Wdr35 Cas9-CKO *Strategy*

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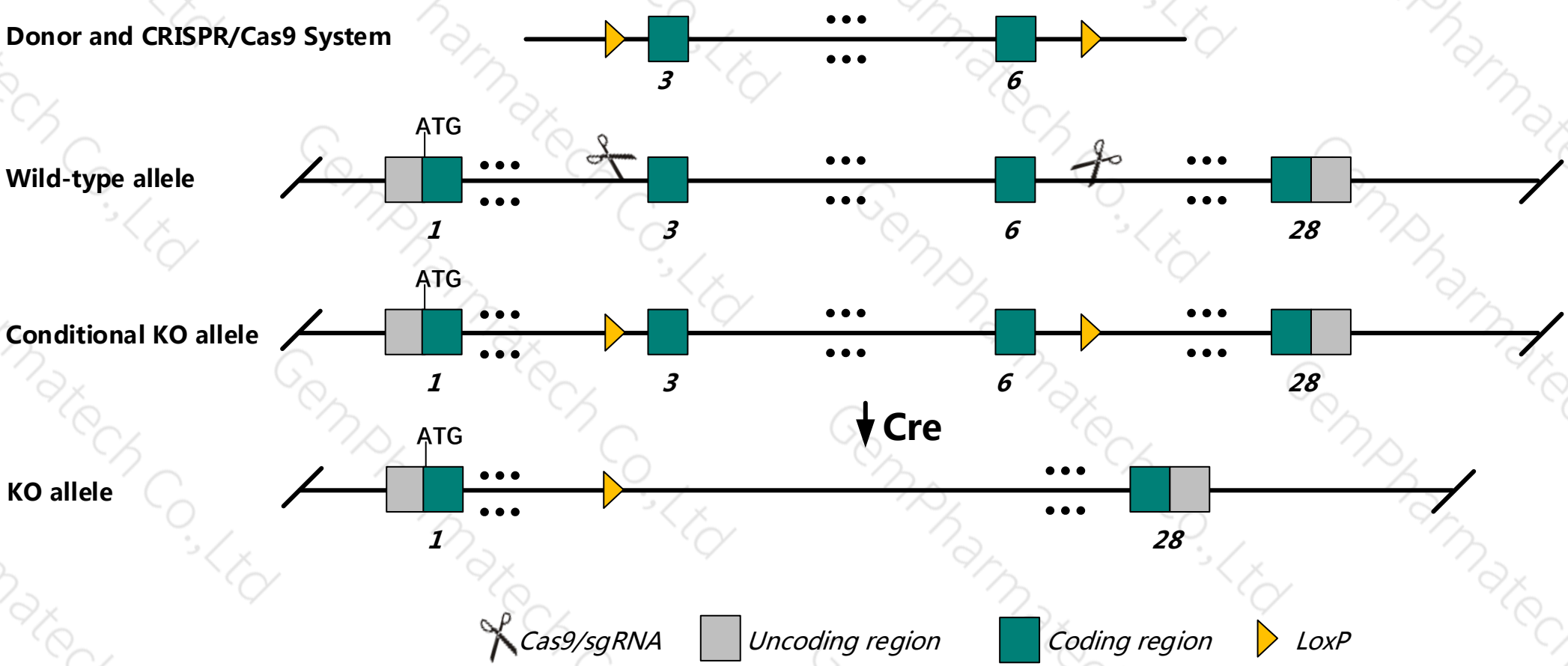
Project Overview

Project Name	***Wdr35***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wdr35* gene. The schematic diagram is as follows:



Technical routes

- The *Wdr35* gene has 7 transcripts.According to the structure of *Wdr35* gene, exon3-exon6 of *Wdr35*-201 (
- ENSMUST00000085745.12) transcript is recommended as the knockout region.The region contains 428bpcoding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Wdr35* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

Notice

- According to the existing MGI data，Mice homozygous for an ENU induced mutation exhibit mid-gestation lethality, heart development defects, turning defects, polysyndactyly, hypoplastic lungs, tracheoesophageal fistula, herniated diaphragm and absent embryonic cilia.

- The *Wdr35* gene is located on the Chr12. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Wdr35 WD repeat domain 35 [*Mus musculus* (house mouse)]

Gene ID: 74682, updated on 13-Mar-2020

Summary

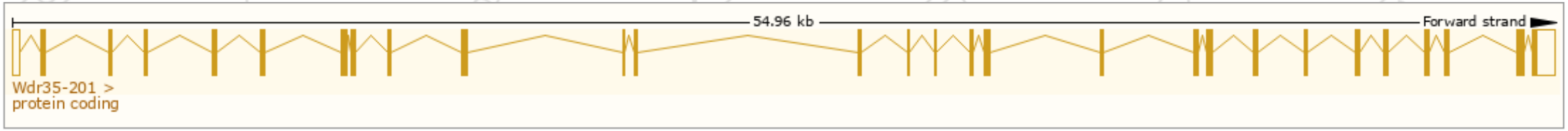
Official Symbol	Wdr35 provided by MGI
Official Full Name	WD repeat domain 35 provided by MGI
Primary source	MGI:MGI:1921932
See related	Ensembl:ENSMUSG00000066643
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	mKIAA1336; 4931430C06; 4930459M12Rik
Expression	Broad expression in testis adult (RPKM 14.6), CNS E18 (RPKM 6.0) and 15 other tissues See more
Orthologs	human all

Transcript information（ Ensembl ）

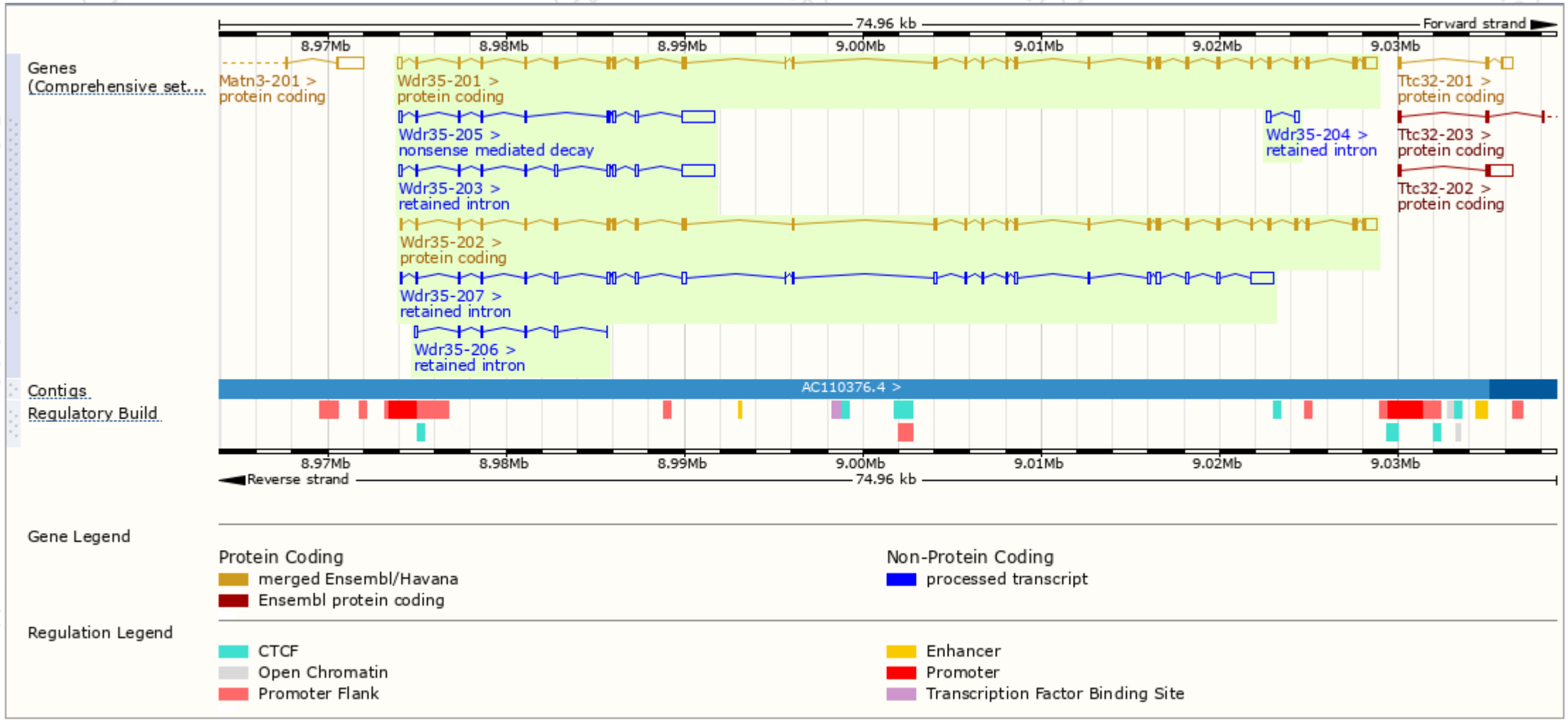
The gene has 7 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wdr35-201	ENSMUST00000085745.12	4501	1181aa	Protein coding	CCDS25807	Q8BND3	TSL:1 GENCODE basic APPRIS P3
Wdr35-202	ENSMUST00000111113.2	4312	1170aa	Protein coding	CCDS49027	Q8BND3	TSL:1 GENCODE basic APPRIS ALT1
Wdr35-205	ENSMUST00000160329.7	2792	186aa	Nonsense mediated decay	-	E0CYD5	TSL:1
Wdr35-207	ENSMUST00000161019.7	3941	No protein	Retained intron	-	-	TSL:1
Wdr35-203	ENSMUST00000159126.7	2921	No protein	Retained intron	-	-	TSL:1
Wdr35-206	ENSMUST00000160873.1	656	No protein	Retained intron	-	-	TSL:2
Wdr35-204	ENSMUST00000159735.1	557	No protein	Retained intron	-	-	TSL:3

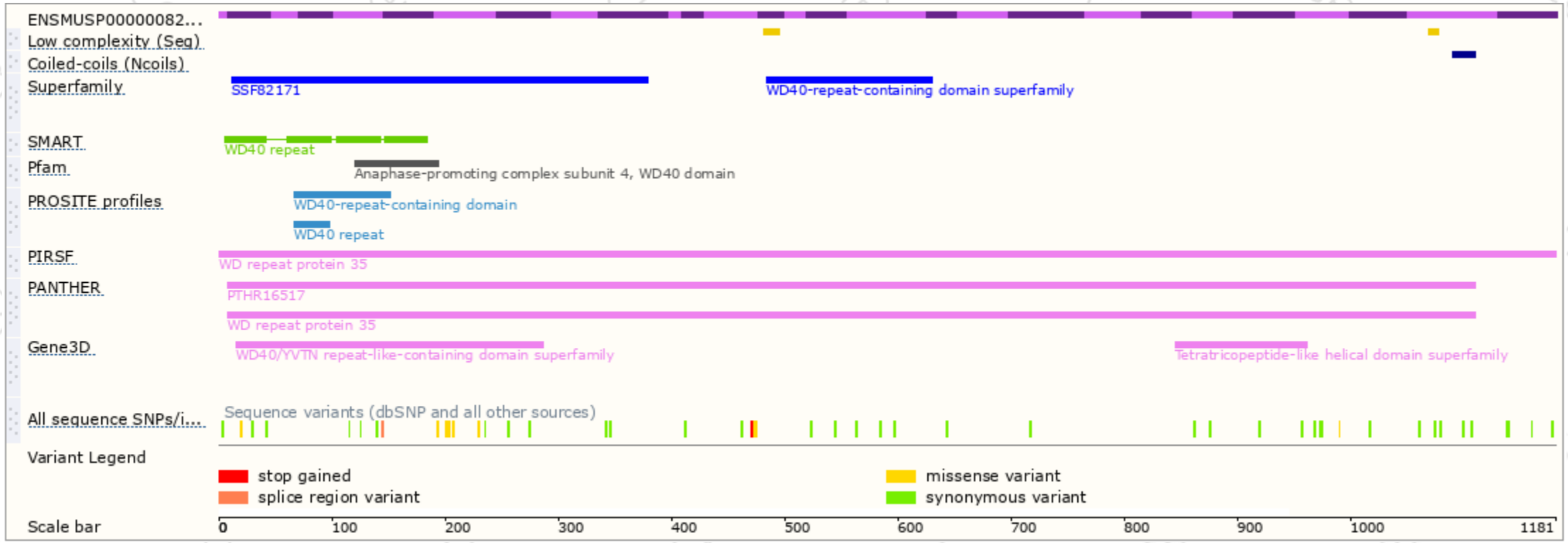
The strategy is based on the design of *Wdr35*-201 transcript,the transcription is shown below:



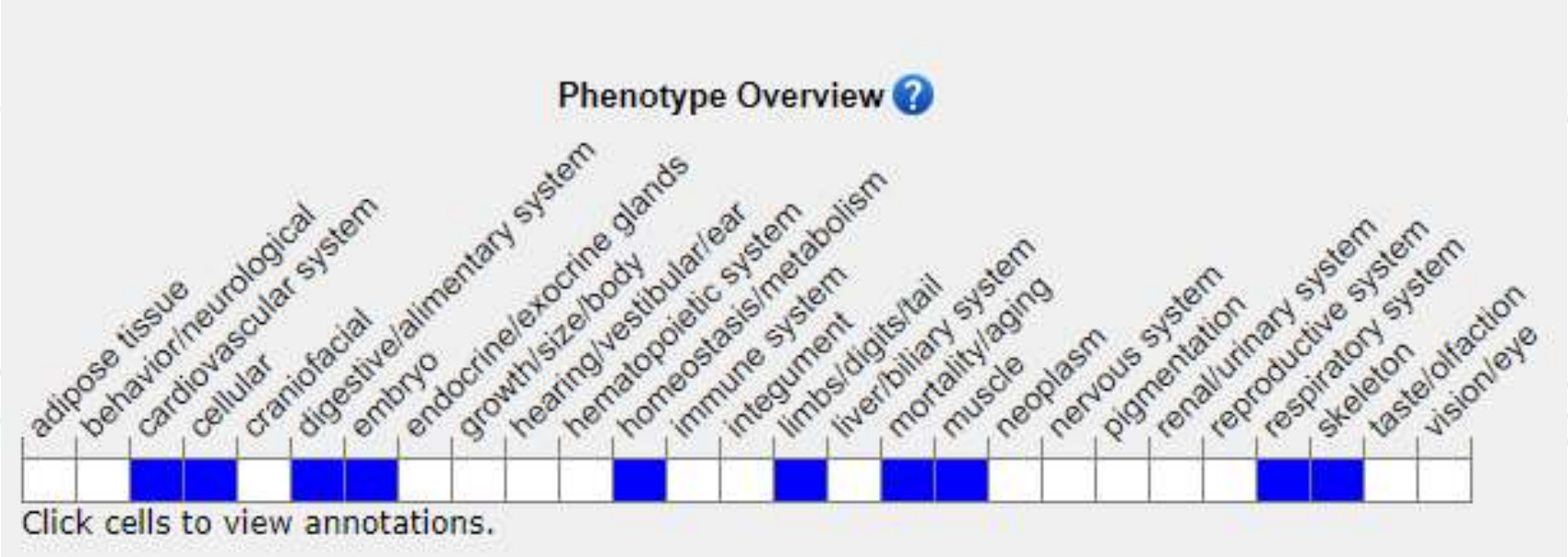
Genomic location distribution



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,mice homozygous for an ENU induced mutation exhibit mid-gestation lethality, heart development defects, turning defects, polysyndactyly, hypoplastic lungs, tracheoesophageal fistula, herniated diaphragm and absent embryonic cilia.

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

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