Dnmt1 Cas9-CKO Strategy

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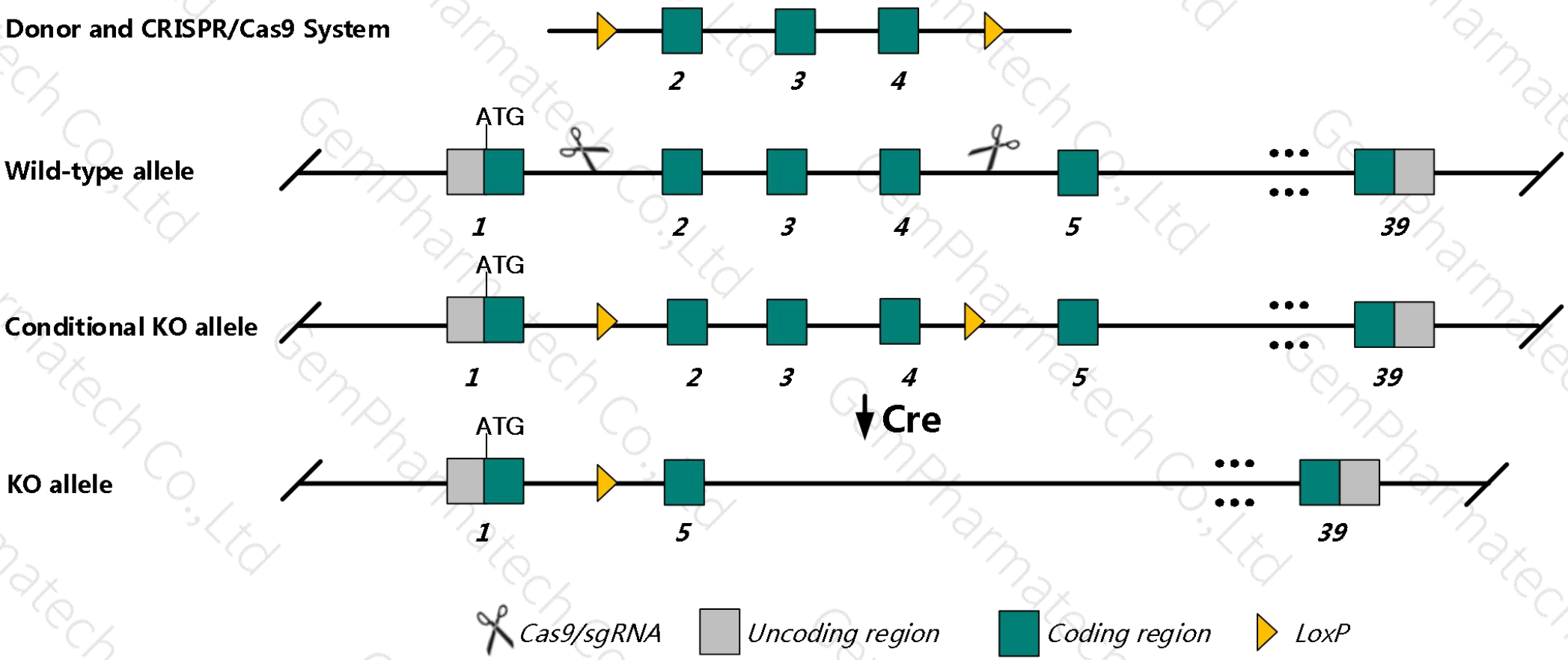
Reviewer:Jia Yu

Project Overview

Project Name	***Dnmt1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

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



Technical routes

- 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, donor vector was constructed.Cas9, sgRNA and Donor 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.

- 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,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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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

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

Summary

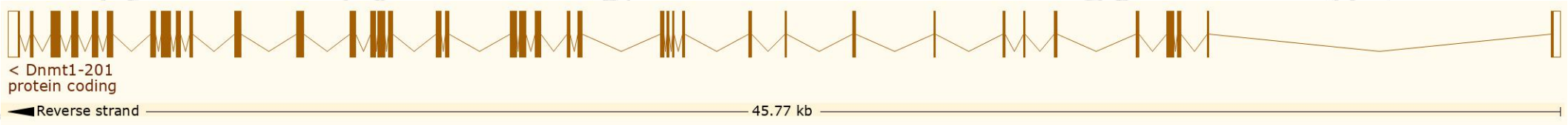
Official Symbol	Dnmt1 provided by MGI
Official Full Name	DNA methyltransferase (cytosine-5) 1 provided by MGI
Primary source	MGI:MGI:94912
See related	Ensembl:ENSMUSG00000004099
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; 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 See more
Orthologs	human all

Transcript information（Ensembl）

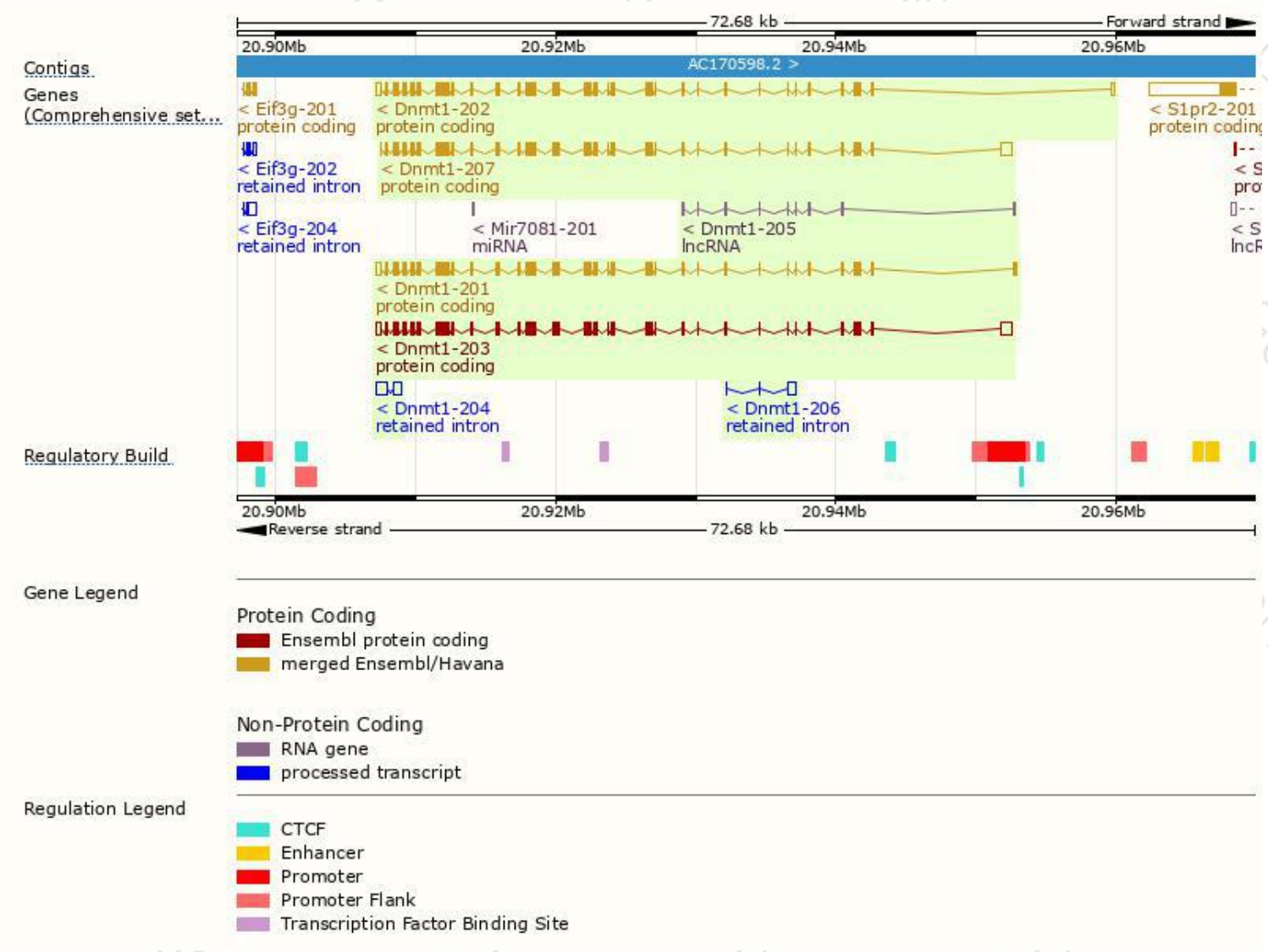
The gene has 7 transcripts,all transcripts are shown below:

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

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

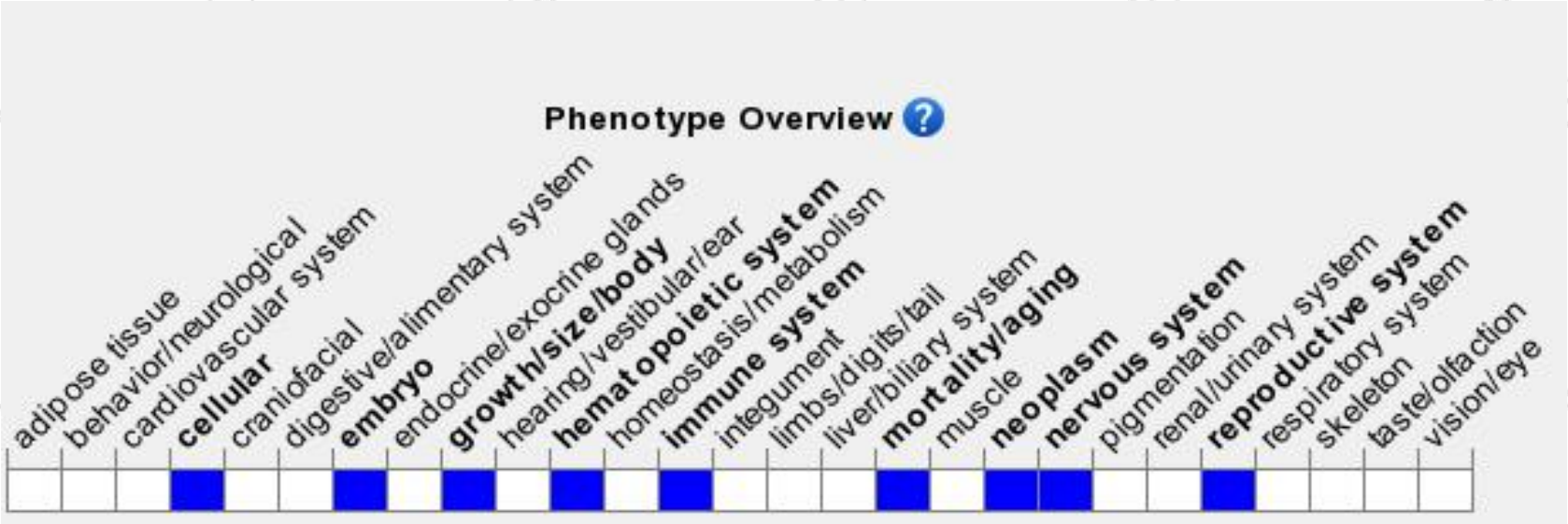


Genomic location distribution



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/).

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

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