

# ***Dnmt1* Cas9-KO Strategy**

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**Reviewer:**

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**Design Date:**

**2019/11/13**



# Project Overview

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**Project Name**

***Dnmt1***

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**Project type**

**Cas9-KO**

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**Strain background**

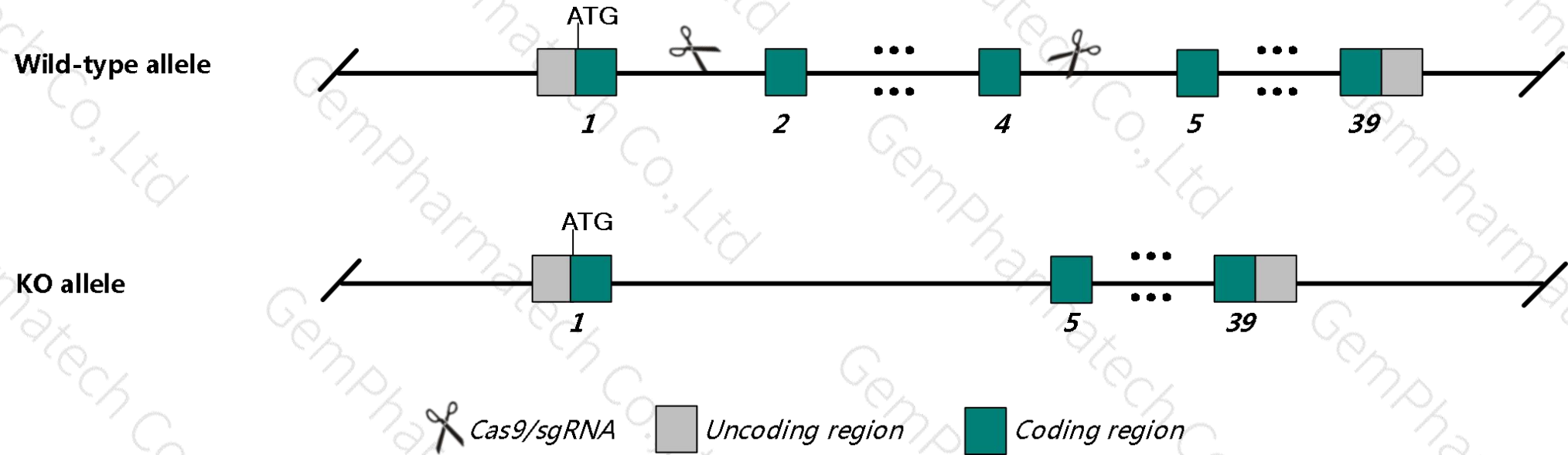
**C57BL/6J**

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# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dnmt1* gene. The schematic diagram is as follows:





- The *Dnmt1* gene has 7 transcripts. According to the structure of *Dnmt1* gene, exon2-exon4 of *Dnmt1-201* (ENSMUST00000004202.16) transcript is recommended as the knockout region. The region contains 356bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dnmt1* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.



- According to the existing MGI data, Mutations causing partial or severe loss of function were homozygous lethal by embryonic day 9.5, with lack of appropriate genomic imprinting observed at several loci.
- The *Dnmt1* gene is located on the Chr9. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



# Gene information (NCBI)

## Dnmt1 DNA methyltransferase (cytosine-5) 1 [ *Mus musculus* (house mouse) ]

Gene ID: 13433, updated on 12-Nov-2019

### Summary

Official Symbol	Dnmt1 provided by MGI
Official Full Name	DNA methyltransferase (cytosine-5) 1 provided by MGI
Primary source	MGI:MGI:94912
See related	Ensembl:ENSMUSG00000004099
Gene type	protein coding
RefSeq status	REVIEWED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Dnmt; MCMT; Met1; Cxxc9; MTase; Met-1; Dnmt1o; m.Mmul; MommeD2
Summary	This gene encodes a methyltransferase that preferentially methylates cytosines of CpG residues in hemimethylated DNA to generate fully methylated CpG base pairs during DNA replication. This enzyme plays roles in diverse cellular processes including cell cycle regulation, DNA repair, and telomere maintenance. The encoded protein is composed of an N-terminal domain with a nuclear localization sequence and replication fork-targeting domain, a DNA-binding CXXC domain, two bromo-adjacent homology domains, and a C-terminal catalytic domain. Mouse embryonic stem cells mutant for this gene are viable, but when introduced into the germ line, cause a recessive lethal phenotype with mutant embryos displaying stunted growth and developmental defects. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2015]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 37.3), thymus adult (RPKM 36.6) and 27 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>



# Transcript information (Ensembl)

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

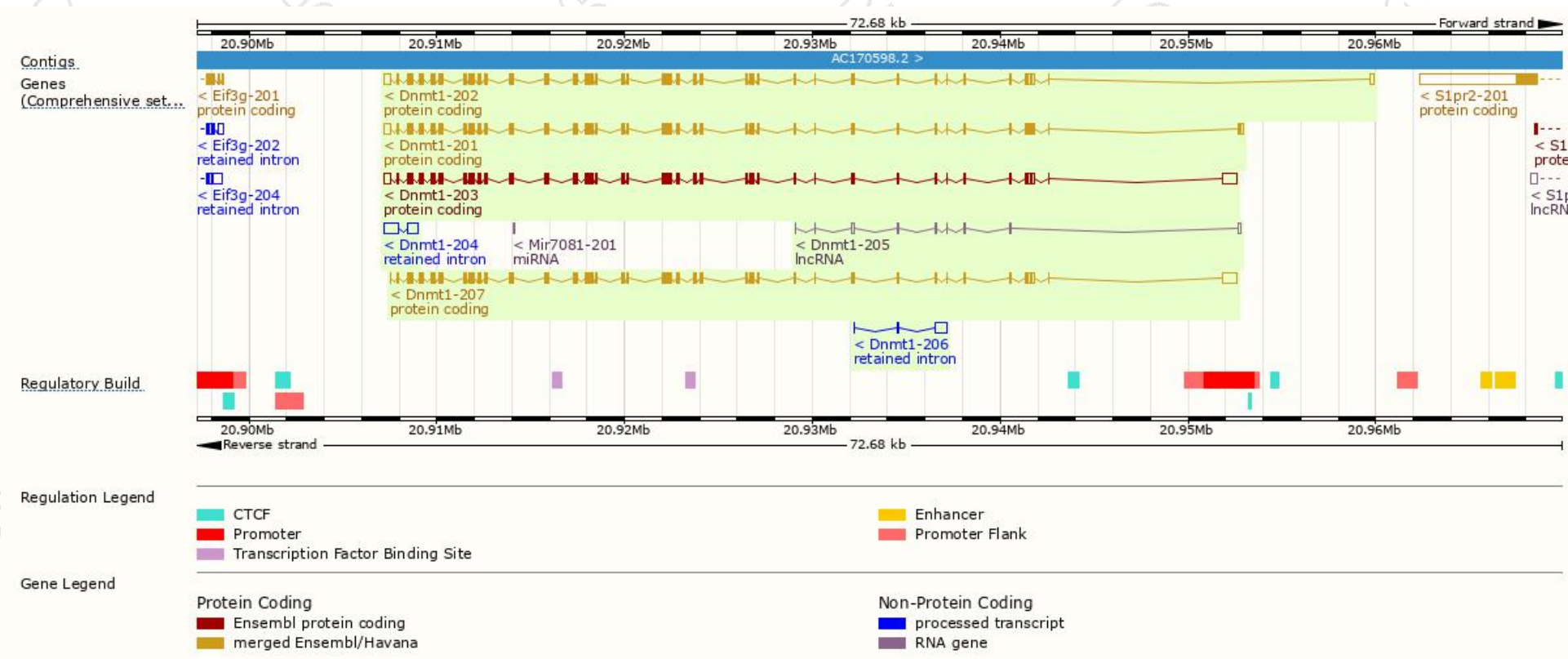
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dnmt1-203	<a href="#">ENSMUST00000178110.2</a>	5870	<a href="#">1501aa</a>	Protein coding	<a href="#">CCDS57653</a>	<a href="#">J3QNW0</a>	TSL:1 GENCODE basic APPRIS ALT2
Dnmt1-207	<a href="#">ENSMUST00000216540.1</a>	5543	<a href="#">1502aa</a>	Protein coding	<a href="#">CCDS57654</a>	<a href="#">P13864</a>	TSL:1 GENCODE basic APPRIS ALT2
Dnmt1-201	<a href="#">ENSMUST00000004202.16</a>	5367	<a href="#">1620aa</a>	Protein coding	<a href="#">CCDS57655</a>	<a href="#">P13864</a>	TSL:1 GENCODE basic APPRIS P4
Dnmt1-202	<a href="#">ENSMUST00000177754.8</a>	5248	<a href="#">1501aa</a>	Protein coding	<a href="#">CCDS57653</a>	<a href="#">J3QNW0</a>	TSL:1 GENCODE basic APPRIS ALT2
Dnmt1-204	<a href="#">ENSMUST00000214964.1</a>	1252	No protein	Retained intron	-	-	TSL:5
Dnmt1-206	<a href="#">ENSMUST00000216135.1</a>	693	No protein	Retained intron	-	-	TSL:3
Dnmt1-205	<a href="#">ENSMUST00000215545.1</a>	607	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Dnmt1-201* transcript,The transcription is shown below



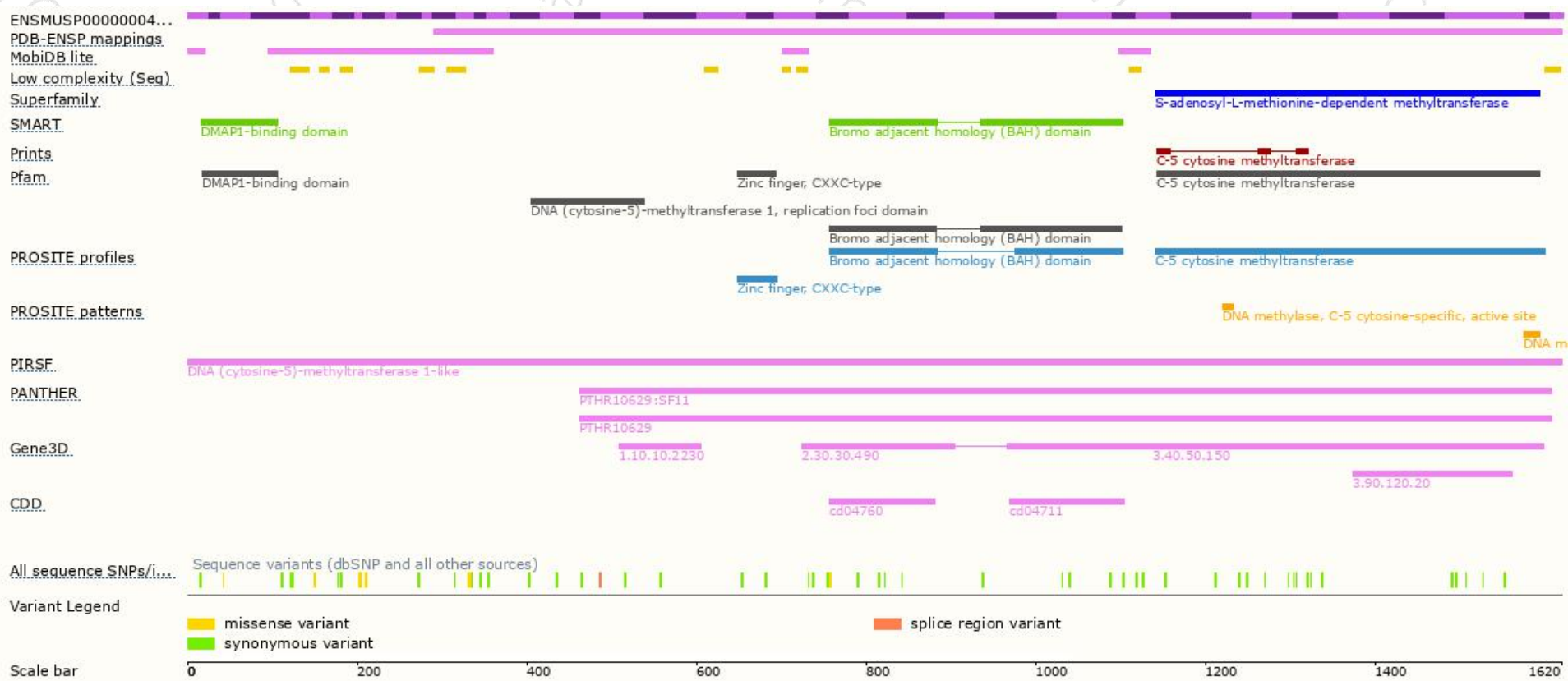


# Genomic location distribution



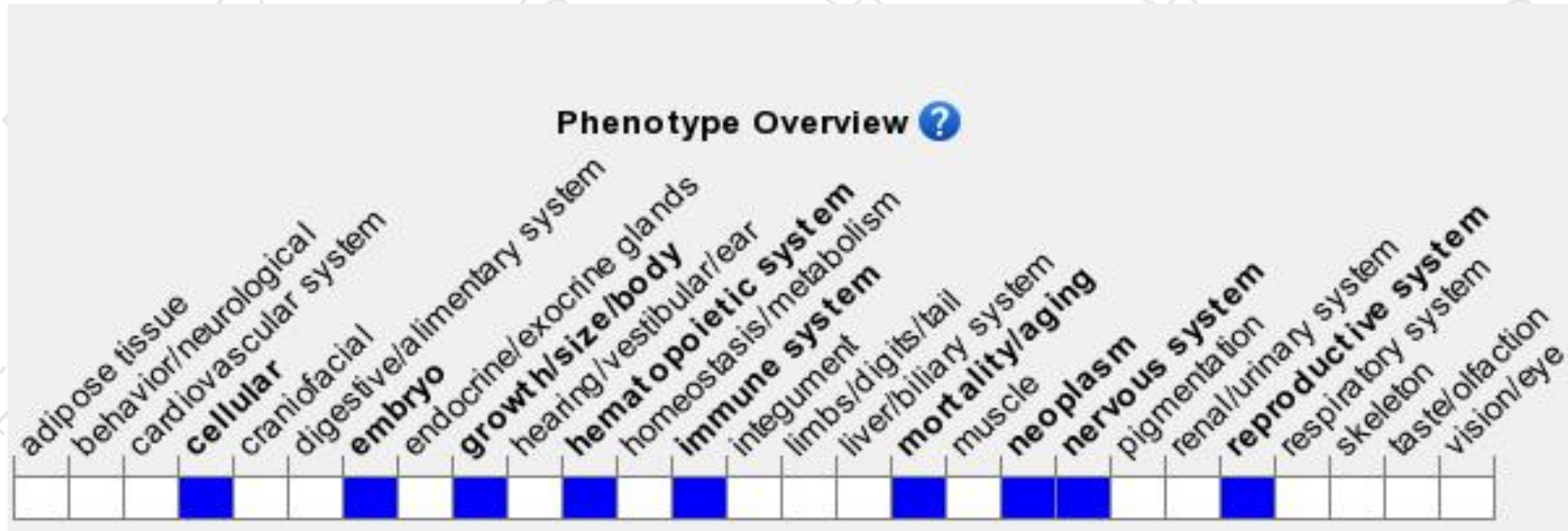


# Protein domain





# 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, Mutations causing partial or severe loss of function were homozygous lethal by embryonic day 9.5, with lack of appropriate genomic imprinting observed at several loci.



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

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