Nbr1 Cas9-CKO Strategy

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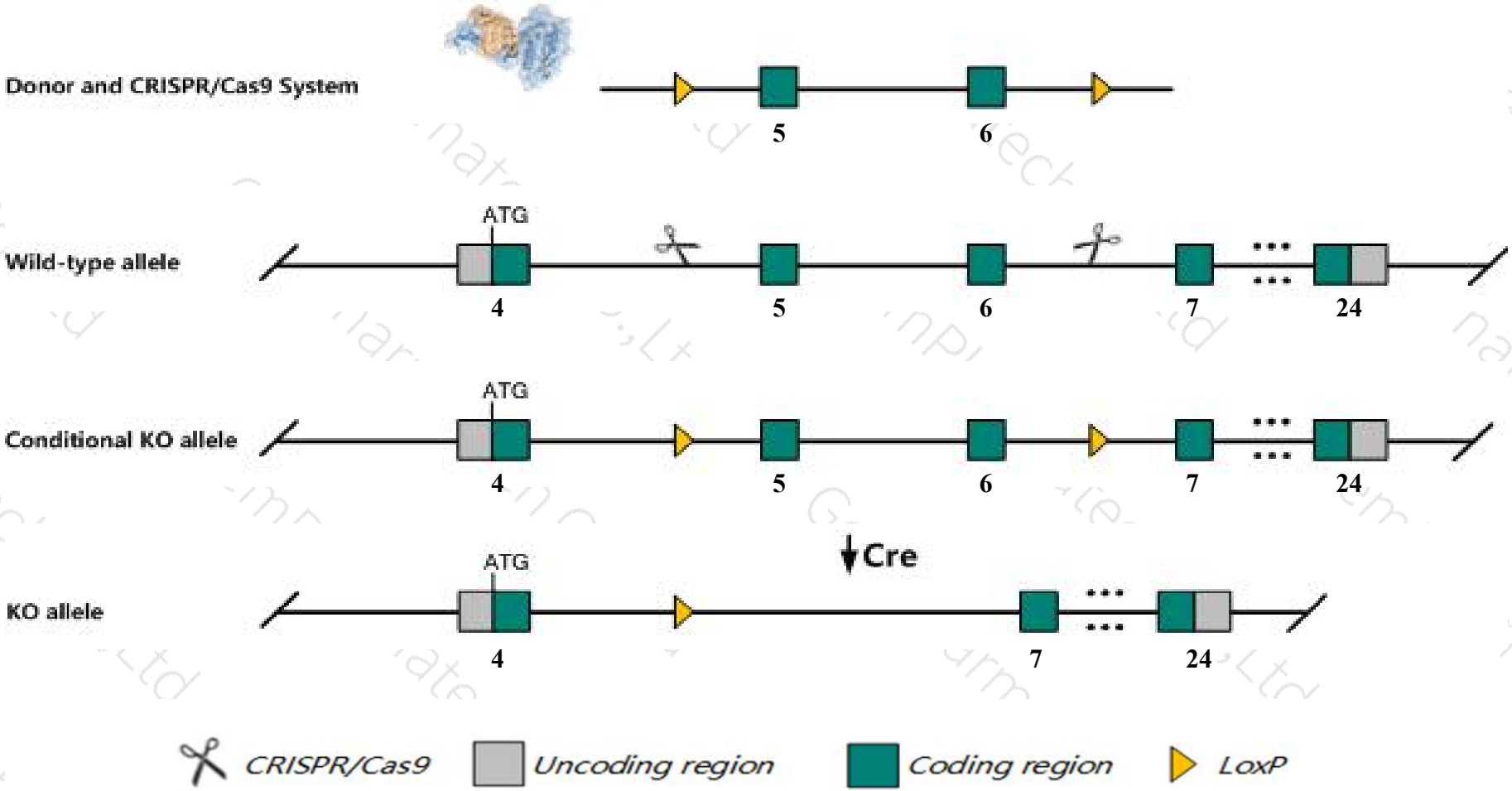
Project Overview

Project Name	***Nbr1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nbr1* gene. The schematic diagram is as follows:



Technical routes

- The *Nbr1* gene has 21 transcripts. According to the structure of *Nbr1* gene, exon5-exon6 of *Nbr1-203* (ENSMUST00000103099.7) transcript is recommended as the knockout region.The region contains 82bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nbr1* 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 of the genetic truncation allele had an age-dependent increase in bone mass and bone mineral density. Mice homozygous for a floxed allele activated in T cells exhibit decreased ovalbumin-induced inflammation and defective Th2 polarization.
- Transcript *Nbr1-217* may not be affected.
- The *Nbr1* gene is located on the Chr11. 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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Nbr1 NBR1, autophagy cargo receptor [Mus musculus (house mouse)]

Gene ID: 17966, updated on 19-Feb-2019

Summary

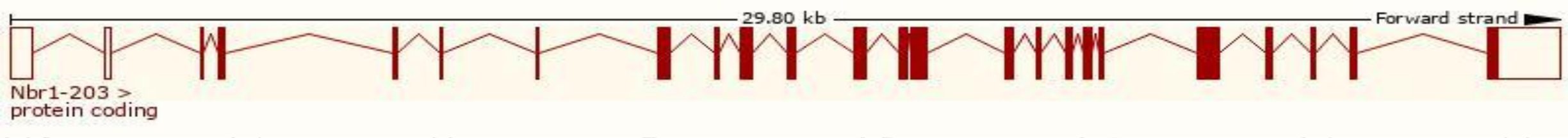
Official Symbol	Nbr1 provided by MGI
Official Full Name	NBR1, autophagy cargo receptor provided by MGI
Primary source	MGI:MGI:108498
See related	Ensembl:ENSMUSG00000017119
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	mKIAA0049
Expression	Ubiquitous expression in testis adult (RPKM 73.0), placenta adult (RPKM 24.7) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

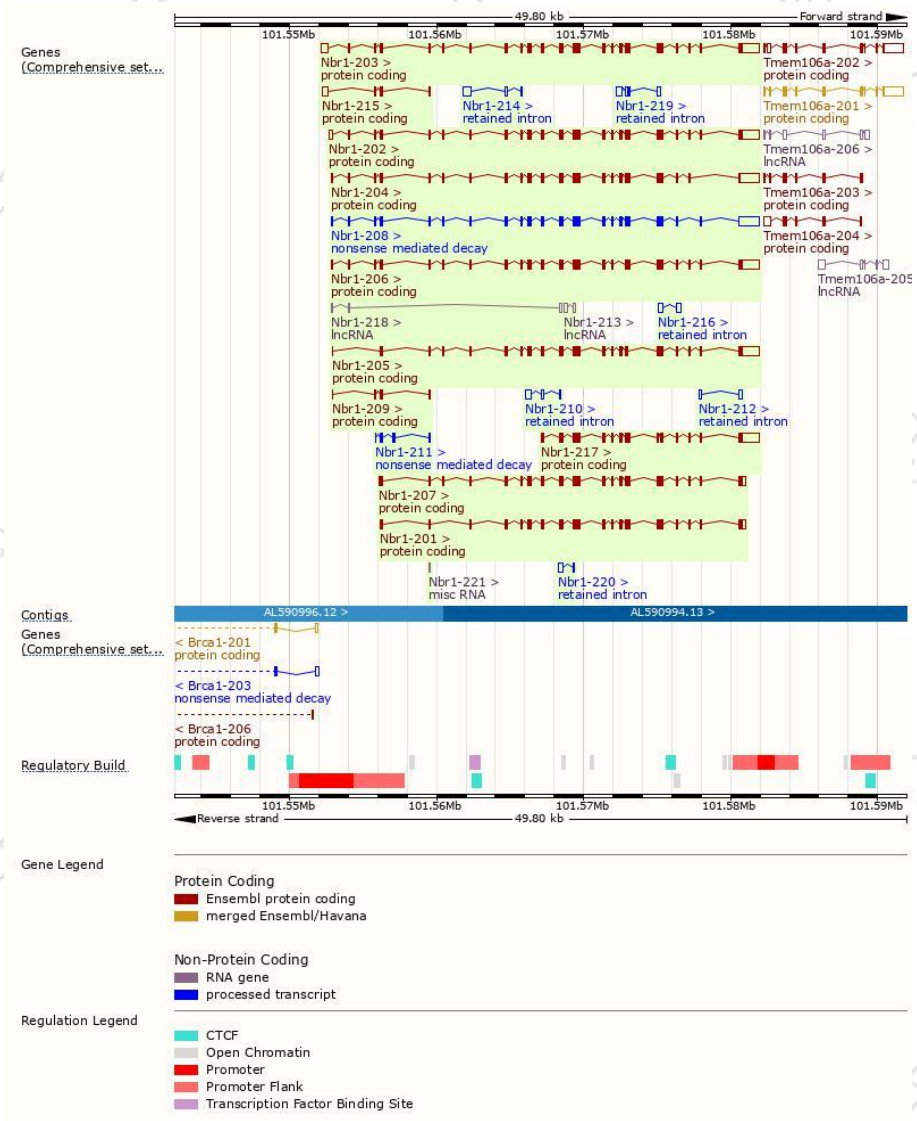
The gene has 21 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nbr1-203	ENSMUST00000103099.7	4789	988aa	Protein coding	CCDS25475	A1L329 P97432	TSL:1 GENCODE basic APPRIS P3
Nbr1-202	ENSMUST00000103098.8	4654	988aa	Protein coding	CCDS25475	A1L329 P97432	TSL:1 GENCODE basic APPRIS P3
Nbr1-204	ENSMUST00000107208.7	4344	906aa	Protein coding	CCDS56810	A2A4N5	TSL:1 GENCODE basic
Nbr1-206	ENSMUST00000107213.7	4339	951aa	Protein coding	CCDS56811	A2A4N8	TSL:1 GENCODE basic APPRIS ALT2
Nbr1-207	ENSMUST00000107218.9	3353	988aa	Protein coding	CCDS25475	A1L329 P97432	TSL:5 GENCODE basic APPRIS P3
Nbr1-205	ENSMUST00000107212.7	4168	963aa	Protein coding	-	A2A4N7	TSL:5 GENCODE basic APPRIS ALT2
Nbr1-217	ENSMUST00000149019.1	3330	712aa	Protein coding	-	F6S397	CDS 5' incomplete TSL:1
Nbr1-201	ENSMUST00000071537.12	3239	988aa	Protein coding	-	K3W4P1	TSL:5 GENCODE basic APPRIS ALT2
Nbr1-215	ENSMUST00000147239.7	563	43aa	Protein coding	-	A2A4R0	CDS 3' incomplete TSL:5
Nbr1-209	ENSMUST00000127421.7	265	43aa	Protein coding	-	A2A4R0	CDS 3' incomplete TSL:3
Nbr1-208	ENSMUST00000123558.7	4392	888aa	Nonsense mediated decay	-	Q05BC8	TSL:1
Nbr1-211	ENSMUST00000136185.1	276	41aa	Nonsense mediated decay	-	G3UZH6	TSL:3
Nbr1-214	ENSMUST00000146452.1	810	No protein	Retained intron	-	-	TSL:3
Nbr1-219	ENSMUST00000172744.1	804	No protein	Retained intron	-	-	TSL:3
Nbr1-216	ENSMUST00000148805.1	639	No protein	Retained intron	-	-	TSL:2
Nbr1-210	ENSMUST00000127871.1	594	No protein	Retained intron	-	-	TSL:2
Nbr1-220	ENSMUST00000174013.1	463	No protein	Retained intron	-	-	TSL:3
Nbr1-212	ENSMUST00000141170.1	418	No protein	Retained intron	-	-	TSL:2
Nbr1-213	ENSMUST00000144517.1	364	No protein	lncRNA	-	-	TSL:2
Nbr1-218	ENSMUST00000149170.1	342	No protein	lncRNA	-	-	TSL:3
Nbr1-221	ENSMUST00000184092.1	63	No protein	misc RNA	-	-	TSL:NA

The strategy is based on the design of *Nbr1-203* transcript,The transcription is shown below

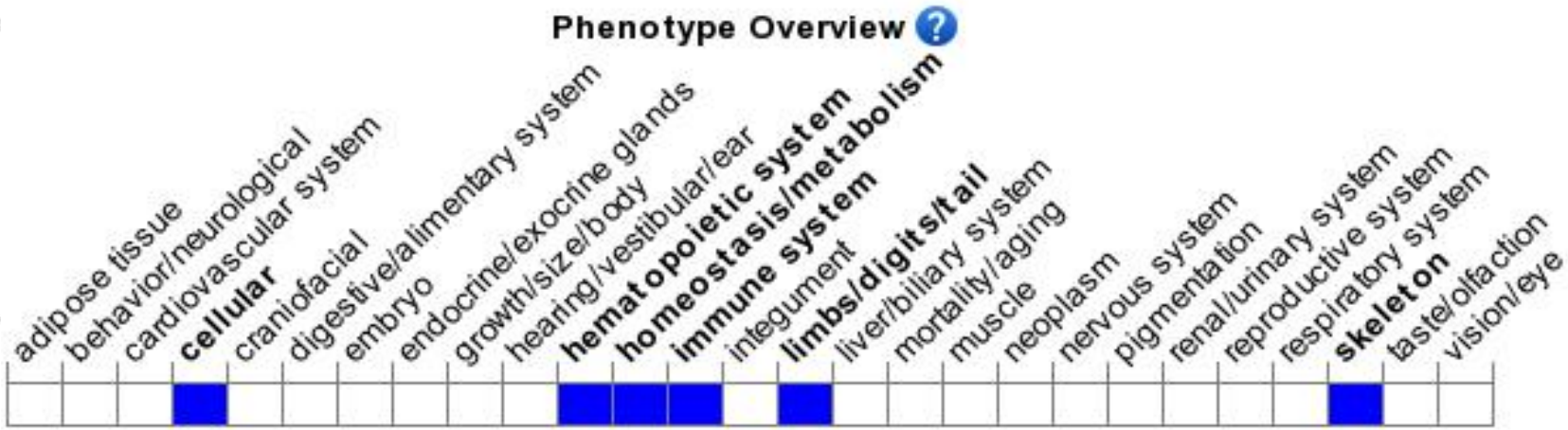


Genomic location distribution



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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