Ncdn Cas9-CKO Strategy

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Design Date: 2021-5-26

Project Overview

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

Conditional Knockout strategy

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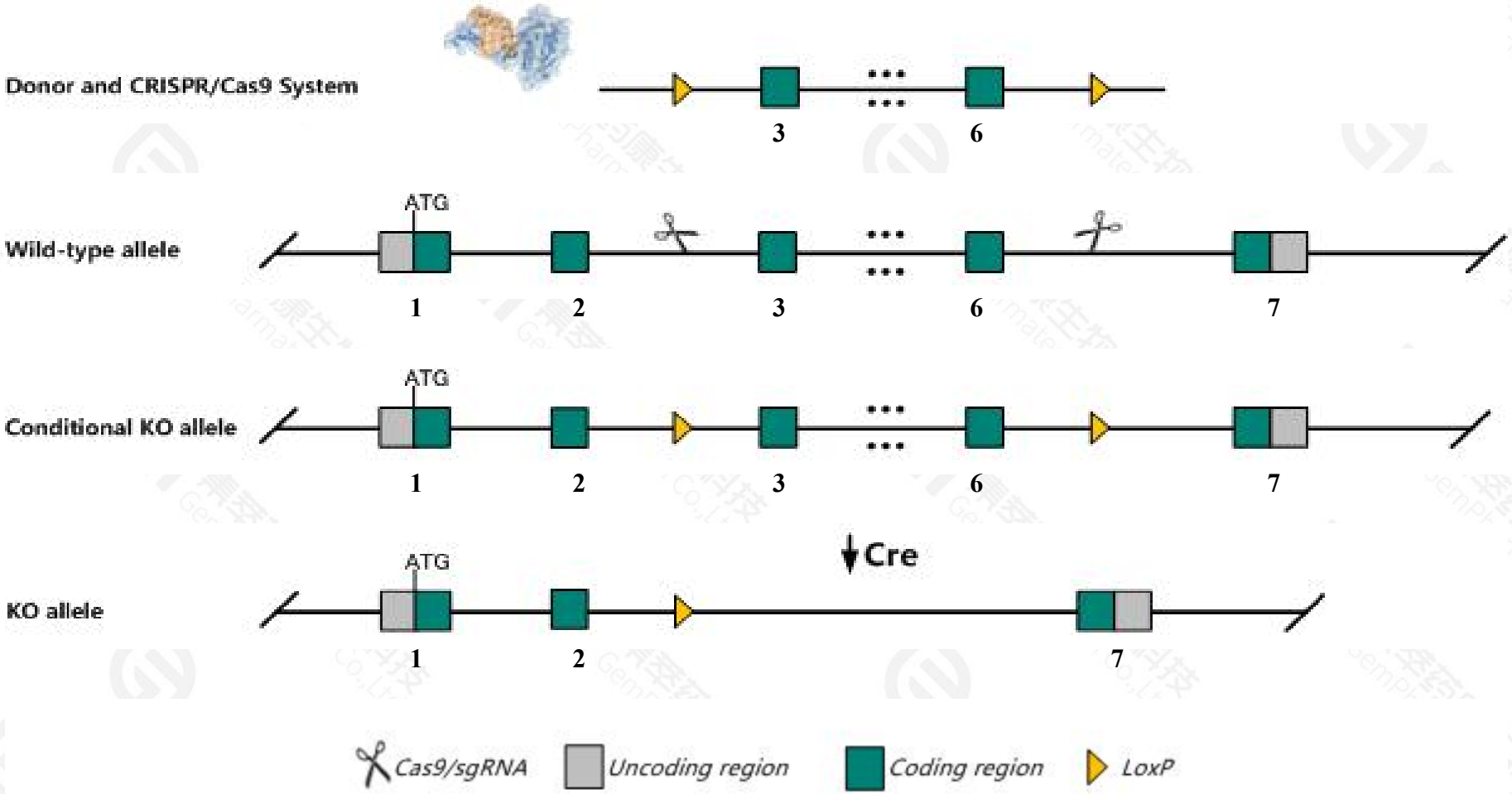
This model will use CRISPR/Cas9 technology to edit the *Ncdn* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ncdn* gene has 3 transcripts. According to the structure of *Ncdn* gene, exon3-exon6 of *Ncdn-201*(ENSMUST00000030637.14) transcript is recommended as the knockout region.The region contains 1579bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ncdn* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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.

Notice

➢ According to the existing MGI data,targeted inactivation of this gene results in early embryonic lethality in the homozygous state and impaired chondrocyte proliferation and differentiation in the heterozygous state. Gene trap mutation resulted in lacrimal gland hypertrophy.

➢Deleted regions may affect splicing at the 5 terminus of the AU040320 gene.

➢ The *Ncdn* 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）

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Ncdn neurochondrin [Mus musculus (house mouse)]

Gene ID: 26562, updated on 3-Jan-2021

Summary

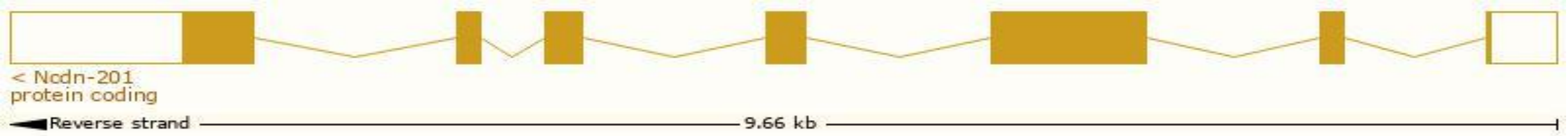
Official Symbol	Ncdn provided by MGI
Official Full Name	neurochondrin provided byMGI
Primary source	MGI:MGI:1347351
See related	Ensembl:ENSMUSG00000028833
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AU042419, MMS10-AE, Ms10ae, mKIAA0607, n, norbin
Expression	Broad expression in frontal lobe adult (RPKM 191.8), cortex adult (RPKM 179.9) and 17 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

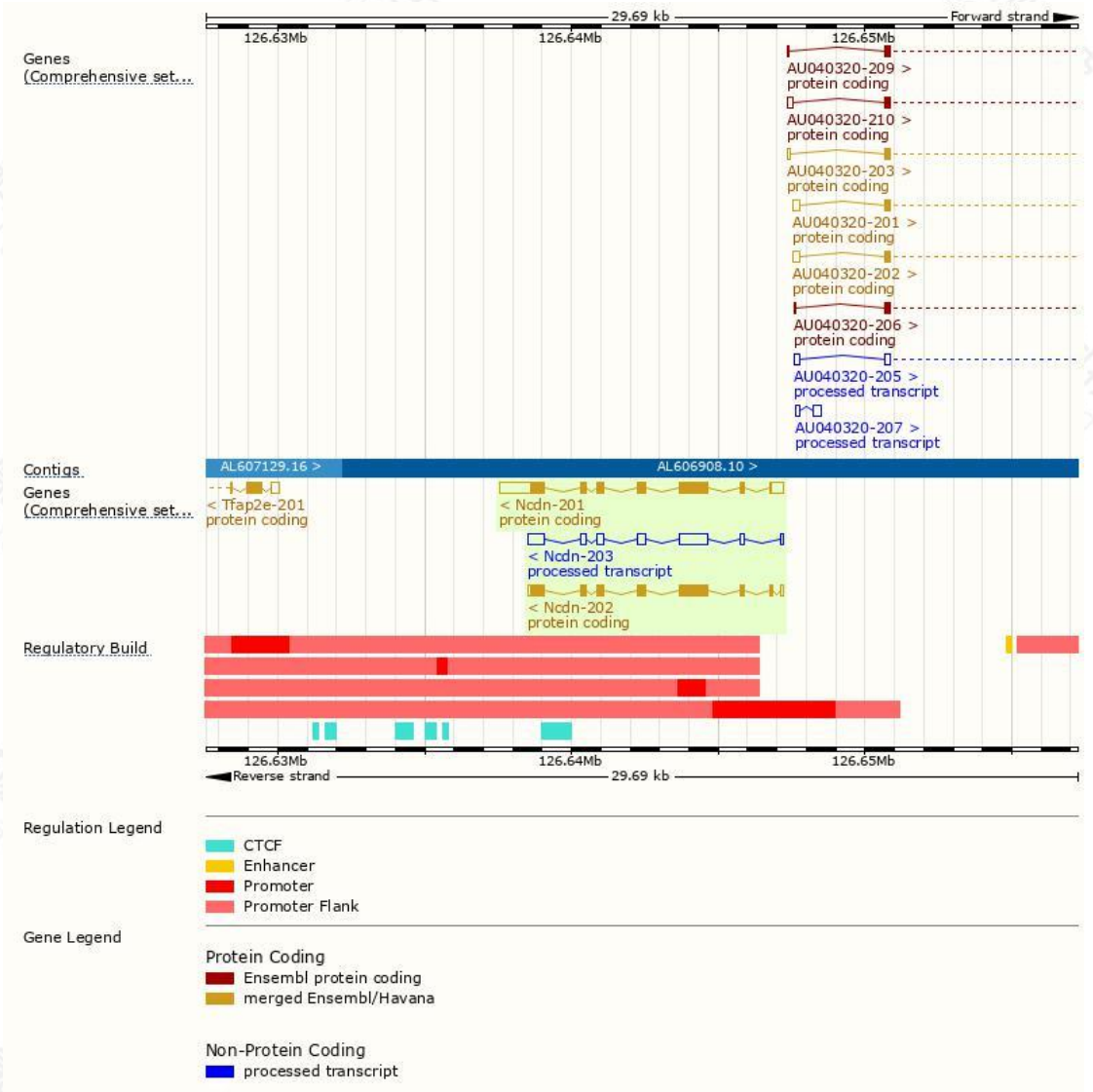
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ncdn-201	ENSMUST00000030637.14	3681	729aa	Protein coding	CCDS18659		TSL:1 , GENCODE basic , APPRIS P1 ,
Ncdn-202	ENSMUST00000106116.2	2434	729aa	Protein coding	CCDS18659		TSL:1 , GENCODE basic , APPRIS P1 ,
Ncdn-203	ENSMUST00000127079.2	2375	No protein	Processed transcript	-		TSL:5 ,

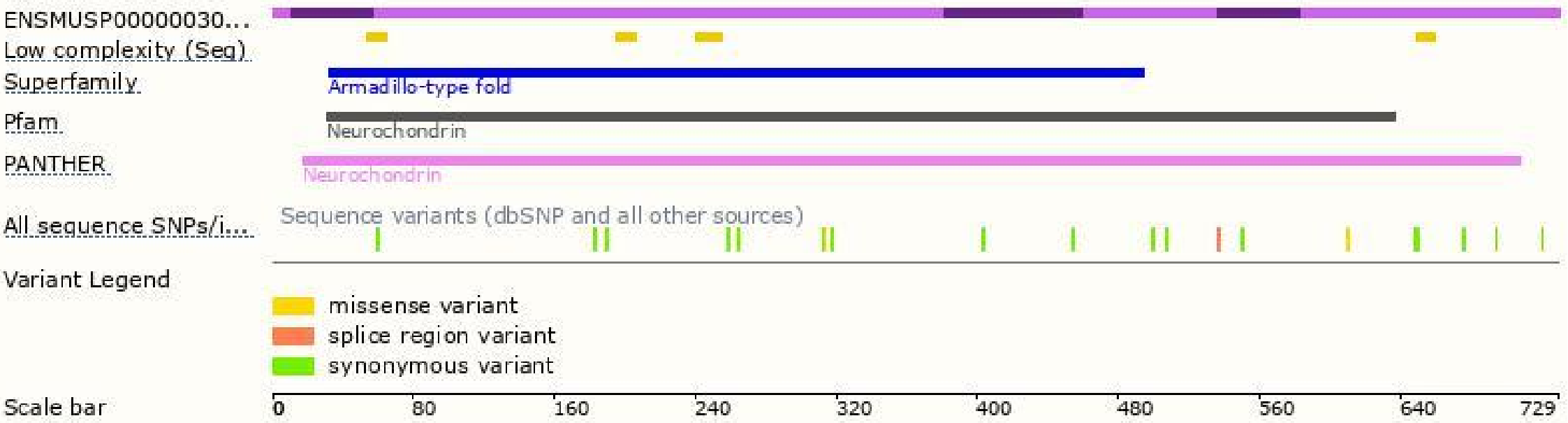
The strategy is based on the design of *Ncdn-201* transcript,the transcription is shown below:



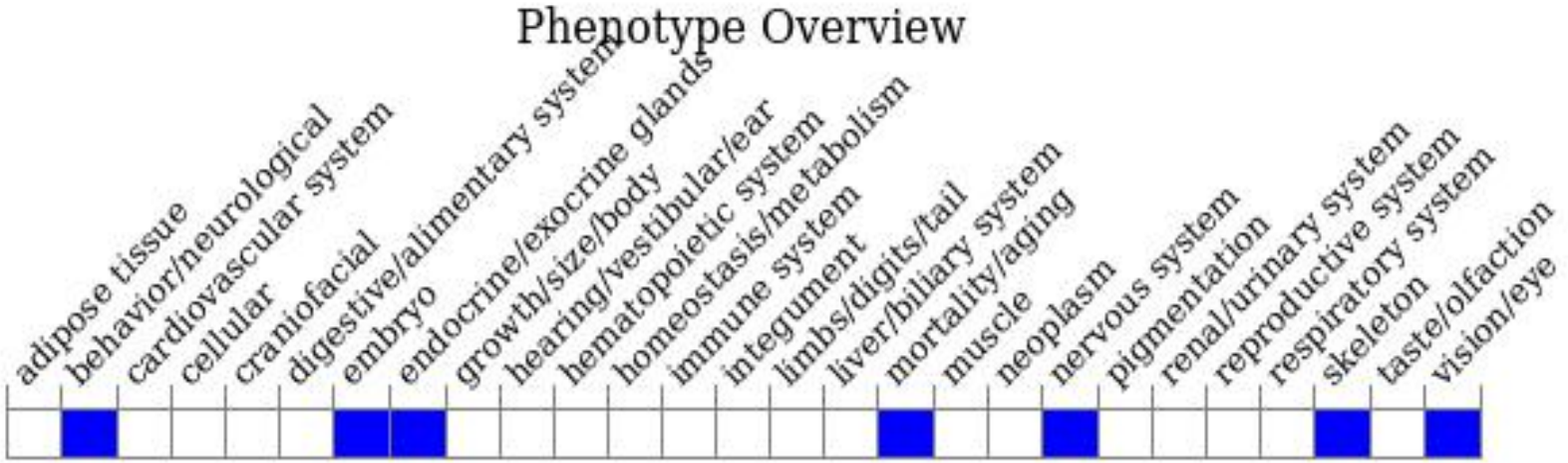
Genomic location distribution



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/).

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

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