

Mettl4 Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer :

Huimin Su

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Project Overview

Project Name

Mettl4

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

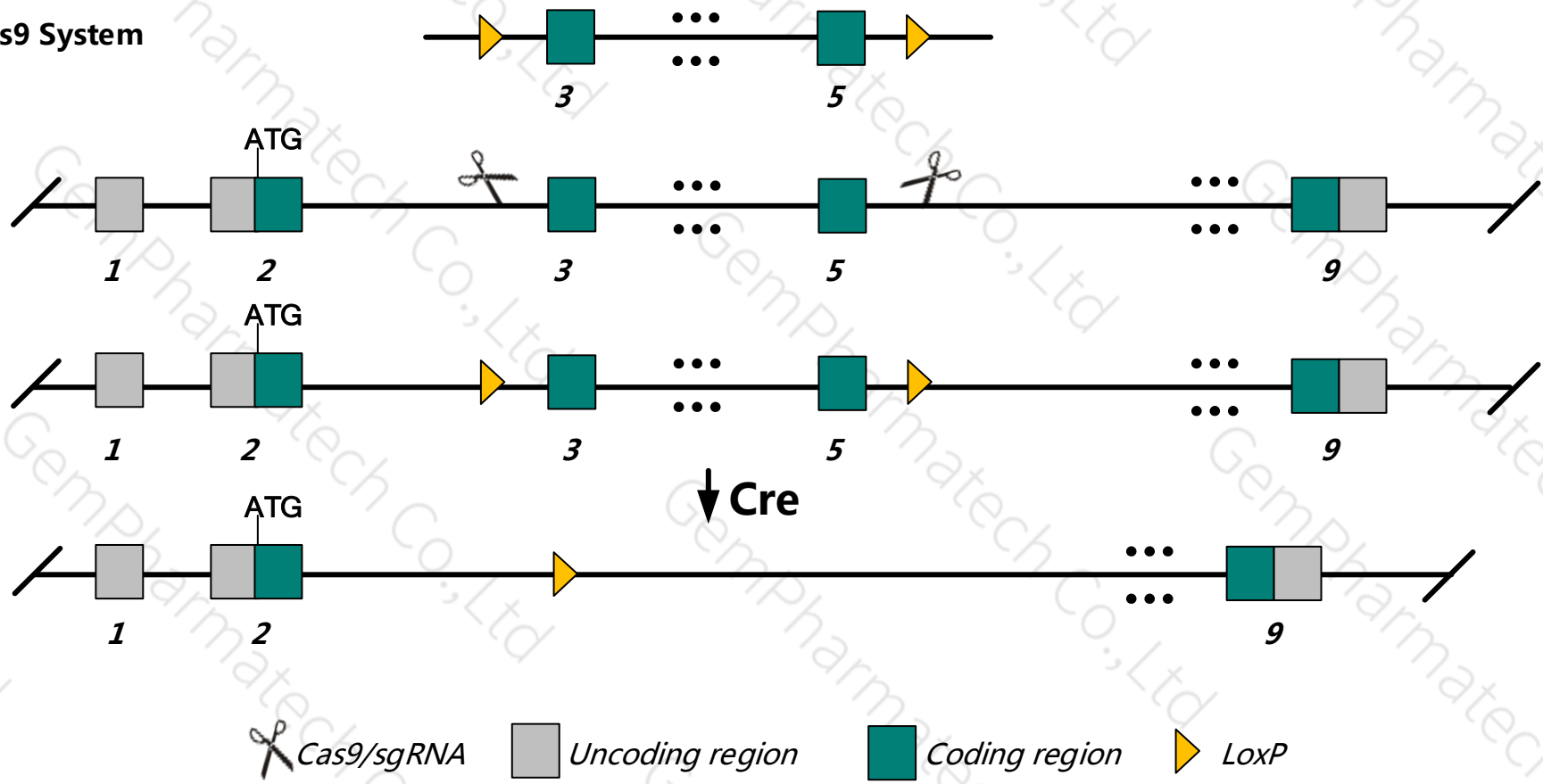
This model will use CRISPR/Cas9 technology to edit the *Mettl4* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Mettl4* gene has 3 transcripts. According to the structure of *Mettl4* gene, exon3-exon5 of *Mettl4*-203 (ENSMUST00000234990.1) transcript is recommended as the knockout region. The region contains 503bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mettl4* 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 or cell types.

- The *Mettl4* gene is located on the Chr17. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Mettl4 methyltransferase like 4 [*Mus musculus* (house mouse)]

Gene ID: 76781, updated on 12-Aug-2018

Summary

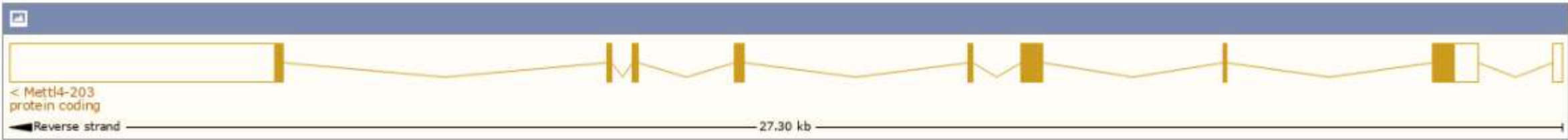
Official Symbol	Mettl4 provided by MGI
Official Full Name	methyltransferase like 4 provided by MGI
Primary source	MGI:MGI:1924031
See related	Ensembl:ENSMUSG00000055660
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	HsT661; AV296509; 2410198H06Rik; A730091E08Rik
Expression	Ubiquitous expression in CNS E14 (RPKM 3.7), CNS E11.5 (RPKM 3.4) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

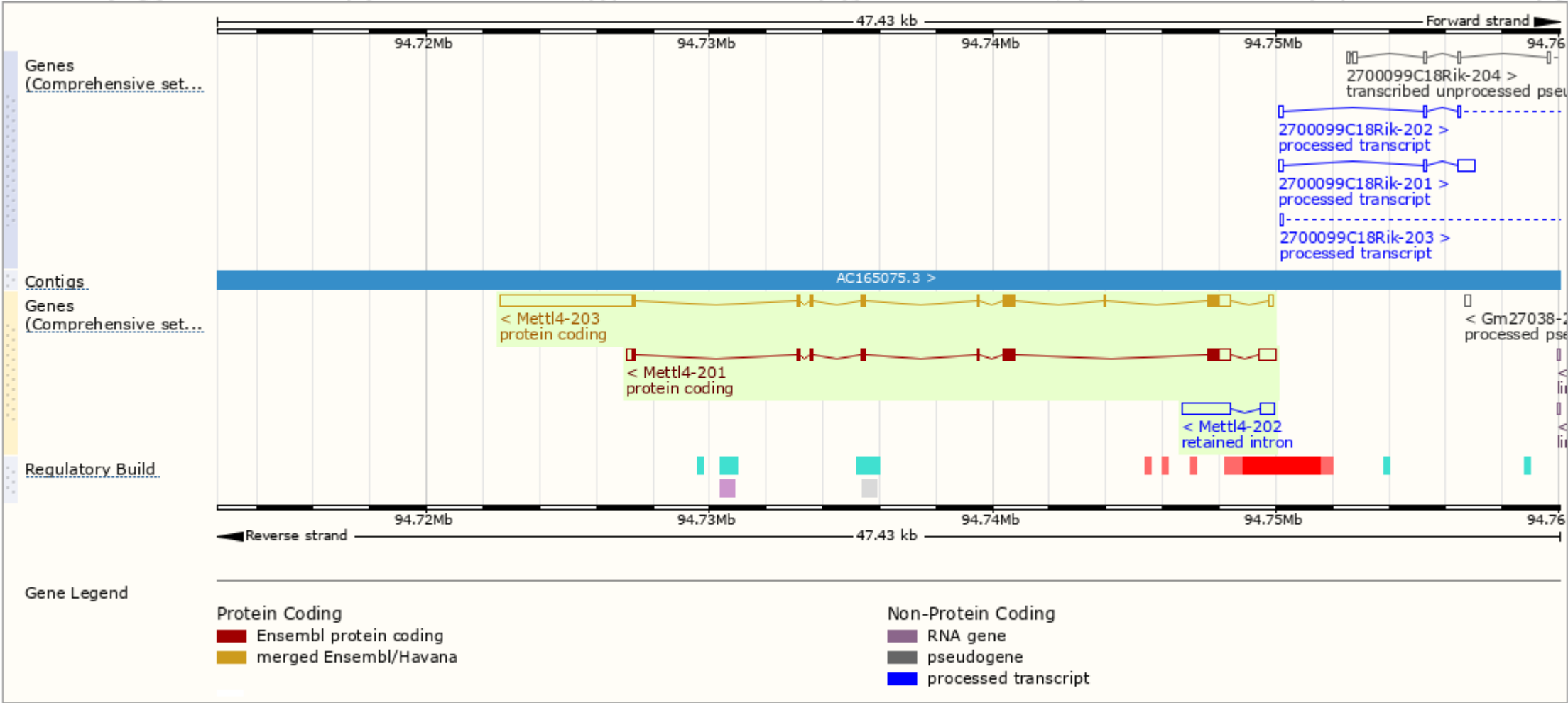
The gene has 3 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden)								Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags	
Mettl4-203	ENSMUST00000234990.1	6640	471aa	Protein coding	-	-	NM_001357135 NM_001357136 NM_176917 NP_001344064 NP_001344065 NP_795891	GENCODE basic	APPRIS P2
Mettl4-201	ENSMUST00000171284.2	2560	450aa	Protein coding	-	A4FTY8 Q3U034	NP_001344066 NP_001344067	TSL:1	GENCODE basic APPRIS ALT2
Mettl4-202	ENSMUST00000234593.1	2224	No protein	Retained intron	-	-	-		

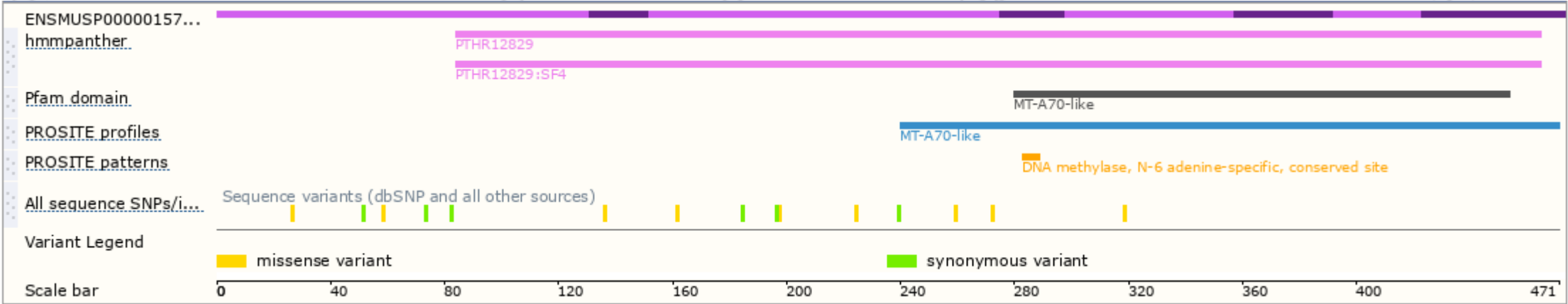
The strategy is based on the design of *Mettl4*-203 transcript, The transcription is shown below



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534



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