

Actr3 Cas9-KO Strategy

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Reviewer: Yanhua Shen

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Project Overview



Project Name Actr3

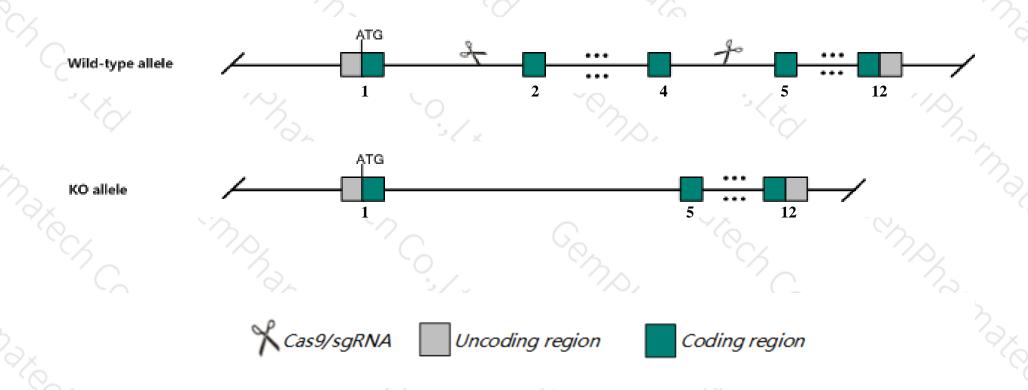
Project type Cas9-KO

Strain background C57BL/6JGpt

Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Actr3* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Actr3* gene has 12 transcripts. According to the structure of *Actr3* gene, exon2-exon4 of *Actr3-202*(ENSMUST00000178474.7) transcript is recommended as the knockout region. The region contains 292bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Actr3* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice



- ➤ According to the existing MGI data, Mice homozygous for a null allele die prior to E4.5 and exhibit abnormal embryogenesis.
- The *Actr3* gene is located on the Chr1. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Actr3 ARP3 actin-related protein 3 [Mus musculus (house mouse)]

Gene ID: 74117, updated on 10-Sep-2019

Summary

Official Symbol Actr3 provided by MGI

Official Full Name ARP3 actin-related protein 3 provided by MGI

Primary source MGI:MGI:1921367

See related Ensembl: ENSMUSG00000026341

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

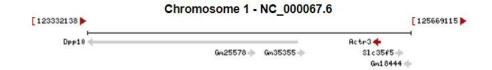
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Arp3; 1200003A09Rik

Expression Ubiquitous expression in placenta adult (RPKM 60.4), CNS E14 (RPKM 36.5) and 28 other tissues See more

Orthologs <u>human</u> all



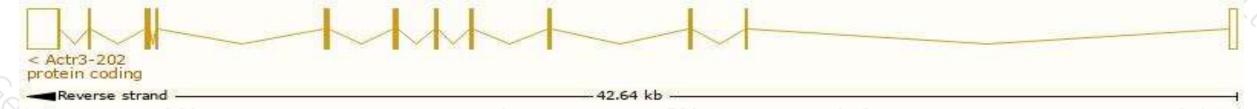
Transcript information (Ensembl)



The gene has 12 transcripts, all transcripts are shown below:

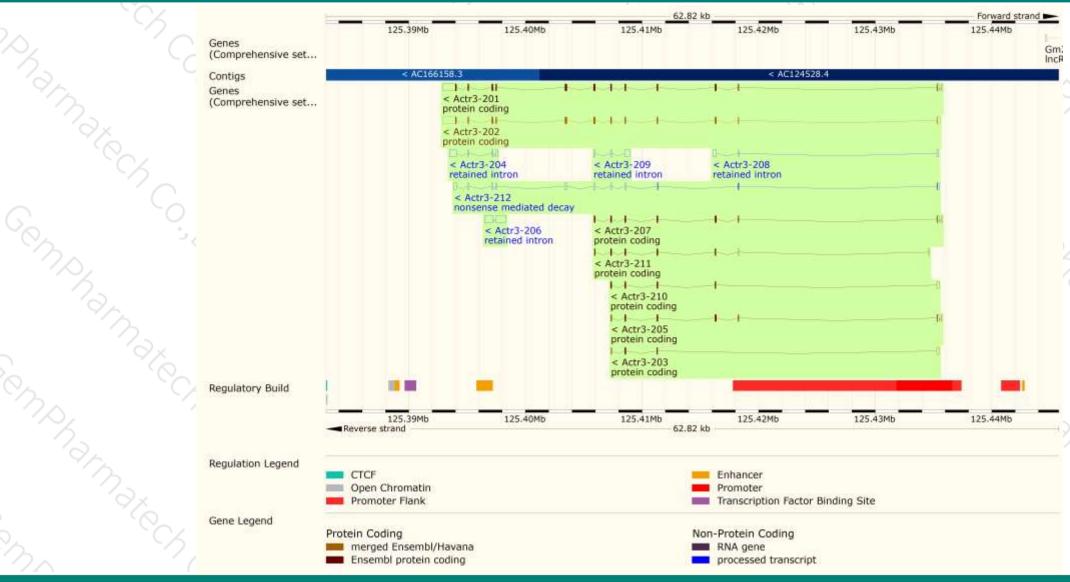
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Name 🍦	Transcript ID 🗼	bp 🌲	Protein 🍦	Translation ID	Biotype	CCDS	UniProt 🝦	Flags	\$
Actr3-202	ENSMUST00000178474.7	2565	<u>418aa</u>	ENSMUSP00000137503.1	Protein coding	CCDS15242 ₺	Q3ULF7₽Q99JY9₽	TSL:1 GENCODE basic	APPRIS P1
Actr3-201	ENSMUST00000027579.16	2554	<u>418aa</u>	ENSMUSP00000027579.10	Protein coding	CCDS15242 ₺	Q3ULF7@Q99JY9@	TSL:5 GENCODE basic	APPRIS P1
Actr3-207	ENSMUST00000188497.6	856	<u>216aa</u>	ENSMUSP00000140535.1	Protein coding	-	<u>A0A087WRA1</u> ₽	CDS 3' incomplete	TSL:3
Actr3-205	ENSMUST00000187460.6	667	<u>161aa</u>	ENSMUSP00000140000.1	Protein coding	-	<u>A0A087WQ14</u> ₽	CDS 3' incomplete	TSL:2
Actr3-211	ENSMUST00000191544.6	654	<u>155aa</u>	ENSMUSP00000139674.1	Protein coding	-	<u>A0A087WP86</u> ₽	CDS 3' incomplete	TSL:3
Actr3-210	ENSMUST00000191004.6	610	<u>129aa</u>	ENSMUSP00000140953.1	Protein coding	-	<u>A0A087WS98</u> ₽	CDS 3' incomplete	TSL:5
Actr3-203	ENSMUST00000185280.1	435	<u>70aa</u>	ENSMUSP00000140082.1	Protein coding	-	<u>A0A087WQ83</u> ₽	CDS 3' incomplete	TSL:5
Actr3-212	ENSMUST00000191578.6	1390	<u>41aa</u>	ENSMUSP00000139886.1	Nonsense mediated decay	-	<u>A0A087WPR6</u> ₽	TSL:5	
Actr3-206	ENSMUST00000188362.1	1609	No protein	-	Retained intron	-	-	TSL:2	
Actr3-204	ENSMUST00000186008.6	978	No protein	-	Retained intron	-	-	TSL:2	
Actr3-209	ENSMUST00000189192.1	658	No protein	-	Retained intron	-	-	TSL:2	
Actr3-208	ENSMUST00000188827.1	517	No protein	-	Retained intron	-	-	TSL:2	

The strategy is based on the design of Actr3-202 transcript, The transcription is shown below



Genomic location distribution





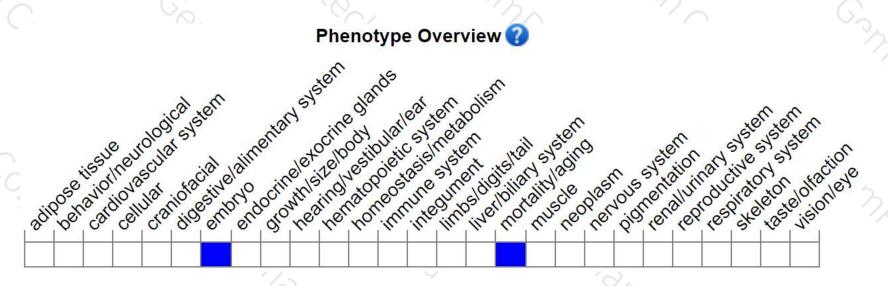
Protein domain





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, Mice homozygous for a null allele die prior to E4.5 and exhibit abnormal embryogenesis.



If you have any questions, you are welcome to inquire.

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