Acan Cas9-CKO Strategy

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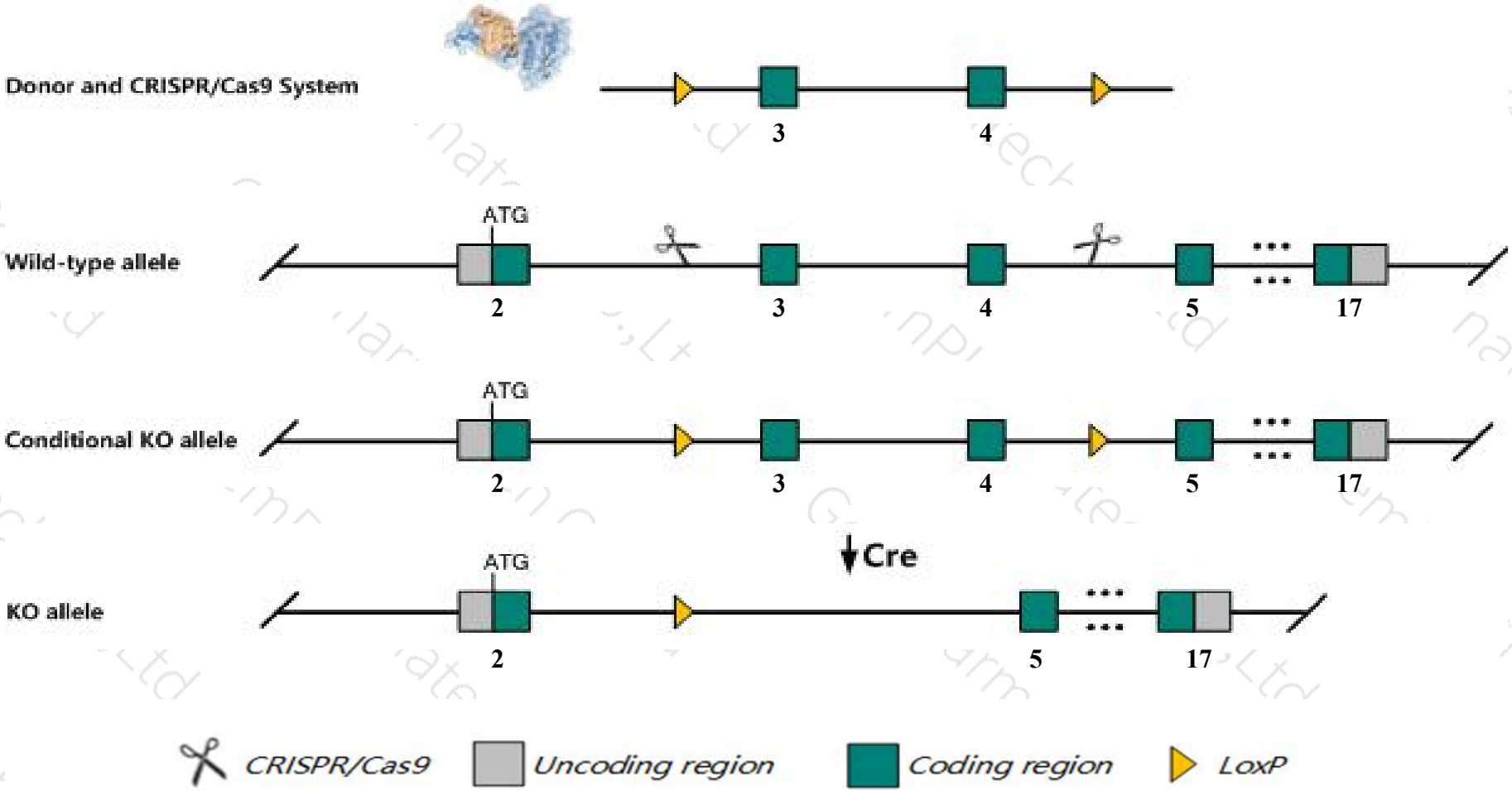
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Acan* gene has 2 transcripts. According to the structure of *Acan* gene, exon3-exon4 of *Acan-201* (ENSMUST00000032835.6) transcript is recommended as the knockout region.The region contains 559bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Acan* 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,Spontaneous mutations in this gene lead to dwarfism, cartilage, skeletal and limb anomalies, craniofacial defects, hearing loss and neonatal death due to respiratory failure. Homozygotes for an ENU-induced allele show cardiomyopathy as well as cleft palate, disproportionate dwarfism and brachypodia.

- The *Acan* 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）

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Acan aggrecan [Mus musculus (house mouse)]

Gene ID: 11595, updated on 2-Apr-2019

Summary

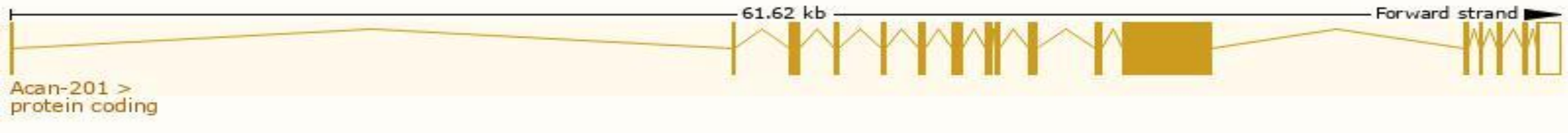
Official Symbol	Acan provided by MGI
Official Full Name	aggrecan provided byMGI
Primary source	MGI:MGI:99602
See related	Ensembl:ENSMUSG00000030607
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Agc, Agc1, CSPCP, Cspg1, b2b183Clo, cmd
Expression	Biased expression in limb E14.5 (RPKM 40.6) and CNS E14 (RPKM 4.1)See more
Orthologs	human all

Transcript information（Ensembl）

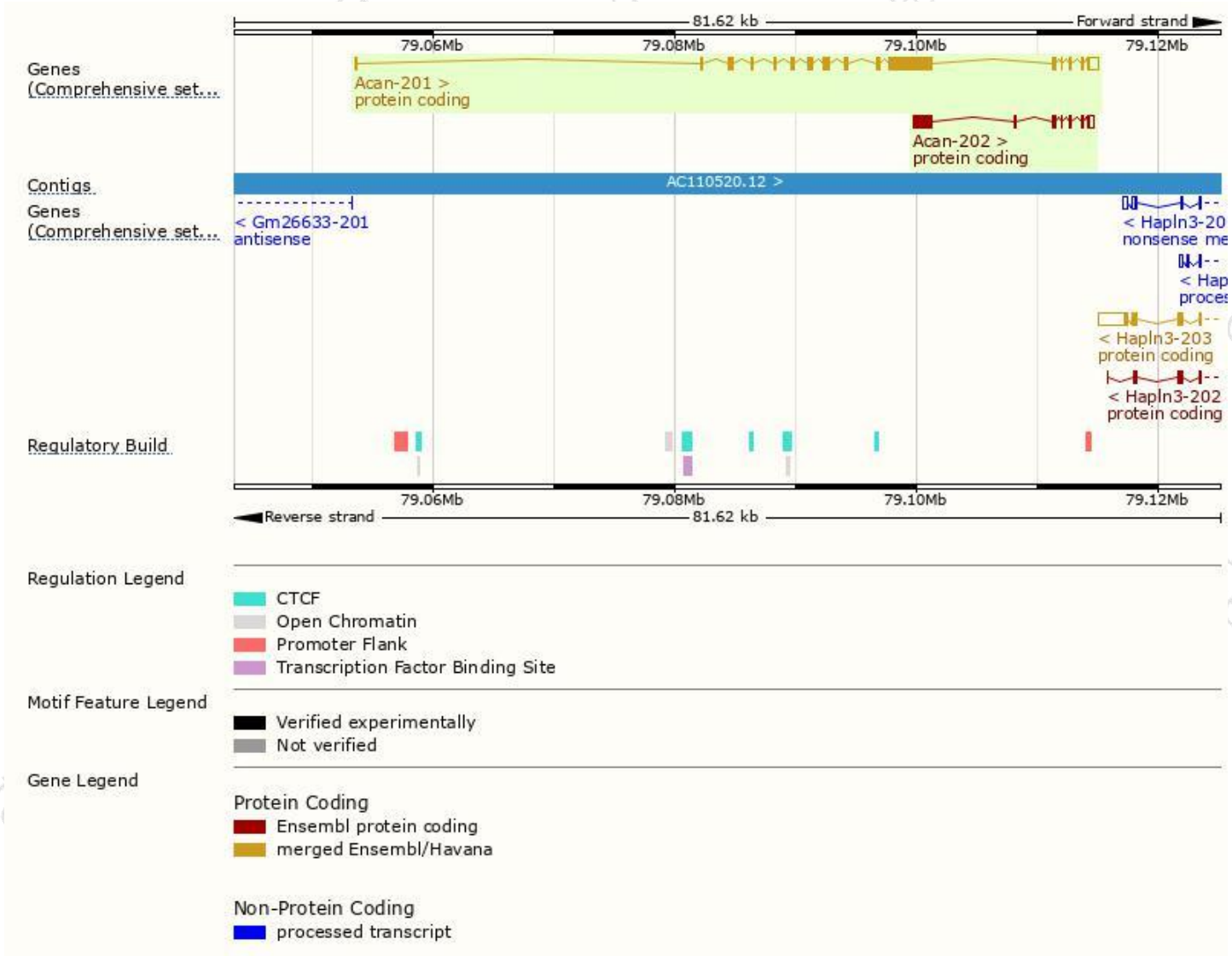
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Acan-201	ENSMUST00000032835.6	7355	2132aa	Protein coding	CCDS21377	Q61282	TSL:1 GENCODE basic APPRIS P1
Acan-202	ENSMUST00000206779.1	2596	748aa	Protein coding	-	A0A0U1RQ88	CDS 5' incomplete TSL:1

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

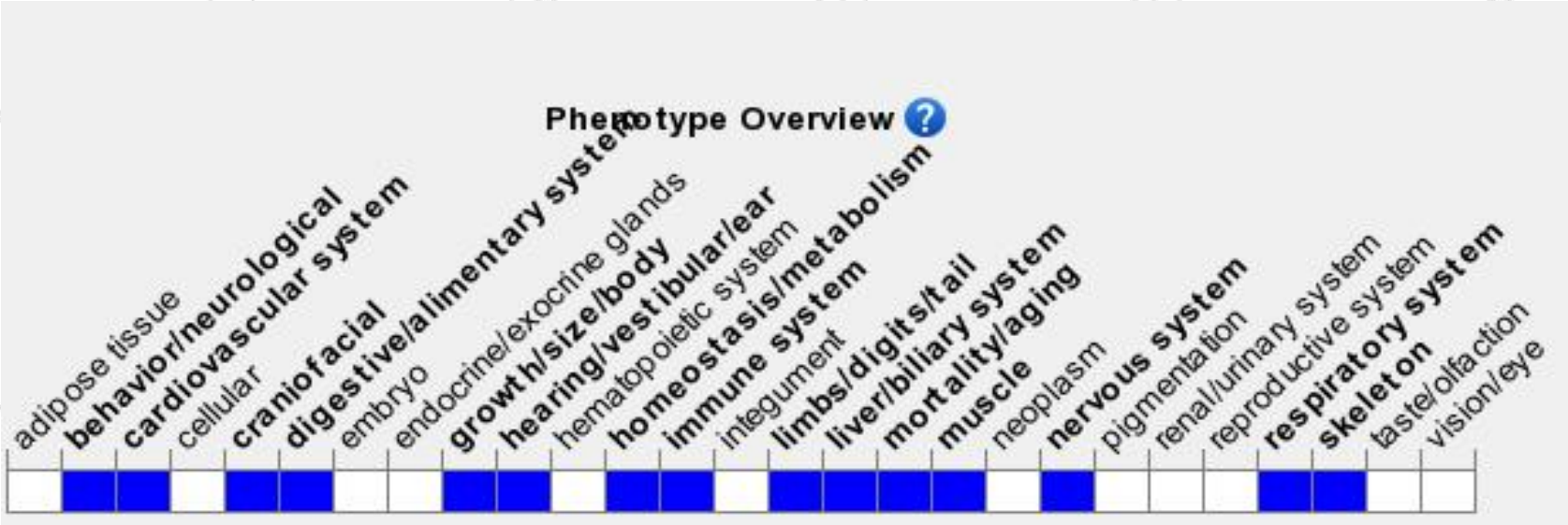


Genomic location distribution



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/).

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

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