

# ***Zeb2* Cas9-CKO Strategy**

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**Reviewer:**

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**Design Date:**

**2019-11-14**

# Project Overview

**Project Name**

***Zeb2***

**Project type**

**Cas9-CKO**

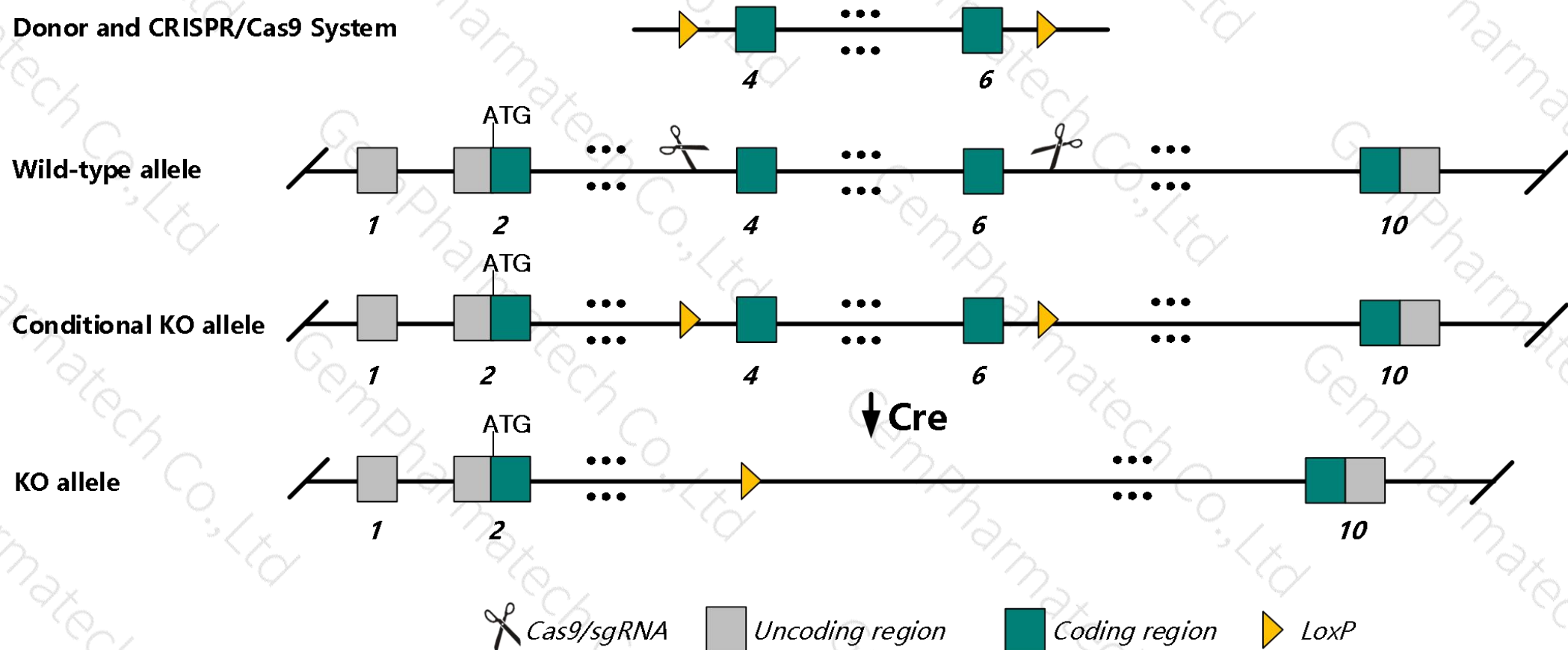
**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Zeb2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System



- The *Zeb2* gene has 30 transcripts. According to the structure of *Zeb2* gene, exon4-exon6 of *Zeb2*-202 (ENSMUST00000068415.10) transcript is recommended as the knockout region. The region contains 476bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Zeb2* 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, Homozygous null mutants exhibit a variety of defects at embryonic day 8.5 and die between embryonic days 9.5 and 10.5.
- The *Zeb2* 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)

## Zeb2 zinc finger E-box binding homeobox 2 [Mus musculus (house mouse)]

Gene ID: 24136, updated on 2-Apr-2019

### Summary



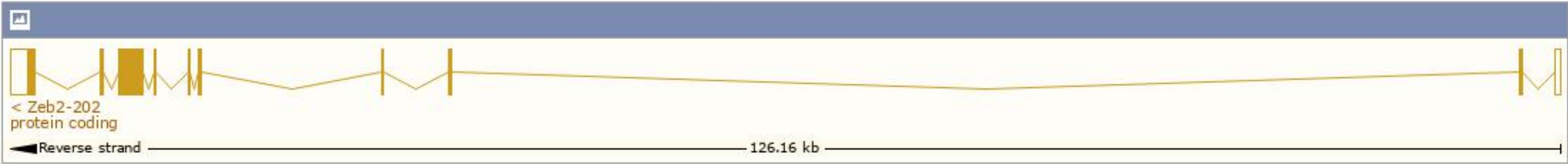
|                           |                                                                                                                                                                           |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Official Symbol</b>    | Zeb2 provided by <a href="#">MGI</a>                                                                                                                                      |
| <b>Official Full Name</b> | zinc finger E-box binding homeobox 2 provided by <a href="#">MGI</a>                                                                                                      |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1344407</a>                                                                                                                                           |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000026872</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>      | 9130203F04Rik, D130016B08Rik, SIP1, Zfhx1b, Zfx1b, Zfxh1b                                                                                                                 |
| <b>Expression</b>         | Ubiquitous expression in frontal lobe adult (RPKM 11.4), CNS E18 (RPKM 9.1) and 23 other tissues <a href="#">See more</a>                                                 |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>                                                                                                                                 |

# Transcript information（Ensembl）

The gene has 30 transcripts,all transcripts are shown below:

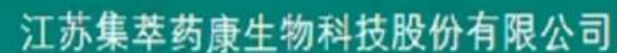
| Name     | Transcript ID          | bp   | Protein    | Biotype         | CCDS      | UniProt    | Flags                                                                                                                              |
|----------|------------------------|------|------------|-----------------|-----------|------------|------------------------------------------------------------------------------------------------------------------------------------|
| Zeb2-212 | ENSMUST000000176438.8  | 9124 | 1215aa     | Protein coding  | CCDS16020 | Q9RP0G7    | TSL5 GENCODE basic APPRIS P2                                                                                                       |
| Zeb2-202 | ENSMUST000000069415.10 | 5624 | 1215aa     | Protein coding  | CCDS16020 | Q9RP0G7    | TSL1 GENCODE basic APPRIS P2                                                                                                       |
| Zeb2-214 | ENSMUST000000177302.7  | 4020 | 1215aa     | Protein coding  | CCDS16020 | Q9RP0G7    | TSL1 GENCODE basic APPRIS P2                                                                                                       |
| Zeb2-201 | ENSMUST000000028229.12 | 5395 | 1258aa     | Protein coding  | -         | A0A0M3HEP2 | TSL1 GENCODE basic                                                                                                                 |
| Zeb2-215 | ENSMUST000000200844.3  | 4064 | 1191aa     | Protein coding  | -         | A0A0J9YV01 | TSL5 GENCODE basic                                                                                                                 |
| Zeb2-203 | ENSMUST000000078836.12 | 3913 | 1214aa     | Protein coding  | -         | H9H9S3     | TSL5 GENCODE basic APPRIS ALT1                                                                                                     |
| Zeb2-221 | ENSMUST00000021804.3   | 2236 | 731aa      | Protein coding  | -         | A0A0J9YVE6 | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-211 | ENSMUST00000153561.5   | 2070 | 37aa       | Protein coding  | -         | H3BRQ1     | TSL1 GENCODE basic                                                                                                                 |
| Zeb2-213 | ENSMUST00000176732.7   | 1762 | 220aa      | Protein coding  | -         | H3BRQ5     | TSL5 GENCODE basic                                                                                                                 |
| Zeb2-220 | ENSMUST000000201623.3  | 868  | 214aa      | Protein coding  | -         | A0A0J9YU94 | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-216 | ENSMUST000000201211.3  | 709  | 164aa      | Protein coding  | -         | A0A0J9YUV9 | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-222 | ENSMUST000000201969.3  | 631  | 141aa      | Protein coding  | -         | A0A0J9YUE5 | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-207 | ENSMUST000000127520.7  | 577  | 63aa       | Protein coding  | -         | F6X889     | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-224 | ENSMUST000000202187.3  | 550  | 91aa       | Protein coding  | -         | A0A0J9YV93 | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-219 | ENSMUST000000201490.1  | 462  | 83aa       | Protein coding  | -         | A0A0J9YU28 | CDS 3' incomplete TSL3                                                                                                             |
| Zeb2-228 | ENSMUST000000202935.3  | 443  | 113aa      | Protein coding  | -         | A0A0J9YTT5 | CDS 3' incomplete TSL5                                                                                                             |
| Zeb2-227 | ENSMUST000000202432.3  | 240  | 80aa       | Protein coding  | -         | A0A0J9YU4  | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL5 |
| Zeb2-204 | ENSMUST00000123037.2   | 3852 | No protein | Retained intron | -         | -          | TSL1                                                                                                                               |
| Zeb2-209 | ENSMUST00000145529.4   | 2924 | No protein | Retained intron | -         | -          | TSL1                                                                                                                               |
| Zeb2-226 | ENSMUST000000202371.3  | 2692 | No protein | Retained intron | -         | -          | TSL1                                                                                                                               |
| Zeb2-205 | ENSMUST00000124942.4   | 2989 | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |
| Zeb2-210 | ENSMUST00000152232.7   | 2690 | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |
| Zeb2-223 | ENSMUST000000201982.3  | 2608 | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |
| Zeb2-225 | ENSMUST000000202345.1  | 644  | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |
| Zeb2-208 | ENSMUST00000131635.7   | 618  | No protein | lncRNA          | -         | -          | TSL3                                                                                                                               |
| Zeb2-229 | ENSMUST000000209076.1  | 584  | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |
| Zeb2-230 | ENSMUST00000238346.1   | 457  | No protein | lncRNA          | -         | -          |                                                                                                                                    |
| Zeb2-218 | ENSMUST000000201413.3  | 453  | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |
| Zeb2-206 | ENSMUST00000126743.1   | 432  | No protein | lncRNA          | -         | -          | TSL1                                                                                                                               |
| Zeb2-217 | ENSMUST000000201298.1  | 370  | No protein | lncRNA          | -         | -          | TSL5                                                                                                                               |

The strategy is based on the design of Zeb2-202 transcript,The transcription is shown below

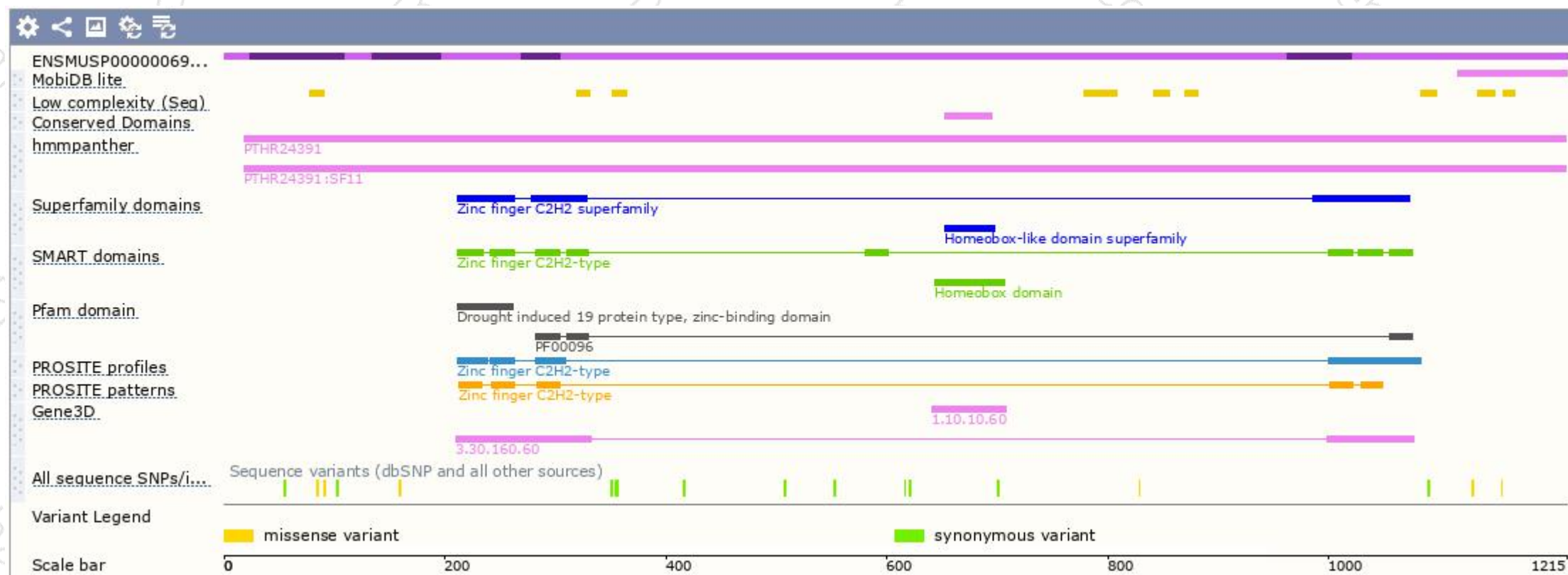




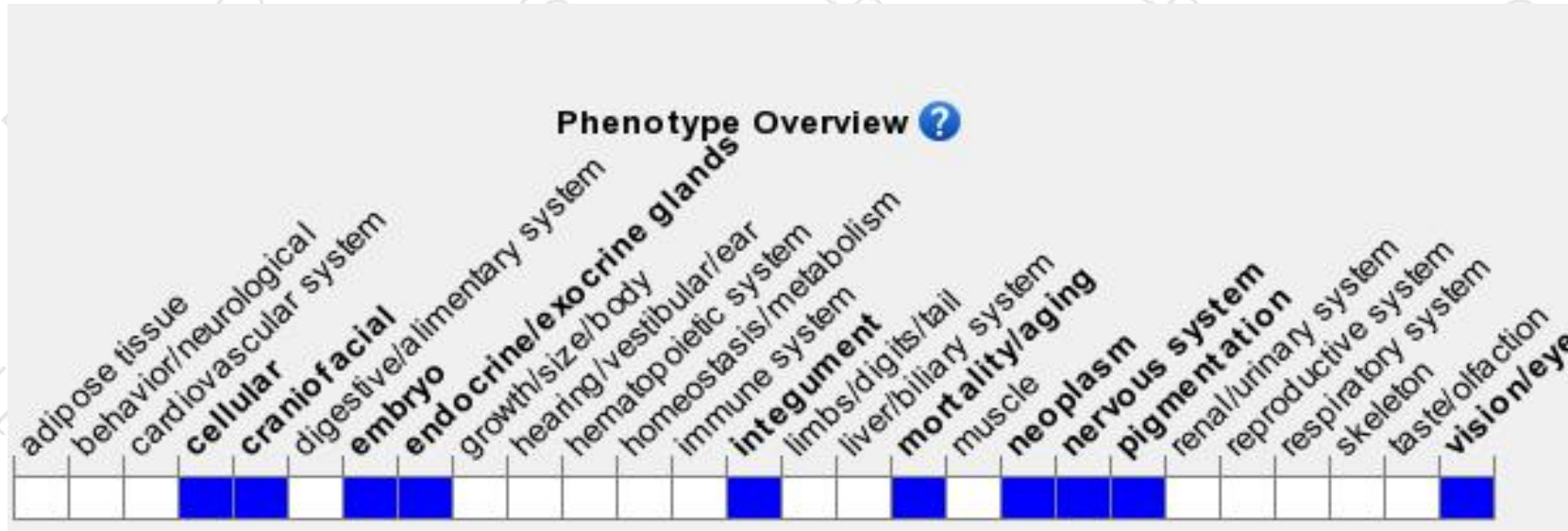
集萃药康  
GemPharmatech



# 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 exhibit a variety of defects at embryonic day 8.5 and die between embryonic days 9.5 and 10.5.

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

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