B3gnt5 Cas9-CKO Strategy

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Project Overview

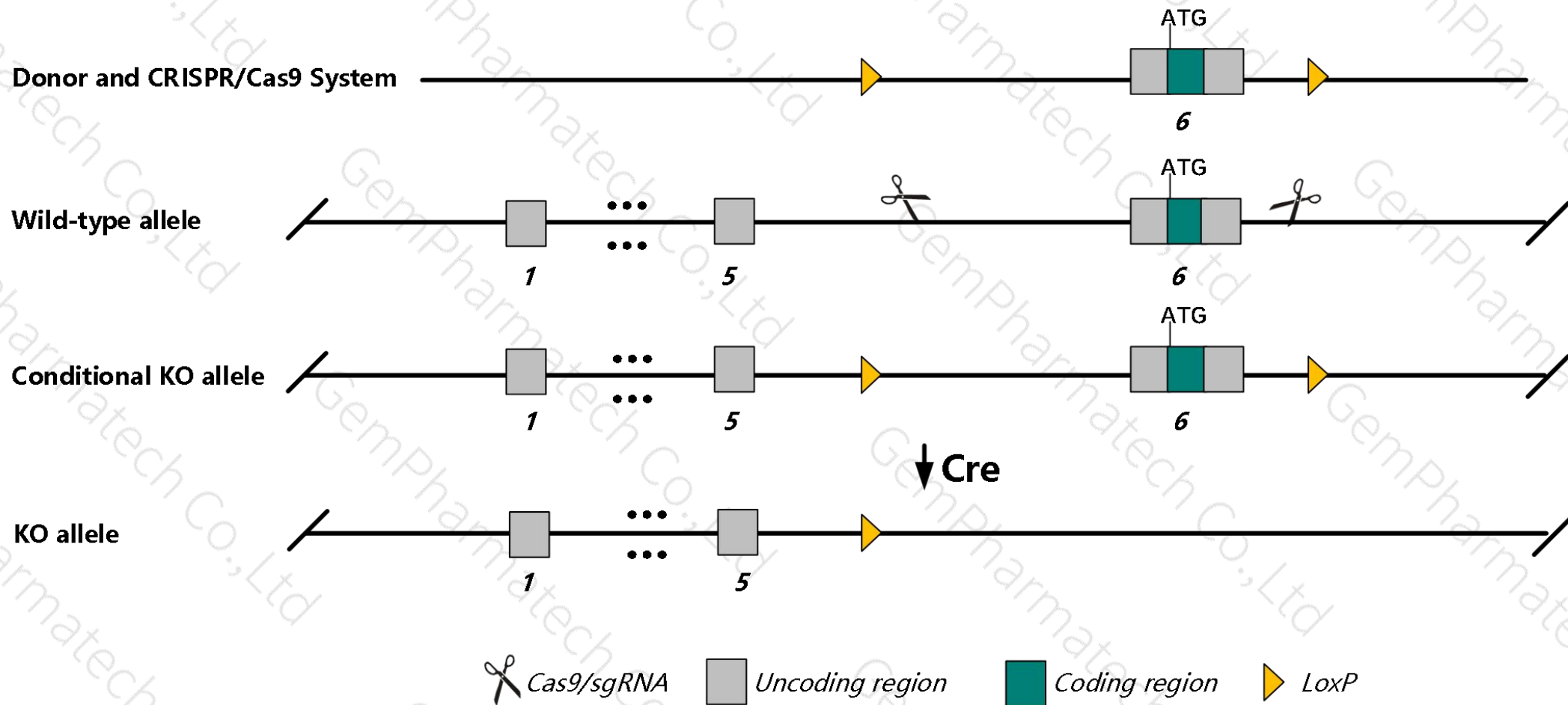|	
Project Name	*B3gnt5*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *B3gnt5* gene. The schematic diagram is as follows:

Cas9/sgRNA   Uncoding region   Coding region   LoxP

Technical routes

➢ The *B3gnt5* gene has 7 transcripts. According to the structure of *B3gnt5* gene, exon6 of *B3gnt5-207* (ENSMUST00000164397.7) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *B3gnt5* 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,Homozygous null mice may show variable types of lethality or no lethality depending on the allele. Mice homozygous for 3 alleles show B cell abnormalities. Mice homozygous or heterozygous for 2 allele show reduced fertility.

➤ The transcripts 205,206 may not be affected.

➤ The *B3gnt5* gene is located on the Chr16. 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）

B3gnt5 UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5 [Mus musculus (house mouse)]

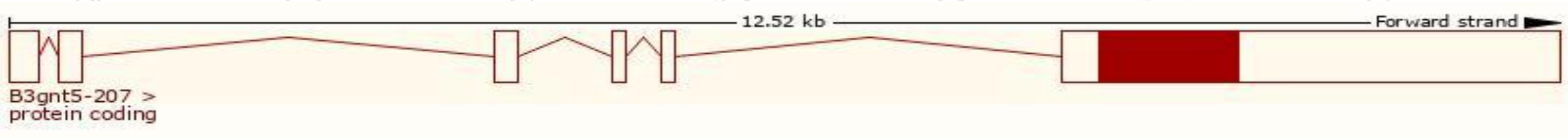Gene ID: 108105, updated on 13-Mar-2020

Summary

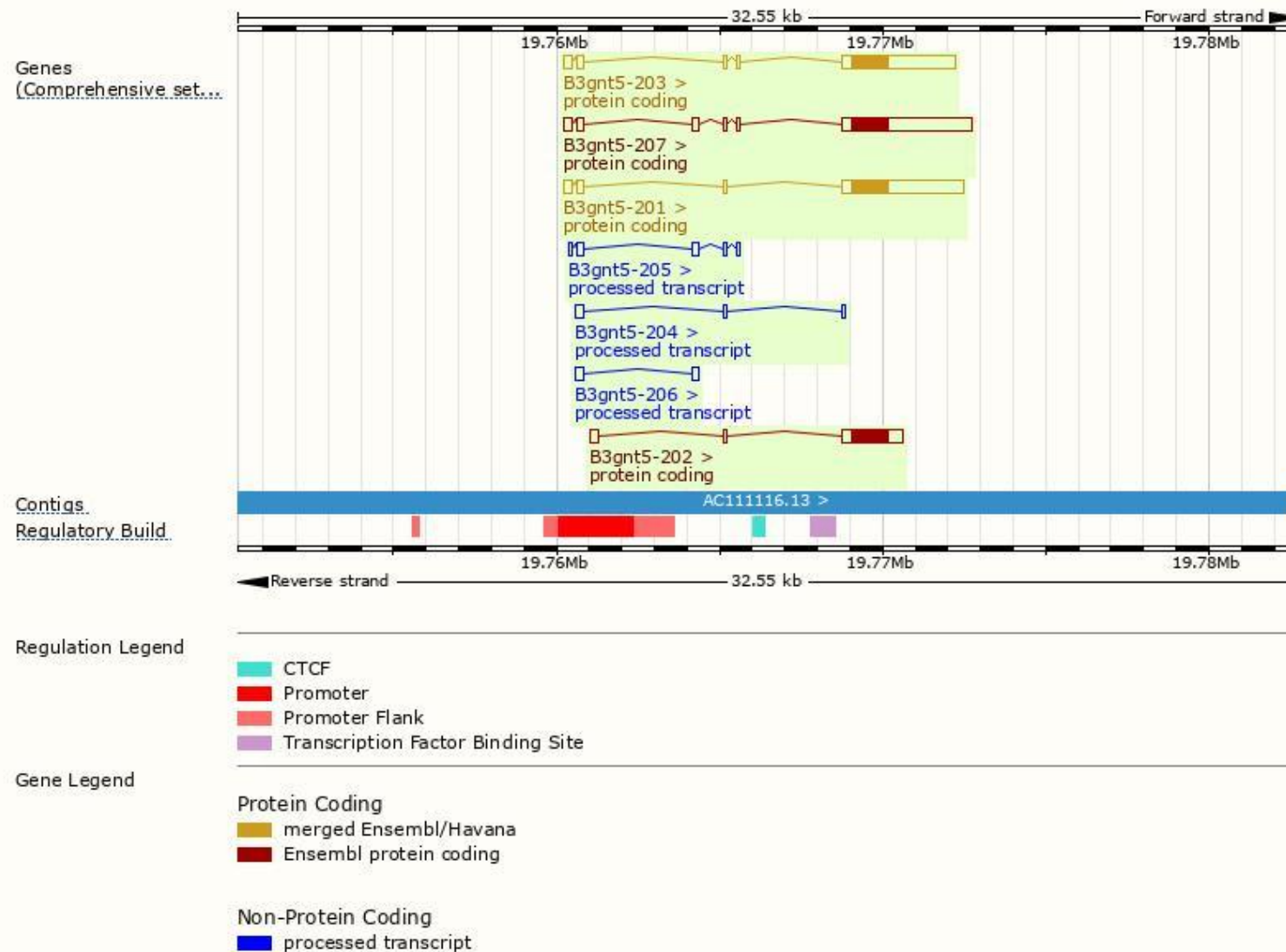|	
Official Symbol	B3gnt5 provided by MGI
Official Full Name	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5 provided by MGI
Primary source	MGI:MGI:2137302
See related	Ensembl:ENSMUSG00000022686
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	beta3GnT5
Expression	Biased expression in CNS E11.5 (RPKM 4.8), whole brain E14.5 (RPKM 4.4) and 12 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
B3gnt5-207	ENSMUST00000164397.7	4872	376aa	Protein coding	CCDS28040	Q8BGY6	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
B3gnt5-201	ENSMUST00000079780.9	4258	376aa	Protein coding	CCDS28040	Q8BGY6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
B3gnt5-203	ENSMUST00000121344.7	4175	376aa	Protein coding	CCDS28040	Q8BGY6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
B3gnt5-202	ENSMUST00000119468.1	2229	376aa	Protein coding	CCDS28040	Q8BGY6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
B3gnt5-205	ENSMUST00000152845.7	698	No protein	Processed transcript	-	-	TSL:3
B3gnt5-206	ENSMUST00000155780.1	446	No protein	Processed transcript	-	-	TSL:3
B3gnt5-204	ENSMUST00000131557.7	444	No protein	Processed transcript	-	-	TSL:2

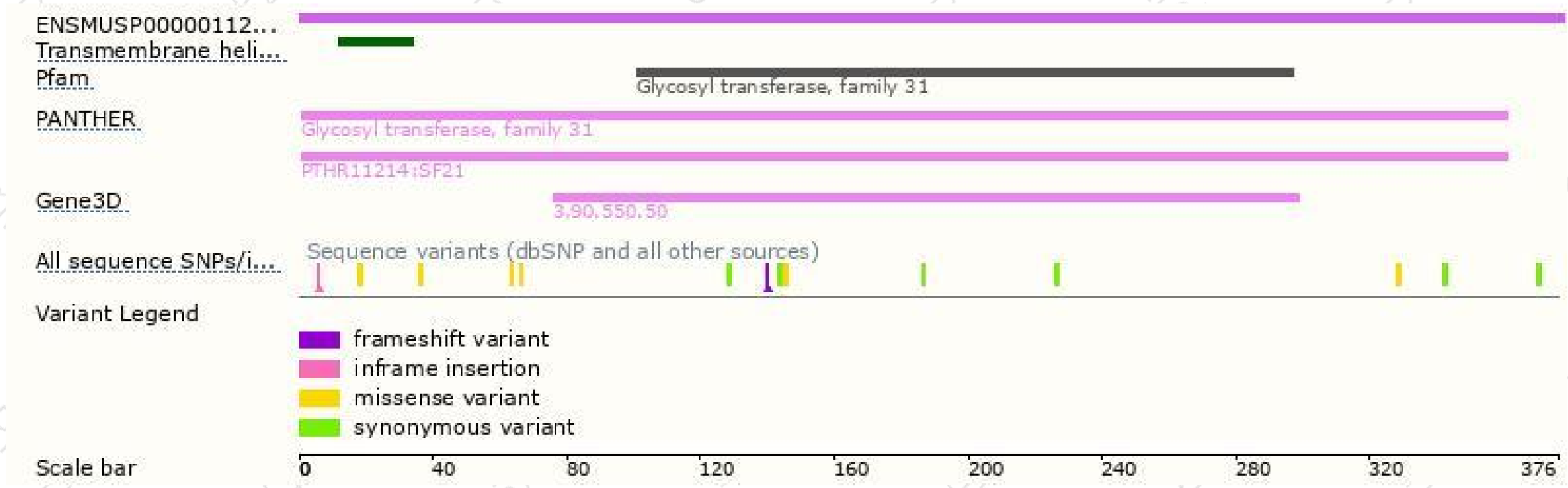The strategy is based on the design of *B3gnt5-207* transcript,The transcription is shown below
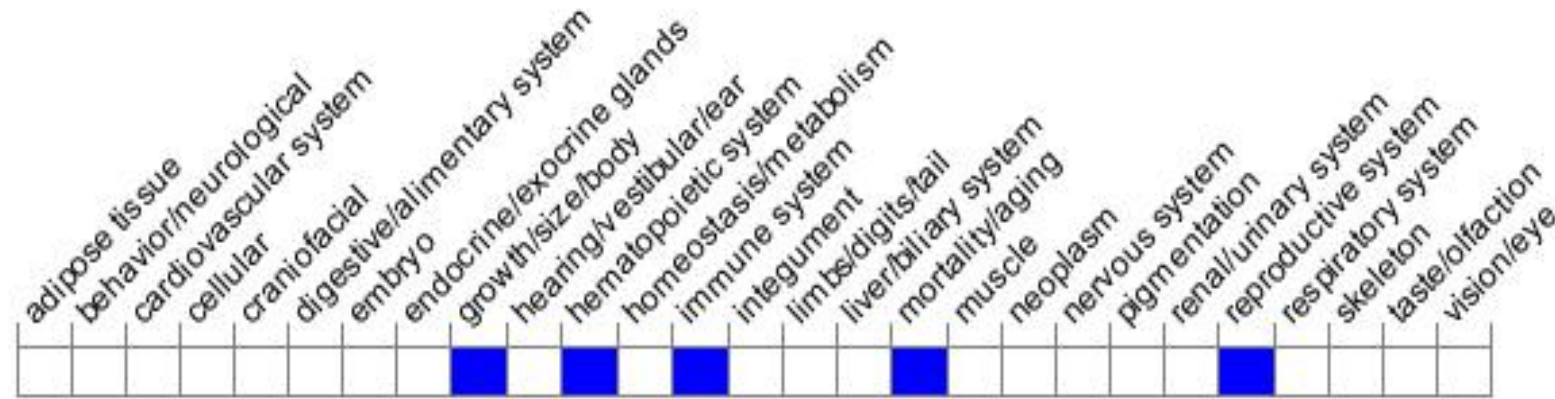
Genomic location distribution

Protein domain

ENSMUSP00000112...

Transmembrane heli...

Pfam.
Glycosyl transferase, family 31

PANTHER
Glycosyl transferase, family 31

PTHR11214:SF21

Gene3D
3.90.550.50

All sequence SNPs/i...
Sequence variants (dbSNP and all other sources)

Variant Legend

- ■ frameshift variant
- ■ inframe insertion
- ■ missense variant
- ■ synonymous variant

Scale bar

| 0 | 40 | 80 | 120 | 160 | 200 | 240 | 280 | 320 | 376 |

Mouse phenotype description(MGI)

Phenotype Overview

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 null mice may show variable types of lethality or no lethality depending on the allele. Mice homozygous for 3 alleles show B cell abnormalities. Mice homozygous or heterozygous for 2 a show reduced fertility.

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