

# Med24 Cas9-CKO Strategy

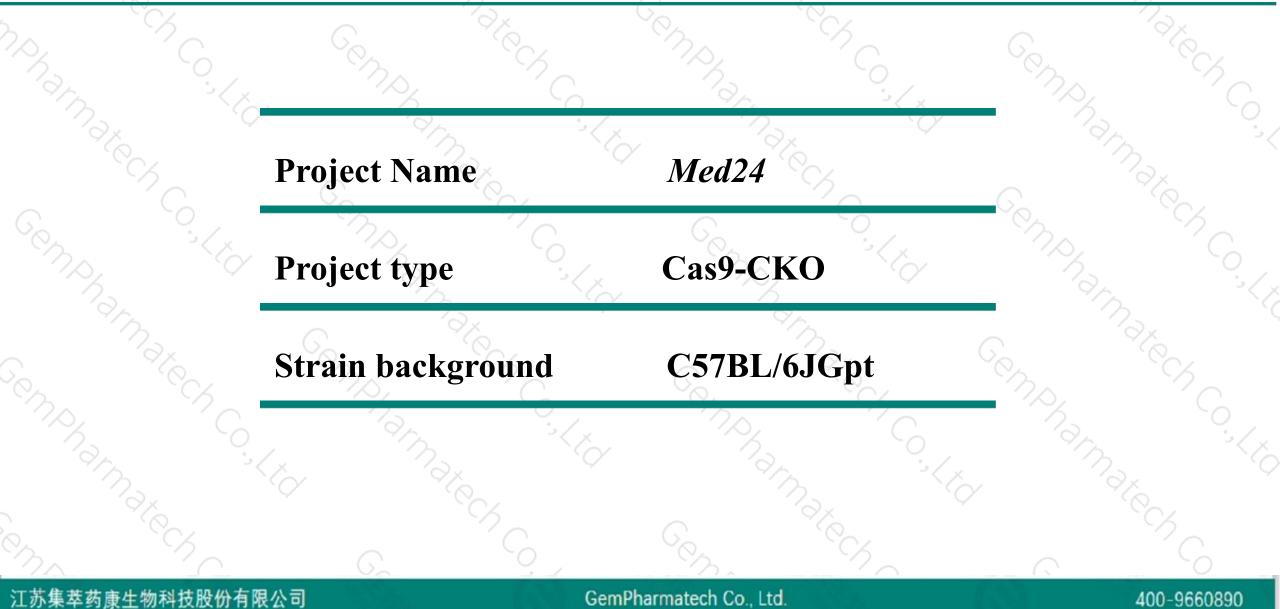
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-9-16

# **Project Overview**



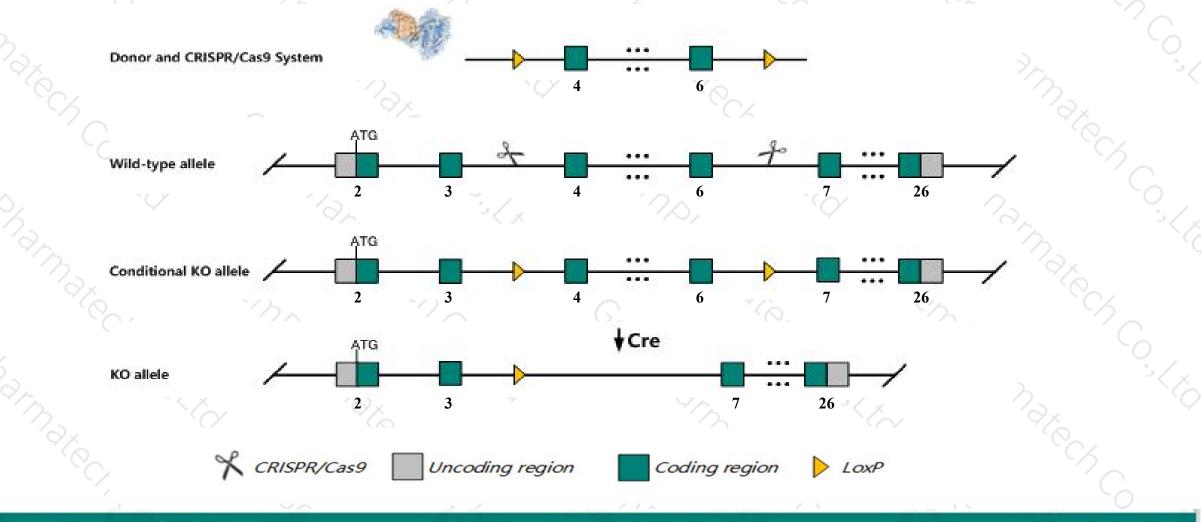


## **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the Med24 gene. The schematic diagram is as follows:



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The Med24 gene has 12 transcripts. According to the structure of Med24 gene, exon4-exon6 of Med24-201 (ENSMUST00000017354.12) transcript is recommended as the knockout region. The region contains 346bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Med24* 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, Homozygous mutant mice die prior to birth exhibiting abnormal heart development, neural tube defects, and anemia.
- The *Med24* 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)



#### Med24 mediator complex subunit 24 [Mus musculus (house mouse)]

Gene ID: 23989, updated on 3-Feb-2019

#### Summary

Muroidea; Muridae; M Also known as 911GSE, AU040102, Summary This gene encodes a complex thought to be	ounit 24 provided by <u>MGI</u> 0000017210
Primary sourceMGI:MGI:1344385See relatedEnsembl:ENSMUSGOGene typeprotein codingRefSeq statusREVIEWEDOrganismMus musculusLineageEukaryota; Metazoa; OAlso known as911GSE, AU040102,SummaryThis gene encodes acomplex thought to be	0000017210
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Summary This gene encodes a complex thought to be	urinae; Mus; Mus
complex thought to be	AW547152, D11Ertd307e, DRIP100, Gse2, Pparb2, Pparbp2, R75526, Thrap4, Trap100
	component of the mediator complex (also known as TRAP, SMCC, DRIP, or ARC), a transcriptional coactivator e required for the expression of almost all genes. The mediator complex is recruited by transcriptional activators or duce gene expression, possibly by interacting with RNA polymerase II and promoting the formation of a transcriptional . The product of this gene may form a submodule of the mediator complex that magnifies the effects of activators on the nachinery. Alternatively spliced transcript variants of this gene have been described, but their full-length nature is not RefSeq, Jul 2008]
Expression Ubiquitous expression	in thymus adult (RPKM 41.8), testis adult (RPKM 39.3) and 28 other tissues <u>See more</u>
Orthologs <u>human</u> all	

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# **Transcript information (Ensembl)**



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

Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000017354.12	3410	<u>987aa</u>	Protein coding	CCDS25361	<u>Q99K74</u>	TSL:1 GENCODE basic APPRIS P2
ENSMUST00000100500.8	3133	<u>1006aa</u>	Protein coding		<u>A6PW47</u>	TSL:5 GENCODE basic APPRIS ALT2
ENSMUST00000126565.1	416	<u>90aa</u>	Protein coding	-	A6PW46	CDS 3' incomplete TSL:5
ENSMUST00000138750.7	3165	<u>70aa</u>	Nonsense mediated decay	12	<u>F6XX22</u>	TSL:1
ENSMUST00000125064.7	4577	No protein	Retained intron	· · · · · · · · · · · · · · · · · · ·	7.4	TSL:1
ENSMUST00000137328.7	2830	No protein	Retained intron		-	TSL:1
ENSMUST00000156378.1	760	No protein	Retained intron	-	20	TSL:5
ENSMUST00000144720.1	685	No protein	Retained intron	12	20	TSL:2
ENSMUST00000144048.1	537	No protein	Retained intron	17	7.0	TSL:1
ENSMUST00000124371.1	478	No protein	Retained intron		-	TSL:3
ENSMUST00000141566.1	453	No protein	IncRNA	-	20	TSL:3
ENSMUST00000139849.1	370	No protein	IncRNA	10 A	20	TSL:3
	ENSMUST0000017354.12 ENSMUST00000100500.8 ENSMUST00000126565.1 ENSMUST00000138750.7 ENSMUST00000125064.7 ENSMUST00000137328.7 ENSMUST00000156378.1 ENSMUST00000144720.1 ENSMUST00000144048.1	ENSMUST0000017354.12 3410   ENSMUST00000126565.1 3133   ENSMUST00000126565.1 416   ENSMUST00000138750.7 3165   ENSMUST00000125064.7 4577   ENSMUST00000156378.1 2830   ENSMUST00000144720.1 685   ENSMUST00000144720.1 537   ENSMUST00000124371.1 478   ENSMUST00000144566.1 453	ENSMUST000001735412 3410 987aa   ENSMUST000001265651 3133 1006aa   ENSMUST00001265651 416 90aa   ENSMUST00001387507 3165 70aa   ENSMUST00001250647 4577 No protein   ENSMUST00001563781 760 No protein   ENSMUST00001447201 685 No protein   ENSMUST00001447201 537 No protein   ENSMUST00001447201 478 No protein   ENSMUST00001443711 478 No protein	ENSMUST0000017354.123410987aaProtein codingENSMUST0000017354.1231331006aaProtein codingENSMUST0000126565.141690aaProtein codingENSMUST0000138750.7316570aaNonsense mediated decayENSMUST0000125064.74577No proteinRetained intronENSMUST0000137328.72830No proteinRetained intronENSMUST0000144720.1685No proteinRetained intronENSMUST0000144720.1685No proteinRetained intronENSMUST0000144720.1478No proteinRetained intronENSMUST0000144048.1537No proteinRetained intronENSMUST0000144048.1478No proteinIntelined intronENSMUST0000144556.1453No proteinIntelined intron	ENSMUST0000017354123410987aaProtein codingCCDS25361ENSMUST0000010500831331006aaProtein coding-ENSMUST0000126565.141690aaProtein coding-ENSMUST0000138750.7316570aaNonsense mediated decay-ENSMUST0000125064.74577No proteinRetained intron-ENSMUST0000137328.72830No proteinRetained intron-ENSMUST0000137328.72830No proteinRetained intron-ENSMUST0000144720.1685No proteinRetained intron-ENSMUST0000144048.1537No proteinRetained intron-ENSMUST0000144048.1478No proteinRetained intron-ENSMUST000014405.1478No proteinRetained intron-ENSMUST0000144048.1537No proteinRetained intron-ENSMUST000014405.1478No proteinRetained intron-ENSMUST000014405.1478No proteinRetained intron-ENSMUST000014405.1478No proteinRetained intron-ENSMUST000014405.1478No proteinIncRNA-	Image: ConstructionImage: ConstructionImage: ConstructionENSMUST0000017354.129410987aaProtein codingCCDS25361099K74ENSMUST000012656.1331331006aaProtein codingImage: ConstructionA6PW47ENSMUST000012656.1441690aaProtein codingImage: ConstructionA6PW46ENSMUST0000125064.7316570aaNonsense mediated decayImage: ConstructionF6XX22ENSMUST0000125064.74577No proteinRetained intronImage: ConstructionImage: ConstructionImage: ConstructionENSMUST0000156378.13760No proteinRetained intronImage: ConstructionImage: ConstructionImage: ConstructionENSMUST000014404.15685No proteinRetained intronImage: ConstructionImage: ConstructionImage: ConstructionENSMUST0000124371.1478No proteinRetained intronImage: ConstructionImage: ConstructionImage: ConstructionENSMUST00001440564573No proteinRetained intronImage: ConstructionImage: ConstructionImage: ConstructionENSMUST0000124371.1478No proteinRetained intronImage: ConstructionImage: ConstructionImage: ConstructionENSMUST00001415664573No proteinImage: ConstructionImage: ConstructionImage: ConstructionImage: ConstructionENSMUST0000112556.134573No proteinImage: ConstructionImage: ConstructionImage: ConstructionImage: ConstructionE

The strategy is based on the design of Med24-201 transcript, The transcription is shown below

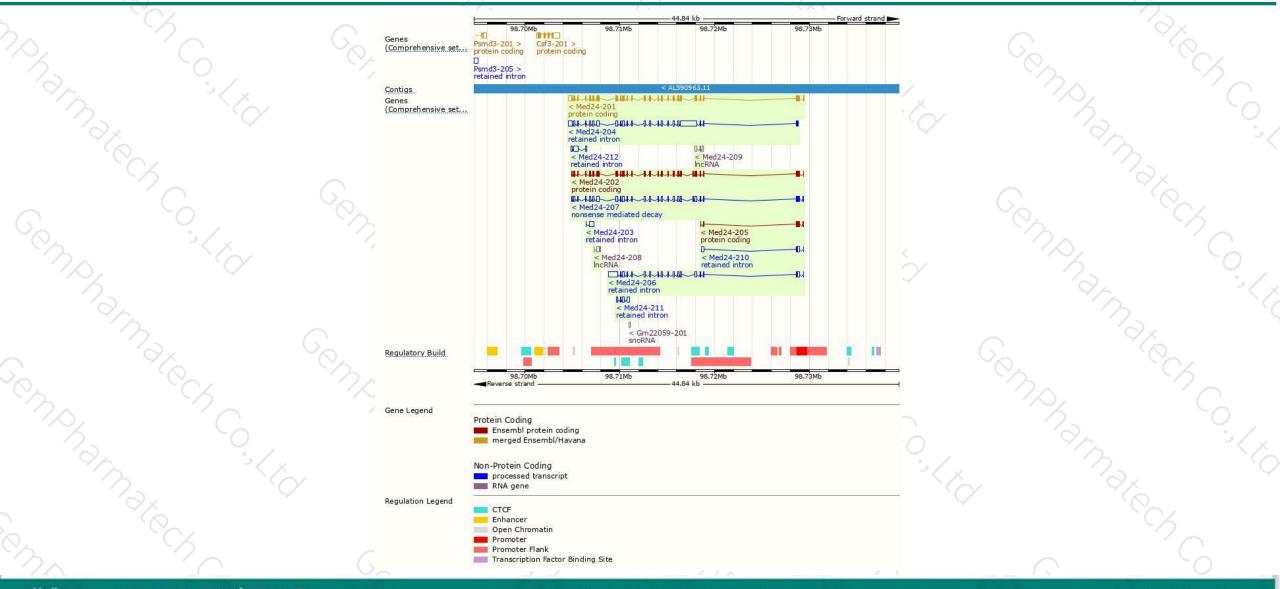
< Med24-201 protein coding

Reverse strand

- 24.84 kb -

### **Genomic location distribution**





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### **Protein domain**



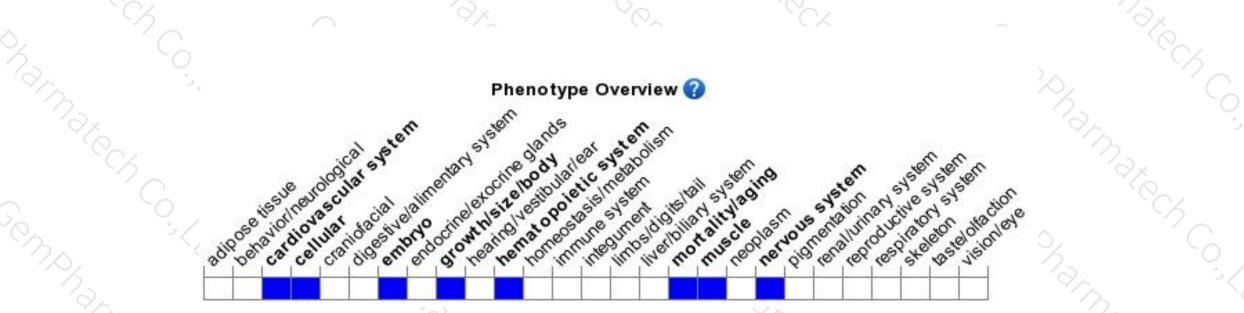


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## Mouse phenotype description(MGI)





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, Homozygous mutant mice die prior to birth exhibiting abnormal heart development, neural tube defects, and anemia.



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



