Npc1 Cas9-CKO Strategy

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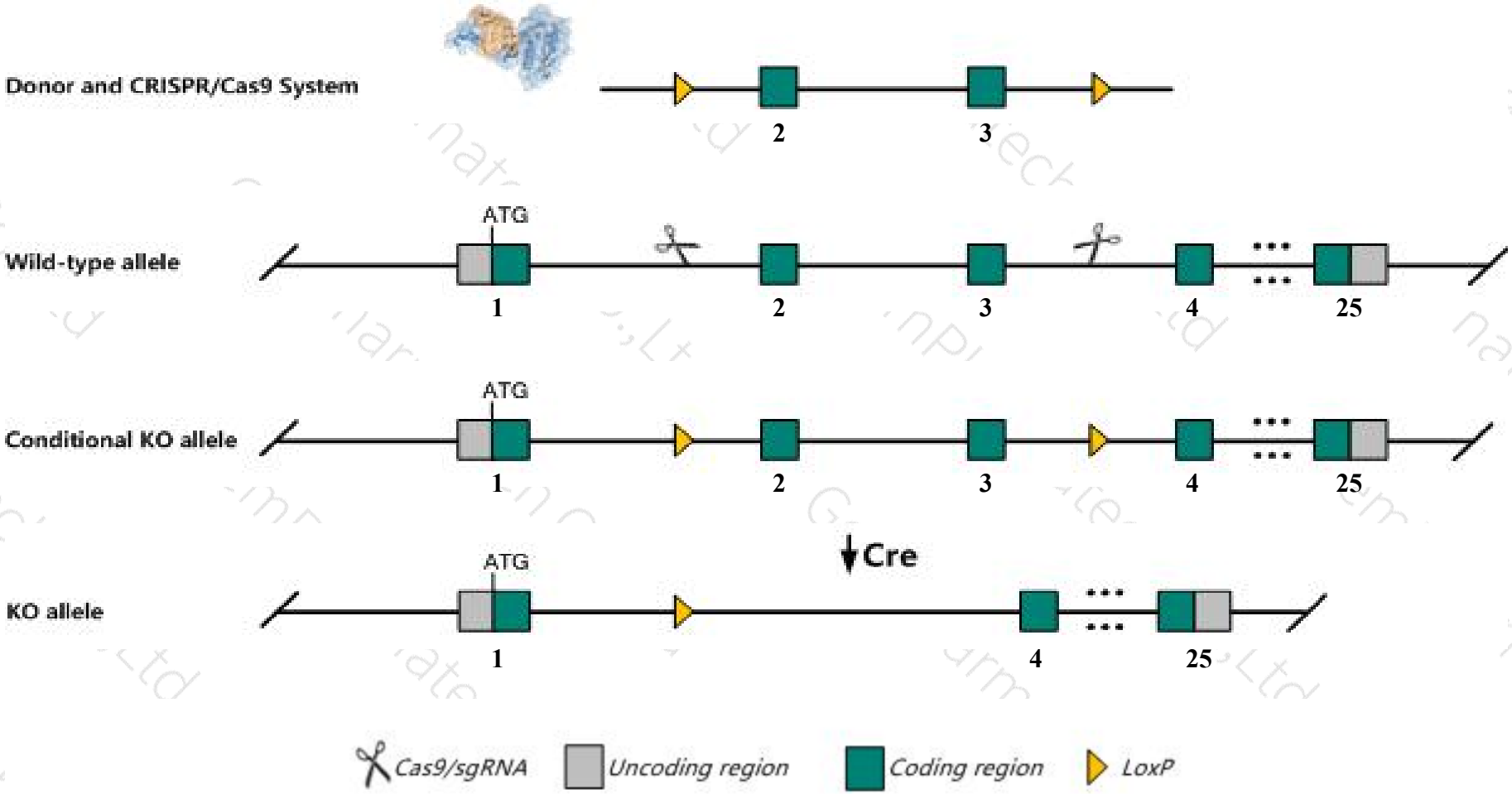
Project Overview

Project Name	***Npc1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Npc1* gene. The schematic diagram is as follows:



Technical routes

- The *Npc1* gene has 7 transcripts. According to the structure of *Npc1* gene, exon2-exon3 of *Npc1-201* (ENSMUST00000025279.5) transcript is recommended as the knockout region.The region contains 230bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Npc1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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,Homozygotes for spontaneous and chemically induced mutations may exhibit lysosomal storage of non-esterified cholesterol, neurodegeneration, ataxia, presence of foam cells, sterility, and shortened lifespan.

➢ The *Npc1* gene is located on the Chr18. 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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Npc1 NPC intracellular cholesterol transporter 1 [Mus musculus (house mouse)]

Gene ID: 18145, updated on 19-Mar-2019

Summary

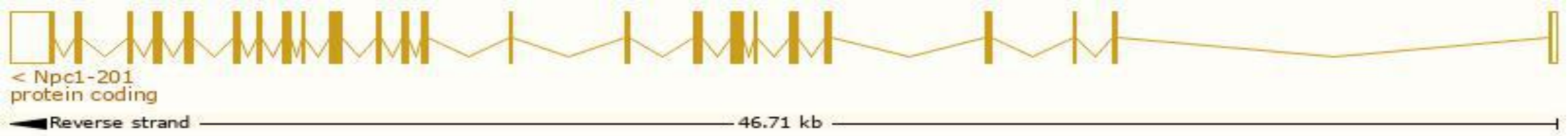
Official Symbol	Npc1 provided by MGI
Official Full Name	NPC intracellular cholesterol transporter 1 provided by MGI
Primary source	MGI:MGI:1097712
See related	Ensembl:ENSMUSG00000024413
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A430089E03Rik, C85354, D18Ertd139e, D18Ertd723e, Icsd, nmf164, spm
Expression	Ubiquitous expression in lung adult (RPKM 23.0), placenta adult (RPKM 16.6) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

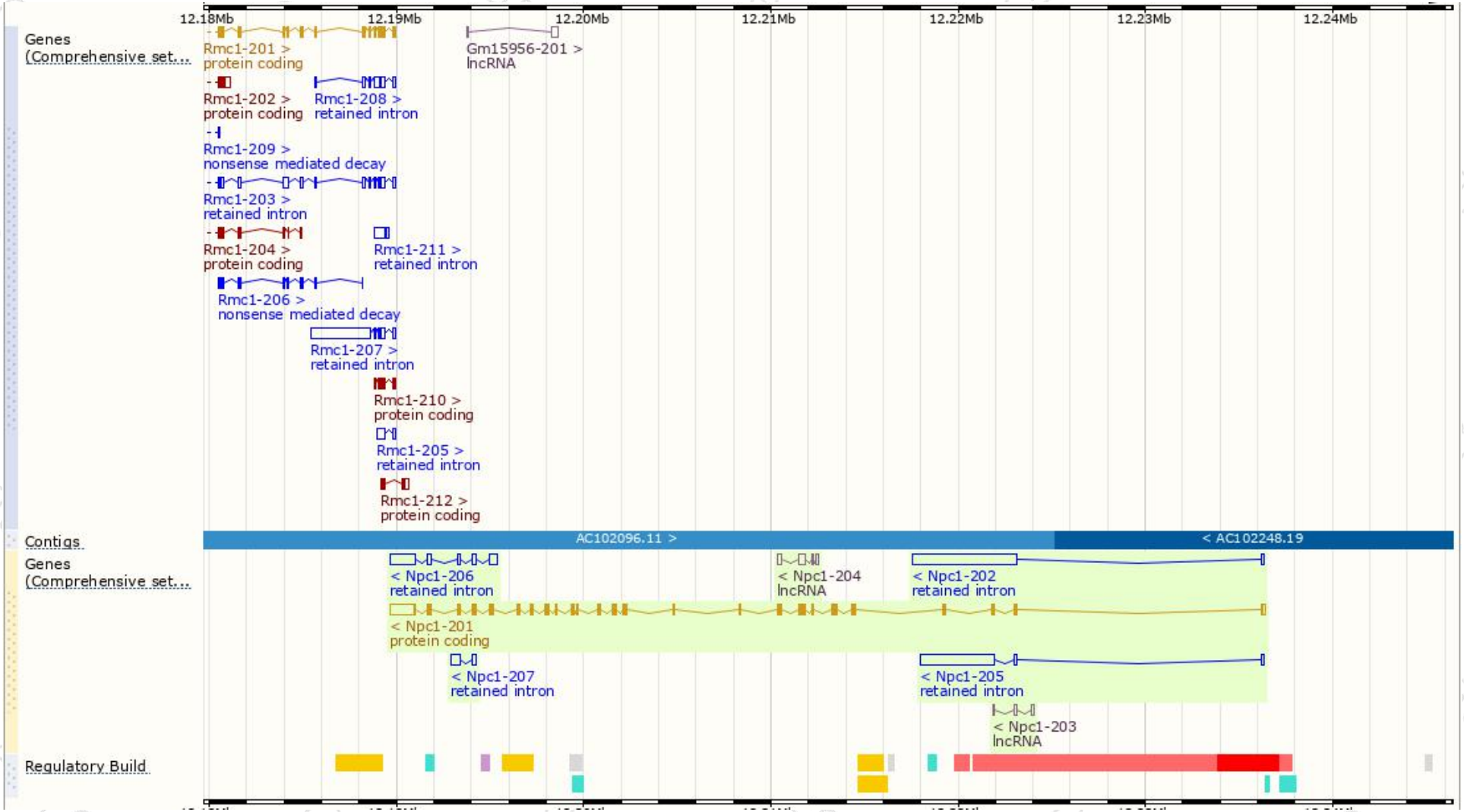
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Npc1-201	ENSMUST00000025279.5	5224	1277aa	Protein coding	CCDS29064	O35604	TSL:1 GENCODE basic APPRIS P1
Npc1-202	ENSMUST00000133112.1	5756	No protein	Retained intron	-	-	TSL:1
Npc1-205	ENSMUST00000149211.7	4188	No protein	Retained intron	-	-	TSL:1
Npc1-206	ENSMUST00000234044.1	2219	No protein	Retained intron	-	-	-
Npc1-207	ENSMUST00000235105.1	715	No protein	Retained intron	-	-	-
Npc1-204	ENSMUST00000145771.1	837	No protein	lncRNA	-	-	TSL:3
Npc1-203	ENSMUST00000144435.1	352	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Npc1-201* transcript,The transcription is shown below

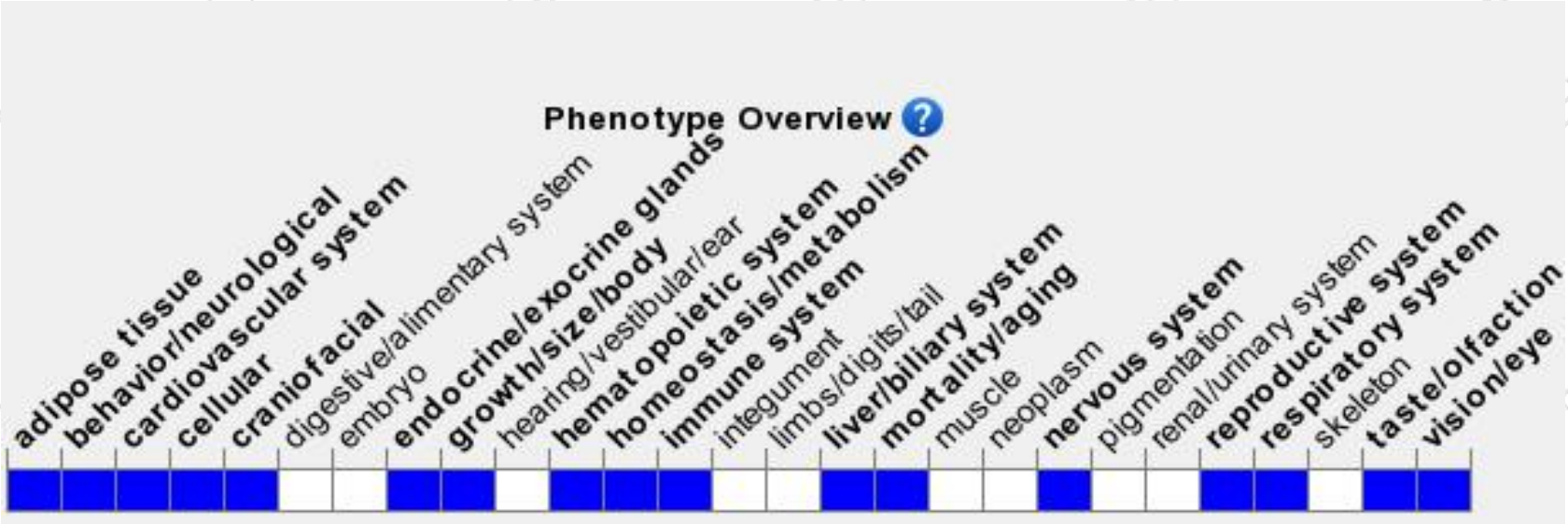


Genomic location distribution



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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