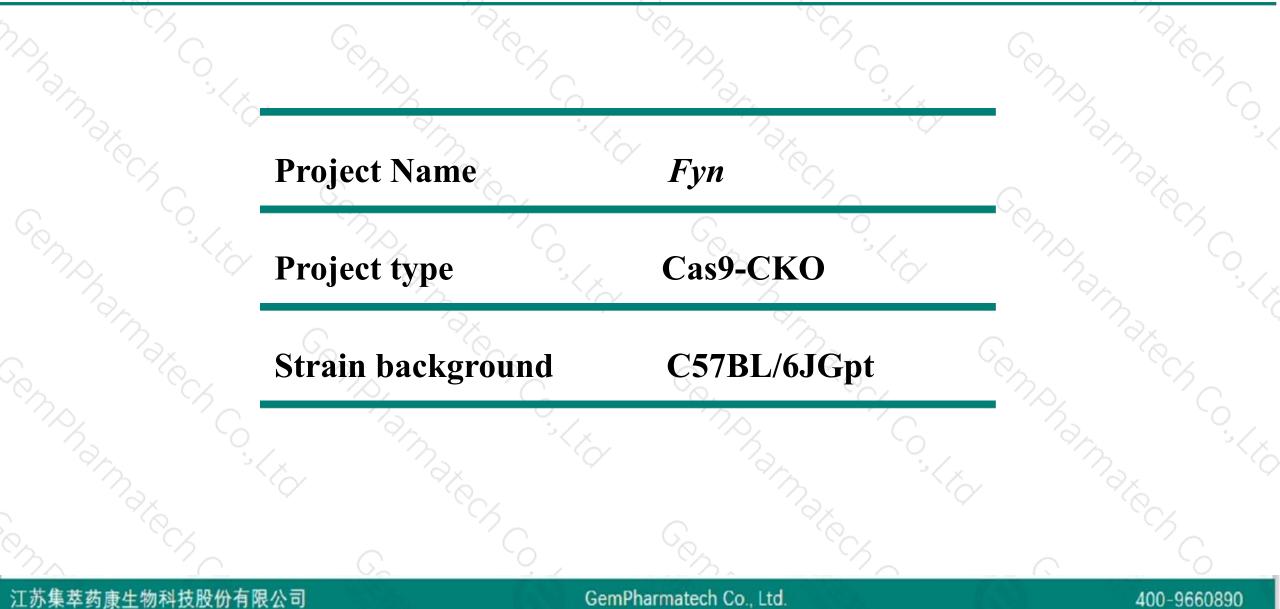


# emphamatech C. NDHarmake Ch Coste **Cas9-CKO Strategy** Fyn Romphamater Coste 6. . < x

Comphannated Co. Designer: Shilei Zhu Emphamater C. Lity

# **Project Overview**

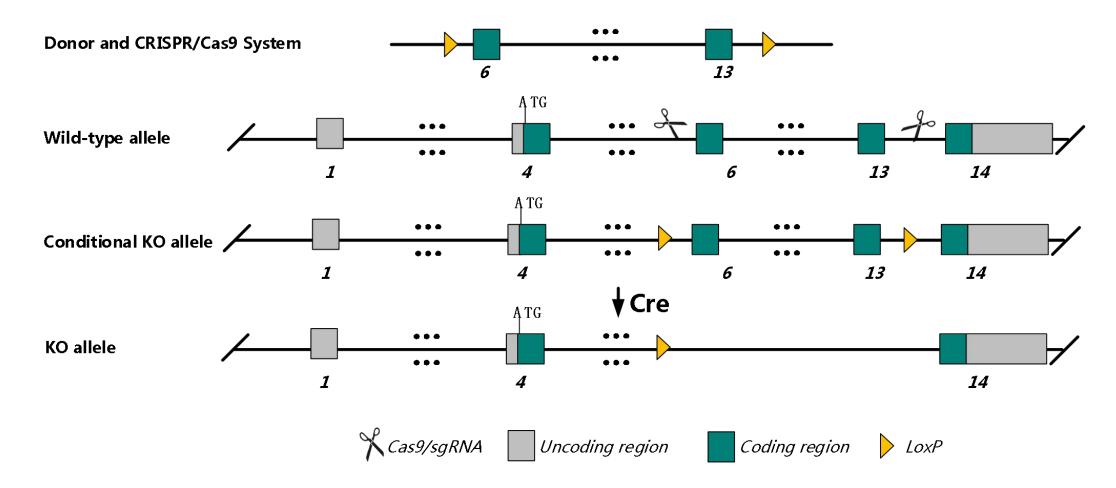




# **Conditional Knockout strategy**



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





The Fyn gene has 9 transcripts. According to the structure of Fyn gene, exon6-exon13 of Fyn-201 (ENSMUST0000063091.12) transcript is recommended as the knockout region. The region contains 1052bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Fyn* 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, Different targeted allele homozygotes show different defects, including seizure susceptibility, anxiety, impaired suckling, myelination, LTP and spatial learning, and defects in immune system, circadian rhythm, testes weight and olfactory bulb formation.
- The Fyn gene is located on the Chr10. 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)



☆ ?

## Fyn Fyn proto-oncogene [Mus musculus (house mouse)]

Gene ID: 14360, updated on 2-Apr-2019

#### Summary

Official SymbolFyn provided by MGIOfficial Full NameFyn proto-oncogene provided by MGIPrimary sourceMGI:MGI:95602Primary sourceInsembl:ENSMUSG0000019843Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso knownasAl448320, AW552119ExpressionUbiquitous expression in CNS E18 (RPKM 23.2), CNS E14 (RPKM 20.7) and 27 other tissuesSee more<br/>human all

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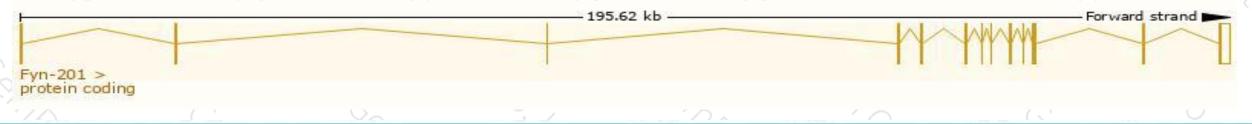
# **Transcript information (Ensembl)**



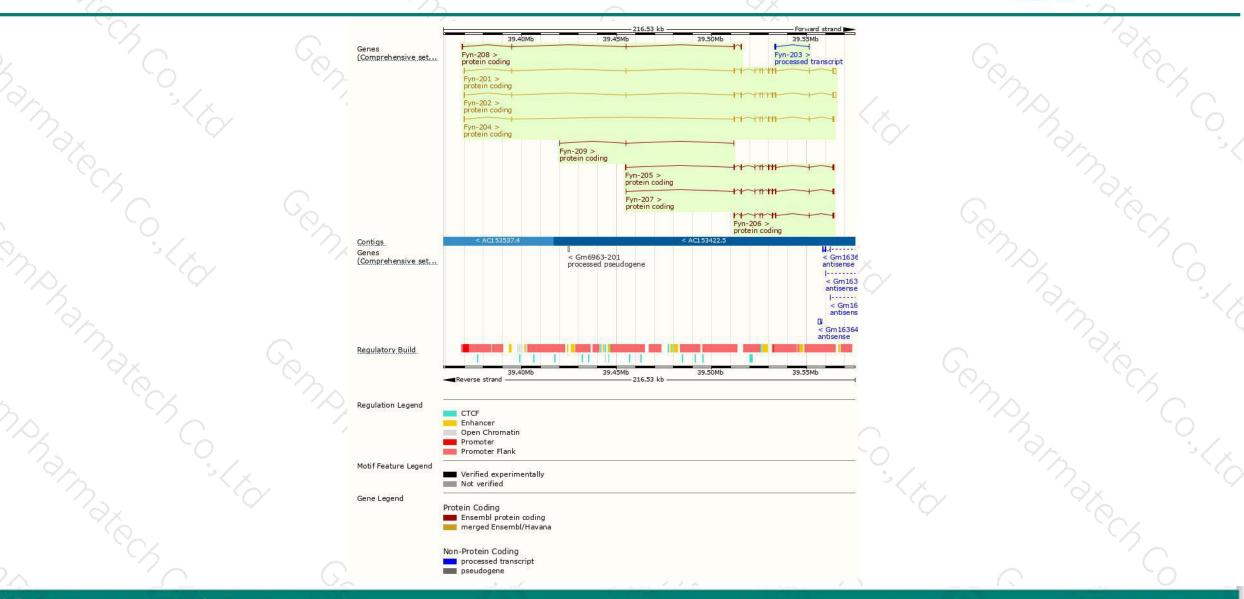
# The gene has 9 transcripts, all transcripts are shown below:

			1				
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fyn-201	ENSMUST0000063091.12	3562	<u>534aa</u>	Protein coding	CCDS23788	P39688	TSL:1 GENCODE basic APPRIS P3
Fyn-202	ENSMUST0000099967.9	3528	<u>537aa</u>	Protein coding	CCDS48538	P39688	TSL:1 GENCODE basic APPRIS ALT1
Fyn-204	ENSMUST00000126486.7	2495	<u>534aa</u>	Protein coding	CCDS23788	P39688	TSL:1 GENCODE basic APPRIS P3
Fyn-207	ENSMUST00000146287.7	2135	<u>534aa</u>	Protein coding	CCDS23788	P39688	TSL:1 GENCODE basic APPRIS P3
Fyn-205	ENSMUST00000135242.7	2114	<u>534aa</u>	Protein coding	CCDS23788	P39688	TSL:1 GENCODE basic APPRIS P3
Fyn-206	ENSMUST00000136659.1	1910	<u>482aa</u>	Protein coding	-	D3YZ57	TSL:5 GENCODE basic
Fyn-208	ENSMUST00000148152.7	675	<u>114aa</u>	Protein coding	2	D3YZA2	CDS 3' incomplete TSL:3
Fyn-209	ENSMUST00000157009.7	441	<u>36aa</u>	Protein coding	2	D3YVZ0	CDS 3' incomplete TSL:2
Fyn-203	ENSMUST00000123205.1	333	No protein	Processed transcript		-	TSL:3

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



# **Genomic location distribution**



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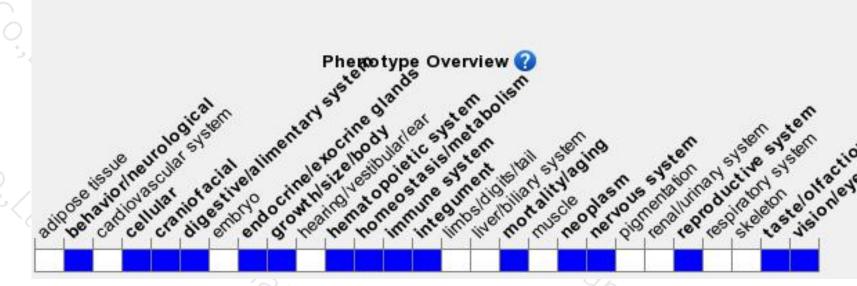
# **Protein domain**





# 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, Different targeted allele homozygotes show different defects, including seizure susceptibility, anxiety, impaired suckling, myelination, LTP and spatial learning, and defects in immune system, circadian rhythm, testes weight and olfactory bulb formation.

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



