

Alyref Cas9-CKO Strategy

Designer: Daohua Xu

Reviewer: Yanhua Shen

Design Date: 2022-8-1

Project Overview

Project Name

Alyref

Project type

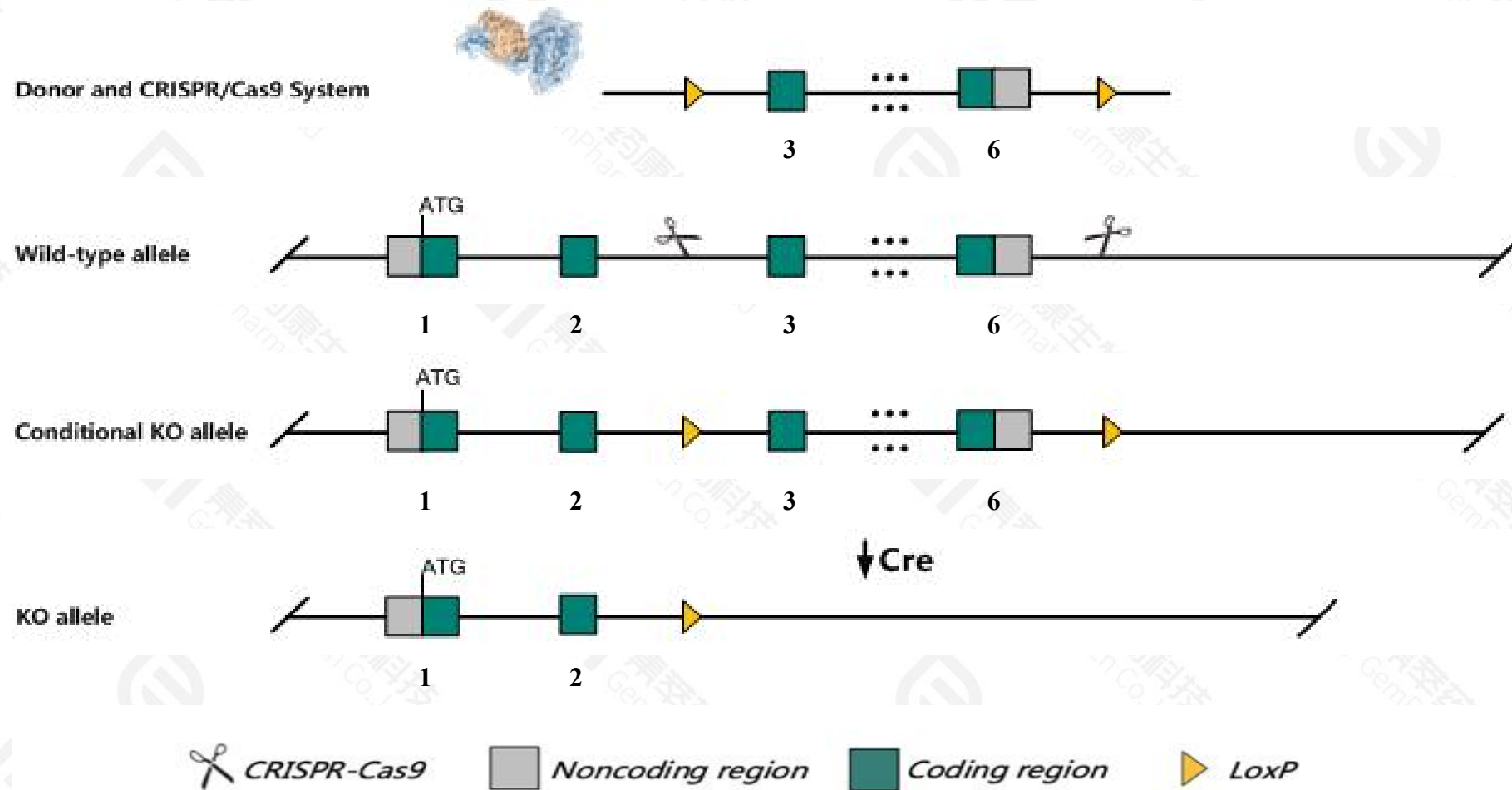
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Alyref* gene has 3 transcripts. According to the structure of *Alyref* gene, exon3-exon6 of *Alyref*-201(ENSMUST00000026125.3) transcript is recommended as the knockout region. The region contains most 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 *Alyref* 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.

- This strategy may affect the regulation of the 5-terminal of *Anapc11* gene.
- The *Alyref* gene is located on the Chr11. 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)

Alyref Aly/REF export factor [*Mus musculus* (house mouse)]

Gene ID: 21681, updated on 24-Apr-2022

[Download Datasets](#)

Summary

Official Symbol Alyref provided by [MGI](#)
Official Full Name Aly/REF export factor provided by [MGI](#)
Primary source [MGI:MGI:1341044](#)
See related [Ensembl:ENSMUSG00000025134](#) [AllianceGenome:MGI:1341044](#)
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 Aly; Ref1; Tho4; Thoc4; Ref1-l; Refbp1
Summary Enables RNA binding activity and single-stranded DNA binding activity. Predicted to be involved in several processes, including mRNA export from nucleus; regulation of nucleobase-containing compound metabolic process; and viral mRNA export from host cell nucleus. Predicted to act upstream of or within RNA splicing; mRNA processing; and mRNA transport. Predicted to be located in cytoplasm. Predicted to be part of catalytic step 2 spliceosome; exon-exon junction complex; and transcription export complex. Predicted to be active in nucleus. Predicted to colocalize with chromosome, telomeric region. Is expressed in several structures, including branchial arch; central nervous system; genitourinary system; hemolymphoid system gland; and sensory organ. Orthologous to human ALYREF (Aly/REF export factor). [provided by Alliance of Genome Resources, Apr 2022]
Expression Broad expression in CNS E11.5 (RPKM 97.1), liver E14 (RPKM 68.0) and 24 other tissues [See more](#)
Orthologs [human](#) [all](#)
NEW Try the new [Gene table](#)
Try the new [Transcript table](#)

Genomic context

Location: 11; 11 E2

[See Alyref in Genome Data Viewer](#)

Exon count: 7

Annotation release	Status	Assembly	Chr	Location
109	current	GRCh39 (GCF_000001635.27)	11	NC_000077.7 (120485330..120489342, complement)
108.20200622	previous assembly	GRCh38.p6 (GCF_000001635.26)	11	NC_000077.6 (120594504..120598399, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	11	NC_000077.5 (120455830..120459679, complement)

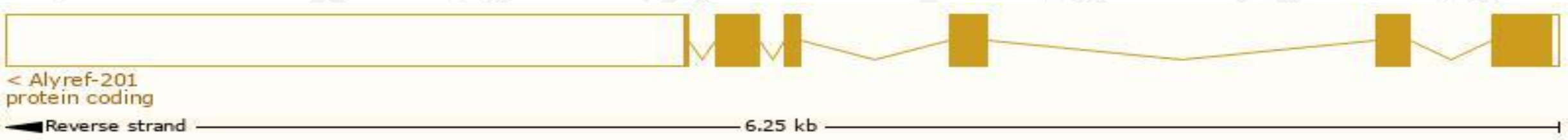


Transcript information (Ensembl)

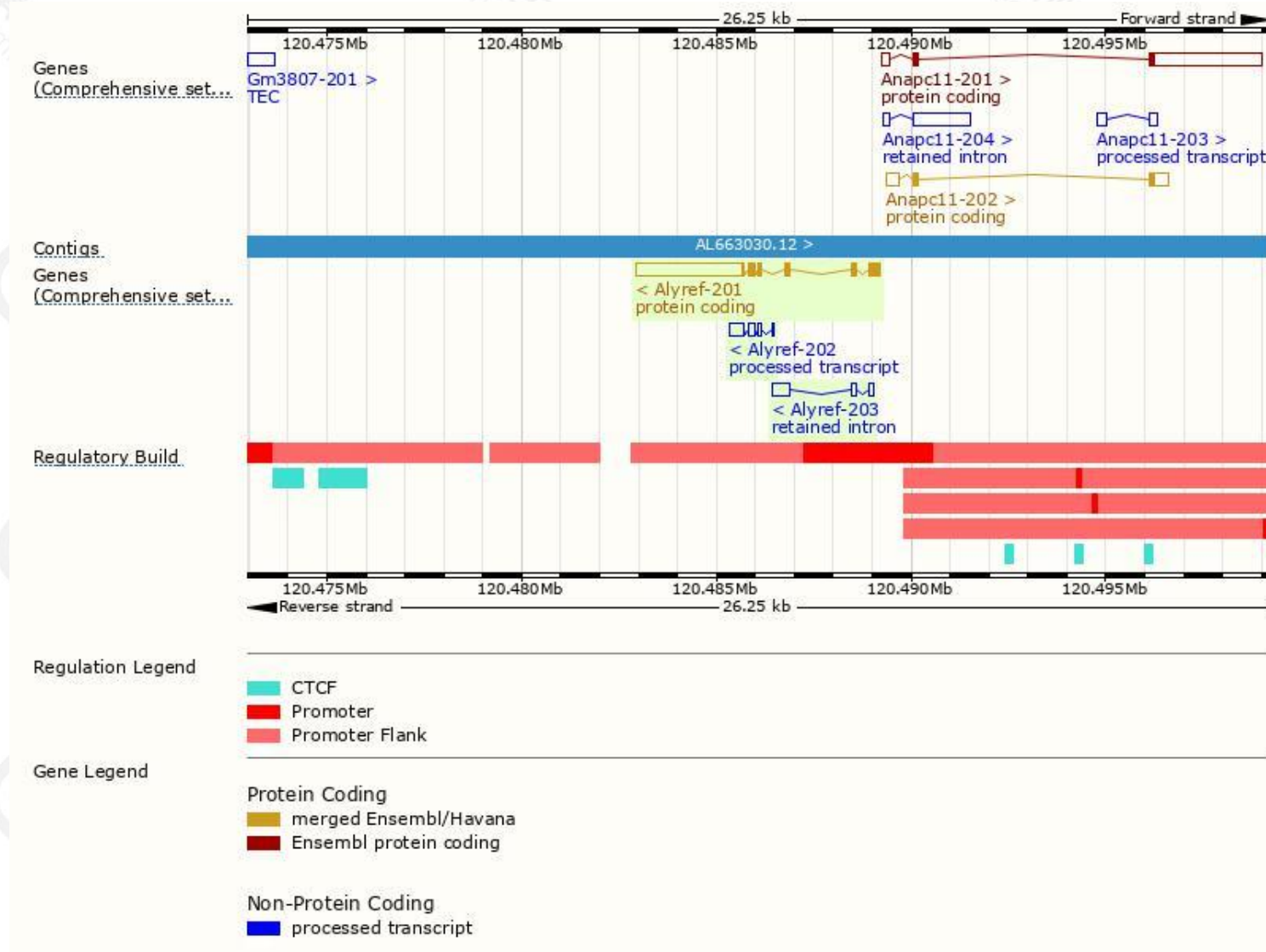
The gene has 3 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags			
ENSMUST00000026125.3	Alyref-201	3527	255aa	Protein coding	CCDS25744	O08583-1	Ensembl Canonical	GENCODE basic	APPRIS P1	TSL:1
ENSMUST00000155325.2	Alyref-202	648	No protein	Processed transcript		-		TSL:3		
ENSMUST00000156685.2	Alyref-203	711	No protein	Retained intron		-		TSL:2		

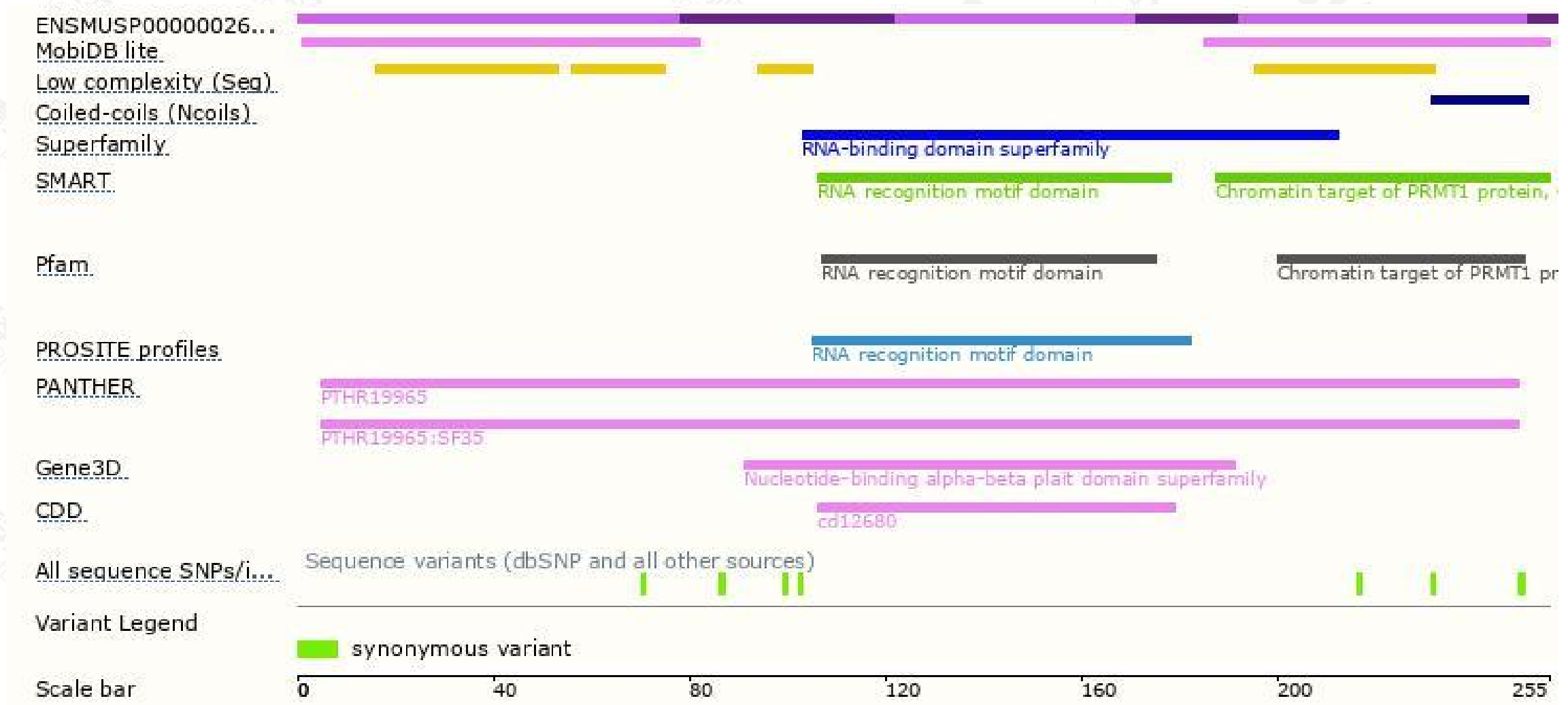
The strategy is based on the design of *Alyref-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 400-9660890

