Smurf1 Cas9-CKO Strategy

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Project Overview

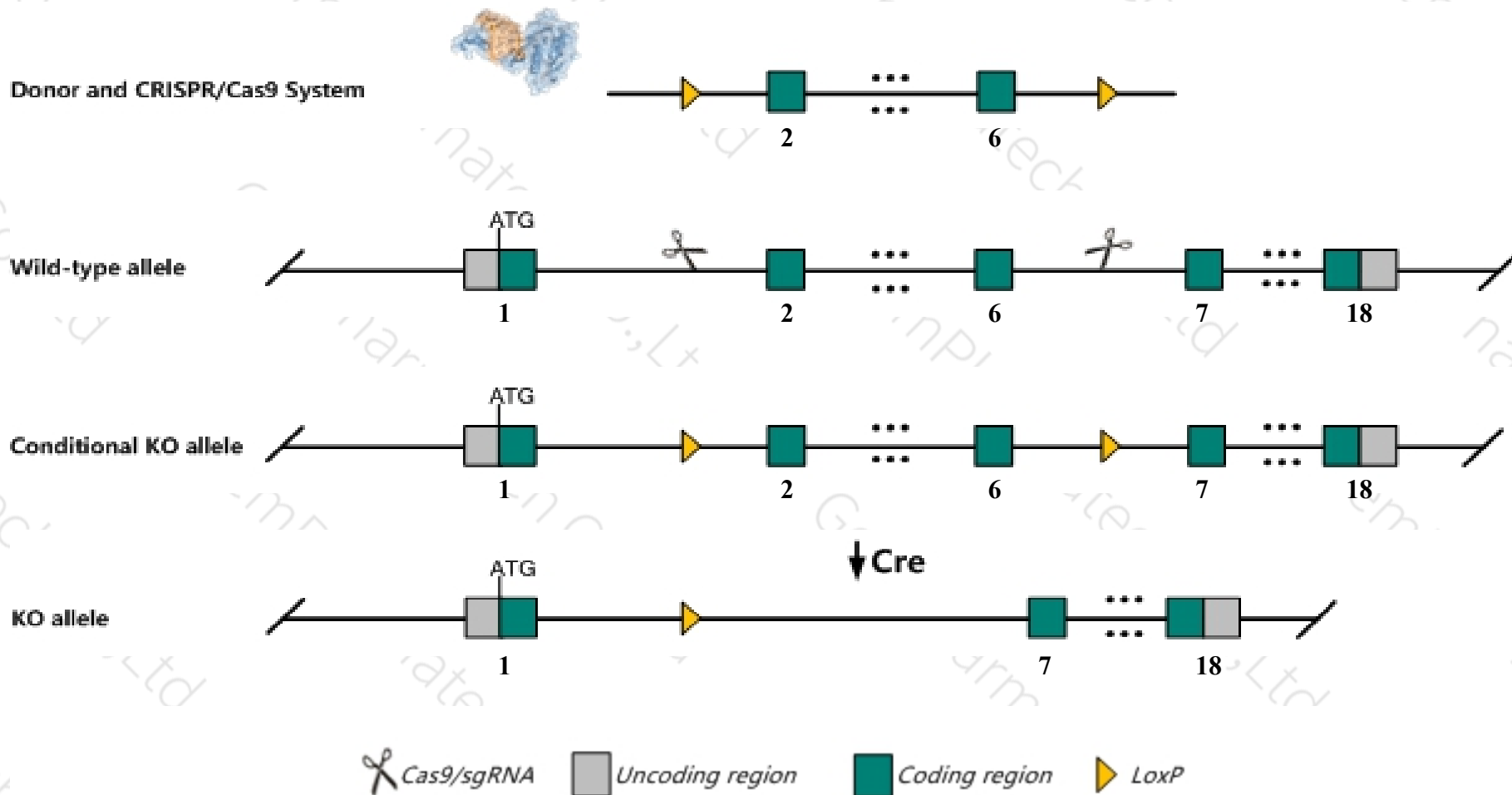|	
Project Name	***Smurf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Smurf1* gene. The schematic diagram is as follows:

Technical routes

➤ The *Smurf1* gene has 5 transcripts. According to the structure of *Smurf1* gene, exon2-exon6 of *Smurf1-201* (ENSMUST00000085684.10) transcript is recommended as the knockout region.The region contains 424bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Smurf1* 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/6J 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/6J 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 and cell types.

Notice

➢ According to the existing MGI data,Mice homozygous for one knock-out allele display increased osteoblast function, bone density, and thickness of the cortical bone in long bones. Mice homozygous for a different knock-out allele are viable and only display gastrulation defects in combination with a Smurf2 knock-out allele.

➢ The *Smurf1* gene is located on the Chr5. 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）

Smurf1 SMAD specific E3 ubiquitin protein ligase 1 [Mus musculus (house mouse)]
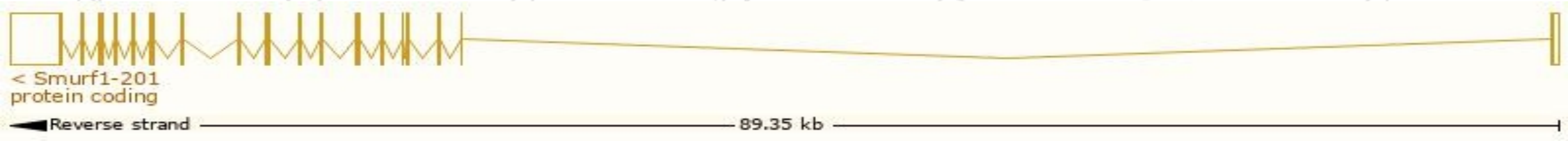
Gene ID: 75788, updated on 25-Mar-2019

▲ Summary

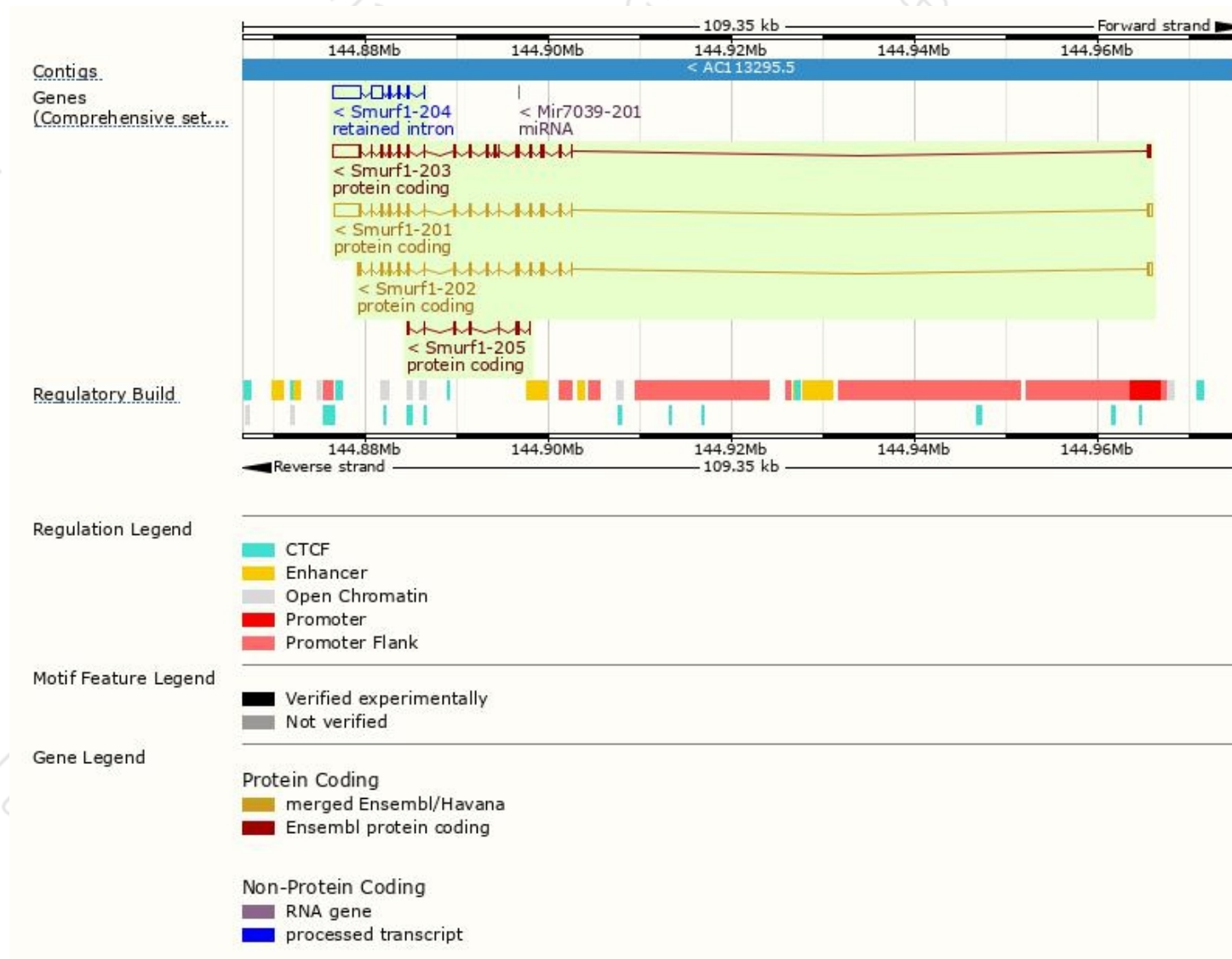|	
Official Symbol	Smurf1 provided by MGI
Official Full Name	SMAD specific E3 ubiquitin protein ligase 1 provided byMGI
Primary source	MGI:MGI:1923038
See related	Ensembl:ENSMUSG00000038780
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4930431E10Rik, mKIAA1625
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 16.3), ovary adult (RPKM 16.0) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 5 transcripts,all transcripts are shown below:

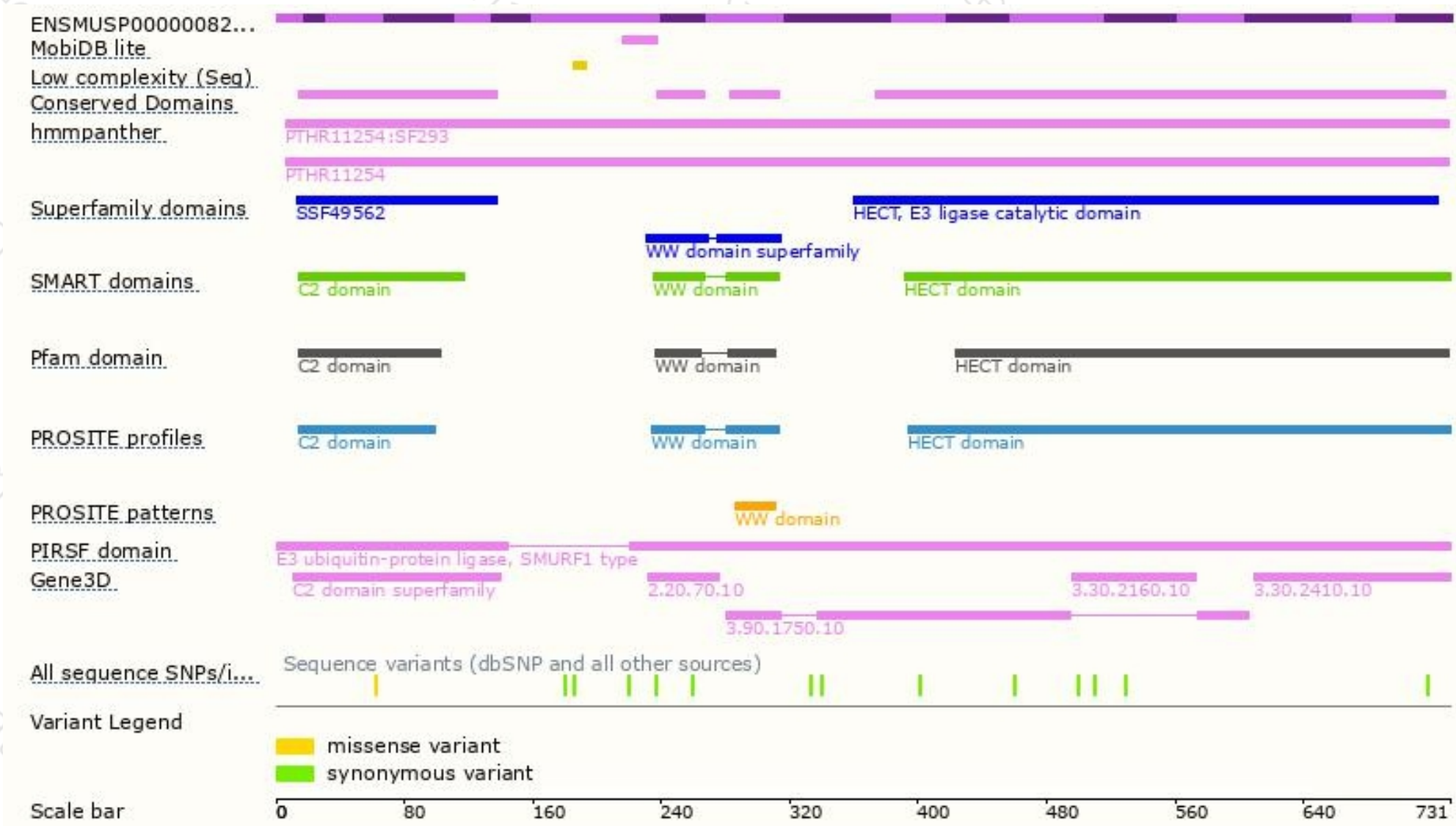| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Smurf1-201	ENSMUST00000085684.10	5343	731aa	Protein coding	CCDS39380	Q9CUN6	TSL:1 GENCODE basic APPRIS P4
Smurf1-202	ENSMUST00000100461.6	2725	728aa	Protein coding	CCDS39381	E9Q4K9	TSL:1 GENCODE basic APPRIS ALT1
Smurf1-203	ENSMUST00000110677.7	5420	757aa	Protein coding	-	E9PYU8	TSL:5 GENCODE basic APPRIS ALT1
Smurf1-205	ENSMUST00000198621.1	927	309aa	Protein coding	-	A0A0G2JEN5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Smurf1-204	ENSMUST00000126822.1	4710	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Smurf1-201* transcript,The transcription is shown below

< Smurf1-201
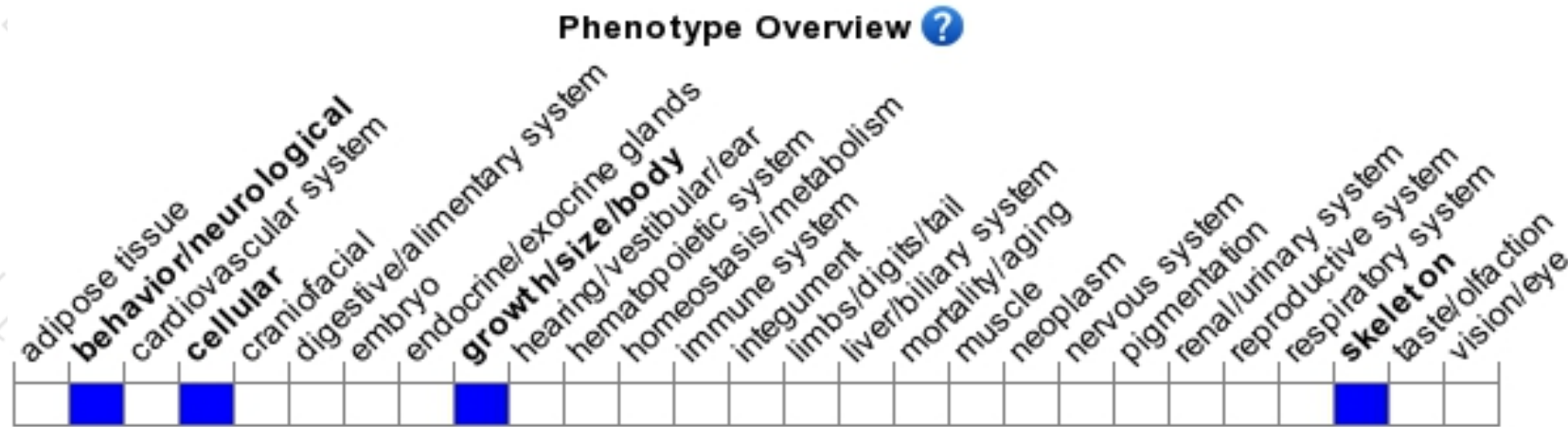protein coding

◄ Reverse strand

89.35 kb

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/).

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