

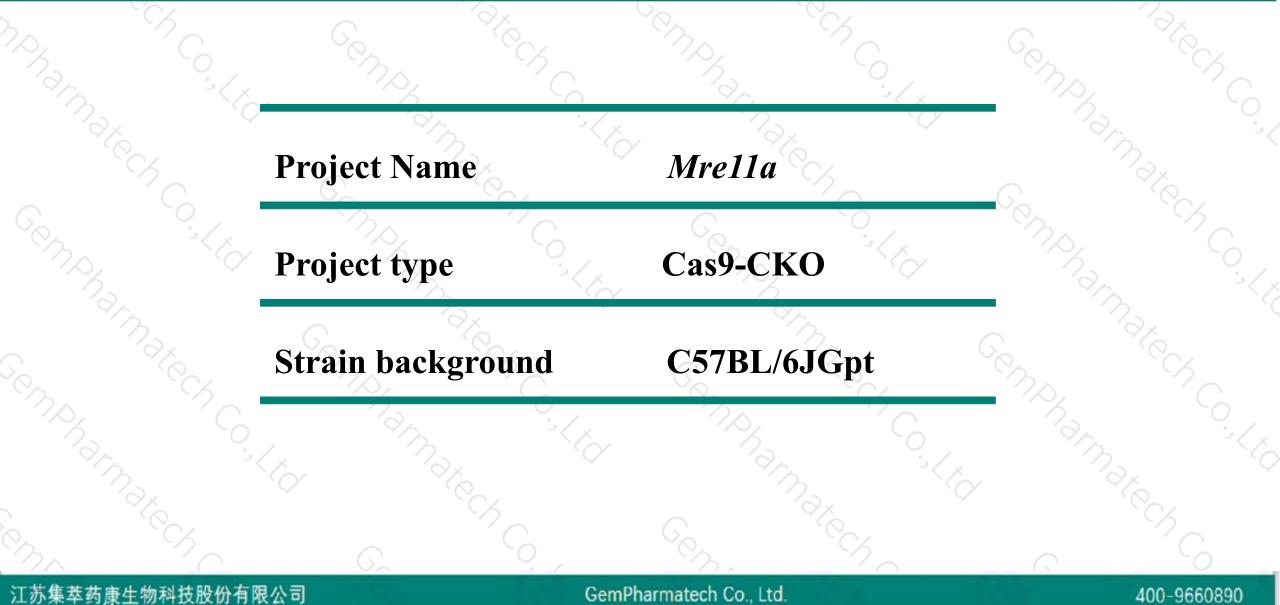
# Emphamated, C. Mrella Cas9-CKO Strategy Romphamater Control

"MBharmarer Comphannated Co. JiaYu

enphamatech (

# **Project Overview**

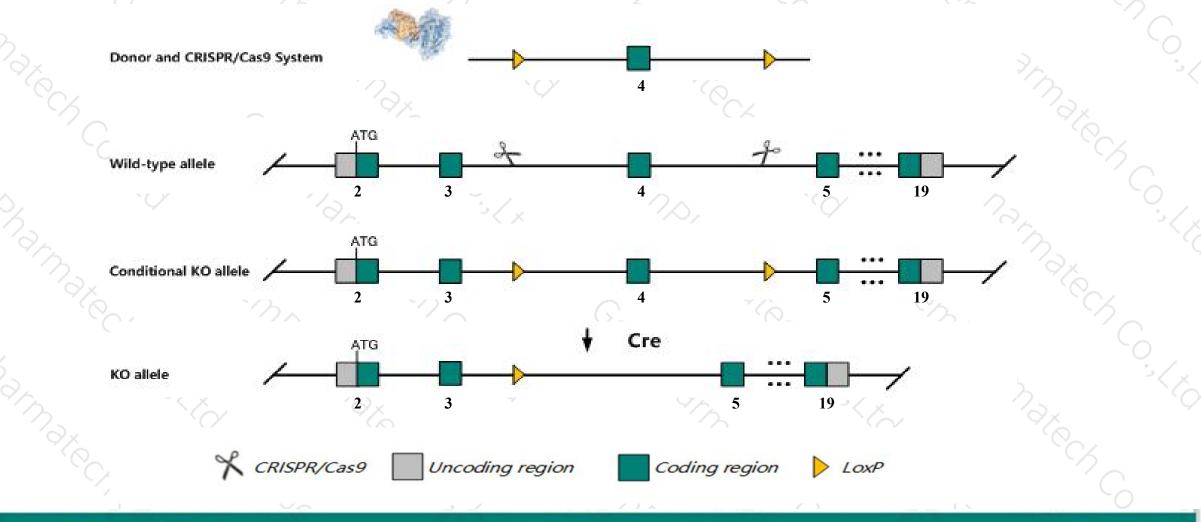




# **Conditional Knockout strategy**



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



江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

400-9660890



The Mrella gene has 6 transcripts. According to the structure of Mrella gene, exon4 of Mrella-201 (ENSMUST00000034405.10) transcript is recommended as the knockout region. The region contains 161bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Mre11a* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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, Though mutation of this locus affected chromosome stability, mutant mice were no more susceptible to tumorigenesis than wild-type mice. Mutant female mice showed reduced fertility.
  - The *Mrella* gene is located on the Chr9. 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)**



\$ ?

Mre11a MRE11A homolog A, double strand break repair nuclease [Mus musculus (house mouse)]

Gene ID: 17535, updated on 7-Apr-2019

#### Summary

Official Symbol	Mre11a provided by MGI
Official Full Name	MRE11A homolog A, double strand break repair nuclease provided by MGI
<b>Primary source</b>	MGI:MGI:1100512
See related	Ensembl:ENSMUSG0000031928
Gene type	protein coding
<b>RefSeq status</b>	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	Mre11, Mre11b
Expression	Ubiquitous expression in CNS E11.5 (RPKM 5.2), liver E14 (RPKM 3.7) and 28 other tissues See more
Orthologs	human all

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890

# **Transcript information (Ensembl)**



#### The gene has 6 transcripts, all transcripts are shown below:

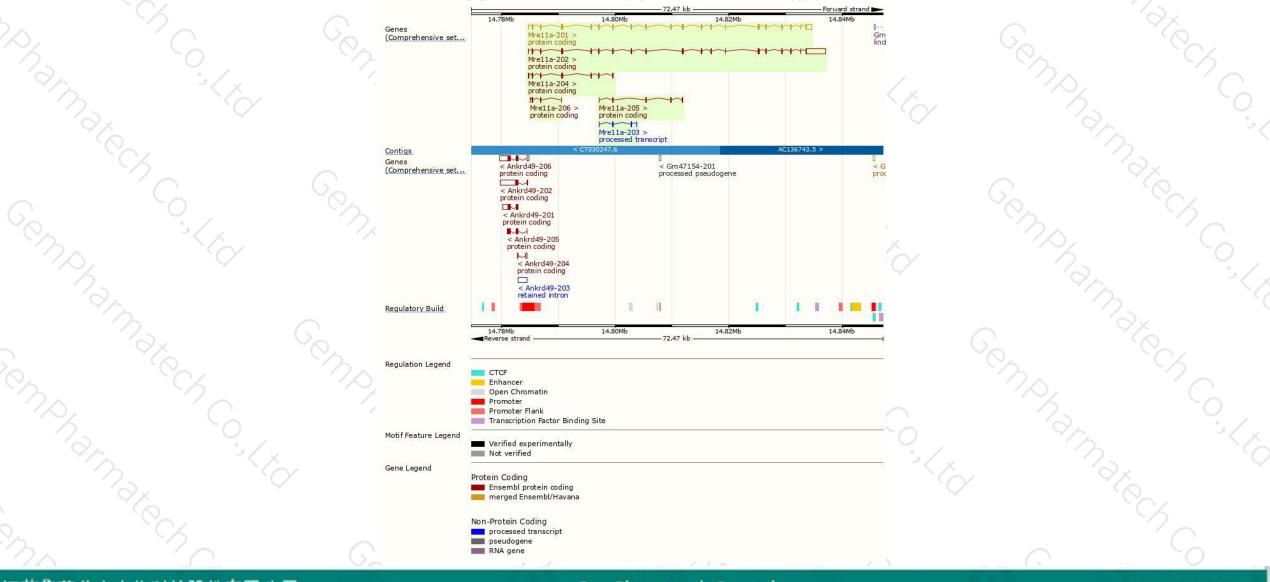
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000115632.9	5660	<u>679aa</u>	Protein coding	CCDS80958	<u>Q61216</u>	TSL:1 GENCODE basic
ENSMUST0000034405.10	3286	<u>706aa</u>	Protein coding	CCDS22827	Q3URU4 Q61216	TSL:1 GENCODE basic APPRIS P1
ENSMUST00000147305.1	793	<u>203aa</u>	Protein coding	2	<u>B2KF76</u>	CDS 3' incomplete TSL:3
ENSMUST00000147676.7	510	<u>170aa</u>	Protein coding	-	<u>F6RX99</u>	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
ENSMUST00000215820.1	274	<u>57aa</u>	Protein coding	-	A0A1L1SQT7	CDS 3' incomplete TSL:5
ENSMUST00000136568.1	409	No protein	Processed transcript	-	-	TSL:5
	ENSMUST00000115632.9 ENSMUST0000034405.10 ENSMUST00000147305.1 ENSMUST00000147676.7 ENSMUST00000215820.1	ENSMUST00000115632.9 5660   ENSMUST0000034405.10 3286   ENSMUST00000147305.1 793   ENSMUST00000147676.7 510   ENSMUST00000215820.1 274	ENSMUST00000115632.9 5660 679aa   ENSMUST0000034405.10 3286 706aa   ENSMUST00000147305.1 793 203aa   ENSMUST00000147676.7 510 170aa   ENSMUST00000215820.1 274 57aa	Transcript IDbpProteinBiotypeENSMUST00000115632.95660679aaProtein codingENSMUST0000034405.103286706aaProtein codingENSMUST00000147305.1793203aaProtein codingENSMUST00000147676.7510170aaProtein codingENSMUST00000147676.727457aaProtein coding	Transcript IDbpProteinBiotypeCCDSENSMUST00000115632.95660679aaProtein codingCCDS80958ENSMUST0000034405.103286706aaProtein codingCCDS22827ENSMUST00000147305.1793203aaProtein coding-ENSMUST00000147676.7510170aaProtein coding-ENSMUST00000215820.127457aaProtein coding-	Transcript IDbpProteinBiotypeCCDSUniProtENSMUST00000115632.95660679aaProtein codingCCDS80958Q61216ENSMUST0000034405.103286706aaProtein codingCCDS22827Q3URU4 Q61216ENSMUST00000147305.1793203aaProtein coding-B2KF76ENSMUST00000147676.7510170aaProtein coding-F6RX99ENSMUST00000215820.127457aaProtein coding-A0A1L1SQT7

The strategy is based on the design of Mre11a-201 transcript, The transcription is shown below

Mre11a-201 >

### **Genomic location distribution**





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

# **Protein domain**



400-9660890

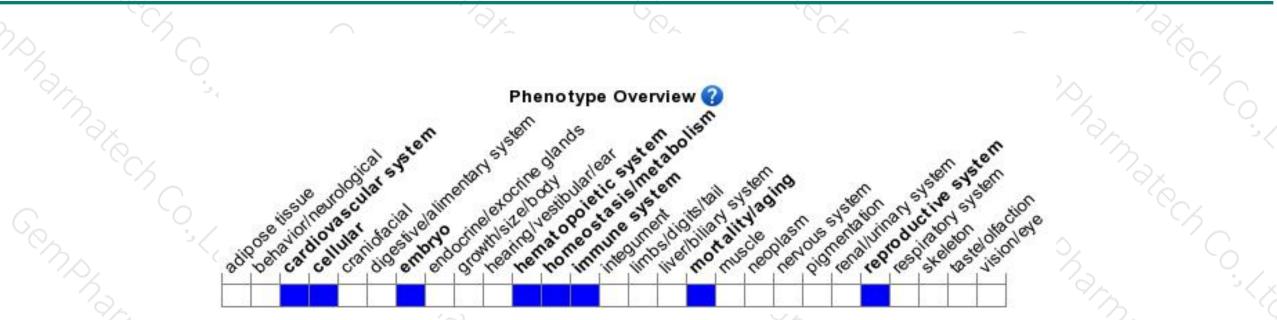
NSMUSP0000034										_	
obiDB lite w complexity (Seg) onserved Domains				-					_	-	
piled-coils (Ncoils) mmpanth <del>er</del>	PTHR10139									-	
GRFAM_domain_	DNA double-strand break repa	ir protein Mre11									
uperfamily domains MART domains	SSF56300			Mart	I, DNA-binding						
am domain.	Calcineurin-like phosphoester	ase domain, ApaH type			1, DNA-binding		-				
	DNA double-strand break repair p	rotein Mreili									- 7
ene3D	Metallo-dependent phosphatase	-like		h	fre11, DNA-binding domain	superfamily					
	Sequence variants (dbSNP and	all other sources)			na (1220)	12.12		12.22		i na na	
l seguence SNPs/i	•	1 1	10		5 <b>1</b>	1.11		· · ·	1.1.1.1.1.1.1.1		
ariant Legend	stop gained splice region variant	1 1		inframe insertion			missense vi		1 1.1		_
ariant Legend	stop gained	120	180	inframe insertion	nt	420			600		706
riant Legend	stop gained splice region variant			inframe insertion	nt		missense vi	ariant	600 600		706
riant Legend	stop gained splice region variant			inframe insertion	nt		missense vi	ariant	600 600	n <sub>ar</sub>	706
riant Legend	stop gained splice region variant			inframe insertion	nt		missense vi	ariant	E00	Na Ke	706

江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

# 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, Though mutation of this locus affected chromosome stability, mutant mice were no more susceptible to tumorigenesis than wild-type mice. Mutant female mice showed reduced fertility.



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



