Necab3 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2020-8-11

Project Overview

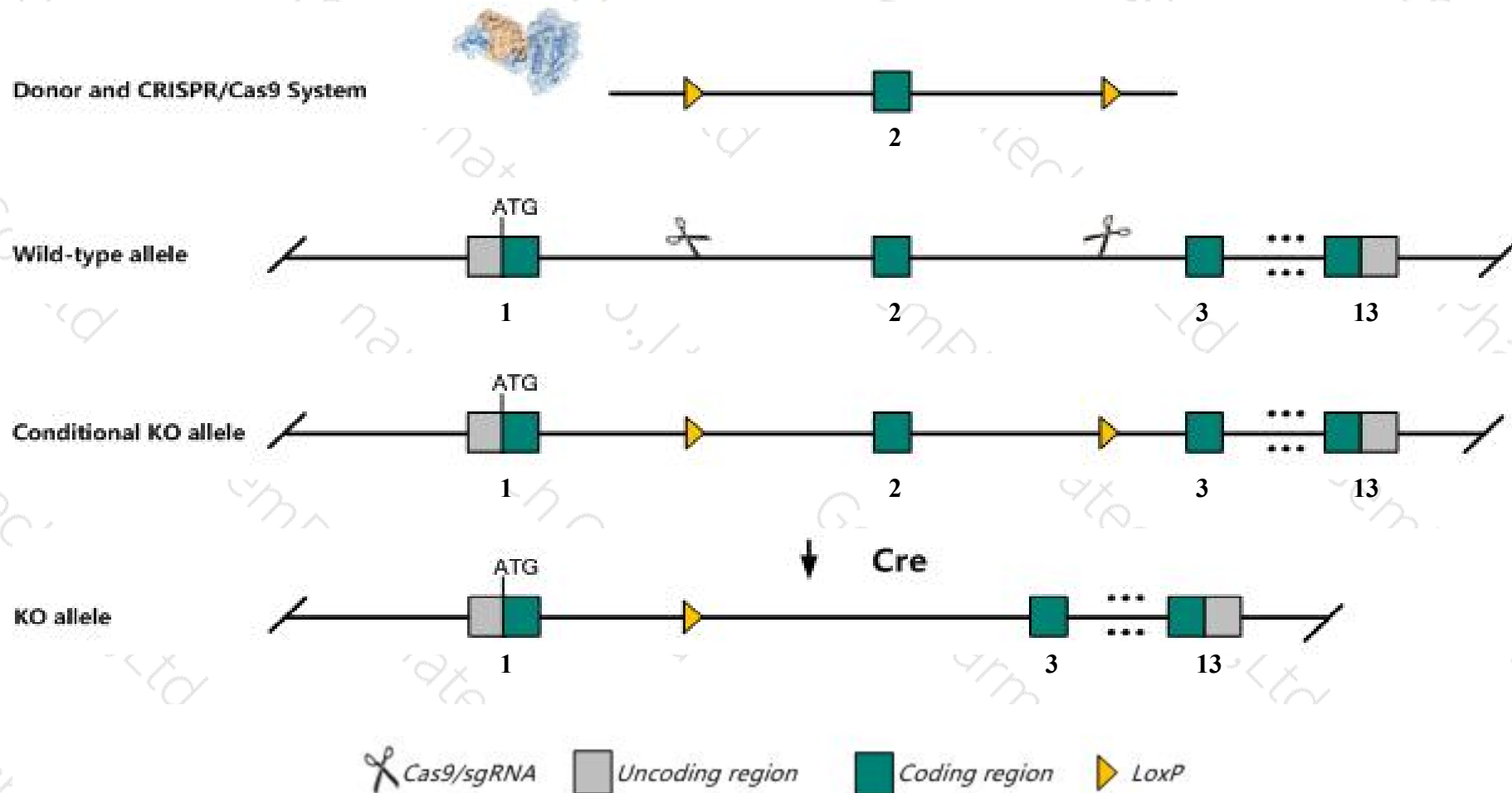|	
Project Name	*Necab3*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Necab3* gene. The schematic diagram is as follows:

Technical routes

➢ The *Necab3* gene has 7 transcripts. According to the structure of *Necab3* gene, exon2 of *Necab3-201*(ENSMUST00000000895.12) transcript is recommended as the knockout region.The region contains 25bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Necab3* 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 *Necab3* 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.

➢ Transcript *Necab3*-205 may not be affected.

➢ 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）

Necab3 N-terminal EF-hand calcium binding protein 3 [Mus musculus (house mouse)]

Gene ID: 56846, updated on 26-Mar-2020

▲Summary

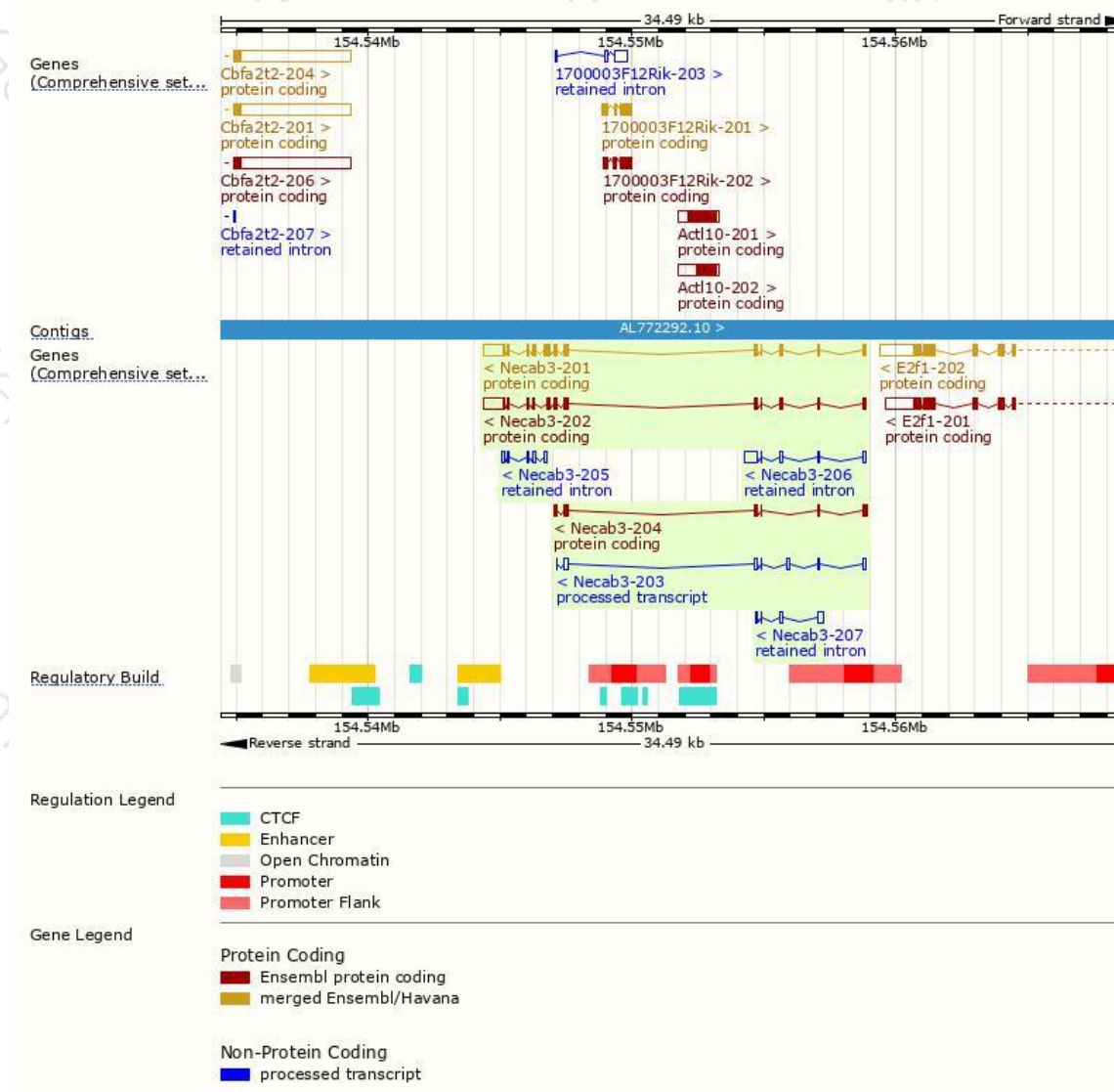|	
Official Symbol	Necab3 provided by MGI
Official Full Name	N-terminal EF-hand calcium binding protein 3 provided byMGI
Primary source	MGI:MGI:1861721
See related	Ensembl:ENSMUSG00000027489
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2900010M17Rik, AI853434, Apba2bp, Nip1, XB51
Expression	Biased expression in frontal lobe adult (RPKM 19.4), cortex adult (RPKM 18.6) and 8 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
Necab3-201	ENSMUST00000000895.12	1829	353aa	Protein coding	CCDS50766	Q9D6J4	TSL:1 GENCODE basic APPRIS P2
Necab3-202	ENSMUST0000109716.8	1742	329aa	Protein coding	-	Q9D6J4	TSL:1 GENCODE basic APPRIS ALT2
Necab3-204	ENSMUST0000125793.1	556	168aa	Protein coding	-	A2AKF0	CDS 3' incomplete TSL:5
Necab3-203	ENSMUST0000124382.7	470	No protein	Processed transcript	-	-	TSL:5
Necab3-206	ENSMUST0000135530.7	730	No protein	Retained intron	-	-	TSL:2
Necab3-205	ENSMUST0000130824.1	531	No protein	Retained intron	-	-	TSL:3
Necab3-207	ENSMUST0000149725.1	435	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Necab3-201* transcript,the transcription is shown below:
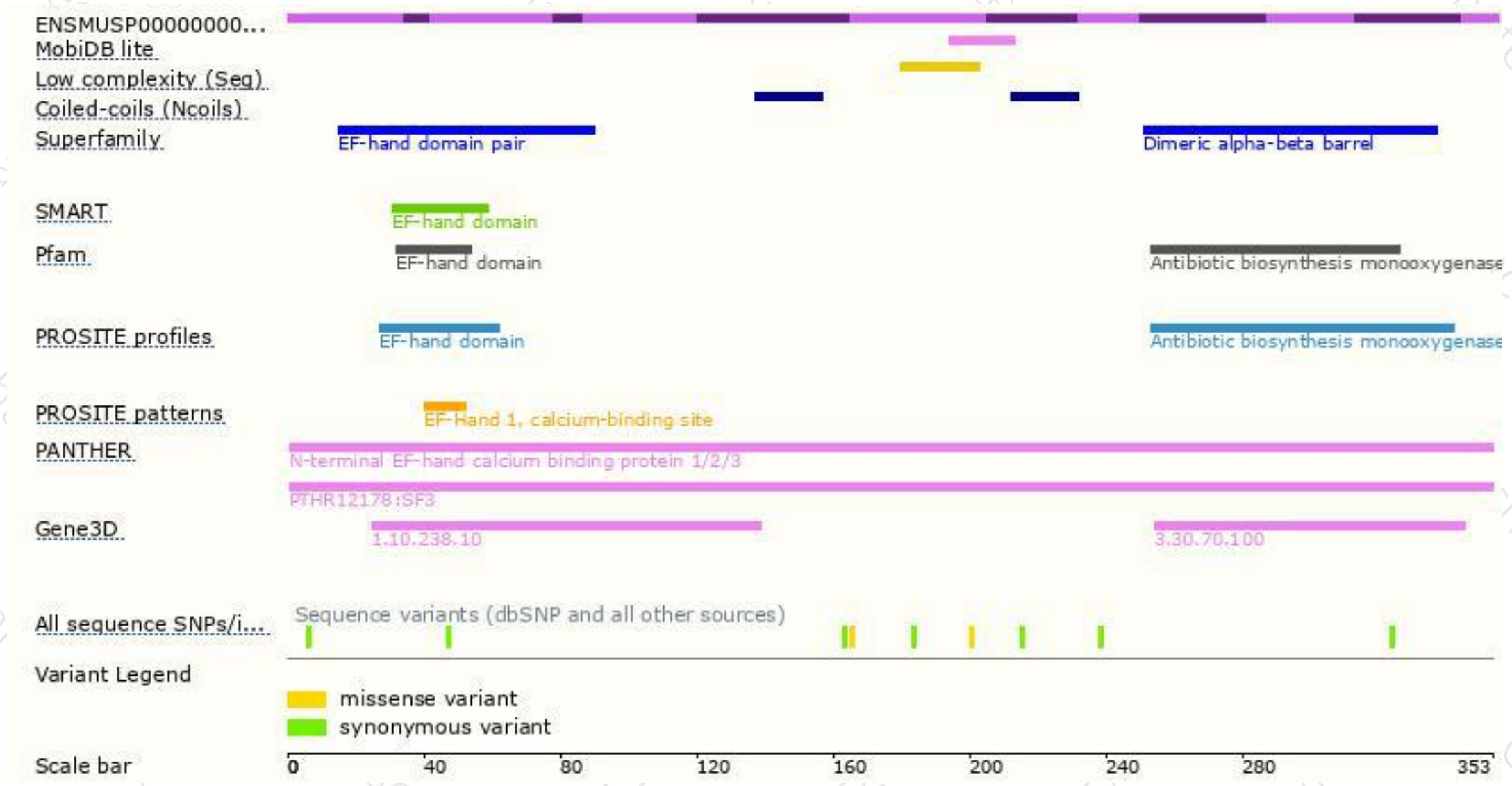

< Necab3-201
protein coding

Reverse strand

14.47 kb
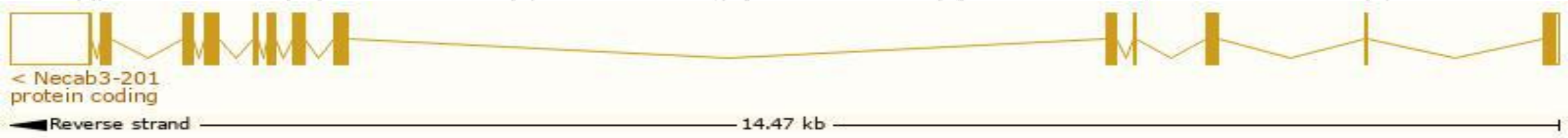
Genomic location distribution

Protein domain

![GemPharmatech 集萃药康 GemPharmatech]

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

集萃药康生物科技
GemPharmatech Co.,Ltd