GemPharmatech™

# Gnmt Cas9-CKO Strategy

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Design Date: 2024-2-19

# Overview

## Target Gene Name

- Gnmt

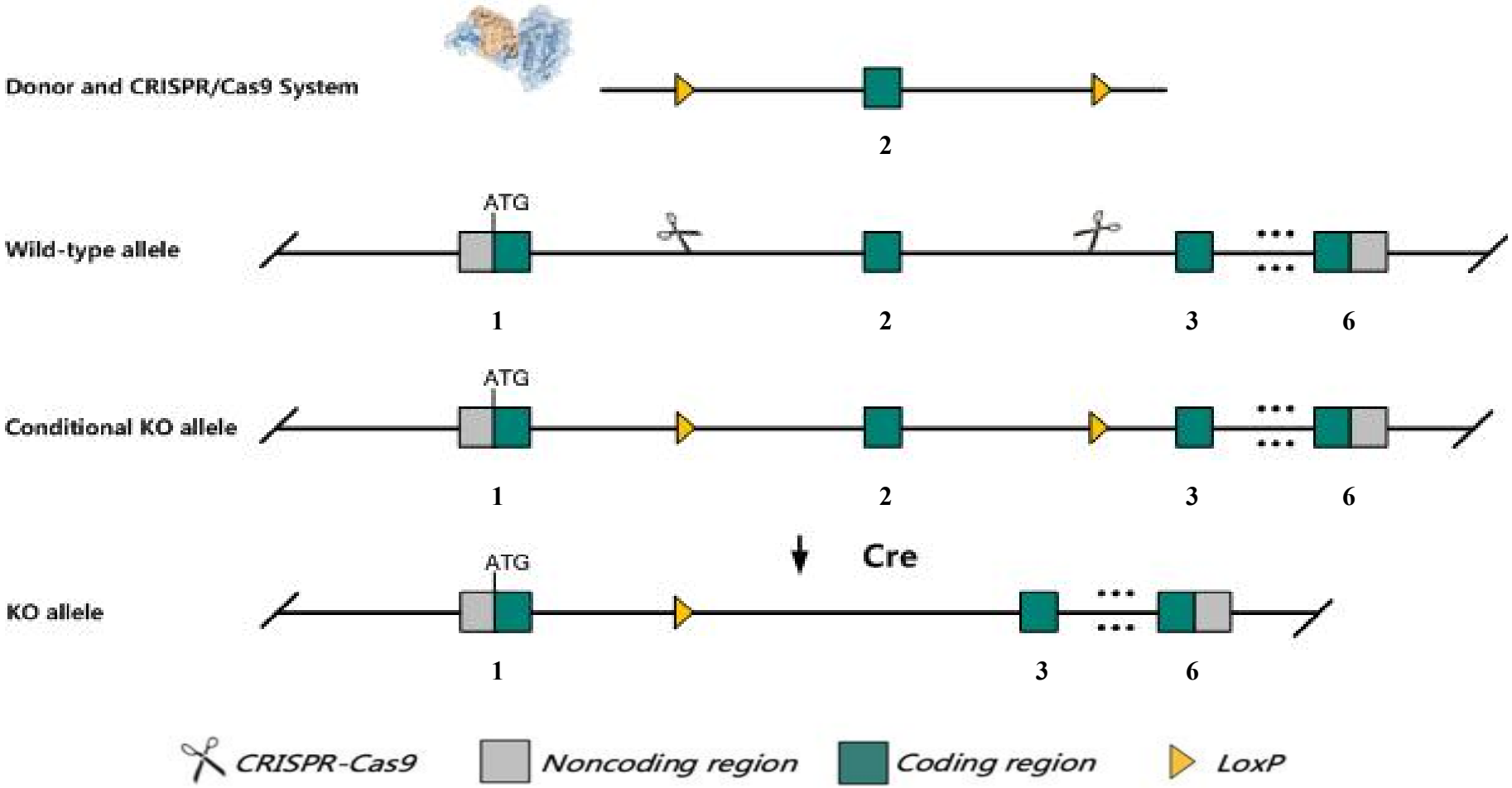
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy




Schematic representation of CRISPR-Cas9 engineering used to edit the *Gnmt* gene.

# Technical Information

- The *Gnmt* gene has 3 transcripts. According to the structure of *Gnmt* gene, exon2 of *Gnmt*-201 (ENSMUST00000002846.9) transcript is recommended as the knockout region. The region contains 128bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Gnmt* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

# Gene Information

**Gnmt glycine N-methyltransferase [Mus musculus (house mouse)]**

Gene ID: 14711, updated on 18-May-2023

**Summary**

| | |
|---|---|
| **Official Symbol** | Gnmt provided by MGI |
| **Official Full Name** | glycine N-methyltransferase provided byMGI |
| **Primary source** | MGI:MGI:1202304 |
| **See related** | Ensembl:ENSMUSG00000002769 |
| **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 |
| **Summary** | Enables glycine N-methyltransferase activity and glycine binding activity. Involved in S-adenosylmethionine metabolic process and protein homotetramerization. Acts upstream of or within several processes, including glycogen metabolic process; one-carbon metabolic process; and regulation of gluconeogenesis. Predicted to be located in nucleus. Predicted to be part of methyltransferase complex. Predicted to be active in cytosol. Is expressed in several structures, including integumental system; limb; nose; pancreas tip epithelium; and skeleton. Used to study glycine N-methyltransferase deficiency and hepatocellular carcinoma. Human ortholog(s) of this gene implicated in glycine N-methyltransferase deficiency. Orthologous to human GNMT (glycine N-methyltransferase). [provided by Alliance of Genome Resources, Apr 2022] |
| **Expression** | Restricted expression toward liver adult (RPKM 1724.9)See more |
| **Orthologs** | human all |

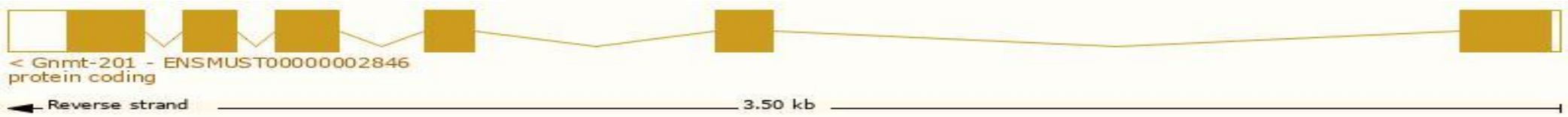
Source: https://www.ncbi.nlm.nih.gov/

# Transcript Information

The gene has 3 transcripts,all transcripts are shown below:

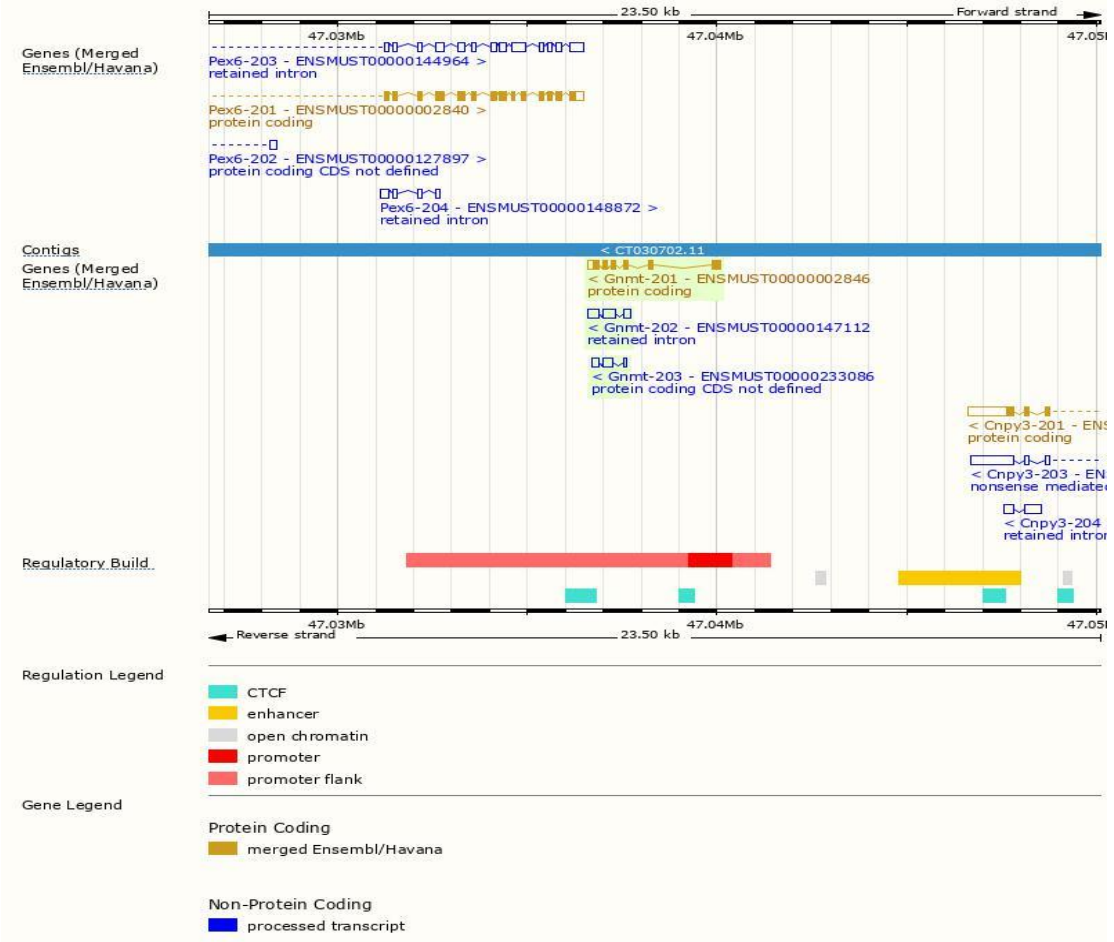
| Transcript ID | Name | bp | Protein | Biotype | CCDS | UniProt Match | Flags |
|---|---|---|---|---|---|---|---|
| ENSMUST00000002846.9 | Gnmt-201 | 1035 | 293aa | Protein coding | CCDS28838 | Q9QXF8 | Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:1 |
| ENSMUST00000233086.2 | Gnmt-203 | 554 | No protein | Protein coding CDS not defined | | - | - |
| ENSMUST00000147112.2 | Gnmt-202 | 855 | No protein | Retained intron | | - | TSL:2 |

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




Source: https://www.ensembl.org

# Genomic Information

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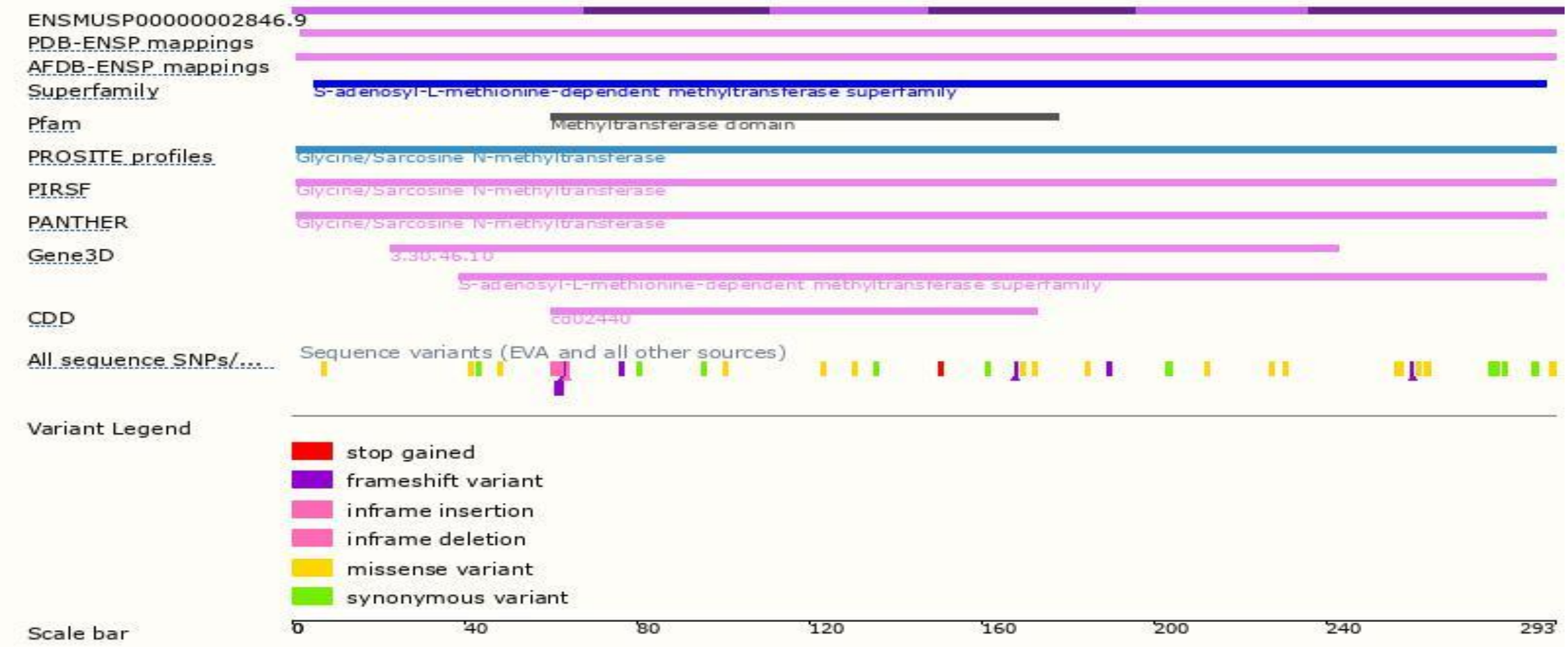
Source: : https://www.ensembl.org

# Protein Information

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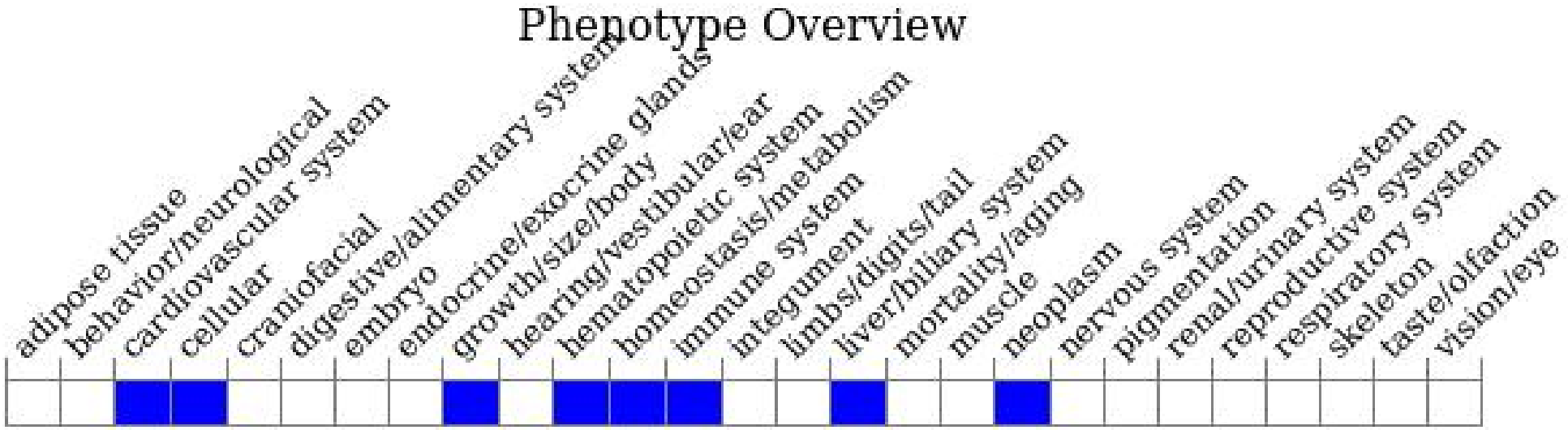
Source: : https://www.ensembl.org

# Mouse Phenotype Information (MGI)




- Mice homozygous for a null mutation display elevated levels of methionine and S-adenosylmethionine in the liver. Mice homozygous for another null allele exhibit hepatitis, increased hepatic glycogen storage, and hepatocellular carcinoma.



Source: https://www.informatics.jax.org

# Important Information

- According to the existing MGI data,mice homozygous for a null mutation display elevated levels of methionine and S-adenosylmethionine in the liver. Mice homozygous for another null allele exhibit hepatitis, increased hepatic glycogen storage, and hepatocellular carcinoma.
- Intron 2-3 of *Gnmt* gene is small,the insertion of loxp may destroy the gene transcription.
- *Gnmt* is located on Chr17. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.