Apoe Cas9-CKO Strategy

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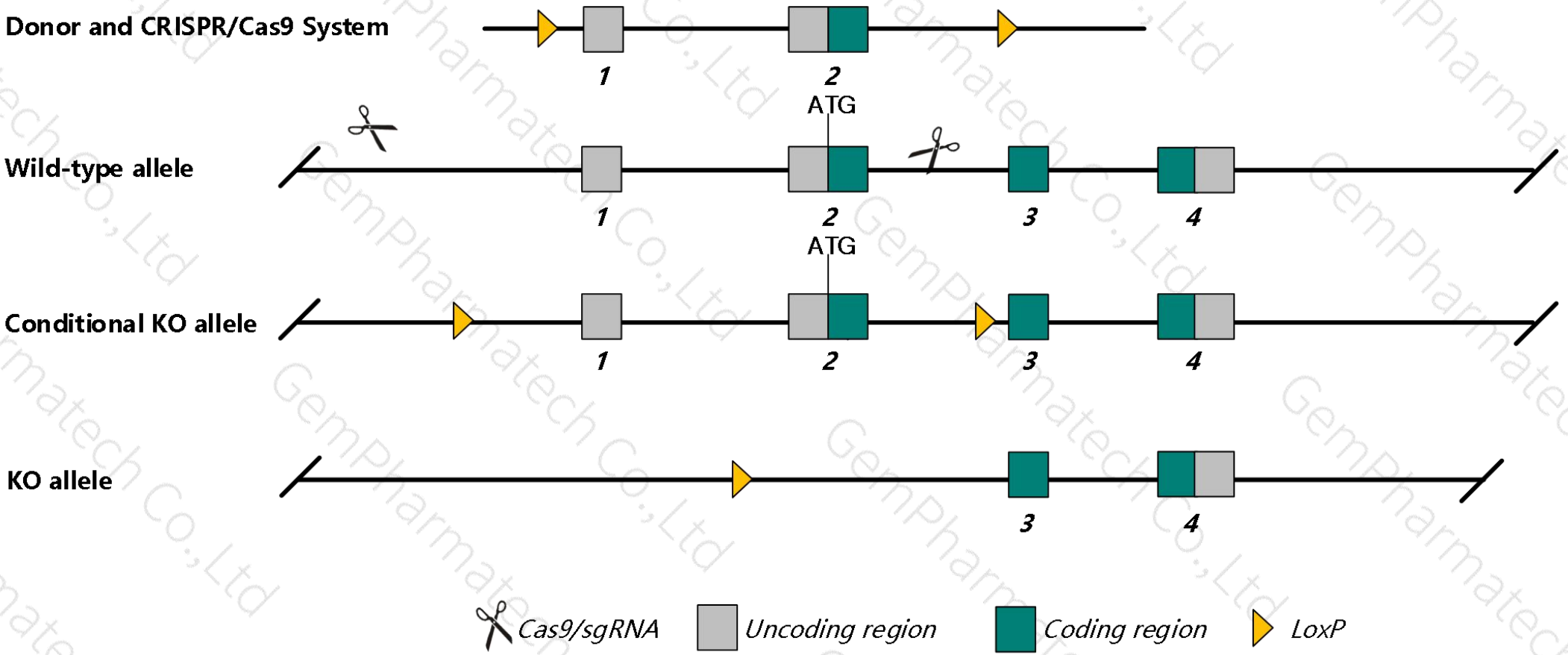
Project Overview

Project Name	***Apoe***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Apoe* gene has 11 transcripts. According to the structure of *Apoe* gene, exon1-exon2 of *Apoe-206* (ENSMUST00000174064.8) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Apoe* 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.

Notice

- According to the existing MGI data,Mutations at this locus cause diet-induced hypercholesterolemia and atherosclerosis. Homozygous null mutants also develop foam-cell rich deposits in proximal aorta, impaired blood-nerve and blood-brain barriers, and many xanthomatous lesions.

- The *Apoe* gene is located on the Chr7. 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）

集萃药康 GemPharmatech

Apoe apolipoprotein E [Mus musculus (house mouse)]

Gene ID: 11816, updated on 9-Apr-2019

Summary

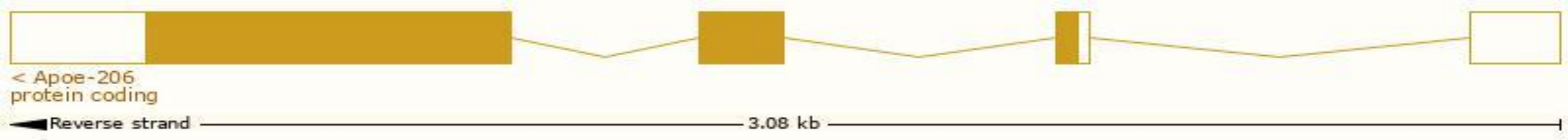
Official Symbol	Apoe provided by MGI
Official Full Name	apolipoprotein E provided byMGI
Primary source	MGI:MGI:88057
See related	Ensembl:ENSMUSG00000002985
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI255918, Apo-E
Summary	This gene encodes a member of the apolipoprotein A1/A4/E family of proteins. This protein is involved in the transport of lipoproteins in the blood. It binds to a specific liver and peripheral cell receptor, and is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. Homozygous knockout mice for this gene accumulate high levels of cholesterol in the blood and develop atherosclerosis. Different alleles of this gene have been associated with either increased risk or a protective effect for Alzheimer's disease in human patients. This gene maps to chromosome 7 in a cluster with the related apolipoprotein C1, C2 and C4 genes. [provided by RefSeq, Apr 2015]
Expression	Biased expression in liver adult (RPKM 9050.0), liver E18 (RPKM 4149.1) and 10 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

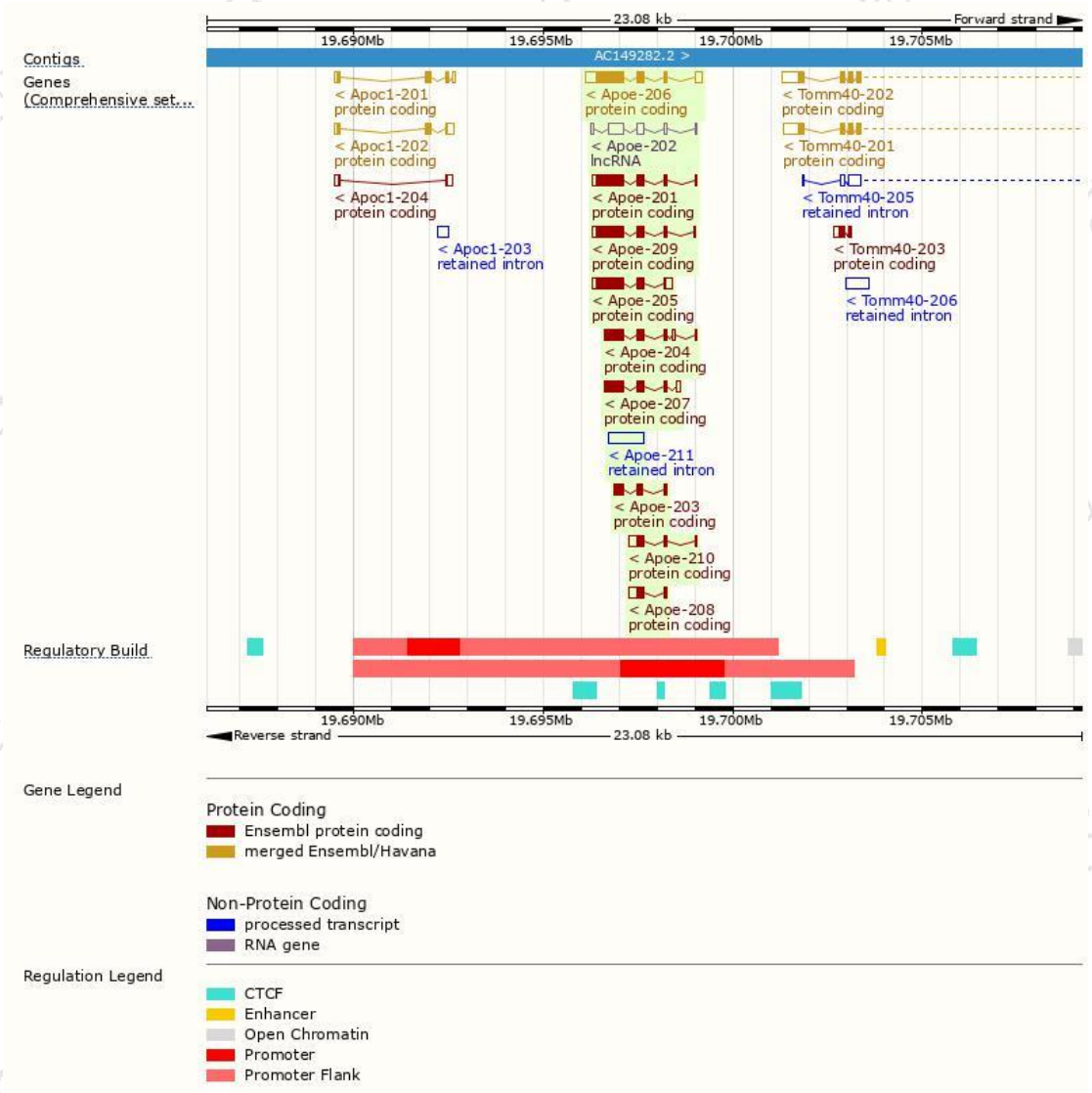
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Apoe-206	ENSMUST00000174064.8	1408	311aa	Protein coding	CCDS20912	P08226 Q3TXU4	TSL:1 GENCODE basic APPRIS P1
Apoe-205	ENSMUST00000173739.7	1221	311aa	Protein coding	CCDS20912	P08226 Q3TXU4	TSL:1 GENCODE basic APPRIS P1
Apoe-201	ENSMUST00000003066.15	1128	311aa	Protein coding	CCDS20912	P08226 Q3TXU4	TSL:5 GENCODE basic APPRIS P1
Apoe-209	ENSMUST00000174355.7	1104	311aa	Protein coding	CCDS20912	P08226 Q3TXU4	TSL:5 GENCODE basic APPRIS P1
Apoe-204	ENSMUST00000172983.7	817	232aa	Protein coding	-	G3UWN5	CDS 3' incomplete TSL:5
Apoe-207	ENSMUST00000174144.7	816	231aa	Protein coding	-	A0A1B0GX15	CDS 3' incomplete TSL:2
Apoe-210	ENSMUST00000174710.1	514	71aa	Protein coding	-	G3UWW2	TSL:1 GENCODE basic
Apoe-208	ENSMUST00000174191.1	472	71aa	Protein coding	-	G3UWW2	TSL:2 GENCODE basic
Apoe-203	ENSMUST00000172808.1	456	146aa	Protein coding	-	G3UZM8	CDS 3' incomplete TSL:3
Apoe-202	ENSMUST00000167646.8	728	No protein	Processed transcript	-	-	TSL:1
Apoe-211	ENSMUST00000207525.1	897	No protein	Retained intron	-	-	TSL:NA

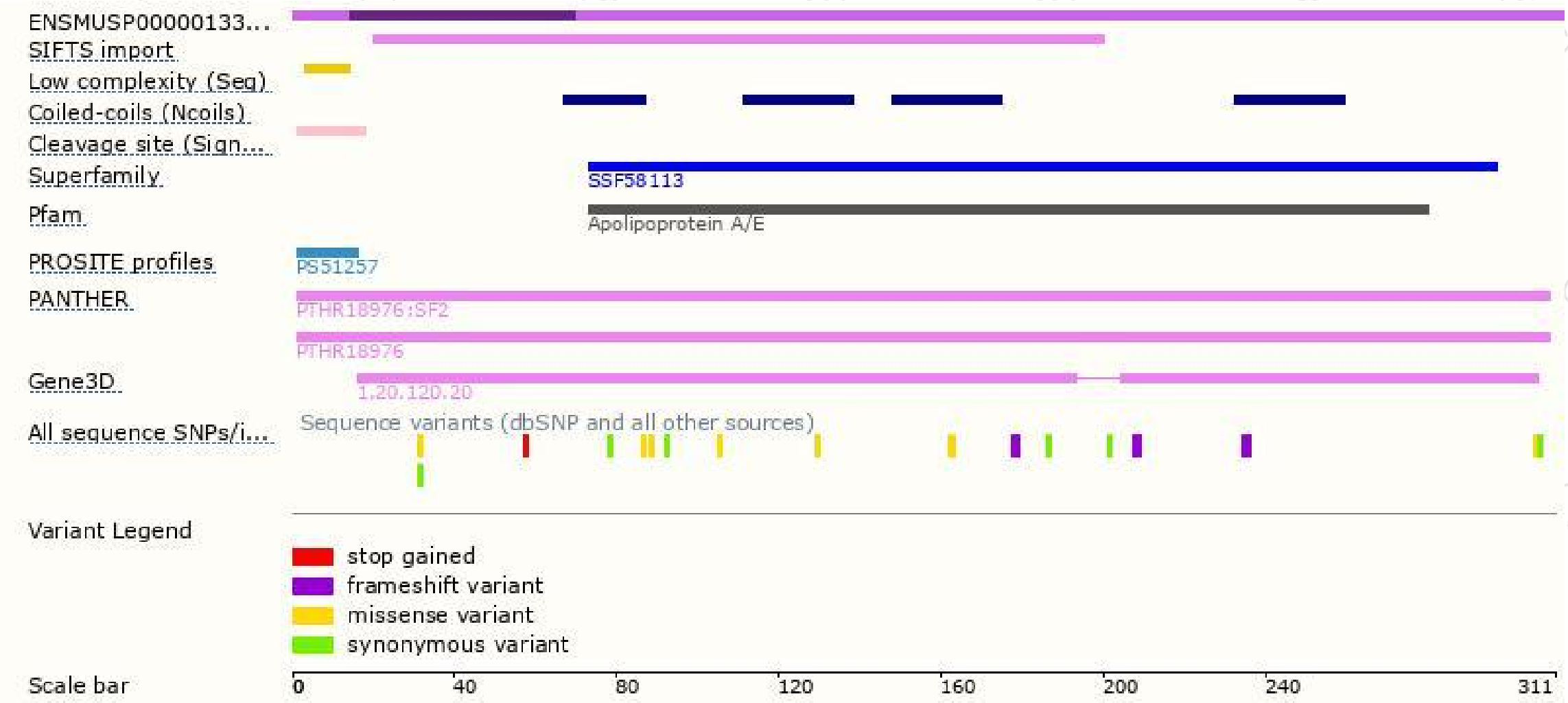
The strategy is based on the design of *Apoe-206* transcript,The transcription is shown below



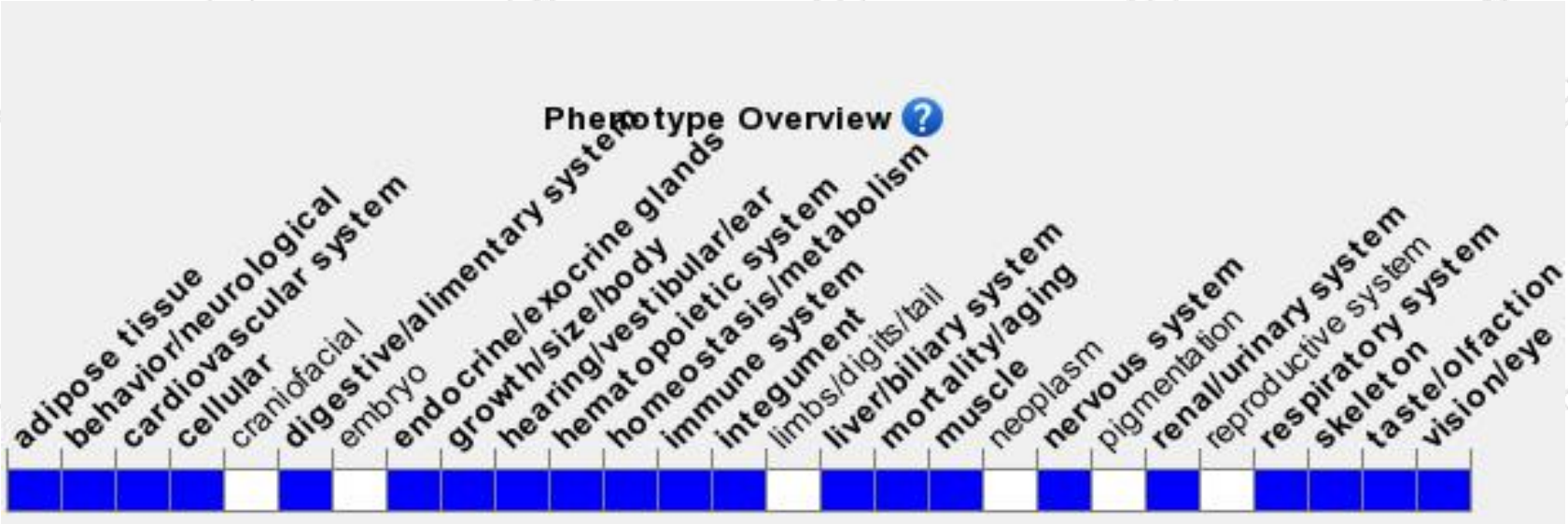
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,Mutations at this locus cause diet-induced hypercholesterolemia and atherosclerosis. Homozygous null mutants also develop foam-cell rich deposits in proximal aorta, impaired blood-nerve and blood-brain barriers, and many xanthomatous lesions.

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

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