

# Nsmce4a Cas9-CKO Strategy

Designer: Jia Yu

Reviewer: Xiaojing Li

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## **Project Overview**



Project Name Nsmce4a

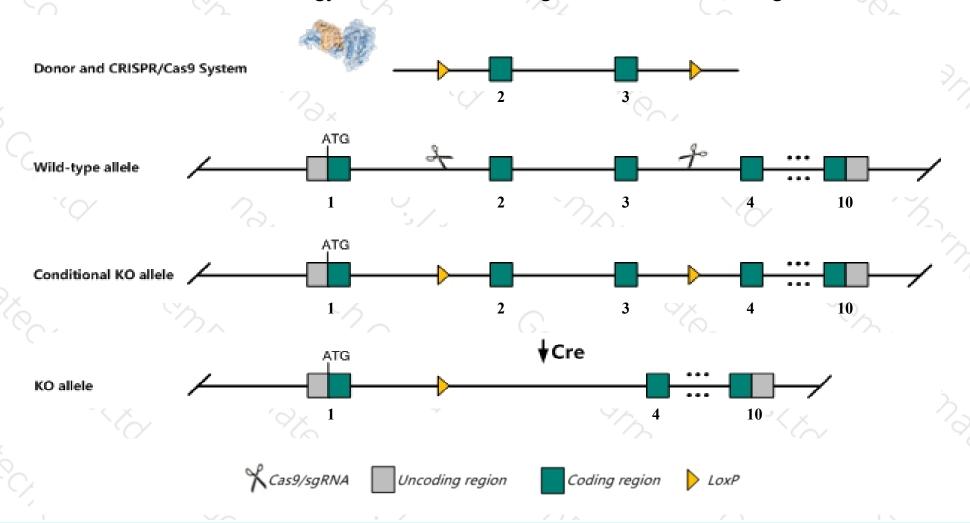
Project type Cas9-CKO

Strain background C57BL/6JGpt

### Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Nsmce4a* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Nsmce4a* gene has 7 transcripts. According to the structure of *Nsmce4a* gene, exon2-exon3 of *Nsmce4a*202(ENSMUST00000160289.8) transcript is recommended as the knockout region. The region contains 209bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Nsmce4a* 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.

### **Notice**



- > The *Nsmce4a* gene is located on the Chr7. 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.
- ➤ The flox region is in the intron of the Fgfr2 gene, which may affect the regulation of this gene.
- 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)



#### Nsmce4a NSE4 homolog A, SMC5-SMC6 complex component [Mus musculus (house mouse)]

Gene ID: 67872, updated on 13-Mar-2020

#### Summary



Official Symbol Nsmce4a provided by MGI

Official Full Name NSE4 homolog A, SMC5-SMC6 complex component provided by MGI

Primary source MGI:MGI:1915122

See related Ensembl:ENSMUSG00000040331

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 2410003A14Rik

Expression Ubiquitous expression in CNS E11.5 (RPKM 50.6), ovary adult (RPKM 38.5) and 28 other tissuesSee more

Orthologs <u>human</u> all

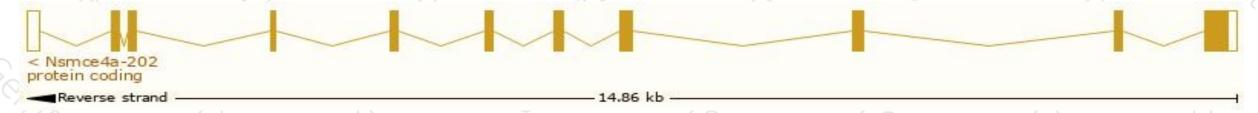
# Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

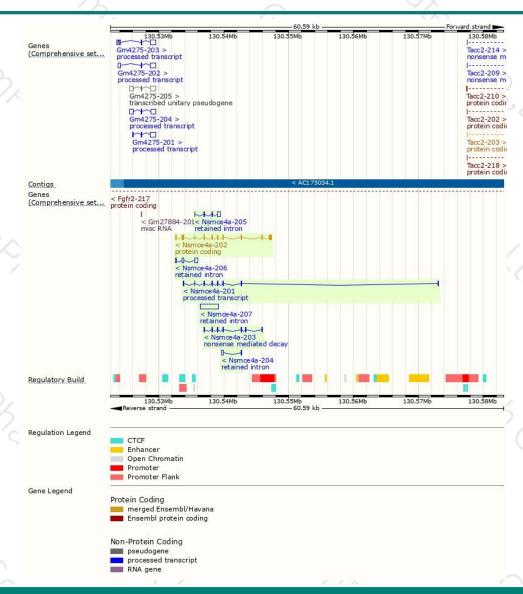
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nsmce4a-202	ENSMUST00000160289.8	1435	<u>381aa</u>	Protein coding	CCDS52415	<u>G3XA30</u>	TSL:1 GENCODE basic APPRIS P1
Nsmce4a-203	ENSMUST00000161076.1	688	<u>71aa</u>	Nonsense mediated decay	-	F6UGP0	CDS 5' incomplete TSL:3
Nsmce4a-201	ENSMUST00000159694.2	803	No protein	Processed transcript	-	-	TSL:3
Nsmce4a-207	ENSMUST00000207276.1	2834	No protein	Retained intron	-	-	TSL:NA
Nsmce4a-206	ENSMUST00000162510.1	895	No protein	Retained intron	-	-	TSL:5
Nsmce4a-205	ENSMUST00000161470.7	707	No protein	Retained intron	-	-	TSL:3
Nsmce4a-204	ENSMUST00000161414.1	370	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Nsmce4a-202* transcript, the transcription is shown below:



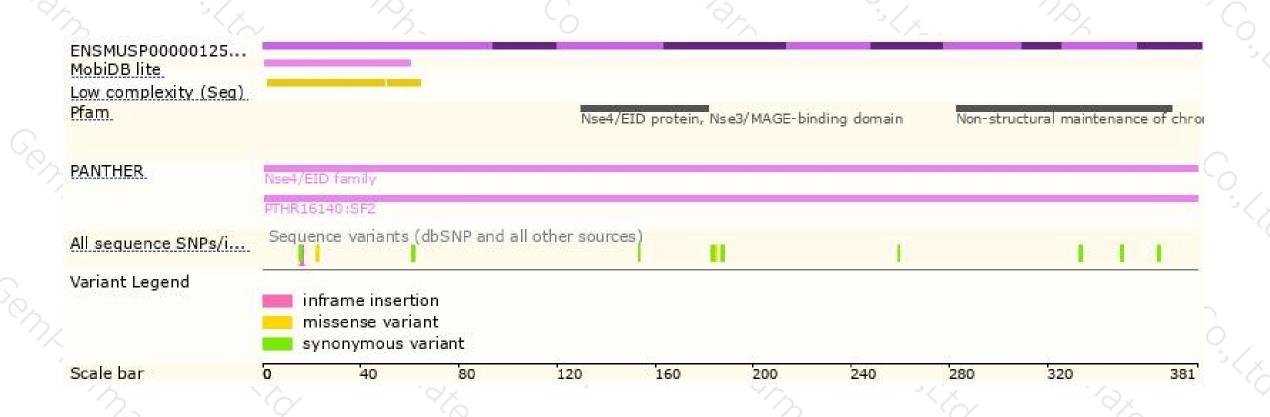
### Genomic location distribution





### Protein domain







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

Tel: 025-5864 1534





