

Dctn1 Cas9-CKO Strategy

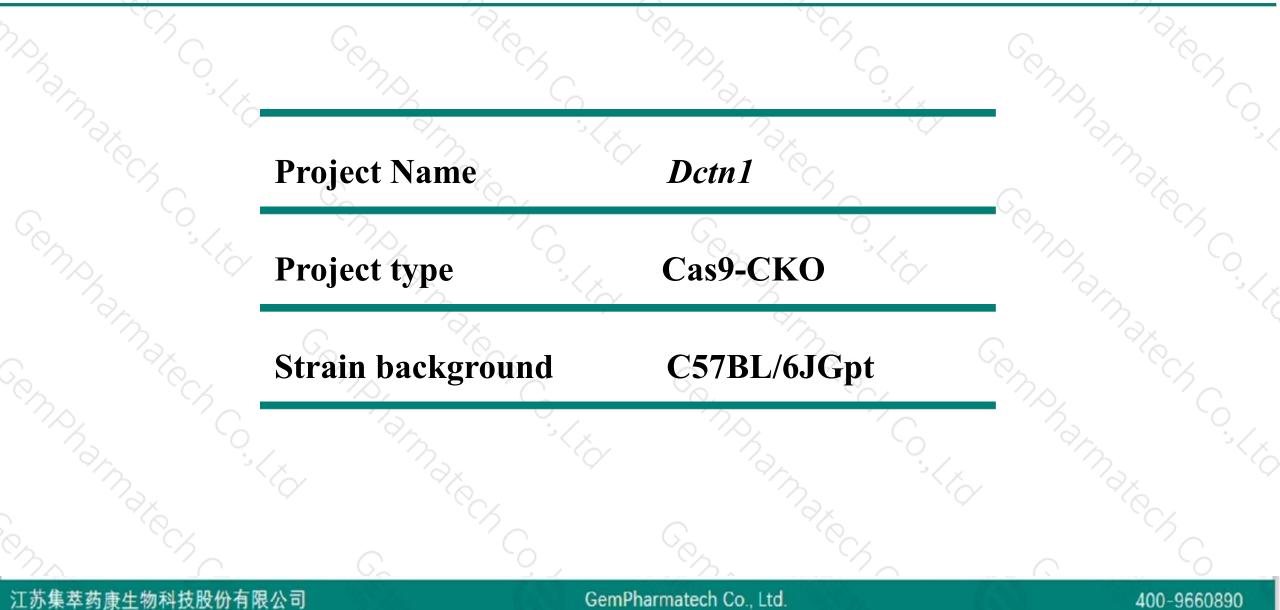
Designer: Huan Fan

Reviewer: Wenjing Li

Design Date: 2018/11/7

Project Overview

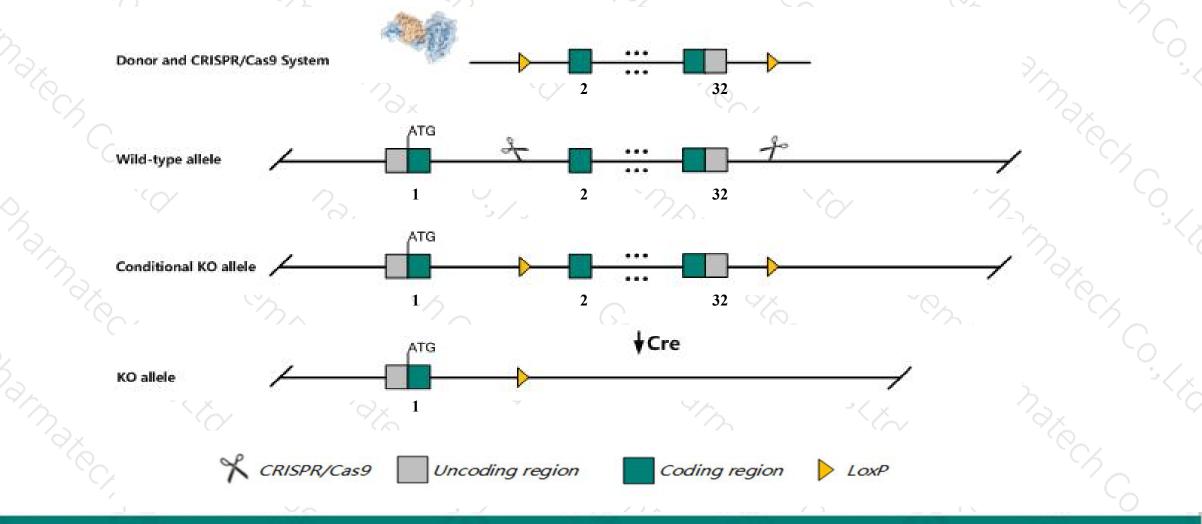




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Dctn1* gene. The schematic diagram is as follows:



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The Dctn1 gene has 12 transcripts. According to the structure of Dctn1 gene, exon2-exon32 of Dctn1-205(ENSMUST00000113919.9) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Dctn1* 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, mice homozygous for a null allele exhibit embryonic lethality and developmental arrest at E7.5 associated with increased apoptosis.
- The *Dctn1* gene is located on the Chr6. 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)



☆ ?

Dctn1 dynactin 1 [Mus musculus (house mouse)]

Gene ID: 13191, updated on 13-Mar-2020

- Summary

Official SymbolDctn1 provided by MGIOfficial Full Namedynactin 1 provided by MGIPrimary sourceMGI:MGI:107745See relatedEnsembl:ENSMUSG0000031865Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asAL022633, DAP-150, DP-150, Glued, p150, p150-glued, p150ExpressionUbiquitous expression in cerebellum adult (RPKM 36.9), frontal lobe adult (RPKM 32.3) and 28 other tissues
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Transcript information (Ensembl)



The gene has 12 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Dctn1-205	ENSMUST00000113919.9	4292	<u>1281aa</u>	Protein coding	CCDS39532	<u>008788</u>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P	1
Dctn1-203	ENSMUST00000113913.7	4163	<u>1264aa</u>	Protein coding	CCDS57432	E9Q3M3	TSL:1 GENCODE basic	7.
Dctn1-201	ENSMUST00000077407.11	4118	<u>1239aa</u>	Protein coding	CCDS57433	E9Q586	TSL:1 GENCODE basic	10
Dctn1-204	ENSMUST00000113918.7	4321	<u>1243aa</u>	Protein coding	25	<u>008788</u>	TSL:5 GENCODE basic	
Dctn1-202	ENSMUST00000113907.1	4205	<u>1142aa</u>	Protein coding	51	D3YX34	TSL:5 GENCODE basic	
Dctn1-210	ENSMUST00000141680.7	696	<u>159aa</u>	Protein coding		D3Z2M9	CDS 3' incomplete TSL:5	
Dctn1-208	ENSMUST00000130212.7	652	<u>189aa</u>	Protein coding	2	D3YYG9	CDS 3' incomplete TSL:3	
Dctn1-211	ENSMUST00000153793.1	839	No protein	Retained intron	25	1028	TSL:3	
Dctn1-206	ENSMUST00000123313.1	718	No protein	Retained intron	-		TSL:1	
Dctn1-209	ENSMUST00000130917.1	717	No protein	Retained intron	-	3.83	TSL:1	
Dctn1-207	ENSMUST00000127824.7	539	No protein	Retained intron	-	120	TSL:2	
Dctn1-212	ENSMUST00000154420.1	481	No protein	Retained intron	26	100	TSL:1	

20.39 kb

The strategy is based on the design of *Dctn1-205* transcript, the transcription is shown below:

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Dctn1-205 > protein coding

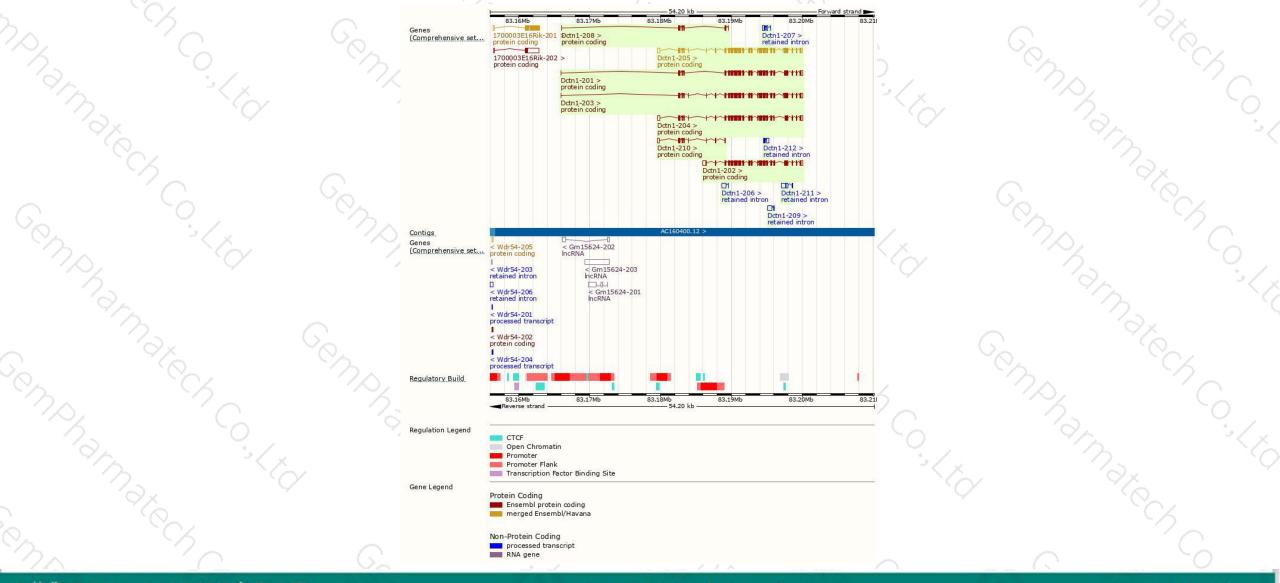
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Forward strand

Genomic location distribution





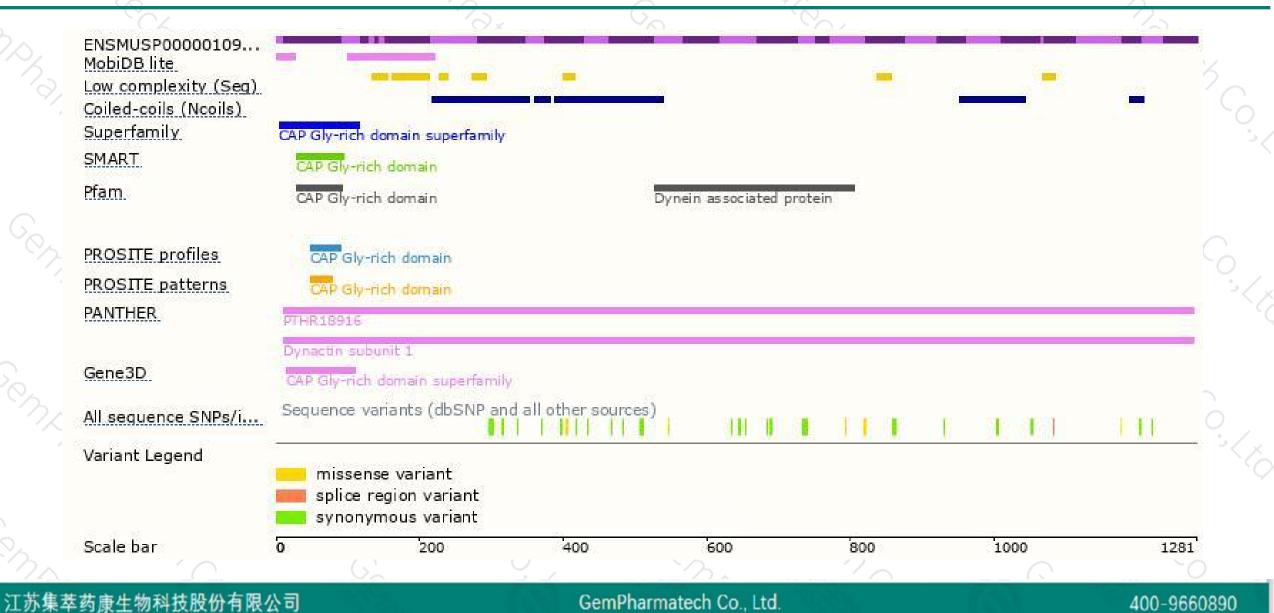
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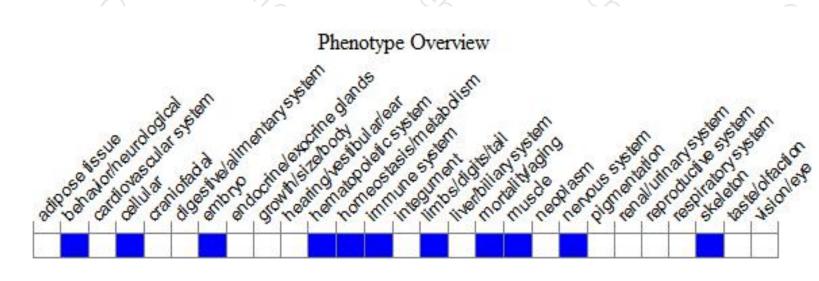
Protein domain





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, mice homozygous for a null allele exhibit embryonic lethality and developmental arrest at E7.5 associated with increased apoptosis.



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



