

Nf2 Cas9-CKO Strategy

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Project Overview

Project Name

Nf2

Project type

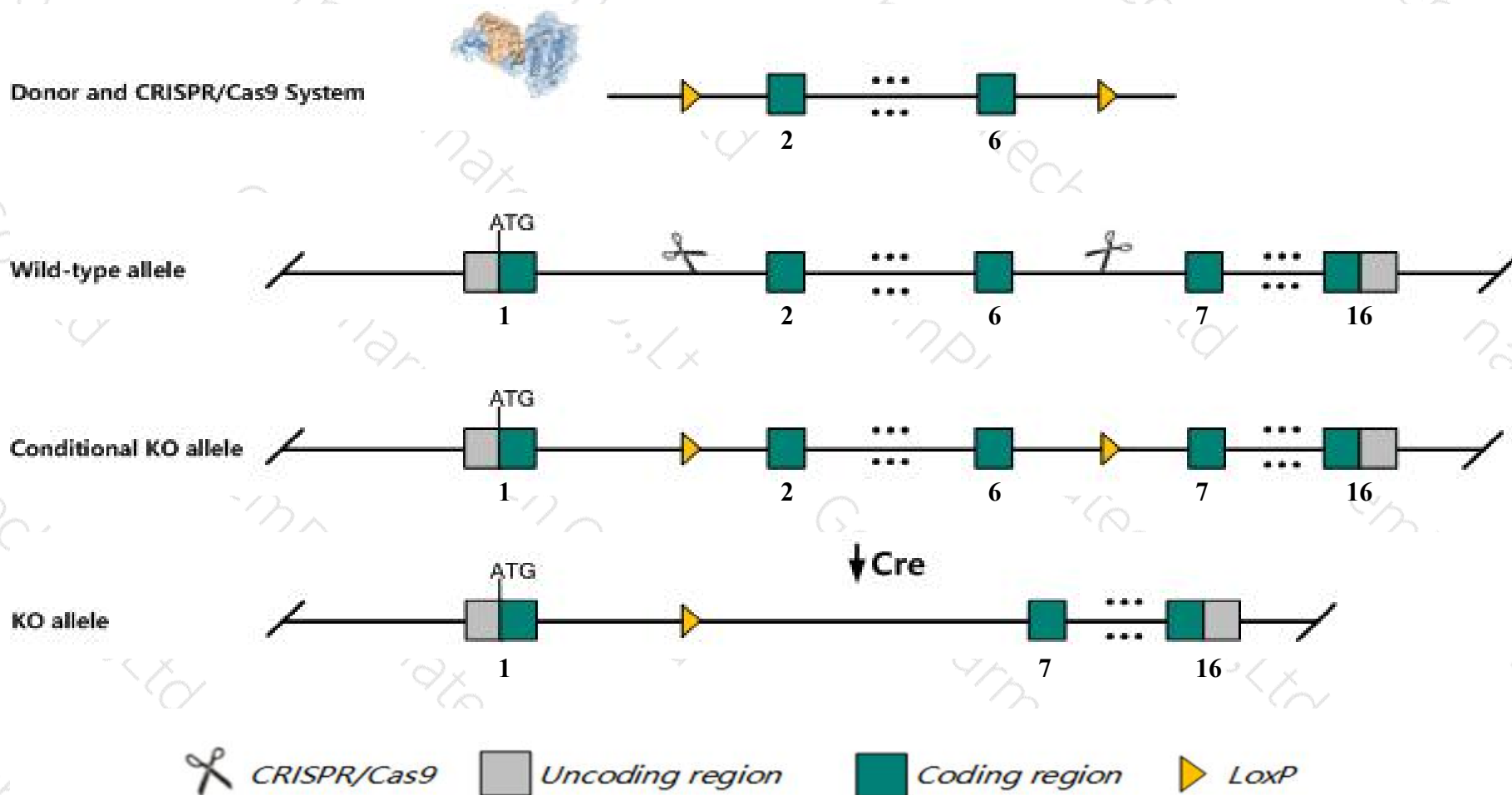
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nf2* gene. The schematic diagram is as follows:



- The *Nf2* gene has 13 transcripts. According to the structure of *Nf2* gene, exon2-exon6 of *Nf2-205* (ENSMUST00000109910.8) transcript is recommended as the knockout region. The region contains 485bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nf2* 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 targeted null mutants lack extraembryonic ectoderm, do not initiate gastrulation and die by embryonic day 7. Heterozygotes develop malignant tumors, especially osteosarcomas. Conditional Schwann cell knockouts resemble neurofibromatosis type 2.
- The *Nf2* gene is located on the Chr11. 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)

Nf2 neurofibromin 2 [Mus musculus (house mouse)]

Gene ID: 18016, updated on 5-Feb-2019

Summary



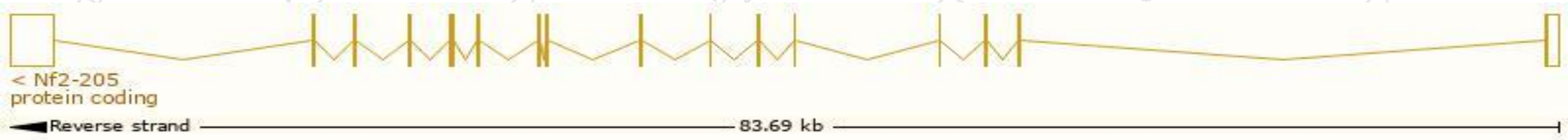
Official Symbol	Nf2 provided by MGI
Official Full Name	neurofibromin 2 provided by MGI
Primary source	MGI:MGI:97307
See related	Ensembl:ENSMUSG00000009073
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	merlin
Expression	Ubiquitous expression in adrenal adult (RPKM 31.2), testis adult (RPKM 20.8) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

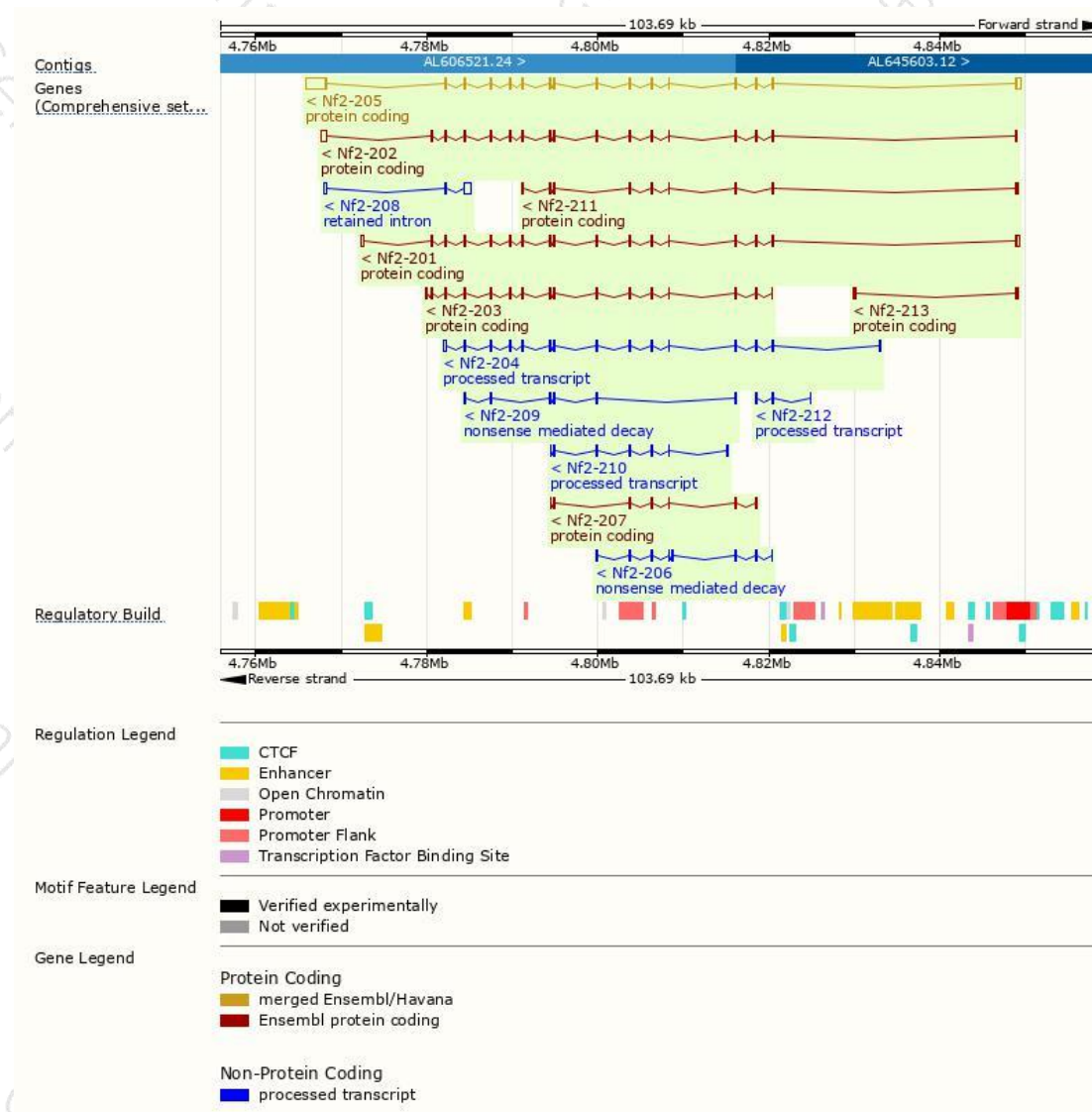
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nf2-205	ENSMUST00000109910.8	4720	596aa	Protein coding	CCDS24391	P46662	TSL:1 GENCODE basic APPRIS P3
Nf2-201	ENSMUST00000053079.12	2470	591aa	Protein coding	CCDS56757	P46662	TSL:1 GENCODE basic APPRIS ALT 1
Nf2-202	ENSMUST00000056290.12	2394	591aa	Protein coding	CCDS56757	P46662	TSL:1 GENCODE basic APPRIS ALT 1
Nf2-203	ENSMUST00000093374.10	1795	521aa	Protein coding	-	F8WJ43	CDS 5' incomplete TSL:5
Nf2-211	ENSMUST00000164190.7	963	251aa	Protein coding	-	E9PW99	CDS 3' incomplete TSL:5
Nf2-213	ENSMUST00000172305.1	533	131aa	Protein coding	-	E9Q7I0	CDS 3' incomplete TSL:3
Nf2-207	ENSMUST00000137926.8	476	159aa	Protein coding	-	F7D6P2	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
Nf2-206	ENSMUST00000124878.2	738	83aa	Nonsense mediated decay	-	F6X089	CDS 5' incomplete TSL:5
Nf2-209	ENSMUST00000152656.7	642	140aa	Nonsense mediated decay	-	F7DBY2	CDS 5' incomplete TSL:5
Nf2-204	ENSMUST00000109908.7	2012	No protein	Processed transcript	-	-	TSL:1
Nf2-210	ENSMUST00000155600.7	627	No protein	Processed transcript	-	-	TSL:3
Nf2-212	ENSMUST00000167480.1	341	No protein	Processed transcript	-	-	TSL:3
Nf2-208	ENSMUST00000141342.2	1159	No protein	Retained intron	-	-	TSL:2

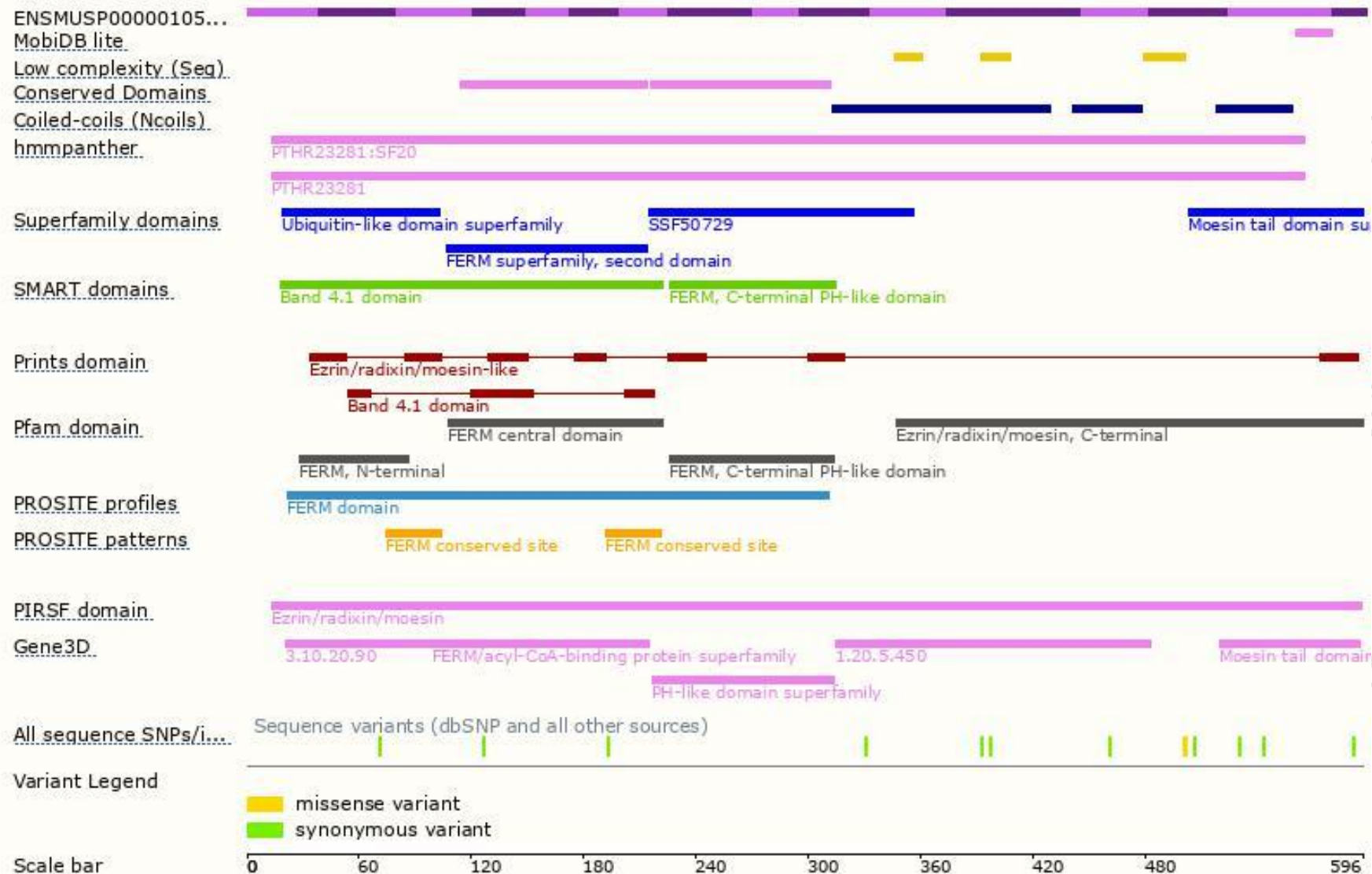
The strategy is based on the design of Nf2-205 transcript,The transcription is shown below



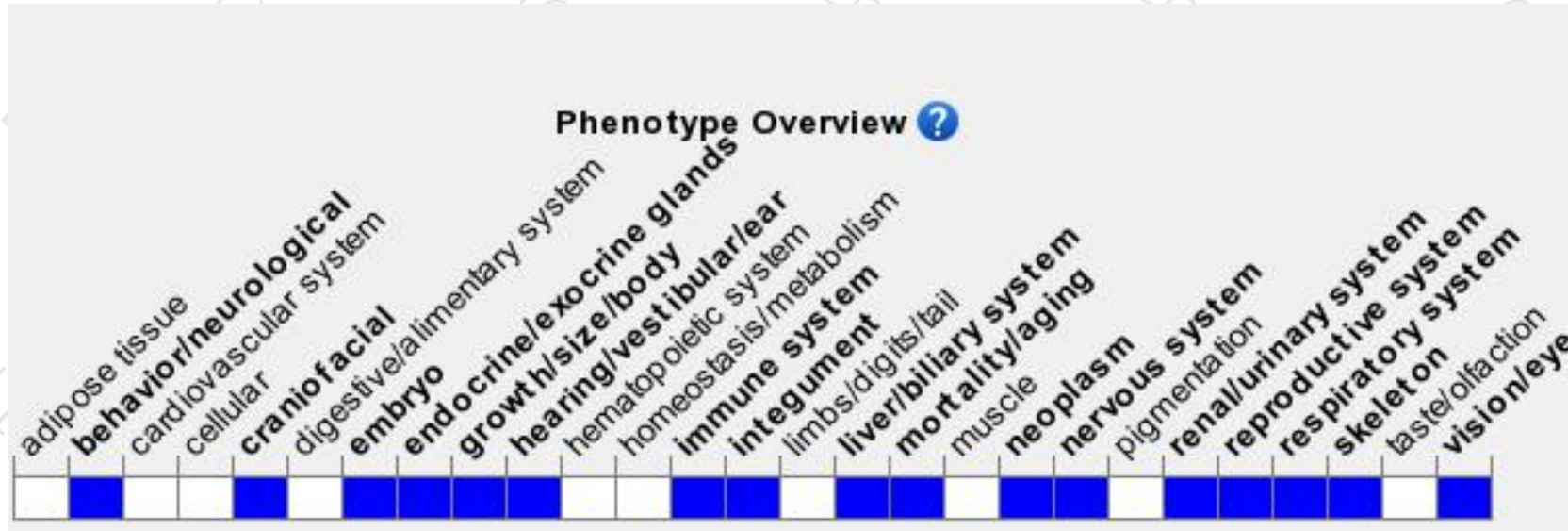
Genomic location distribution



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, Homozygous targeted null mutants lack extraembryonic ectoderm, do not initiate gastrulation and die by embryonic day 7. Heterozygotes develop malignant tumors, especially osteosarcomas. Conditional Schwann cell knockouts resemble neurofibromatosis type 2.

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

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