Ldlr-p.D622N Mouse Model Strategy

-CRISPR/Cas9 technology

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Reviewer **Xiaojing Li**

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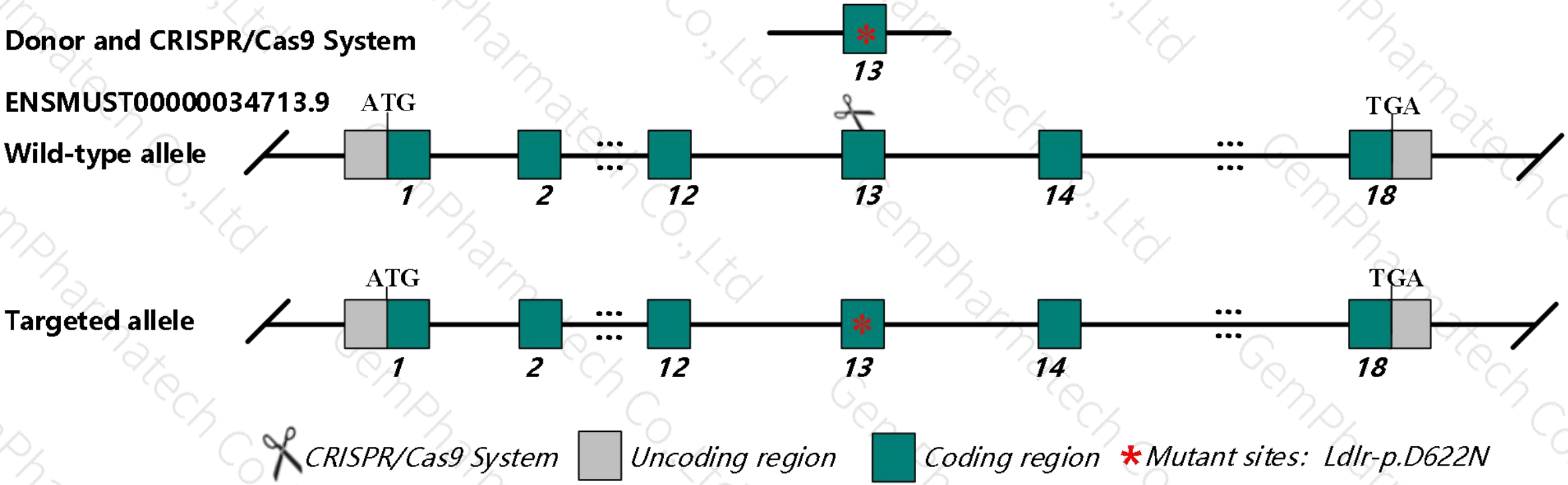
Project Overview

Project Name	Ldlr-p.D622N
Project Type	Cas9-KI(PM)
Background	C57BL/6JGpt
Project Cycle	5-8 months

Strategy

This model uses CRISPR/Cas9 technology to edit the *Ldlr* gene and the schematic diagram is as follow:



Technical Description

- According to the data of Ensembl, mouse *Ldlr* gene has 7 transcripts.
- The mouse model will introduce *p.D622N* point mutation in exon13 of *Ldlr*-201 (ENSMUST00000034713.9), the 622th amino acid of *Ldlr* is mutated from D(Asp) to N(Asn).
- *Ldlr*-201 has 18 exons,the translation start codon ATG is located in exon1, and the translation stop codon TGA is located in exon18, which encodes 862 amino acids.
- In this project, *Ldlr* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and gRNA were constructed in vitro, Cas9,donor and gRNA were microinjected into the fertilized eggs of C57BL/6JGpt mice, and obtained positive F0 generation mice. The F0 positive mice were mated with C57BL/6JGpt mice,the pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the data of MGI, homozygous targeted mutants exhibit 2X higher total plasma cholesterol and 7-9X higher IDL and LDL levels on a normal diet compared to controls. On a high cholesterol diet, mutant effects dramatically increase and mice develop xanthomatosis and atherosclerosis.
- In addition to the target mutation of p.D622N, it may be necessary to introduce 1~2 amino acid synonymous mutations on exon13.
- Mouse *Ldlr* gene is located on Chr9. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr9, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

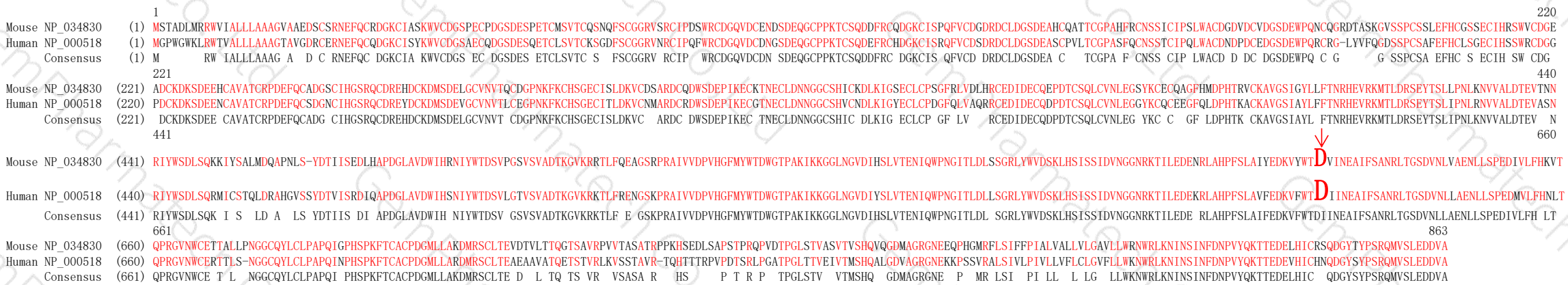
Sequence homology Analysis

Human *LDLR* gene has 5 protein coding transcripts.

Human LDLR Transcripts	Amino Acids
NM_000527.5 → NP_000518.1	860aa
NM_001195798.2 → NP_001182727.1	858aa
NM_001195799.2 → NP_001182728.1	819aa
NM_001195800.2 → NP_001182729.1	692aa
NM_001195803.2 → NP_001182732.1	682aa

Mouse *Ldlr* gene has 3 protein coding transcript.

Mouse Ldlr Transcripts	Amino Acids
NM_001252658.1 → NP_001239587.1	810aa
NM_001252659.1 → NP_001239588.1	861aa
NM_010700.3 → NP_034830.2	862aa

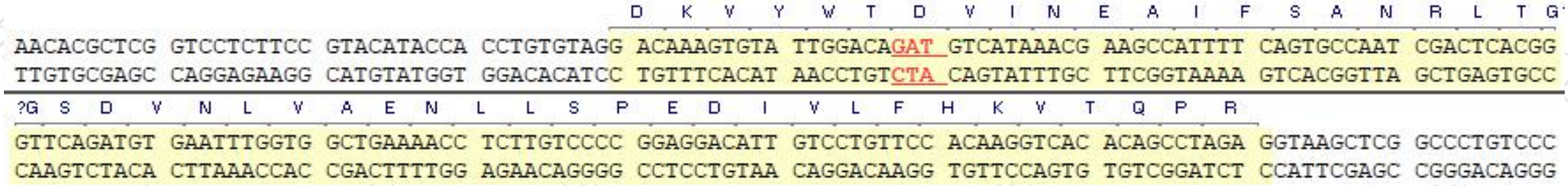


Based on the data of NCBI, there are 5 transcripts of human *LDLR* gene, which encode amino acids of different lengths, and the mouse *Ldlr* gene has 3 transcripts. The homology of human NP_000518.1 and mouse NP_034830.2 is about 78.0%.

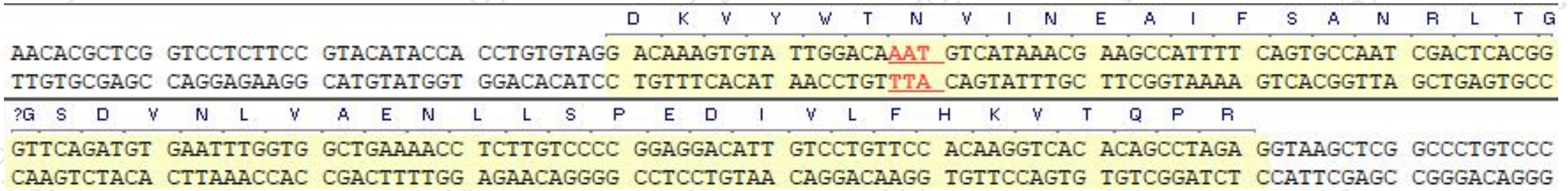
Mutation Site

集萃药康 GemPharmatech

Before mutation



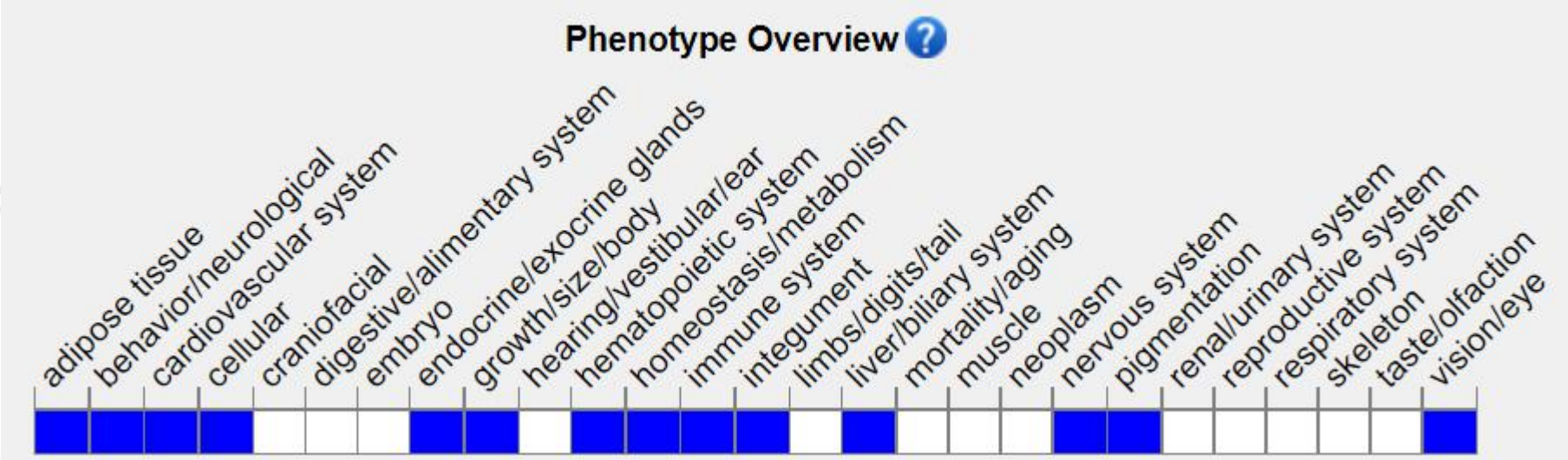
After mutation



The yellow region is exon13 of *Ldlr-201,* and the red region represents the p.D622N mutation site.

Mouse phenotype description(MGI)

http://www.informatics.jax.org/marker/MGI:96765



Homozygous targeted mutants exhibit 2X higher total plasma cholesterol and 7-9X higher IDL and LDL levels on a normal diet compared to controls. On a high cholesterol diet, mutant effects dramatically increase and mice develop xanthomatosis and atherosclerosis.

Gene name and location （NCBI)

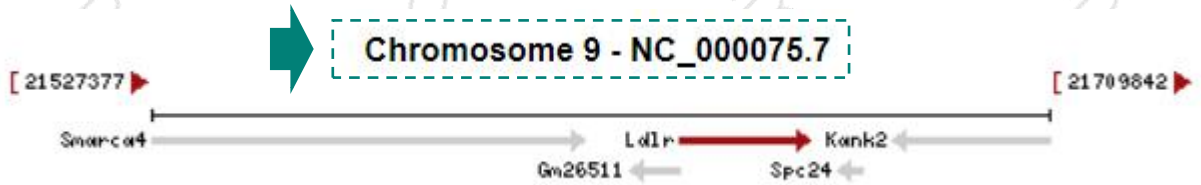
Ldlr low density lipoprotein receptor [*Mus musculus* (house mouse)]

Gene ID: 16835, updated on 6-Sep-2021

Download Datasets

Summary

Official Symbol	Ldlr provided by MGI
Official Full Name	low density lipoprotein receptor provided by MGI
Primary source	MGI:MGI:96765
See related	Ensembl:ENSMUSG00000032193
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	Hlb301
Expression	Ubiquitous expression in colon adult (RPKM 56.3), adrenal adult (RPKM 52.3) and 27 other tissues See more
Orthologs	human all

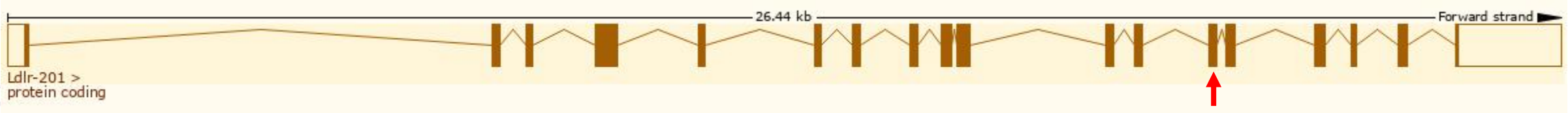


Transcript information （Ensembl）

The gene has 7 transcripts, and all transcripts are shown below:

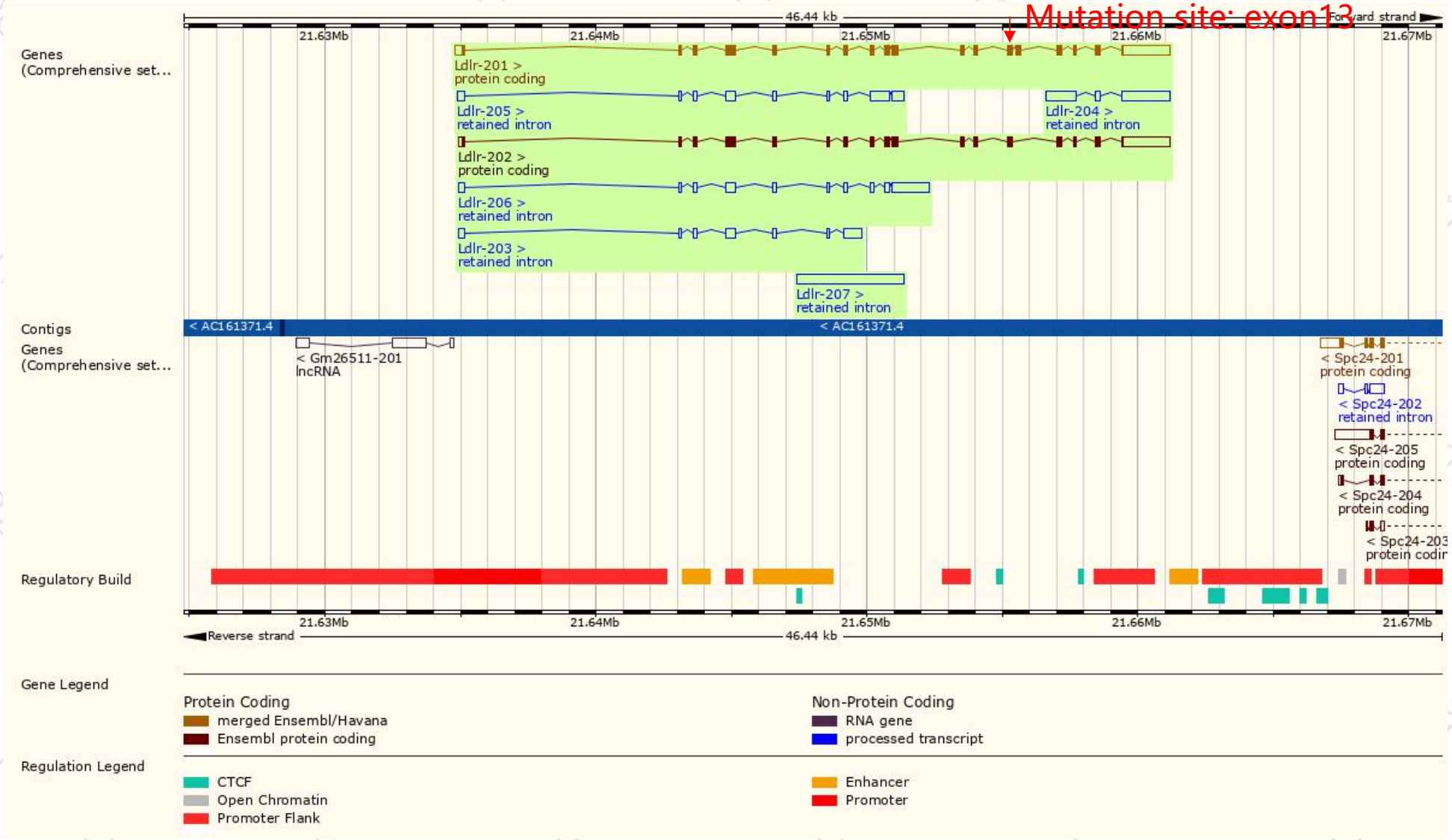
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match ▲	Flags
Ldlr-202	ENSMUST00000213114.2	4318	810aa	Protein coding	-	A0A1L1SRE8	GENCODE basic TSL:1
Ldlr-201	ENSMUST00000034713.9	4627	862aa	Protein coding	CCDS22910	P35951	GENCODE basic APPRIS P1 TSL:1
Ldlr-207	ENSMUST00000217613.2	3980	No protein	Retained intron	-	-	TSL:NA
Ldlr-204	ENSMUST00000214549.2	3049	No protein	Retained intron	-	-	TSL:1
Ldlr-206	ENSMUST00000217111.2	2854	No protein	Retained intron	-	-	TSL:1
Ldlr-205	ENSMUST00000215917.2	2383	No protein	Retained intron	-	-	TSL:1
Ldlr-203	ENSMUST00000214359.2	1739	No protein	Retained intron	-	-	TSL:1

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



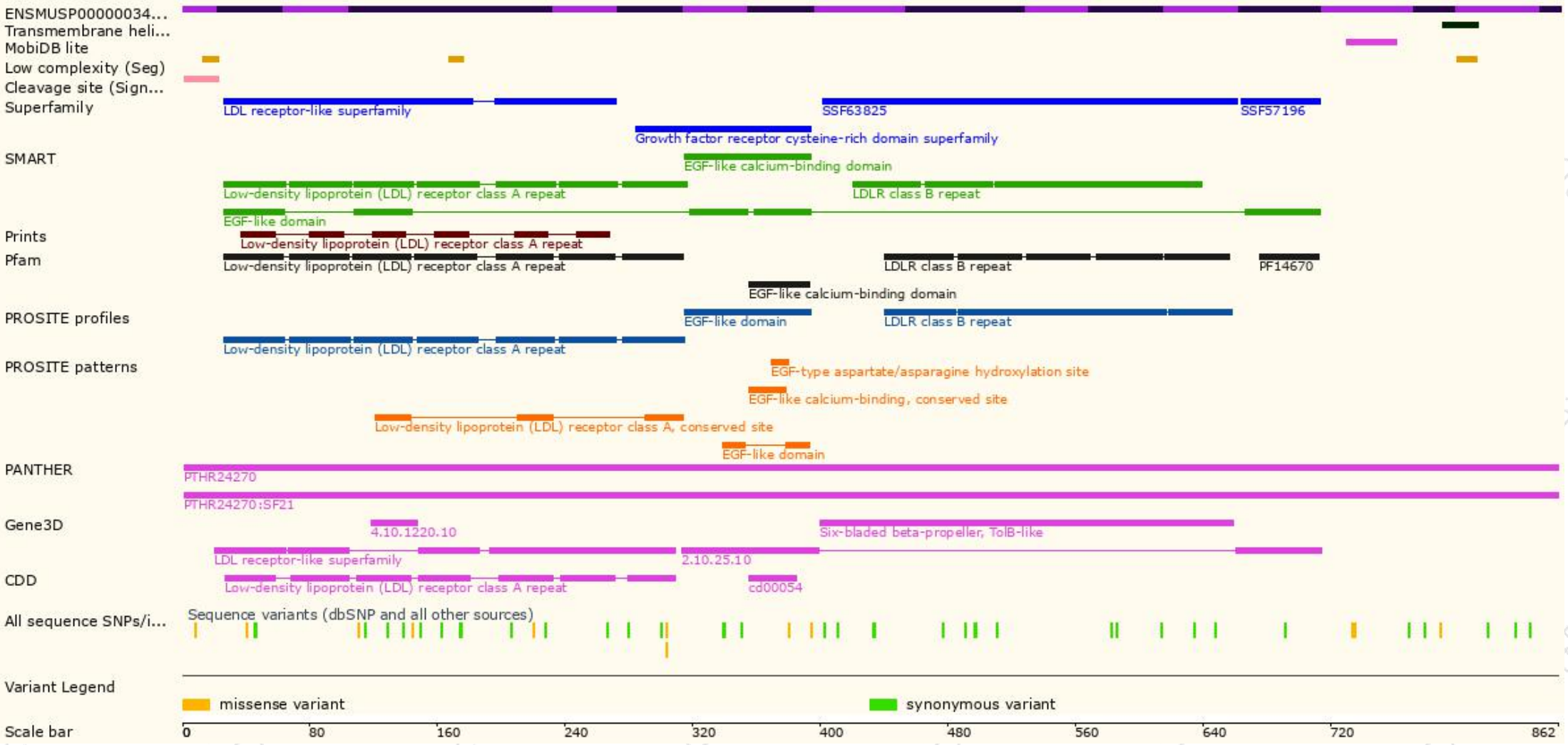
Mutation site：exon13

Genomic location distribution



Protein domain

Protein domains for ENSMUSP00000034713.8



If you have any questions, please feel free to contact us.
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