

Shroom3 Cas9-CKO Strategy

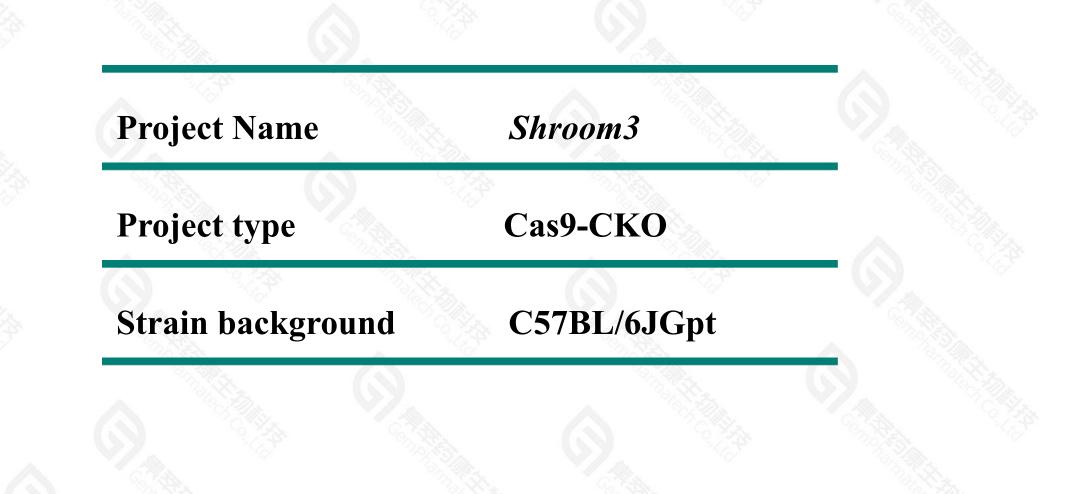
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Reviewer: Shanhong Tao

Design Date: 2021-6-16

Project Overview





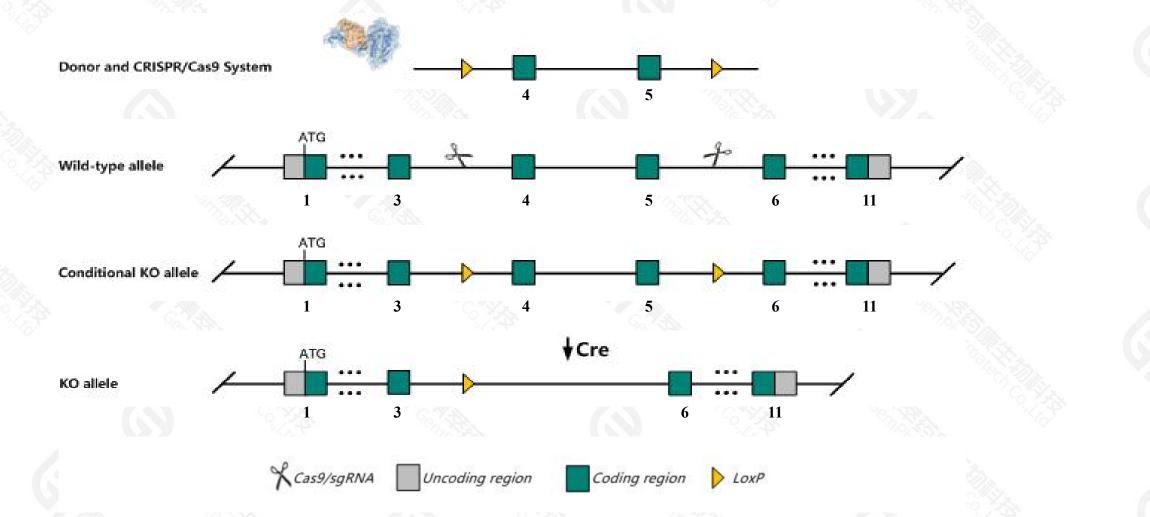
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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the Shroom3 gene. The schematic diagram is as follows:



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➤ The Shroom3 gene has 11 transcripts. According to the structure of Shroom3 gene, exon4-exon5 of Shroom3-203(ENSMUST00000113055.9) transcript is recommended as the knockout region. The region contains 3295bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Shroom3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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,homozygous mutation of this locus results in failed neural tube closure leading to exencephaly, acrania, facial clefting, and spina bifida. Homozygotes develop to term but die either at birth or shortly thereafter.

> The *Shroom3* gene is located on the Chr5. 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.

Shroom3 shroom family member 3 [Mus musculus (house mouse)] Gene ID: 27428, updated on 9-Feb-2021

Summary

Official Symbol	Shroom3 provided by MGI
Official Full Name	shroom family member 3 provided by MGI
Primary source	MGI:MGI:1351655
See related	Ensembl:ENSMUSG0000029381
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	AL022960, D5Ertd287, D5Ertd287e, Sh, Shr, Shrm, Shrm3
Expression	Broad expression in colon adult (RPKM 39.5), small intestine adult (RPKM 32.7) and 15 other tissuesSee more
Orthologs	human all

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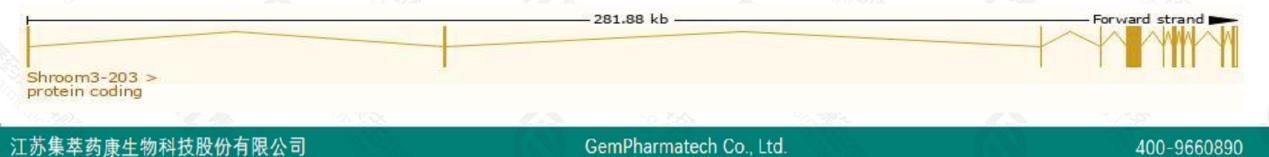
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Transcript information (Ensembl)

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

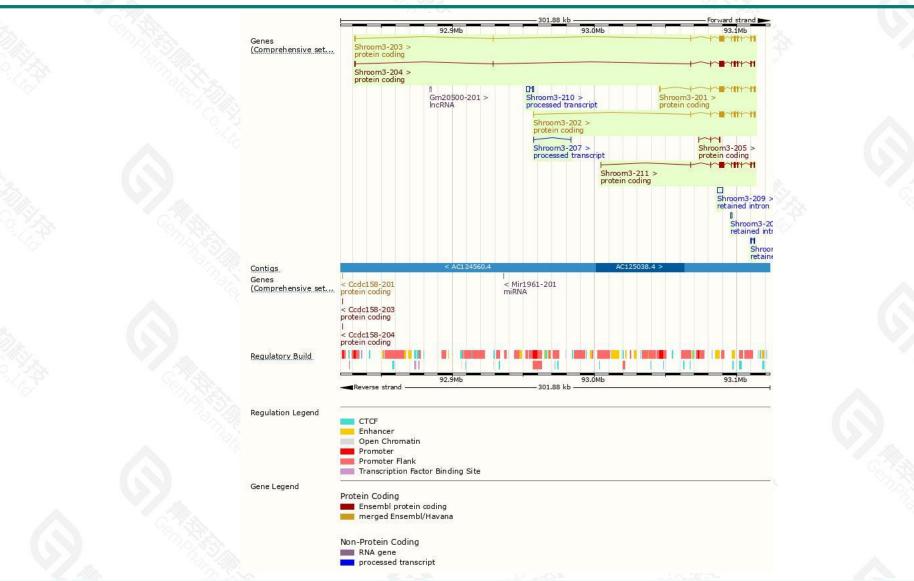
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Shroom3-203	ENSMUST00000113055.9	6765	<u>1980aa</u>	Protein coding	CCDS39154		TSL:1 , GENCODE basic , APPRIS P1
Shroom3-201	ENSMUST00000113051.9	6435	<u>1805aa</u>	Protein coding	CCDS39155		TSL:1 , GENCODE basic ,
Shroom3-202	ENSMUST00000113054.9	6401	<u>1805aa</u>	Protein coding	CCDS39155		TSL:1 , GENCODE basic ,
Shroom3-211	ENSMUST00000225438.2	5742	<u>1899aa</u>	Protein coding			GENCODE basic ,
Shroom3-204	ENSMUST00000168878.8	5630	<u>1849aa</u>	Protein coding	12		TSL:1 , GENCODE basic ,
Shroom3-205	ENSMUST00000172706.2	687	<u>179aa</u>	Protein coding	े 		CDS 3' incomplete , TSL:2 ,
Shroom3-210	ENSMUST00000202767.2	3307	No protein	Processed transcript	-		TSL:5 ,
Shroom3-207	ENSMUST00000172849.2	271	No protein	Processed transcript	2 2		TSL:3 ,
Shroom3-209	ENSMUST00000201800.2	3611	No protein	Retained intron	-		TSL:NA ,
Shroom3-206	ENSMUST00000172752.2	1027	No protein	Retained intron	-		TSL:1,
Shroom3-208	ENSMUST00000200869.2	911	No protein	Retained intron	10 NO		TSL:NA ,

The strategy is based on the design of *Shroom3-203* transcript, the transcription is shown below:



Genomic location distribution





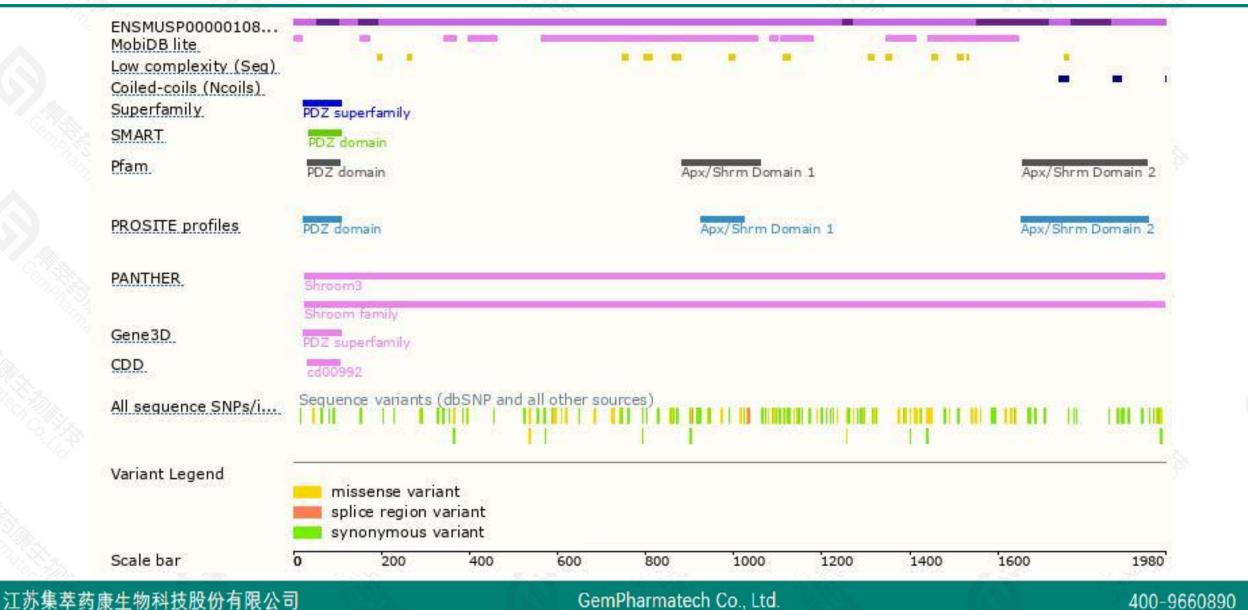
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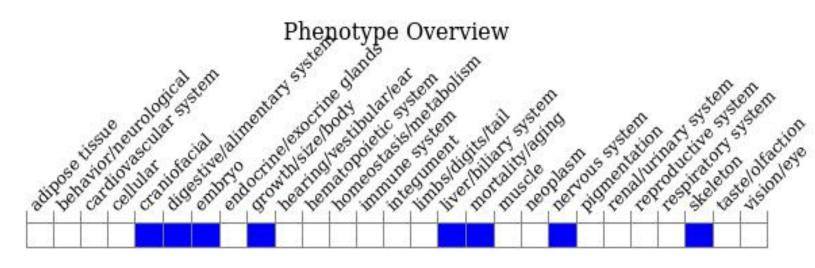
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Protein domain





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, homozygous mutation of this locus results in failed neural tube closure leading to exencephaly, acrania, facial clefting, and spina bifida. Homozygotes develop to term but die either at birth or shortly thereafter.



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



