Ankrd45 Cas9-CKO Strategy

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Design Date: 2020-11-25

Project Overview

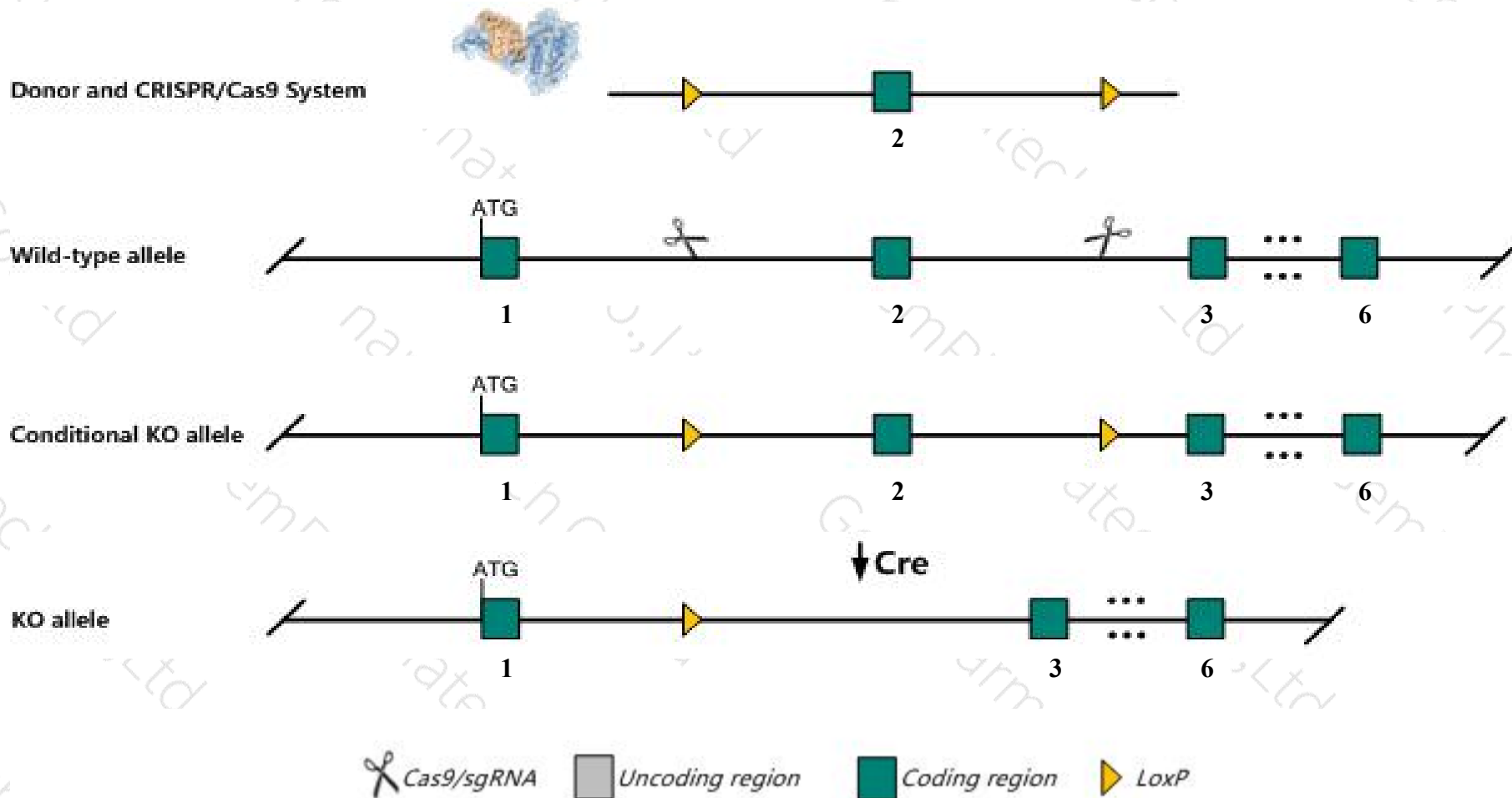|	
Project Name	*Ankrd45*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ankrd45* gene. The schematic diagram is as follows:

Technical routes

➤ The *Ankrd45* gene has 5 transcripts. According to the structure of *Ankrd45* gene, exon2 of *Ankrd45-201*(ENSMUST00000052245.8) transcript is recommended as the knockout region.The region contains Ens 353bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Ankrd45* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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

➢ The *Ankrd45* gene is located on the Chr1. 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）

Ankrd45 ankyrin repeat domain 45 [Mus musculus (house mouse)]

Gene ID: 73844, updated on 13-Mar-2020

▲ Summary

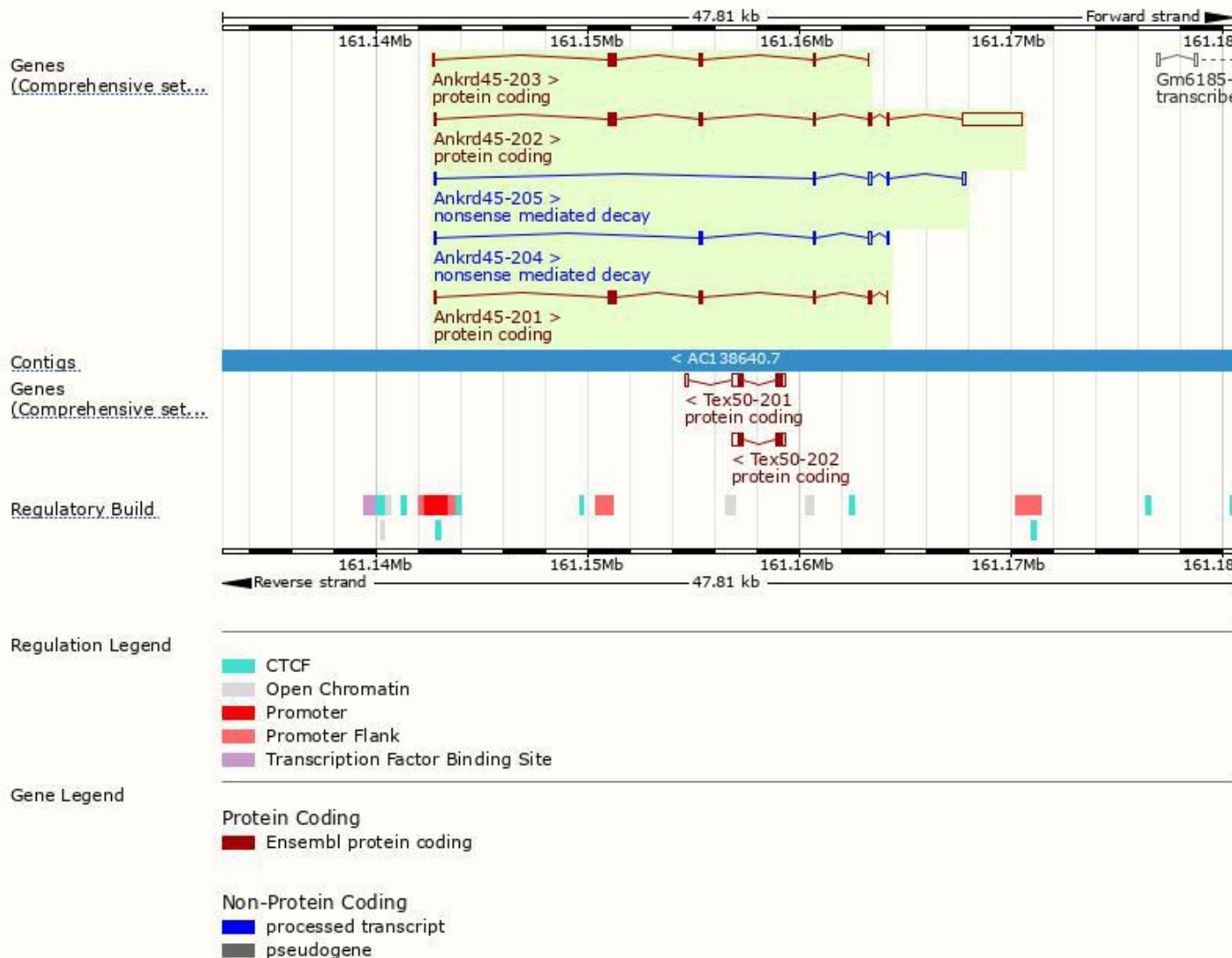|	
Official Symbol	Ankrd45 provided by MGI
Official Full Name	ankyrin repeat domain 45 provided by MGI
Primary source	MGI:MGI:1921094
See related	Ensembl:ENSMUSG00000044835
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4933409K03Rik
Expression	Biased expression in frontal lobe adult (RPKM 11.2), CNS E18 (RPKM 7.8) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 5 transcripts,all transcripts are shown below:

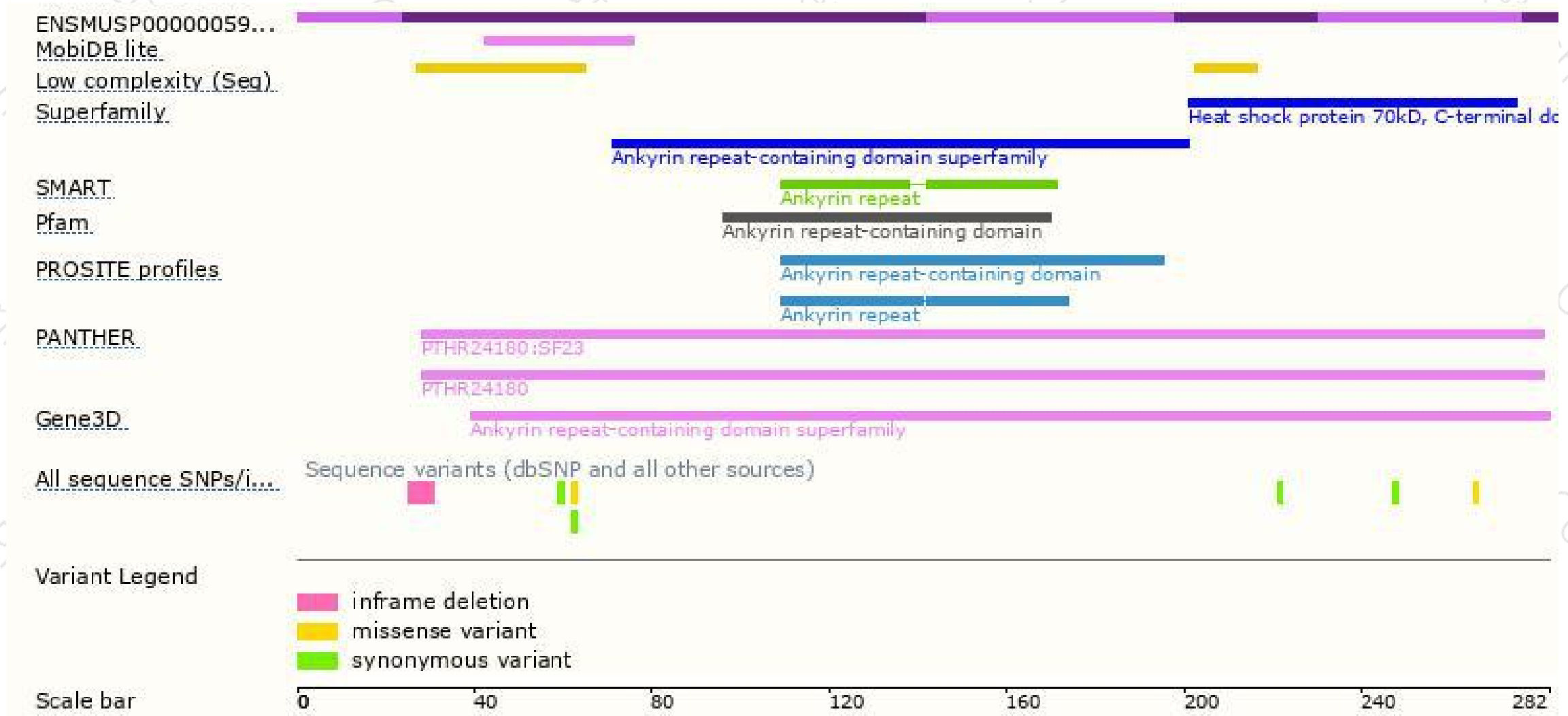| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ankrd45-201	ENSMUST00000052245.8	849	282aa	Protein coding	CCDS48414	G3X9E0	TSL:5 GENCODE basic APPRIS P2
Ankrd45-202	ENSMUST00000111608.7	3714	248aa	Protein coding	-	Q810N6	TSL:1 GENCODE basic APPRIS ALT2
Ankrd45-203	ENSMUST00000125018.7	673	205aa	Protein coding	-	D3YVW9	CDS 3' incomplete TSL:5
Ankrd45-204	ENSMUST00000150721.3	546	49aa	Nonsense mediated decay	-	A0A0A6YY30	TSL:3
Ankrd45-205	ENSMUST00000192384.5	523	42aa	Nonsense mediated decay	-	A0A0A6YVY8	TSL:3

The strategy is based on the design of *Ankrd45-201* transcript,the transcription is shown below:

Genomic location distribution

Protein domain

ENSMUSP00000059...		
MobiDB lite		
Low complexity (Seg)		
Superfamily		Heat shock protein 70kD, C-terminal do
	Ankyrin repeat-containing domain superfamily	
SMART	Ankyrin repeat	
Pfam	Ankyrin repeat-containing domain	
PROSITE profiles	Ankyrin repeat-containing domain	
	Ankyrin repeat	
PANTHER	PTHR24180:SF23	
	PTHR24180	
Gene3D	Ankyrin repeat-containing domain superfamily	
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)	

Variant Legend

- inframe deletion
- missense variant
- synonymous variant

Scale bar 0   40   80   120   160   200   240   282

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