

Manba Cas9-CKO Strategy

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Project Overview



Project Name

17s, 16

Project type

Strain background

Manba

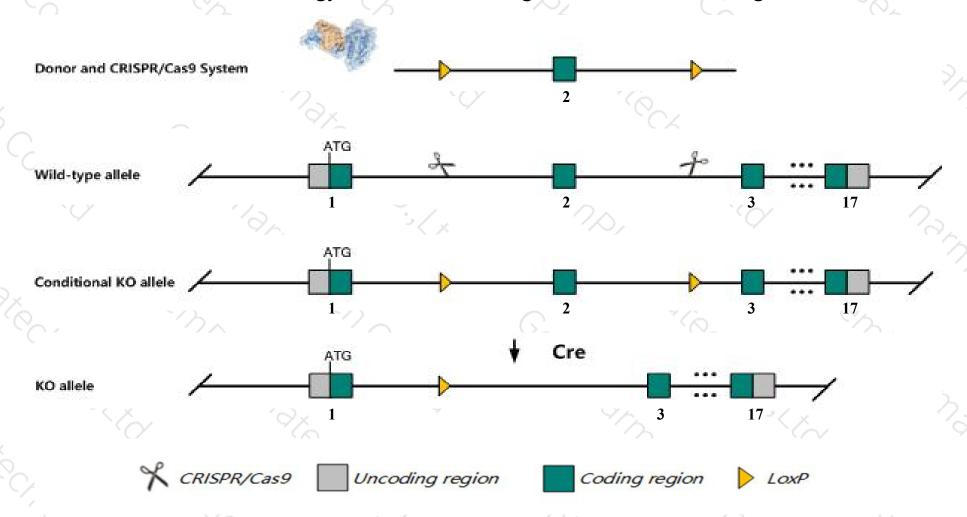
Cas9-CKO

C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- ➤ The *Manba* gene has 11 transcripts. According to the structure of *Manba* gene, exon2 of *Manba-201*(ENSMUST00000029814.9) transcript is recommended as the knockout region. The region contains 95bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Manba* 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 mutation results in no dysmorphology or overt neurological problems. Homozygotes show no beta-mannosidase activity and display consistent cytoplasmic vacuolation in the central nervous system and minimal vacuolation in most visceral organs.
- > Transcript *Manba*-202&203&205&207&210&211 may not be affected.
- > The *Manba* gene is located on the Chr3. 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)



Manba mannosidase, beta A, lysosomal [Mus musculus (house mouse)]

Gene ID: 110173, updated on 10-Oct-2019

Summary

☆ ?

Official Symbol Manba provided by MGI

Official Full Name mannosidase, beta A, lysosomal provided by MGI

Primary source MGI:MGI:88175

See related Ensembl: ENSMUSG00000028164

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 Bmn; 2410030007Rik; B930014J03Rik

Expression Ubiquitous expression in placenta adult (RPKM 10.2), kidney adult (RPKM 8.8) and 28 other tissues See more

Orthologs <u>human</u> all

Genomic context



Location: 3 G3; 3 62.65 cM

See Manba in Genome Data Viewer

Exon count: 19

Annotation release	Status	Assembly	Chr	Location	
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (135485611135571404)	
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (135148575135234368)	

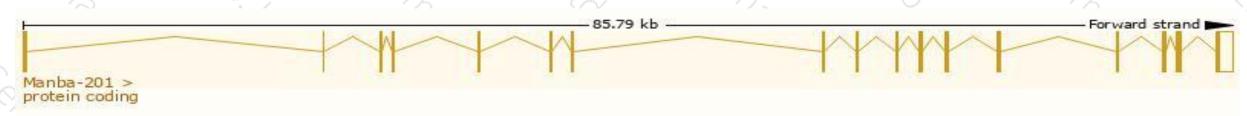
Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

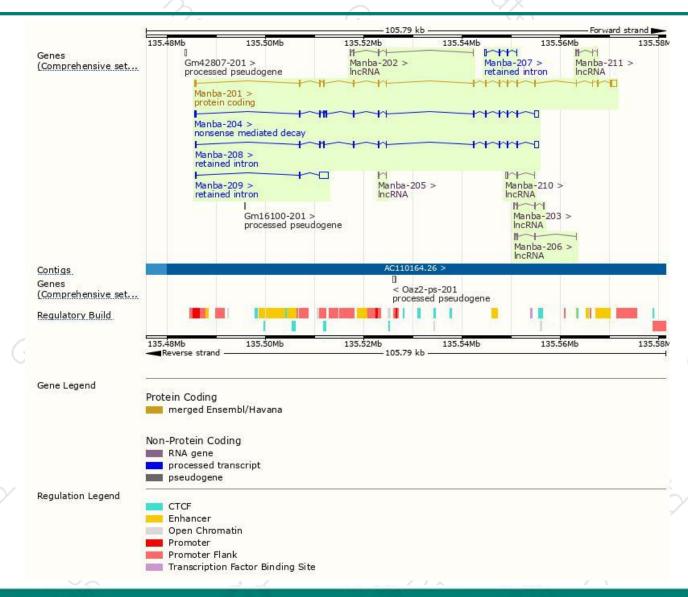
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Manba-201	ENSMUST00000029814.9	3675	879aa	Protein coding	CCDS17857	A0A0R4J092	TSL:1 GENCODE basic APPRIS P1		
Manba-204	ENSMUST00000131610.7	2683	<u>186aa</u>	Nonsense mediated decay	8	D6RGR1	TSL:1		
Manba-208	ENSMUST00000140893.7	2534	No protein	Retained intron	2	ē.	TSL:1		
Manba-209	ENSMUST00000144654.1	2183	No protein	Retained intron	24	<u> </u>	TSL:1		
Manba-207	ENSMUST00000140496.7	535	No protein	Retained intron	5	5	TSL:2		
Manba-202	ENSMUST00000123061.1	638	No protein	IncRNA	*	-	TSL:5		
Manba-203	ENSMUST00000130147.7	634	No protein	IncRNA	24	9	TSL:3		
Manba-210	ENSMUST00000147872.7	604	No protein	IncRNA	20	<u> </u>	TSL:5		
Manba-206	ENSMUST00000134478.1	599	No protein	IncRNA	-	8	TSL:3		
Manba-211	ENSMUST00000153412.1	511	No protein	IncRNA	*	-	TSL:3		
Manba-205	ENSMUST00000134095.1	276	No protein	IncRNA	2	9	TSL:3		
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The strategy is based on the design of Manba-201 transcript, The transcription is shown below



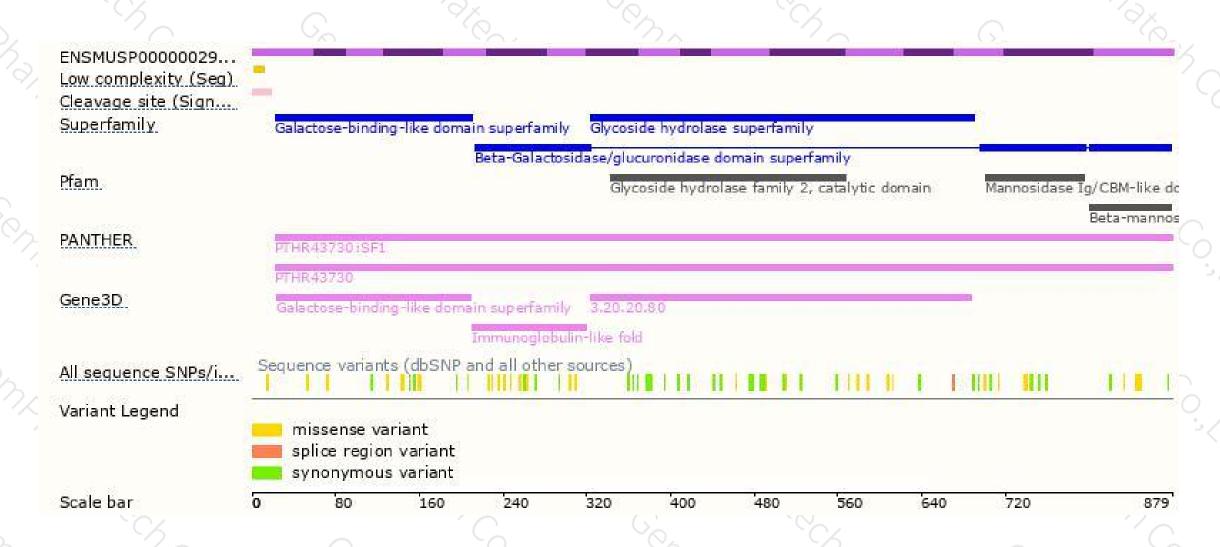
Genomic location distribution





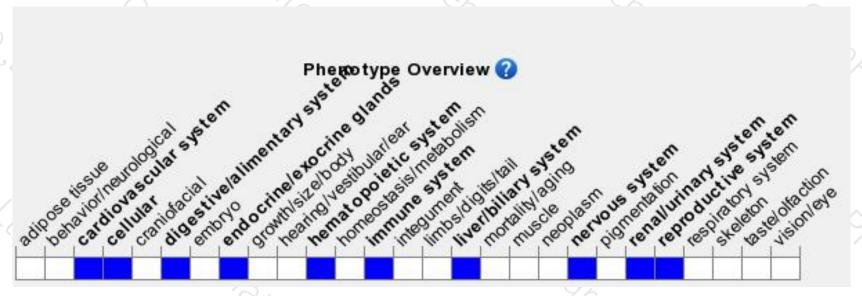
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Homozygous mutation results in no dysmorphology or overt neurological problems. Homozygotes show no beta-mannosidase activity and display consistent cytoplasmic vacuolation in the central nervous system and minimal vacuolation in most visceral organs.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





