

Cenps Cas9-CKO Strategy

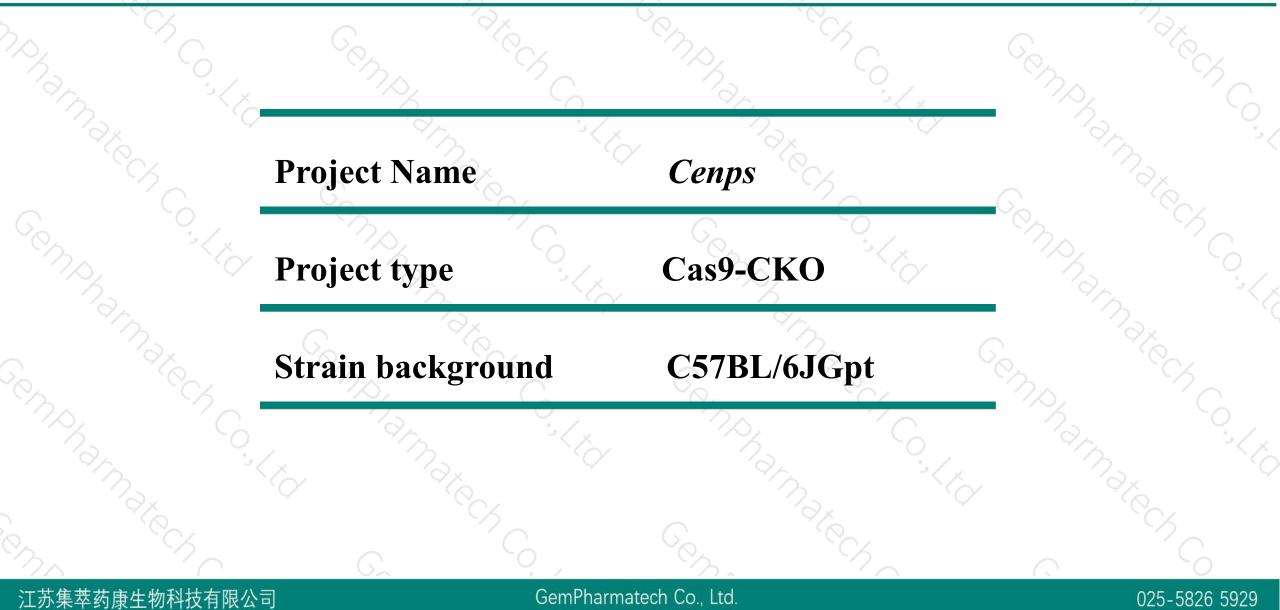
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Reviewer: Wenjing Li

Design Date: 2020-8-7

Project Overview



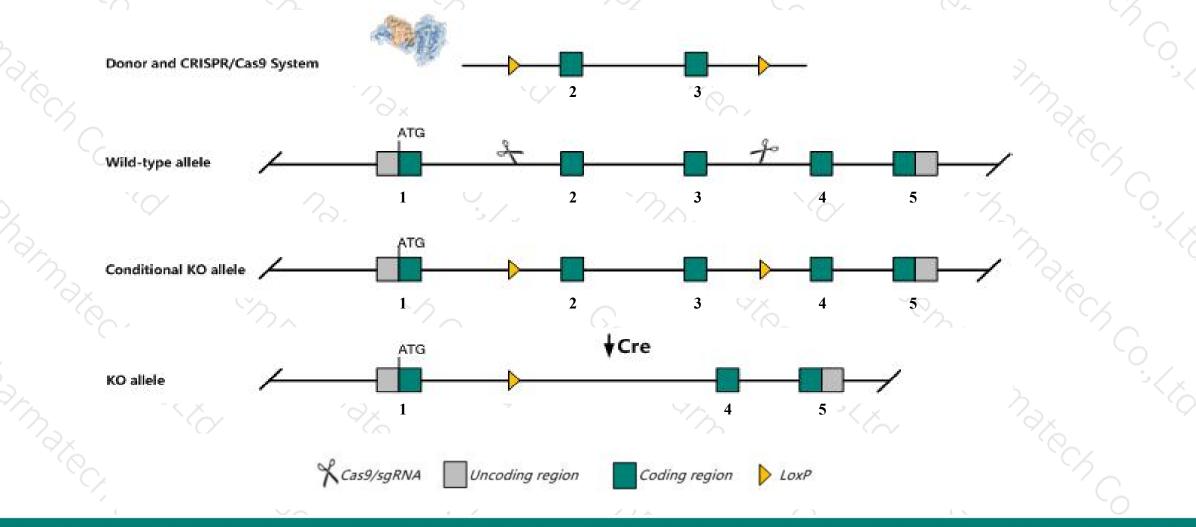


Conditional Knockout strategy



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



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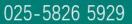
The Cenps gene has 7 transcripts. According to the structure of Cenps gene, exon2-exon3 of Cenps-201(ENSMUST00000030813.9) transcript is recommended as the knockout region. The region contains 158bp coding sequence. Knock out the region will result in disruption of protein function.

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



- > The *Cenps* gene is located on the Chr4. 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)



\$?

Cenps centromere protein S [Mus musculus (house mouse)]

Gene ID: 69928, updated on 13-Mar-2020

Summary

Official Symbol	Cenps provided by MGI
Official Full Name	centromere protein S provided by <u>MGI</u>
Primary source	MGI:MGI:1917178
See related	Ensembl:ENSMUSG0000073705
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	2610040C18Rik, 2810407L01Rik, Apitd1, CENP-S
Expression	Broad expression in CNS E11.5 (RPKM 11.2), liver E14 (RPKM 8.1) and 16 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cenps-201	ENSMUST0000030813.9	1110	<u>142aa</u>	Protein coding	CCDS18954	Q9D084	TSL:1 GENCODE basic APPRIS P1
Cenps-207	ENSMUST00000177408.1	689	<u>74aa</u>	Protein coding	£	H3BKU9	TSL:2 GENCODE basic
Cenps-202	ENSMUST00000105695.1	544	<u>76aa</u>	Protein coding	2	Q9D084	TSL:1 GENCODE basic
Cenps-206	ENSMUST00000176124.7	518	<u>77aa</u>	Protein coding		A0A140T8U2	CDS 3' incomplete TSL:3
Cenps-205	ENSMUST00000150150.2	349	<u>58aa</u>	Protein coding	2	A6PWQ1	CDS 3' incomplete TSL:3
Cenps-203	ENSMUST00000105696.7	412	<u>102aa</u>	Nonsense mediated decay	5	A6PWQ2	TSL:5
Cenps-204	ENSMUST00000132672.1	686	No protein	Retained intron	E.	-	TSL:2

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

< Cenps-201 protein coding

Reverse strand

— 9.43 kb -

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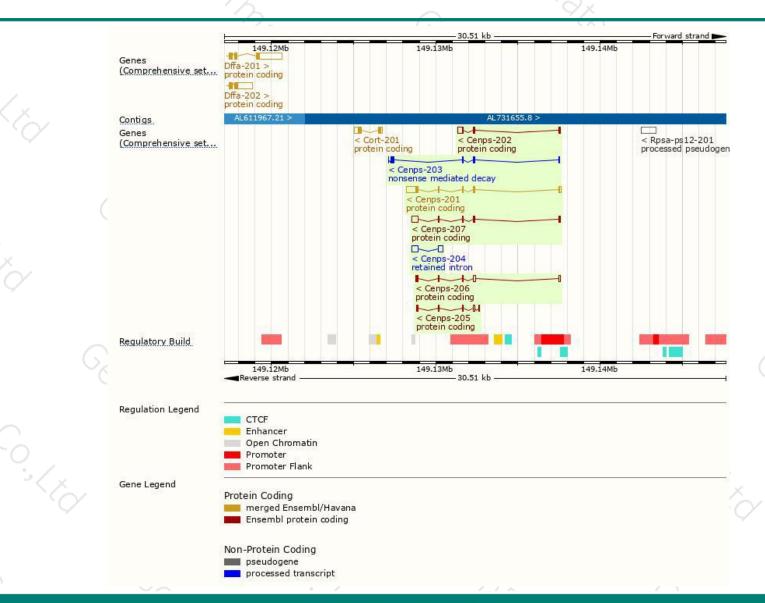
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Genomic location distribution



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Protein domain

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47	ENSMUSP0000030 MobiDB lite				10		-		
	Low complexity (Seg) Superfamily	Histone-fold							
	Pfam.	CENP-S/Mhf1							
	PANTHER	Centromere protein S PTHR22980						S	
	Gene3D	Historie-fold							
	All sequence SNPs/i	Sequence variants (dbSN	P and all other sou	irces)	1 =	1.1			
	Variant Legend	splice donor variant missense variant synonymous variant stop retained varian							
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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



