Nle1 Cas9-CKO Strategy

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Project Overview

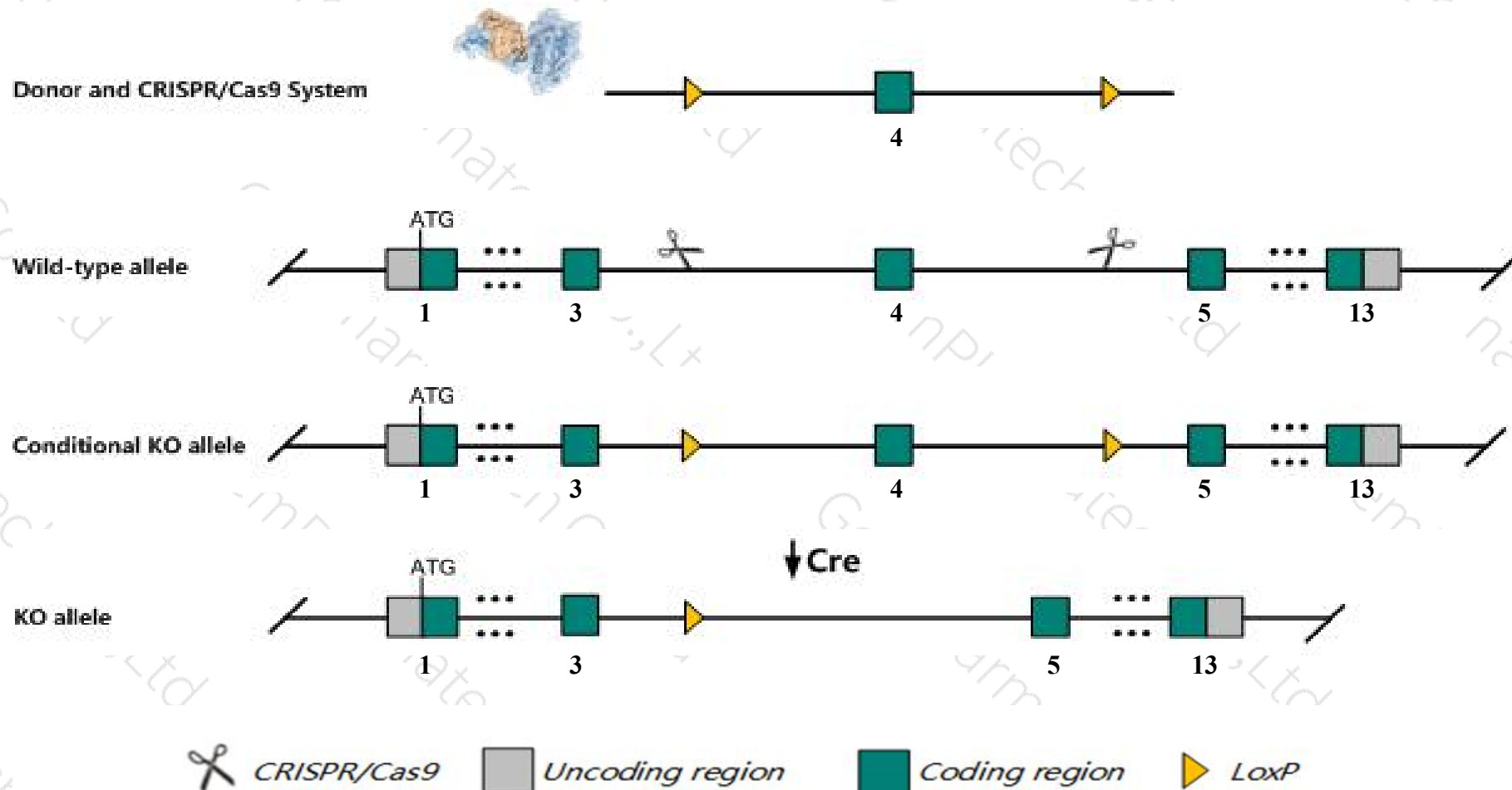|	
Project Name	*Nle1*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nle1* gene. The schematic diagram is as follows:

Technical routes

➤ The *Nle1* gene has 7 transcripts. According to the structure of *Nle1* gene, exon4 of *Nle1-201* (ENSMUST00000103213.9) transcript is recommended as the knockout region.The region contains Ens 80bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Nle1* 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,Homozygous null mice display embryonic lethality before somite

  formation, most blastocysts fail to hatch out of the zona pellucida, and apoptosis is increased in the inner cell mass.

➢ The *Nle1* gene is located on the Chr11. 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）

Nle1 notchless homolog 1 [Mus musculus (house mouse)]
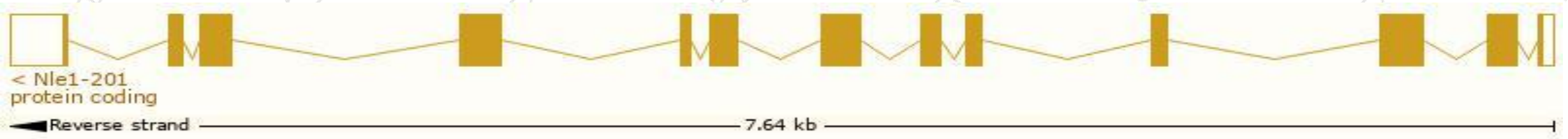
Gene ID: 217011, updated on 9-Apr-2019

Summary

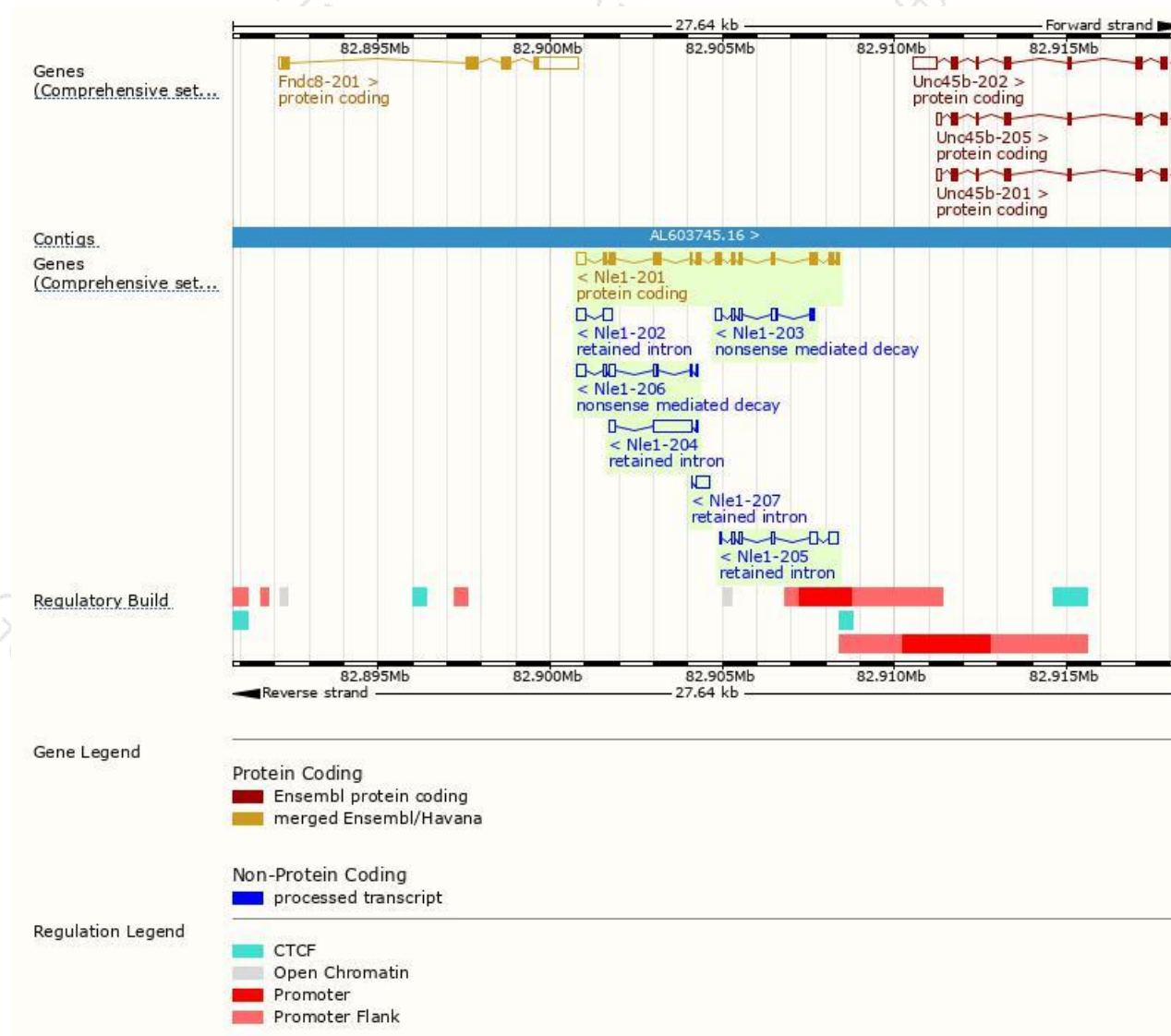|	
Official Symbol	Nle1 provided by MGI
Official Full Name	notchless homolog 1 provided byMGI
Primary source	MGI:MGI:2429770
See related	Ensembl:ENSMUSG00000020692
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AL022765, BC018399, Nle, I11Jus1, I11Jus4
Expression	Ubiquitous expression in ovary adult (RPKM 23.0), thymus adult (RPKM 15.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nle1-201	ENSMUST00000103213.9	1783	485aa	Protein coding	CCDS25152	Q8VEJ4	TSL:1 GENCODE basic APPRIS P1
Nle1-206	ENSMUST00000167196.1	738	42aa	Nonsense mediated decay	-	F7BBL8	CDS 5' incomplete TSL:3
Nle1-203	ENSMUST00000126202.7	696	57aa	Nonsense mediated decay	-	F6QAD9	CDS 5' incomplete TSL:5
Nle1-204	ENSMUST00000140318.1	1301	No protein	Retained intron	-	-	TSL:5
Nle1-205	ENSMUST00000147915.1	787	No protein	Retained intron	-	-	TSL:3
Nle1-202	ENSMUST00000124109.1	539	No protein	Retained intron	-	-	TSL:2
Nle1-207	ENSMUST00000170815.1	428	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Nle1-201* transcript,The transcription is shown below

< Nle1-201
protein coding
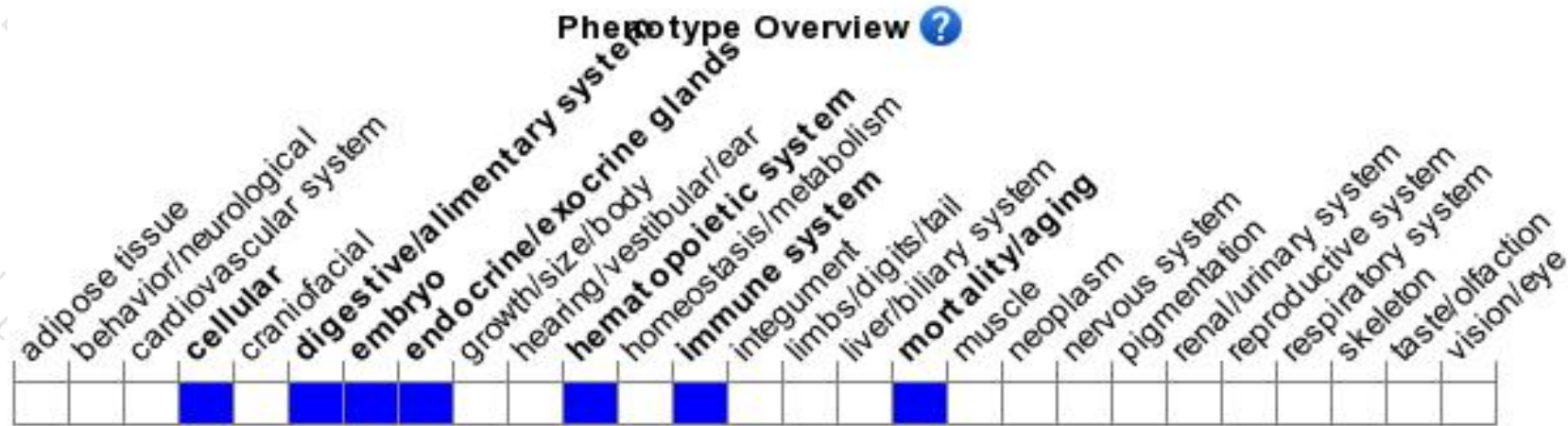◄ Reverse strand
7.64 kb

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,Homozygous null mice display embryonic lethality before somite formation, most blastocysts fail to hatch out of the zona pellucida, and apoptosis is increased in the inner cell mass.

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