

***Fancd2* Cas9-CKO Strategy**

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Design Date:

Project Overview

Project Name

Fancd2

Project type

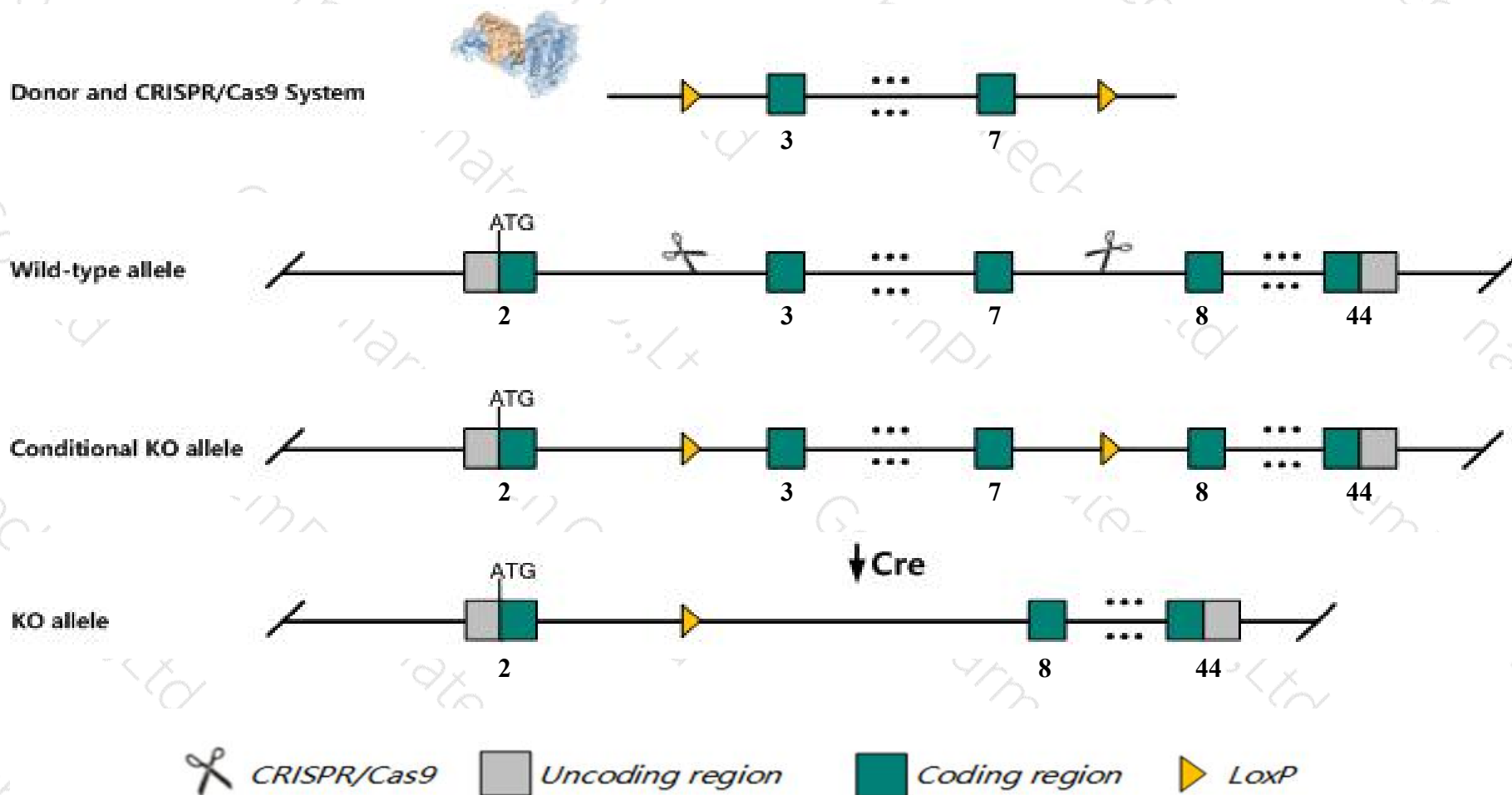
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Fancd2* gene. The schematic diagram is as follows:



Technical routes

- The *Fancd2* gene has 8 transcripts. According to the structure of *Fancd2* gene, exon3-exon7 of *Fancd2*-201 (ENSMUST00000036340.11) transcript is recommended as the knockout region. The region contains 421bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Fancd2* 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 mutant mice exhibit defects observed in human patients with Fanconi anemia (FA) meiotic defects and germ cell loss. In addition, mutant mice display perinatal lethality, susceptibility of epithelial cancer, and microphthalmia.
- The *Fancd2* gene is located on the Chr6. 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)

Fancd2 Fanconi anemia, complementation group D2 [Mus musculus (house mouse)]

Gene ID: 211651, updated on 31-Jan-2019

Summary



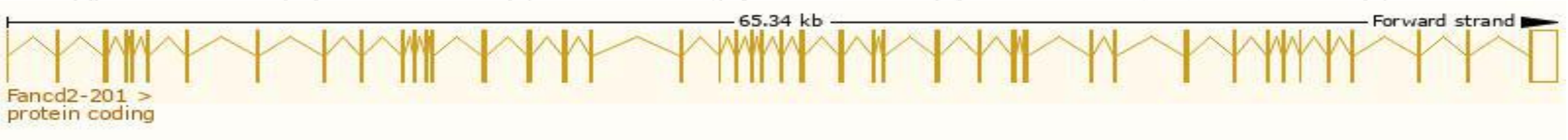
Official Symbol	Fancd2 provided by MGI
Official Full Name	Fanconi anemia, complementation group D2 provided by MGI
Primary source	MGI:MGI:2448480
See related	Ensembl:ENSMUSG00000034023
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410150O07Rik, AU015151, BB137857, FA-D2, FA4, FACD, FAD, FANCD
Expression	Broad expression in CNS E11.5 (RPKM 4.2), liver E14 (RPKM 4.2) and 20 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

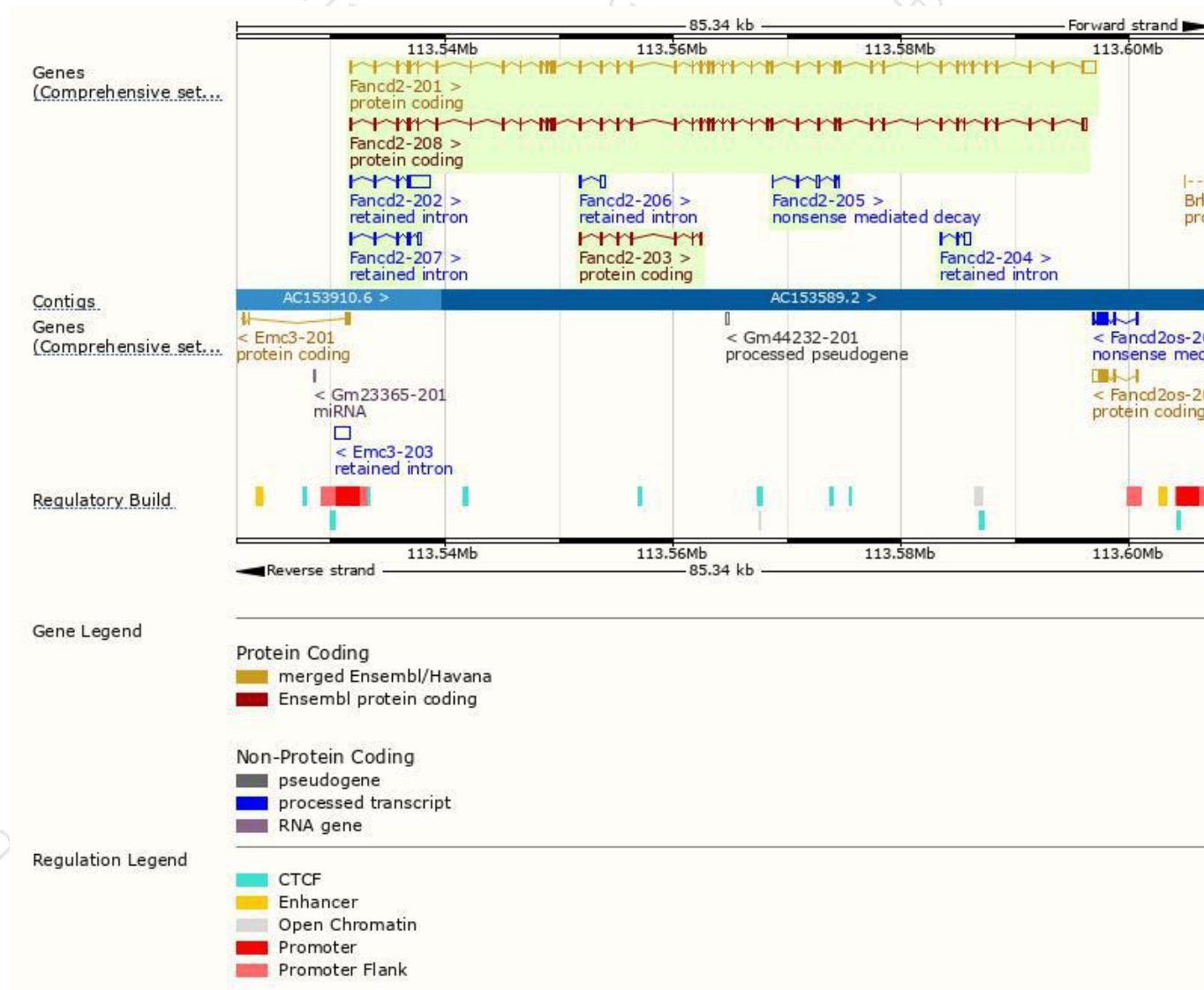
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fancd2-201	ENSMUST00000036340.11	5512	1450aa	Protein coding	CCDS20426	B2RSU4_Q80V62	TSL:1 GENCODE basic APPRIS P3
Fancd2-208	ENSMUST00000204827.2	4707	1437aa	Protein coding	CCDS85123	A0A0N4SV29	TSL:1 GENCODE basic APPRIS ALT2
Fancd2-203	ENSMUST00000123738.1	738	246aa	Protein coding	-	F7CAP1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Fancd2-205	ENSMUST00000129462.2	564	84aa	Nonsense mediated decay	-	A0A0N4SVS0	CDS 5' incomplete TSL:3
Fancd2-202	ENSMUST00000101051.4	2142	No protein	Retained intron	-	-	TSL:1
Fancd2-204	ENSMUST00000124262.1	764	No protein	Retained intron	-	-	TSL:2
Fancd2-207	ENSMUST00000143535.7	680	No protein	Retained intron	-	-	TSL:2
Fancd2-206	ENSMUST00000142453.1	391	No protein	Retained intron	-	-	TSL:2

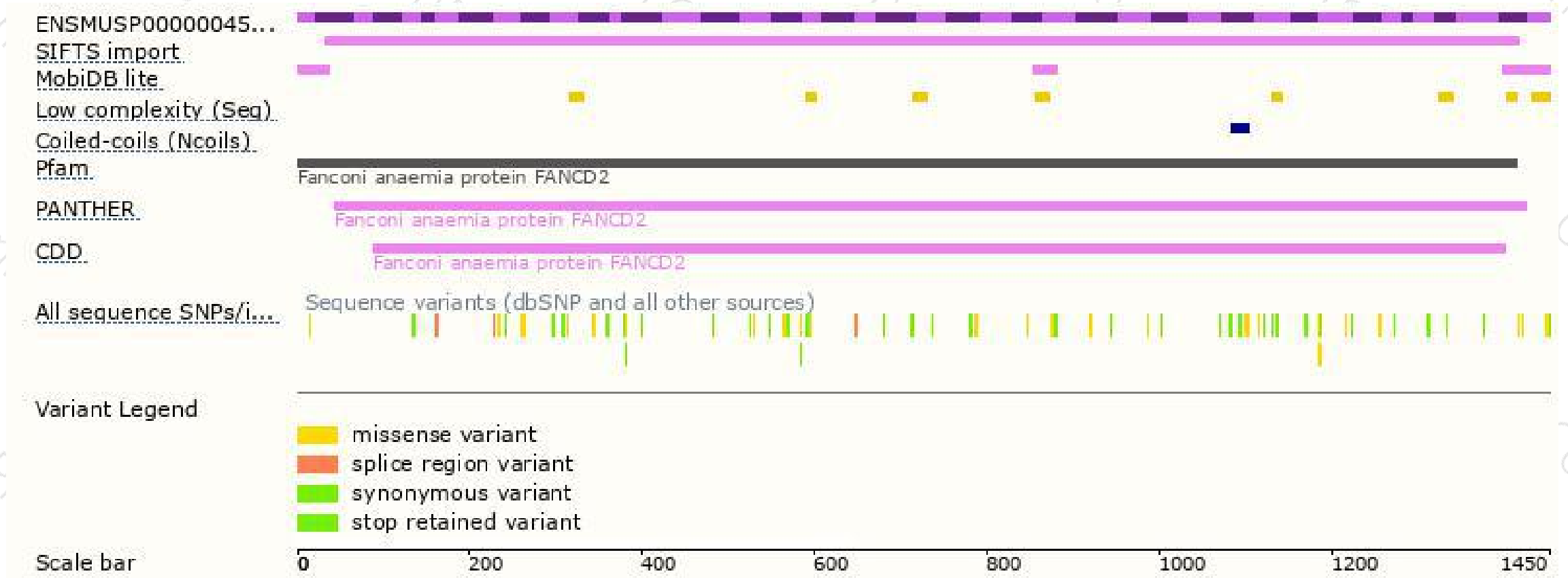
The strategy is based on the design of *Fancd2-201* transcript,The transcription is shown below



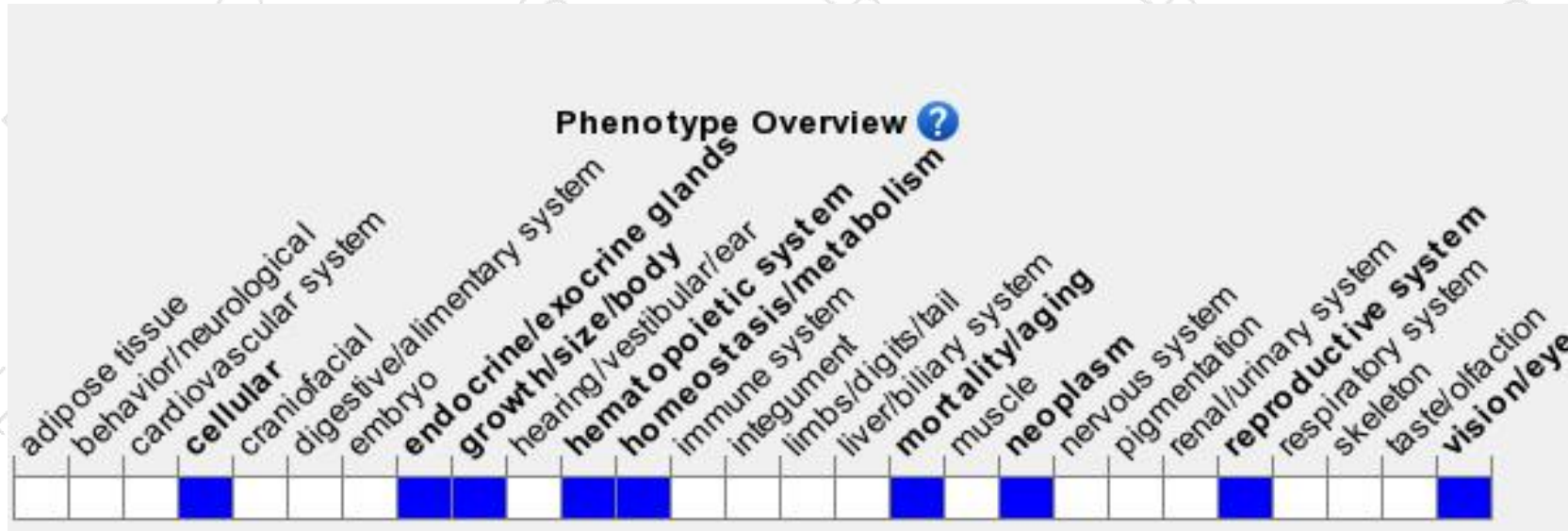
Genomic location distribution



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, Homozygous mutant mice exhibit defects observed in human patients with Fanconi anemia (FA) meiotic defects and germ cell loss. In addition, mutant mice display perinatal lethality, susceptibility to epithelial cancer, and microphthalmia.

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

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