Mnt Cas9-KO Strategy

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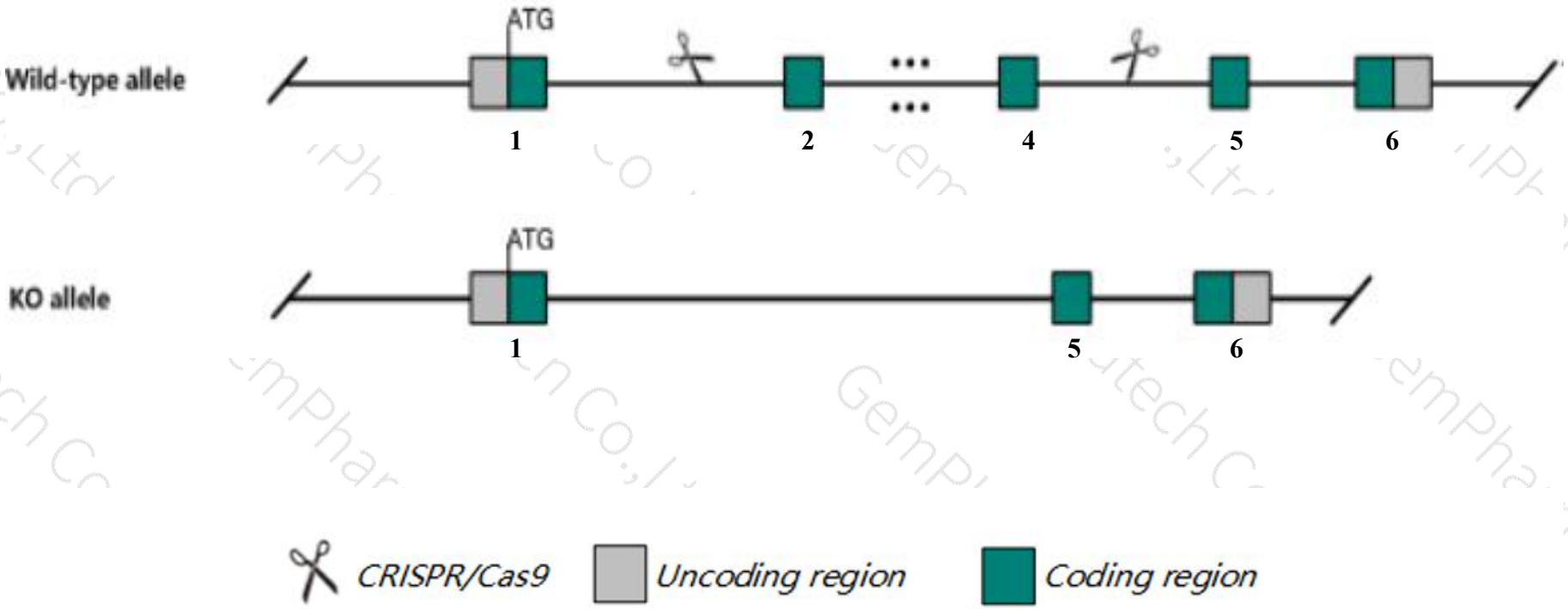
Design Date: 2020-7-24

Project Overview

Project Name	***Mnt***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

➢ The *Mnt* gene has 3 transcripts. According to the structure of *Mnt* gene, exon2-exon4 of *Mnt-201*(ENSMUST00000000291.8) transcript is recommended as the knockout region.The region contains 740bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mnt* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,most homozygotes for a targeted null mutation are runted at birth and die within a few days, while mutant fibroblasts show abnormal cell cycling. Those homozygotes that survive are fertile and attain normal Heterozygotes for a conditional mammary epithelial specific knockout develop adenocarcinomas.

➢ The *Mnt* gene is located on the Chr11. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Mnt max binding protein [Mus musculus (house mouse)]

Gene ID: 17428, updated on 13-Mar-2020

Summary

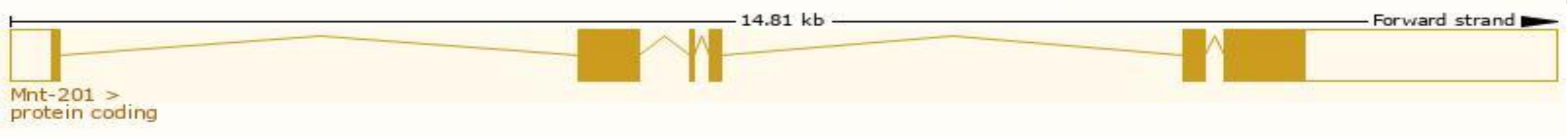
Official Symbol	Mnt provided by MGI
Official Full Name	max binding protein provided by MGI
Primary source	MGI:MGI:109150
See related	Ensembl:ENSMUSG00000000282
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Rox, bHLHd3
Expression	Ubiquitous expression in testis adult (RPKM 15.8), adrenal adult (RPKM 10.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

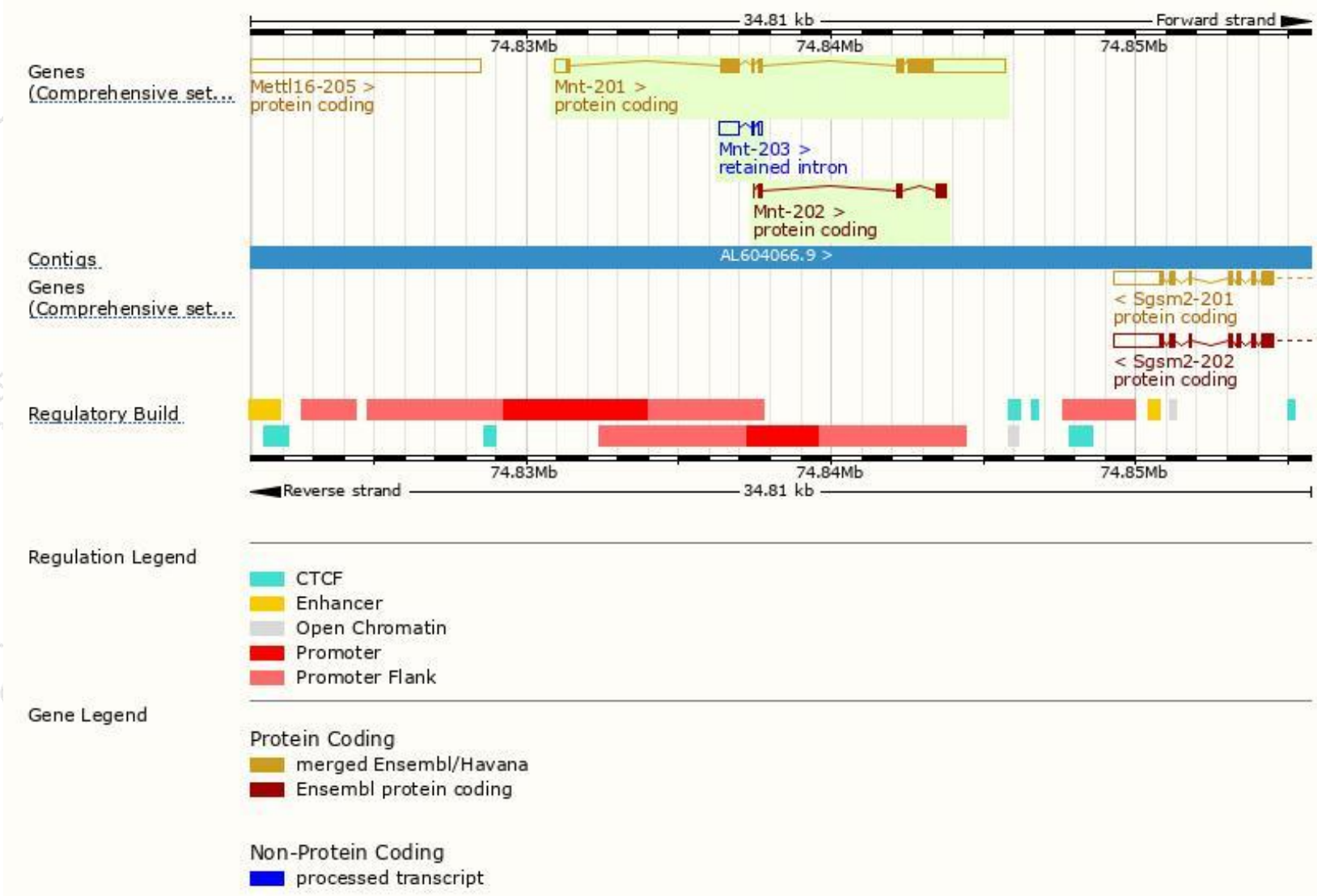
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mnt-201	ENSMUST00000000291.8	4590	591aa	Protein coding	CCDS25037	O08789	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
Mnt-202	ENSMUST00000132150.1	622	207aa	Protein coding	-	Q5SWE2	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Mnt-203	ENSMUST00000133217.1	777	No protein	Retained intron	-	-	TSL:3

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

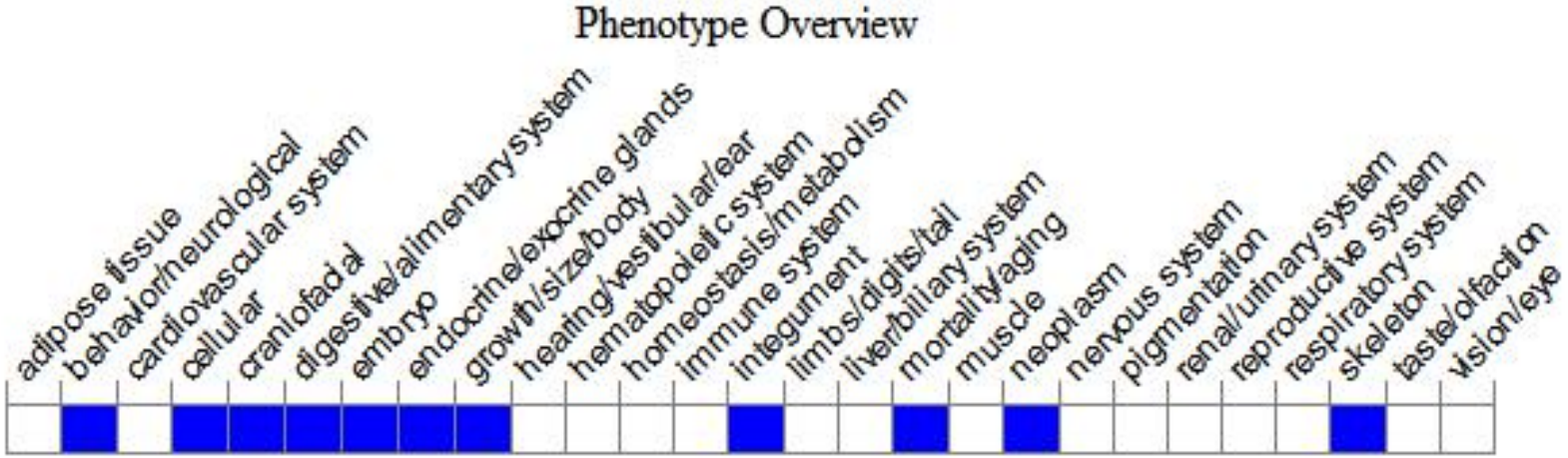


Genomic location distribution



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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