Ntmt1 Cas9-CKO Strategy

Designer: Lingyan Wu

Reviewer: Miaomiao Cui

Design Date: 2021-6-15

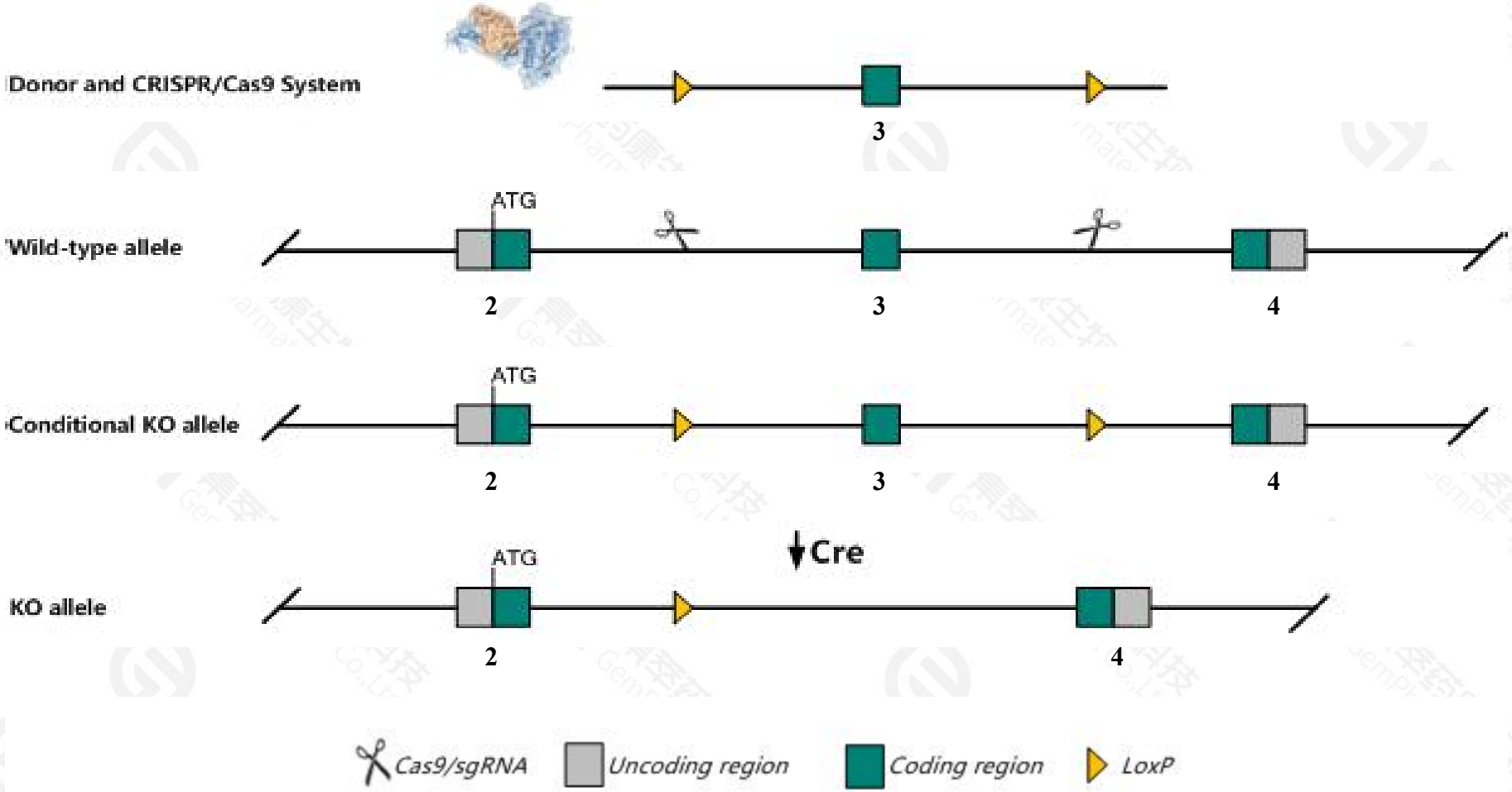
Project Overview

Project Name	***Ntmt1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

集萃药康 GemPharmatech

This model will use CRISPR/Cas9 technology to edit the *Ntmt1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ntmt1* gene has 9 transcripts. According to the structure of *Ntmt1* gene, exon3 of *Ntmt1-201*(ENSMUST00000041830.10) transcript is recommended as the knockout region.The region contains 253bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ntmt1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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,mice homozygous for a knock-out allele exhibit preweaning lethality and premature death associated with premature aging, decreased body size and weight, skin thinning, liver degeneration, increased sensitivity to oxidative stress and female infertility.

➢ The *Ntmt1* gene is located on the Chr2. 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）

集萃药康 GemPharmatech

Ntmt1 N-terminal Xaa-Pro-Lys N-methyltransferase 1 [Mus musculus (house mouse)]

Gene ID: 66617, updated on 17-Dec-2020

Summary

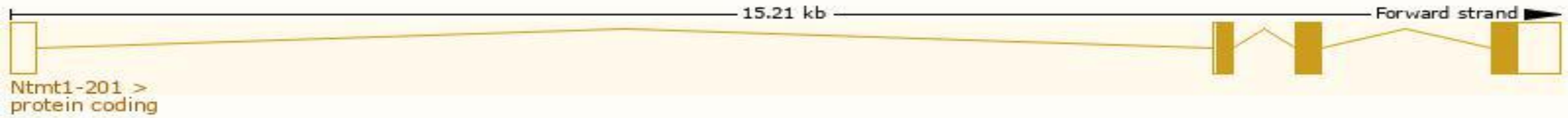
Official Symbol	Ntmt1 provided by MGI
Official Full Name	N-terminal Xaa-Pro-Lys N-methyltransferase 1 provided by MGI
Primary source	MGI:MGI:1913867
See related	Ensembl:ENSMUSG00000026857
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2610205E22Rik, AL033331, AL033332, Mettl1, Mettl11a, NTM1A
Expression	Ubiquitous expression in ovary adult (RPKM 27.0), adrenal adult (RPKM 21.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

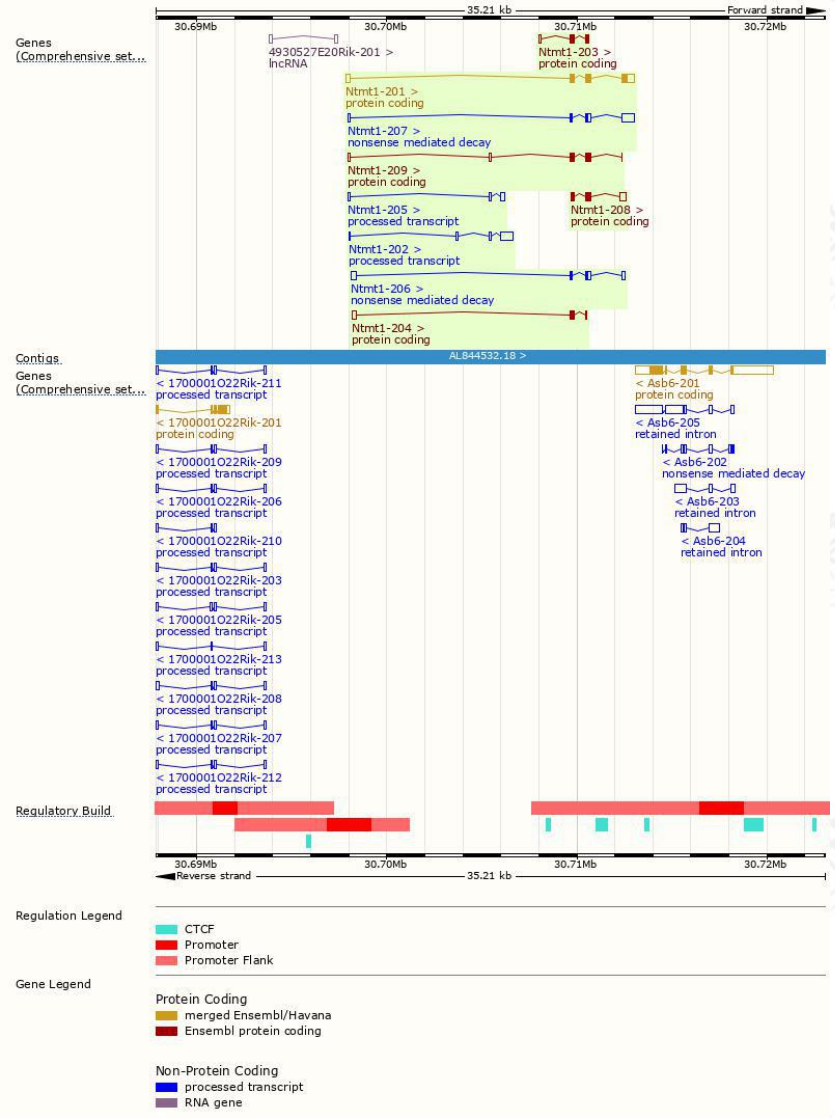
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ntmt1-201	ENSMUST00000041830.10	1387	223aa	Protein coding	CCDS15886		TSL:1 , GENCODE basic , APPRIS P1 ,
Ntmt1-209	ENSMUST00000152374.8	763	146aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Ntmt1-208	ENSMUST00000143970.3	715	144aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Ntmt1-203	ENSMUST00000127566.3	558	118aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Ntmt1-204	ENSMUST00000128303.7	453	78aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Ntmt1-207	ENSMUST00000138889.8	1192	70aa	Nonsense mediated decay	-		TSL:1 ,
Ntmt1-206	ENSMUST00000129712.3	837	70aa	Nonsense mediated decay	-		TSL:3 ,
Ntmt1-202	ENSMUST00000126037.2	981	No protein	Processed transcript	-		TSL:1 ,
Ntmt1-205	ENSMUST00000128509.8	445	No protein	Processed transcript	-		TSL:2 ,

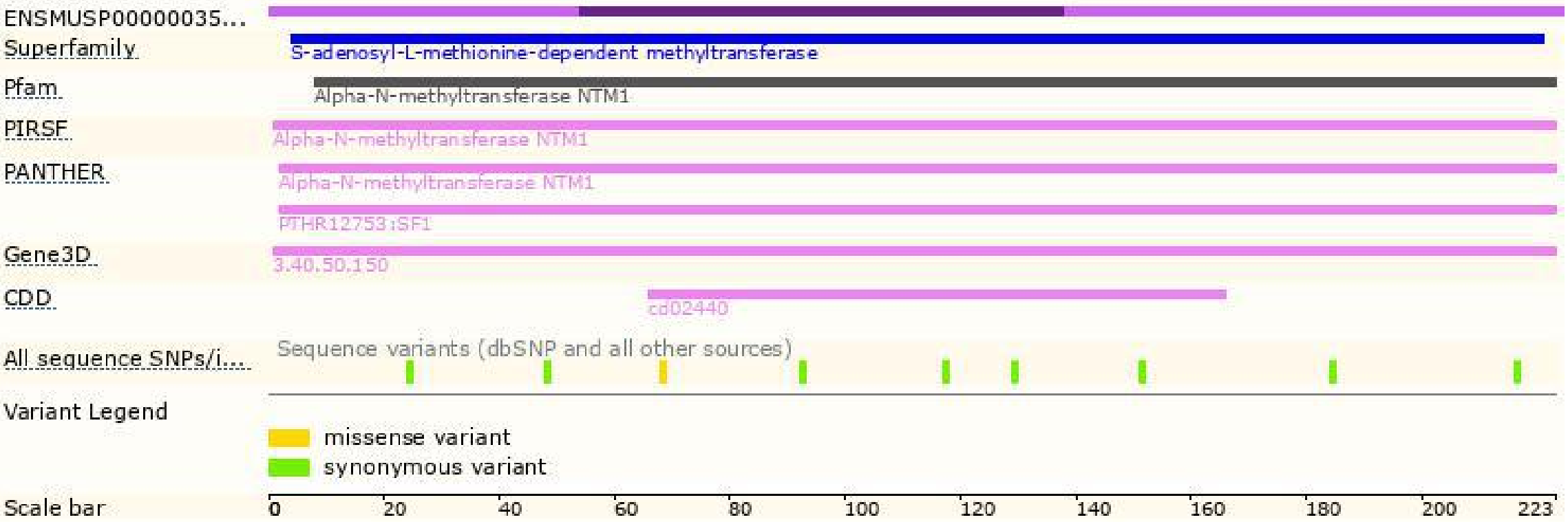
The strategy is based on the design of *Ntmt1-201* transcript,the transcription is shown below:



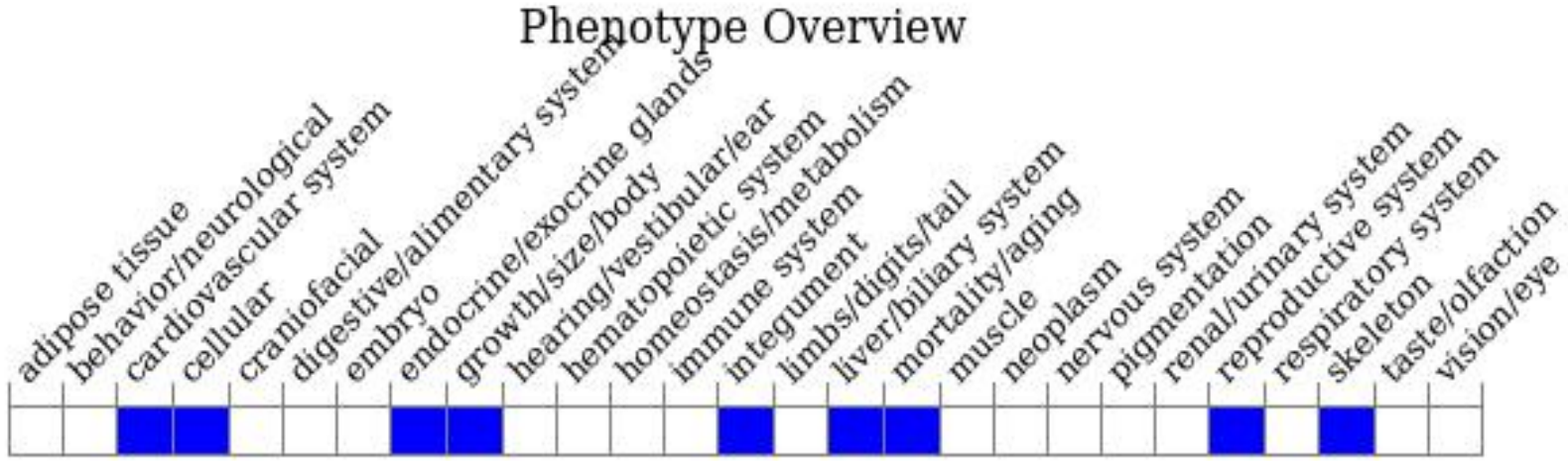
Genomic location distribution



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/).

According to the existing MGI data,mice homozygous for a knock-out allele exhibit preweaning lethality and premature death associated with premature aging, decreased body size and weight, skin thinning, liver degeneration, increased sensitivity to oxidative stress and female infertility.

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd