# *Mettl8* Cas9-CKO *Strategy*

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# Project Overview

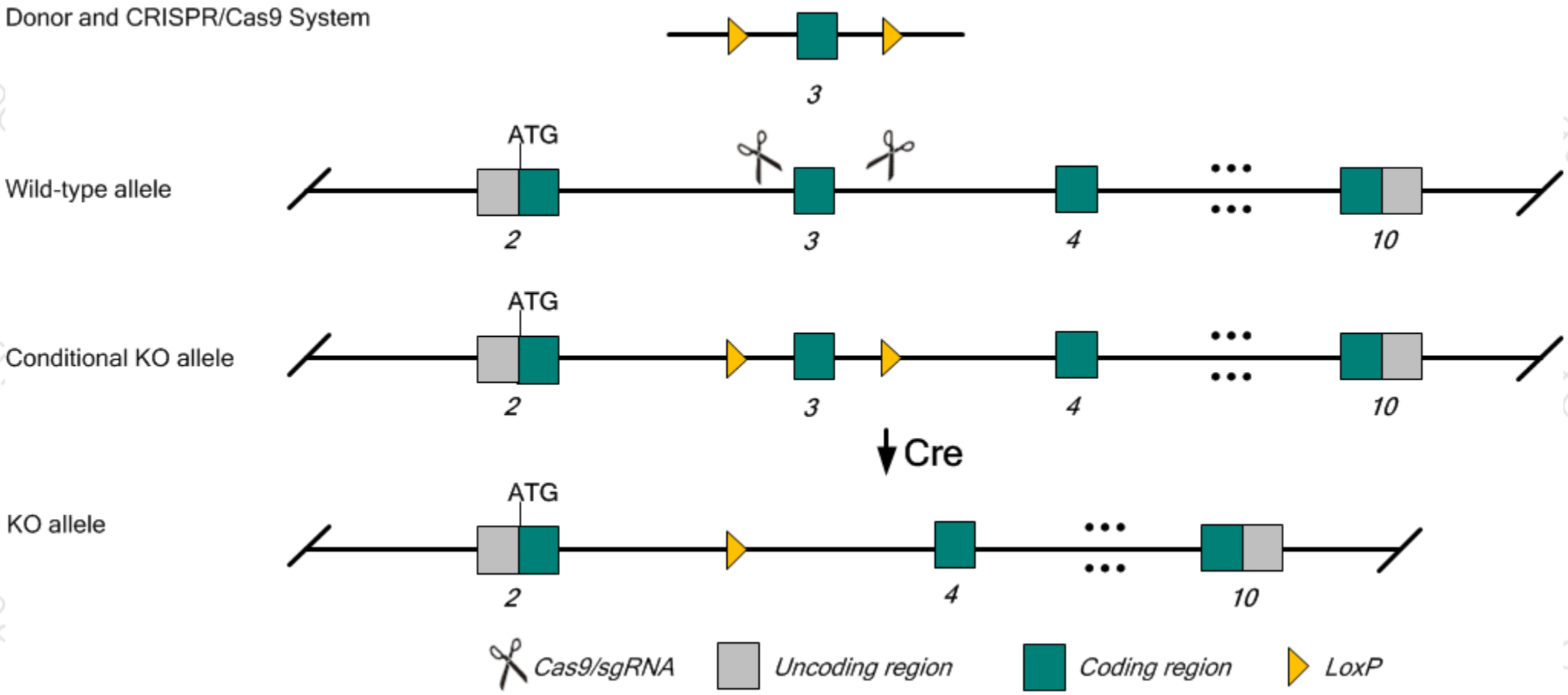| | |
|---|---|
| **Project Name** | *Mettl8* |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mettl8* gene. The schematic diagram is as follows:

# Technical routes

- The Mettl8 gene has 11 transcripts, According to the structure of *Mettl8* gene, exon3 of Mettl8-*204* transcript is recommended as the knockout region.The region contains the 92bp coding seqence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mettl8* 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.

# Notice

- According to the existing MGI data，Mice homozygous for a knock-out allele exhibit reduced 3-methylcytidine (m3C) methyltransferases modification of mRNA.

- Transcript Mettl8-*209* may not be affected.

- The *Mettl8* gene is located in 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information（NCBI）

**Mettl8   methyltransferase like 8 [ *Mus musculus* (house mouse) ]**

Gene ID: 228019, updated on 8-Dec-2018

▲ **Summary**                                                                      ⌃ ?

| | |
|---|---|
| **Official Symbol** | Mettl8 provided by MGI |
| **Official Full Name** | methyltransferase like 8 provided by MGI |
| **Primary source** | MGI:MGI:2385142 |
| **See related** | Ensembl:ENSMUSG00000041975 |
| **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** | Tip; BC004636 |
| **Summary** | This locus encodes a member of the methyltransferase family, and is involved in chromatin remodeling. Transcripts from this locus can be induced or inhibited by cell stretch and affect cell differentiation in the myogenic or adipogenic pathways. Multiple transcript variants encoding different isoforms have been found for this gene. Additional splice variants have been described in the literature but they meet nonsense-mediated decay (NMD) criteria and are likely to be degraded as soon as they are transcribed. [provided by RefSeq, Jul 2008] |
| **Expression** | Ubiquitous expression in thymus adult (RPKM 2.3), CNS E11.5 (RPKM 2.1) and 28 other tissues See more |
| **Orthologs** | human    all |

# Transcript information（Ensembl）

The gene has 11 transcripts, and all transcripts are shown below :

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | RefSeq | Flags |
|------|---------------|-----|---------|---------|------|---------|--------|-------|
| Mettl8-204 | ENSMUST00000112186.8 | 2347 | 388aa | Protein coding | CCDS50600 | A2AUU0 | NM_001110512<br>NM_145524<br>NP_001103982<br>NP_663499 | TSL:2  GENCODE basic  APPRIS P2 |
| Mettl8-202 | ENSMUST00000100037.8 | 2254 | 341aa | Protein coding | - | B0R0U1 | - | TSL:1  GENCODE basic  APPRIS ALT2 |
| Mettl8-201 | ENSMUST00000090849.5 | 1842 | 163aa | Protein coding | - | Q8CA70 | - | TSL:1  GENCODE basic |
| Mettl8-205 | ENSMUST00000121586.7 | 1151 | 281aa | Protein coding | - | A2AUU0 | - | TSL:5  GENCODE basic |
| Mettl8-209 | ENSMUST00000140293.1 | 592 | 110aa | Protein coding | - | F6W103 | - | CDS 5' incomplete  TSL:3 |
| Mettl8-210 | ENSMUST00000148876.7 | 2473 | 304aa | Nonsense mediated decay | - | A2AUU0 | - | TSL:1 |
| Mettl8-203 | ENSMUST00000112179.8 | 2297 | 206aa | Nonsense mediated decay | - | A2AUU0 | - | TSL:2 |
| Mettl8-211 | ENSMUST00000149181.7 | 743 | 148aa | Nonsense mediated decay | - | F6Q7P7 | - | CDS 5' incomplete  TSL:5 |
| Mettl8-206 | ENSMUST00000124208.1 | 1597 | No protein | Retained intron | - | - | - | TSL:1 |
| Mettl8-208 | ENSMUST00000124781.1 | 833 | No protein | Retained intron | - | - | - | TSL:5 |
| Mettl8-207 | ENSMUST00000124273.1 | 390 | No protein | Retained intron | - | - | - | TSL:5 |

The strategy is based on the design of *Mettl8-204* transcript,The transcription is shown below



< Mettl8-204
protein coding

◀ Reverse strand ─────────────────── 91.02 kb ───────────────────
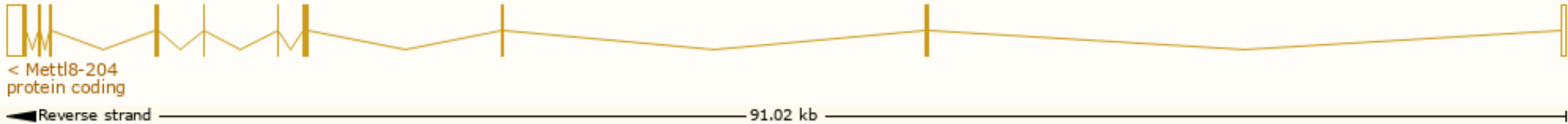
# Genomic location distribution
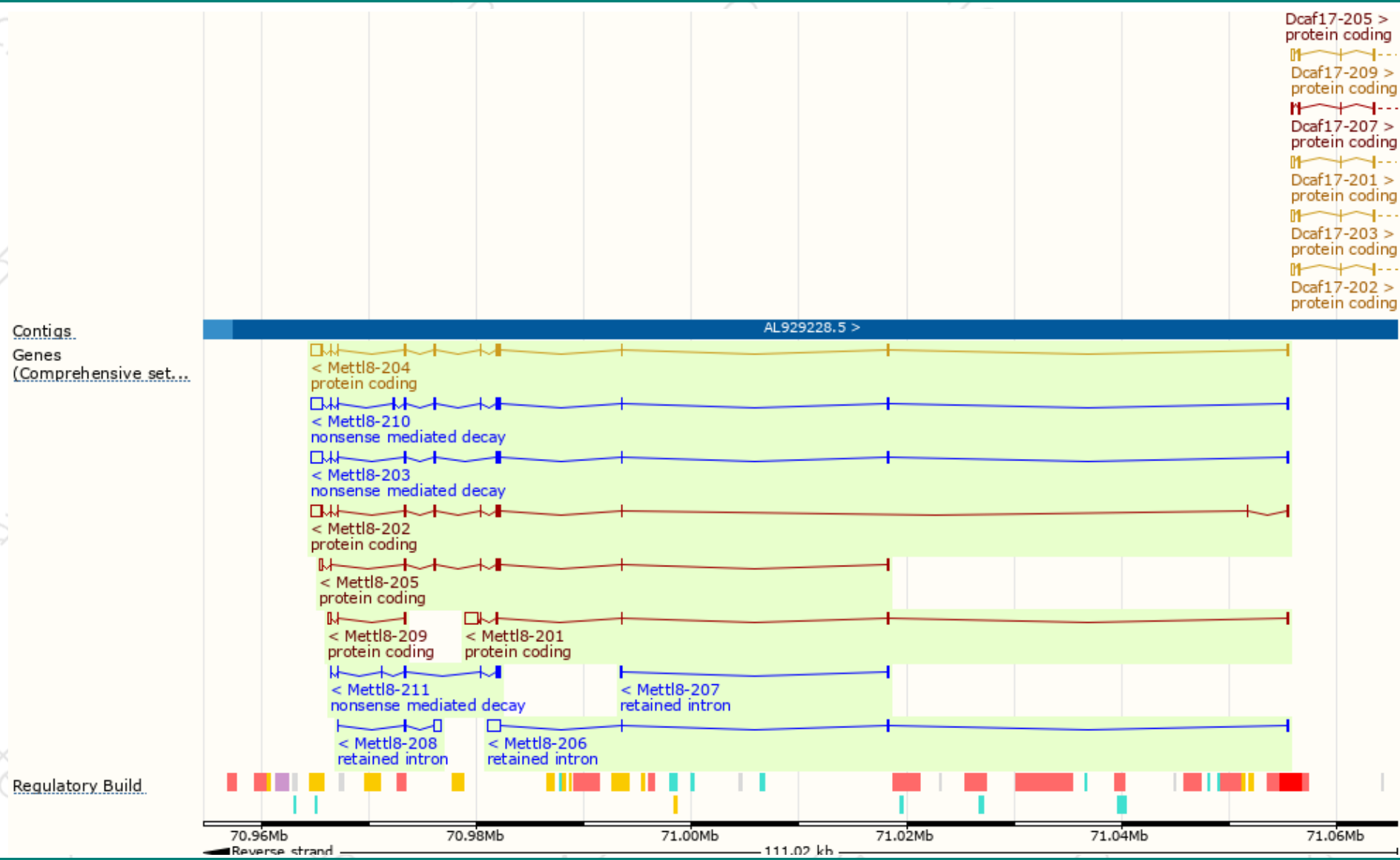
# Protein domain
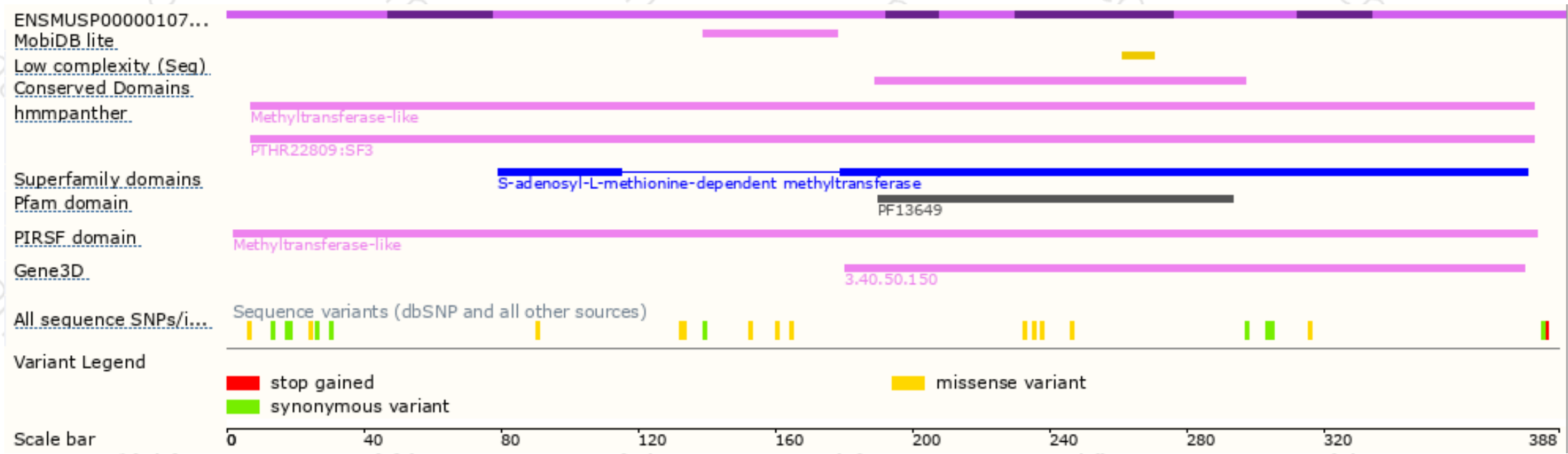
If you have any questions, you are welcome to inquire.
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