# *Asmt* Cas9-CKO Strategy

**Designer:** Yanhua Shen

**Reviewer:** Xueting Zhang

**Design Date:** 2020-4-29

# Project Overview

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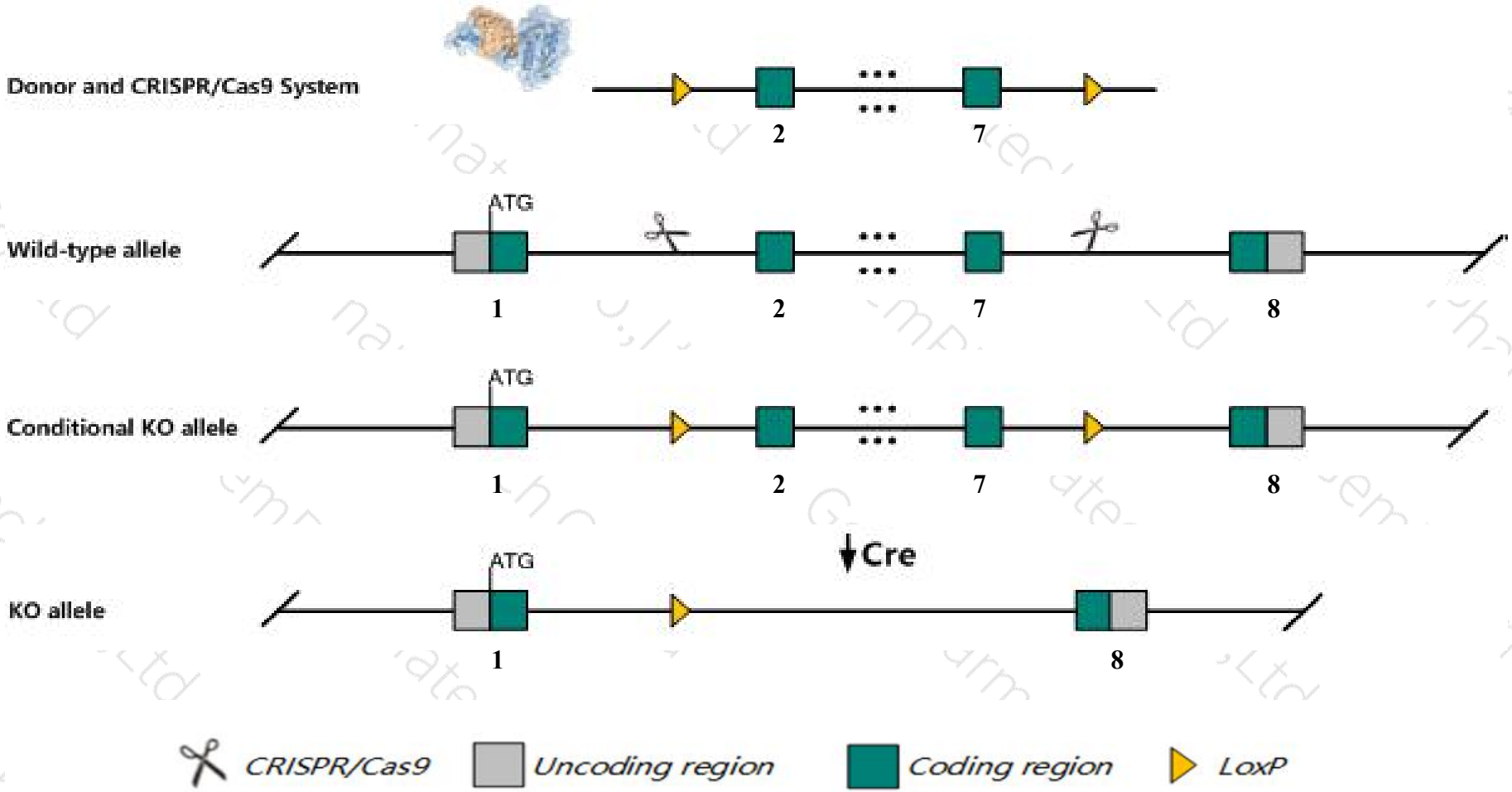
| **Project Name** | ***Asmt*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

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



# Technical routes

- The *Asmt* gene has 1 transcript. According to the structure of *Asmt* gene, exon2-exon7 of *Asmt-201* (ENSMUST00000178693.1) transcript is recommended as the knockout region.The region contains 772bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Asmt* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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,pineal melatonin synthesis requires enzymes encoded by Asmt and Aanat. C57BL/6, BALB/c, AKR/J, NZB/Bl, IS/Cam, and CAST/Ei carry the a allele of Asmt and lack melatonin. SK/Cam, SF/Cam, PERU, and FDS carry the b allele and have normal melatonin levels.
- The *Asmt* gene is located on the ChrX. 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）

集萃药康 GemPharmatech

## Asmt acetylserotonin O-methyltransferase [Mus musculus (house mouse)]

Gene ID: 107626, updated on 13-Mar-2020

### Summary

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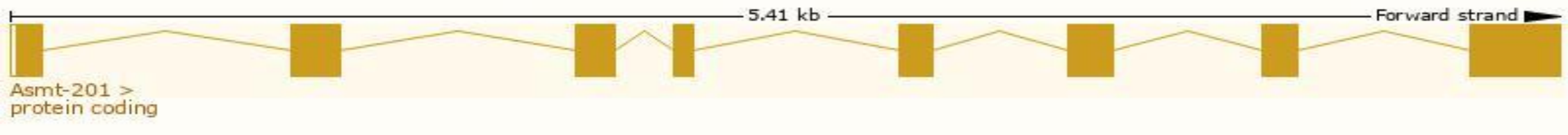
|---|---|
| **Official Symbol** | Asmt provided by MGI |
| **Official Full Name** | acetylserotonin O-methyltransferase provided by MGI |
| **Primary source** | MGI:MGI:96090 |
| **See related** | Ensembl:ENSMUSG00000093806 |
| **Gene type** | protein coding |
| **RefSeq status** | REVIEWED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | Hiomt |
| **Summary** | This gene belongs to the methyltransferase superfamily and is located in the pseudoautosomal region (PAR) of the X and Y chromosomes. The encoded enzyme catalyzes the final reaction in the synthesis of melatonin and is abundant in the pineal gland. Two amino acid substitutions (R78G and R242C) are present in the encoded protein derived from the reference strain, C57BL/6J, and this protein shows low enzyme activity relative to the protein derived from other strains. [provided by RefSeq, May 2015] |
| **Expression** | Low expression observed in reference datasetSee more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

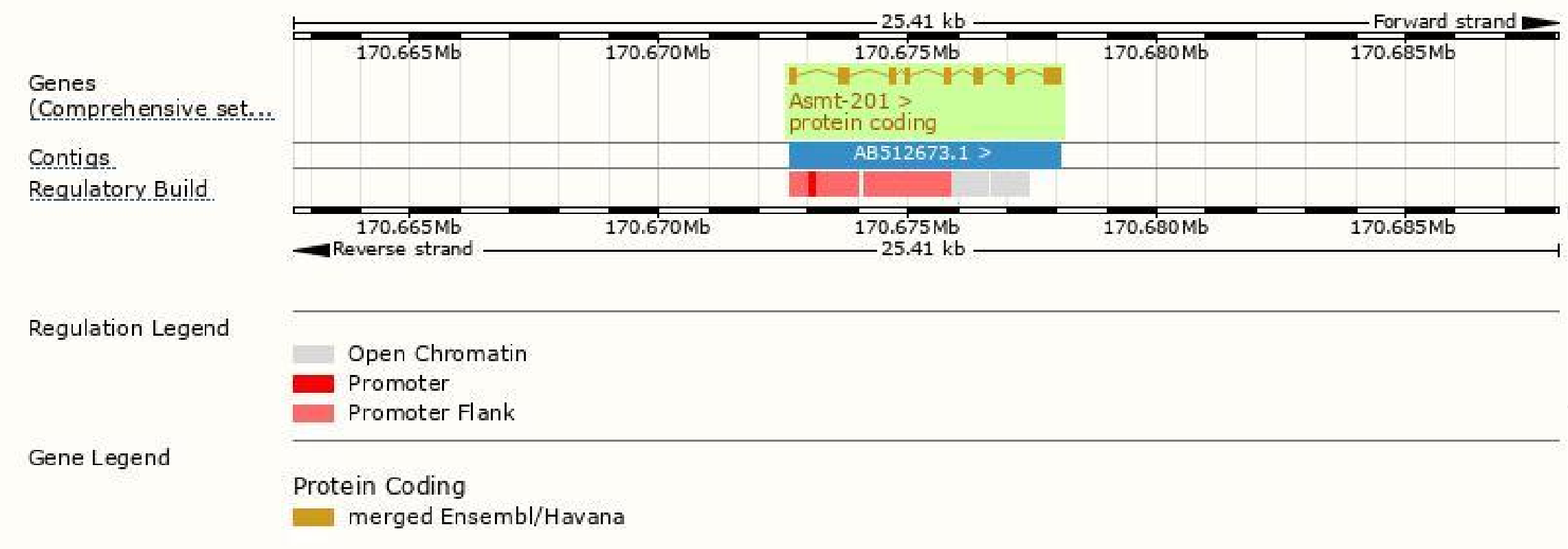
The gene has 1 transcript,and the transcript is shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Asmt-201 | ENSMUST00000178693.1 | 1191 | 387aa | Protein coding | CCDS85832 | A0A0R4J285 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1 |

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

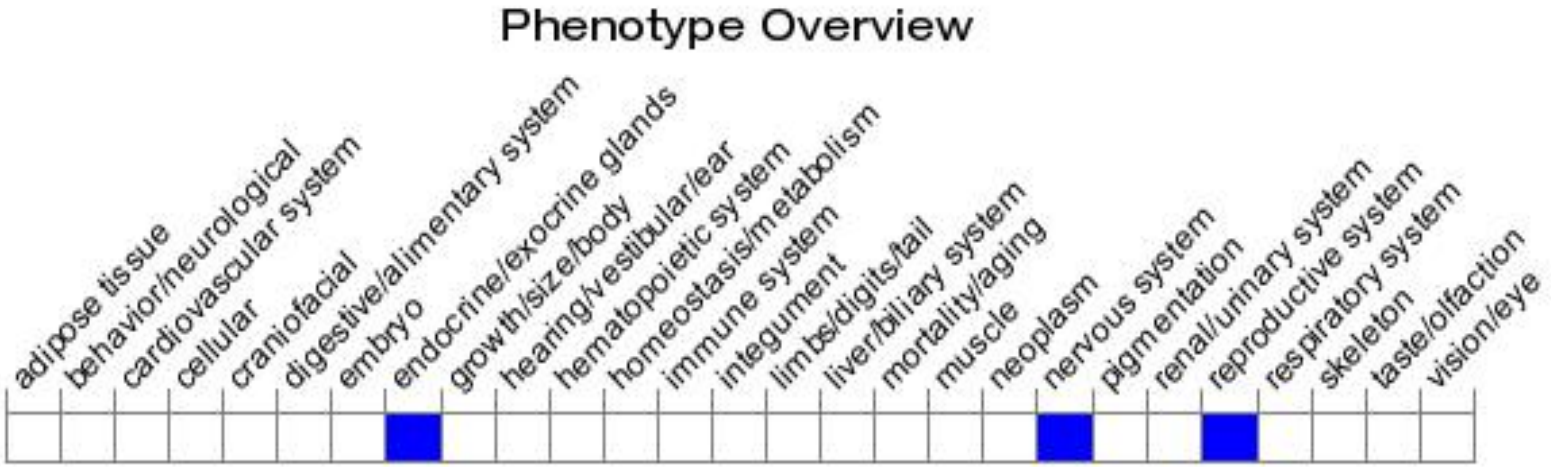


# Genomic location distribution



# 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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集萃药康
GemPharmatech

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd