

# Cmtr1 Cas9-CKO Strategy

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**Reviewer: Lu Chen** 

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## **Project Overview**

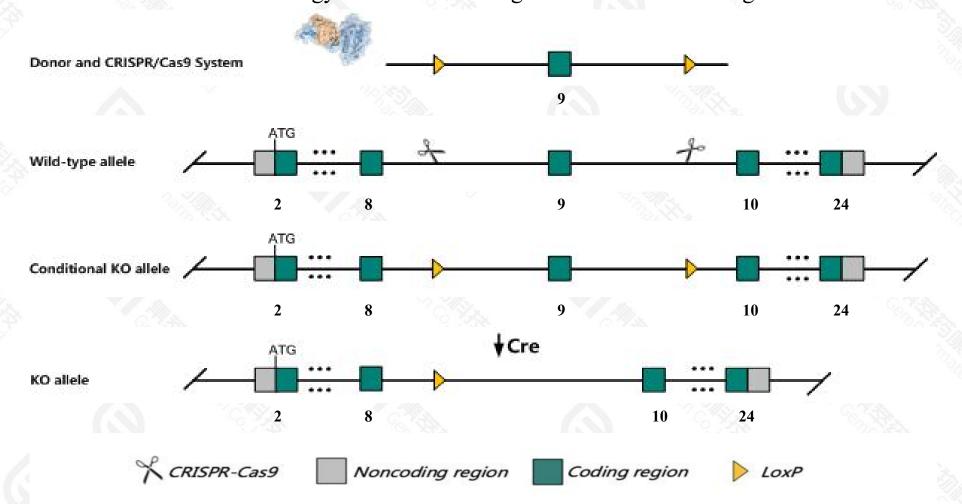


Project Name	Cmtr1
Project type	Cas9-CKO
Strain background	C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR-Cas9 technology to edit the *Cmtr1* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Cmtr1* gene has 17 transcripts. According to the structure of *Cmtr1* gene, exon9 of *Cmtr1*201(ENSMUST00000024816.13) transcript is recommended as the knockout region. The region contains 199bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR-Cas9 technology to modify *Cmtr1* 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, homozygous knockout is early embryonic lethal. Conditional homozygous KO in neurons leads to reduced neocortex size and neurite dysmorphology.
- > The transcripts of Cmtr1-204 and Cmtr1-206 are not affected and their effects are unknown.
- > This strategy will destroy Gm28043 gene while knocking out the target gene, and the effect is unknown.
- This strategy may affect the 3-terminal regulation of *Ccdc167* gene.
- > The *Cmtr1* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

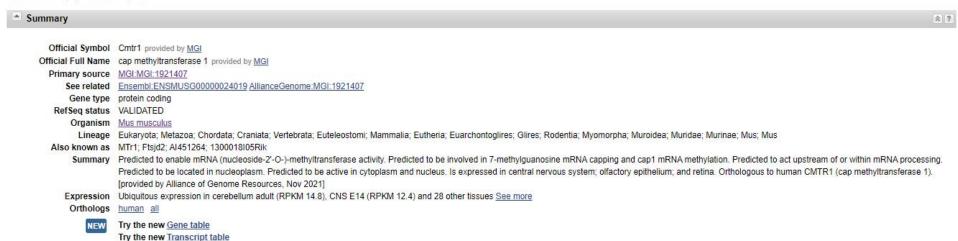
## Gene information (NCBI)



#### Cmtr1 cap methyltransferase 1 [ Mus musculus (house mouse) ]

**▲** Download Datasets

Gene ID: 74157, updated on 3-Apr-2022



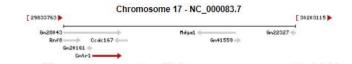
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See Cmtr1 in Genome Data Viewer

Location: 17; 17 A3.3

Exon count: 24

Annotation release	Status	Assembly	Chr	Location	
109	current	GRCm39 (GCF 000001635.27)	17	NC_000083.7 (2987956929924953)	
108.20200622	previous assembly	GRCm38.p6 (GCF 000001635.26)	17	NC_000083.6 (2966059529705979)	
Build 37.2	previous assembly	MGSCv37 (GCF 000001635.18)	17	NC_000083.5 (2979754629840304)	



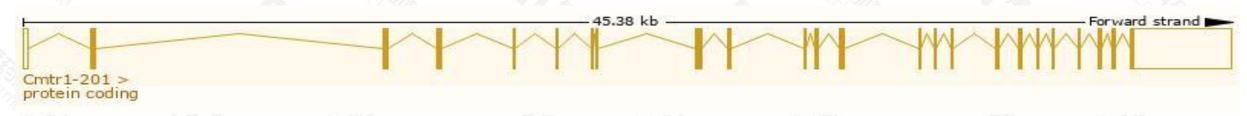
## Transcript information (Ensembl)



#### The gene has 17 transcripts, all transcripts are shown below:

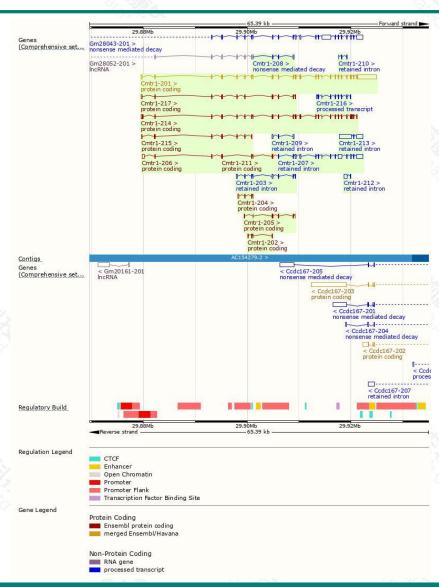
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cmtr1-201	ENSMUST00000024816.13	6380	837aa	Protein coding	CCDS37539		TSL:1 , GENCODE basic , APPRIS P1
Cmtr1-214	ENSMUST00000234911.2	2815	<u>844aa</u>	Protein coding	-		CDS 3' incomplete ,
Cmtr1-217	ENSMUST00000235031.2	1271	369aa	Protein coding	-		CDS 3' incomplete ,
Cmtr1-215	ENSMUST00000235014.2	771	225aa	Protein coding	-		CDS 3' incomplete ,
Cmtr1-206	ENSMUST00000130423.4	655	<u>99aa</u>	Protein coding	2		CDS 3' incomplete , TSL:3 ,
Cmtr1-205	ENSMUST00000130052.9	615	<u>165aa</u>	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Cmtr1-211	ENSMUST00000234388.2	440	<u>147aa</u>	Protein coding	-		CDS 5' and 3' incomplete ,
Cmtr1-202	ENSMUST00000127695.2	375	<u>72aa</u>	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Cmtr1-204	ENSMUST00000129864.2	367	<u>68aa</u>	Protein coding	-		CDS 3' incomplete , TSL:2 ,
Cmtr1-208	ENSMUST00000172516.9	495	<u>37aa</u>	Nonsense mediated decay	-		CDS 5' incomplete , TSL:3 ,
Cmtr1-216	ENSMUST00000235028.2	792	No protein	Processed transcript	2		
Cmtr1-213	ENSMUST00000234716.2	3600	No protein	Retained intron	-		
Cmtr1-207	ENSMUST00000138939.2	3075	No protein	Retained intron	2		TSL:2,
Cmtr1-203	ENSMUST00000128410.8	959	No protein	Retained intron	-		TSL:5,
Cmtr1-212	ENSMUST00000234666.2	702	No protein	Retained intron	-		
Cmtr1-209	ENSMUST00000172610.2	557	No protein	Retained intron	-		TSL:2,
Cmtr1-210	ENSMUST00000234278.2	232	No protein	Retained intron	-		

The strategy is based on the design of *Cmtr1-201* transcript, the transcription is shown below:



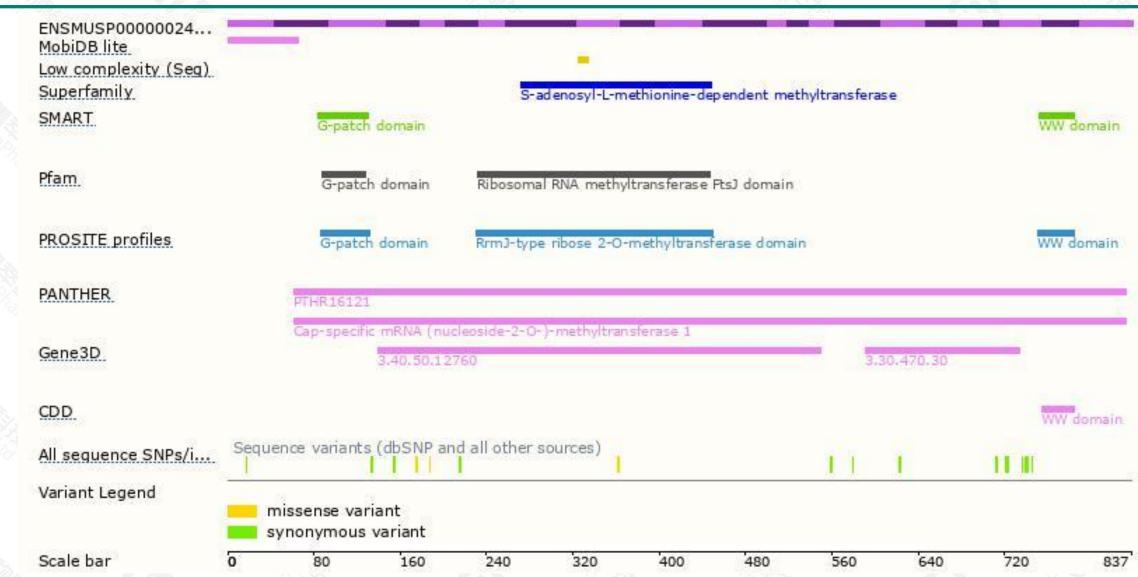
### Genomic location distribution





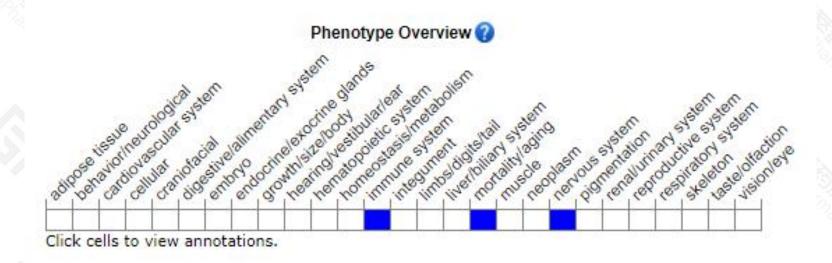
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, homozygous knockout is early embryonic lethal. Conditional homozygous KO in neurons leads to reduced neocortex size and neurite dysmorphology.



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

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