

# *Nt5c2* Cas9-CKO Strategy

**Designer:** Daohua Xu

**Reviewer:** Huimin Su

**Design Date:** 2020-5-20

# Project Overview

**Project Name**

*Nt5c2*

**Project type**

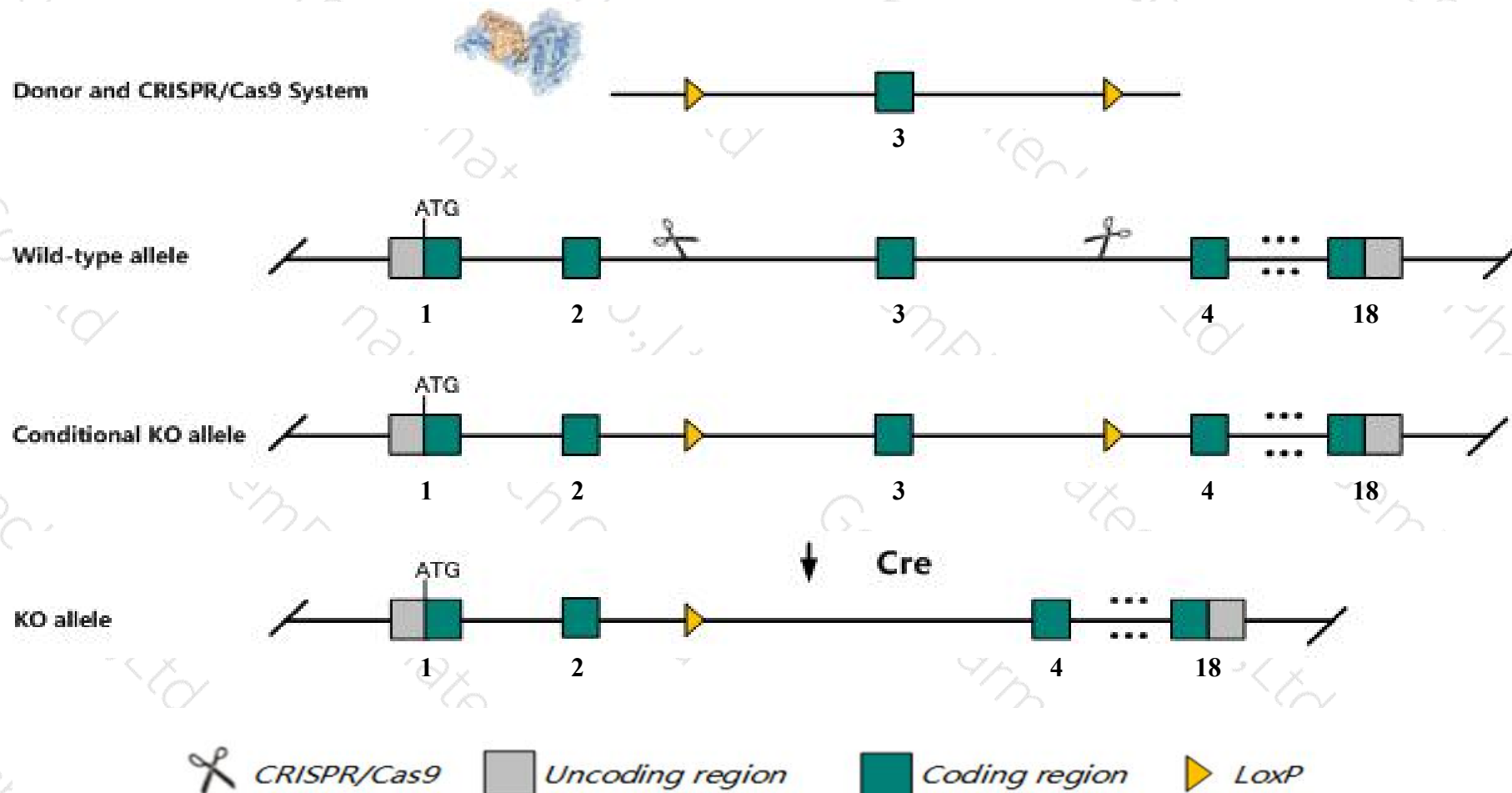
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nt5c2* gene. The schematic diagram is as follows:



- The *Nt5c2* gene has 15 transcripts. According to the structure of *Nt5c2* gene, exon3 of *Nt5c2-203* (ENSMUST00000172239.2) transcript is recommended as the knockout region. The region contains 74bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nt5c2* 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.

- According to the existing MGI data, bone marrow cells with a nucleotide substitution allele used in a transplantation experiment following tamoxifen-induction produce notch1-induced tumors that are resistant to 6-mercaptopurine chemotherapy.
- Transcript Nt5c2-205, Nt5c2-202, Nt5c2-209, Nt5c2-212, Nt5c2-215 may not be affected.
- The *Nt5c2* gene is located on the Chr19. 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)

## Nt5c2 5'-nucleotidase, cytosolic II [Mus musculus (house mouse)]

Gene ID: 76952, updated on 13-Mar-2020

### Summary



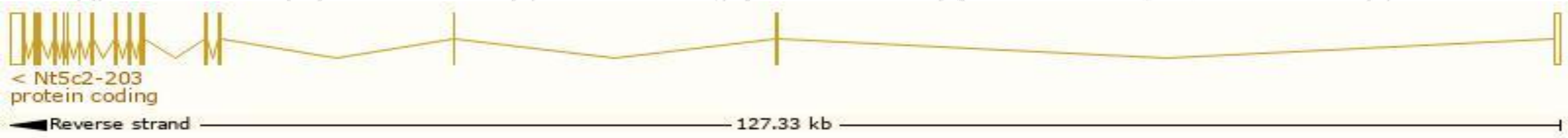
<b>Official Symbol</b>	Nt5c2 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	5'-nucleotidase, cytosolic II provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:2178563</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000025041</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	2010002I23Rik, Cnll, Gm9751, Gmp, Nt5b, Pnt5, cN-II
<b>Expression</b>	Ubiquitous expression in whole brain E14.5 (RPKM 17.2), CNS E14 (RPKM 14.7) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

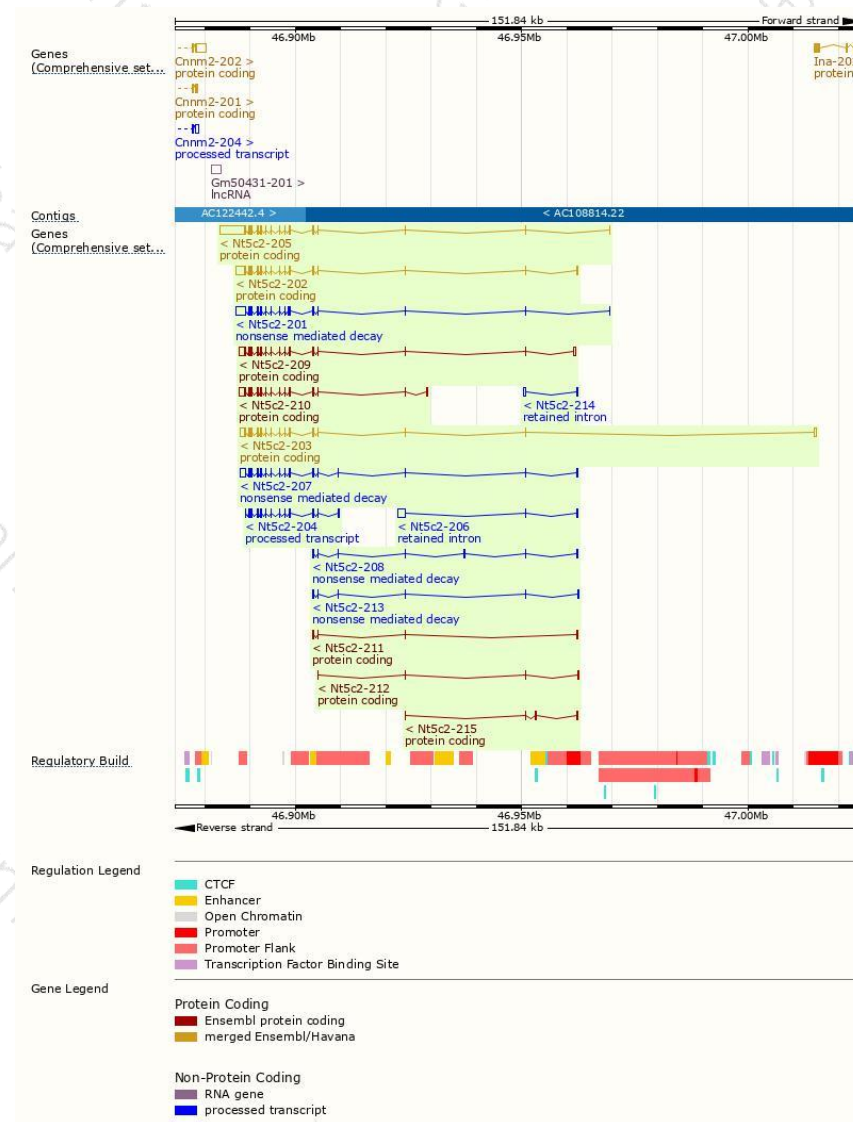
The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nt5c2-205	<a href="#">ENSMUST00000235234.1</a>	7353	<a href="#">585aa</a>	Protein coding	<a href="#">CCDS50461</a>	<a href="#">A0A494B9X3</a>	GENCODE basic
Nt5c2-202	<a href="#">ENSMUST00000168536.8</a>	3728	<a href="#">560aa</a>	Protein coding	<a href="#">CCDS29883</a>	<a href="#">Q3V1L4</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
Nt5c2-209	<a href="#">ENSMUST00000236501.1</a>	3183	<a href="#">560aa</a>	Protein coding	<a href="#">CCDS29883</a>	<a href="#">Q3V1L4</a>	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
Nt5c2-203	<a href="#">ENSMUST00000172239.2</a>	3148	<a href="#">586aa</a>	Protein coding	<a href="#">CCDS50462</a>	<a href="#">E9Q9M1</a>	TSL:5 GENCODE basic
Nt5c2-210	<a href="#">ENSMUST00000236727.1</a>	2920	<a href="#">528aa</a>	Protein coding	-	<a href="#">A0A494BBP6</a>	
Nt5c2-212	<a href="#">ENSMUST00000236924.1</a>	449	<a href="#">97aa</a>	Protein coding	-	<a href="#">A0A494BBM7</a>	CDS 3' incomplete
Nt5c2-215	<a href="#">ENSMUST00000238106.1</a>	362	<a href="#">59aa</a>	Protein coding	-	<a href="#">A0A494BBK9</a>	CDS 3' incomplete
Nt5c2-211	<a href="#">ENSMUST00000236783.1</a>	325	<a href="#">87aa</a>	Protein coding	-	<a href="#">A0A494BBI8</a>	CDS 3' incomplete
Nt5c2-201	<a href="#">ENSMUST00000086961.14</a>	3939	<a href="#">251aa</a>	Nonsense mediated decay	-	<a href="#">G3X9J6</a>	TSL:5
Nt5c2-207	<a href="#">ENSMUST00000235596.1</a>	2768	<a href="#">62aa</a>	Nonsense mediated decay	-	<a href="#">A0A494BAU4</a>	
Nt5c2-213	<a href="#">ENSMUST00000237639.1</a>	606	<a href="#">62aa</a>	Nonsense mediated decay	-	<a href="#">A0A494BAU4</a>	
Nt5c2-208	<a href="#">ENSMUST00000235926.1</a>	599	<a href="#">78aa</a>	Nonsense mediated decay	-	<a href="#">A0A494BAN2</a>	
Nt5c2-204	<a href="#">ENSMUST00000174731.2</a>	1404	No protein	Processed transcript	-	-	TSL:5
Nt5c2-206	<a href="#">ENSMUST00000235296.1</a>	1779	No protein	Retained intron	-	-	
Nt5c2-214	<a href="#">ENSMUST00000237658.1</a>	635	No protein	Retained intron	-	-	

The strategy is based on the design of *Nt5c2-203* transcript,The transcription is shown below

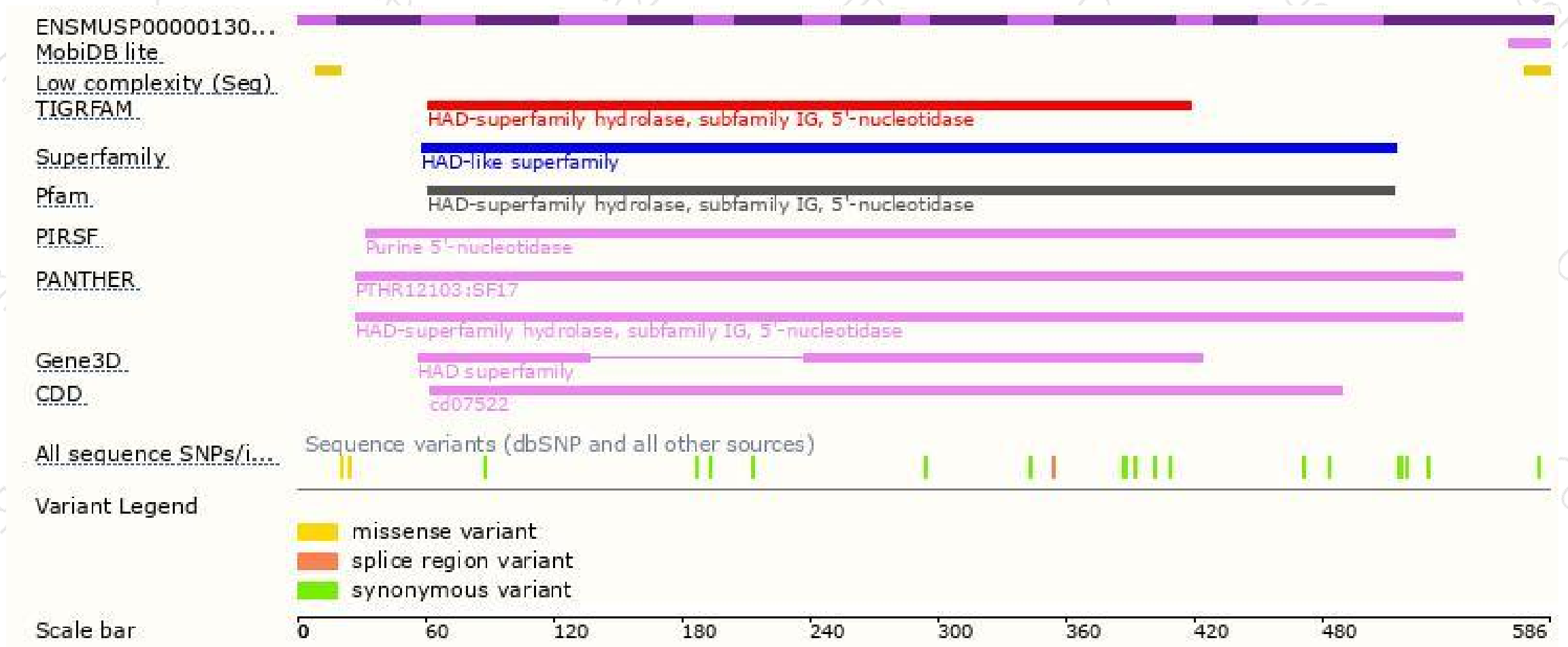


# Genomic location distribution

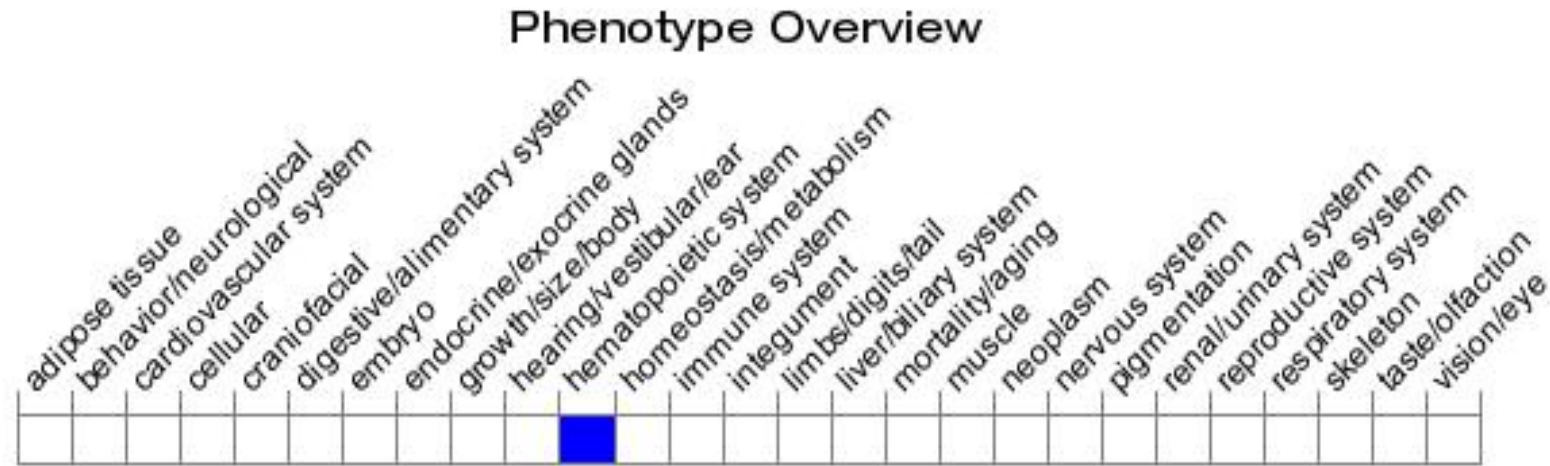




# Protein domain



# 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, Bone marrow cells with a nucleotide substitution allele used in a transplantation experiment following tamoxifen-induction produce NOTCH1-induced tumors that are resistant to 6-mercaptopurine chemotherapy.

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

Tel: 400-9660890

