

Aqp5-IRES-CreERT2-EGFP cas9-ki Mouse Model Strategy

-CRISPR/Cas9 technology

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Project Overview

Project Name

Aqp5-IRES-CreERT2-EGFP

Project Type

cas9-ki

Background

C57BL/6JGpt

Project Cycle

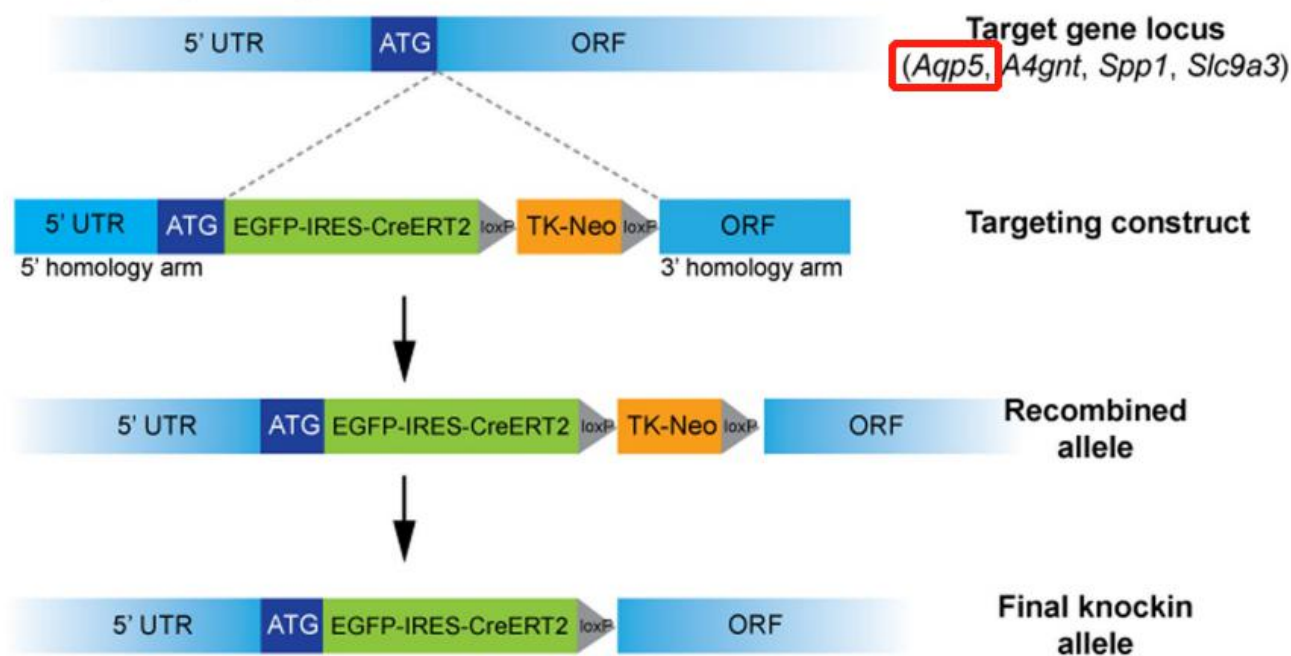
6-8 months

Technical Description

- The mouse *Aqp5* gene has 6 transcripts.
- According to the structure of *Aqp5* gene and clients' requirement ,the element CreERT2-IRES-EGFP-P2A will be inserted at the translation start codon(ATG) of *Aqp5*-202(ENSMUST00000169082.2), the length of inserted fragment is about 3.4kb.
- In this project, *Aqp5* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR, sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

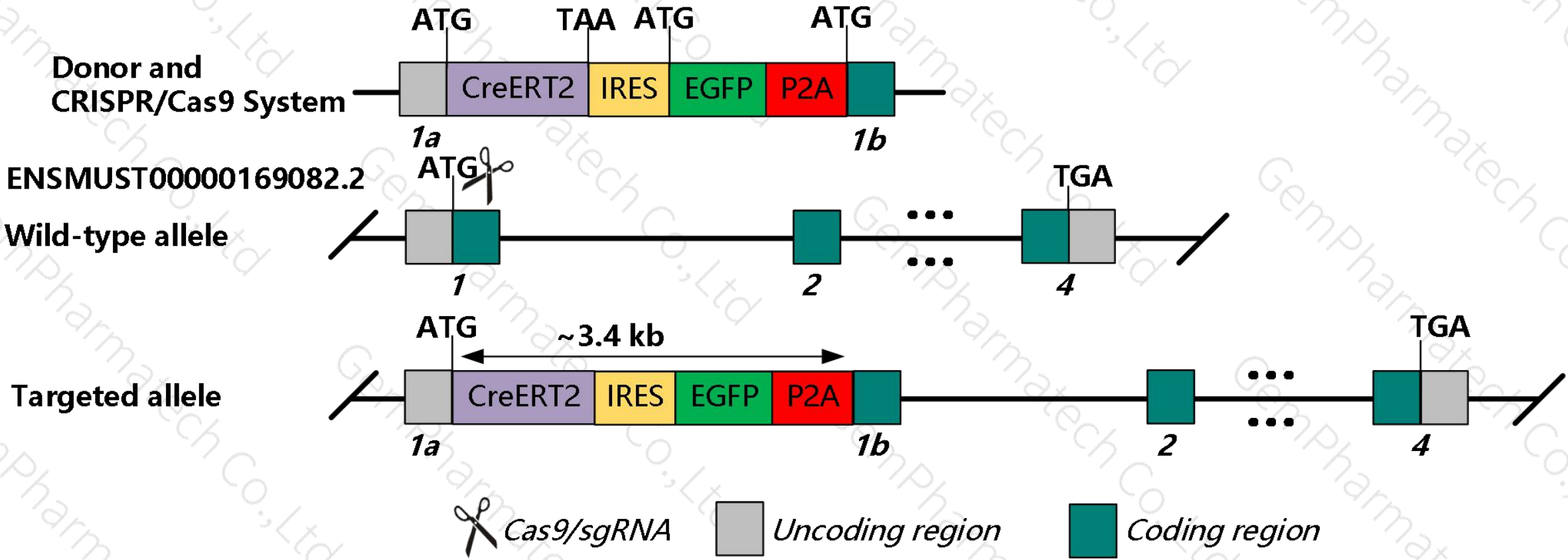
Tan SH, et al., AQP5 enriches for stem cells and cancer origins in the distal stomach. Nature. 2020 Feb;578(7795):437-443

a Targeting strategy for EGFP-IRES-CreERT2 construct



▼ Mutation details: The following construct was inserted immediately after the ATG start codon: the EGFP fluorescent reporter gene, IRES sequence, a cre-ERT2 fusion gene, and a loxP site flanked neomycin resistance gene cassette. The neo cassette was removed through subsequent cre-mediated recombination. ([J:292580](#))

This model uses CRISPR/Cas9 technology to edit the *Aqp5* gene and the schematic diagram is as follow:



- According to the existing MGI data, homozygous null mutants exhibit reduced growth on solid food and secrete diminished amounts of hypertonic, viscous saliva.
- There may be 1 to 2 amino acid synonymous mutation in exon1 of *Aqp5* gene in this strategy.
- If the two genes are linked with IRES, and they will be transcribed together and then be translated two protein separately. Otherwise, IRES may produce lower amounts of the downstream protein in relation to the upstream protein.
- If the two genes are linked with P2A, and these two genes will be transcribed together and then be translated two protein separately. Otherwise, P2A may produce lower amounts of the downstream protein in relation to the upstream protein.
- Mouse *Aqp5* gene is located on Chr15. Please take the loci in consideration when breeding this knockin mice with other gene modified (e.g., Tg, iCre) strains, if the other gene is also on Chr15, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 5'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene name and location (NCBI)

Aqp5 aquaporin 5 [*Mus musculus* (house mouse)]

Gene ID: 11830, updated on 15-Dec-2020

Summary

- Official Symbol

Aqp5 provided by [MGI](#)
- Official Full Name

aquaporin 5 provided by [MGI](#)
- Primary source

[MGI:MGI:106215](#)
- See related

[Ensembl:ENSMUSG00000044217](#)
- 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
- Expression

Biased expression in lung adult (RPKM 187.0), mammary gland adult (RPKM 72.8) and 4 other tissues [See more](#)
- Orthologs

[human](#) [all](#)

Genomic context

Location: 15 F1; 15 56.13 cM

See Aqp5 in [Genome Data Viewer](#)

Exon count: 4

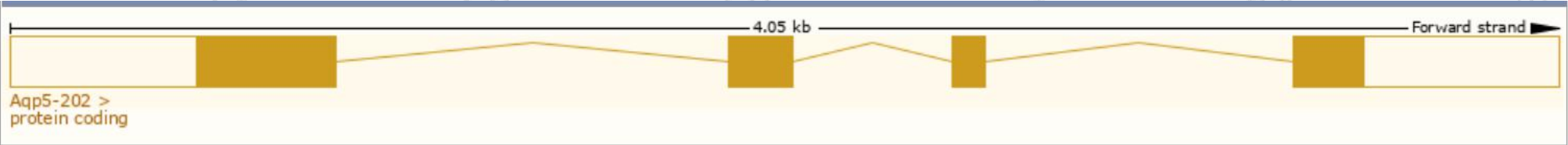
| Annotation release | Status | Assembly | Chr | Location |
|---------------------|-------------------|--|-----|----------------------------------|
| 109 | current | GRCm39 (GCF_000001635.27) | 15 | NC_000081.7 (99488909..99492710) |
| 108.20200622 | previous assembly | GRCm38.p6 (GCF_000001635.26) | 15 | NC_000081.6 (99591028..99594829) |
| Build 37.2 | previous assembly | MGSCv37 (GCF_000001635.18) | 15 | NC_000081.5 (99421459..99425260) |

Transcript information (Ensembl)

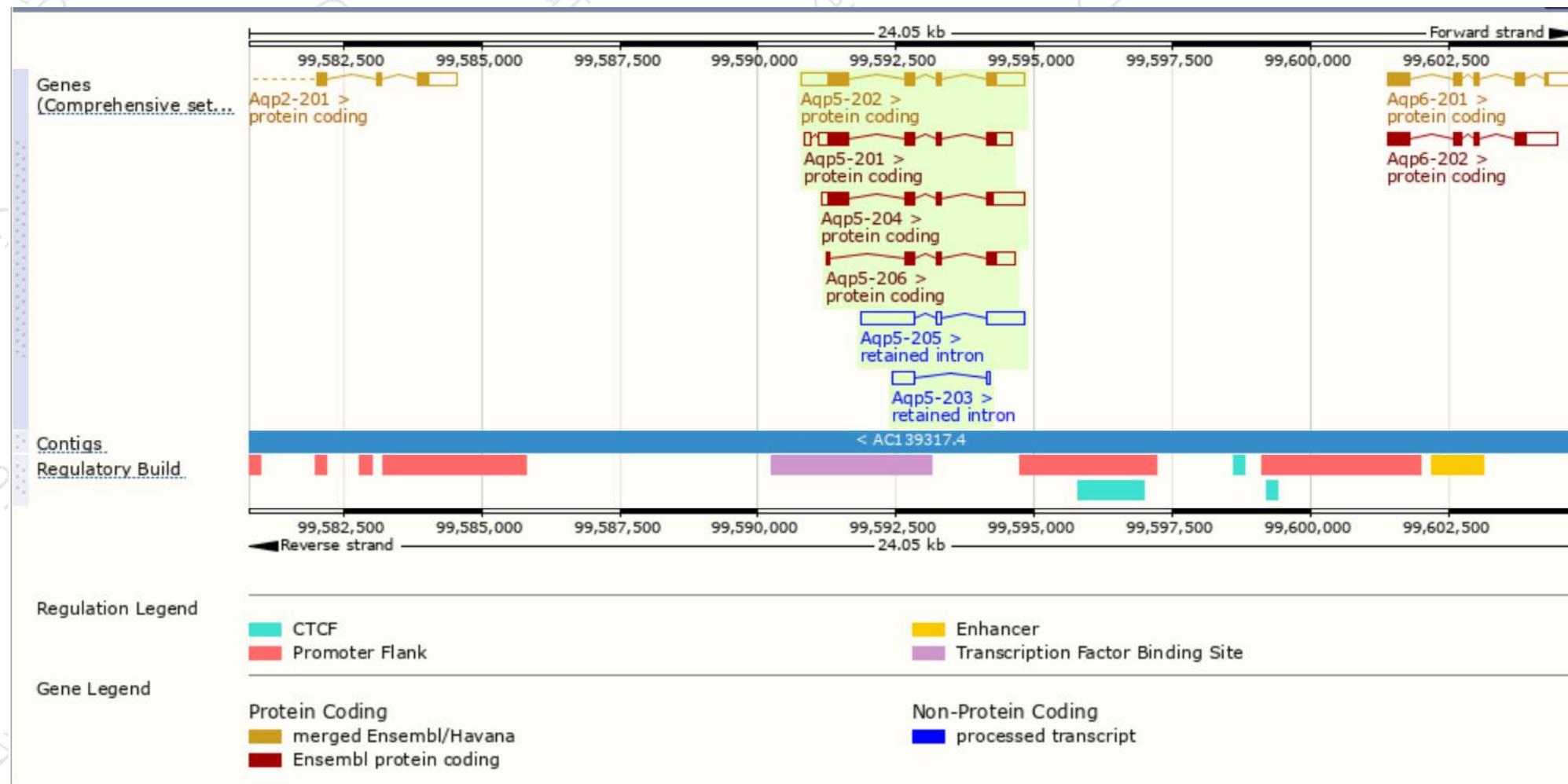
The gene has 6 transcripts, and all the transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt Match | Flags |
|----------|---------------------------------------|------|-----------------------|-----------------|---------------------------|----------------------------|-------------------------------|
| Aqp5-202 | ENSMUST00000169082.2 | 1797 | 265aa | Protein coding | CCDS27823 | Q9WTY4 | TSL:1 GENCODE basic APPRIS P1 |
| Aqp5-201 | ENSMUST00000088200.12 | 1376 | 265aa | Protein coding | CCDS27823 | Q9WTY4 | TSL:1 GENCODE basic APPRIS P1 |
| Aqp5-204 | ENSMUST00000229728.1 | 1415 | 241aa | Protein coding | - | A0A2R8VI16 | GENCODE basic |
| Aqp5-206 | ENSMUST00000231163.1 | 831 | 148aa | Protein coding | - | A0A2R8VHL2 | GENCODE basic |
| Aqp5-205 | ENSMUST00000230998.1 | 1739 | No protein | Retained intron | - | - | - |
| Aqp5-203 | ENSMUST00000229264.1 | 443 | No protein | Retained intron | - | - | - |

The strategy is based on the design of *Aqp5-202* transcript,the transcription is shown below:



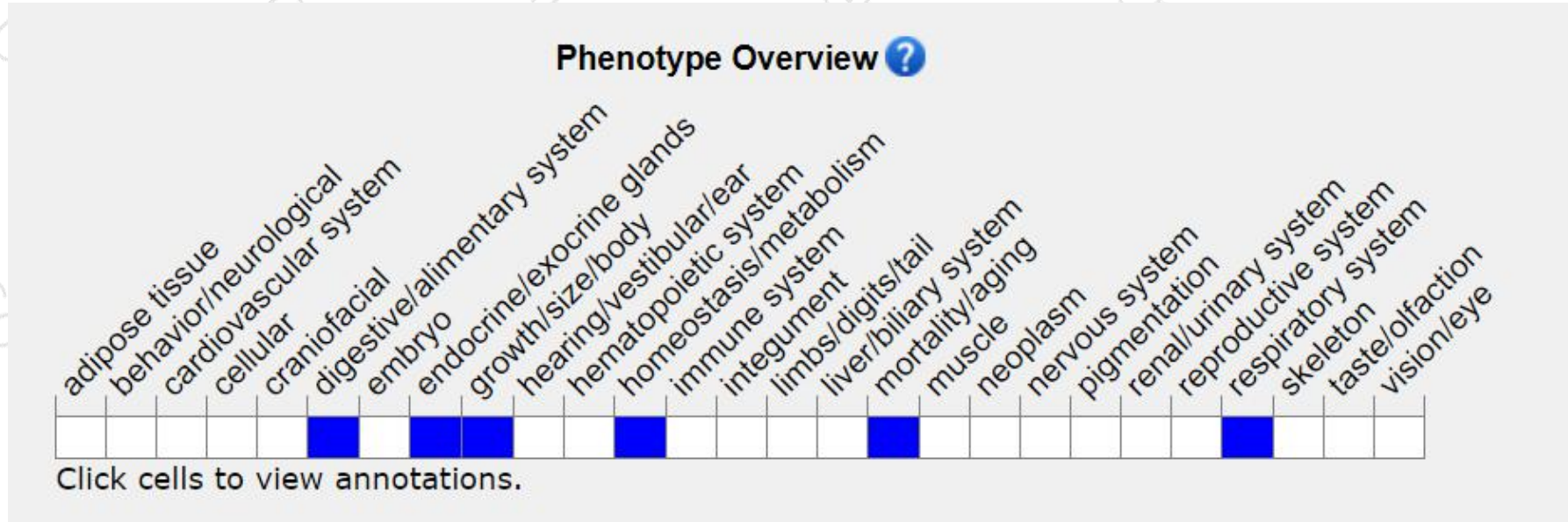
Genomic location distribution



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/marker/MGI:106215>) .

Homozygous null mutants exhibit reduced growth on solid food and secrete diminished amounts of hypertonic, viscous saliva.

If you have any questions, please feel free to contact us.
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