

Sh2d4a Cas9-CKO Strategy

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Project Overview

Project Name

Sh2d4a

Project type

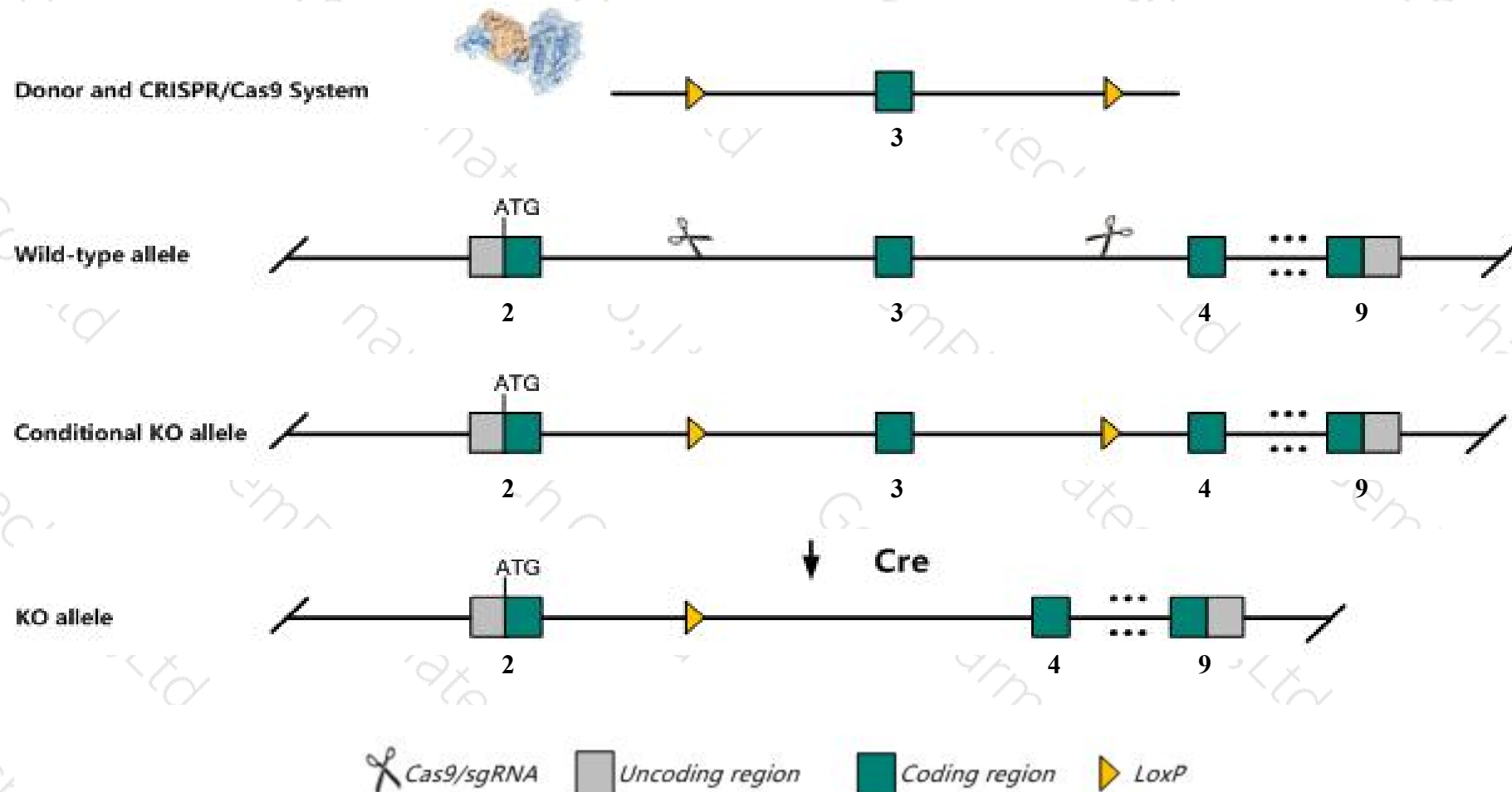
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sh2d4a* gene. The schematic diagram is as follows:



- The *Sh2d4a* gene has 2 transcripts. According to the structure of *Sh2d4a* gene, exon3 of *Sh2d4a-201*(ENSMUST00000066594.3) transcript is recommended as the knockout region. The region contains 157bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Sh2d4a* 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.

- According to the existing MGI data, mice homozygous for a knock-out allele display normal T cell development, homeostasis, proliferation, and function.
- The floxed region is near to the N-terminal of *Gm45739* gene, this strategy may influence the regulatory function of the N-terminal of *Gm45739* gene.
- The *Sh2d4a* gene is located on the Chr8. 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)

Sh2d4a SH2 domain containing 4A [Mus musculus (house mouse)]

Gene ID: 72281, updated on 13-Mar-2020

Summary



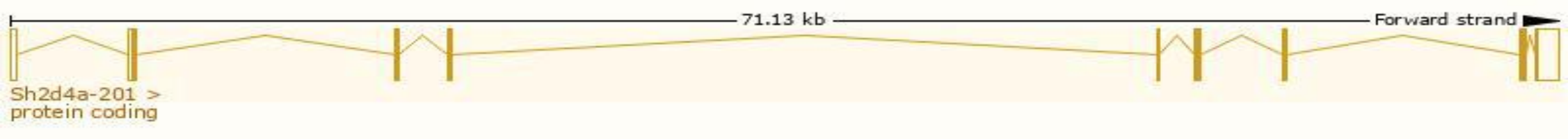
Official Symbol	Sh2d4a provided by MGI
Official Full Name	SH2 domain containing 4A provided by MGI
Primary source	MGI:MGI:1919531
See related	Ensembl:ENSMUSG00000053886
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2210402M20Rik, SH2A
Expression	Broad expression in bladder adult (RPKM 6.9), stomach adult (RPKM 6.8) and 22 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

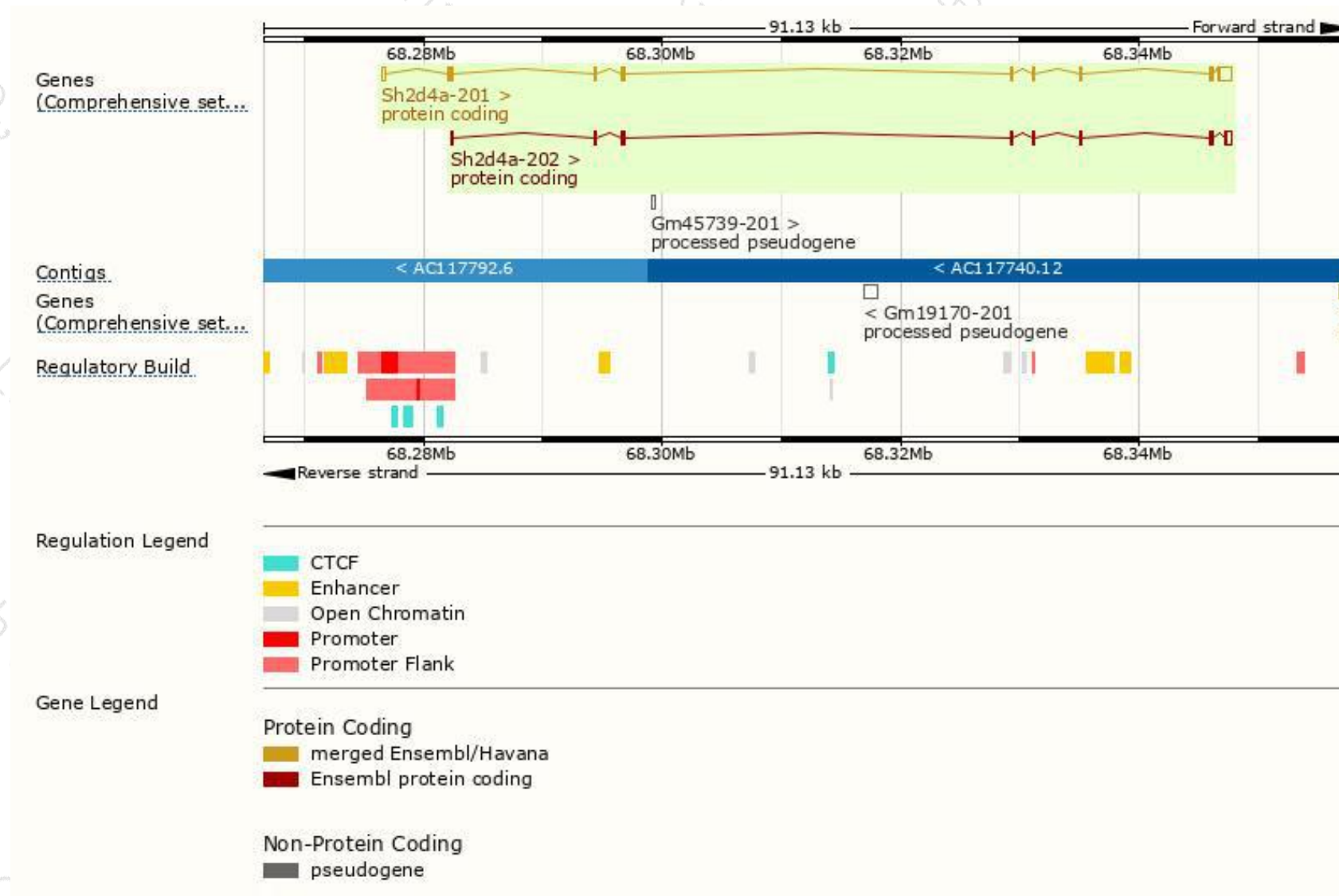
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sh2d4a-201	ENSMUST00000066594.3	2718	421aa	Protein coding	CCDS52564	Q9D7V1	TSL:1 GENCODE basic APPRIS P1
Sh2d4a-202	ENSMUST00000212166.1	1600	390aa	Protein coding	-	A0A1D5RMA9	CDS 5' incomplete TSL:1

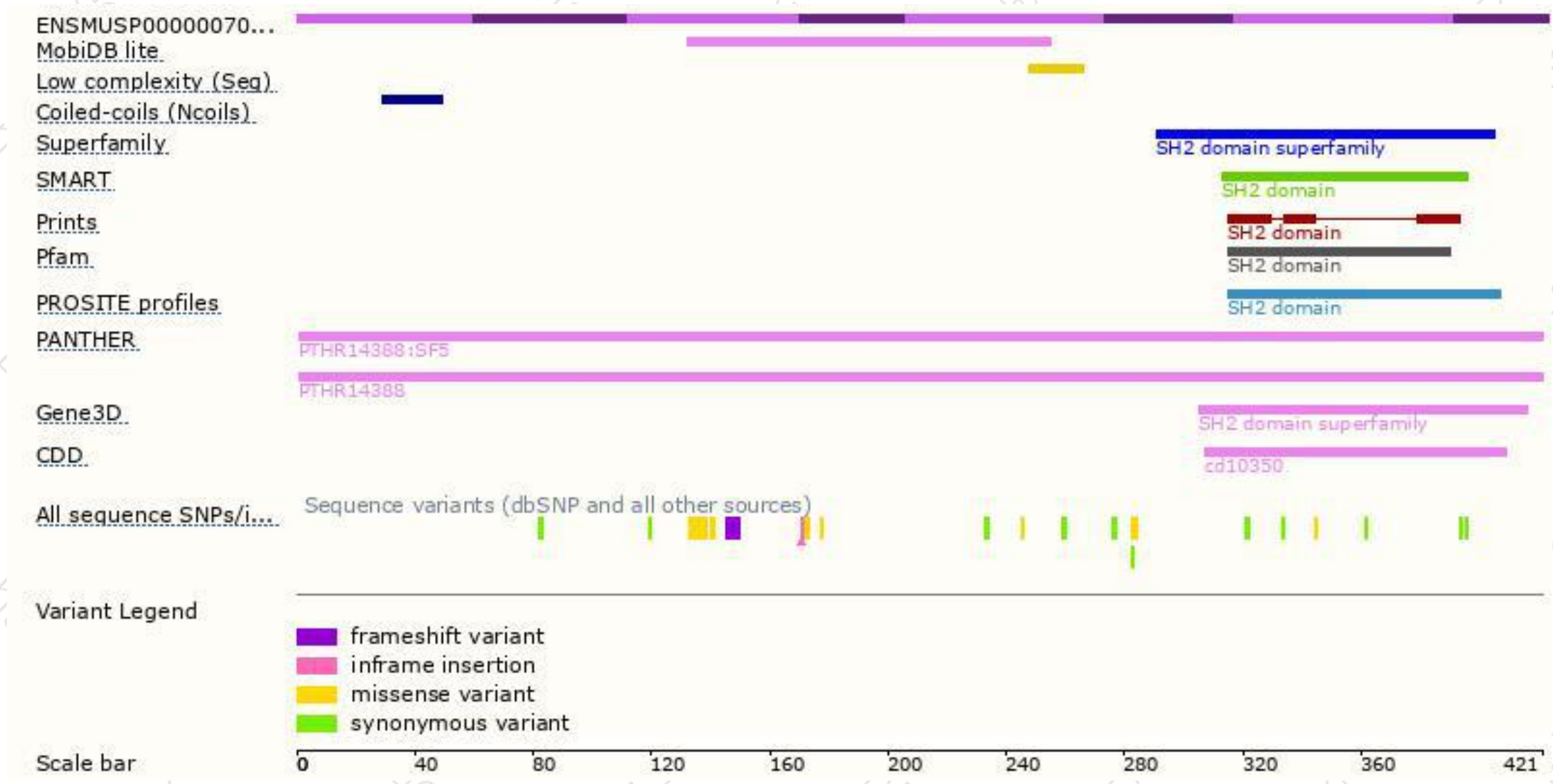
The strategy is based on the design of *Sh2d4a-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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