

Eef2kmt Cas9-CKO Strategy

Designer:

Reviewer:

Design Date:

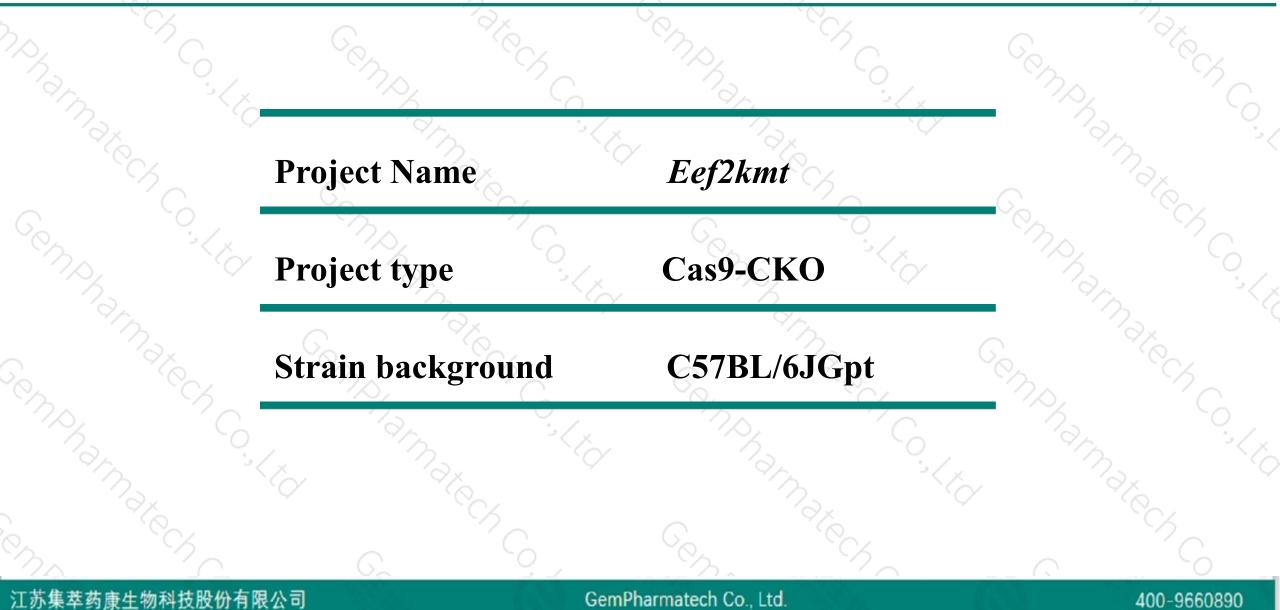
Yanhua Shen

Xueting Zhang

2020-4-29

Project Overview

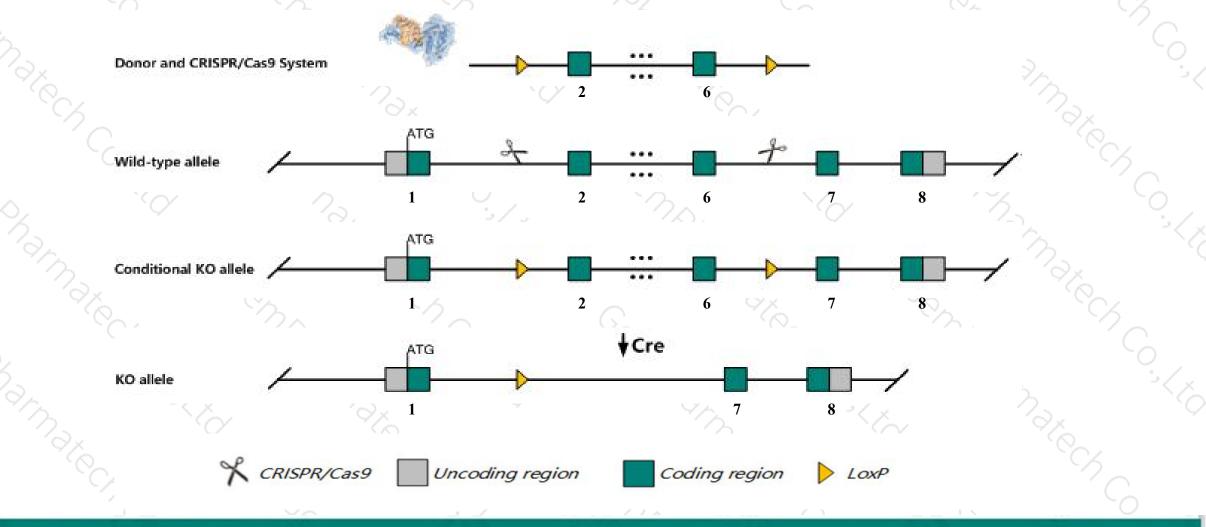




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Eef2kmt* gene. The schematic diagram is as follows:



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The *Eef2kmt* gene has 6 transcripts. According to the structure of *Eef2kmt* gene, exon2-exon6 of *Eef2kmt-201* (ENSMUST00000064635.11) transcript is recommended as the knockout region. The region contains 646bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Eef2kmt* 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.



- > The flox region is about 1.5 kb away from the 5th end of the Gm15983-201 gene, and its effect is unknown.
- The *Eef2kmt* gene is located on the Chr16. 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)



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Eef2kmt eukaryotic elongation factor 2 lysine methyltransferase [Mus musculus (house mouse)]

Gene ID: 70511, updated on 13-Mar-2020

Summary

Official Symbol	Eef2kmt provided by MGI											
Official Full Name	ukaryotic elongation factor 2 lysine methyltransferase provided by <u>MGI</u>											
Primary source	MGI:MGI:1917761											
See related	Ensembl:ENSMUSG0000022544											
Gene type	protein coding											
RefSeq status	PROVISIONAL											
Organism	Mus musculus											
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;											
	Muroidea; Muridae; Murinae; Mus; Mus											
Also known as	5730409G15Rik, Fam86, Fam86a, eEF2-KMT											
Expression	Ubiquitous expression in ovary adult (RPKM 25.4), adrenal adult (RPKM 22.4) and 28 other tissues See more											
Orthologs	human all											

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Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Eef2kmt-201	ENSMUST0000064635.11	2170	<u>335aa</u>	Protein coding	CCDS27935	Q3UZW7	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 F
Eef2kmt-204	ENSMUST00000139584.1	987	<u>322aa</u>	Protein coding	-	D3YYE2	CDS 3' incomplete TSL:1
Eef2kmt-206	ENSMUST00000230238.1	604	No protein	Processed transcript	-	2	
Eef2kmt-203	ENSMUST00000132225.7	1490	No protein	Retained intron	-	2	TSL:1
Eef2kmt-205	ENSMUST00000140718.2	695	No protein	Retained intron			TSL:2
Eef2kmt-202	ENSMUST00000129729.1	519	No protein	Retained intron			TSL:2

The strategy is based on the design of *Eef2kmt-201* transcript, the transcription is shown below:

< Eef2kmt-201 protein coding

Reverse strand

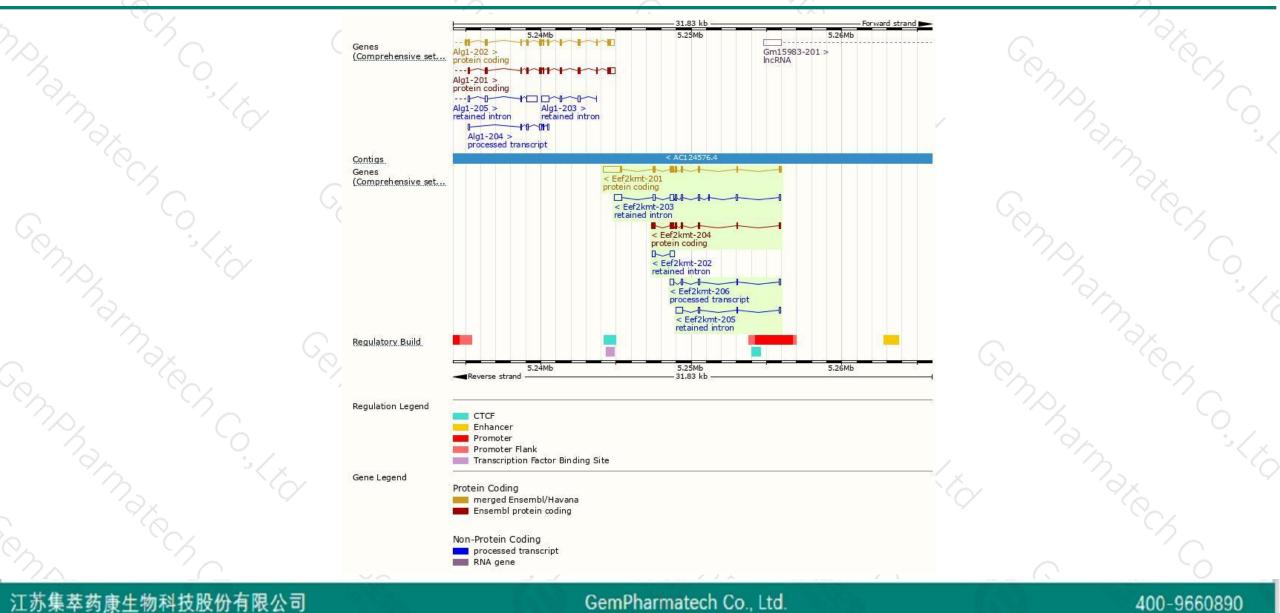
— 11.83 kb -

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Genomic location distribution





Protein domain



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ENSMUSP00000068 Superfamily		- · · · · ·						
<u>Pfam</u>	FAM86		Ly	sine methyltransfer	ase		d):	
PANTHER	PTHR14614							
Gene3D	PTHR14614;SF							6
CDD		3.40.	50.150	cd02440			-	5
All sequence SNPs/i	Sequence va	riants (dbSNP and a	ll other sources)		0 1	5	0.9	0.0
Variant Legend		e variant nous variant						
Scale bar	0	40 80	120	160	200	240	280	335
								~~~
10	6	~		<u>`</u> ``				0

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



