# *Igf2* Cas9-CKO Strategy

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**Reviewer:** Jia Yu

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# Project Overview

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|---|---|
| **Project Name** | ***Igf2*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

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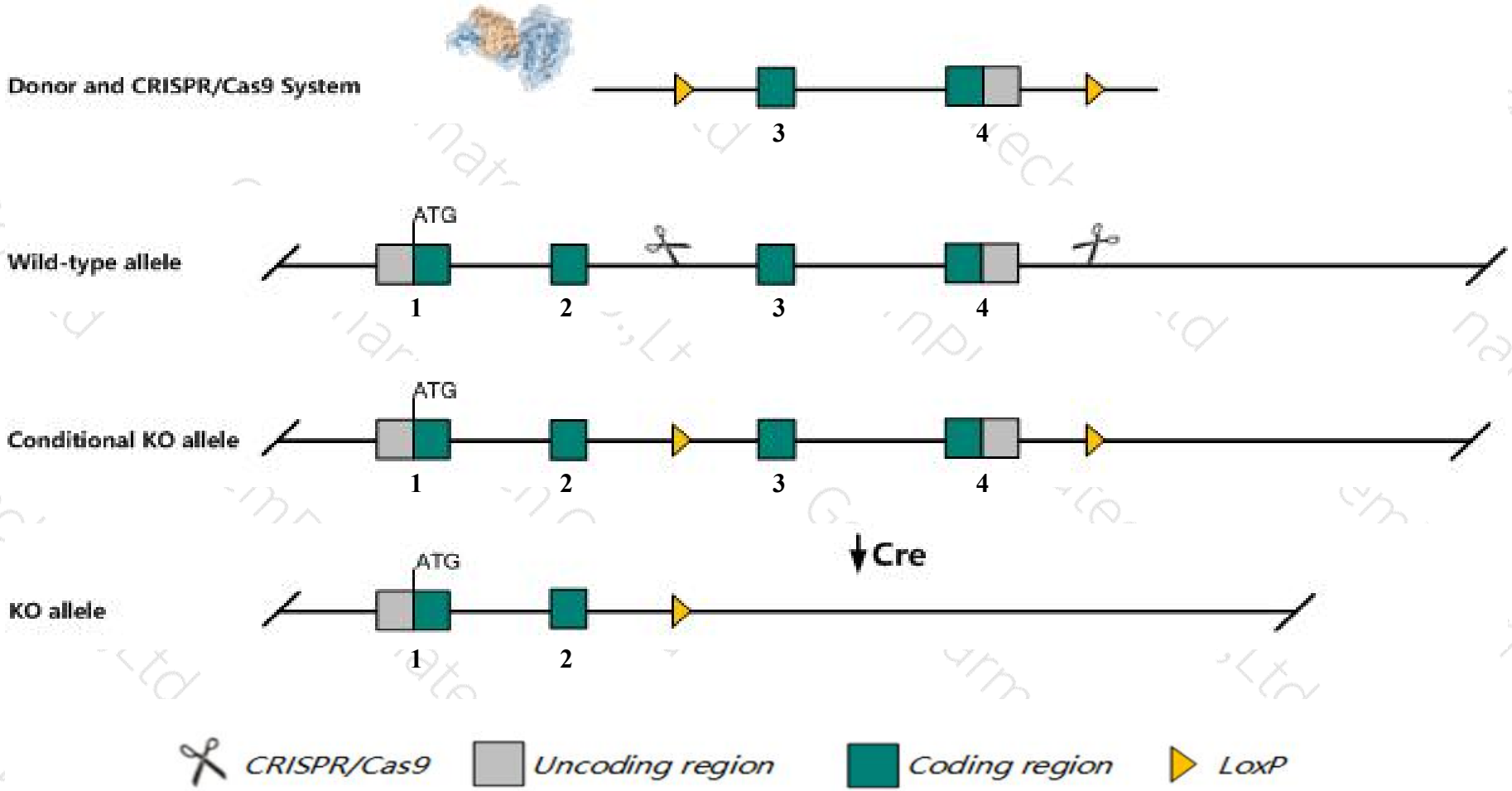
This model will use CRISPR/Cas9 technology to edit the *Igf2* gene. The schematic diagram is as follows:



# Technical routes

- The *Igf2* gene has 9 transcripts. According to the structure of *Igf2* gene, exon3-exon4 of *Igf2-205* (ENSMUST00000121128.7) transcript is recommended as the knockout region.The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Igf2* 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.

# Notice

- According to the existing MGI data,Mutations that are paternally transmitted result in growth deficiency. Heterozygous mice inheriting a mutant allele from their mother appear to be phenotypically normal.
- The effect on transcripts 207,209 is unknown.Transcript 206 may not be affected.
- The floxed region is near to the *Mir483-201* gene,the effect on *Mir483-201* is unknown
- *Gm94394* gene will be deleted together in this strategy.
- The N-terminal will remain some amino acid,it may remain the partial function.
- The *Igf2* gene is located on the Chr7. 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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## Igf2 insulin-like growth factor 2 [ *Mus musculus* (house mouse) ]

Gene ID: 16002, updated on 12-Nov-2019

### Summary

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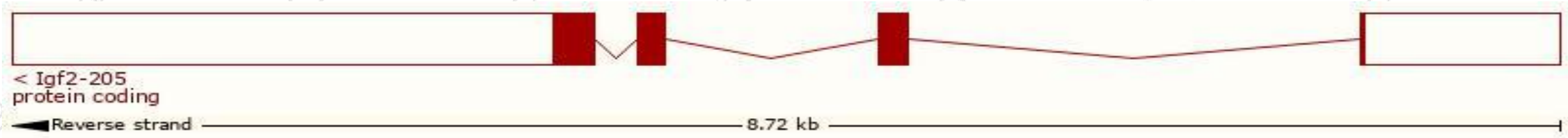
|---|---|
| **Official Symbol** | Igf2 provided by MGI |
| **Official Full Name** | insulin-like growth factor 2 provided by MGI |
| **Primary source** | MGI:MGI:96434 |
| **See related** | Ensembl:ENSMUSG00000048583 |
| **Gene type** | protein coding |
| **RefSeq status** | REVIEWED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | Mpr; M6pr; Peg2; Igf-2; Igf-II; AL033362 |
| **Summary** | This gene encodes a member of the insulin-like growth factor (IGF) family of proteins that promote growth and development during fetal and postnatal life. It is an imprinted gene that is expressed only from the paternal allele. The encoded protein undergoes proteolytic processing to generate a mature peptide. The transgenic overexpression of this gene in mice results in prenatal overgrowth, polyhydramnios, fetal and neonatal lethality, disproportionate organ overgrowth including tongue enlargement, and skeletal abnormalities. Mice lacking the encoded protein exhibit growth deficiency. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar processing to generate mature protein. [provided by RefSeq, Oct 2015] |
| **Expression** | Biased expression in limb E14.5 (RPKM 601.7), liver E18 (RPKM 468.8) and 7 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

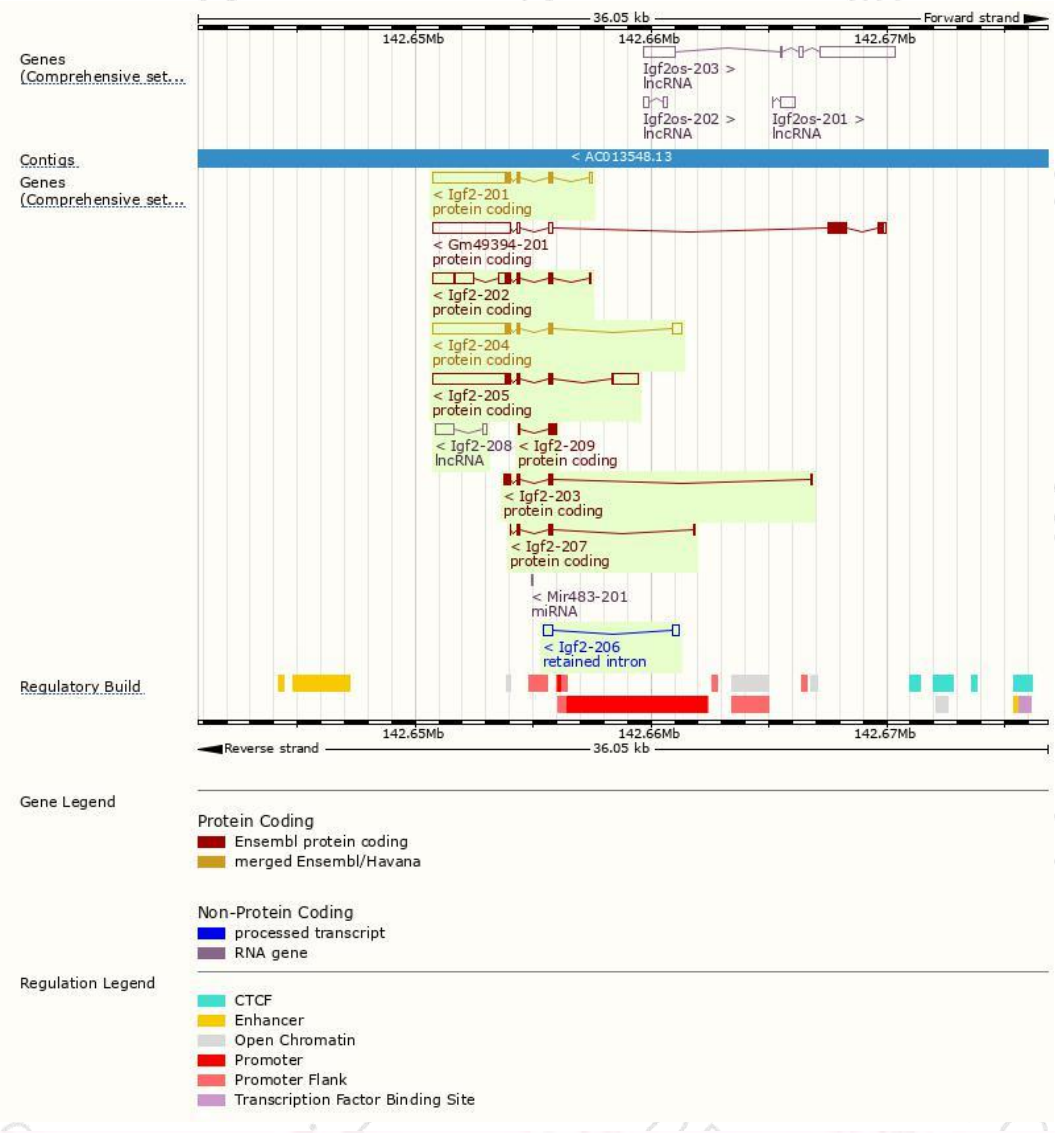
The gene has 9 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Igf2-205 | ENSMUST00000121128.7 | 4722 | 191aa | Protein coding | CCDS22033 | P09535 | TSL:2 GENCODE basic |
| Igf2-204 | ENSMUST00000105936.7 | 3970 | 180aa | Protein coding | CCDS52458 | P09535 | TSL:2 GENCODE basic APPRIS P1 |
| Igf2-201 | ENSMUST00000000033.11 | 3708 | 180aa | Protein coding | CCDS52458 | P09535 | TSL:1 GENCODE basic APPRIS P1 |
| Igf2-202 | ENSMUST00000097936.8 | 2503 | 180aa | Protein coding | CCDS52458 | P09535 | TSL:5 GENCODE basic APPRIS P1 |
| Igf2-203 | ENSMUST00000105935.7 | 650 | 180aa | Protein coding | CCDS52458 | P09535 | TSL:3 GENCODE basic APPRIS P1 |
| Igf2-209 | ENSMUST00000178921.1 | 462 | 121aa | Protein coding | - | J3QJZ9 | CDS 3' incomplete TSL:5 |
| Igf2-207 | ENSMUST00000145896.2 | 376 | 103aa | Protein coding | - | D3Z4N4 | CDS 3' incomplete TSL:5 |
| Igf2-206 | ENSMUST00000143666.2 | 649 | No protein | Retained intron | - | - | TSL:3 |
| Igf2-208 | ENSMUST00000163148.2 | 958 | No protein | lncRNA | - | - | TSL:3 |

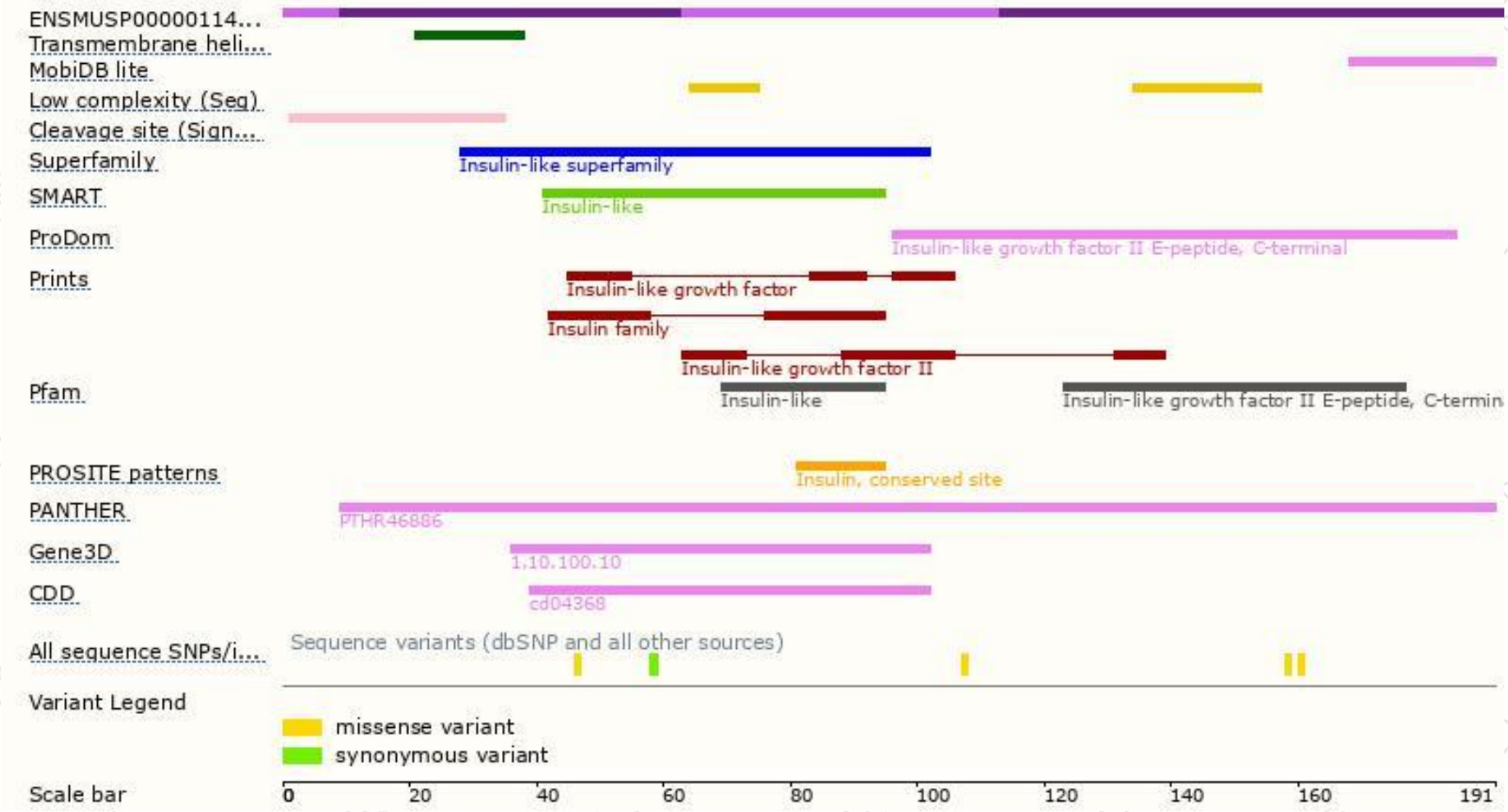
The strategy is based on the design of *Igf2-205* transcript,The transcription is shown below



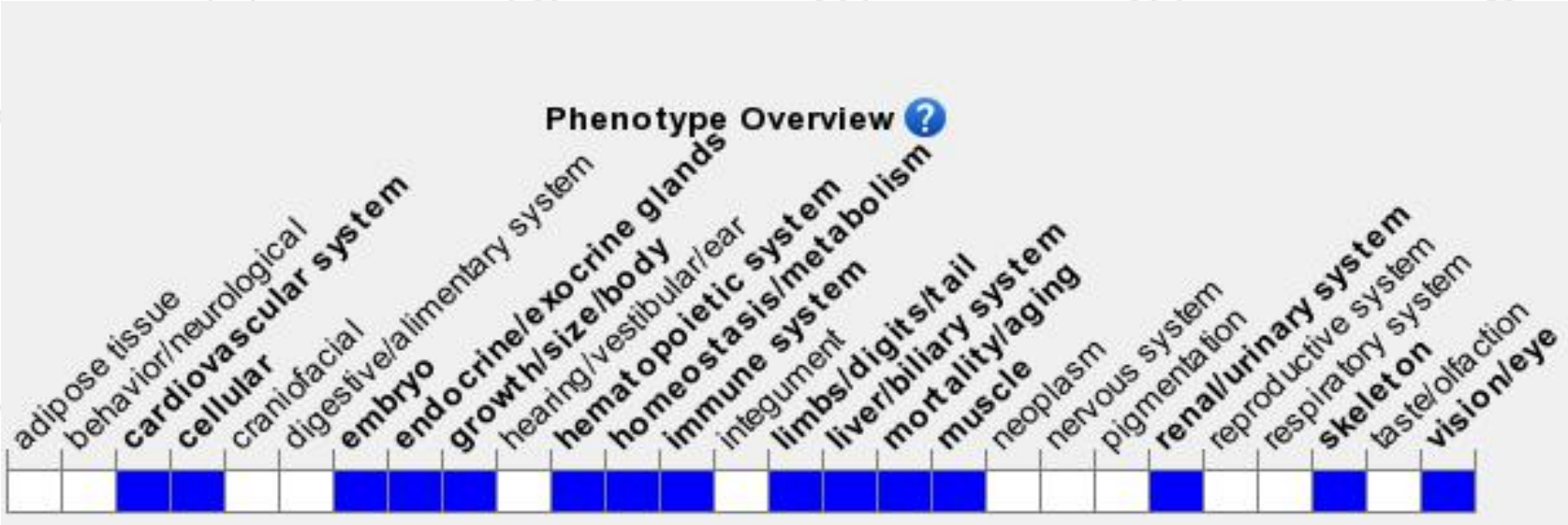
# Genomic location distribution



# 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,Mutations that are paternally transmitted result in growth deficiency.

Heterozygous mice inheriting a mutant allele from their mother appear to be phenotypically normal.

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

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