Nr1d2 Cas9-CKO Strategy

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Project Overview

Project Name	***Nr1d2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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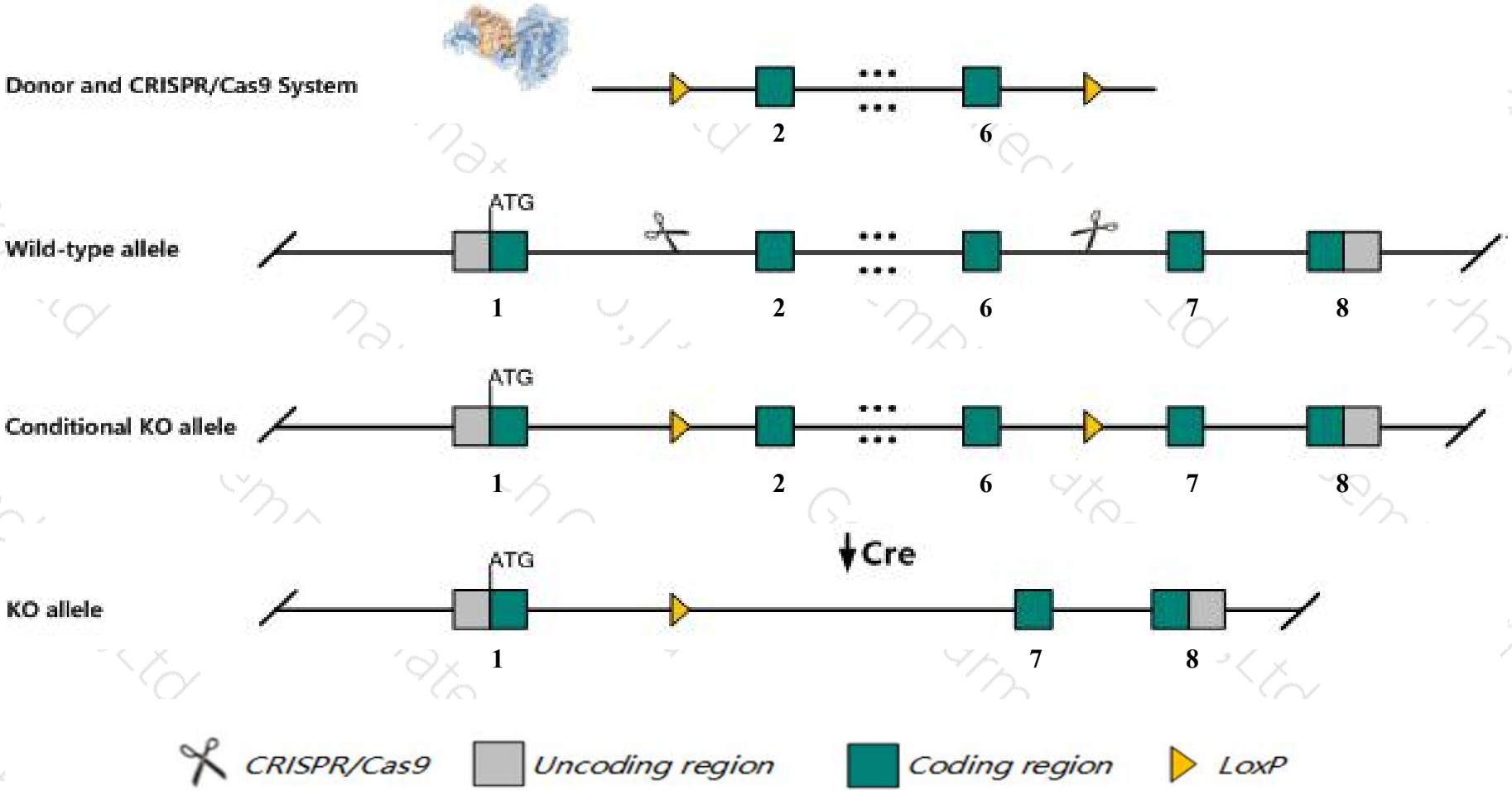
This model will use CRISPR/Cas9 technology to edit the *Nr1d2* gene. The schematic diagram is as follows:



Technical routes

- The *Nr1d2* gene has 4 transcripts. According to the structure of *Nr1d2* gene, exon2-exon6 of *Nr1d2-201* (ENSMUST00000090543.5) transcript is recommended as the knockout region.The region contains 1307bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nr1d2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,mice homozygous for a knock-out allele exhibit no gross abnormalities. mice homozygous for a different knock-out allele display an increased anxiety-related response. a subset of mice homozygous for a third knock-out allele show neonatal lethality, atrioventricular septal defects (avsds) and related cardiovascular malformations.
- The *Nr1d2* gene is located on the Chr14. 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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Nr1d2 nuclear receptor subfamily 1, group D, member 2 [Mus musculus (house mouse)]

Gene ID: 353187, updated on 13-Mar-2020

Summary

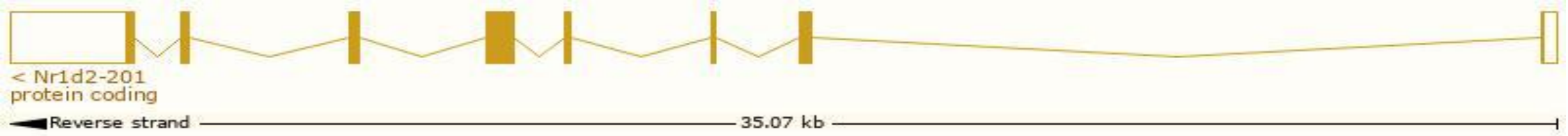
Official Symbol	Nr1d2 provided by MGI
Official Full Name	nuclear receptor subfamily 1, group D, member 2 provided byMGI
Primary source	MGI:MGI:2449205
See related	Ensembl:ENSMUSG00000021775
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	RVR, Rev-erb
Summary	This gene encodes a member of the nuclear hormone receptor family, specifically the NR1 subfamily of receptors. The encoded protein functions as a transcriptional repressor and may play a role in circadian rhythms and carbohydrate and lipid metabolism. [provided by RefSeq, Feb 2014]
Expression	Broad expression in bladder adult (RPKM 22.5), cerebellum adult (RPKM 19.3) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

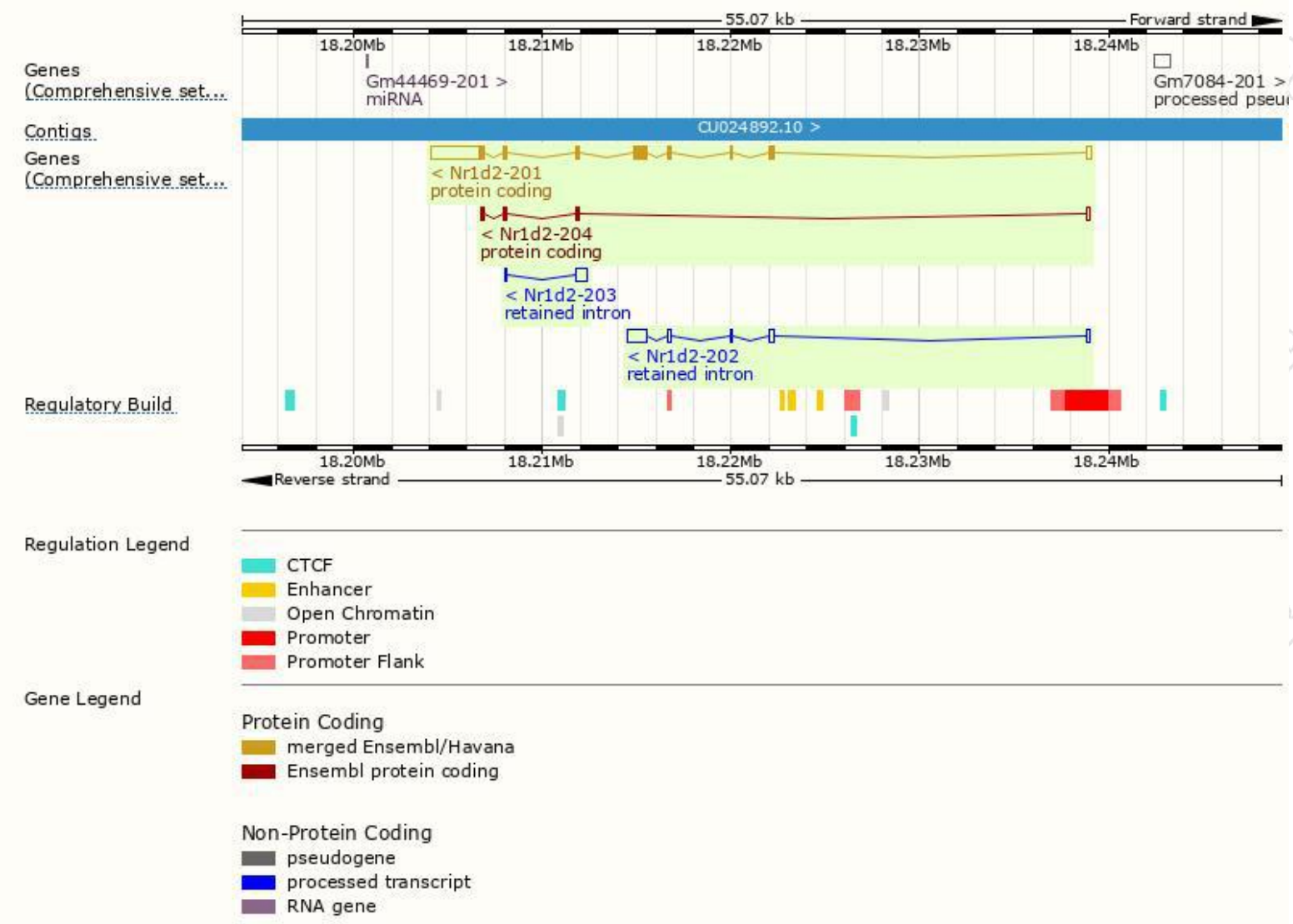
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nr1d2-201	ENSMUST00000090543.5	4663	576aa	Protein coding	CCDS36811	Q4VAB7 Q60674	TSL:1 GENCODE basic APPRIS P1
Nr1d2-204	ENSMUST00000225491.1	737	175aa	Protein coding	-	A0A286YD98	CDS 3' incomplete
Nr1d2-202	ENSMUST00000143308.1	1680	No protein	Retained intron	-	-	TSL:1
Nr1d2-203	ENSMUST00000225308.1	687	No protein	Retained intron	-	-	

The strategy is based on the design of *Nr1d2-201* transcript,the transcription is shown below:

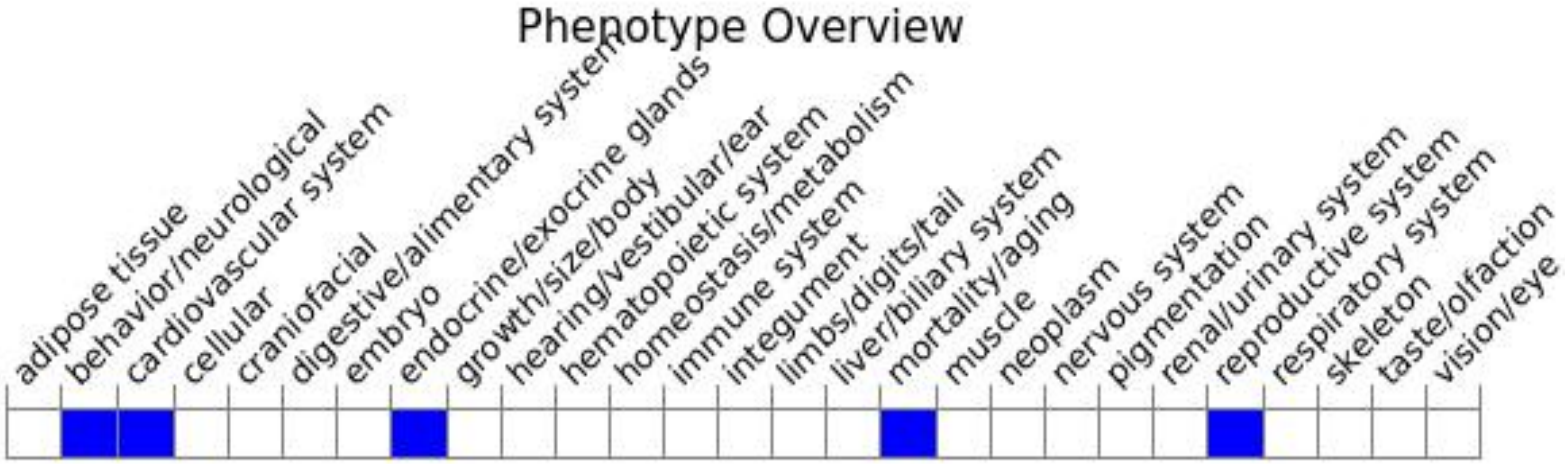


Genomic location distribution



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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