Xiap Cas9-CKO Strategy

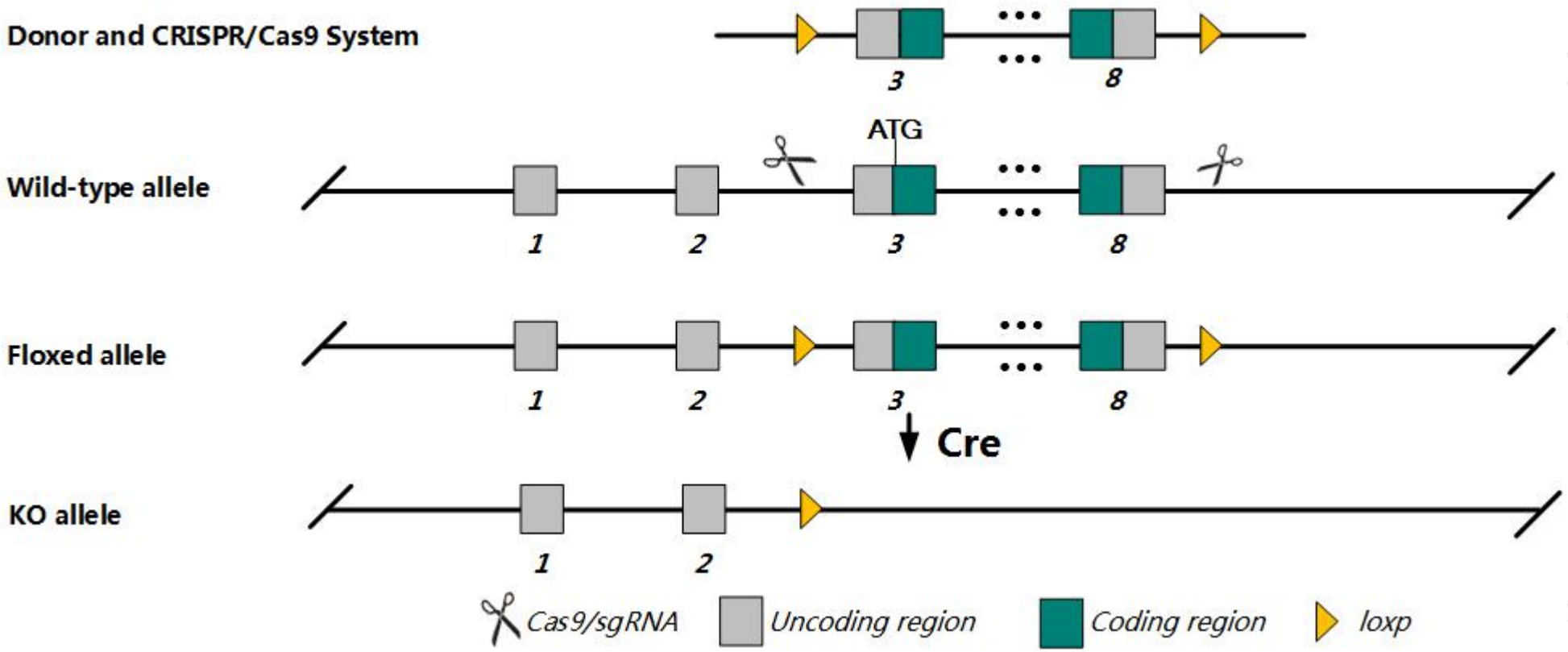
Designer: Xiaojing Li
Design Date: 2019-8-15

Project Overview

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

Conditional Knockout strategy

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



Technical routes

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- The *Xiap* gene has 11 transcripts. According to the structure of *Xiap* gene, exon3-exon8 of *Xiap-203* (ENSMUST00000115094.7) transcript is recommended as the knockout region.The region contains All coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Xiap* 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 mutants are indistinguishable from normal littermates, but increased levels of protein from other Birc gene family members suggest a compensatory mechanism in the absence of the Birc4 genes product.

- The *Xiap* gene is located on the ChrX. 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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Xiap X-linked inhibitor of apoptosis [Mus musculus (house mouse)]

Gene ID: 11798, updated on 7-Apr-2019

Summary

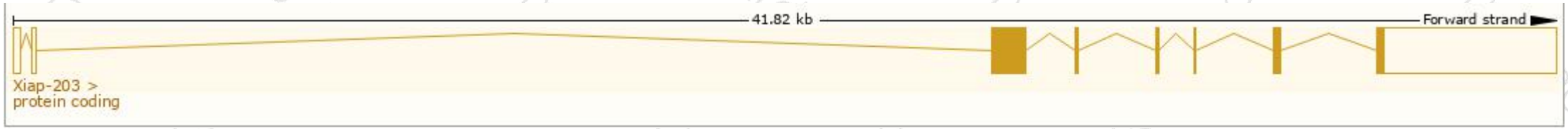
Official Symbol	Xiap provided by MGI
Official Full Name	X-linked inhibitor of apoptosis provided byMGI
Primary source	MGI:MGI:107572
See related	Ensembl:ENSMUSG00000025860
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110015C02Rik, Aipa, Api3, Birc4, IAP3, ILP-1, MIHA
Summary	The protein encoded by this gene is a member of the inhibitor of apoptosis (IAP) family of proteins. While first identified for its role in blocking apoptosis, this protein modulates many other signaling processes including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathways and inflammatory responses. This protein blocks apoptosis by binding and inhibiting target caspases after they have been activated. Binding occurs to some, but not all, caspases. This protein has several conserved regions, including baculoviral IAP repeat (BIR) motifs and a RING finger E3 ligase domain. In humans, mutations in this gene are linked to immunodeficiency in X-linked lymphoproliferative syndrome type-2 (XLP-2). A pseudogene of this gene is found on chromosome 7. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Aug 2014]
Expression	Ubiquitous expression in cerebellum adult (RPKM 7.1), bladder adult (RPKM 6.9) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

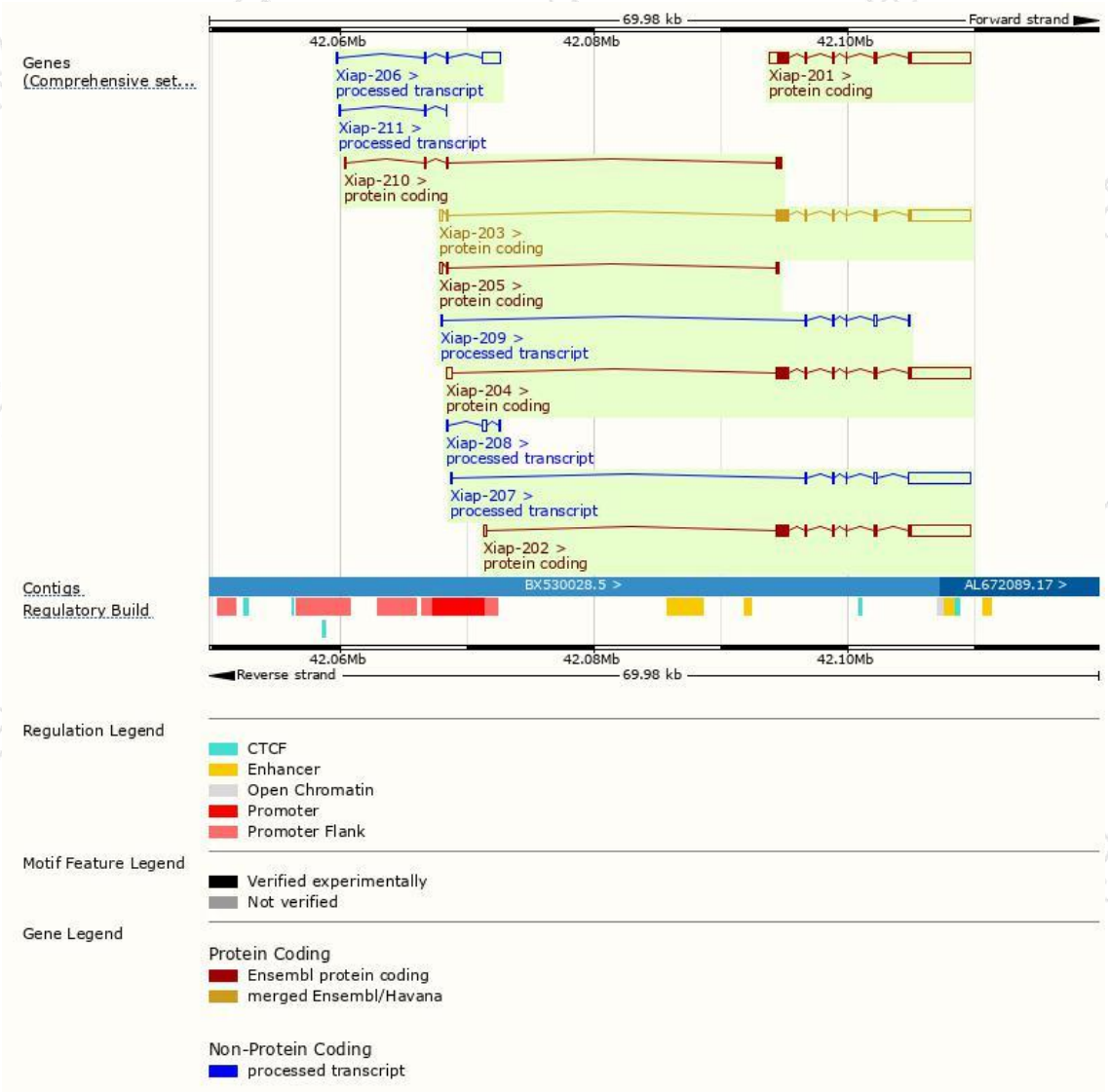
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Xiap-201	ENSMUST00000026978.6	6834	496aa	Protein coding	CCDS30098	Q60989	TSL:1 GENCODE basic APPRIS P1
Xiap-204	ENSMUST00000115095.8	6670	496aa	Protein coding	CCDS30098	Q60989	TSL:1 GENCODE basic APPRIS P1
Xiap-203	ENSMUST00000115094.7	6542	496aa	Protein coding	CCDS30098	Q60989	TSL:5 GENCODE basic APPRIS P1
Xiap-202	ENSMUST00000055483.9	6374	496aa	Protein coding	CCDS30098	Q60989	TSL:1 GENCODE basic APPRIS P1
Xiap-210	ENSMUST00000224454.1	693	106aa	Protein coding	-	A0A286YE63	CDS 3' incomplete
Xiap-205	ENSMUST00000126375.1	487	52aa	Protein coding	-	A2BGY5	CDS 3' incomplete TSL:3
Xiap-207	ENSMUST00000145065.1	5407	No protein	Processed transcript	-	-	TSL:3
Xiap-206	ENSMUST00000141316.7	1817	No protein	Processed transcript	-	-	TSL:2
Xiap-209	ENSMUST00000150635.7	591	No protein	Processed transcript	-	-	TSL:5
Xiap-208	ENSMUST00000146874.2	500	No protein	Processed transcript	-	-	TSL:3
Xiap-211	ENSMUST00000225851.1	284	No protein	Processed transcript	-	-	

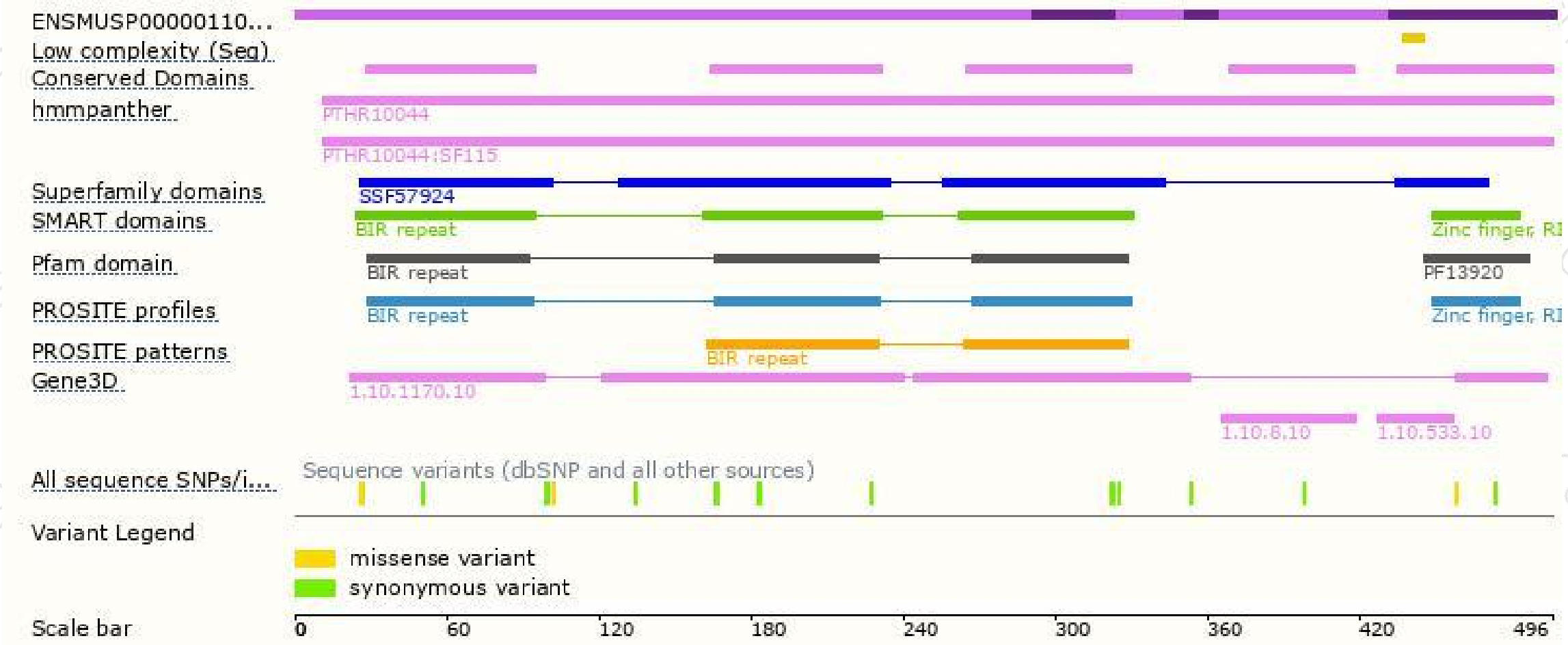
The strategy is based on the design of *Xiap-203* transcript,The transcription is shown below



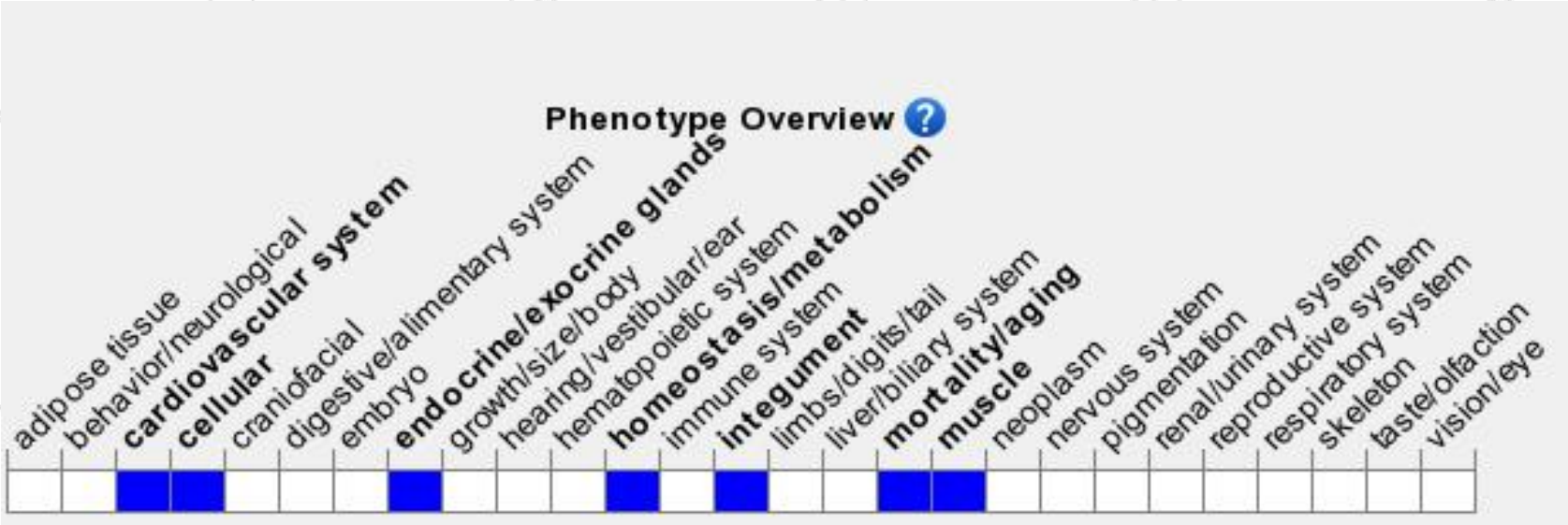
Genomic location distribution



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 mutants are indistinguishable from normal littermates, but increased levels of protein from other Birc gene family members suggest a compensatory mechanism in the absence of the Birc4 genes product.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd