

# ***Itch Cas9-CKO Strategy***

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**Reviewer: Longyun Hu**

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# Project Overview

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**Project Name**

*Itch*

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**Project type**

**Cas9-CKO**

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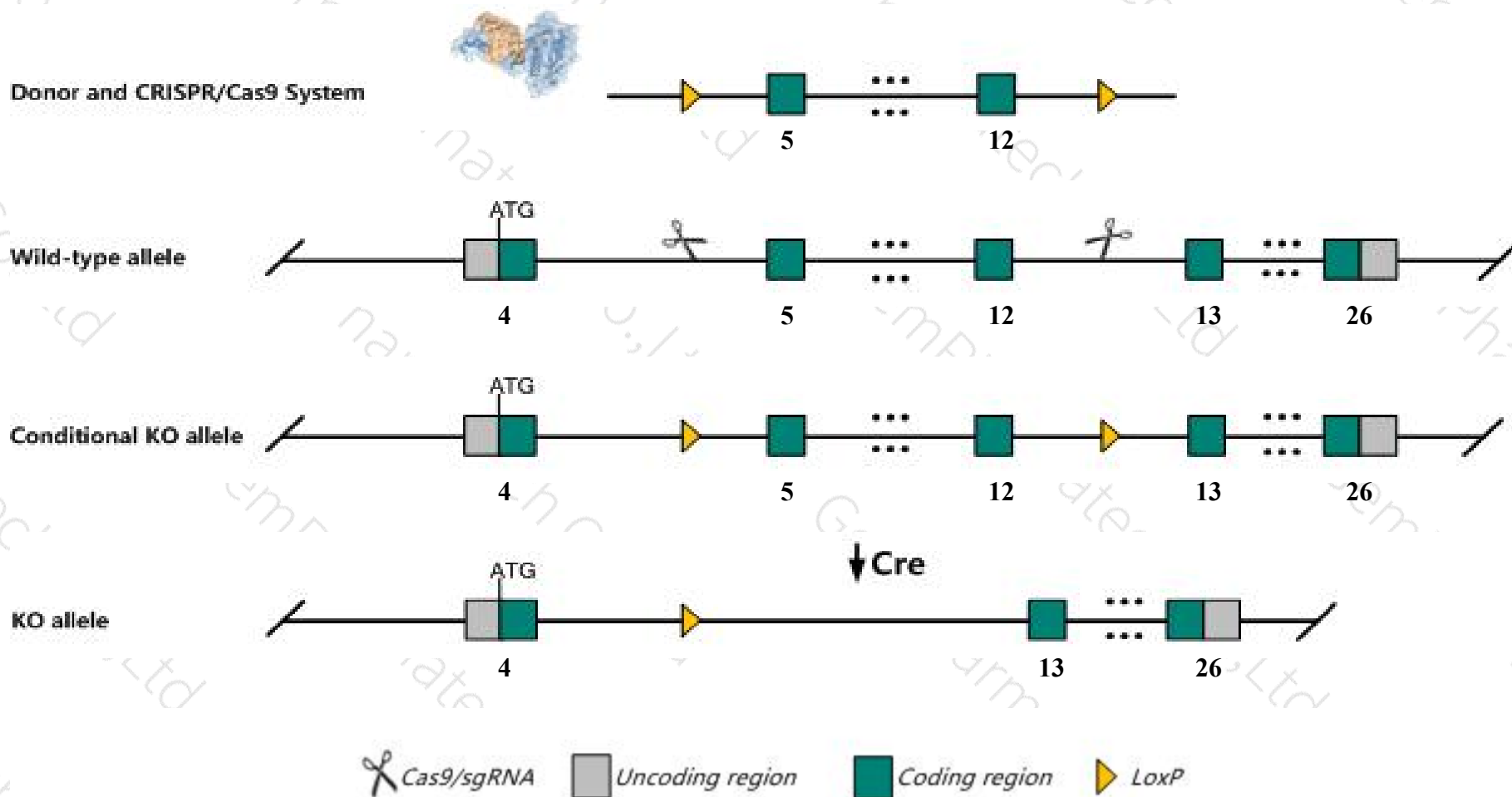
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

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



- The *Itch* gene has 7 transcripts. According to the structure of *Itch* gene, exon5-exon12 of *Itch*-202(ENSMUST00000109685.7) transcript is recommended as the knockout region. The region contains 1076bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Itch* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was 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.

- According to the existing MGI data, mice homozygous for an ENU mutation exhibit increased total IgE levels in the peripheral blood and an enhanced IgE response to the cysteine protease allergen, papain.
- The *Itch* gene is located on the Chr2. 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)

## Itch itchy, E3 ubiquitin protein ligase [Mus musculus (house mouse)]

Gene ID: 16396, updated on 13-Mar-2020

### Summary

**Official Symbol** Itch provided by [MGI](#)

**Official Full Name** itchy, E3 ubiquitin protein ligase provided by [MGI](#)

**Primary source** [MGI:MGI:1202301](#)

**See related** [Ensembl:ENSMUSG00000027598](#)

**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** 6720481N21Rik, 8030492O04Rik, A130065M08, AIP4, C230047C07Rik

**Expression** Ubiquitous expression in limb E14.5 (RPKM 9.0), CNS E11.5 (RPKM 8.0) and 28 other tissues [See more](#)

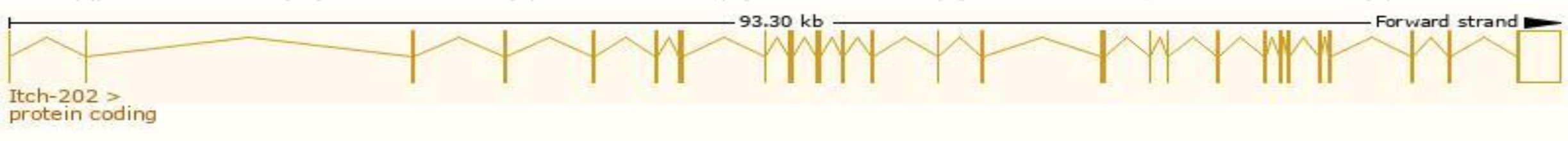
**Orthologs** [human](#) [all](#)

# Transcript information (Ensembl)

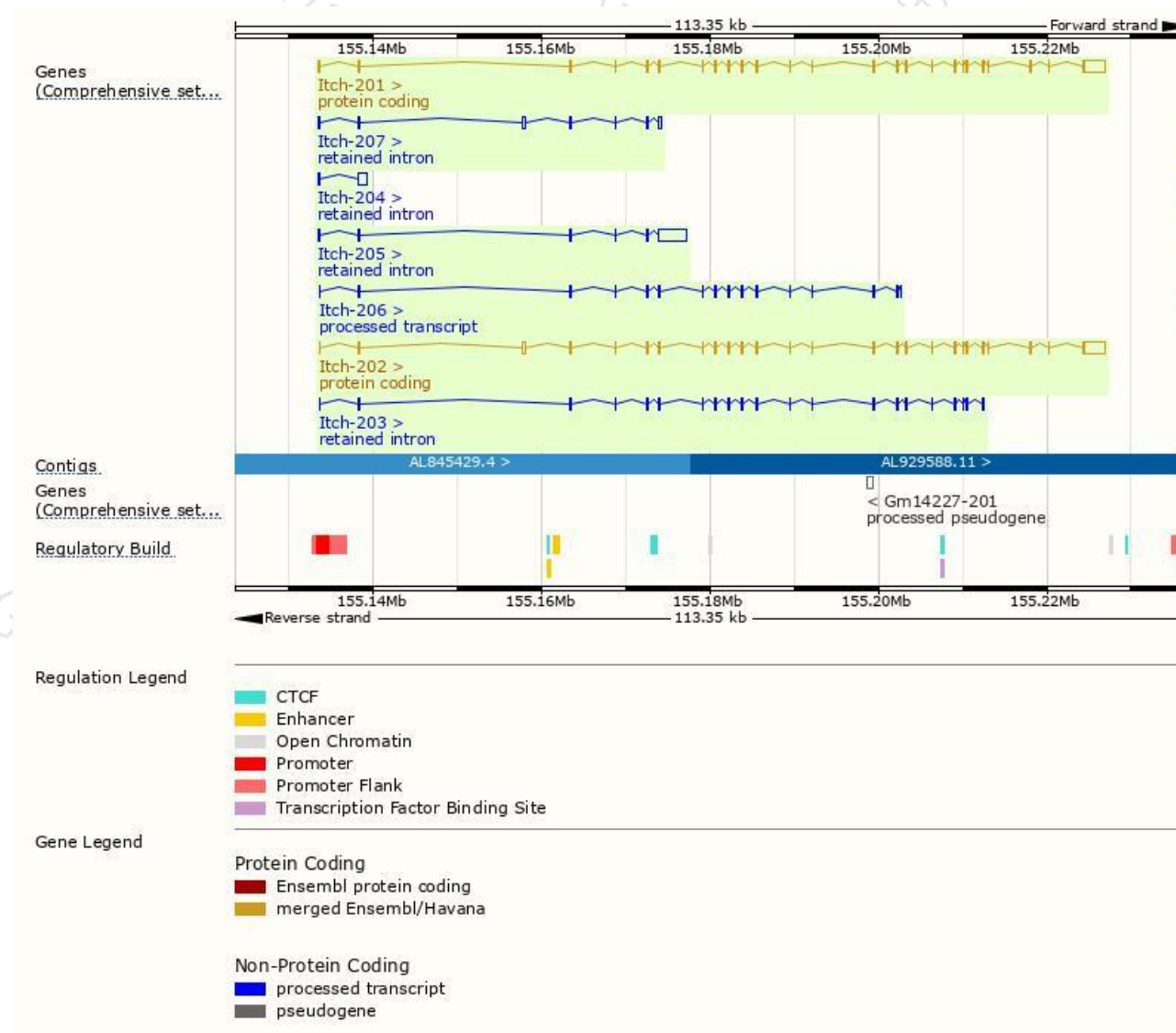
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itch-202	<a href="#">ENSMUST00000109685.7</a>	5309	<a href="#">864aa</a>	Protein coding	<a href="#">CCDS38293</a>	<a href="#">Q8C863</a>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Itch-201	<a href="#">ENSMUST00000029126.14</a>	5159	<a href="#">864aa</a>	Protein coding	<a href="#">CCDS38293</a>	<a href="#">Q8C863</a>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Itch-206	<a href="#">ENSMUST00000142147.7</a>	1774	No protein	Processed transcript	-	-	TSL:1
Itch-205	<a href="#">ENSMUST00000138468.7</a>	3711	No protein	Retained intron	-	-	TSL:1
Itch-203	<a href="#">ENSMUST00000123497.1</a>	2480	No protein	Retained intron	-	-	TSL:1
Itch-204	<a href="#">ENSMUST00000126648.7</a>	1171	No protein	Retained intron	-	-	TSL:1
Itch-207	<a href="#">ENSMUST00000155360.7</a>	1070	No protein	Retained intron	-	-	TSL:1

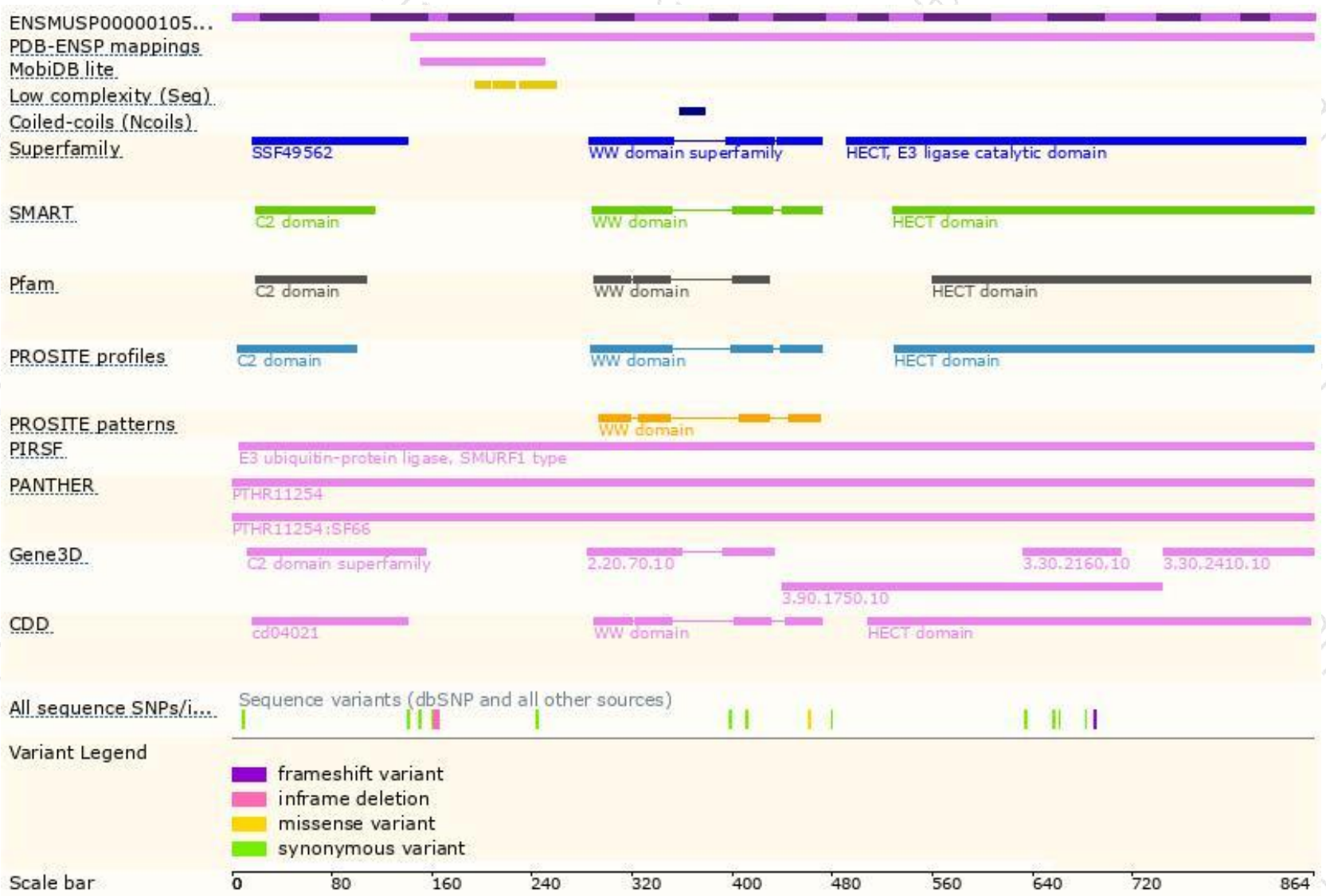
The strategy is based on the design of *Itch-202* transcript,the transcription is shown below:



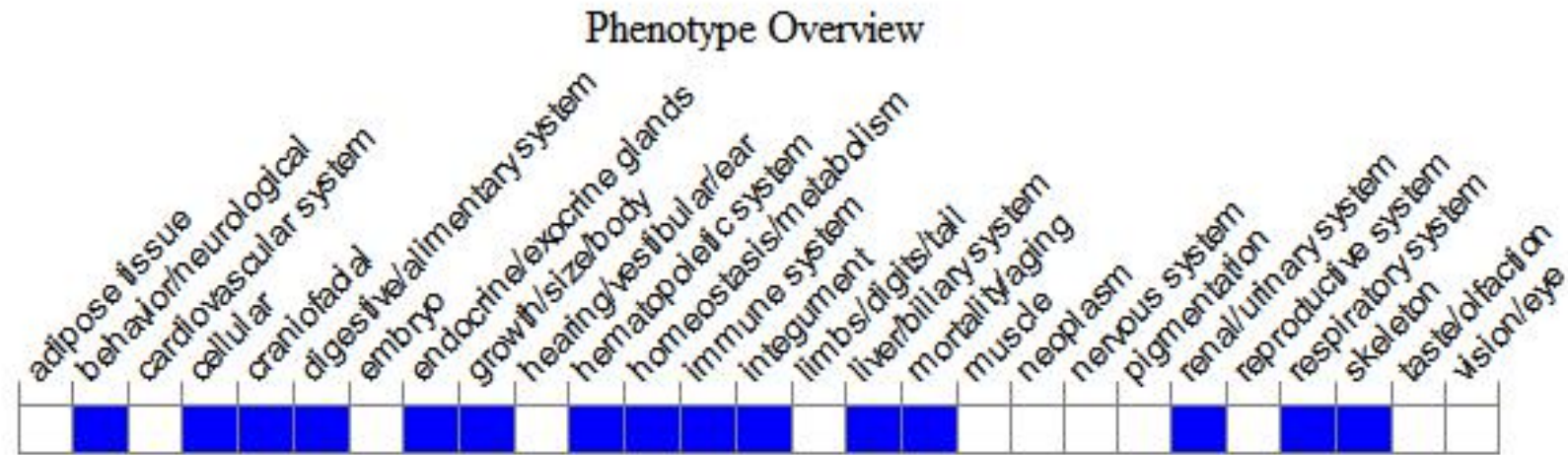
# Genomic location distribution



# 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, mice homozygous for an ENU mutation exhibit increased total IgE levels in the peripheral blood and an enhanced IgE response to the cysteine protease allergen, papain.

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

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