Ctnnb1 Cas9-CKO Strategy

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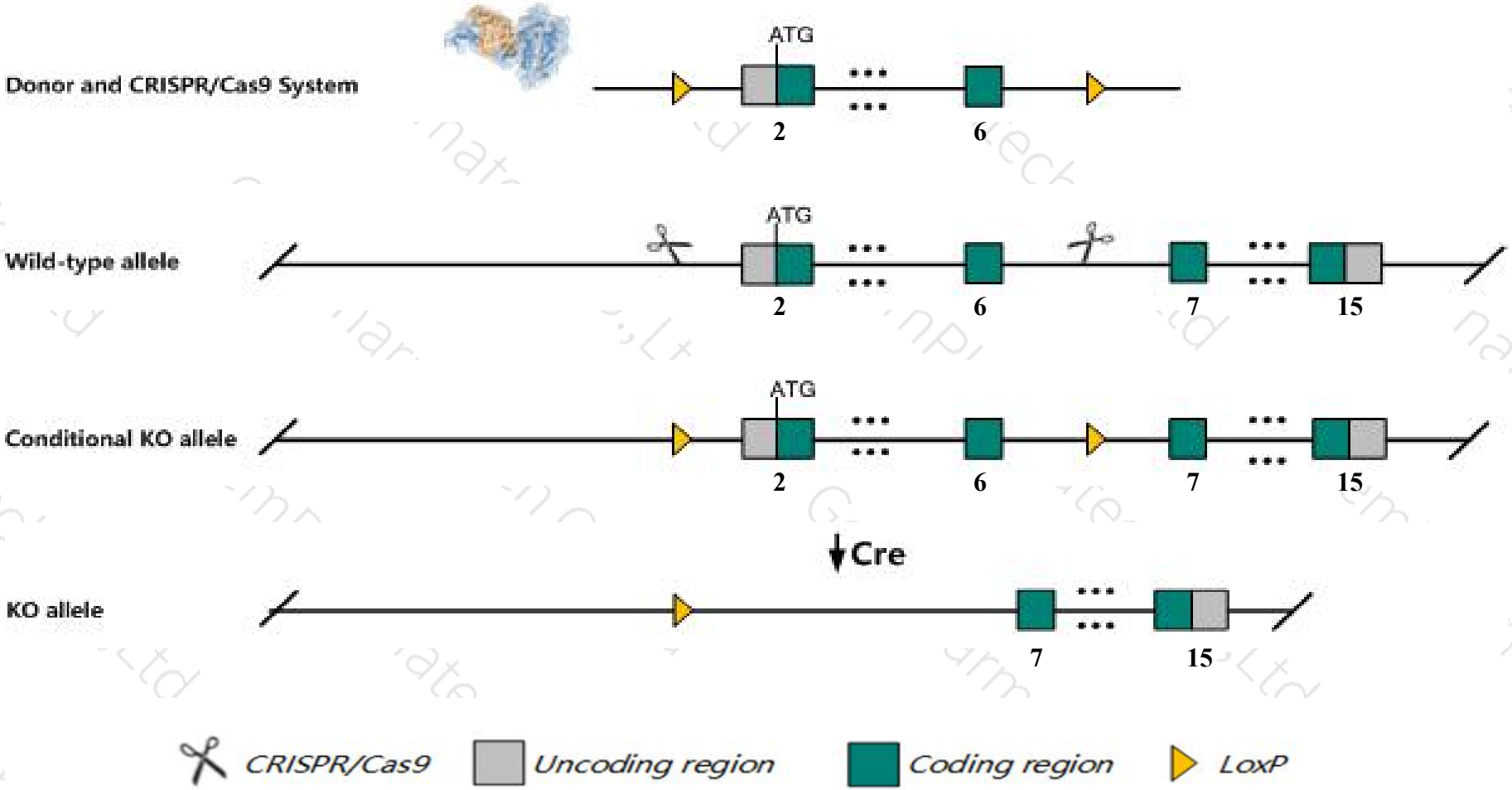
Project Overview

Project Name	***Ctnnb1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ctnnb1* gene. The schematic diagram is as follows:



Technical routes

- The *Ctnnb1* gene has 15 transcripts. According to the structure of *Ctnnb1* gene, exon2-exon6 of *Ctnnb1-201* (ENSMUST00000007130.14) 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 *Ctnnb1* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,Homozygous null embryos show anterior-posterior axis formation anomalies, but develop to E7. Multiple conditional mutations have shown defects in distinct stem cell types that result in proliferation defects, such as intestinal polyps, brain and spinal cord size anomalies, etc.
- Transcript *Ctnnb1-212,205* may not be affected.
- The *Ctnnb1* gene is located on the Chr9. 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）

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Ctnnb1 catenin (cadherin associated protein), beta 1 [Mus musculus (house mouse)]

Gene ID: 12387, updated on 9-Apr-2019

Summary

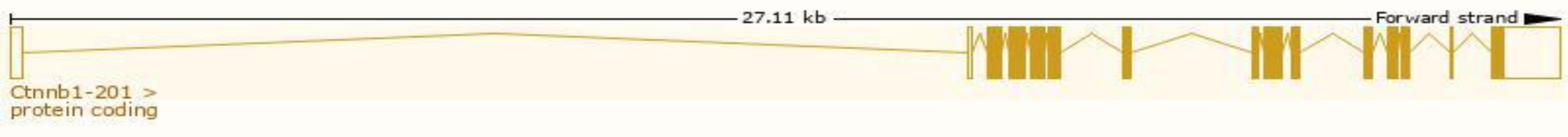
Official Symbol	Ctnnb1 provided by MGI
Official Full Name	catenin (cadherin associated protein), beta 1 provided by MGI
Primary source	MGI:MGI:88276
See related	Ensembl:ENSMUSG00000006932
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bfc, Catnb, Mesc
Summary	This gene encodes not only an important cytoplasmic component of the classical cadherin adhesion complex that forms the adherens junction in epithelia and mediates cell-cell adhesion in many other tissues but also a key signaling molecule in the canonical Wnt signaling pathway that controls cell growth and differentiation during both normal development and tumorigenesis. The gene product contains a central armadillo-repeat containing domain through which it binds the cytoplasmic tail of classical cadherins; meanwhile, it also binds alpha-catenin, which further links the cadherin complex to the actin cytoskeleton either directly or indirectly. Beta-catenin is therefore necessary for the adhesive function of classical cadherins. Another key function of this protein is to mediate the canonical Wnt signaling pathway and regulate gene transcription. Without Wnt signal, cytoplasmic beta-catenin that is not associated with the cadherin complex is quickly phosphorylated at the N-terminal Ser/Thr residues by the so called degradation complex containing axin, adenomatous polyposis coli (APC), casein kinase I, and GSK3B, then ubiquitylated by beta-TrCP, and degraded by the proteasome. However, in the presence of Wnt signal, the degradation complex is disrupted and the stabilized cytoplasmic beta-catenin translocates into the nucleus, where it binds various transcription factors and, together with these factors, regulates the transcription of many downstream genes. Mutations of this gene have been linked with various types of tumors. Alternatively spliced variants have been found for this gene. [provided by RefSeq, Sep 2009]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 116.8), limb E14.5 (RPKM 110.5) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

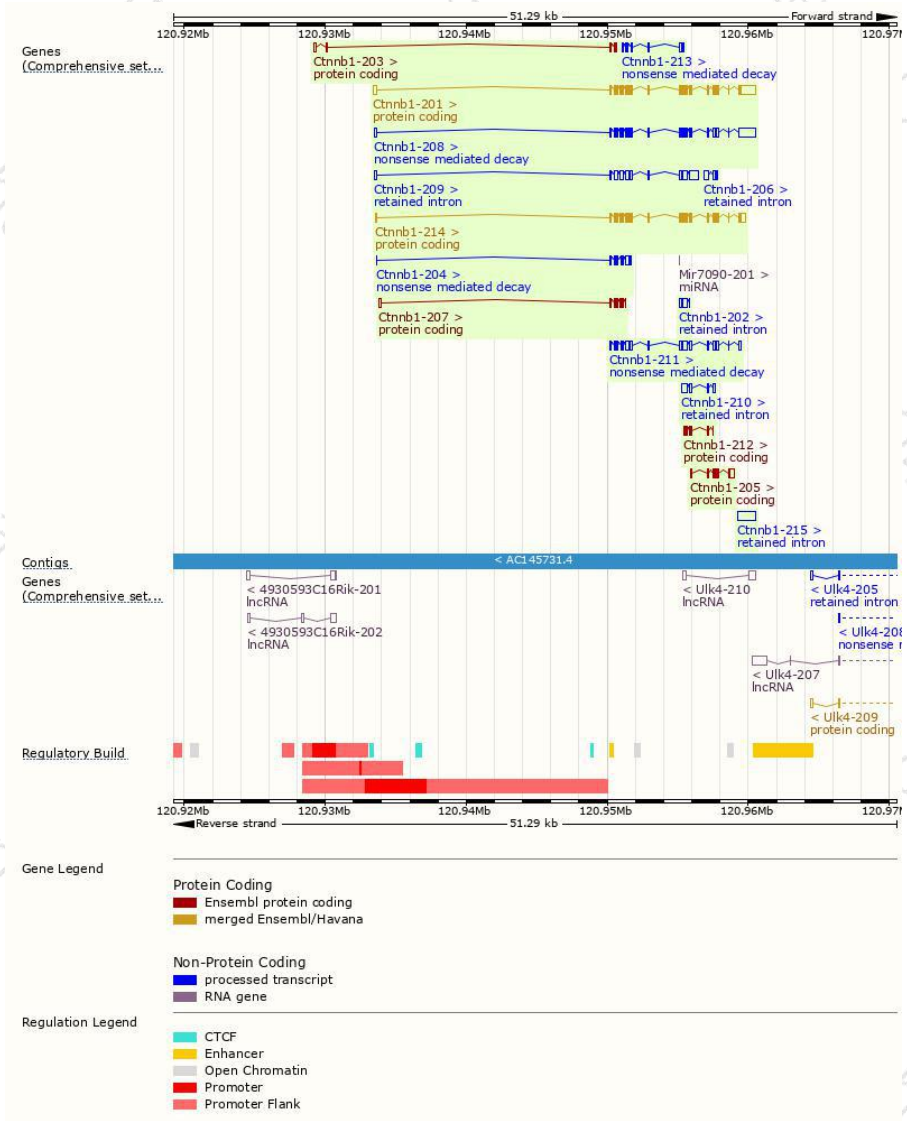
The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctnnb1-201	ENSMUST00000007130.14	3623	781aa	Protein coding	CCDS23630	Q02248	TSL:1 GENCODE basic APPRIS P1
Ctnnb1-214	ENSMUST00000178812.8	2702	781aa	Protein coding	CCDS23630	Q02248	TSL:1 GENCODE basic APPRIS P1
Ctnnb1-205	ENSMUST00000133689.1	886	174aa	Protein coding	-	F7CRC6	CDS 5' incomplete TSL:2
Ctnnb1-207	ENSMUST00000145093.1	714	174aa	Protein coding	-	D3YUH4	CDS 3' incomplete TSL:3
Ctnnb1-212	ENSMUST00000169931.7	505	169aa	Protein coding	-	F7BAC9	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Ctnnb1-203	ENSMUST00000130466.7	364	42aa	Protein coding	-	D3Z7S6	CDS 3' incomplete TSL:3
Ctnnb1-208	ENSMUST00000154356.7	3548	607aa	Nonsense mediated decay	-	E9Q6A9	TSL:5
Ctnnb1-211	ENSMUST00000163844.7	2222	90aa	Nonsense mediated decay	-	E9PW26	TSL:5
Ctnnb1-204	ENSMUST00000130845.8	795	83aa	Nonsense mediated decay	-	D3Z5Q1	TSL:5
Ctnnb1-213	ENSMUST00000170729.1	692	145aa	Nonsense mediated decay	-	F6QZ47	CDS 5' incomplete TSL:5
Ctnnb1-209	ENSMUST00000154687.7	2359	No protein	Retained intron	-	-	TSL:1
Ctnnb1-215	ENSMUST00000215573.1	1292	No protein	Retained intron	-	-	TSL:NA
Ctnnb1-210	ENSMUST00000156911.1	773	No protein	Retained intron	-	-	TSL:2
Ctnnb1-206	ENSMUST00000139138.1	577	No protein	Retained intron	-	-	TSL:1
Ctnnb1-202	ENSMUST00000126633.1	494	No protein	Retained intron	-	-	TSL:2

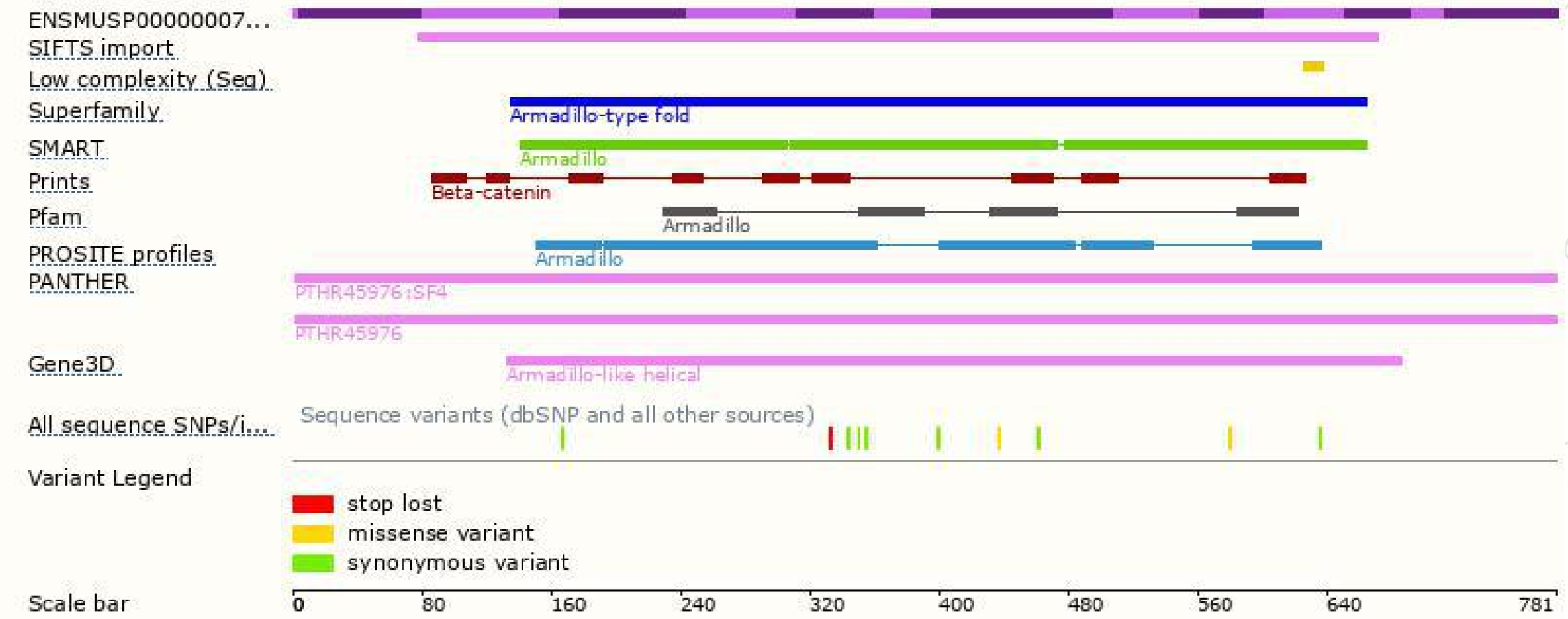
The strategy is based on the design of *Ctnnb1-201* transcript,The transcription is shown below



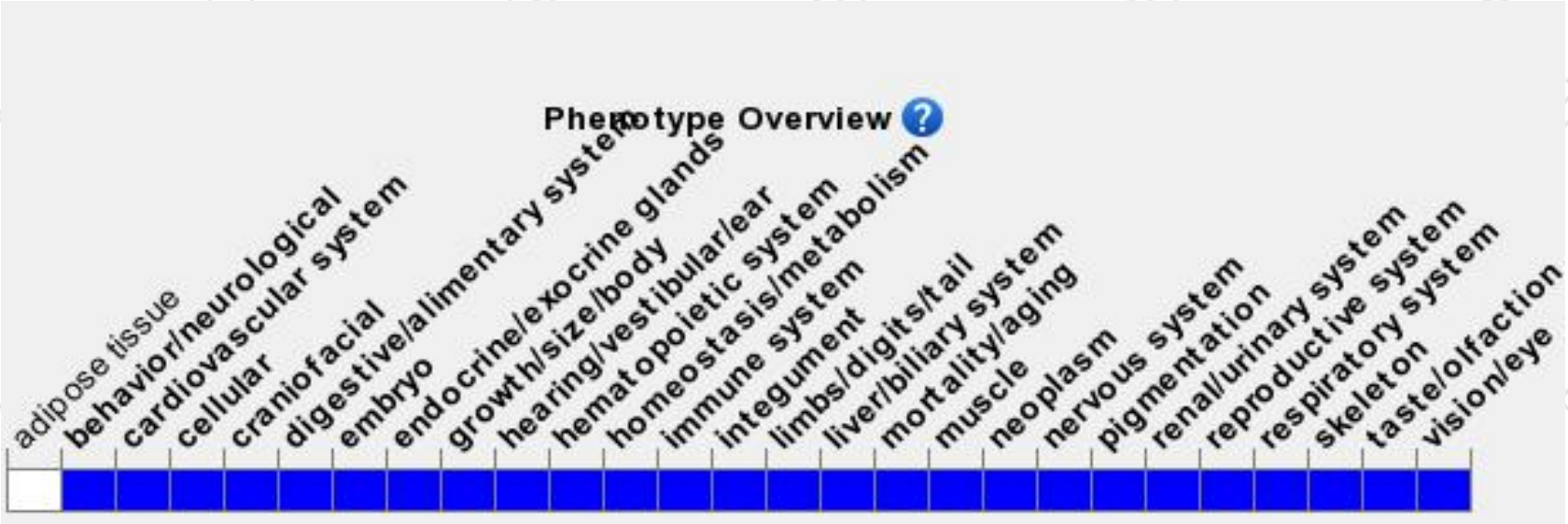
Genomic location distribution



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,Homozygous null embryos show anterior-posterior axis formation anomalies, but develop to E7. Multiple conditional mutations have shown defects in distinct stem cell types that result in proliferation defects, such as intestinal polyps, brain and spinal cord size anomalies, etc.

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

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