Calca Cas9-KO Strategy

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Reviewer: Fengjuan Wang

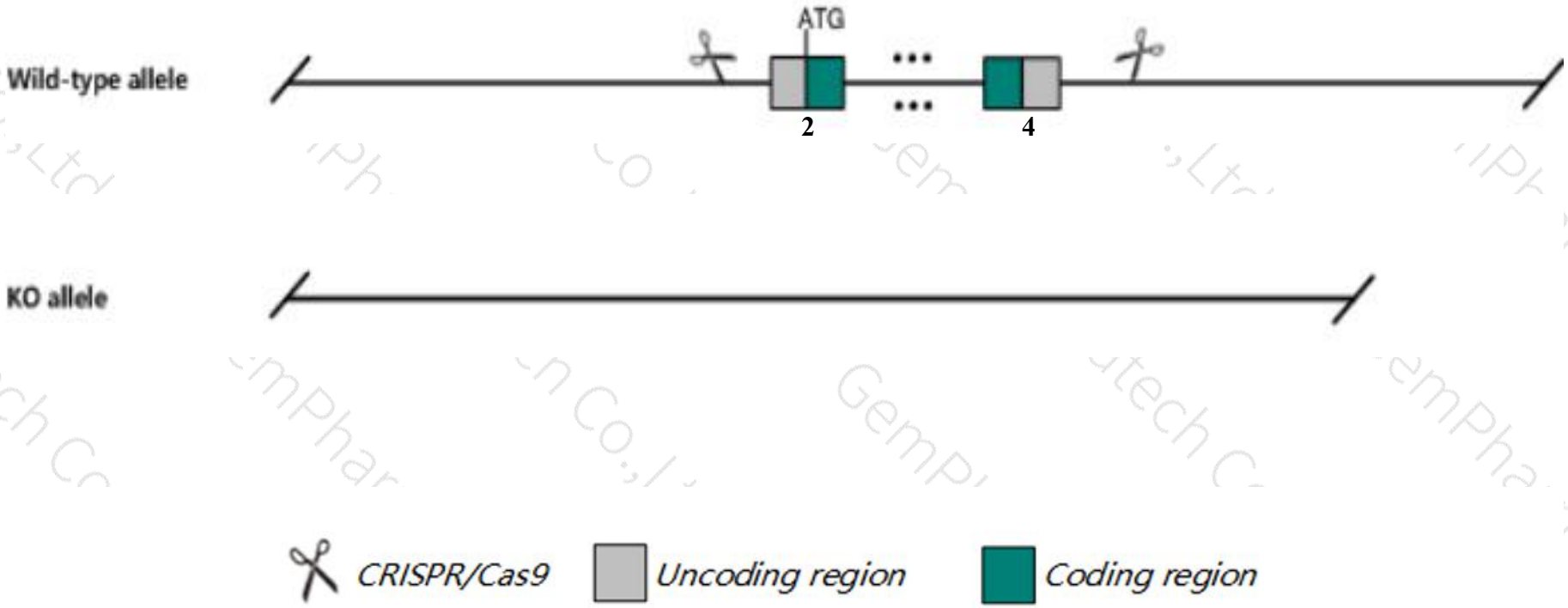
Design Date: 2018-10-25

Project Overview

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

Knockout strategy

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



Technical routes

➢ The *Calca* gene has 7 transcripts. According to the structure of *Calca* gene, exon2-exon4 of *Calca-201*(ENSMUST00000032906.10) transcript is recommended as the knockout region.The region contains all 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 *Calca* 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,two separate peptides, calcitonin and calcitonin gene related peptide-alpha (CGRP-alpha), are derived from this locus by alternative splicing. Mice homozygous null for CGRP-alpha have changes in the vascular and nervous system. Mice lacking calcitonin have increased bone mineralization.

➢ The Intron4 is only 510bp,loxp insertion may affect mRNA splicing.

➢ The *Calca* 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 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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Calca calcitonin/calcitonin-related polypeptide, alpha [Mus musculus (house mouse)]

Gene ID: 12310, updated on 13-Mar-2020

Summary

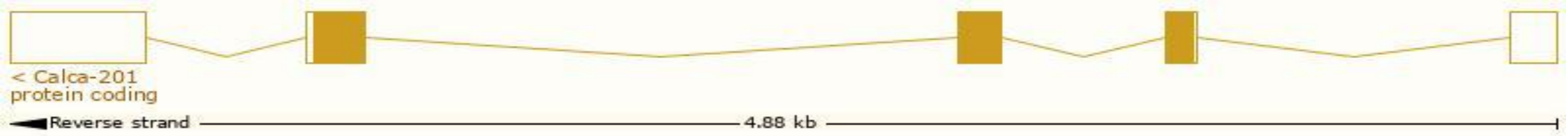
Official Symbol	Calca provided by MGI
Official Full Name	calcitonin/calcitonin-related polypeptide, alpha provided byMGI
Primary source	MGI:MGI:2151253
See related	Ensembl:ENSMUSG00000030669
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CA, CGRP-1, CGRP1, Calc, Calc1, Cgrp, Ct, Ctn
Summary	This gene encodes the peptide hormones calcitonin, calcitonin gene-related peptide (CGRP) and katacalcin. Alternative splicing of the mRNA results in multiple variants that encode either calcitonin or CGRP preproproteins. Post-translational processing of the calcitonin and CGRP propeptides results in either calcitonin and katacalcin, or CGRP, respectively. Calcitonin and katacalcin modulate calcium levels in the blood stream. CGRP can function as a vasodilator and play a role in the transmission of pain. The human homolog of CGRP was found to have antimicrobial activity. [provided by RefSeq, Mar 2015]
Expression	Biased expression in CNS E11.5 (RPKM 1.9), cerebellum adult (RPKM 1.7) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

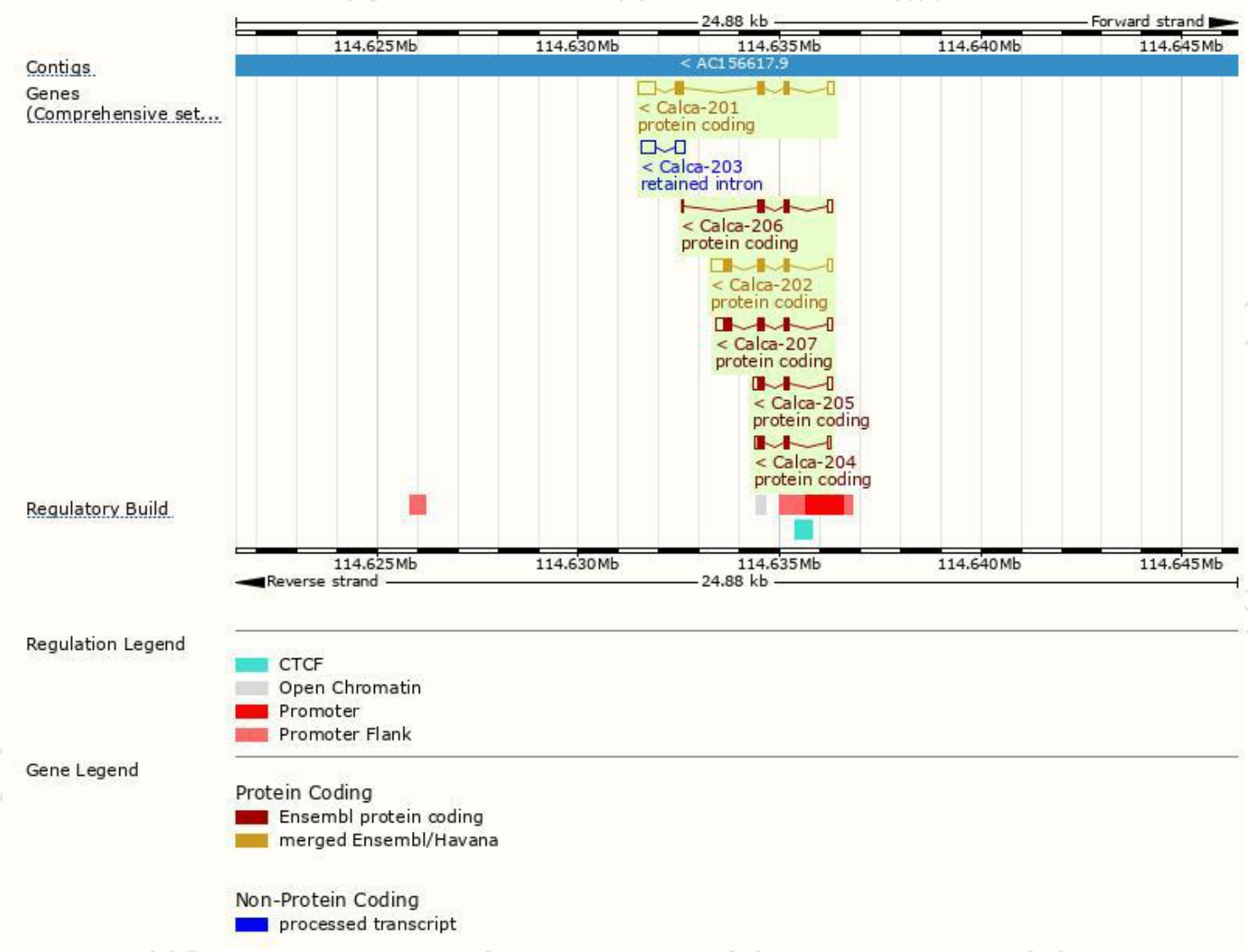
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Calca-201	ENSMUST00000032906.10	994	128aa	Protein coding	CCDS21762	Q99JA0	TSL:1 GENCODE basic APPRIS P1
Calca-202	ENSMUST00000032907.8	830	136aa	Protein coding	CCDS21763	P70160	TSL:1 GENCODE basic
Calca-207	ENSMUST00000206853.1	720	136aa	Protein coding	CCDS21763	P70160	TSL:2 GENCODE basic
Calca-205	ENSMUST00000205933.1	458	76aa	Protein coding	-	A0A0U1RNG0	TSL:3 GENCODE basic
Calca-204	ENSMUST00000205714.1	416	76aa	Protein coding	-	A0A0U1RNG0	TSL:2 GENCODE basic
Calca-206	ENSMUST00000206156.1	404	91aa	Protein coding	-	A0A0U1RNZ0	CDS 3' incomplete TSL:3
Calca-203	ENSMUST00000205560.1	568	No protein	Retained intron	-	-	TSL:2

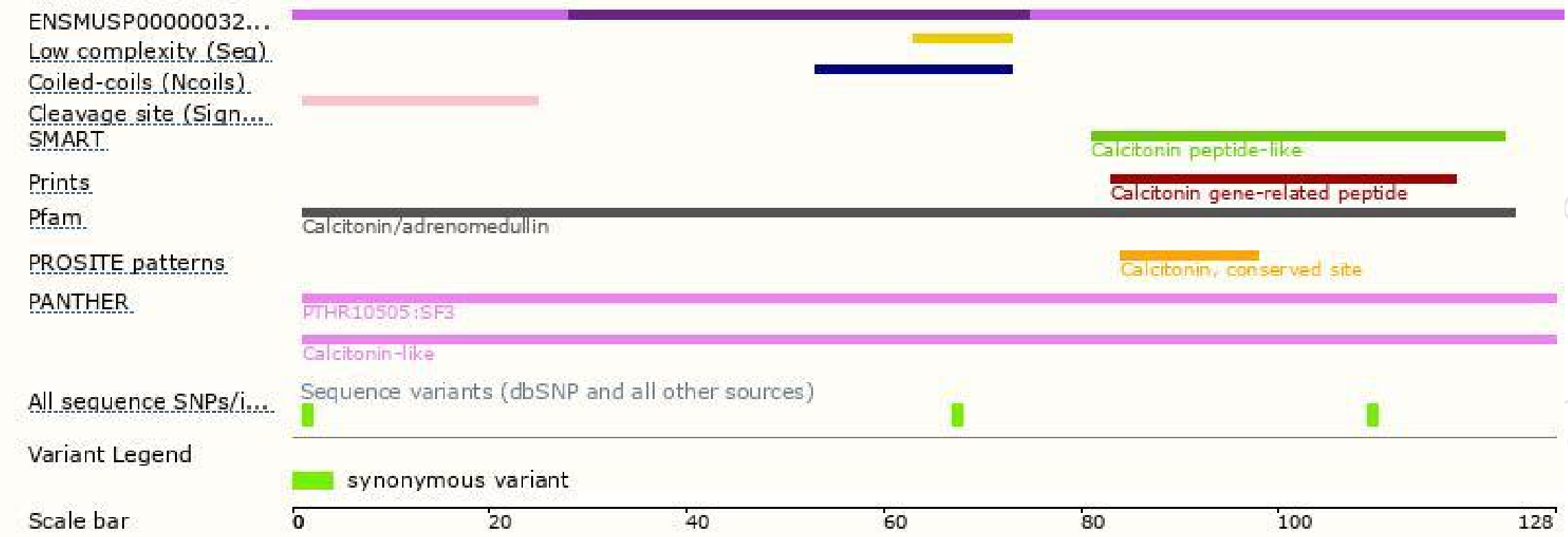
The strategy is based on the design of *Calca-201* transcript,the transcription is shown below:



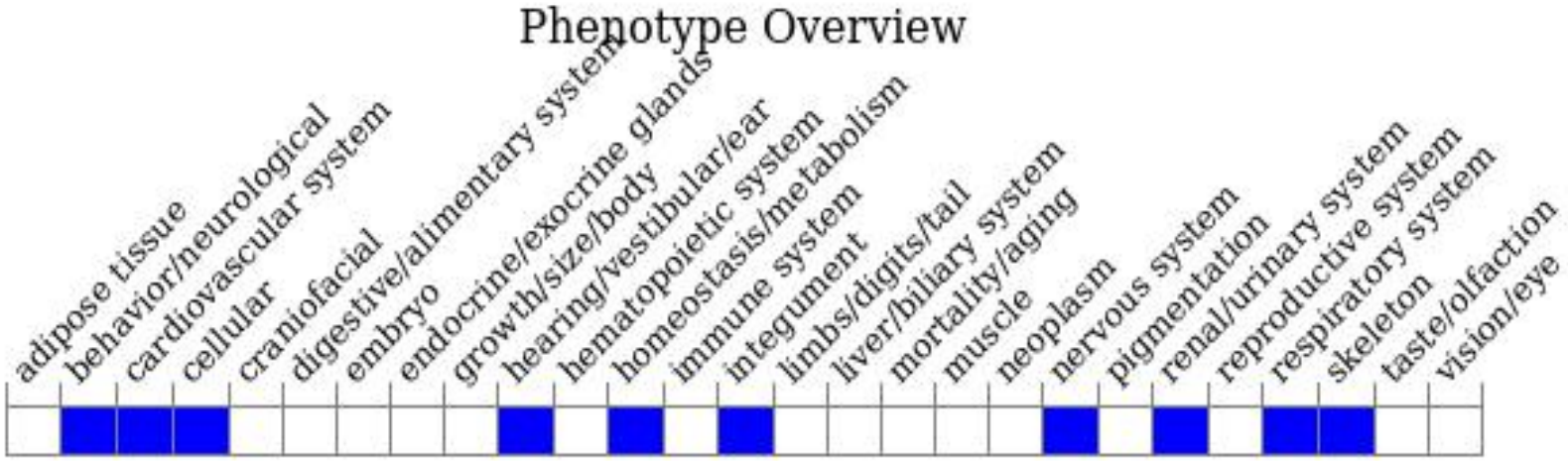
Genomic location distribution



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,two separate peptides, calcitonin and calcitonin gene related peptide-alpha (CGRP-alpha), are derived from this locus by alternative splicing. Mice homozygous null for CGRP-alpha have changes in the vascular and nervous system. Mice lacking calcitonin have increased bone mineralization.

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

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