

Mars1 Cas9-CKO Strategy

Designer: Yanhua Shen

Reviewer: Xueting Zhang

Design Date: 2020-5-7

Project Overview



Project Name Mars1

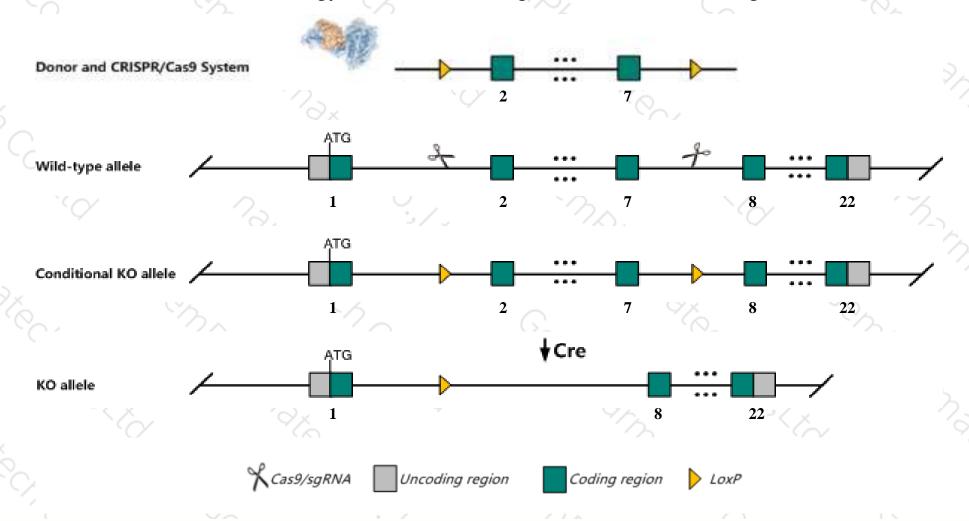
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Mars1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Mars1* gene has 6 transcripts. According to the structure of *Mars1* gene, exon2-exon7 of *Mars-206*(ENSMUST00000171564.7) transcript is recommended as the knockout region. The region contains 667bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Mars1* 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 effect of transcript 203 is unknown.
- ➤ The *Mars1* gene is located on the Chr10. 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)



Mars1 methionine-tRNA synthetase 1 [Mus musculus (house mouse)]

Gene ID: 216443, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Mars1 provided by MGI

Official Full Name methionine-tRNA synthetase 1 provided by MGI

Primary source MGI:MGI:1345633

See related Ensembl: ENSMUSG00000040354

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 Mars, Metrs, Mtrns

Summary The encoded protein belongs to the class I family of tRNA synthetases, a class of enzymes that charge tRNAs with their cognate amino

acids. The related human gene product is essential for the translation initiation of mRNAs. This gene has an overlapping 3' UTR tail-to-tail arrangement with an adjacent gene on the opposite strand that encodes an inhibitor of the CCAAT/enhancer-binding protein's DNA binding activity. This arrangement, conserved in human and mouse, may be involved in mRNA stability and possible functional and regulatory interaction of these adjacent overlapping genes. Alternative splicing results in multiple transcript variants.[provided by RefSeq, Jan 2010]

Expression Ubiquitous expression in adrenal adult (RPKM 23.2), ovary adult (RPKM 19.8) and 28 other tissuesSee more

Orthologs <u>human</u> <u>all</u>

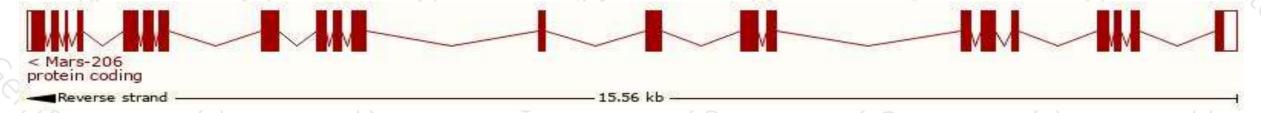
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

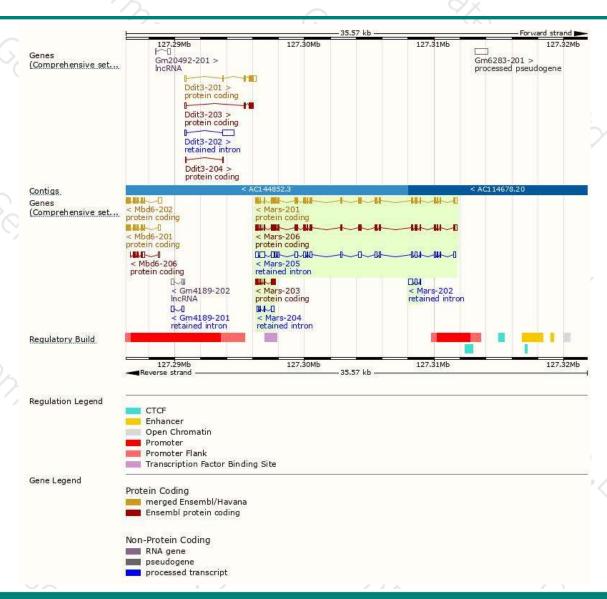
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mars-206	ENSMUST00000171564.7	2962	<u>910aa</u>	Protein coding	CCDS48716	E9QB02	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Mars-201	ENSMUST00000037290.11	2939	<u>902aa</u>	Protein coding	CCDS24237	Q68FL6	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 P3
Mars-203	ENSMUST00000134778.2	621	<u>184aa</u>	Protein coding	-	F6W0G8	CDS 5' incomplete TSL:3
Mars-205	ENSMUST00000145275.7	3065	No protein	Retained intron	-	-	TSL:2
Mars-202	ENSMUST00000128369.1	574	No protein	Retained intron	-	-	TSL:3
Mars-204	ENSMUST00000139683.1	467	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Mars-206* transcript, the transcription is shown below:



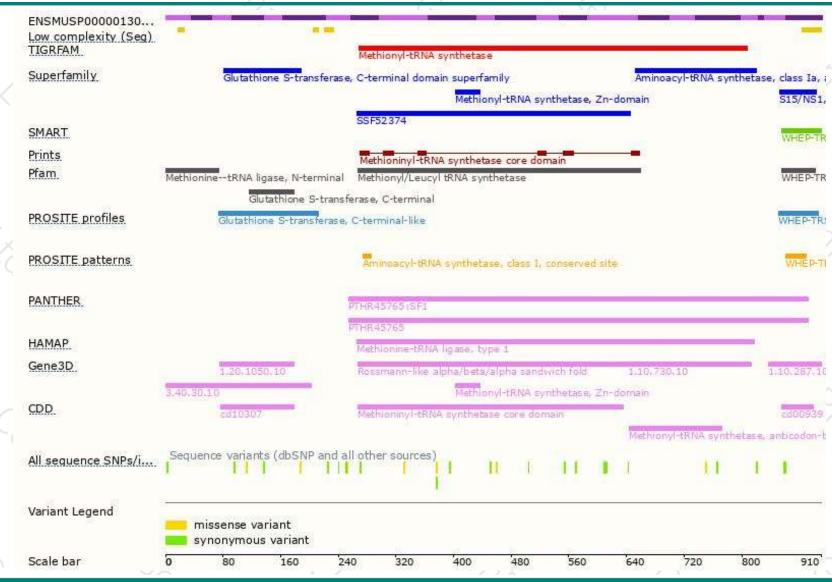
Genomic location distribution





Protein domain







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

Tel: 025-5864 1534





