Ank Cas9-CKO Strategy

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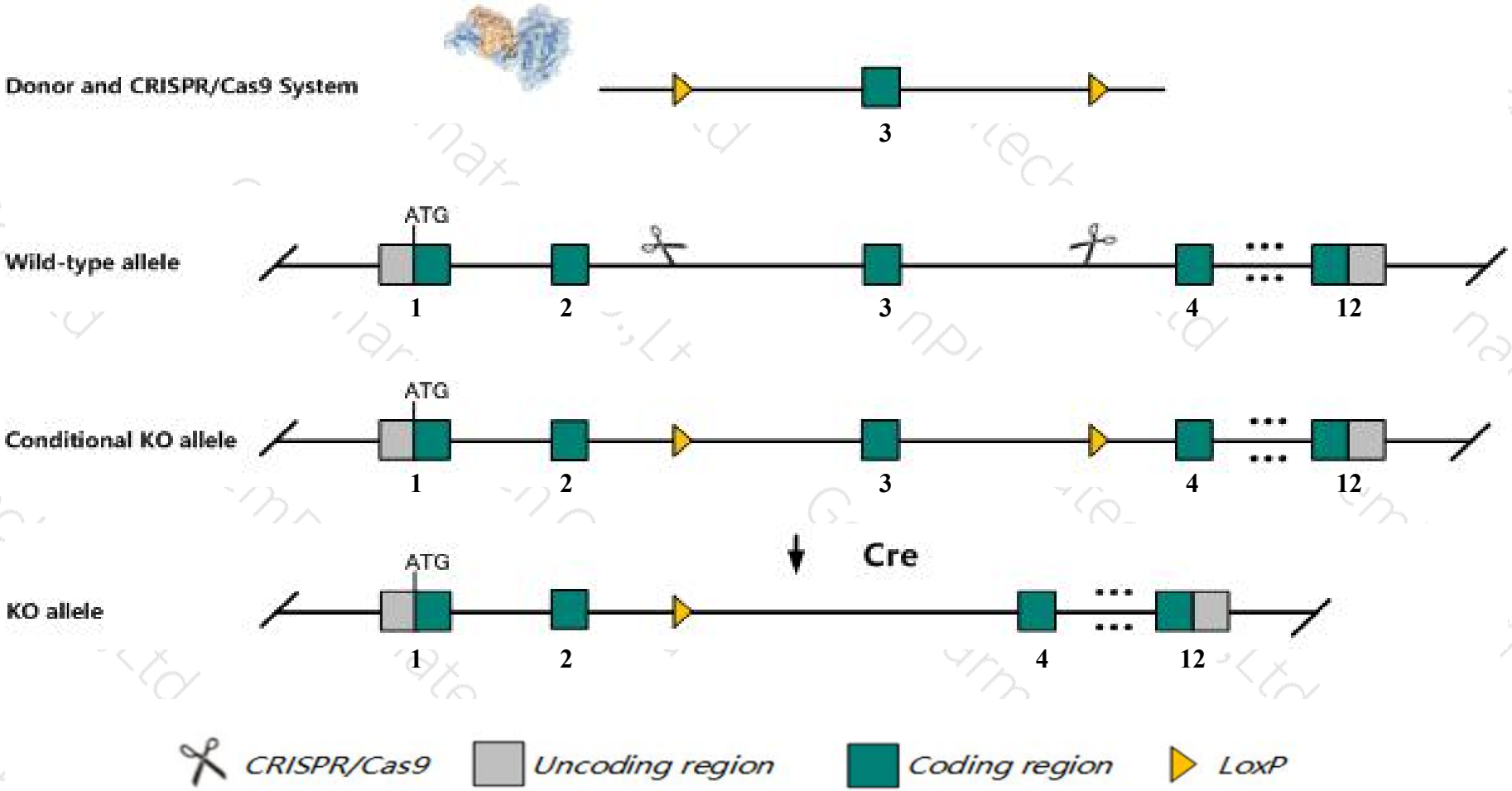
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Ank* gene has 2 transcripts. According to the structure of *Ank* gene, exon3 of *Ank-201* (ENSMUST00000022875.6) transcript is recommended as the knockout region.The region contains 119bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ank* 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,Homozygous mutant animals exhibit joint stiffness due to increased calcium deposits in calcified cartilages and die prematurely. Hyperostosis of craniofacial bones and the mandible has been reported in other mutants as well.

- The *Ank* gene is located on the Chr15. 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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Ank progressive ankylosis [Mus musculus (house mouse)]

Gene ID: 11732, updated on 31-Jan-2019

Summary

Official Symbol	Ank provided by MGI
Official Full Name	progressive ankylosis provided by MGI
Primary source	MGI:MGI:3045421
See related	Ensembl:ENSMUSG00000022265
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	Ankh, D15Ertd221e, ank, mKIAA1581
Expression	Ubiquitous expression in heart adult (RPKM 65.4), cerebellum adult (RPKM 43.1) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

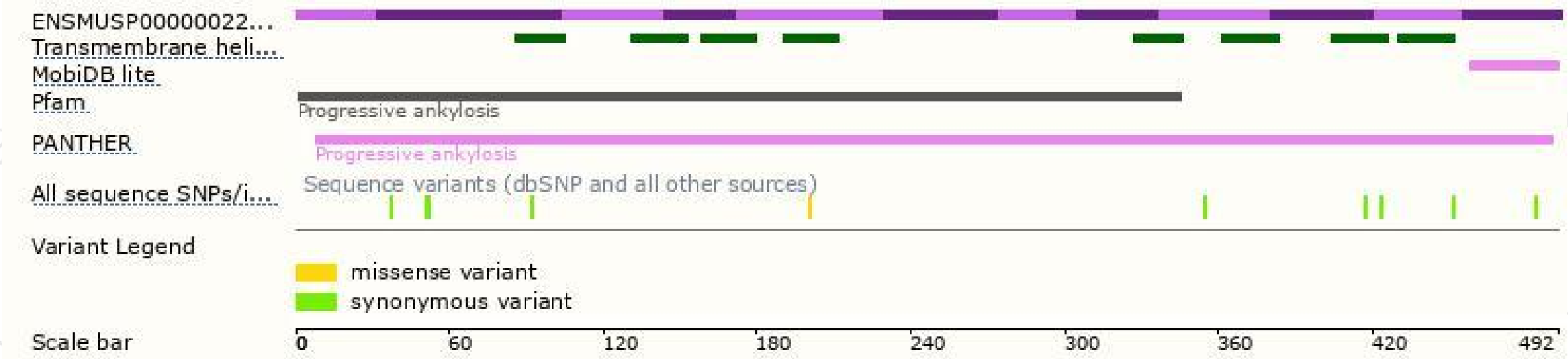
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ank-201	ENSMUST00000022875.6	3498	492aa	Protein coding	CCDS27402	Q3UG85 Q9JHZ2	TSL:1 GENCODE basic APPRIS P1
Ank-202	ENSMUST00000134004.2	1911	No protein	Retained intron	-	-	TSL:1

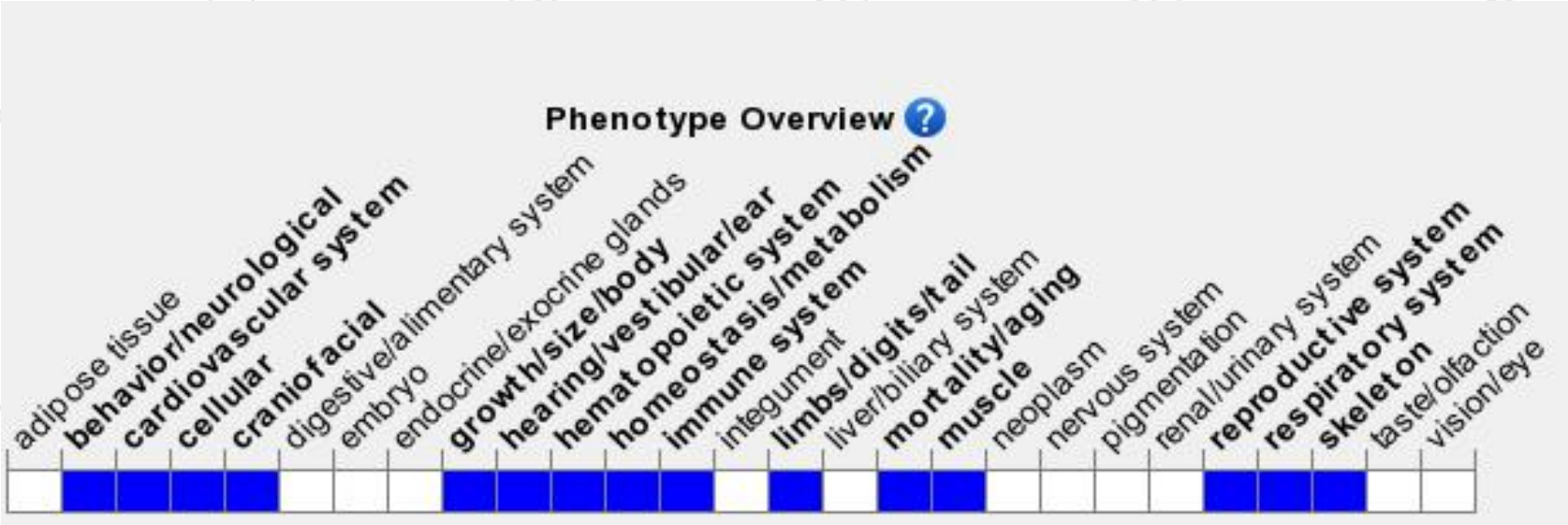
The strategy is based on the design of *Ank-201* transcript,The transcription is shown below

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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