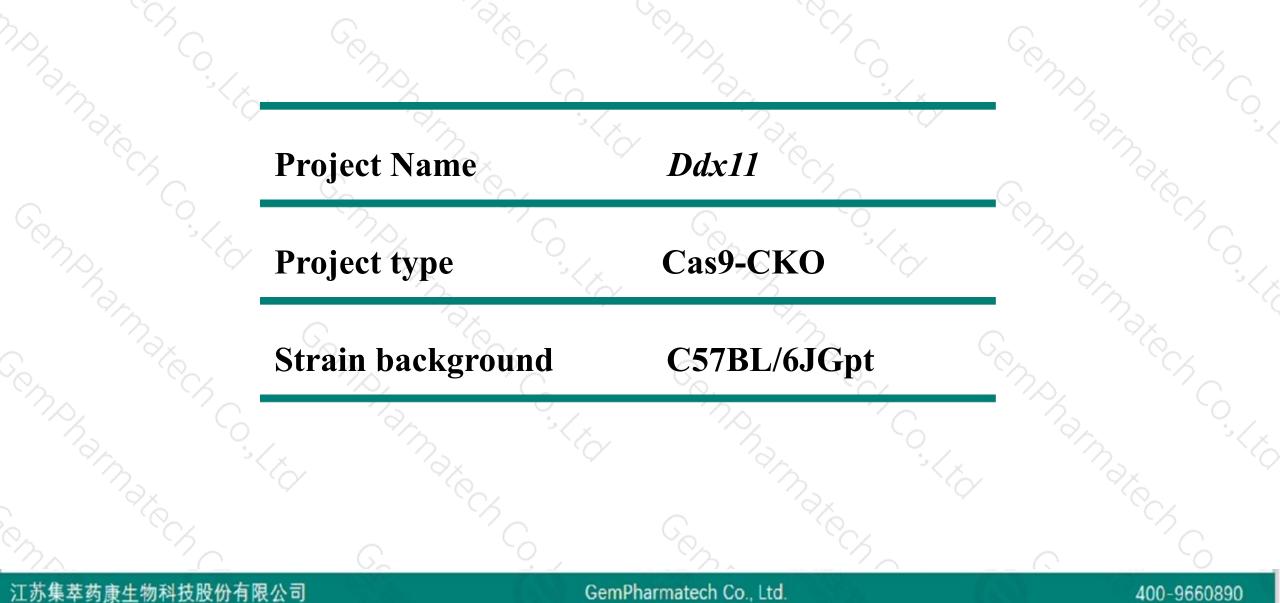


# Ddx11 Cas9-CKO Strategy

Designer:Xiaojing Li Reviewer:JiaYu Design Date:2020-3-12

## **Project Overview**



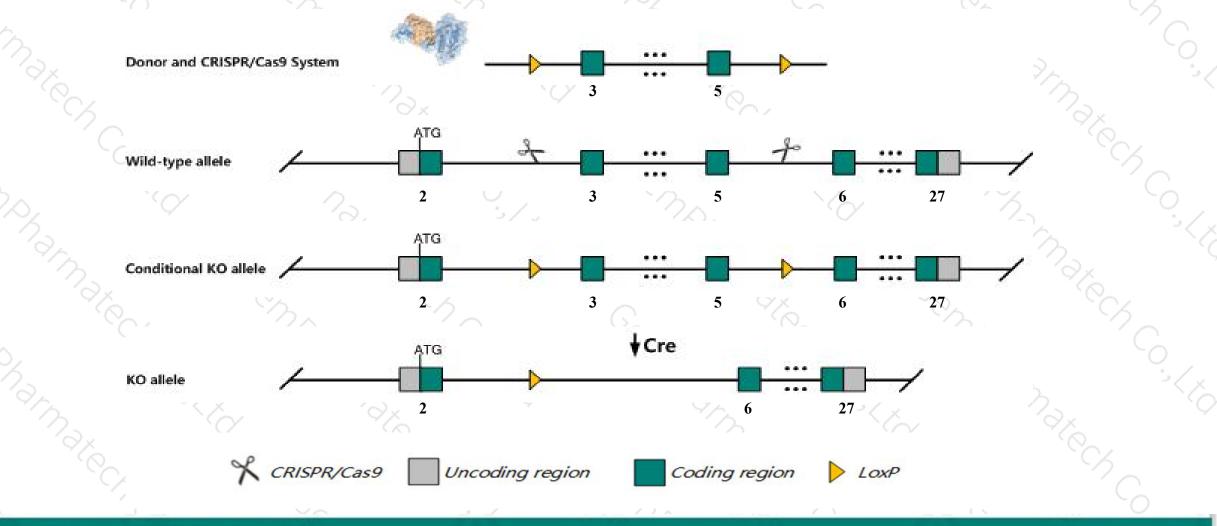


## **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Ddx11* gene. The schematic diagram is as follows:



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The Ddx11 gene has 9 transcripts. According to the structure of Ddx11 gene, exon3-exon5 of Ddx11-201 (ENSMUST00000163605.2) transcript is recommended as the knockout region. The region contains 410bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ddx11* 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 lethality before E11.5 with growth retardation, failure of chorioallantoic fusion, poor placental labyrinth development, and embryonic cell physiology.
- > The Ddx11 gene is located on the Chr17. 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)



### Ddx11 DEAD/H (Asp-Glu-Ala-Asp/His) box helicase 11 [ Mus musculus (house mouse) ]

Gene ID: 320209, updated on 27-Feb-2020

### Summary

Official Symbol Ddx11 provided by MGI Official Full Name DEAD/H (Asp-Glu-Ala-Asp/His) box helicase 11 provided by MGI MGI:MGI:2443590 Primary source Ensembl:ENSMUSG0000035842 See related protein coding Gene type **RefSeg status** VALIDATED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Lineage Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as CHL1; KRG2; CHLR1; essa15a; 4732462I11Rik Ubiguitous expression in CNS E11.5 (RPKM 7.2), limb E14.5 (RPKM 5.7) and 27 other tissues See more Expression Orthologs human all

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## **Transcript information (Ensembl)**



### The gene has 9 transcripts, all transcripts are shown below:

		L. A.				I have
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000163605.2	4149	<u>880aa</u>	Protein coding	CCDS28945	A0A2Y9CZM3	TSL:5 GENCODE basic APPRIS P2
ENSMUST00000224497.1	4141	<u>906aa</u>	Protein coding	6 <del>7</del> 9	Q6AXC6	GENCODE basic APPRIS ALT2
ENSMUST00000225956.1	681	<u>168aa</u>	Protein coding	144	A0A286YCB9	CDS 3' incomplete
ENSMUST00000224903.1	3520	<u>393aa</u>	Nonsense mediated decay	10 <u>1</u> 0	A0A286YDY6	
ENSMUST00000225687.1	4480	No protein	Retained intron	1753	7	
ENSMUST00000223801.1	805	No protein	Retained intron	6731		
ENSMUST00000223805.1	662	No protein	Retained intron	144	-	
ENSMUST00000226095.1	618	No protein	Retained intron	( <u>1</u> 2)	2	
ENSMUST00000223600.1	576	No protein	Retained intron	1751	17	
	ENSMUST00000163605.2 ENSMUST00000224497.1 ENSMUST00000225956.1 ENSMUST00000224903.1 ENSMUST00000225687.1 ENSMUST00000223801.1 ENSMUST00000223805.1	ENSMUST0000163605.2 4149   ENSMUST0000224497.1 4141   ENSMUST0000225956.1 681   ENSMUST0000224903.1 3520   ENSMUST0000225687.1 4480   ENSMUST0000223801.1 805   ENSMUST0000223805.1 662   ENSMUST0000226095.1 618	ENSMUST00000163605.2 4149 880aa   ENSMUST00000224497.1 4141 906aa   ENSMUST00000225956.1 681 168aa   ENSMUST00000224903.1 3520 393aa   ENSMUST00000225687.1 4480 No protein   ENSMUST00000223801.1 805 No protein   ENSMUST00000223805.1 662 No protein	ENSMUST00000163605.24149880aaProtein codingENSMUST0000224497.14141906aaProtein codingENSMUST0000225956.1681168aaProtein codingENSMUST0000224903.13520393aaNonsense mediated decayENSMUST0000223801.1805No proteinRetained intronENSMUST0000223805.1662No proteinRetained intronENSMUST0000223805.1662No proteinRetained intronENSMUST0000223805.1662No proteinRetained intronENSMUST0000223805.1618No proteinRetained intron	ENSMUST000016360524149880aaProtein codingCCDS28945ENSMUST0000224497.14141906aaProtein coding-ENSMUST0000225956.1681168aaProtein coding-ENSMUST0000224903.13520393aaNonsense mediated decay-ENSMUST0000223801.1805No proteinRetained intron-ENSMUST0000223805.1662No proteinRetained intron-ENSMUST0000223805.1662No proteinRetained intron-ENSMUST0000223805.1618No proteinRetained intron-	ENSMUST0000016360524149880aaProtein codingCCDS28945A0A2Y9CZM3ENSMUST0000224497.14141906aaProtein coding-Q6AXC6ENSMUST0000225956.1681168aaProtein coding-A0A286YCB9ENSMUST0000224903.13520393aaNonsense mediated decay-A0A286YDY6ENSMUST0000225687.14480No proteinRetained intronENSMUST0000223801.1805No proteinRetained intronENSMUST0000226095.1662No proteinRetained intronENSMUST0000223805.1618No proteinRetained intron

The strategy is based on the design of Ddx11-201 transcript, The transcription is shown below

U Ddx11-201 > protein coding

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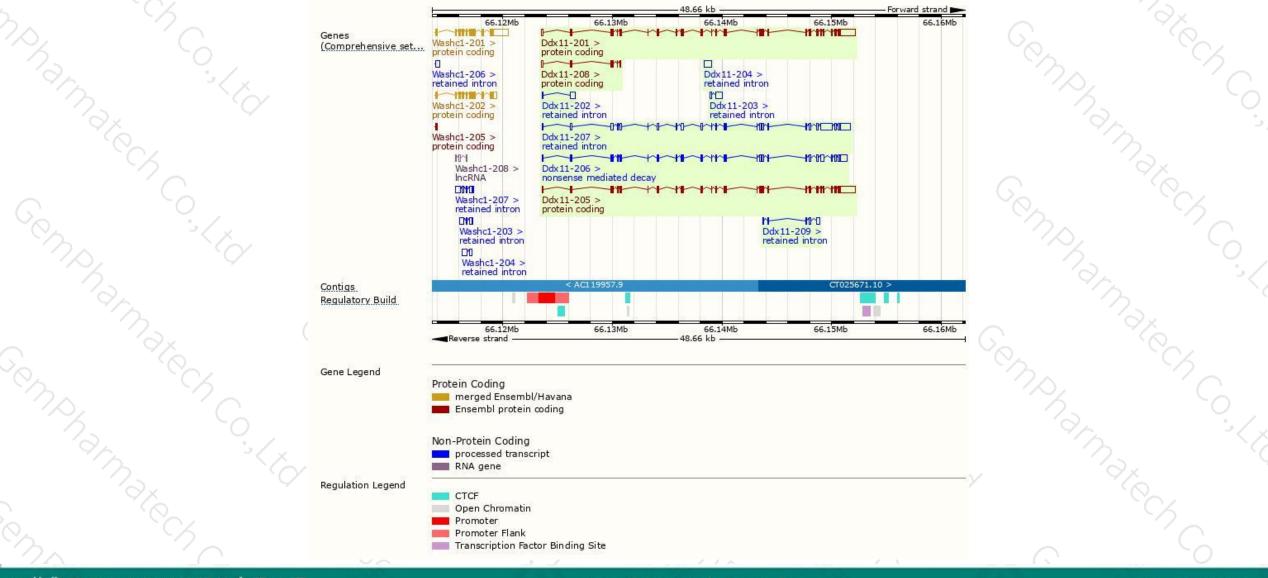
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### 400-9660890

Forward strand

## **Genomic location distribution**





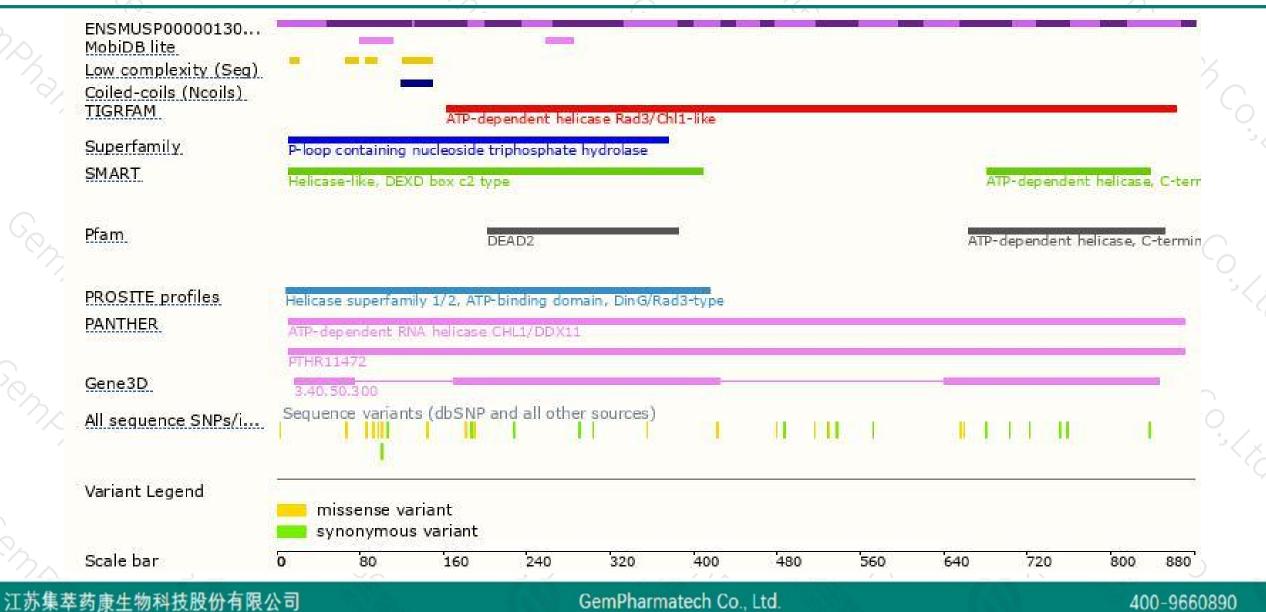
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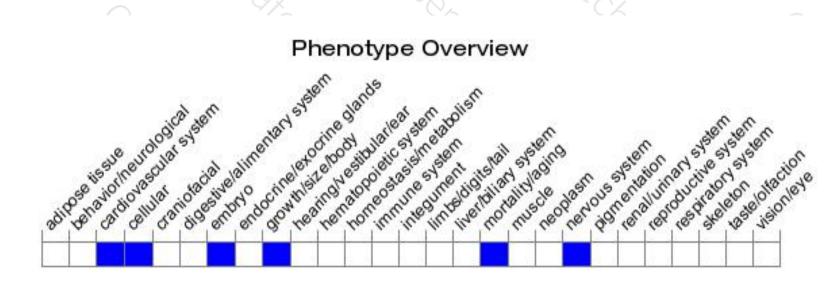
### **Protein domain**





## 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 lethality before E11.5 with growth retardation, failure of chorioallantoic fusion, poor placental labyrinth development, and embryonic cell physiology.



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



