H11-Fabp4-iCre-ployA Cas9-KI Strategy

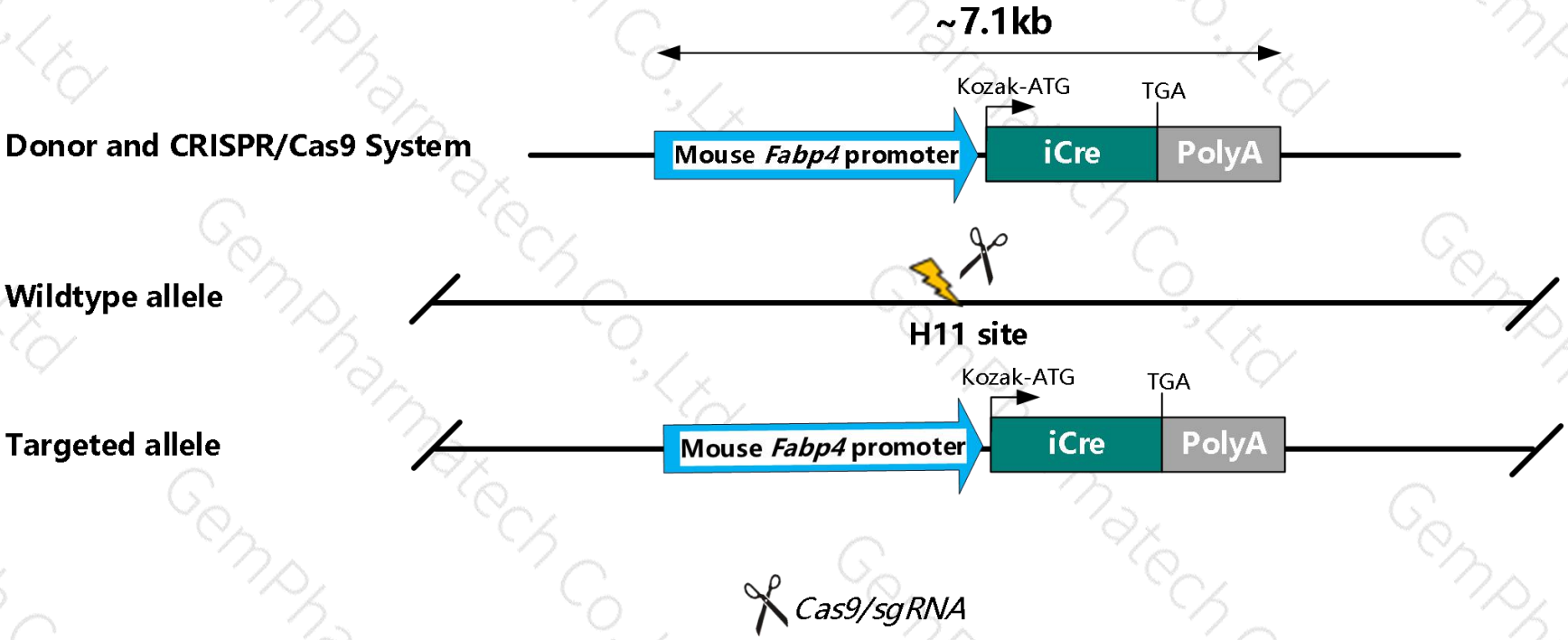
Designer:	**JiaYu**
Design Date:	**2019-8-15**
Reviewer	**Huimin Su**

Project Overview

Project Name	***H11-Fabp4-iCre-ployA***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

The *Fabp4-iCre-ployA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of mouse *Fabp4* promoter [1]

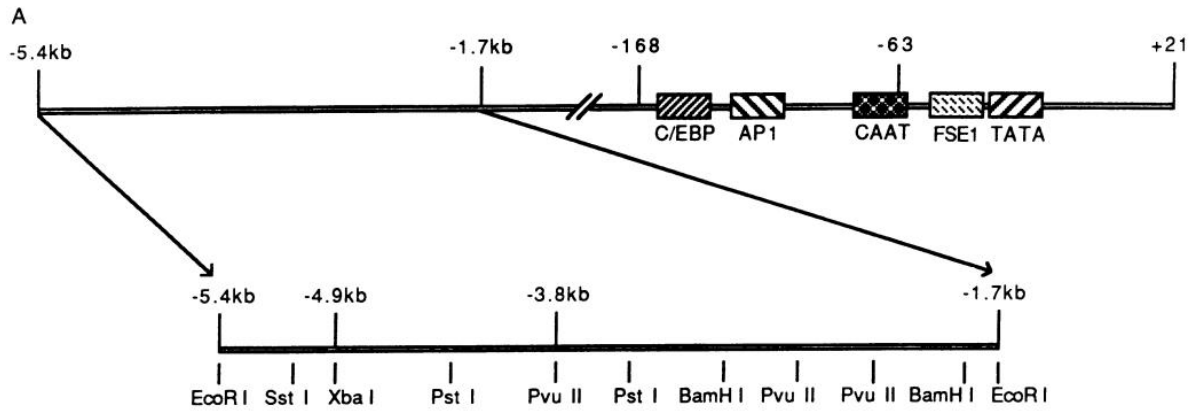


FIG. 1. (*A*) Map of the aP2 5′ flanking region used to construct transgenes. FSE1, fat-specific element 1. (*B*) Map of the −168aP2, −247aP2, −1.7aP2, and −5.4aP2 constructs used to make transgenic mice. Open box, aP2 sequences; line, CAT/SV40 sequences. The putative GRE at bp −363 is represented by a solid bar, and the 540-bp sequence containing the 5′ distal enhancer is represented by a stippled box.

Construction of Transgenes. The plasmids used for the 168aP2CAT and 247aP2CAT transgenes have been described (7). For the 1.7aP2CAT transgene, an *Eco*RI (kb −1.7) to *Pst* I (bp +21) fragment was ligated upstream of CAT, and the 5.4aP2CAT transgene was generated by ligating an *Eco*RI fragment containing kb −1.7 to kb −5.4 into the 1.7aP2CAT transgene (see Fig. 1*A*). Deletion constructs of the fragment between kb −5.4 and kb −1.7 were made by blunt-end ligation of the various restriction fragments (see Fig. 1*A*) into *Hin*dIII-digested and filled-in −63aP2CAT (7).

expression and hormone sensitivity. We find that the specific expression of the gene for aP2 in adipocytes derives from an enhancer element at kilobase (kb) −5.4 that directs linked chloramphenicol acetyltransferase (CAT) marker gene expression very strongly and specifically to fat tissue. This

1、SUSAN R. Ross*, REED A. GRAVESt, AMY GREENSTEIN*, KENNETH A. PLATT*, HAI-LUN SHYU*,BARRY MELLOVITZ*, AND BRUCE M. SPIEGELMANt A fat-specific enhancer is the primary determinant of gene expression for adipocyte P2 in vivo. Proc.Nail. Acad. Sci. USA Vol. 87, pp. 9590-9594, December 1990.Biochemistry.

The promoter Sequence of Mouse *Fabp4*(5548bp)

aattcccagcaggaatcaggtagctggagaatcgcacagagccatgcggattcttggcaagccatgcgacaaaggcagaaatgcacatttcacccagagagaagggattgtagtcagcaggaagtcaccacccagagagcaaatggagttcccagatgcctga
catttgccttcttactggatcagagttcactagtggaagtgtcacagcccaaacactcccccaaaggctcagcccttccttgccttgtaacaatcaagccgctcctggatgaactgctccgccctctgtctctttggcagggttggagcccactgtggcctgagcgacttc
tatggctccctttctgtgattttcatggtttctgagctcttttccccgctttatgattttctctttttgtctctctcttgctaaacctccttcgtatatgccctctcaggtttcatttctgaatcatctactgtgaactattcccattgtttgccagaagccccctggttcttccttctagaagtt
caggccaggagtcagaaccataaccacccaccacaactgtaatggacaaaaaaatgaagtgaaaccctggtctttaagtccagtcctaggctacaccctgtaaggctggtgattaaatacattttacttactcagttcccgtttctgaaagcaggggctcaggttaaa
gcaagtgggggactcagagtgtctaagattacaggcagagtggttccagtgtctgtttctagcctttctctggaaacccacacaagctgtttctcctactagaatgccaccgtttacatagtggtggcctgatttctactttcagaacccttgagcagtttctgtatgccatgc
ccttgctgcgcgcccactgataaaagctctgtgaactggtttctcagagcatcccgtggttcttaagatcttttctgctttgttgtgttttgattttggttttggtttttatcaggatggggtttctctggctgtcttgtaactctgtagactaggctggcctctaactcaagacctgaat
gcacctgcttcaaatacatggccaccttccgactggttcttaagatctttatgacccgatagtgatctctgcagccctaacccttctgtcttagatatatttaagacattagatatcttcaaatgctatgcctgtcctggctcagctctgagattccgagatatgtcctcagcccc
atatccgaaggcaaagccaggctgctcccattgtagtccttttctccctggctctgaggaaatactcagcctgctgtgtctgatttttttgcttgtttgttttgtttatttgttgtctggctcttttaaaatttaacacagaacatctttttttttttttttttttttttgtatctgcacatcact
atgtaccaagcacaactggcacttactagggatttaatgtctttttttgtatttaggtctatttgtagaaagtaagttatatatgtactcaggcttaaaaatatataagatgcaagcagttgagatggtgaaagtaataagacacaaacagagcaaatgtgaagtgtgtgtttgtg
tgtgtttttgtgtgtgtgtgtgtgtgggtgtgtgggtgcacacacatgtgcttatgctcatgcatgtgtgcttgtacgtggttgcatatctgccctcatggagatatgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgttggcaggggggttacatggtgcagctggcctgc
tggtaagttcttctctgctttttttctggtagagtgtcaccttgcttccacttaattcctgatgggtttgctttatgaaatgaagacaaagtaggtcacagtttatctgcagaaagcactttgaggtcccctccccccaccgtgggtccctgcttgcacaccctgagctatgtgtgaa
gaggttcttttatgagcaagaaggcaaatgaagaagttatctaagagccttcaaagcactctcagtctgtctttacctgaggcagagtctgtggcttactcattctttgctaaacggttgccatcttgagatcaaggagtcagtgcttagtcatcggaaccatgcatgtaaag
aagaacatactggcctagctggacaggggacctaggacagtcctggtaagtgacctcatcagggagagaaggaaaaaaaatgtgcctgcatttcctgttcagggctcaccttacagttgccagcaagcacccgaatgcacttagaaagcagcattaacaaagtaa
gtttgcctccaataaaaggggatcctggatgtgaaccctcaccttagctggcctgtacaagctggaagctctccctgccaacggggctccgtcgttactcttcattatctagagtggggtctgtttcctcactaattaaaaattgtcagaaatgatattctcatttcctccgaa
agaagtaaacacaggcaaaggttagaaggatgcattccacagaaacggcccattgaggagcaaagtgcaaggcaaacagcctggaccagagggcctgccctgtcagccctgagattctgagatgtgacctcagccccacatccccacagccaagccaggct
gttcccactgcagacccttccctcctccagctttcaggctgttcccactgcagacccttccctcctccagctttcaggctgttcccactgcagacccttccctcctccagctttcaggctgttcccactgcagacccttccctcctccagctttcaggctgttcccatt
gcagacccttccccacggctctgaggaagtactcagccagctgtgtctgtgtggctgttgttgggcatcccaggttcttttgtaacttttaatttggagacacactcattgctaaattagctaggatggccttggactcactctgtggctcaggagagcagcgaacttaggg
gattctcctggctcagcctcctgtggtgtttcttgtttgttttttgttttactttgctttaacaggaccctcaggagcctggtctgttaagattcccttggtatatcttatgggatatccgattgtatctcatagtgaatgaatctggctaaaagttactgcctgacggttggtctttgctt
actaggaacaaactcacacgtttgccctgatttacagatacccccatggccatagttgagtgaaattaactgcacatggcactaagccagccatcttgtagttcctcggttccccagtgttgtggcggttgtcatctatcttcagctgacaattcctttgttctggaaggcc
cttctggtgtcatgtcagcttacattgtgcggatgctctggacagtgggtccctaactgtcccctgtacaccgtctttcccagtgtgggaaacatgttccttgctccgtggatccccagtggtcagcctgtgcctaaggcatgtgtgaataaacgggtggacttggtgtcact
gtgctgcgctaccagttccaagagaaacagagcacagagcacagagcacatgtgaaattctagcaaccacattagaaaagtaaaaacaaacaaacaagcaaataaaaacaaattagttttagtaaaatatttcacttggcttgccttaattatggttattccaacatata
ttcaatgtaagaaattttcacgatgcattgcattttatattaacttcaactgtgatacatactgtacactaacagtcatcttgagtcagactctttttttgaaatacttgttcattatttaaagttcacaaaaattatagttaaaaaatttagattgatgaattcaagttgtttcacacatttt
atttttacatttaattaaagttgagctttcttgttatattagccacctgtcggaggcagaagtagttgtgtaagtgcagaagtttacatgggctggctctacagcacgtctaacccatagcactataatccacatgcagtcagcacagcgggctaatcgcttgagaacatgtct
aagttagggatcagcatacgtttccaaataaaacaatttagaacattatcaaaattattcctgcattaaaatcgcttatttttatttgagttgcaatccaggggtcttatccagtaggaagcctgagtctgagagtagtccatagtgggtgaaagttgttcccctgacactagc
agatgcgattaaaagttgttactgtgagttaaatttatcaaacttttacagtagcccattttcctacagaaaatatcatgtttcagagtctgtttccccagactttgggggctggtagggacttgggagcaggtacttctcagcgagcccctgtacagcagtccatctctctcta
acaccctgaaatgtgggtgtgctcgcaggcactggtgtacatgtgaacatctcccatcattctgctgtgaggcagggaggctgagaaactggattacttttataaaagctatctcagatgagtcactgtgtcaaagaagaaaacaggttttgataattcatgaccacattac
ttctgatcattcacataaacccacaatctttaggtctgcatgaaattaaaacttacaatatatttcaaaaatgaaatgagggacatattttgcattgggacccatttttgaagggtctgtaaaaatgagtactacatggctatttacgaatgggaagaataaggcttaacttttaat
gtaattaaatctgtgcactacattcctgagcaatgagcagaatgtattttaagtatgtattaaatatttgaaattataaatctaggccgctgtagcccgcatccagaggcaggggcagctaggtttctttgagttagagaccagcctggtctaggtagtaagttccaggacta
ccagagctgtgcagcgagactgtctcaaaaaccaaaccaaaccaaaaacccaacccaaaccaaacaaagccaaacaacaacaacaaaaacaaaaaaacccaccaaaaaaaccaaggaaacaaaacaaccaaaaatcaaaaaactaggctactttaaaatgt
cattatttattttgttaaaattcctgagataaacactattctaacaaaagagccattaagactaagaatctctaagatagttttatgttctcaaattcagaagaactaaacacattattgcagtattaataaaataaaaactcaagataagaaggtcaaatgtgtccaagataatt
gtctcctccacaatgaggcaaatccataaggaataatgggggaagttcaatgcattagcttttgacagtcaaaacaggaacctttaaaatactctgttcatggttaaaaataatttgtactctaagtccagtgatcattgccagggagaaccaaagttgagaaatttctatt
aaaaacatgactcagaggaaaacatacagggtctggtcatgaaggaaatgatctggccccccattggtcactcctacagtcacatggtcagggcatctttaaaagtgagctatctggacttcagaggctcatagcaccctcctgtgctgca

Promoter sequence : The promoter fragment extended from nucleotides −5.4k to +21bp

Technical routes

- The *Fabp4* gene has 2 transcripts.According to the structure of *Fabp4* gene, *Fabp4-201*(ENSMUST00000029041.5) is selected for presentation of the recommended strategy.

- *Fabp4-201* gene has 4 exons, with the ATG start codon in exon1 and TGA stop codon in exon4.

- The *Fabp4* promoter is from article, the length is about 5.5kb.

- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.

- In this study, the *Fabp4-iCre-ployA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9,donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

Notice

- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, 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.

Gene information (NCBI)

集萃药康 GemPharmatech

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

Gene ID: 11770, updated on 12-Aug-2019

Summary

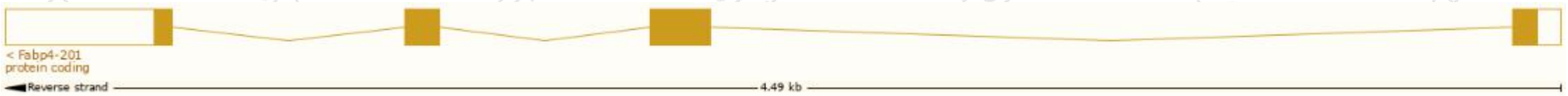
Official Symbol	Fabp4 provided by MGI
Official Full Name	fatty acid binding protein 4, adipocyte provided by MGI
Primary source	MGI:MGI:88038
See related	Ensembl:ENSMUSG00000062515
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; 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

Transcript information （Ensembl）

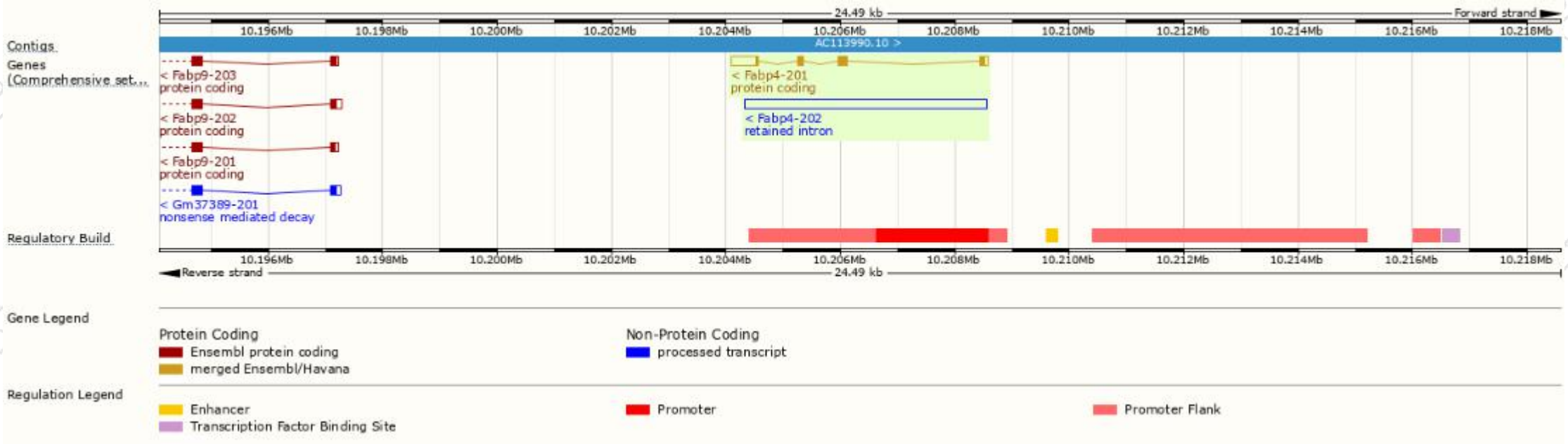
The gene has 2 transcript, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fabp4-201	ENSMUST00000029041.5	896	132aa	Protein coding	CCDS17238	P04117 Q542H7	TSL:1 GENCODE basic APPRIS P1
Fabp4-202	ENSMUST00000191757.1	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