

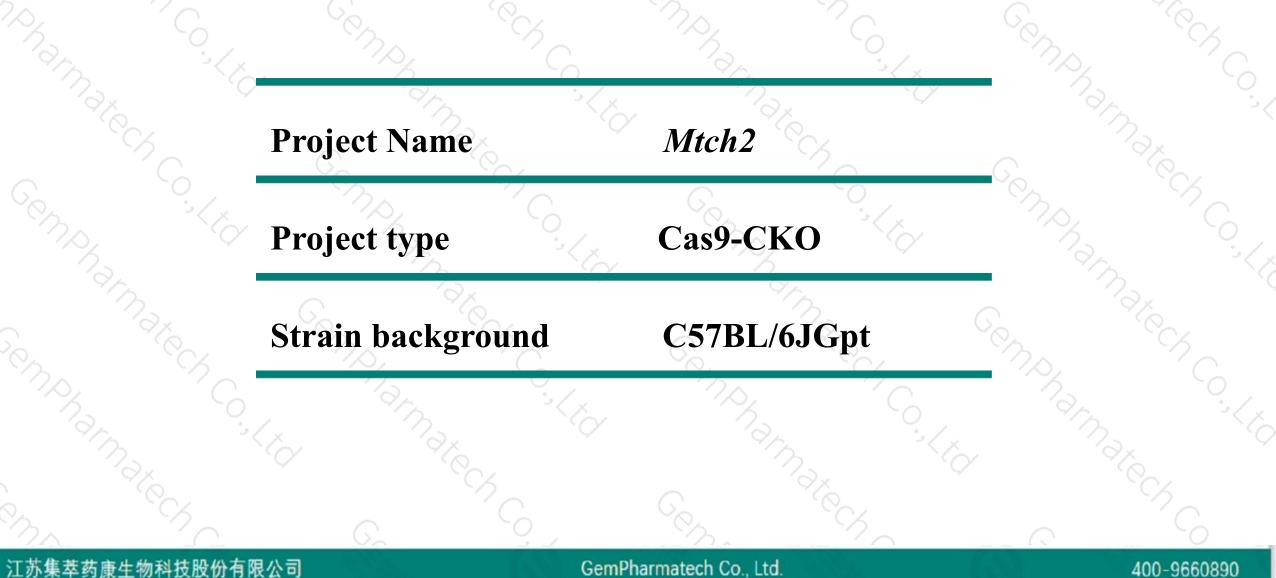
# Mtch2 Cas9-CKO Strategy

CMPharmatect

Designer: Yanhua Shen Design Date: 2019-7-24

# **Project Overview**





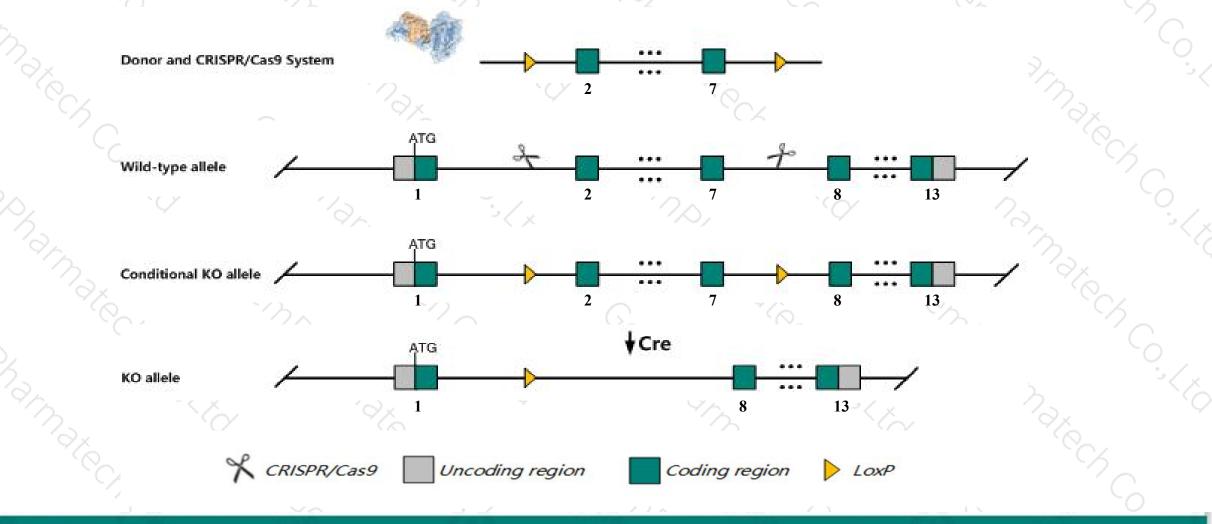
江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

# **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Mtch2 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 *Mtch2* gene has 11 transcripts. According to the structure of *Mtch2* gene, exon2-exon7 of *Mtch2-205* (ENSMUST00000136872.7) transcript is recommended as the knockout region. The region contains 392bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Mtch2* 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 knock-out allele exhibit abnormal mesoderm development, disorganized extraembryonic tissue, lack of amnion and chorion formation, decreased embryo size, and lethality at around E7.5.

Non-coding transcripts 204, 206 and 210 may not be affected.

The *Mtch2* gene is located on the Chr2. 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.

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

# Gene information (NCBI)



#### Mtch2 mitochondrial carrier 2 [Mus musculus (house mouse)]

Gene ID: 56428, updated on 5-Mar-2019

#### Summary

[☆][?

400-9660890

Official Symbol	Mtch2 provided by MGI
Official Full Name	mitochondrial carrier 2 provided by MGI
Primary source	MGI:MGI:1929260
See related	Ensembl:ENSMUSG0000027282
Gene type	protein coding
<b>RefSeq status</b>	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2310034D24Rik, 4930539J07Rik, HSPC032
Summary	This gene encodes a member of the SLC25 family of nuclear-encoded transporters that are localized in the inner mitochondrial membrane.
	Members of this superfamily are involved in many metabolic pathways and cell functions. Genome-wide association studies in human have identified single-nucleotide polymorphisms in several loci associated with obesity. This gene is one such locus, which is highly expressed in white adipose tissue and adipocytes, and thought to play a regulatory role in adipocyte differentiation and biology. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. A recent study showed this gene to be an authentic stop codon readthrough target that can produce two isoforms from the same mRNA by use of alternative in-frame translation termination codons. [provided by RefSeq, Dec 2017]
Expression	Ubiquitous expression in testis adult (RPKM 96.2), large intestine adult (RPKM 54.2) and 27 other tissues See more
Orthologs	human all

#### GemPharmatech Co., Ltd.

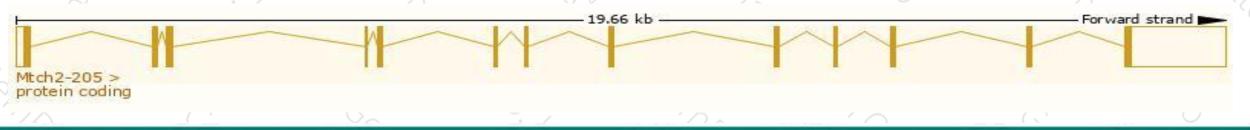
# **Transcript information (Ensembl)**



#### The gene has 11 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Mtch2-205	ENSMUST00000136872.7	2596	<u>303aa</u>	Protein coding	CCDS16416	<u>Q791V5</u>	TSL:1 GENCODE basic APPRIS P	
Mtch2-211	ENSMUST00000150232.7	2377	<u>312aa</u>	Protein coding	CCDS84547	Q9D050	TSL:1 GENCODE basic	
Mtch2-202	ENSMUST00000111467.3	2281	<u>294aa</u>	Protein coding CCDS84548		A2AFW6	TSL:5 GENCODE basic	
Mtch2-203	ENSMUST00000111468.9	1066	<u>45aa</u>	Nonsense mediated decay	( <u>*</u>	D6RCZ1	TSL:1	
Mtch2-201	ENSMUST00000057216.14	1047	No protein	Processed transcript	17		TSL:1	
Mtch2-210	ENSMUST00000148936.1	998	No protein	Processed transcript	87		TSL:1	
Mtch2-208	ENSMUST00000144696.1	3103	No protein	Retained intron	Retained intron -		TSL:2	
Mtch2-209	ENSMUST00000146392.7	2241	No protein	Retained intron	Retained intron		TSL:1	
Mtch2-204	ENSMUST00000135026.1	574	No protein	Retained intron	Retained intron		TSL:2	
Mtch2-207	ENSMUST00000143773.1	457	No protein	Retained intron	Retained intron -		TSL:5	
Mtch2-206	ENSMUST00000142350.1	412	No protein	Retained intron	<u>2</u>	-	TSL:2	
4			1.6	/ >		V //~		

The strategy is based on the design of Mtch2-205 transcript, The transcription is shown below

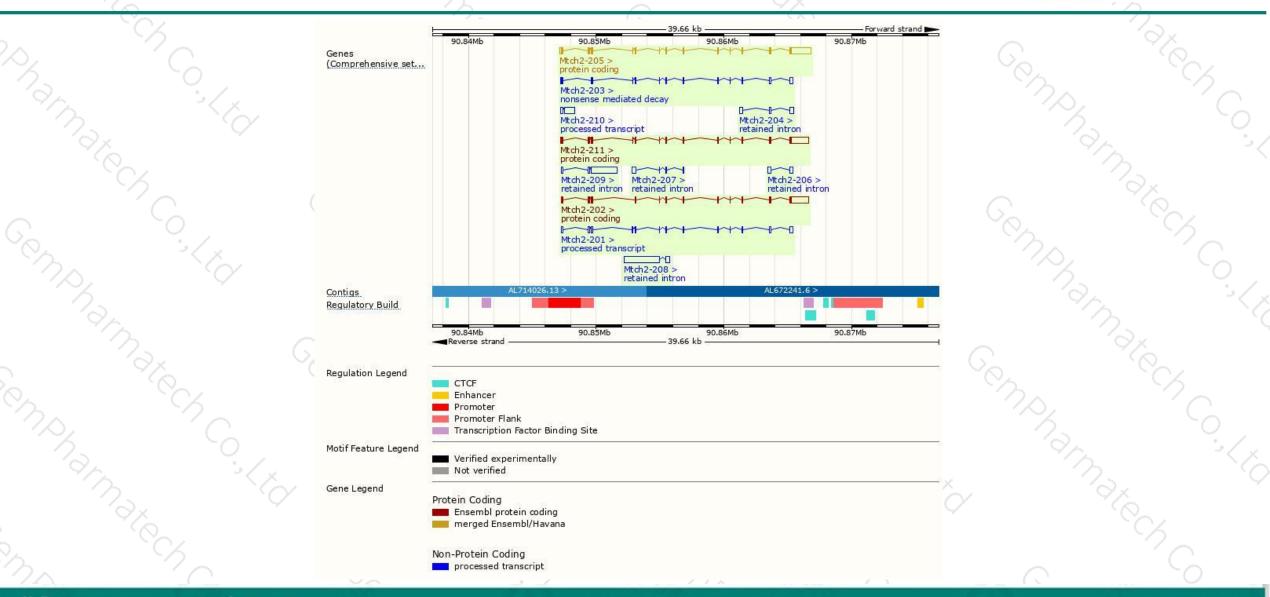


#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890

### **Genomic location distribution**



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

**集萃药康** GemPharmatech

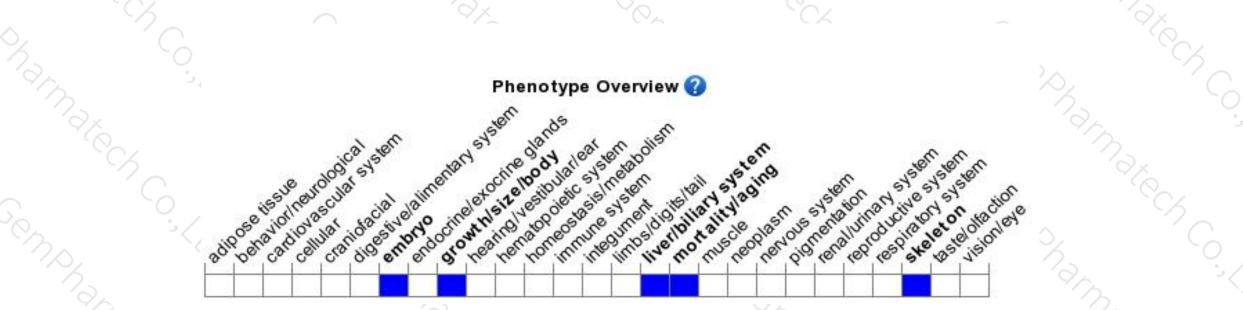
# **Protein domain**



10/0	6		`°°		DD -		C C C C C C C C C C C C C C C C C C C		
417	s its		5	G.	2th				
	ENSMUSP00000121 hmmpanther	PTHR10780:SF20			W.				
	Superfamily domains	PTHR10780 Mitochondrial carr	ier domain superf	amily				<u> </u>	
	Pfam domain				Mitochondrial substra	te/solute carrier			S
· · ·	PROSITE profiles	Mitochondrial subs	strate/solute carrie	er en		3			36
	Gene3D		rier domain superf						
	All sequence SNPs/i	Sequence varian	its (dbSNP and a	ll other sources)	1	- <b>R</b> - 1	í í	11	
S CNX	Variant Legend	missense v synonymou							6
	Scale bar	0 4	0 80	) 12	0 160	200	240	303	
	nax and	× V			Strong Strong	>د ب			
Con .	°°%		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~					C C	
江苏集萃	药康生物科技股份有限公	公司	6	GemPhar	matech Co., Ltd.		13	400-966	0890

## 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 knock-out allele exhibit abnormal mesoderm development, disorganized extraembryonic tissue, lack of amnion and chorion formation, decreased embryo size, and lethality at around E7



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



