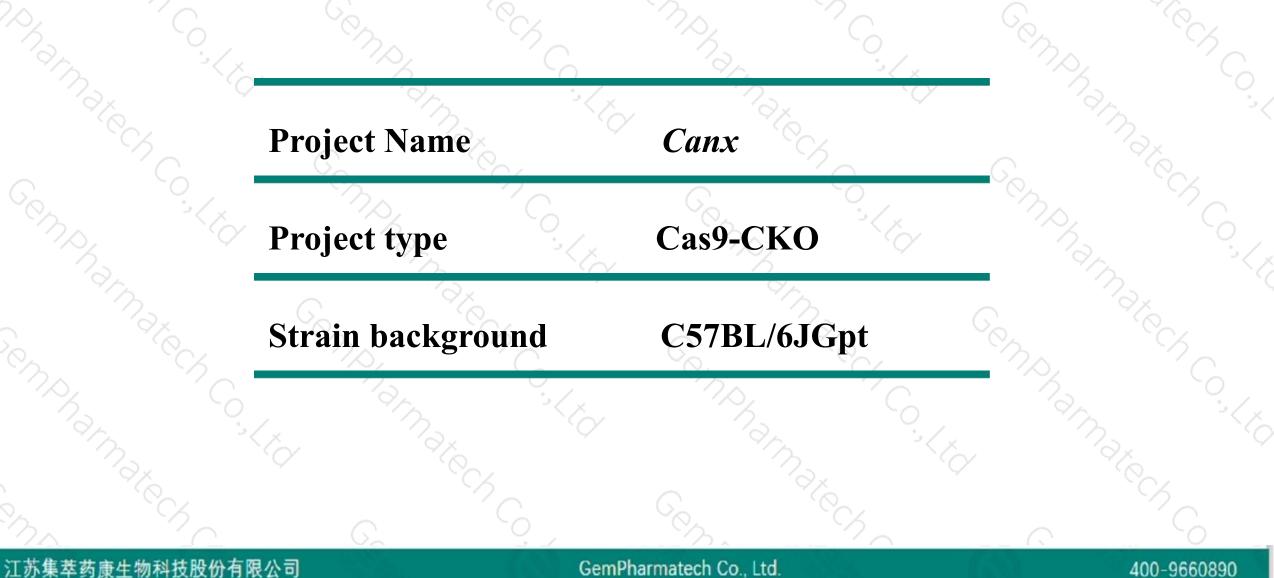


Canx Cas9-CKO Strategy

Designer: Reviewer: Design Date: Yang Zeng Jing Jin 2019-10-15

Project Overview





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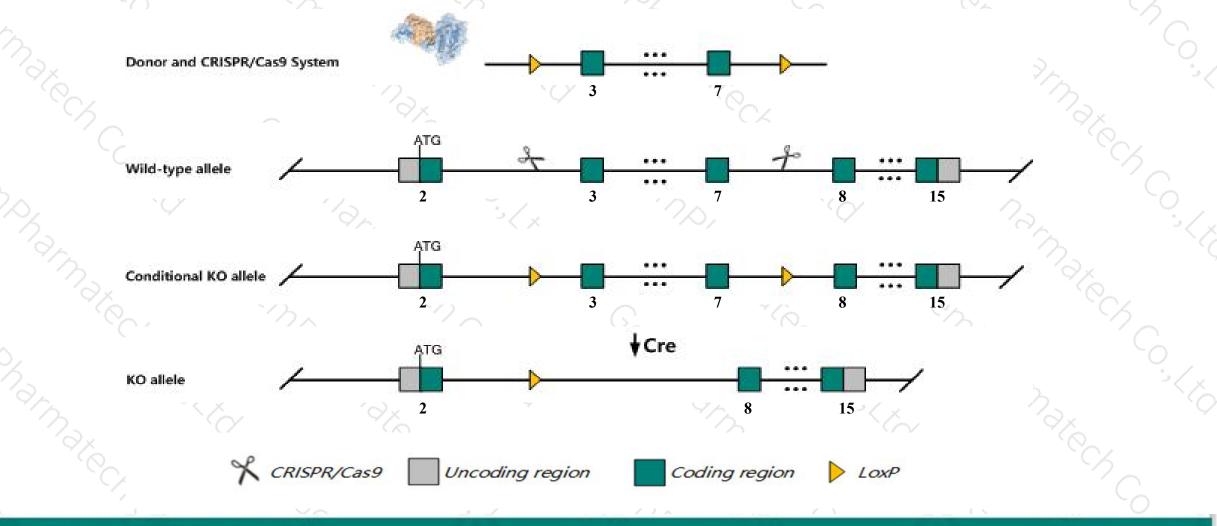
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Conditional Knockout strategy



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



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The Canx gene has 5 transcripts. According to the structure of Canx gene, exon3-exon7 of Canx-205 (ENSMUST00000179865.7) transcript is recommended as the knockout region. The region contains 550bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Canx* 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.



According to the existing MGI data, Homozygotes for a targeted null mutation exhibit motor defects, loss of large myelinated nerve fibers, small size, and very high mortality between birth and 4 weeks of age.

Transcript *Canx-202/203/204* lncRNA may not be affected.

The Canx 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.

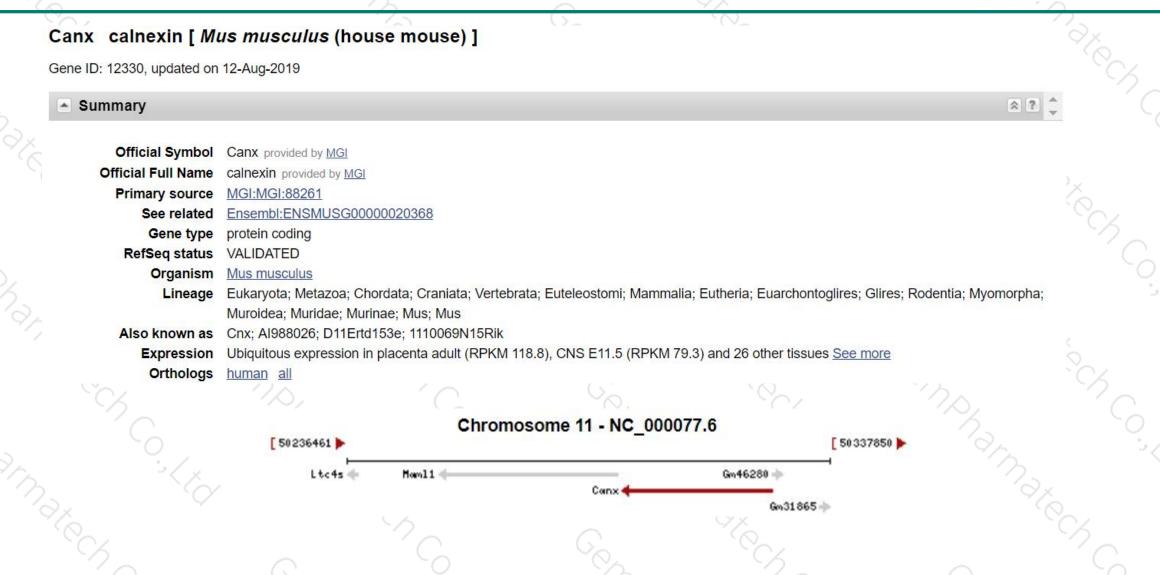
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Gene information (NCBI)





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Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

1		and the second		Press.							
Name 🍦	Transcript ID	bp 🝦 Protein 🖕		Translation ID	Biotype 🍦	CCDS	UniProt 🖕	Flags			
Canx-205	ENSMUST00000179865.7	4281	<u>591aa</u>	ENSMUSP00000137440.1	Protein coding	<u>CCDS24633</u> &	P35564 & Q5SUC3 &	TSL:1 GENCODE basic APPRIS P1			
Canx-201	ENSMUST00000020637.8	3779	<u>591aa</u>	ENSMUSP00000020637.8	Protein coding	CCDS24633	P35564 & Q5SUC3 &	TSL:1 GENCODE basic APPRIS P1			
Canx-202	ENSMUST00000146979.1	708	No protein	-	IncRNA	-	1.5	TSL:2			
Canx-204	ENSMUST00000155801.1	600	No protein	1070	IncRNA	(19 7 8)	1.51	TSL:3			
Canx-203	ENSMUST00000153068.1	234	No protein	12	IncRNA	12	12	TSL:5			

The strategy is based on the design of Canx-205 transcript, The transcription is shown below

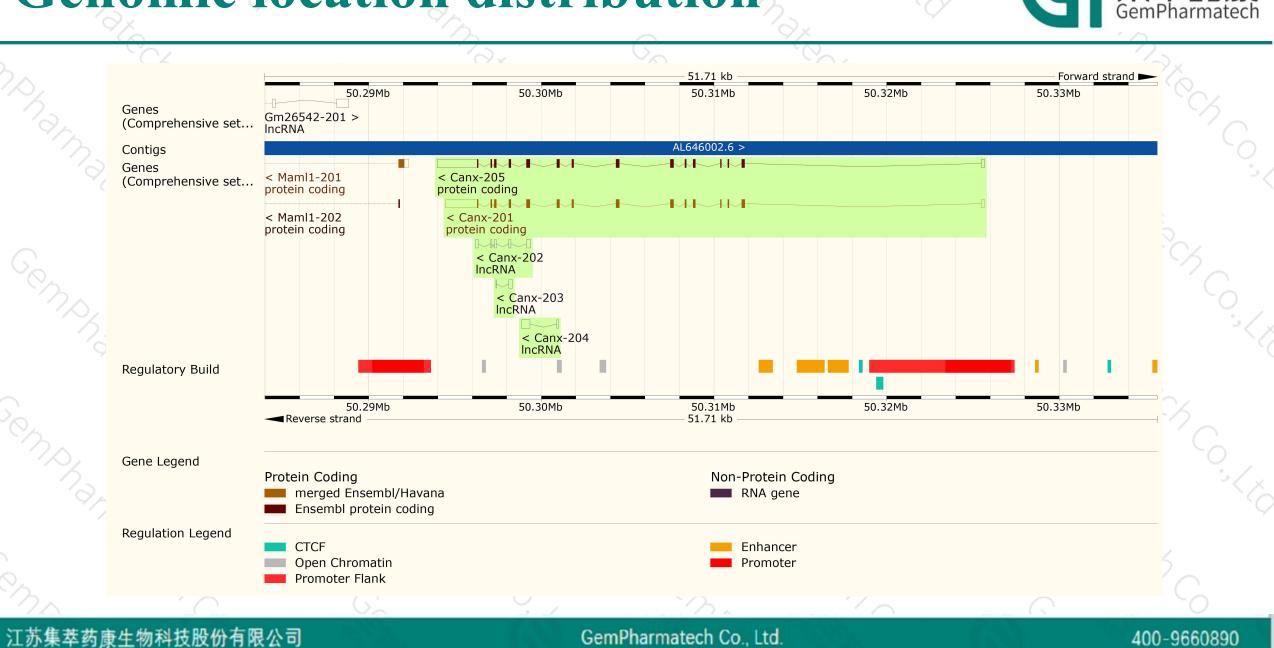
< Canx-205

protein coding

Reverse strand –

— 31.71 kb —

Genomic location distribution



Protein domain



Scale bar	0	60	120	180	240	300	360	420	480	10	591	
Variant Legend	sync	gained onymous variant	1.00				se variant					
All sequence SNPs/i	Sequence	2.60.120.2 e variants (dbSNP a	00 and all other sourc	es)		I	I	I	1 1	I	I I	
Gene3D	Calreticulir	n/calnexin			Calro	eticulin/calnexin, F	? domain superfamily					
PANTHER	PTHR1107	3:SF11			lin/calnexin, conserved	site						
PROSITE patterns		Calretic	ulin/calnexin	alreticulin/calnexin	, conserved site	Ca	Ireticulin/calnexin, co	nserved site				
Prints Pfam				nase domain super Calreticulin/calnexii					I			
ENSMUSP00000137 Transmembrane heli MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Cleavage site (Sign Superfamily		-			Calre	ticulin/calnexin, P	domain superfamily	_		=	=	0.0

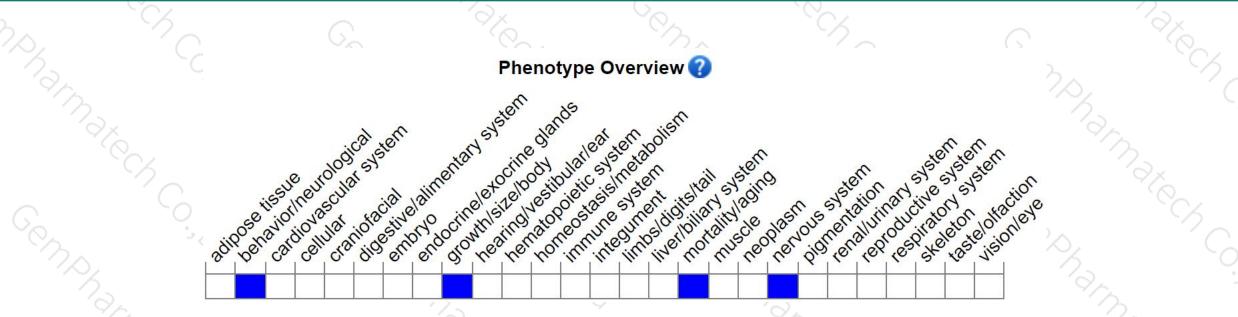
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Mouse phenotype description(MGI)





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, Homozygotes for a targeted null mutation exhibit motor defects, loss of large myelinated nerve fibers, small size, and very high mortality between birth and 4 weeks of age.



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



