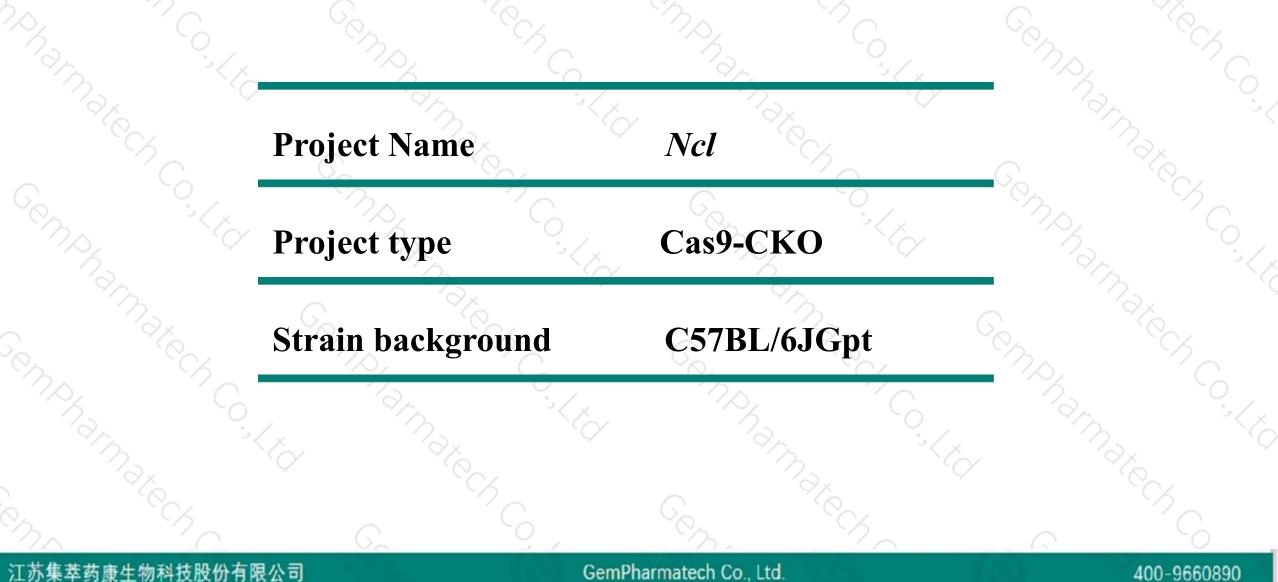


Ncl Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-10-16

Project Overview





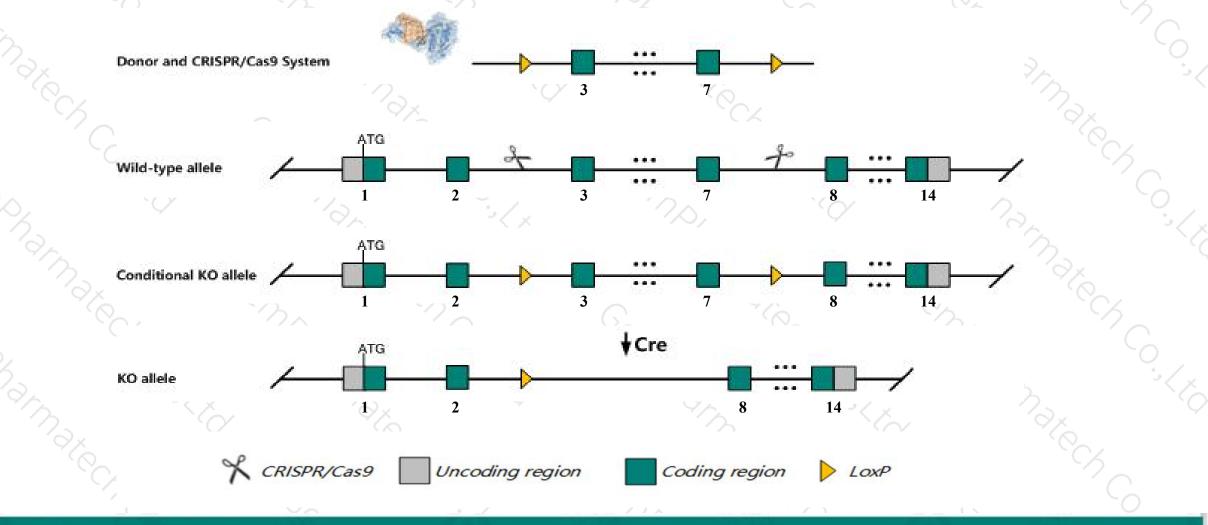
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Conditional Knockout strategy



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



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 The Ncl gene has 6 transcripts. According to the structure of Ncl gene, exon3-exon7 of Ncl-201 (ENSMUST00000027438.7) transcript is recommended as the knockout region. The region contains 1036bp coding sequence. Knock out the region will result in disruption of protein function.

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



- ➤ The floxed region is near to the N-terminal of *Snora* and *Mir3535* and *Gm24148* gene, this strategy may influence the regulatory function of the N-terminal of these genes.
- Transcript *Ncl*-204&205&206 may not be affected. And the effect on transcript *Ncl*-203 is unknown.
- > The *Snord82* gene will be deleted together in this strategy.
- The Ncl gene is located on the Chr1. 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)



Icl nucleolin [<i>Mu</i>	s <i>musculus</i> (house mouse)]		`~	
ene ID: 17975, updated on	10-Oct-2019			
 Summary 				× ?
Official Symbol	Ncl provided by MGI			
Official Full Name	nucleolin provided by MGI			
Primary source	MGI:MGI:97286			
See related	Ensembl:ENSMUSG00000026234			
Gene type	protein coding			
RefSeq status	VALIDATED			
Organism	Mus musculus			
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Verte Myomorpha; Muroidea; Muridae; Murinae; Mus		Mammalia; Eutheria; Euarchontoglir	es; Glires; Rodentia;
Also known as	C23; Nucl; D0Nds28; D1Nds28; B530004O11R	<u> </u>		
Expression	Biased expression in CNS E11.5 (RPKM 52.4),	, liver E14 (RPKM 41.	.1) and 11 other tissues See more	
Orthologs	human all			
 Genomic context 				≈ ?
Location: 1 C5; 1 43.94	4 cM			See Ncl in Genome Data Viewer
Exon count: 15				

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (8634471986359455, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC_000067.5 (8824129488256030, complement)

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ncl-201	ENSMUST00000027438.7	8202	<u>707aa</u>	Protein coding	CCDS35646	<u>P09405</u>	TSL:1 GENCODE basic APPRIS P1
NcI-203	ENSMUST00000185785.1	661	<u>76aa</u>	Protein coding		A0A087WRM5	CDS 3' incomplete TSL:2
Ncl-202	ENSMUST00000185676.6	3072	No protein	Retained intron	20	2	TSL:1
Ncl-204	ENSMUST00000186050.1	1054	No protein	Retained intron	2	-	TSL:2
NcI-205	ENSMUST00000188682.1	601	No protein	Retained intron			TSL:2
Ncl-206	ENSMUST00000189504.1	502	No protein	Retained intron		<i>1</i> 2	TSL:2

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

< Nd-201 protein coding

Reverse strand

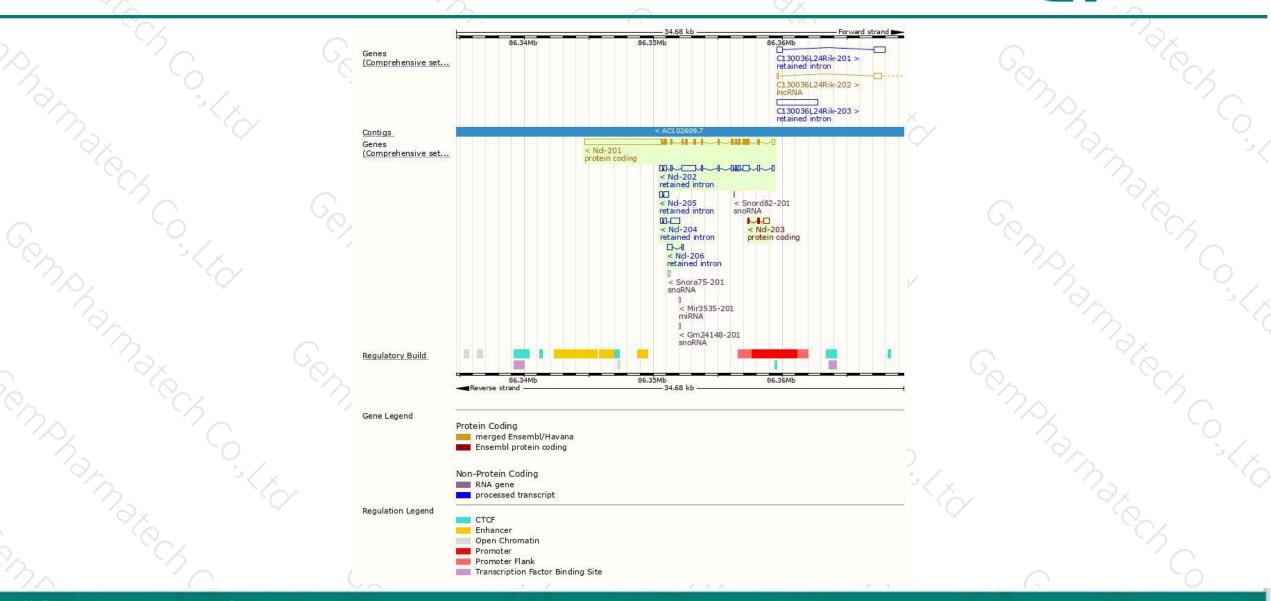
– 14.68 kb –

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Genomic location distribution



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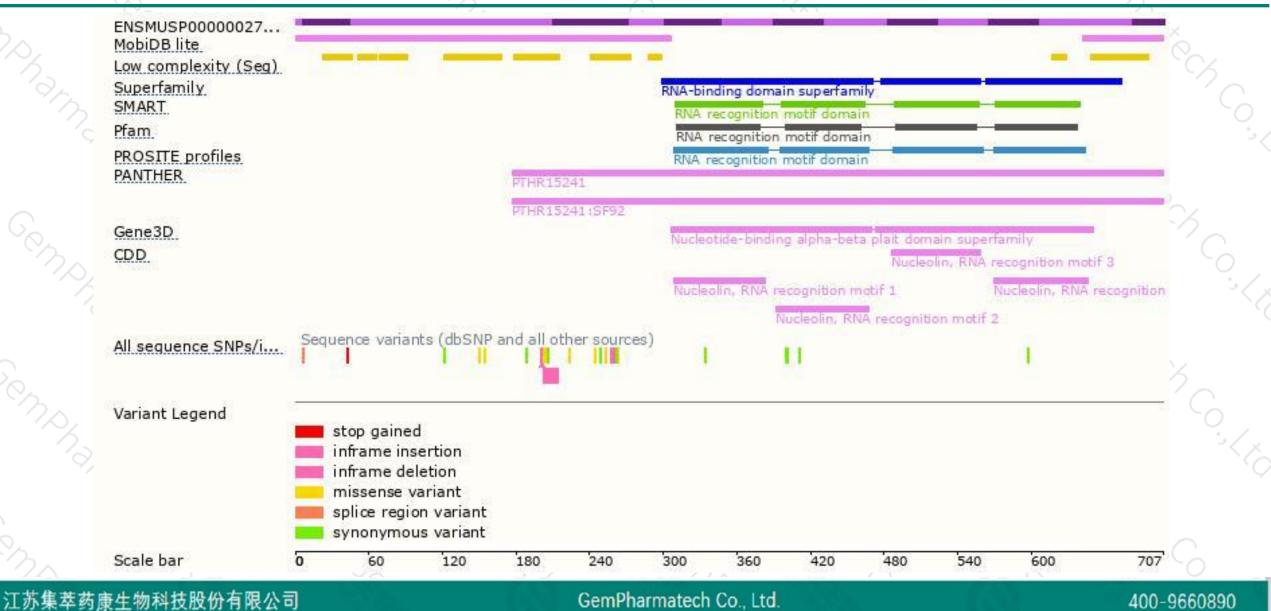
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Protein domain







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



