B3gnt8 Cas9-CKO Strategy

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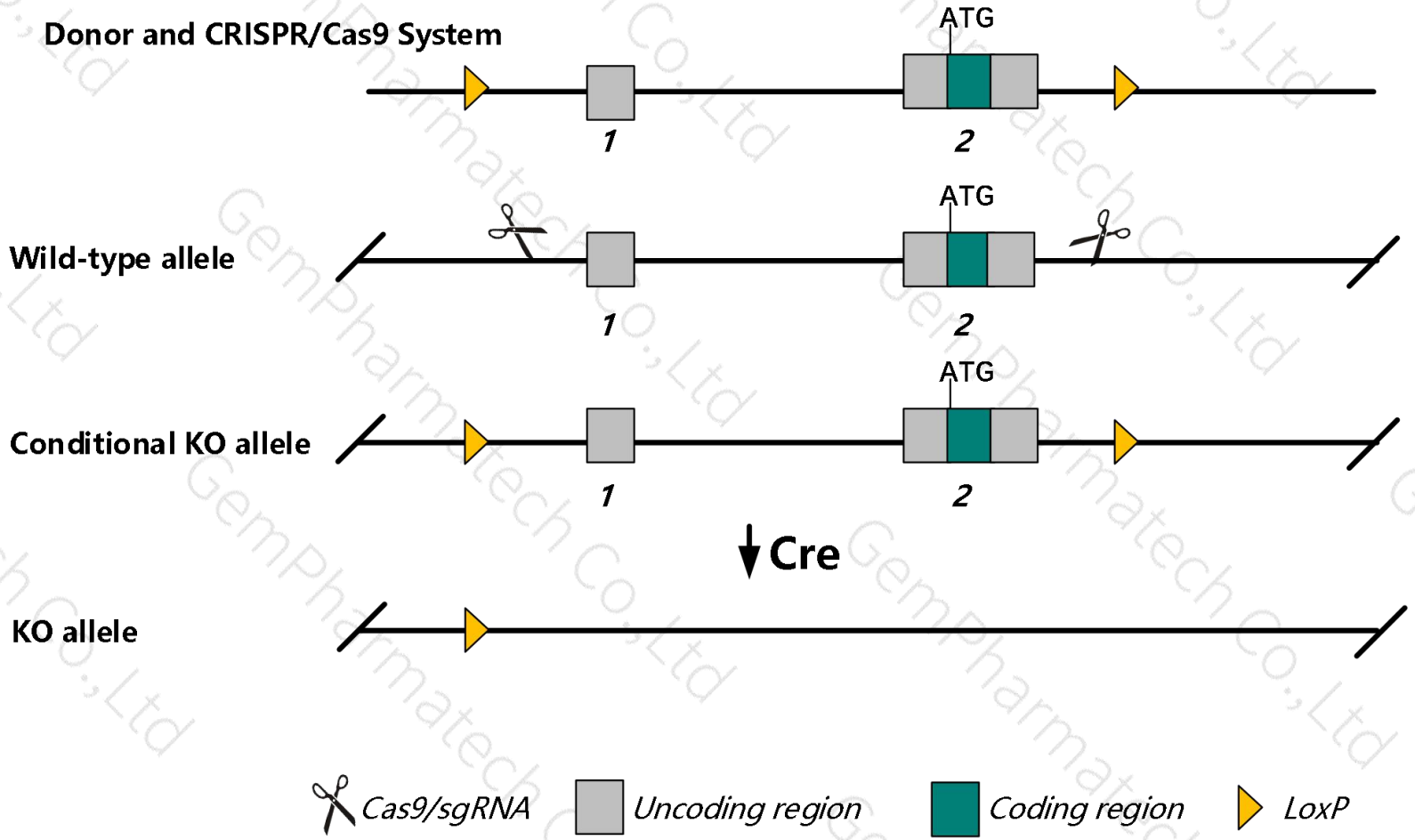
Project Overview

Project Name	***B3gnt8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *B3gnt8* gene. The schematic diagram is as follows:



Technical routes

- The *B3gnt8* gene has 4 transcripts. According to the structure of *B3gnt8* gene, exon1-exon2 of *B3gnt8-201* (ENSMUST00000076034.7) 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 *B3gnt8* 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

➢The flox region is about 10 kb away from the 5th end of the *Erich4* gene, and its effect is unknown.

➢The flox region is about 0.4 kb and 0.9kb away from the 3th end of the *Bckdha* and *Dmac2* gene, and effects is unknown.

➢The transcript 204 will be direct destroyed.

➢ The *B3gnt8* gene is located on the Chr7. 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）

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B3gnt8 UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8 [Mus musculus (house mouse)]

Gene ID: 232984, updated on 20-Mar-2020

Summary

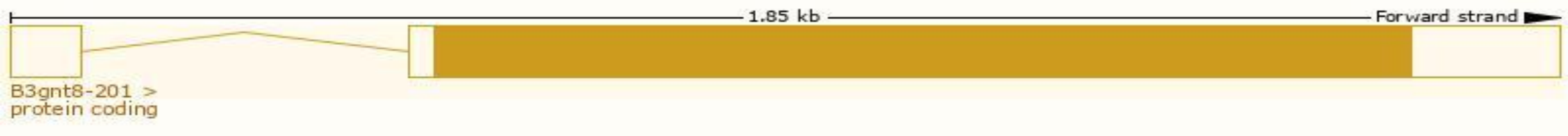
Official Symbol	B3gnt8 provided by MGI
Official Full Name	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8 provided by MGI
Primary source	MGI:MGI:2385269
See related	Ensembl:ENSMUSG00000059479
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	B3galt7, B7galt7, BC025206
Expression	Broad expression in spleen adult (RPKM 26.8), ovary adult (RPKM 11.3) and 18 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

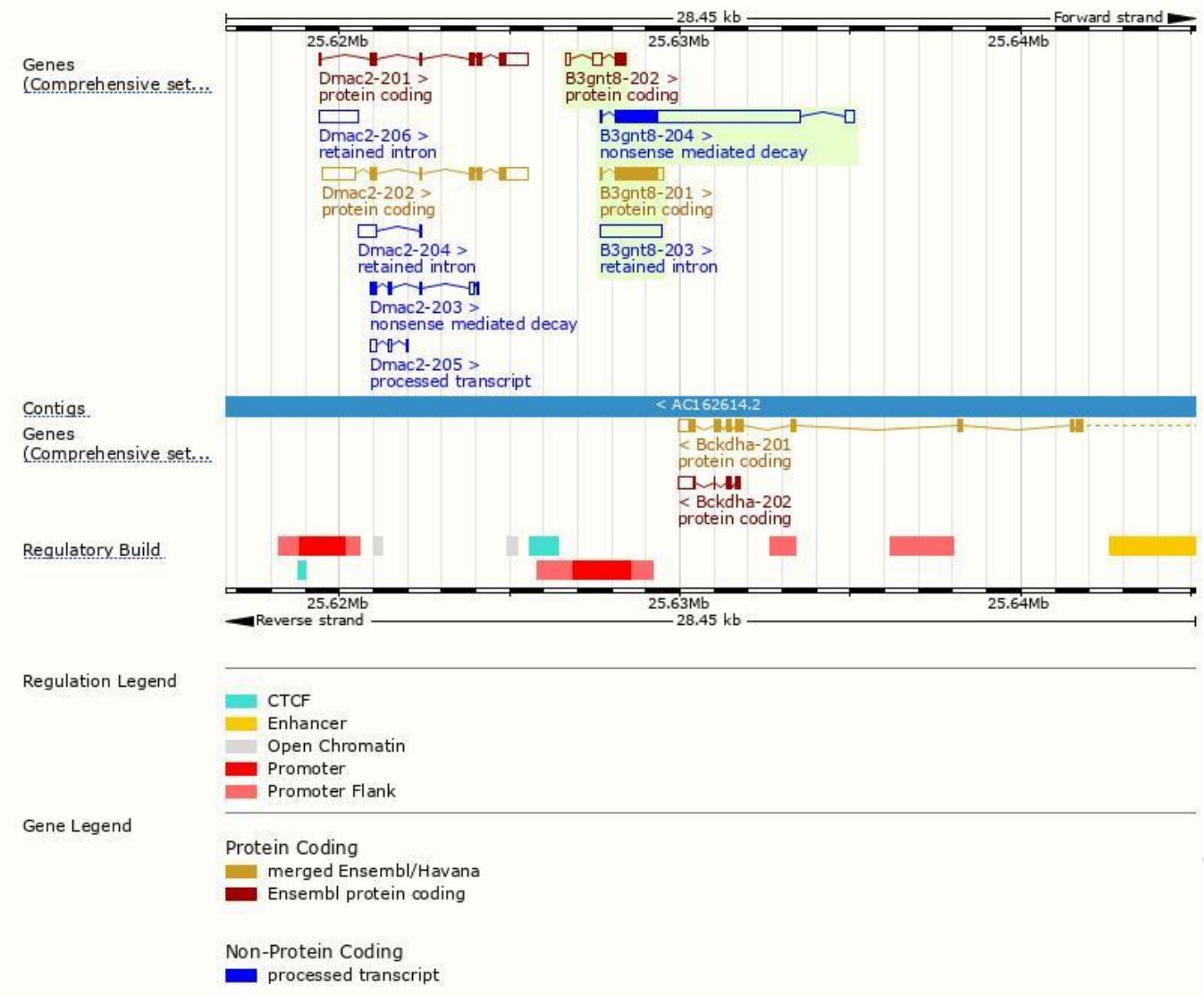
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
B3gnt8-201	ENSMUST00000076034.7	1463	389aa	Protein coding	CCDS20989	Q8R3I9	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
B3gnt8-202	ENSMUST00000205281.1	723	93aa	Protein coding	-	A0A0U1RPU4	CDS 3' incomplete TSL:3
B3gnt8-204	ENSMUST00000206940.1	5756	389aa	Nonsense mediated decay	-	Q8R3I9	TSL:1
B3gnt8-203	ENSMUST00000206824.1	1844	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *B3gnt8-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain

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

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