Fuca1 Cas9-CKO Strategy

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Project Overview

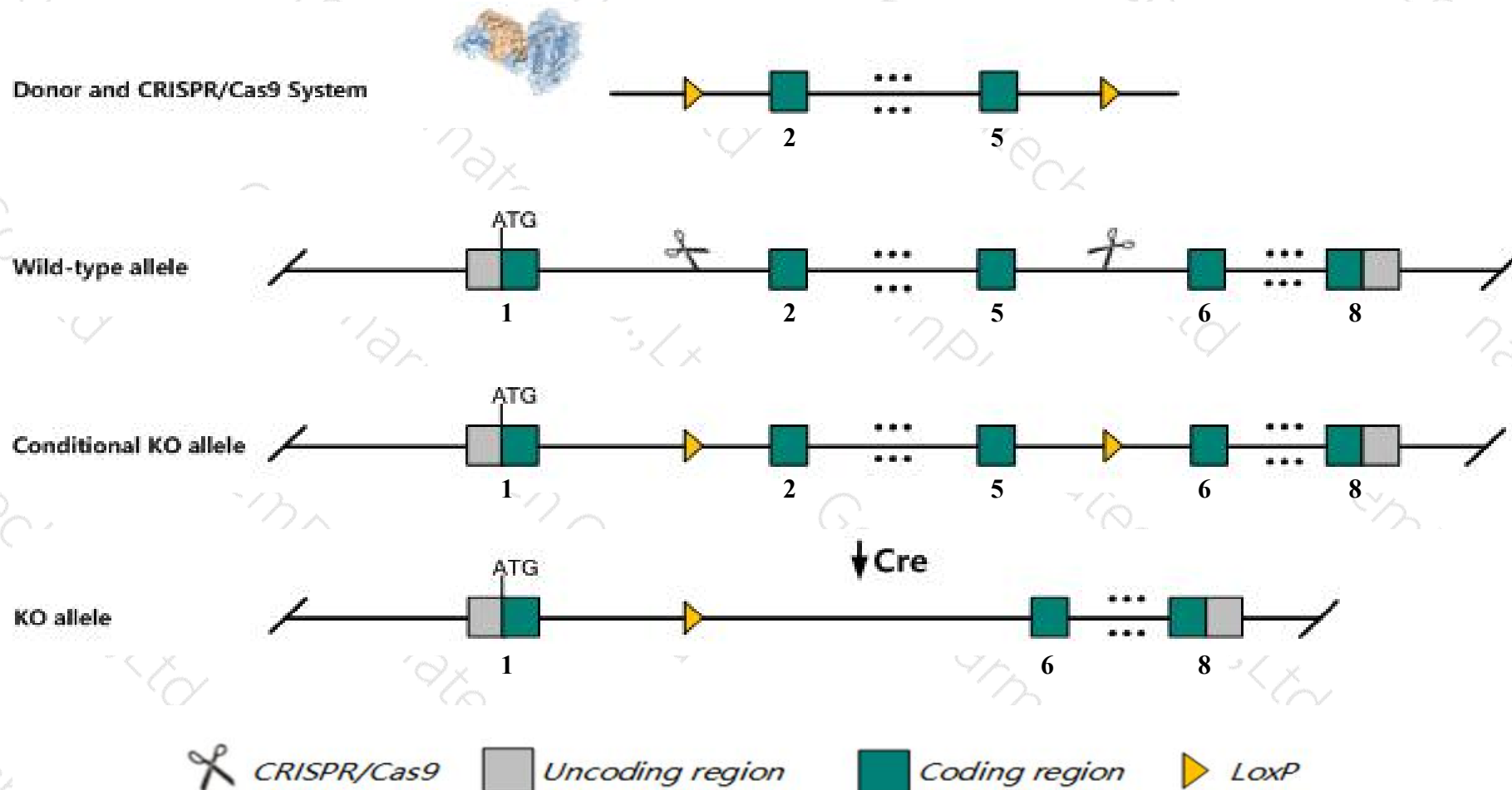|	
Project Name	*Fuca1*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Fuca1* gene. The schematic diagram is as follows:

Technical routes

➤ The *Fuca1* gene has 5 transcripts. According to the structure of *Fuca1* gene, exon2-exon5 of *Fuca1-201* (ENSMUST00000030434.4) transcript is recommended as the knockout region.The region contains Ens 580bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Fuca1* 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.

Notice

➢ According to the existing MGI data,Strain differences are probably due to a structural variant in Fuca1.

Strains A/J, BDP, LP, P, SEA/Gn and 129/J have high FUCA activity and high heat stability; C57BL/6, C3H/He,

DBA/2, BALB/c and 22 other strains have low activity and low heat stability.

➢ Transcript *Fuca1*-203 may not be affected.

➢ The N-terminal of *Fuca1* gene will remain several amino acids ,it may remain the partial function of *Fuca1* gene.

➢ The *Fuca1* gene is located on the Chr4. 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）

Fuca1 fucosidase, alpha-L- 1, tissue [*Mus musculus* (house mouse)]

Gene ID: 71665, updated on 12-Aug-2019

▲ Summary

Official Symbol	Fuca1 provided by MGI
Official Full Name	fucosidase, alpha-L- 1, tissue provided by MGI
Primary source	MGI:MGI:95593
See related	Ensembl:ENSMUSG00000028673
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	Afuc; Fuca; 0610006A03Rik; 9530055J05Rik
Expression	Ubiquitous expression in colon adult (RPKM 71.1), kidney adult (RPKM 50.6) and 28 other tissues See more
Orthologs	human all

▲ Genomic context

Location: 4 D3; 4 68.01 cM

See Fuca1 in Genome Data Viewer

Exon count: 8

| Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	4	NC_000070.6 (135920726..135940300)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	4	NC_000070.5 (135476641..135496215)

Transcript information（Ensembl）

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

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fuca1-201	ENSMUST00000030434.4	2525	452aa	Protein coding	CCDS18794	Q99LJ1	TSL:1 GENCODE basic APPRIS P1
Fuca1-204	ENSMUST00000146290.1	628	No protein	lncRNA	-	-	TSL:2
Fuca1-203	ENSMUST00000142111.1	595	No protein	lncRNA	-	-	TSL:2
Fuca1-202	ENSMUST00000142033.7	436	No protein	lncRNA	-	-	TSL:2
Fuca1-205	ENSMUST00000153592.1	401	No protein	lncRNA	-	-	TSL:2

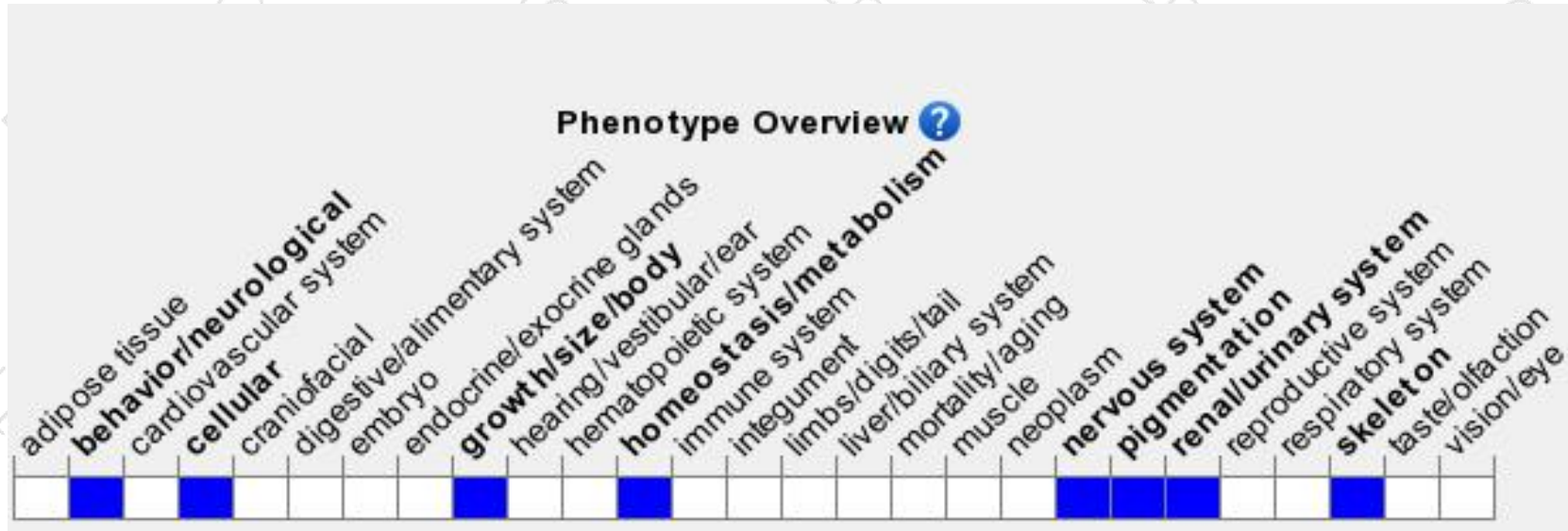The strategy is based on the design of *Fuca1-201* transcript,The transcription is shown below
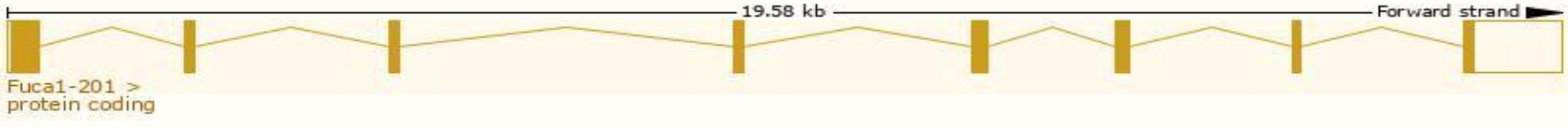
Genomic location distribution
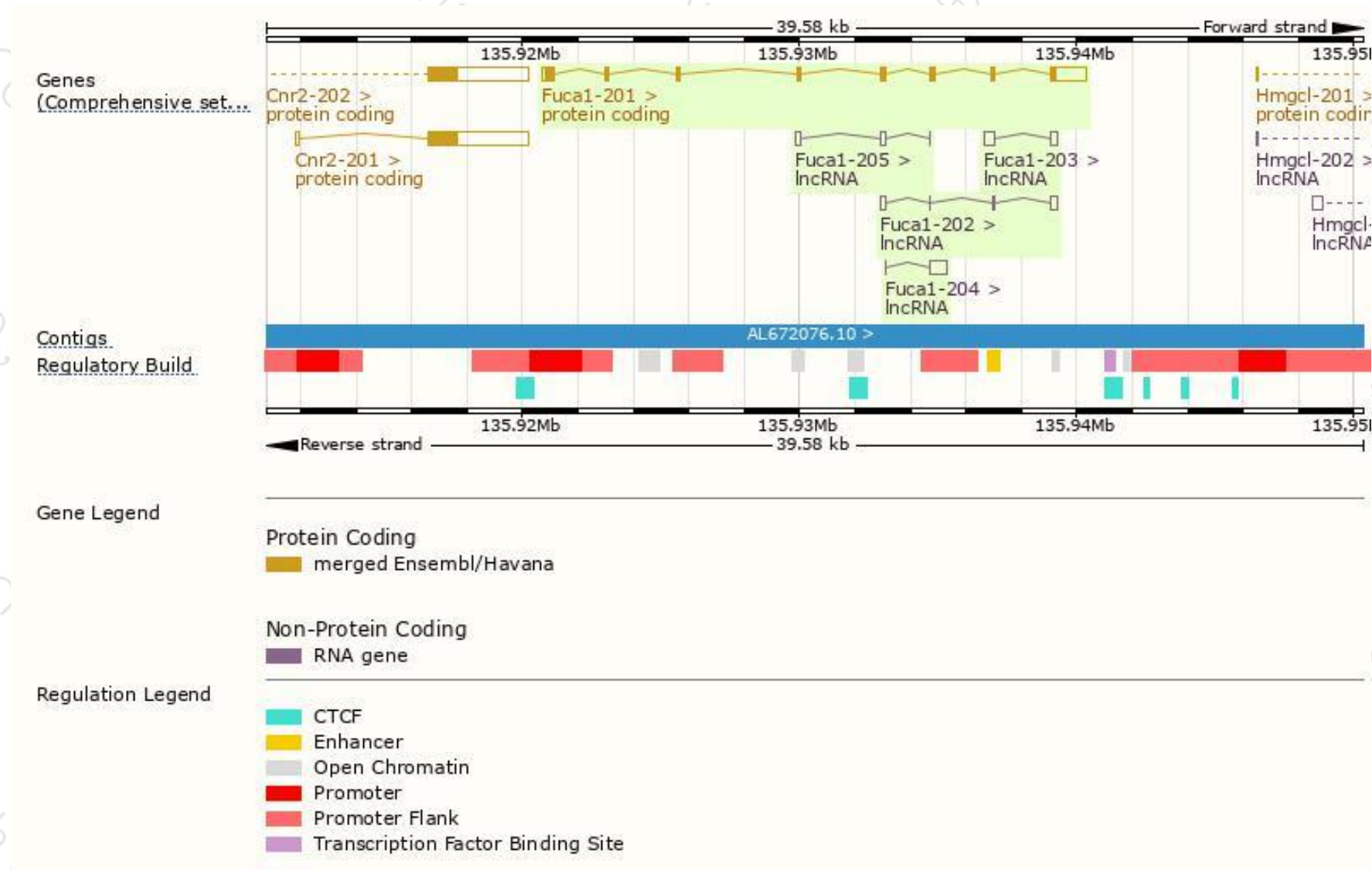
Protein domain
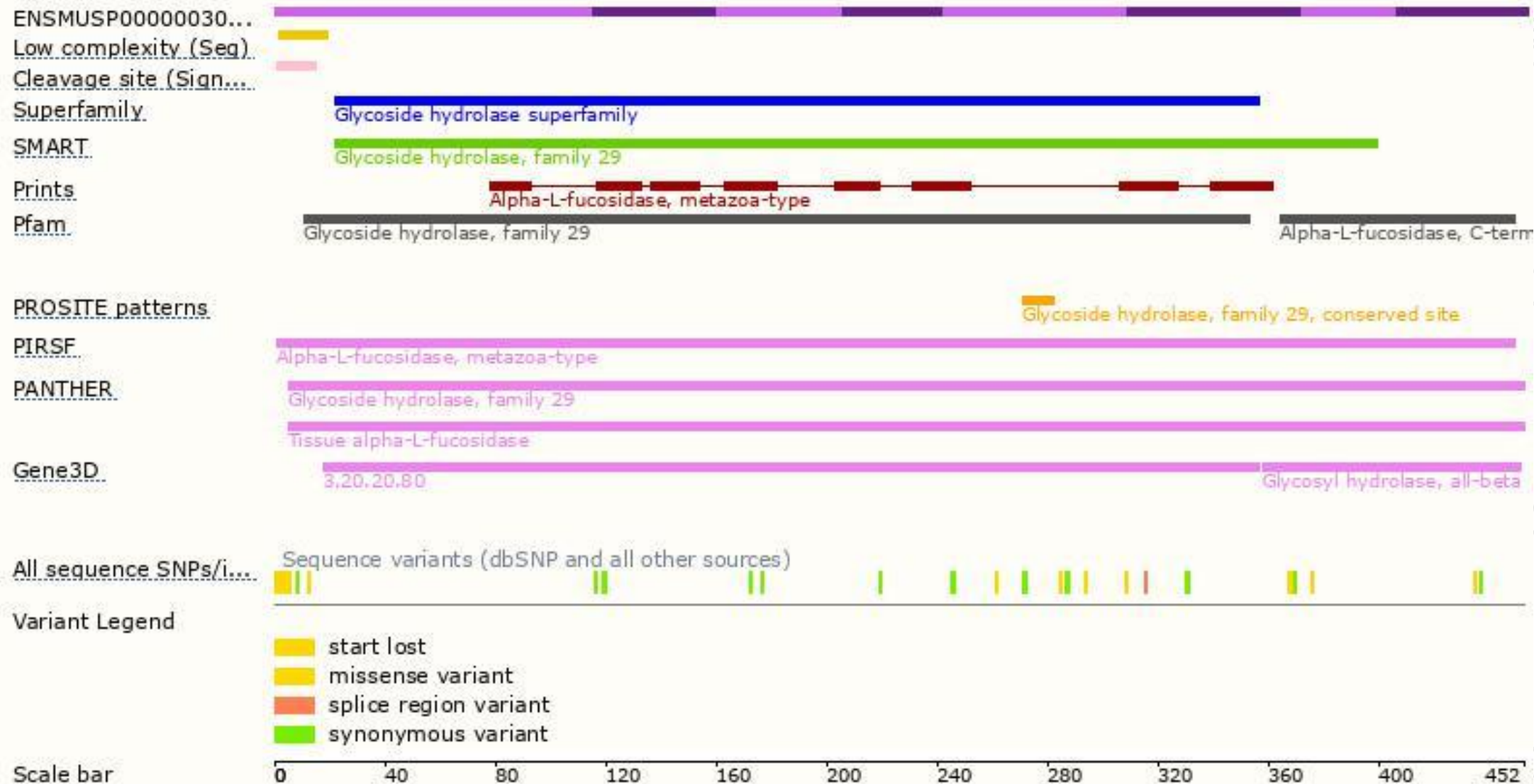
Mouse phenotype description(MGI)

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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