Gca Cas9-KO Strategy

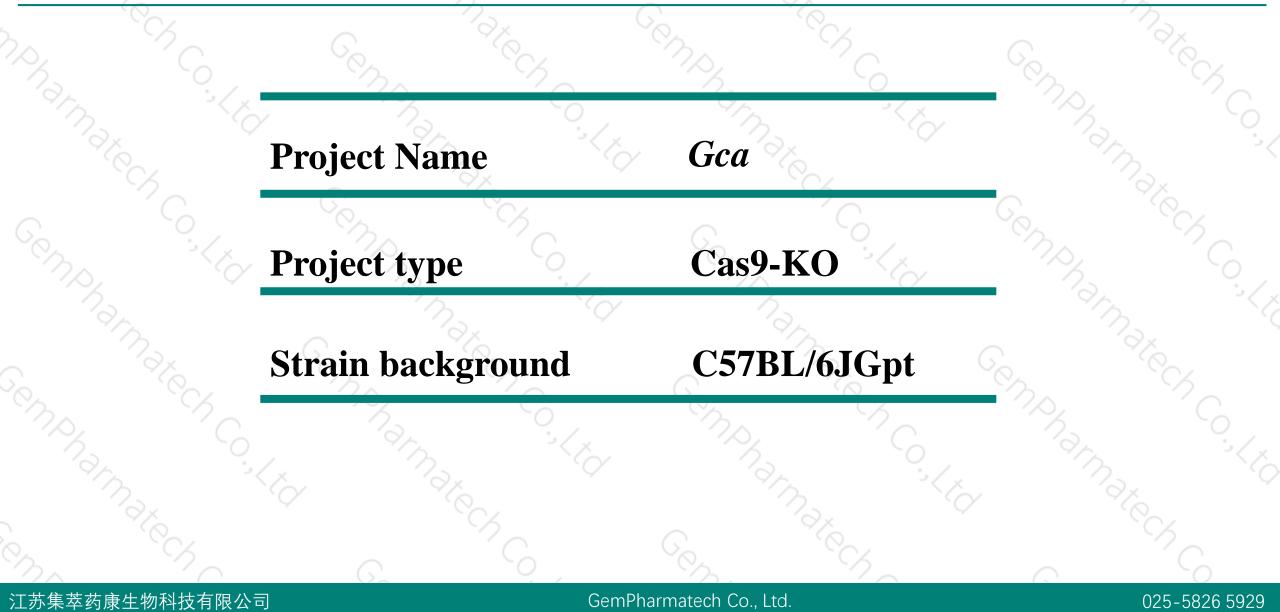
Designer: Reviewer :

Design Date:

Daohua Xu Huimin Su 2020-3-23

Project Overview





Knockout strategy



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



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Technical routes



- The *Gca* gene has 2 transcripts.According to the structure of *Gca* gene, exon3 of *Gca*-201 (
 ENSMUST0000028257.2) transcript is recommended as the knockout region.The region contains 70bp coding sequence.Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gca* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9, sgRNA 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.



- According to the existing MGI data, Mice homozygous for disruptions in this gene are essentially normal. However they do demonstrate an increased resistance to endotoxic shock.
- The Gca gene is located on the Chr2. 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.

Gene information (NCBI)



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Gca grancalcin [Mus musculus (house mouse)]

Gene ID: 227960, updated on 13-Mar-2020

Summary

Official SymbolGca provided by MGIOfficial Full Namegrancalcin provided by MGIPrimary sourceMGI:MGI:1918521See relatedEnsembl:ENSMUSG0000026893Gene typeprotein codingRefSeq statusPROVISIONALOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asAl573844; 5133401E04RikExpressionUbiquitous expression in cortex adult (RPKM 6.2), frontal lobe adult (RPKM 5.7) and 28 other tissues See more
huma_all

Transcript information (Ensembl)



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

1		~ ()	<u> </u>								_ /	
	Name 🖕	Transcript ID 💧	bp 🖕	Protein 🖕	Biotype	CCDS 🖕	UniProt 🖕		Flags		12	
	Gca-201	ENSMUST0000028257.2	3330	<u>220aa</u>	Protein coding	<u>CCDS16069</u> @	<u>Q8VC88</u> &	TSL:1	GENCODE basic	APPRIS P1		
	Gca-202	ENSMUST00000148083.1	382	No protein	Processed transcript	-	-		TSL:2			

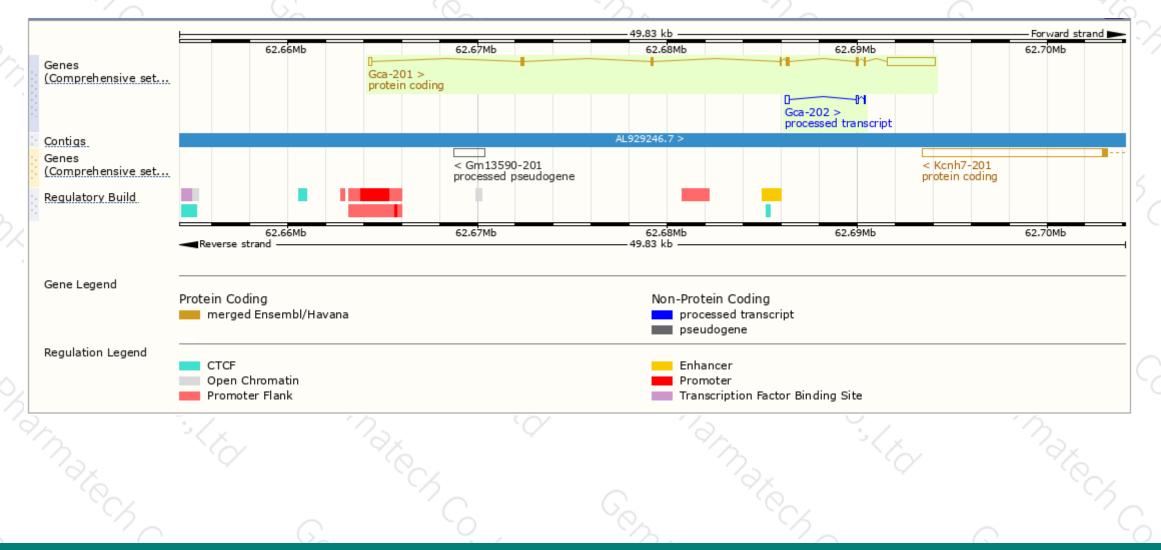
The strategy is based on the design of Gca-201 transcript, The transcription is shown below



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Genomic location distribution





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025-5826 5929

Protein domain



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im.			PF138	333							
OSITE profiles			EF-ha	and domain							
OSITE patterns							EF-Hand 1, calciu	im-binding site			
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Mouse phenotype description(MGI)



Phenotype Overview 🕜 cardiovascular sys hearnghestipu nematopoletic Innune Syste homeoslasis renallumary growthiste reproductive Impologies integunent pigmentatio endocrinele respiratory INSIDILARY nerrous sy. daniofaci digestivelt motalityle tasteloftat neoplash exeleton embryo muscle

Click cells to view annotations.

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 disruptions in this gene are essentially normal. However they do demonstrate an increased resistance to endotoxic shock.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



