Pomgnt1 Cas9-CKO Strategy

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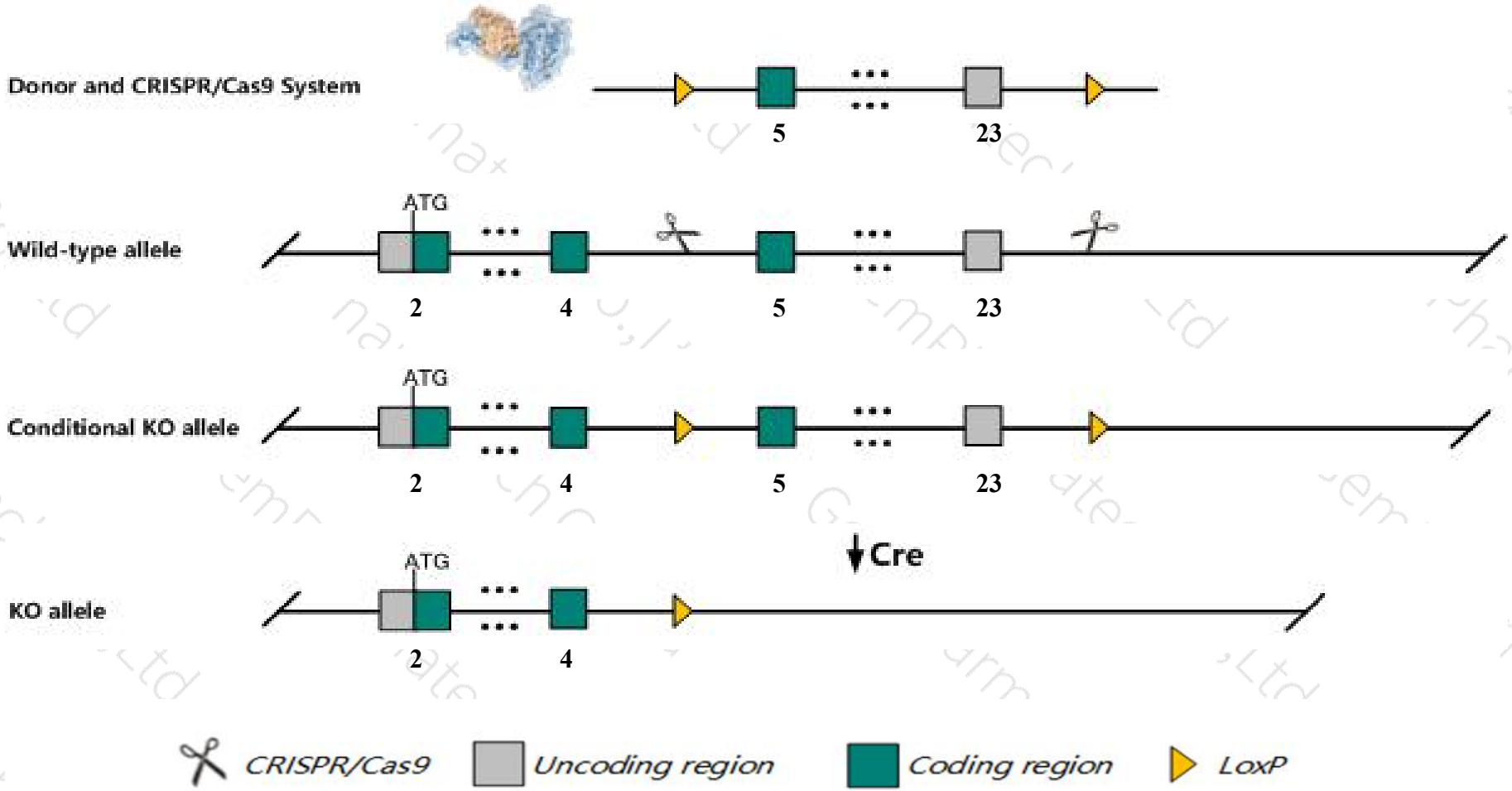
Project Overview

Project Name	***Pomgnt1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pomgnt1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pomgnt1* gene has 12 transcripts. According to the structure of *Pomgnt1* gene, exon5-exon23 of *Pomgnt1-204*(ENSMUST00000120083.7) transcript is recommended as the knockout region.The region contains most 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 *Pomgnt1* 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,surviving homozygous null mice display a mild dystrophy despite a reduced muscle mass and myofiber number, impaired muscle regeneration and low proliferative activity of satellite cells. Mice homozygous for a gene trap allele show reduced fertility and multiple defects in muscle, eye and brain.

➢ The Intron4 is only 513bp,loxp insertion may affect mRNA splicing.

➢ The *Pomgnt1* 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）

Pomgnt1 protein O-linked mannose beta 1,2-N-acetylglucosaminyltransferase [Mus musculus (house mouse)]

Gene ID: 68273, updated on 13-Mar-2020

Summary

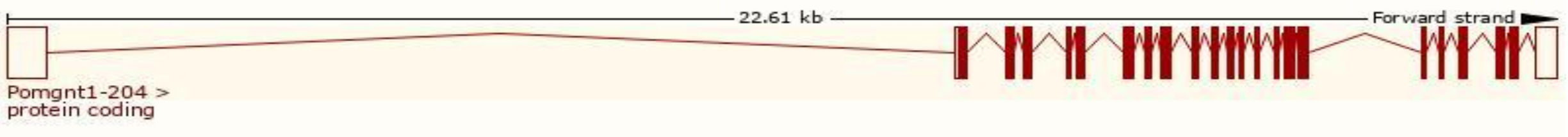
Official Symbol	Pomgnt1 provided by MGI
Official Full Name	protein O-linked mannose beta 1,2-N-acetylglucosaminyltransferase provided byMGI
Primary source	MGI:MGI:1915523
See related	Ensembl:ENSMUSG00000028700
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	0610016I07Rik, 4930467B06Rik
Expression	Ubiquitous expression in testis adult (RPKM 35.5), ovary adult (RPKM 18.0) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

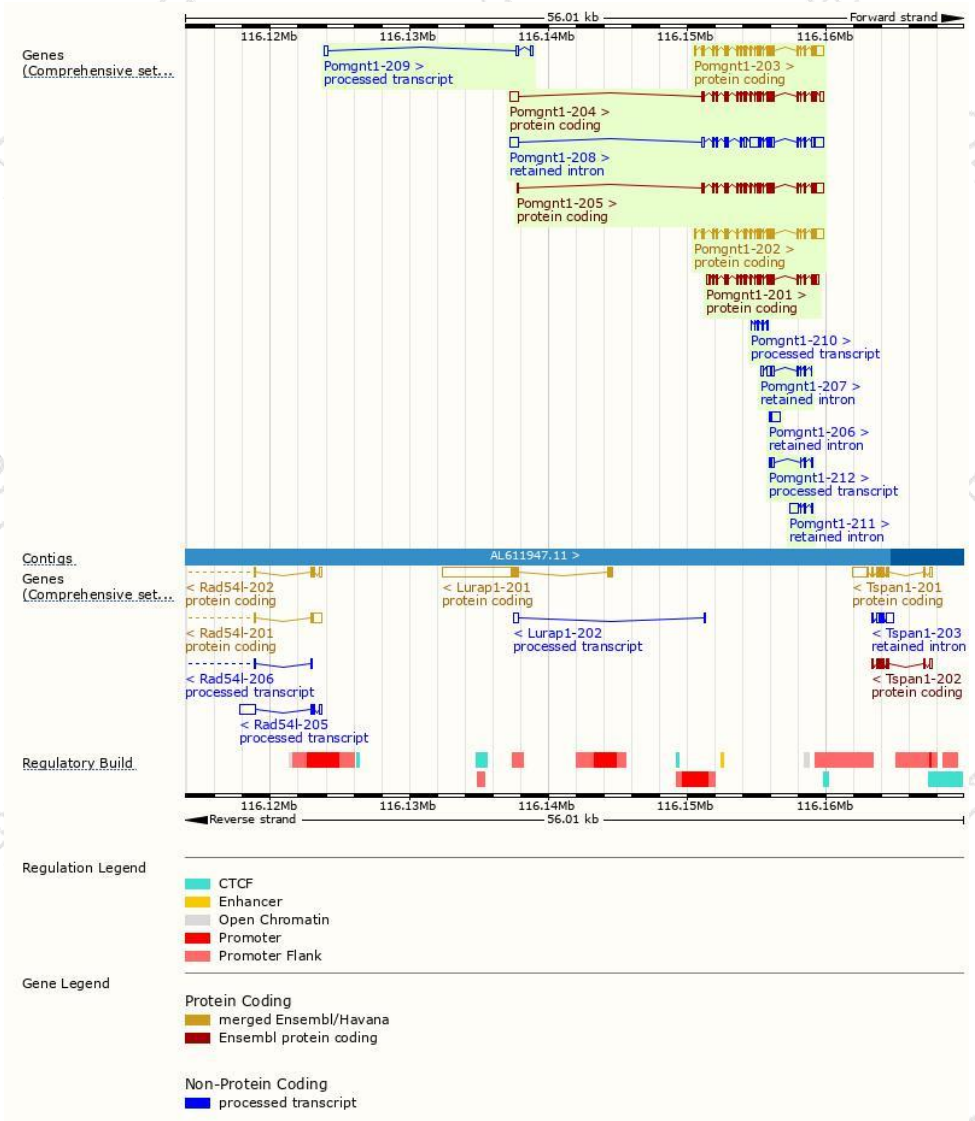
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pomgnt1-204	ENSMUST00000120083.7	2953	660aa	Protein coding	CCDS18506	Q91X88	TSL:5 GENCODE basic APPRIS P1
Pomgnt1-203	ENSMUST00000106498.7	2719	660aa	Protein coding	CCDS18506	Q91X88	TSL:1 GENCODE basic APPRIS P1
Pomgnt1-205	ENSMUST00000121052.7	2693	660aa	Protein coding	CCDS18506	Q91X88	TSL:5 GENCODE basic APPRIS P1
Pomgnt1-202	ENSMUST00000106496.7	2613	627aa	Protein coding	CCDS18507	Q91X88	TSL:1 GENCODE basic
Pomgnt1-201	ENSMUST00000106494.2	2226	638aa	Protein coding	CCDS71450	Q91X88	TSL:1 GENCODE basic
Pomgnt1-209	ENSMUST00000144311.1	557	No protein	Processed transcript	-	-	TSL:1
Pomgnt1-212	ENSMUST00000155718.7	449	No protein	Processed transcript	-	-	TSL:2
Pomgnt1-210	ENSMUST00000147612.1	393	No protein	Processed transcript	-	-	TSL:3
Pomgnt1-208	ENSMUST00000136855.7	3448	No protein	Retained intron	-	-	TSL:5
Pomgnt1-207	ENSMUST00000133838.7	870	No protein	Retained intron	-	-	TSL:5
Pomgnt1-211	ENSMUST00000151325.1	790	No protein	Retained intron	-	-	TSL:3
Pomgnt1-206	ENSMUST00000127426.1	636	No protein	Retained intron	-	-	TSL:1

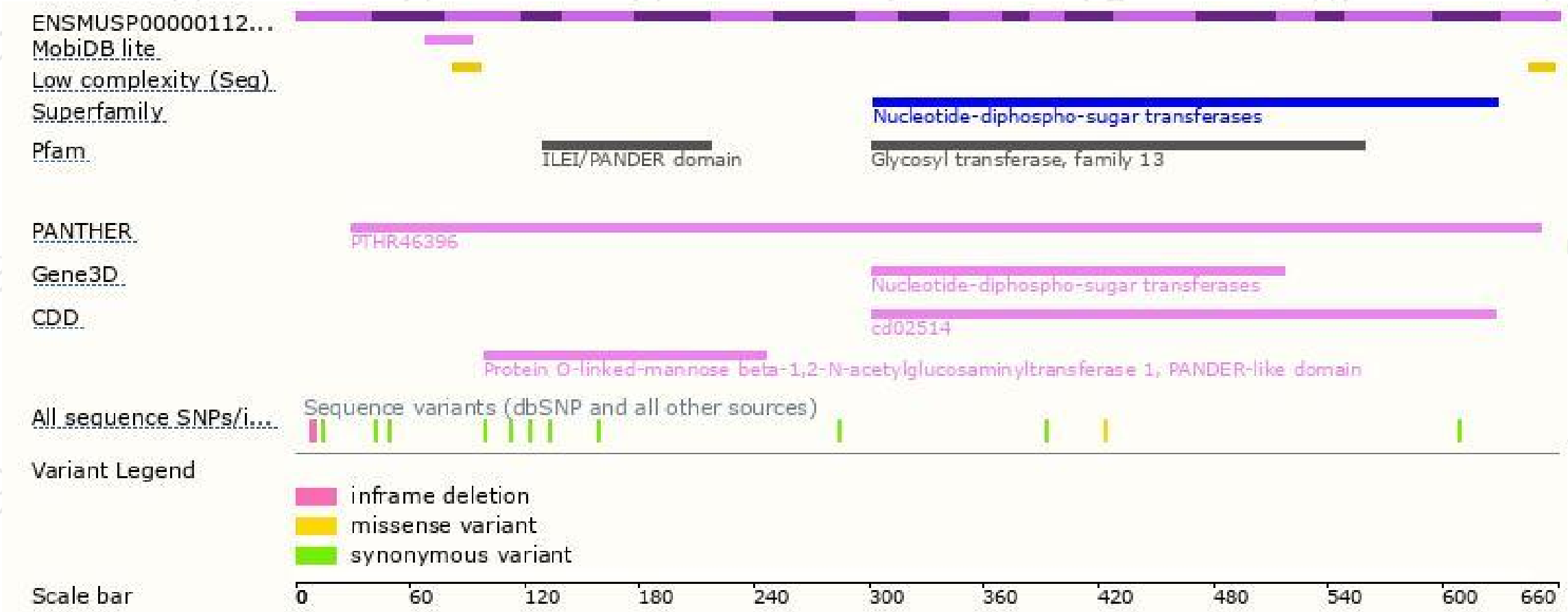
The strategy is based on the design of *Pomgnt1-204* transcript,the transcription is shown below:



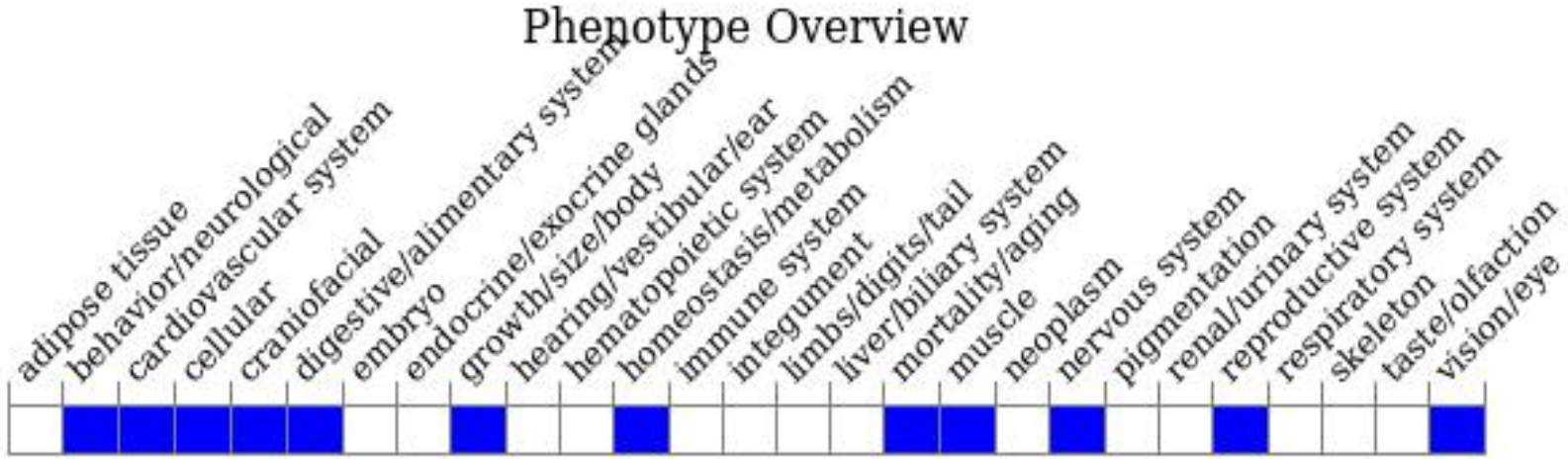
Genomic location distribution



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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