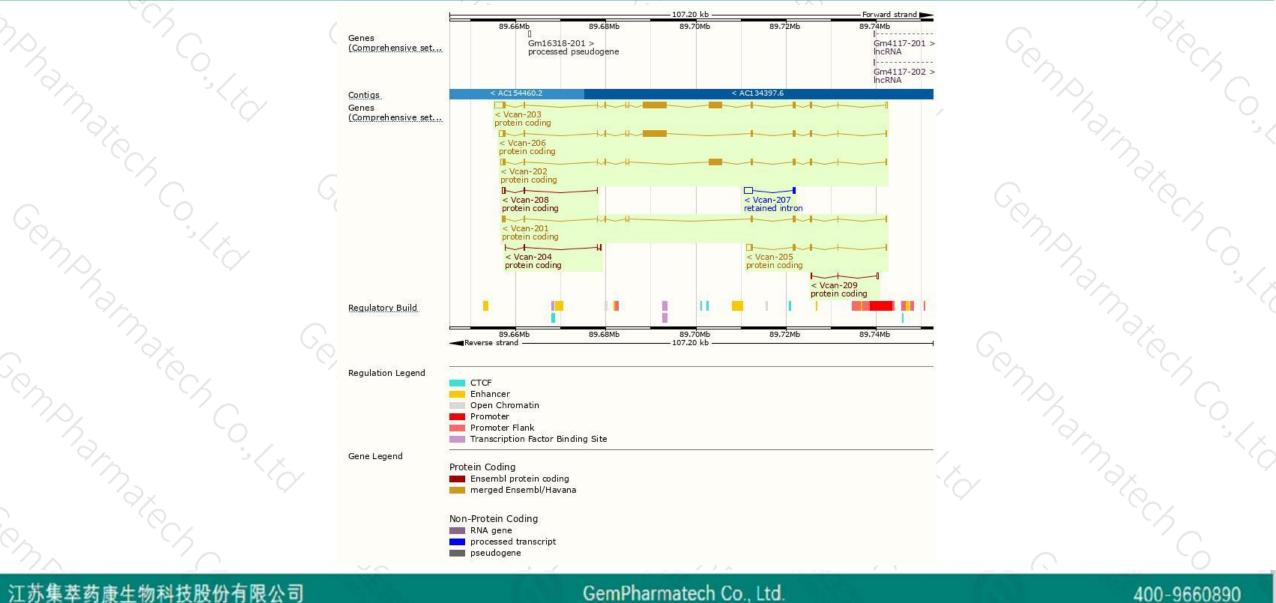
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Genomic location distribution





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Protein domain





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Coiled-coils (Ncoils)					
Cleavage site (Sign	(B)				
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	C-type lectin fold			Sush	
SMART	Link domain			C-type lec	
	Immunoglobulin subtype			Sush	
	Immunoglobulin V-set domain			EGF-like dom	
				EGF-like calci	
Prints	Link domain				
Pfam.	Link domain			EGF-like dom	
	Immunoglobulin V-set domain			Sush	
				C-type lee	
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	Immunoglobulin-like domain			C-type lec	
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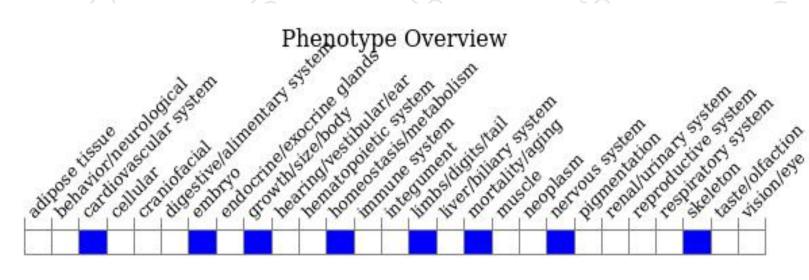
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Mouse phenotype description(MGI)





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



