

***Mettl25* Cas9-CKO Strategy**

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Design Date: 2020-11-24

Project Overview

Project Name

Mettl25

Project type

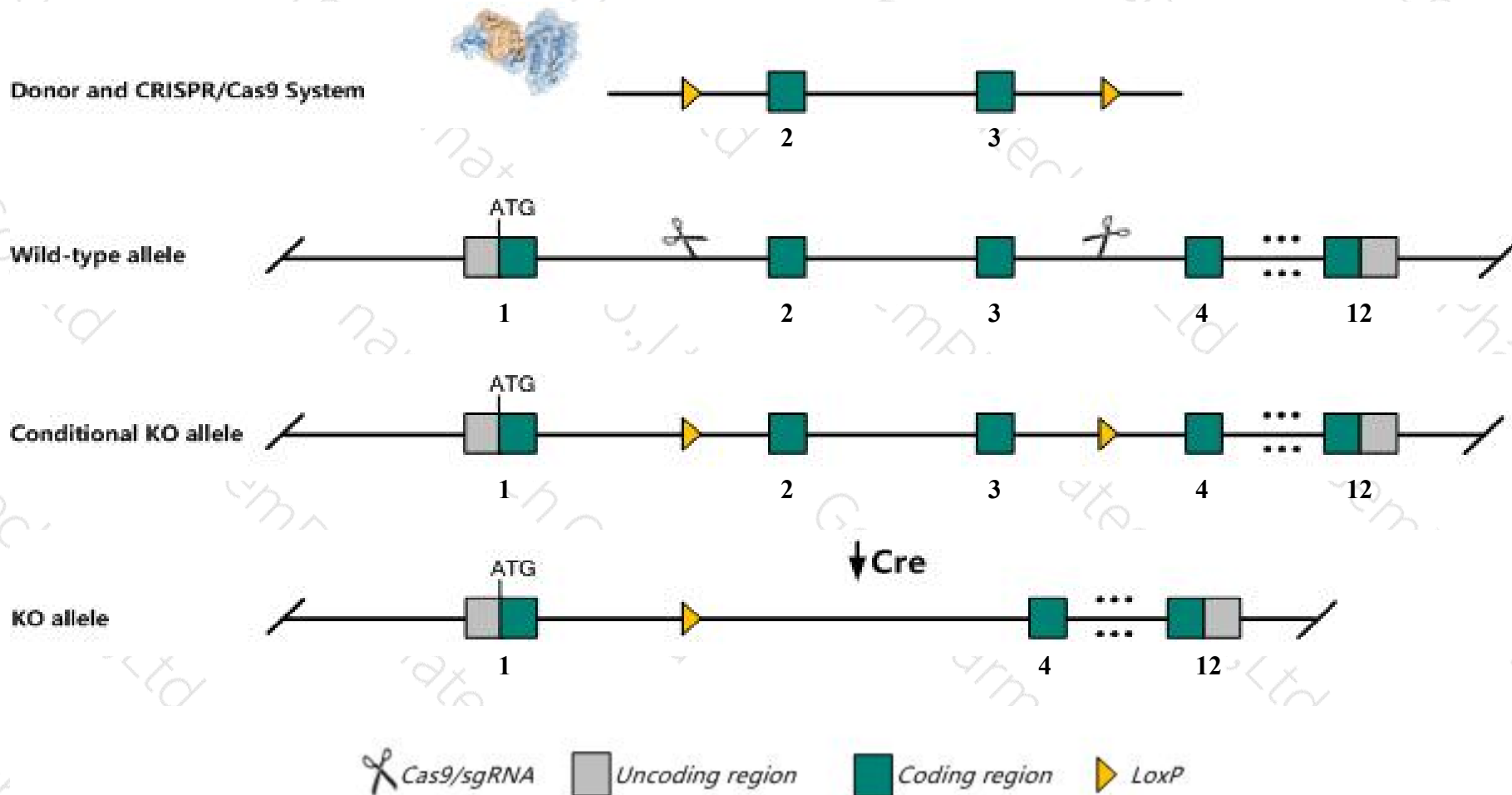
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mettl25* gene. The schematic diagram is as follows:



Technical routes

- The *Mettl25* gene has 4 transcripts. According to the structure of *Mettl25* gene, exon2-exon3 of *Mettl25-201*(ENSMUST00000046638.9) transcript is recommended as the knockout region. The region contains 272bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mettl25* 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/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.

- The *Mettl25* gene is located on the Chr10. 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)

Mettl25 methyltransferase like 25 [Mus musculus (house mouse)]

Gene ID: 216292, updated on 13-Mar-2020

Summary

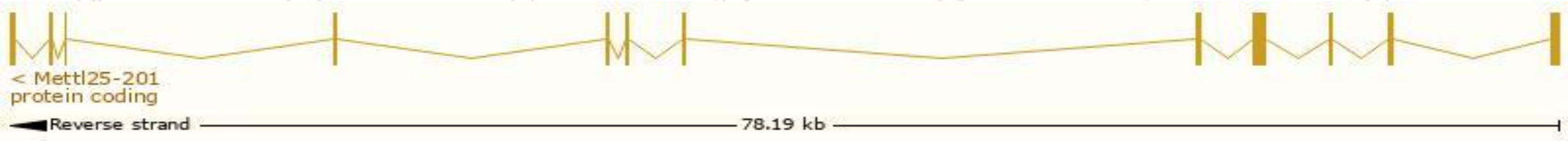
Official Symbol	Mettl25 provided by MGI
Official Full Name	methyltransferase like 25 provided by MGI
Primary source	MGI:MGI:3041259
See related	Ensembl:ENSMUSG00000036009
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in CNS E14 (RPKM 2.9), whole brain E14.5 (RPKM 2.4) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

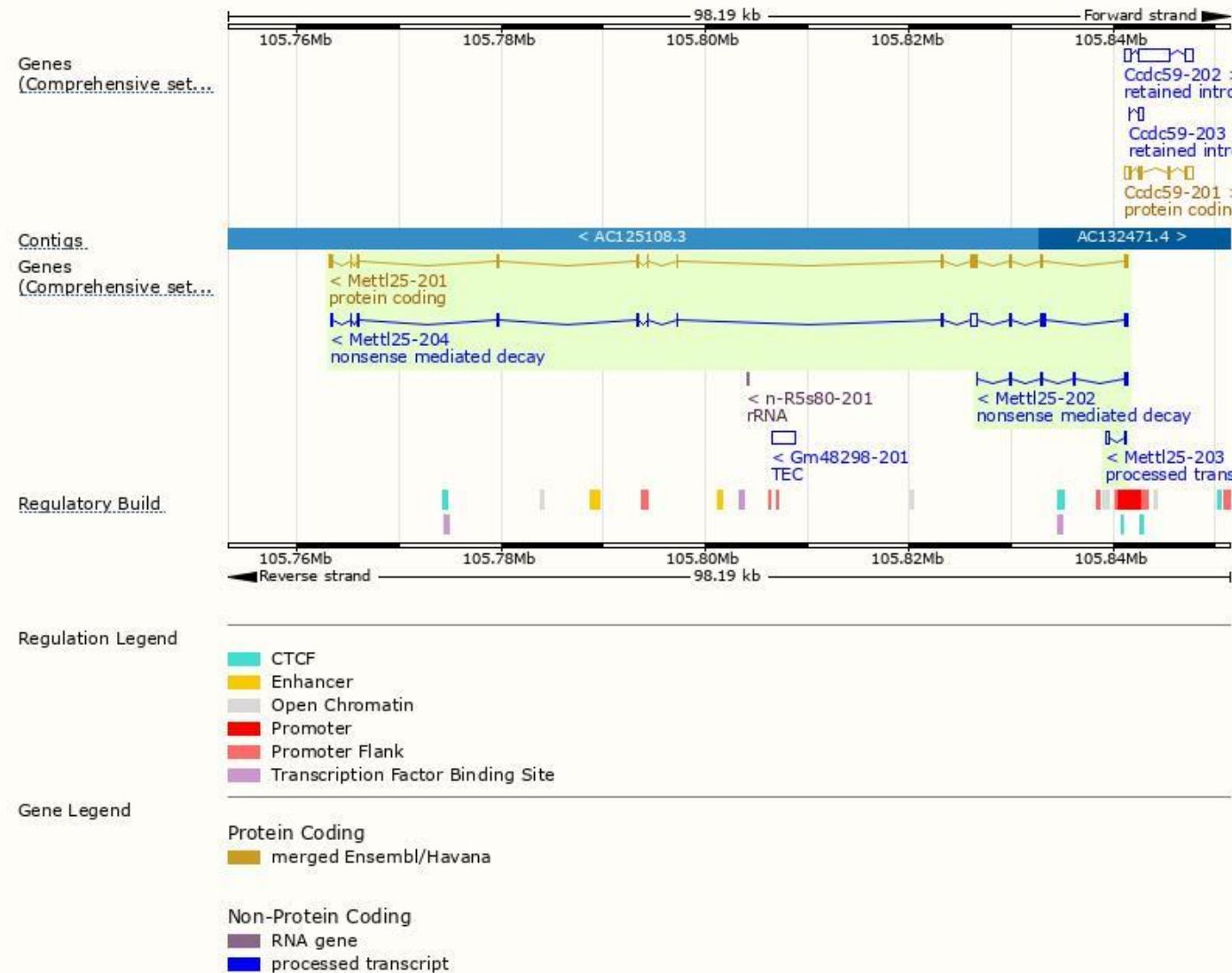
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mettl25-201	ENSMUST00000046638.9	2033	600aa	Protein coding	CCDS36049	A0A0R4J0G2	TSL:1 GENCODE basic APPRIS P1
Mettl25-204	ENSMUST00000176924.7	2145	98aa	Nonsense mediated decay	-	H3BJ85	TSL:1
Mettl25-202	ENSMUST00000176040.1	681	90aa	Nonsense mediated decay	-	H3BK52	TSL:3
Mettl25-203	ENSMUST00000176830.1	519	No protein	Processed transcript	-	-	TSL:2

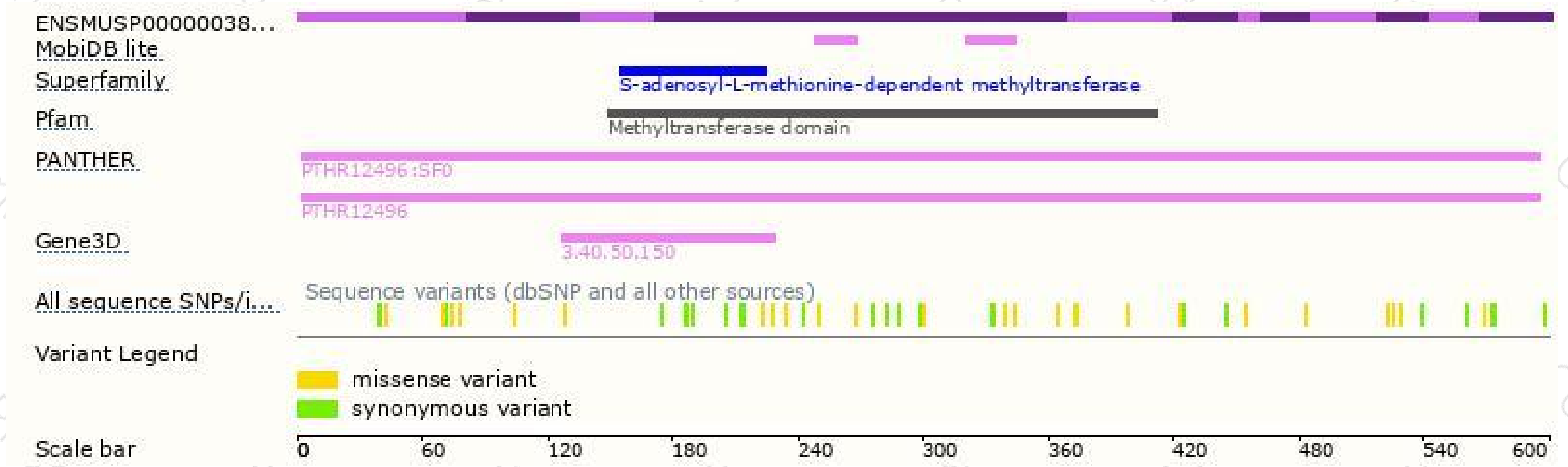
The strategy is based on the design of *Mettl25-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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