Cnot1 Cas9-CKO Strategy

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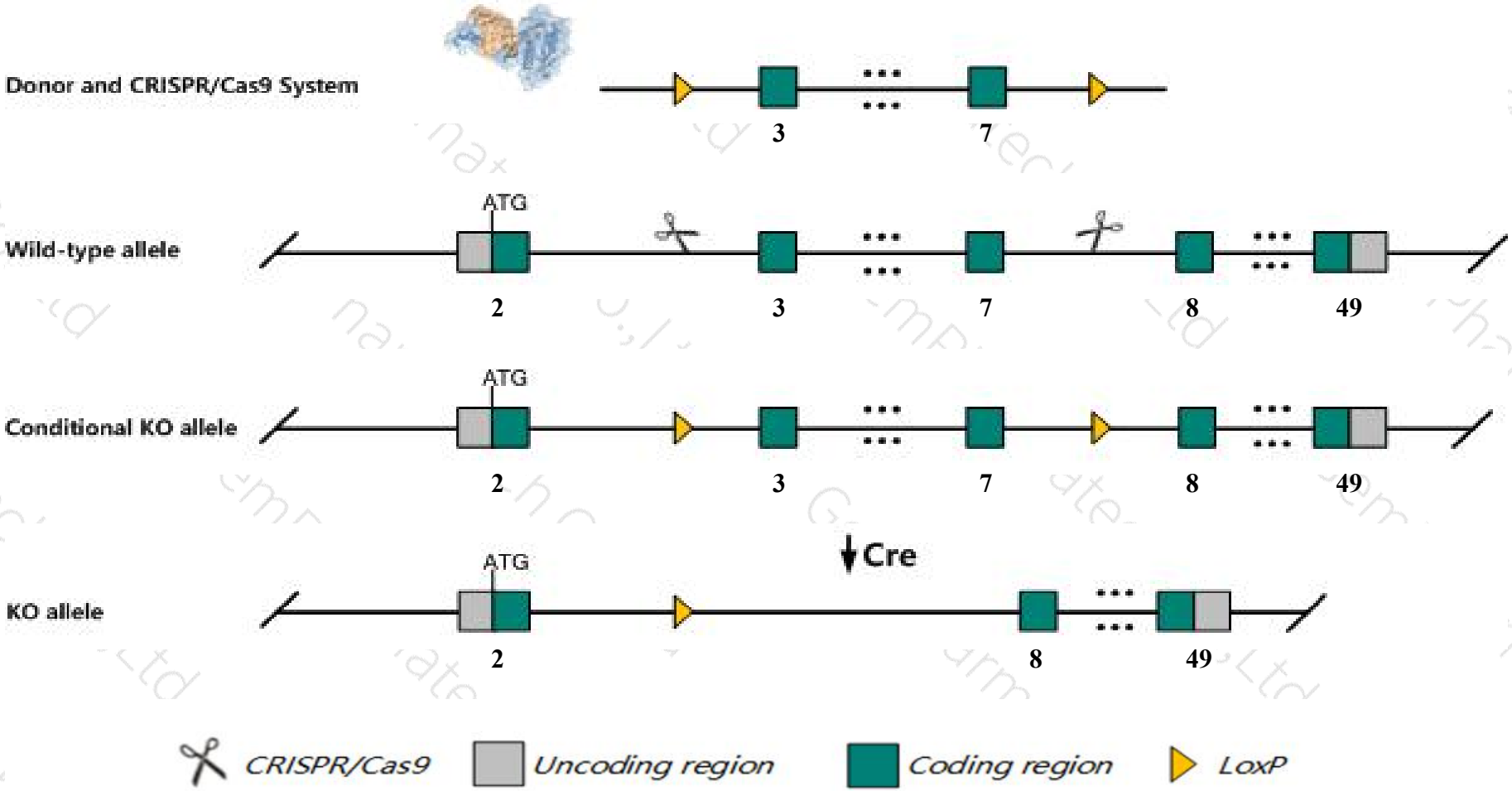
Project Overview

Project Name	***Cnot1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cnot1* gene. The schematic diagram is as follows:



Technical routes

- The *Cnot1* gene has 17 transcripts. According to the structure of *Cnot1* gene, exon3-exon7 of *Cnot1-203* (ENSMUST00000211887.1) transcript is recommended as the knockout region.The region contains 529bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cnot1* 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,mice hmozygous for a conditional allele activated in cardiomyocytes exhibit postnatal lethality, decreased cardiac muscle contractility, prolonged qt interval and cardiac muscle cell death.
- The 3-regulation of *Gm45762-201* may be affect.
- *Cnot1-217* and *Cnot1-205* transcripts are unknown, and the effect on these two transcripts are unknown.
- The *Cnot1* gene is located on the Chr8. 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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Cnot1 CCR4-NOT transcription complex, subunit 1 [*Mus musculus* (house mouse)]

Gene ID: 234594, updated on 8-Jun-2020

Summary

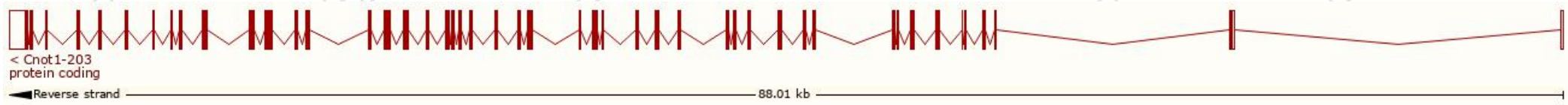
Official Symbol	Cnot1 provided by MGI
Official Full Name	CCR4-NOT transcription complex, subunit 1 provided by MGI
Primary source	MGI:MGI:2442402
See related	Ensembl:ENSMUSG00000036550
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA815922; D830048B13; 6030411K04Rik
Expression	Ubiquitous expression in testis adult (RPKM 29.9), placenta adult (RPKM 21.5) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

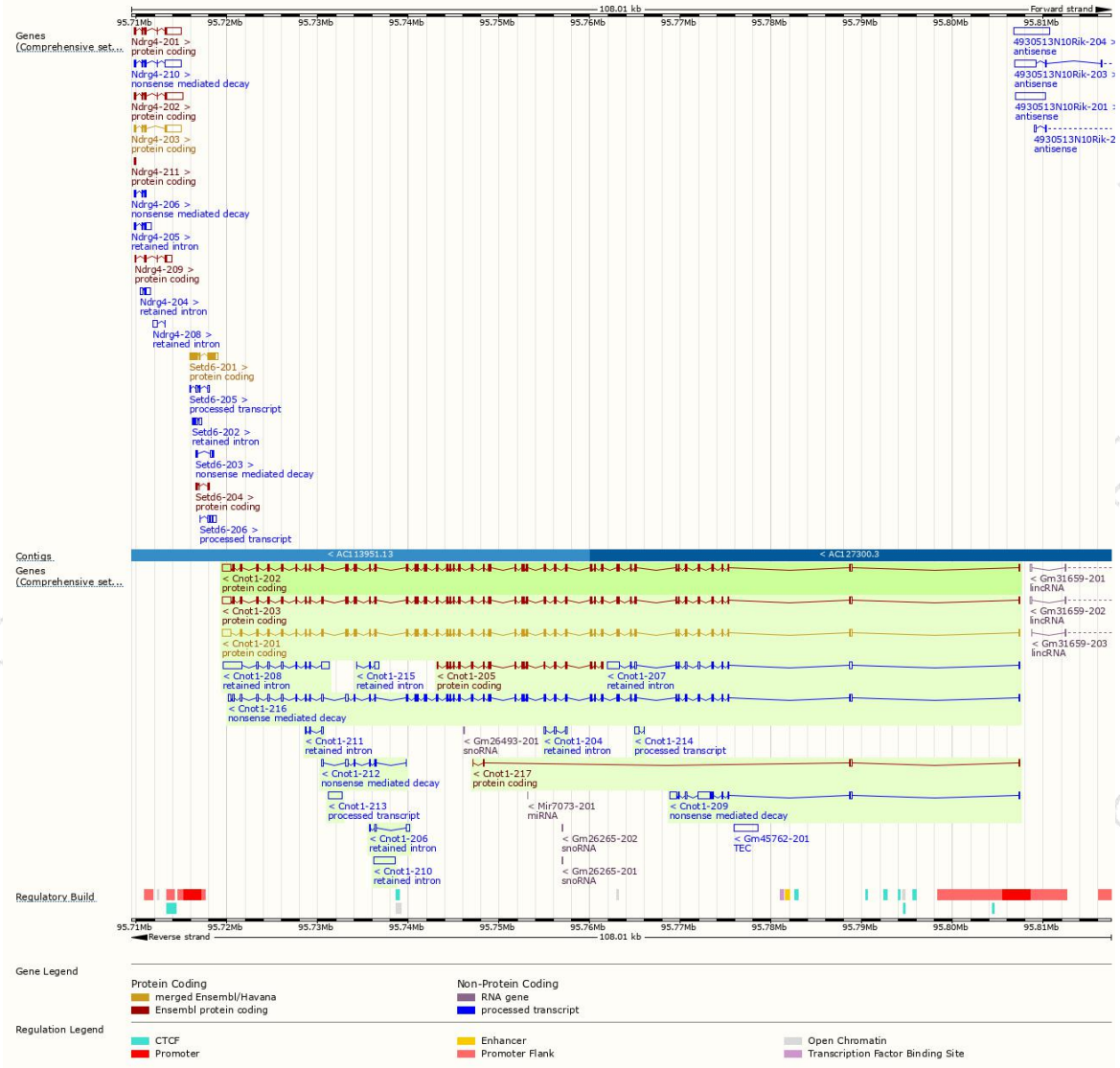
The gene has 17 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cnot1-203	ENSMUST00000211887.1	8364	2369aa	Protein coding	CCDS85589	A0A1D5RMJ8	TSL:1 GENCODE basic APPRIS P2
Cnot1-201	ENSMUST00000068452.9	8152	2326aa	Protein coding	CCDS57635	B7ZWL1	TSL:1 GENCODE basic
Cnot1-202	ENSMUST00000098473.10	8390	2376aa	Protein coding	-	Q6ZQ08	TSL:5 GENCODE basic APPRIS ALT1
Cnot1-205	ENSMUST00000211973.1	2469	823aa	Protein coding	-	A0A1D5RML9	CDS 5' and 3' incomplete TSL:5
Cnot1-217	ENSMUST00000213046.1	550	92aa	Protein coding	-	A0A1D5RMB6	CDS 3' incomplete TSL:3
Cnot1-216	ENSMUST00000213006.1	7479	1614aa	Nonsense mediated decay	-	A0A1D5RMD8	TSL:1
Cnot1-209	ENSMUST00000212323.1	3277	213aa	Nonsense mediated decay	-	A0A1D5RM03	TSL:2
Cnot1-212	ENSMUST00000212415.1	956	122aa	Nonsense mediated decay	-	A0A1D5RM04	CDS 5' incomplete TSL:5
Cnot1-213	ENSMUST00000212535.1	1546	No protein	Processed transcript	-	-	TSL:NA
Cnot1-214	ENSMUST00000212556.1	521	No protein	Processed transcript	-	-	TSL:5
Cnot1-208	ENSMUST00000212302.1	3809	No protein	Retained intron	-	-	TSL:1
Cnot1-207	ENSMUST00000212228.1	2925	No protein	Retained intron	-	-	TSL:1
Cnot1-210	ENSMUST00000212340.1	2381	No protein	Retained intron	-	-	TSL:NA
Cnot1-206	ENSMUST00000212195.1	696	No protein	Retained intron	-	-	TSL:3
Cnot1-215	ENSMUST00000212712.1	656	No protein	Retained intron	-	-	TSL:3
Cnot1-204	ENSMUST00000211937.1	500	No protein	Retained intron	-	-	TSL:3
Cnot1-211	ENSMUST00000212369.1	479	No protein	Retained intron	-	-	TSL:2

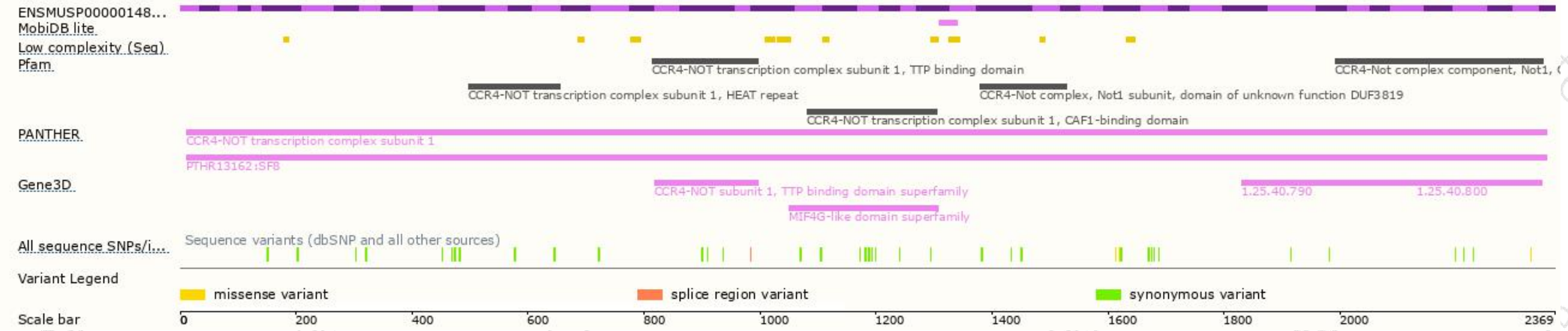
The strategy is based on the design of *Cnot1-203* transcript,the transcription is shown below:



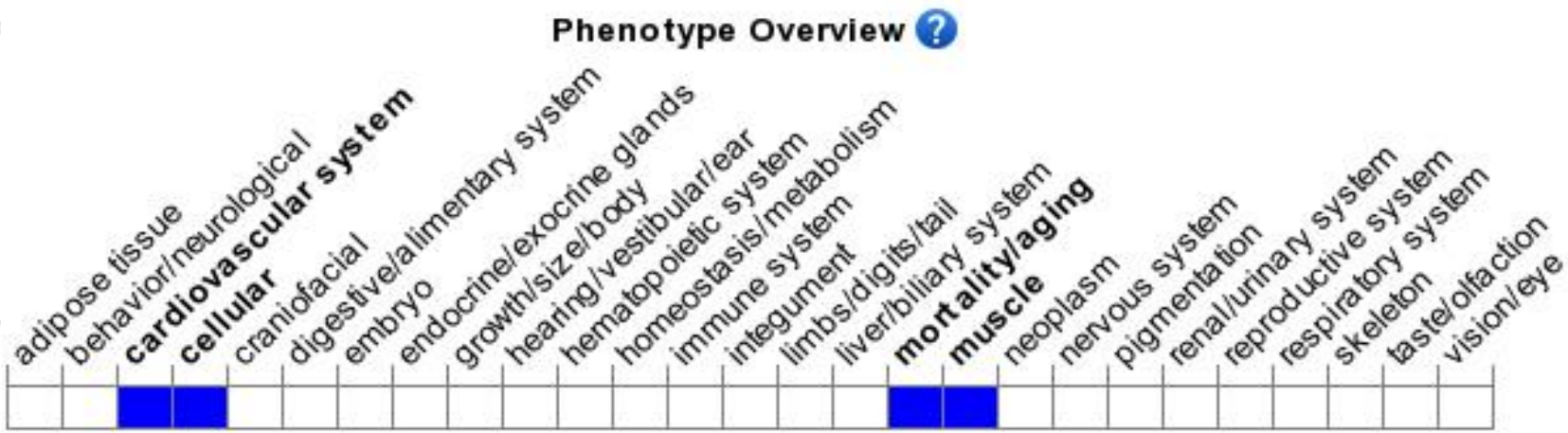
Genomic location distribution



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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