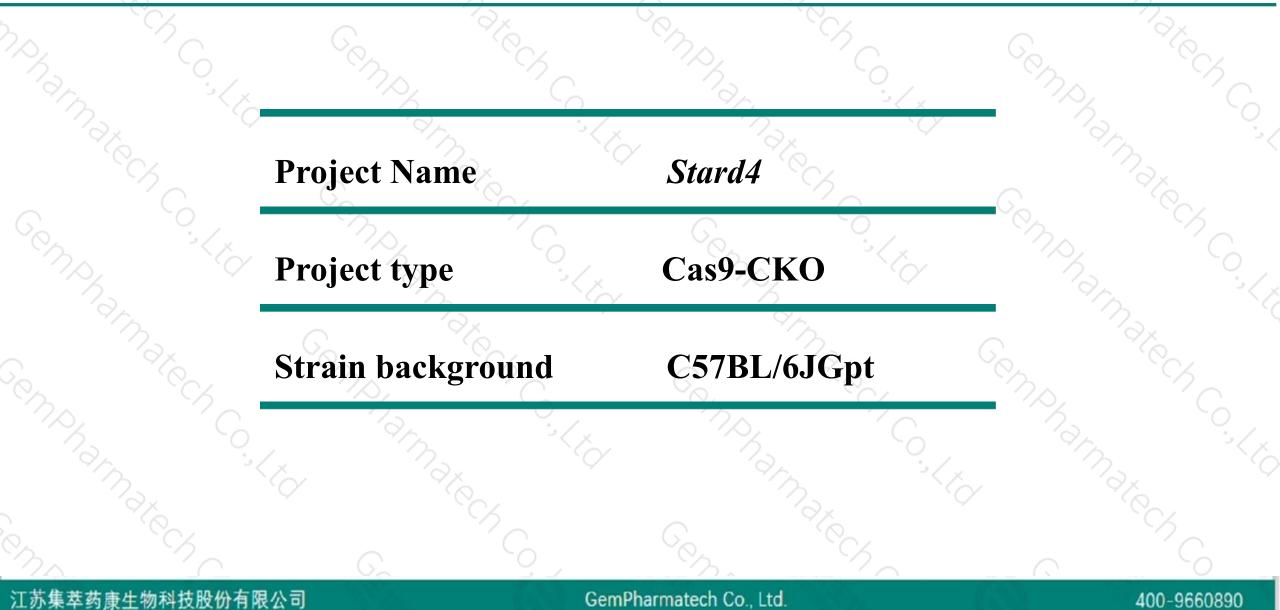


# Stard4 Cas9-CKO Strategy

Designer: Xueting Zhang Reviewer:Yanhua Shen Date:2020-02-19

# **Project Overview**

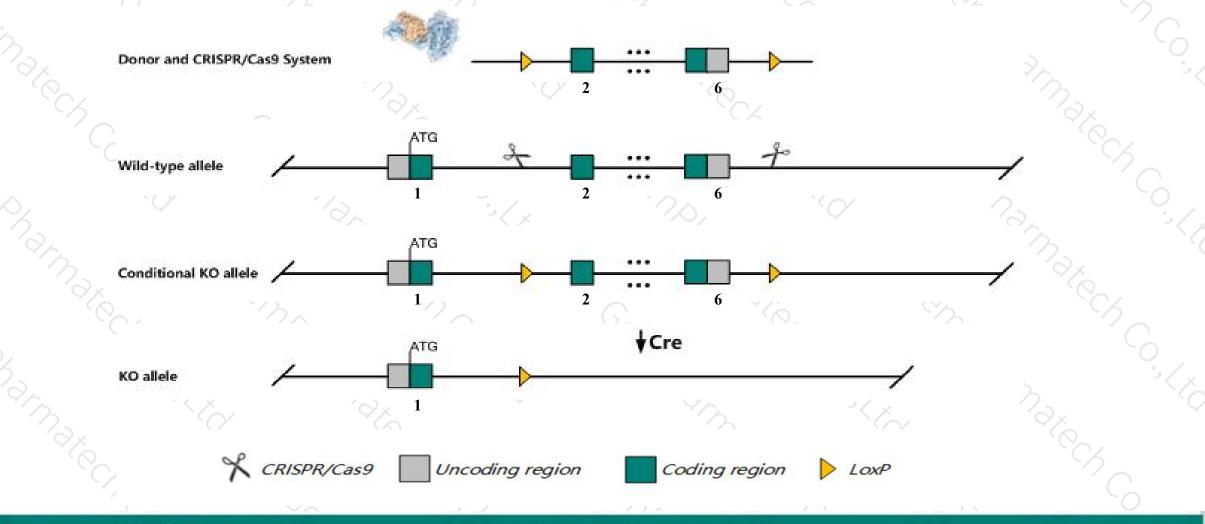




# **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Stard4* gene. The schematic diagram is as follows:



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The Stard4 gene has 4 transcripts. According to the structure of Stard4 gene, exon2-exon6 of Stard4-201 (ENSMUST00000025236.8) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Stard4* 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.

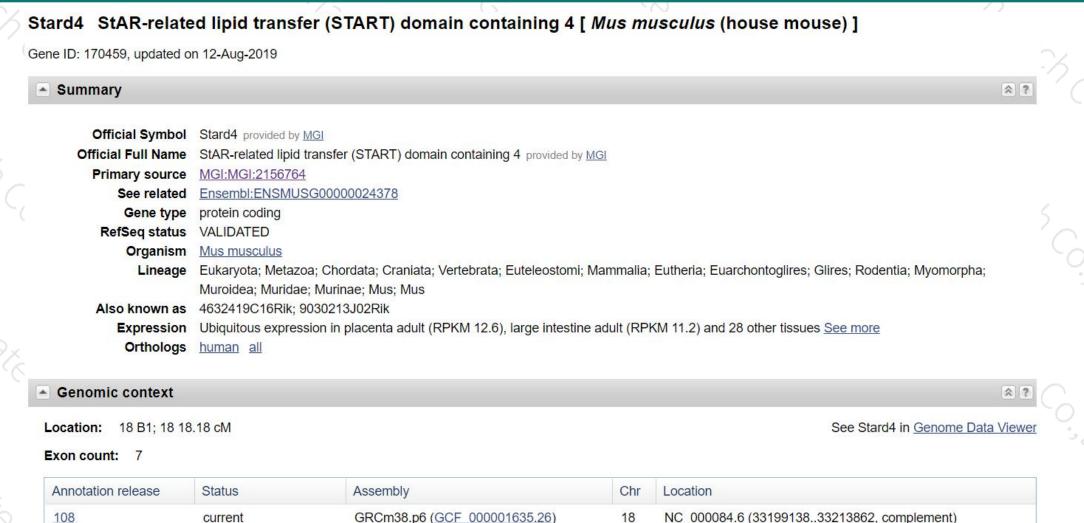
> The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit decreased liver weight, body weight, and body length. Female mice homozygous for this allele exhibit decreased circulating cholesterol when fed a low cholesterol diet and altered bile composition when fed standard chow.
- The Stard4 gene is located on the Chr18. 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)





Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	18	NC_000084.6 (3319913833213862, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	18	NC_000084.5 (3336107533373470, complement)

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# **Transcript information (Ensembl)**



The gene has 4 transcripts, all transcripts are shown below:

1 m		A		No			l hus.
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Stard4-201	ENSMUST0000025236.8	5097	<u>224aa</u>	Protein coding	CCDS29123	<u>Q80SX0</u>	TSL:1 GENCODE basic APPRIS P1
Stard4-203	ENSMUST00000119991.7	941	<u>146aa</u>	Protein coding	CCDS84369	D3YW30	TSL:5 GENCODE basic
Stard4-202	ENSMUST00000118990.1	648	<u>111aa</u>	Protein coding	8 <u>4</u>	D3YW24	TSL:2 GENCODE basic
Stard4-204	ENSMUST00000141617.1	979	No protein	Retained intron	( <u> </u>	-	TSL:1

The strategy is based on the design of Stard4-201 transcript, The transcription is shown below

#### < Stard4-201 protein coding

Reverse strand

- 14.51 kb --

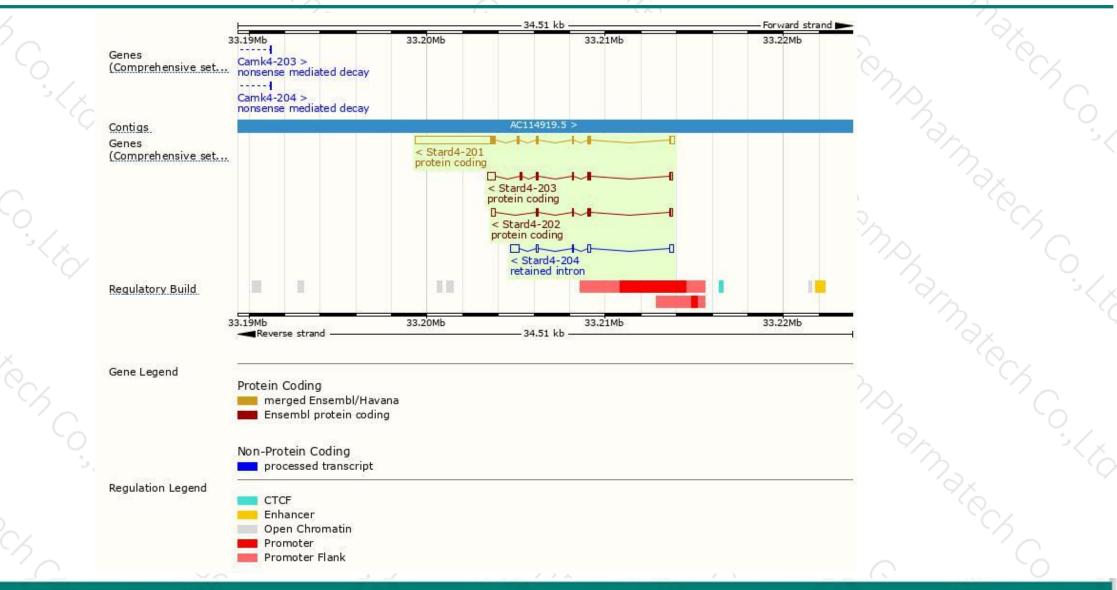
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## **Genomic location distribution**



400-9660890



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# **Protein domain**



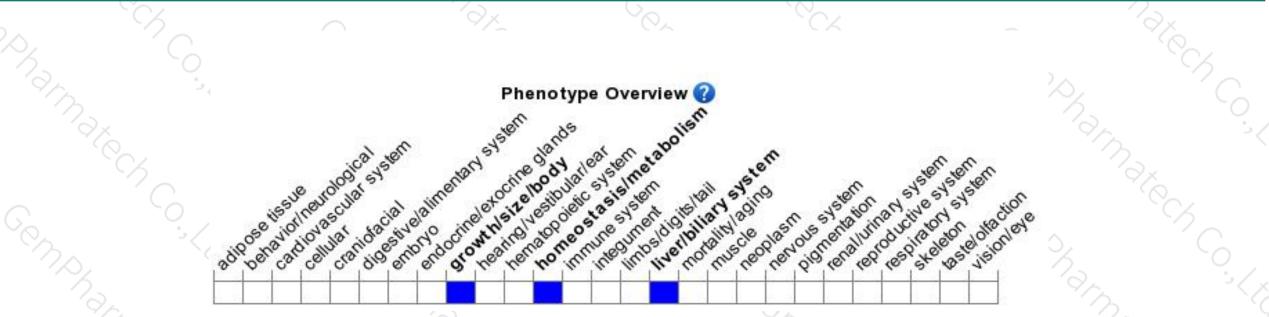
Superfamily		SSF55	961									
SMART		START	domain									
Pfam		ST	ART domai	n								
PROSITE profiles		S	TART dom	ain								4
PANTHER	8	PTHR47006	5									10
Gene3D		CTA PT	lika damai	n superfami								
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# Mouse phenotype description(MGI)





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, Mice homozygous for a knock-out allele exhibit decreased liver weight, body weight, and body length. Female mice homozygous for this allele exhibit decreased circulating cholesterol when fed a low cholesterol diet and altered bile composition when fed standard chow.

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



