

Samhd1 Cas9-CKO Strategy

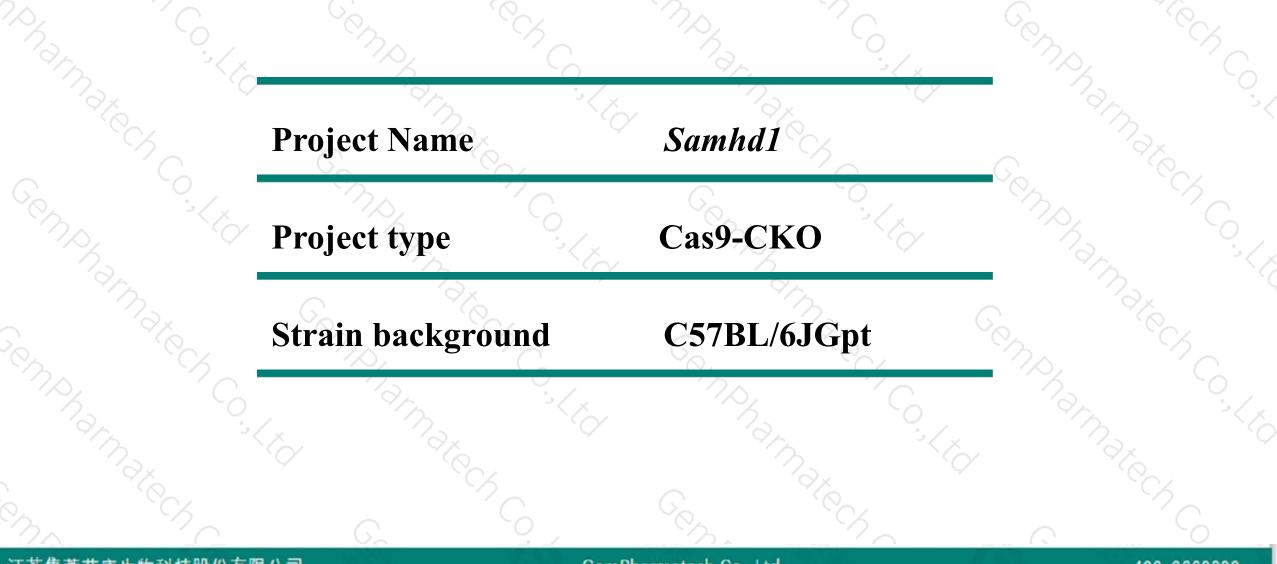
Designer: Reviewer:

Design Date:

Huan Fan Huan Wang 2020-1-13

Project Overview





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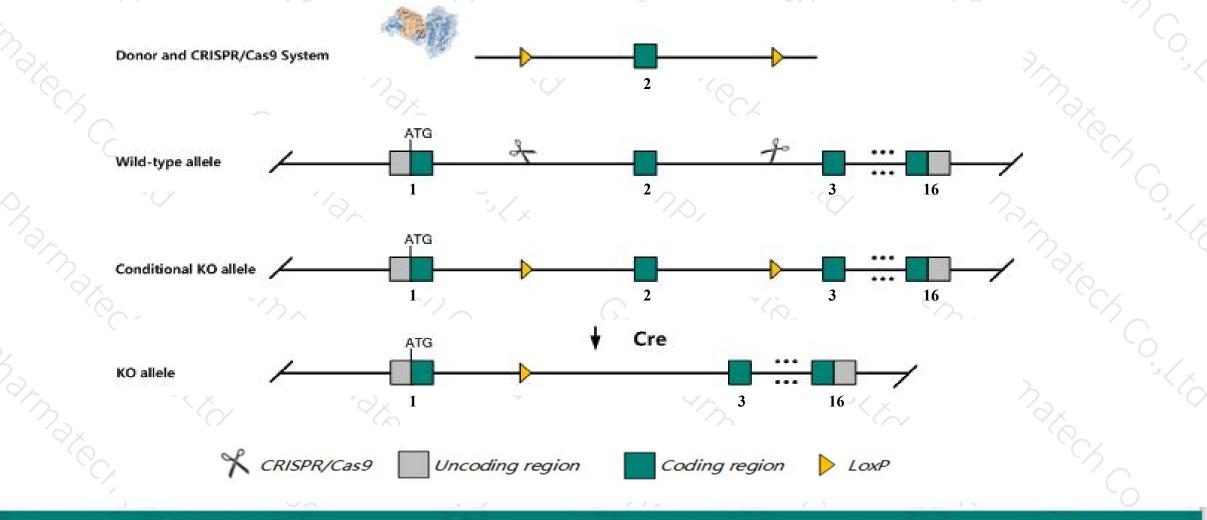
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Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Samhd1* gene. The schematic diagram is as follows:



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 The Samhd1 gene has 10 transcripts. According to the structure of Samhd1 gene, exon2 of Samhd1-201 (ENSMUST00000057725.9) transcript is recommended as the knockout region. The region contains 67bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Samhd1 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 increased susceptibility to infection with pseudotyped HIV-1.
 - > The Samhd1 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.

Gene information (NCBI)



\$?

Samhd1 SAM domain and HD domain, 1 [Mus musculus (house mouse)]

Gene ID: 56045, updated on 19-Mar-2019

Summary

Official Symbol	Samhd1 provided by MGI
Official Full Name	SAM domain and HD domain, 1 provided by MGI
Primary source	MGI:MGI:1927468
See related	Ensembl:ENSMUSG0000027639
Gene type	protein coding
RefSeq status	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	E330031J07Rik, Mg11, mSAMHD1
Expression	Ubiquitous expression in spleen adult (RPKM 38.6), lung adult (RPKM 21.3) and 26 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 10 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Samhd1-201	ENSMUST00000057725.9	3926	<u>658aa</u>	Protein coding	CCDS16973	<u>Q60710</u>	TSL:1 GENCODE basic APPRIS P3
Samhd1-202	ENSMUST0000088523.10	1996	<u>651aa</u>	Protein coding	CCDS50783	<u>Q60710</u>	TSL:5 GENCODE basic APPRIS ALT2
Samhd1-203	ENSMUST00000109549.2	3245	<u>582aa</u>	Protein coding	2	E9PYG9	TSL:1 GENCODE basic APPRIS ALT2
Samhd1-206	ENSMUST00000139263.7	2036	<u>631aa</u>	Protein coding	-	F6TVP2	CDS 5' incomplete TSL:1
Samhd1-204	ENSMUST00000123932.7	3753	<u>150aa</u>	Nonsense mediated decay	5	E0CXZ5	TSL:2
Samhd1-208	ENSMUST00000149313.7	1375	No protein	Retained intron	, ÷	6.48	TSL:1
Samhd1-210	ENSMUST00000160213.1	681	No protein	Retained intron	-	120	TSL:3
Samhd1-205	ENSMUST00000139149.1	678	No protein	Retained intron	-	828	TSL:2
Samhd1-209	ENSMUST00000152866.1	542	No protein	Retained intron	5	1753	TSL:2
Samhd1-207	ENSMUST00000142508.1	473	No protein	Retained intron		6.57	TSL:3

The strategy is based on the design of Samhd1-201 transcript, The transcription is shown below

< Samhd1-201 protein coding

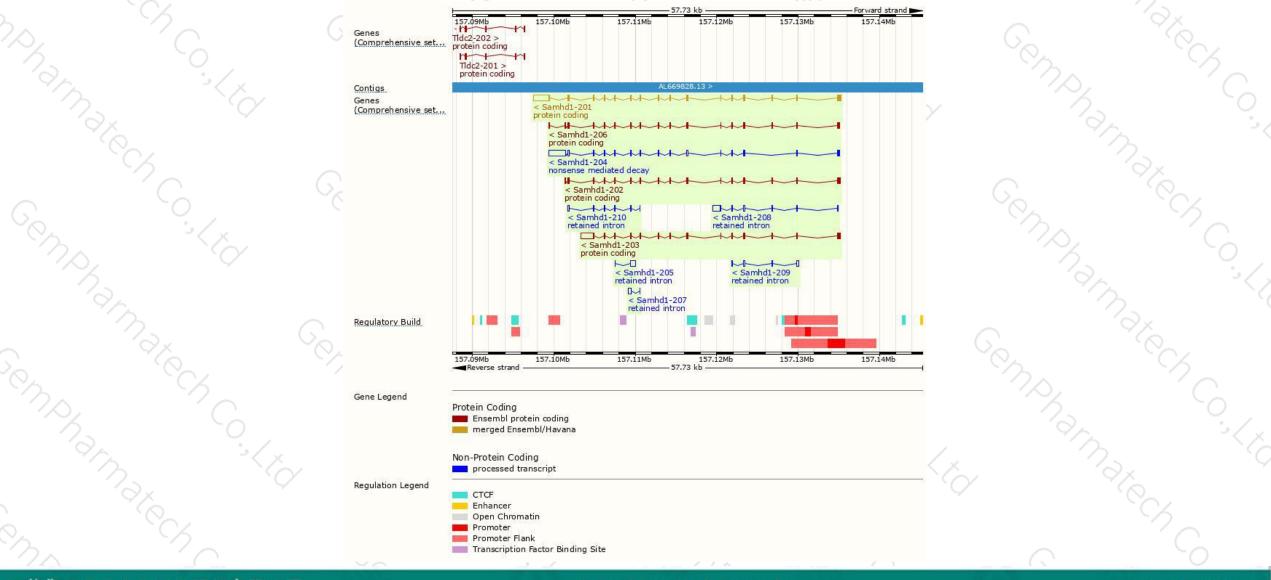
Reverse strand

- 37.73 kb -

Genomic location distribution



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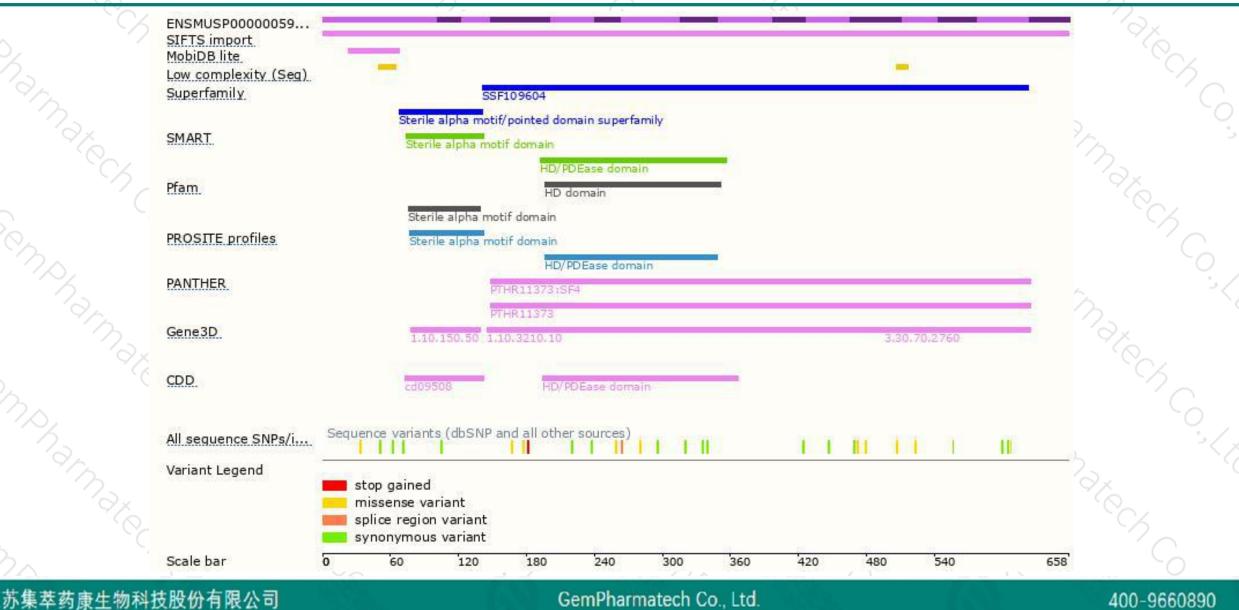


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Protein domain

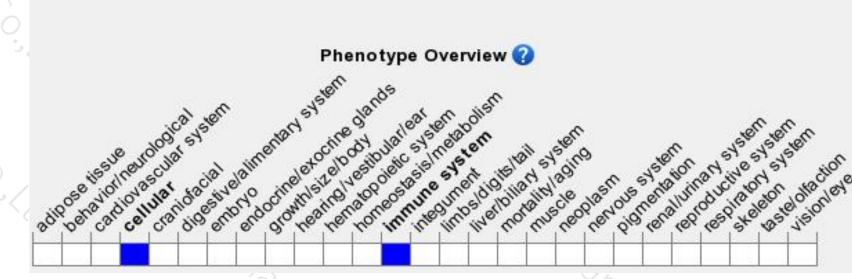




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Mouse phenotype description(MGI)





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, Mice homozygous for a knock-out allele exhibit increased susceptibility to infection with pseudotyped HIV-1.



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



