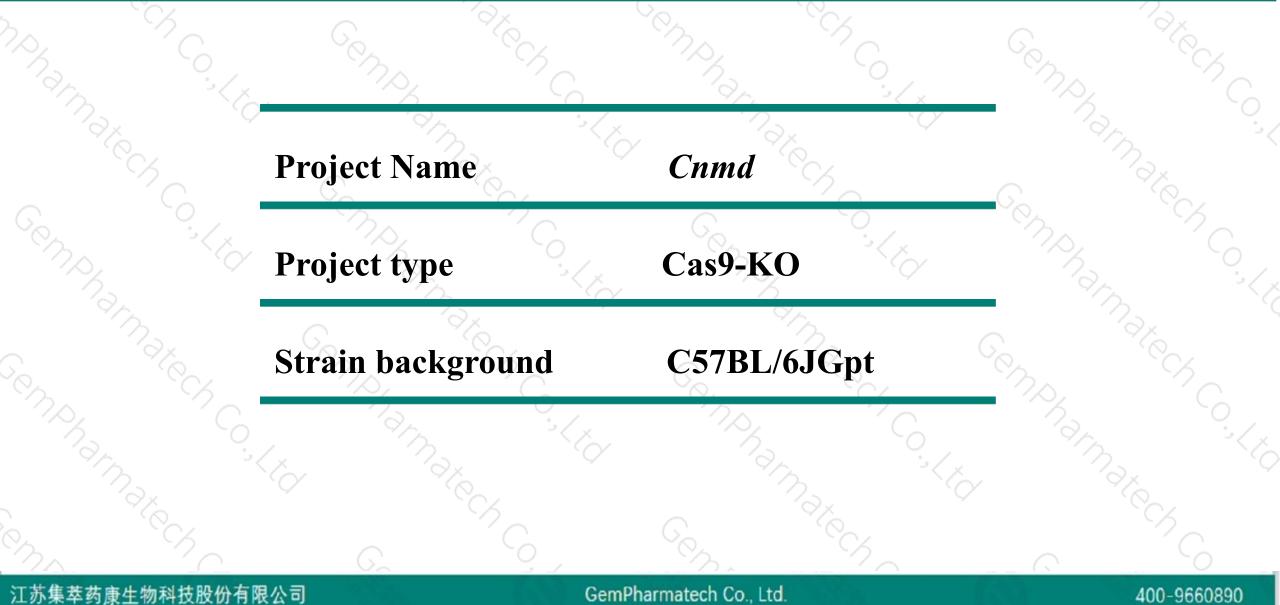


Cnmd Cas9-KO Strategy

Designer:Xueting Zhang Reviewer;Yanhua Shen Date:2020-1-13

Project Overview

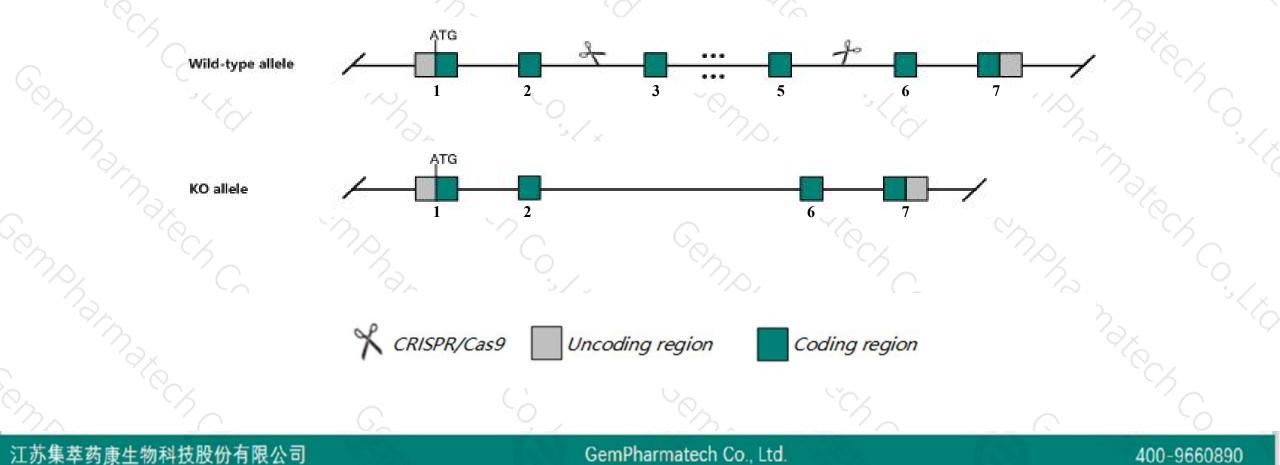




Knockout strategy



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





- The Cnmd gene has 5 transcripts. According to the structure of Cnmd gene, exon3-exon5 of Cnmd-201 (ENSMUST0000022603.7) transcript is recommended as the knockout region. The region contains 406bp coding sequence. Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify Cnmd gene. The brief process is as follows: CRISPR/Cas9 system

- According to the existing MGI data, Homozygous mutant mice are viable and show no gross morphologic defects. While cartilage development and embryonic endochondral bone formation were found to be normal in mutant mice, one line of targeted mutants showed increased bone density and impairedbone resorption.
 Transcript *Cnmd*-202 may not be affected.
- The Cnmd gene is located on the Chr14. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

Gene information (NCBI)

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Cnmd chondromodulin [Mus musculus (house mouse)] Gene ID: 16840, updated on 12-Aug-2019 Summary ☆ ? Official Symbol Cnmd provided by MGI Official Full Name chondromodulin provided by MGI Primary source MGI:MGI:1341171 See related Ensembl:ENSMUSG0000022025 Gene type protein coding RefSeg 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 Chmd; ChM-I; Lect1; Bricd3 Expression Biased expression in limb E14.5 (RPKM 98.5), ovary adult (RPKM 23.0) and 1 other tissue See more Orthologs human all Genomic context ☆ ? Location: 14; 14 D3 See Cnmd in Genome Data Viewer Exon count: 7

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	14	NC_000080.6 (7963768279662194, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	14	NC_000080.5 (8003749780061977, complement)

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Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Cnmd-201	201 ENSMUST00000022603.7		<u>334aa</u>	Protein coding	CCDS27302	Q9Z1F6	TSL:1 GENCODE basic APPRIS P3	
Cnmd-203	ENSMUST00000165835.7	1362	<u>338aa</u>	Protein coding	CCDS79348	<u>G5E8Z6</u>	TSL:1 GENCODE basic APPRIS ALT	
Cnmd-202	ENSMUST00000165204.1	659	No protein	Retained intron	(a)	-	TSL:2	
Cnmd-204	ENSMUST00000167524.1	372	No protein	Retained intron	120	-	TSL:3	
Cnmd-205	ENSMUST00000172331.1	459	No protein	IncRNA	850		TSL:3	

The strategy is based on the design of Cnmd-201 transcript, The transcription is shown below

< Cnmd-201 protein coding

Reverse strand

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24.48 kb

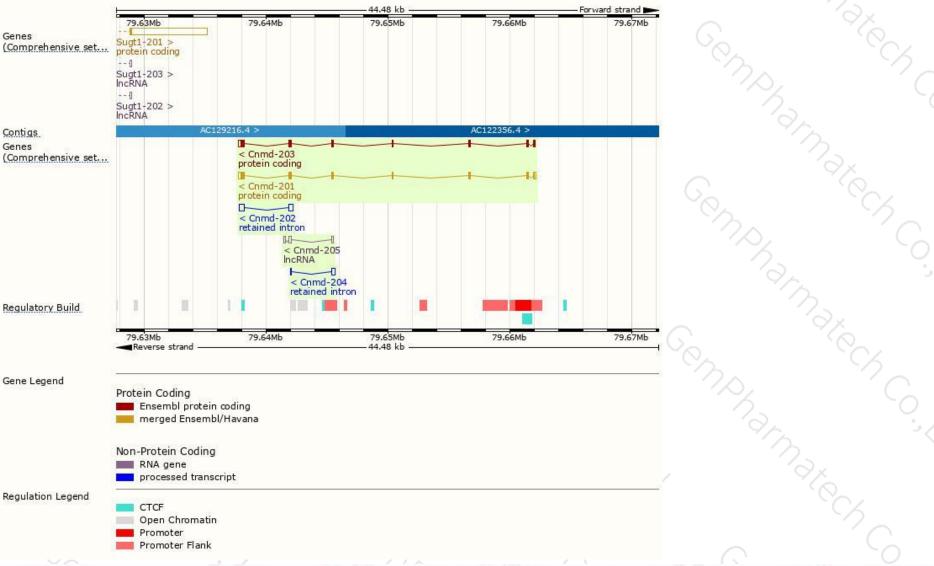
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Genomic location distribution



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Protein domain



ENSMUSP00000022 Transmembrane heli MobiDB lite	<u>-</u>									
SMART Pfam				BRICHOS dom						
				BRICHOS do	main					
PROSITE profiles				BRICHOS dom	ain					
PANTHER	PTHR.14064:	SF6								- C
Gene3D	PTHR14064		2	20.25.460						
All sequence SNPs/i	Sequence v	ariants (dbS	NP and all o	ther sources)	in in			n n		
Variant Legend		nse variant ymous varia	nt							0
Scale bar	0	40	80	120	160	200	240	280	334	

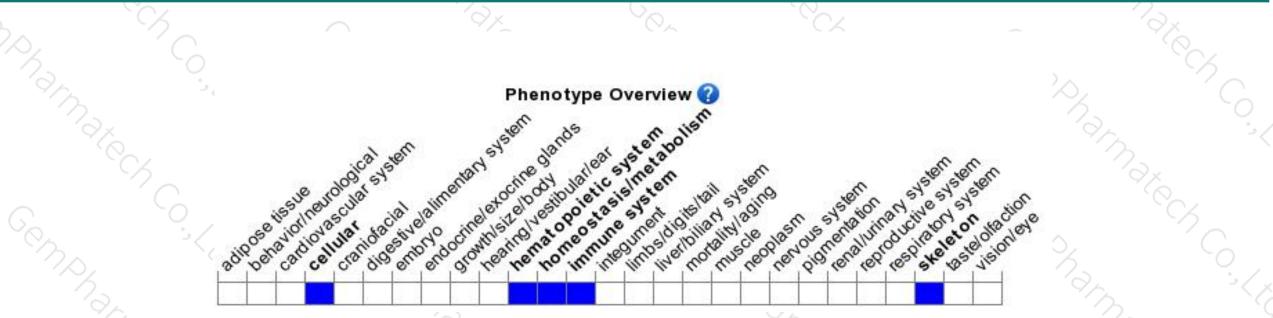
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Mouse phenotype description(MGI)





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 mutant mice are viable and show no gross morphologic defects. While cartilage development and embryonic endochondral bone formation were found to be normal in mutant mice, one line of targe mutants showed increased bone density and impaired bone resorption.

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



