Dnm1 Cas9-CKO Strategy

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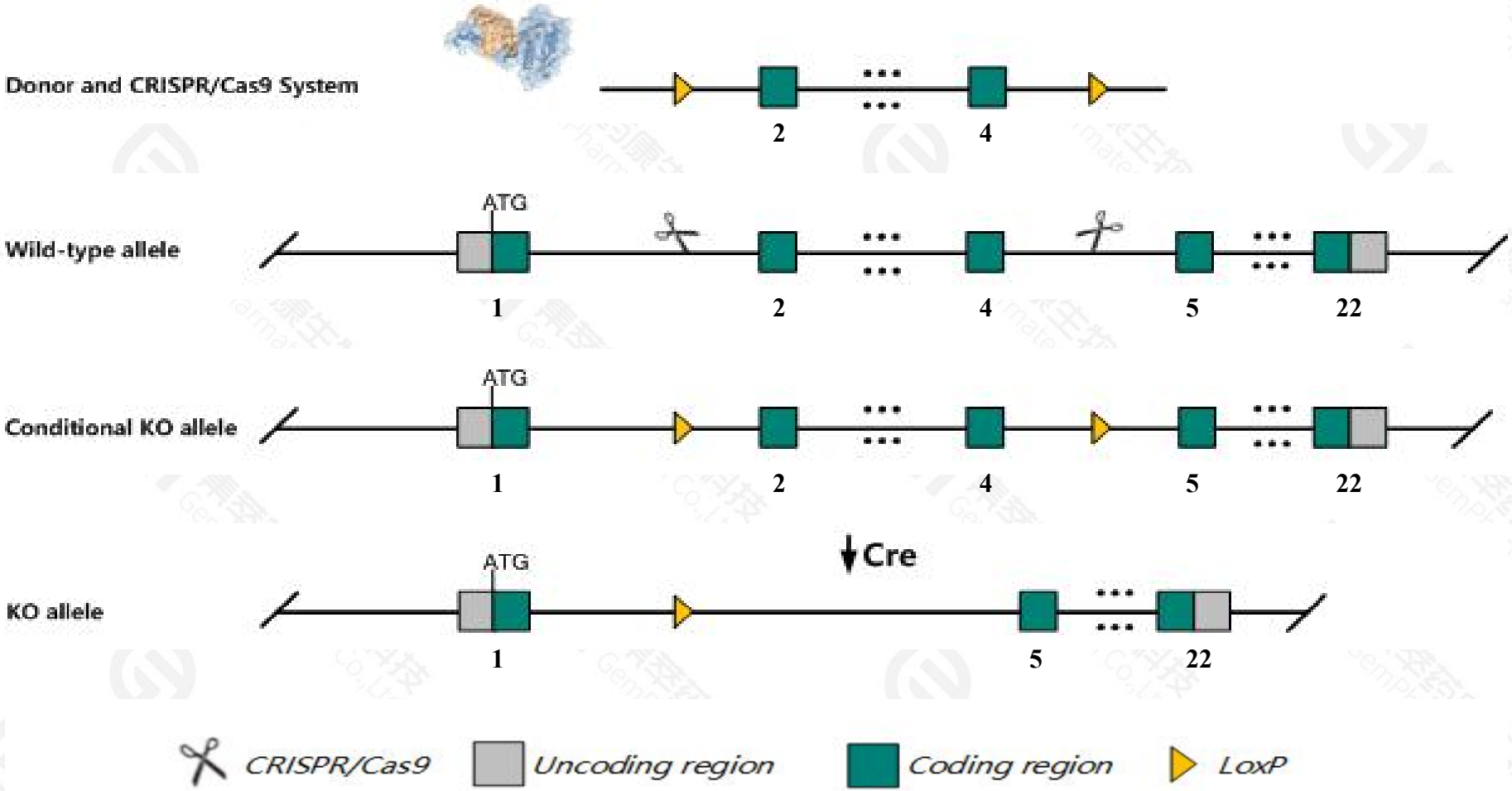
Project Overview

Project Name	***Dnm1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dnm1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Dnm1* gene has 17 transcripts. According to the structure of *Dnm1* gene, exon2-exon4 of *Dnm1-201*(ENSMUST00000078352.12) transcript is recommended as the knockout region.The region contains 428bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dnm1* 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 mice display reduced postnatal viability. Null mutation of this gene results in abnormal synaptic vesicle morphology, and recycling during neuronal activity. Other alleles are associated with seizures.

➢ The *Dnm1* gene is located on the Chr2. 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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Dnm1 dynamin 1 [Mus musculus (house mouse)]

Gene ID: 13429, updated on 23-Feb-2021

Summary

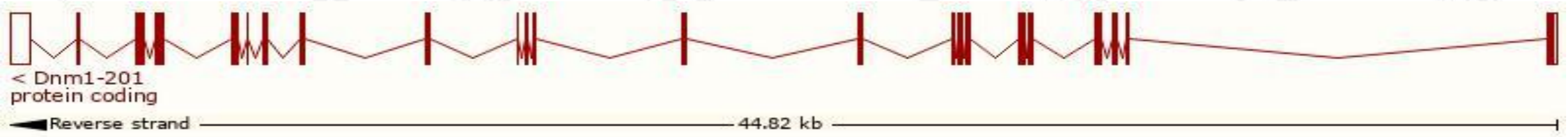
Official Symbol	Dnm1 provided by MGI
Official Full Name	dynamin 1 provided byMGI
Primary source	MGI:MGI:107384
See related	Ensembl:ENSMUSG00000026825
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI838169, Dnm, Ftf, Ftfl, mKIAA4093
Summary	This gene encodes a member of the dynamin subfamily of GTP-binding proteins. The encoded protein is a GTPase which is required for membrane recycling, including vesicle endocytosis in neurons. It may also be involved in cellular fission via association with microtubules and actin filaments. Mutations in this gene have been shown to cause seizures. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2014]
Expression	Biased expression in cerebellum adult (RPKM 188.3), cortex adult (RPKM 160.9) and 14 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

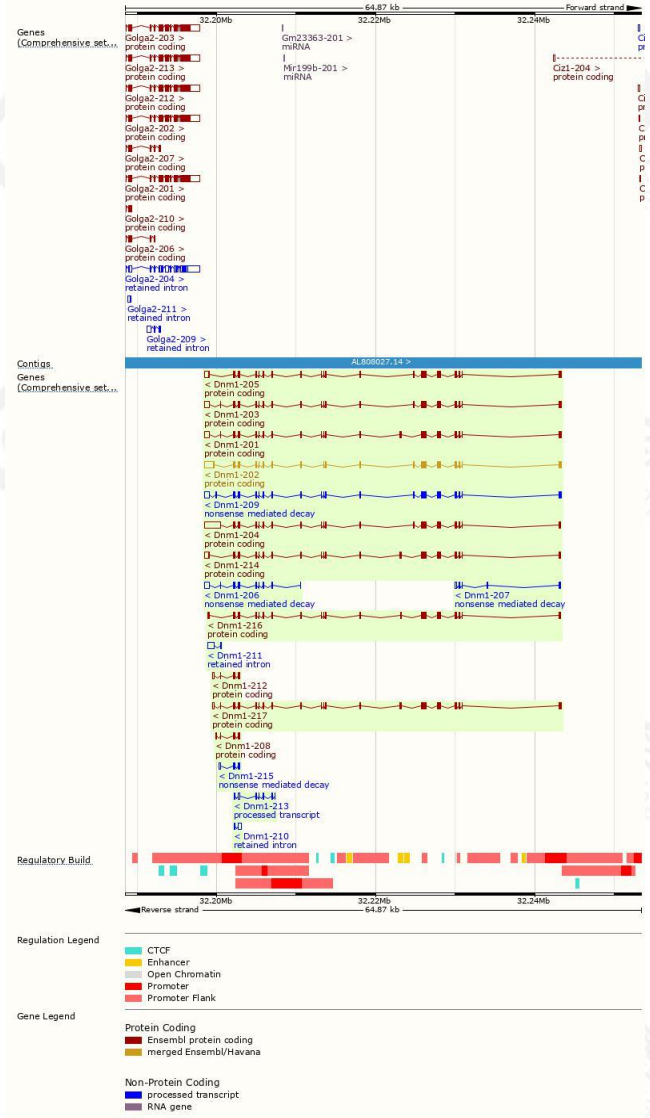
The gene has 17 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dnm1-202	ENSMUST00000091089.12	3764	864aa	Protein coding	CCDS38102		TSL:1 , GENCODE basic ,
Dnm1-201	ENSMUST00000078352.12	3246	851aa	Protein coding	CCDS79772		TSL:1 , GENCODE basic , APPRIS P2 ,
Dnm1-217	ENSMUST00000202578.4	2820	851aa	Protein coding	CCDS79772		TSL:5 , GENCODE basic , APPRIS P2 ,
Dnm1-204	ENSMUST00000113352.9	4605	851aa	Protein coding	-		TSL:2 , GENCODE basic , APPRIS ALT1 ,
Dnm1-203	ENSMUST00000113350.8	3258	851aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Dnm1-205	ENSMUST00000113365.8	3257	864aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Dnm1-214	ENSMUST00000201433.4	3146	864aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Dnm1-216	ENSMUST00000201494.4	2732	835aa	Protein coding	-		TSL:5 , GENCODE basic ,
Dnm1-212	ENSMUST00000155269.8	656	159aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Dnm1-208	ENSMUST00000139291.3	641	149aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Dnm1-209	ENSMUST00000139624.8	3337	867aa	Nonsense mediated decay	-		TSL:1 ,
Dnm1-206	ENSMUST00000129156.5	1520	283aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Dnm1-207	ENSMUST00000139238.2	695	59aa	Nonsense mediated decay	-		TSL:3 ,
Dnm1-215	ENSMUST00000201440.2	621	110aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Dnm1-213	ENSMUST00000156866.2	810	No protein	Processed transcript	-		TSL:3 ,
Dnm1-211	ENSMUST00000145885.2	899	No protein	Retained intron	-		TSL:3 ,
Dnm1-210	ENSMUST00000141633.2	495	No protein	Retained intron	-		TSL:2 ,

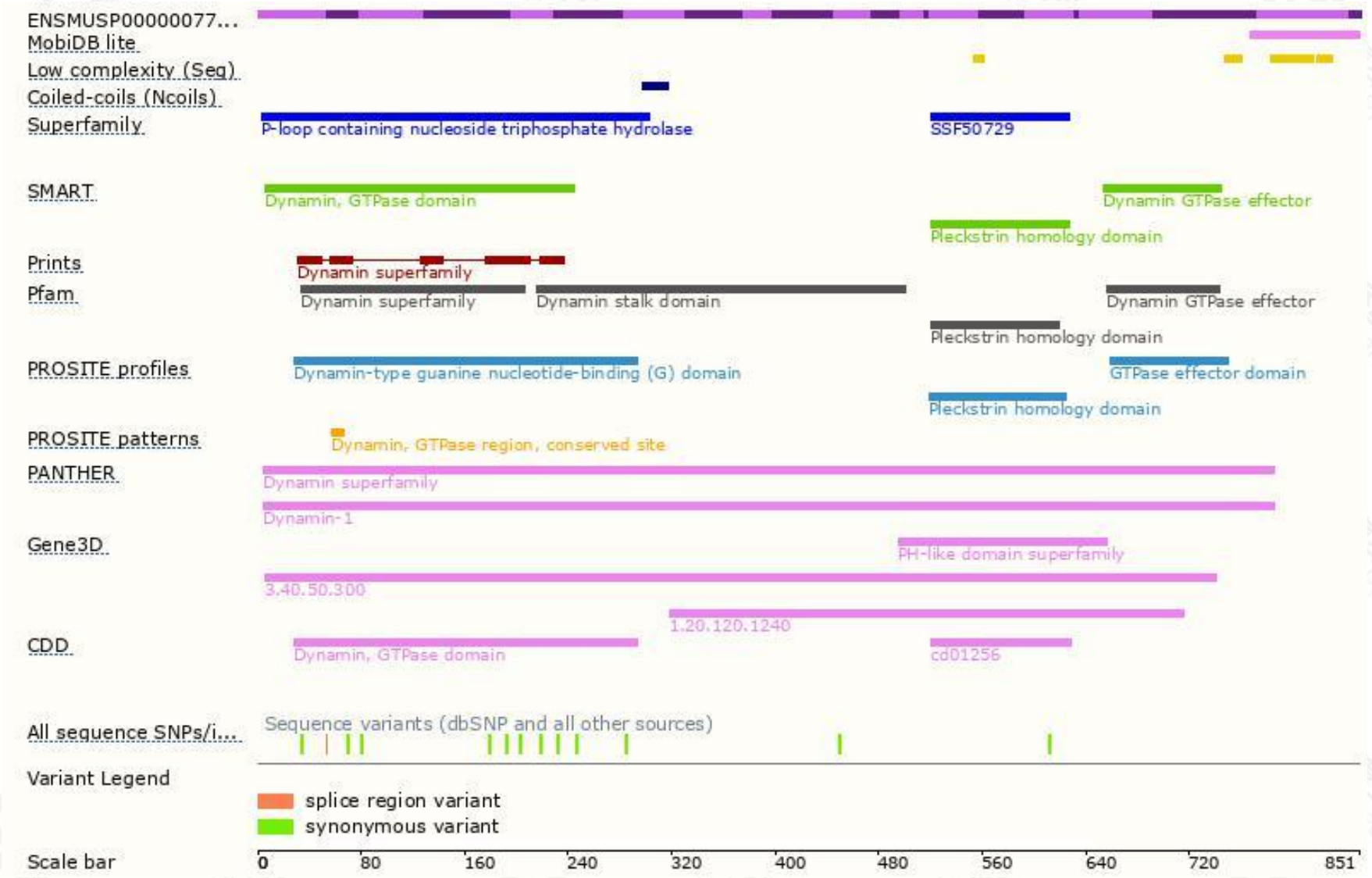
The strategy is based on the design of *Dnm1-201* transcript,the transcription is shown below:



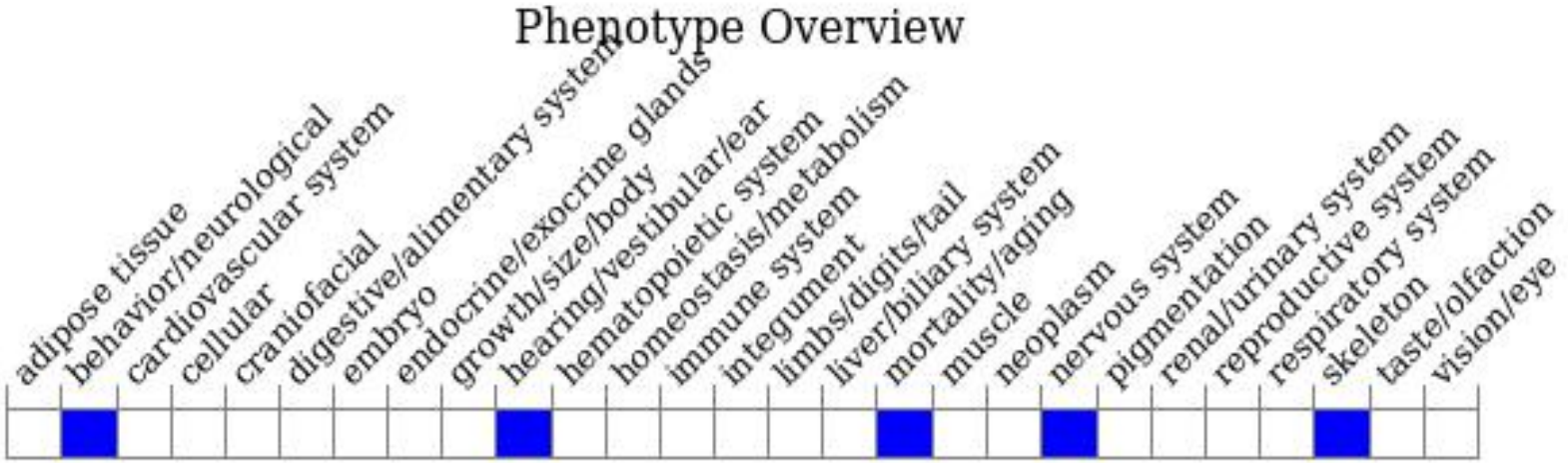
Genomic location distribution



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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