

# Nup214 Cas9-CKO Strategy

**Designer: Xueting Zhang** 

Reviewer: Yanhua Shen

**Design Date: 2021-9-26** 

# **Project Overview**

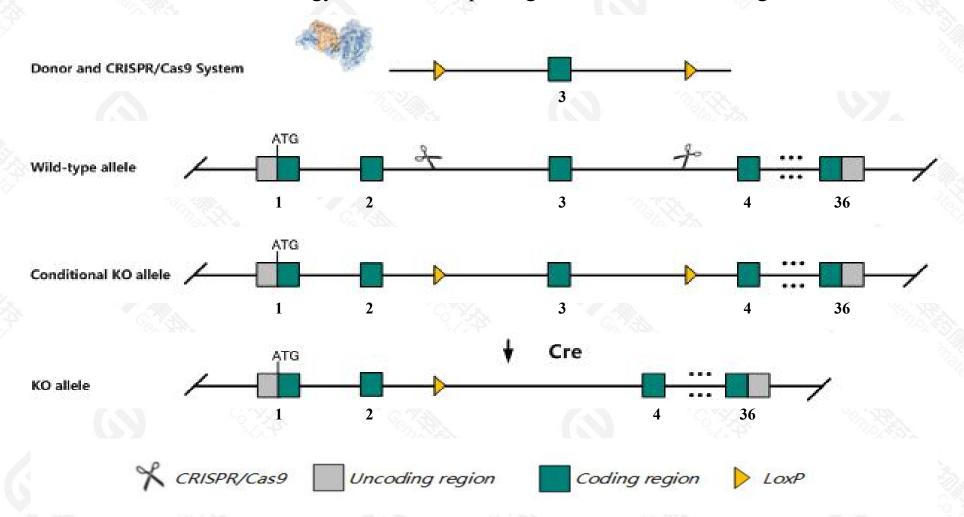


Project Name	Nup214			
Project type	Cas9-CKO			
Strain background	C57BL/6JGpt			

# Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Nup214 gene. The schematic diagram is as follows:



### **Technical routes**



- > The Nup214 gene has 11 transcripts. According to the structure of Nup214 gene, exon3 of Nup214-201(ENSMUST00000065398.13) transcript is recommended as the knockout region. The region contains 152bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Nup214* 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**



- ➤ According to the existing MGI data, embryos homozygous for a null mutation die between 4.0 and 4.5 dpc, following depletion of maternally-derived gene product. In vitro, cultured 3.5-dpc mutant embryos arrest in the G2 phase, and show blastocoel contraction with defects in NLS-mediated protein import and polyadenylated RNA export.
- ➤ Transcript *Nup214*-204&208&210 may not be affected.
- ➤ The effect on transcript *Nup214*-202&203&205&206&209&211 is unknown.
- > The Nup214 gene is located on the Chr2. 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)



#### Nup214 nucleoporin 214 [Mus musculus (house mouse)]

Gene ID: 227720, updated on 17-Dec-2020

#### Summary

☆ ?

Official Symbol Nup214 provided by MGI

Official Full Name nucleoporin 214 provided by MGI

Primary source MGI:MGI:1095411

See related Ensembl:ENSMUSG00000001855

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 BC039282, C, CAN, D2H9S46, D2H9S46E

Expression Ubiquitous expression in testis adult (RPKM 23.6), thymus adult (RPKM 16.1) and 28 other tissuesSee more

Orthologs <u>human all</u>

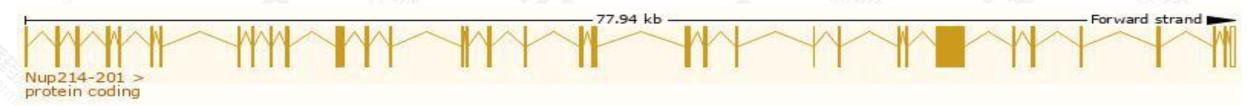
# Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

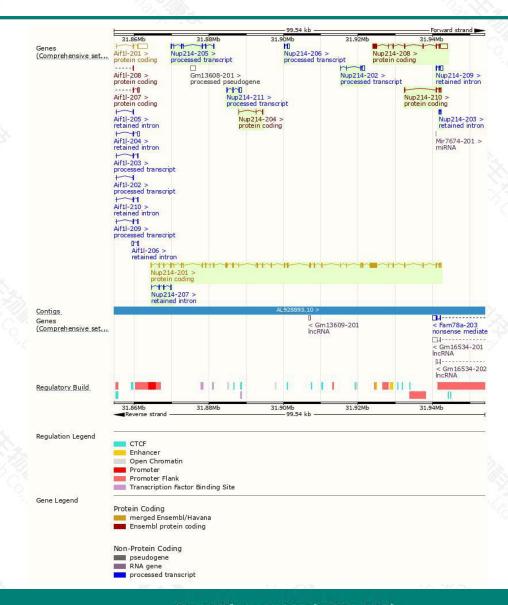
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nup214-201	ENSMUST00000065398.13	6605	2085aa	Protein coding	CCDS38098		TSL:1 , GENCODE basic , APPRIS P1 ,
Nup214-208	ENSMUST00000138012.2	3569	<u>581aa</u>	Protein coding	-		CDS 5' incomplete , TSL:1 ,
Nup214-210	ENSMUST00000152791.4	466	<u>147aa</u>	Protein coding	9		CDS 5' incomplete , TSL:3 ,
Nup214-204	ENSMUST00000126301.3	295	<u>98aa</u>	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Nup214-211	ENSMUST00000153096.2	925	No protein	Processed transcript	2		TSL:3,
Nup214-202	ENSMUST00000123062.2	667	No protein	Processed transcript			TSL:5 ,
Nup214-206	ENSMUST00000130185.2	629	No protein	Processed transcript	-		TSL:3,
Nup214-205	ENSMUST00000129496.2	628	No protein	Processed transcript	-		TSL:2,
Nup214-209	ENSMUST00000146138.3	915	No protein	Retained intron	-		TSL:2,
Nup214-207	ENSMUST00000130975.2	738	No protein	Retained intron	-		TSL:2,
Nup214-203	ENSMUST00000124455.2	354	No protein	Retained intron	0		TSL:2,

The strategy is based on the design of *Nup214-201* transcript, the transcription is shown below:



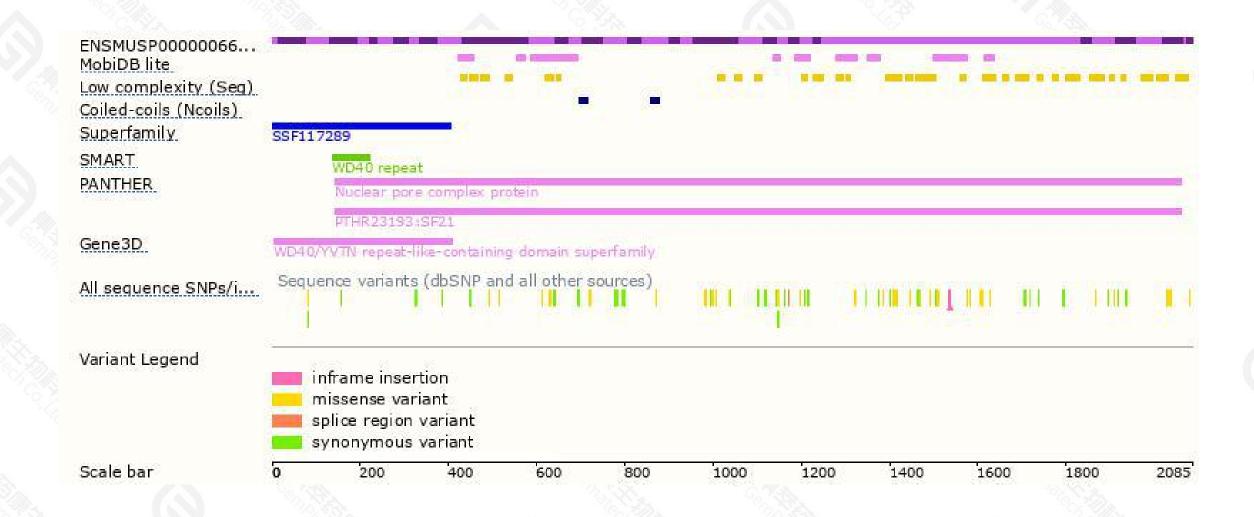
### Genomic location distribution





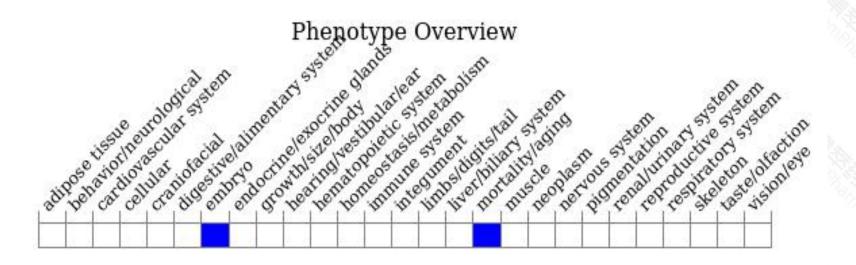
### Protein domain





# 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, embryos homozygous for a null mutation die between 4.0 and 4.5 dpc, following depletion of maternally-derived gene product. In vitro, cultured 3.5-dpc mutant embryos arrest in the G2 phase, and show blastocoel contraction with defects in NLS-mediated protein import and polyadenylated RNA export.



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

Tel: 400-9660890





