Fabp4-LSL-P2A-DTR Cas9-KI Mouse Model Strategy

Designer: Hui Li

Reviewer: Daohua Xu

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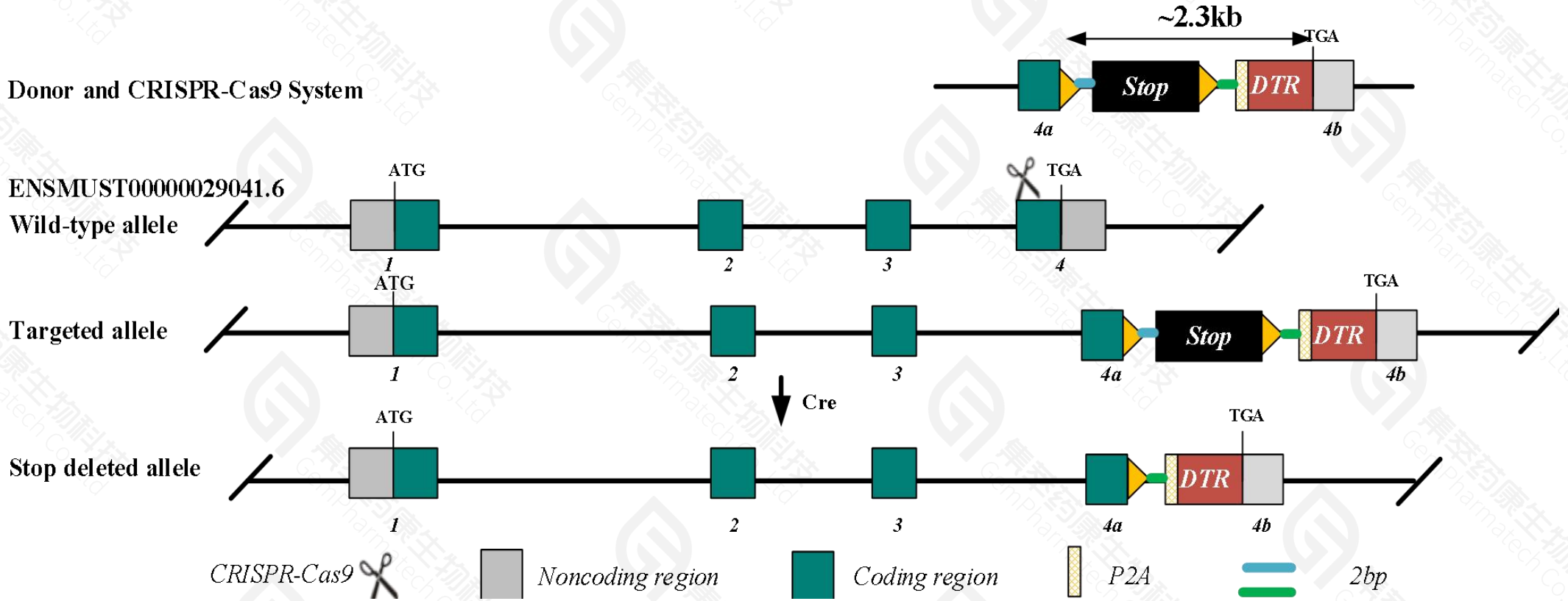
Project Overview

Project Name	**Fabp4-LSL-P2A-DTR**
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR-Cas9 technology to edit the *Fabp4* gene. The schematic diagram is as follows:



The 2bp marked in blue and loxp in the figure form a stop codon; the 2bp marked in green and loxp do not form a stop codon.

Technical routes

➢ Ensembl data show, the *Fabp4* gene has 2 transcripts.

➢ According to the structure of *Fabp4* gene, the element *LSL-P2A-DTR* will be inserted at the translation stop codon of *Fabp4*-201(ENSMUST00000029041.6), the length of inserted fragment is about 2.3 kb.

➢ The mouse *Fabp4*-201 transcript contains 4 exons. The translation initiation site ATG is located at exon 1, and the translation termination site TGA is located at exon 4, encoding 132 aa.

➢ In this project we use CRISPR-Cas9 technology to modify *Fabp4* 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.

Notice

- According to the MGI data，Homozygotes for a targeted null mutation exhibit susceptibility to diet-induced obesity, attenuated dibutyryl cAMP-induced adipocyte release of glycerol and free fatty acid, and reduced acute insulin secretion in response to beta-adrenergic stimulation.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Before breeding with Cre mice, the C-terminus of *Fabp4* protein will have loxp-translated polypeptides, and after breeding with Cre mice, the C-terminus of *Fabp4* protein will have loxp and P2A-translated polypeptides.
- It may be necessary to introduce 1-2 amino acid synonymous mutations in the coding region of exon 4.
- The insertion site is about 6.8 kb from the 5-terminal of *Fabp9* and *GM37389* genes, and this strategy may affect the regulation of the 5-terminal.
- The *Fabp4* gene is located on the Chr 3. 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.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

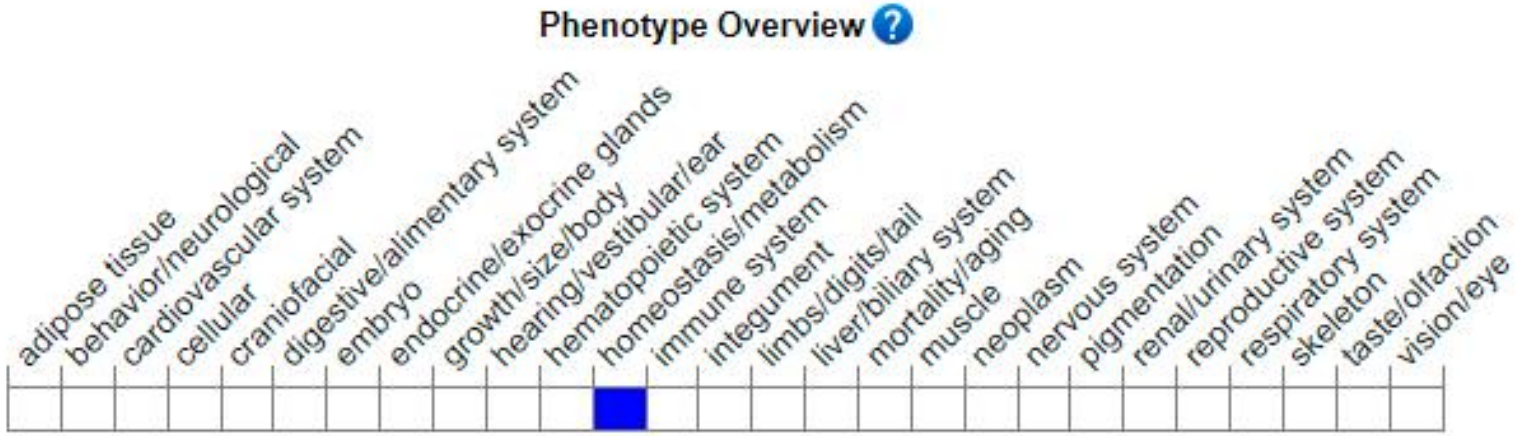
Existing model information

http://www.informatics.jax.org/allele/summary?markerId=MGI:88038&alleleType=Targeted

Export: Text File | Excel File

Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
Fabp4$^{tm1.2Mrl}$ fatty acid binding protein 4, adipocyte; targeted mutation 1.2, Merck Research Laboratory	3		Targeted (Null/knockout)		
Fabp4^{tm1Brsp} fatty acid binding protein 4, adipocyte; targeted mutation 1, Bruce M Spiegelman	3	A-FABP/aP2, aP2-	Targeted (Null/knockout)	homeostasis	
Fabp4$^{tm2.2Mrl}$ fatty acid binding protein 4, adipocyte; targeted mutation 2.2, Merck Research Laboratory	3		Targeted (Null/knockout)		
Gt(ROSA)26Sor$^{tm3(RNAi:Fabp4)Mrl}$ gene trap ROSA 26, Philippe Soriano; targeted mutation 3, Merck Research Laboratory	6	shFABP4	Targeted Involves 1 genes (Fabp4) View all	adipose, behavior, growth/size/body	
Fabp4$^{tm1(KOMP)Vlcg}$ fatty acid binding protein 4, adipocyte; targeted mutation 1, Velocigene	3		Targeted (Null/knockout, Reporter) **(Cell Line)**		
Fabp4$^{tm1a(KOMP)Wtsi}$ fatty acid binding protein 4, adipocyte; targeted mutation 1a, Wellcome Trust Sanger Institute	3		Targeted (Conditional ready, Null/knockout, Reporter) **(Cell Line)**		
Fabp4$^{tm1e(KOMP)Wtsi}$ fatty acid binding protein 4, adipocyte; targeted mutation 1e, Wellcome Trust Sanger Institute	3		Targeted (Null/knockout, Reporter) **(Cell Line)**		
Fabp4$^{tm2a(KOMP)Wtsi}$ fatty acid binding protein 4, adipocyte; targeted mutation 2a, Wellcome Trust Sanger Institute	3		Targeted (Conditional ready, Null/knockout, Reporter) **(Cell Line)**		

Mouse phenotype description(MGI)



http://www.informatics.jax.org/marker/MGI:88038

Homozygotes for a targeted null mutation exhibit susceptibility to diet-induced obesity, attenuated dibutyryl cAMP-induced adipocyte release of glycerol and free fatty acid, and reduced acute insulin secretion in response to beta-adrenergic stimulation.

Information of *Fabp4* Gene

Target Gene	*Fabp4*
Gene ID(NCBI)	11770
Link(NCBI)	https://www.ncbi.nlm.nih.gov/gene/11770
Link(Ensembl)	http://uswest.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000062515;r=3:10269148-10273636;t=ENSMUST00000029041
Chromosome Location	Chr 3

Gene information (NCBI)

Fabp4 fatty acid binding protein 4, adipocyte [*Mus musculus* (house mouse)]

Download Datasets

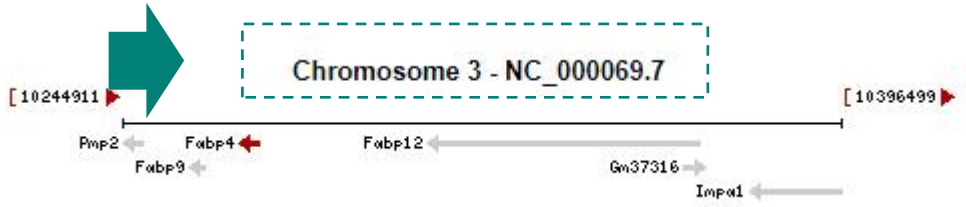
Gene ID: 11770, updated on 28-Oct-2021

Summary

Official Symbol	Fabp4 provided by MGI
Official Full Name	fatty acid binding protein 4, adipocyte provided by MGI
Primary source	MGI:MGI:88038
See related	Ensembl:ENSMUSG00000062515
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	Ap2; P15; ALBP; Lbpl; AFABP; 422/aP2; ALBP/Ap2
Expression	Biased expression in subcutaneous fat pad adult (RPKM 1709.3), genital fat pad adult (RPKM 1107.4) and 4 other tissues See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table



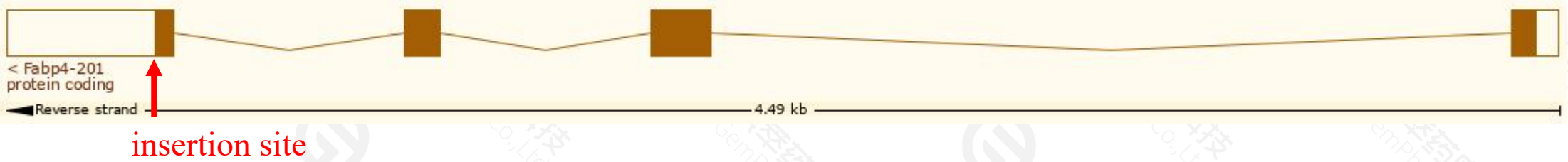
Transcript information（Ensembl）

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

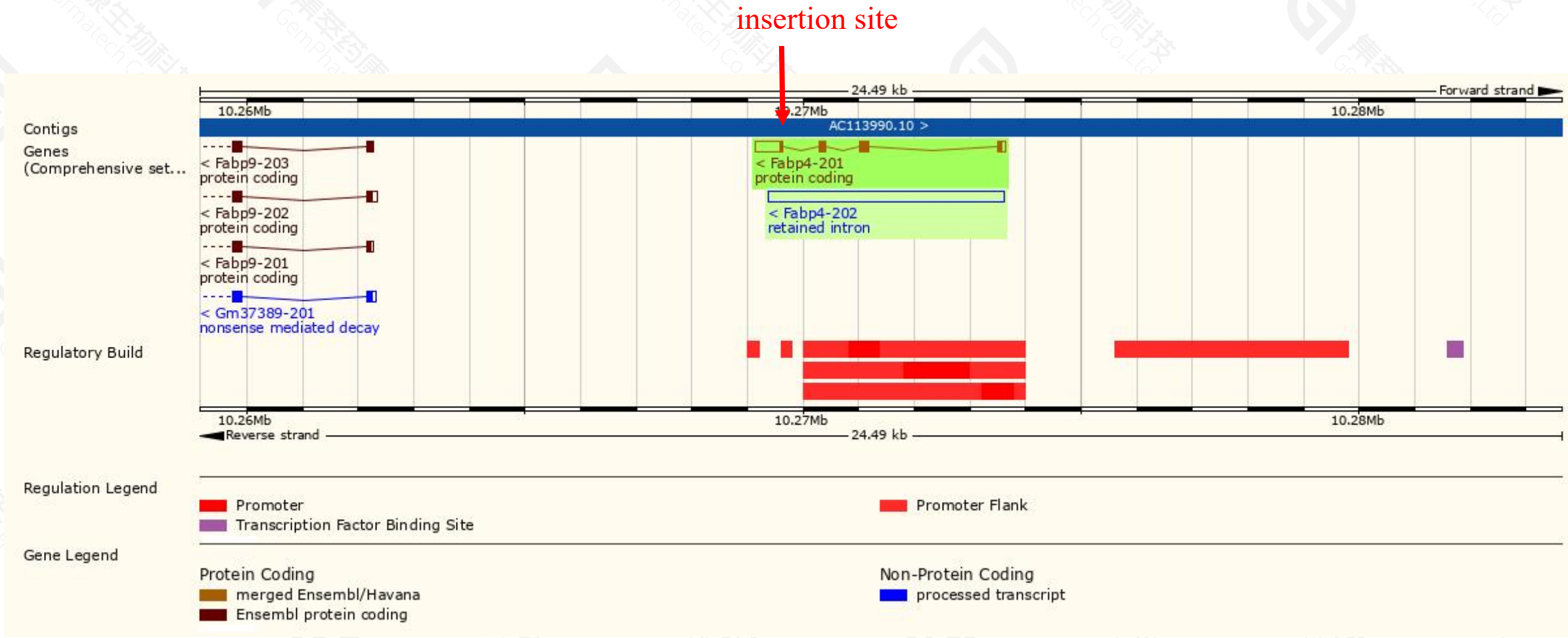
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Fabp4-201	ENSMUST00000029041.6	896	132aa	Protein coding	CCDS17238	P04117 Q542H7	GENCODE basic APPRIS P1 TSL:1
Fabp4-202	ENSMUST00000191757.2	4231	No protein	Retained intron	-	-	TSL:NA

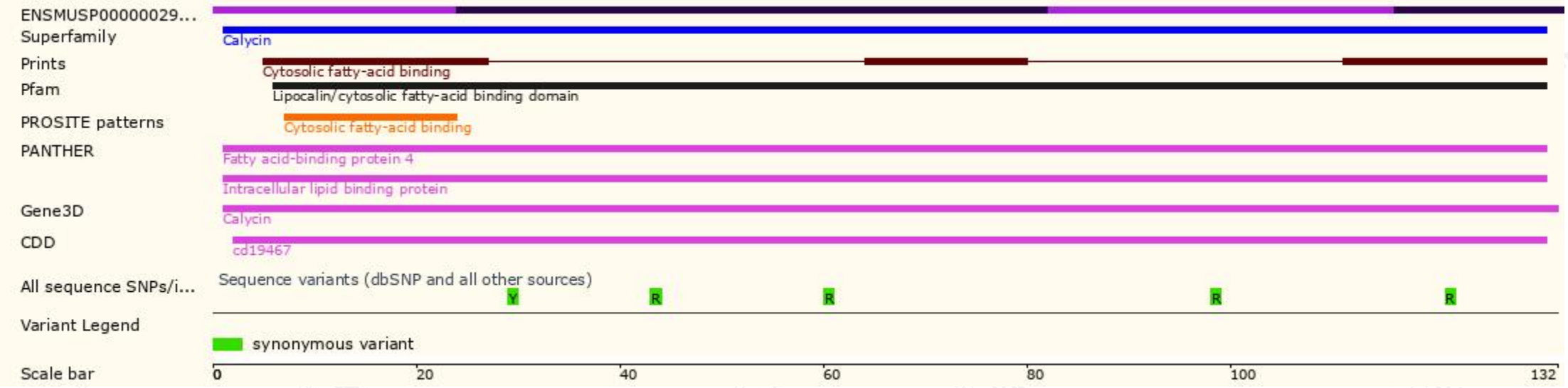
The strategy is based on the design of *Fabp4* -201 transcript,the transcription is shown below:



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 400-966 0890

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