

Arrb2 Cas9-KO Strategy

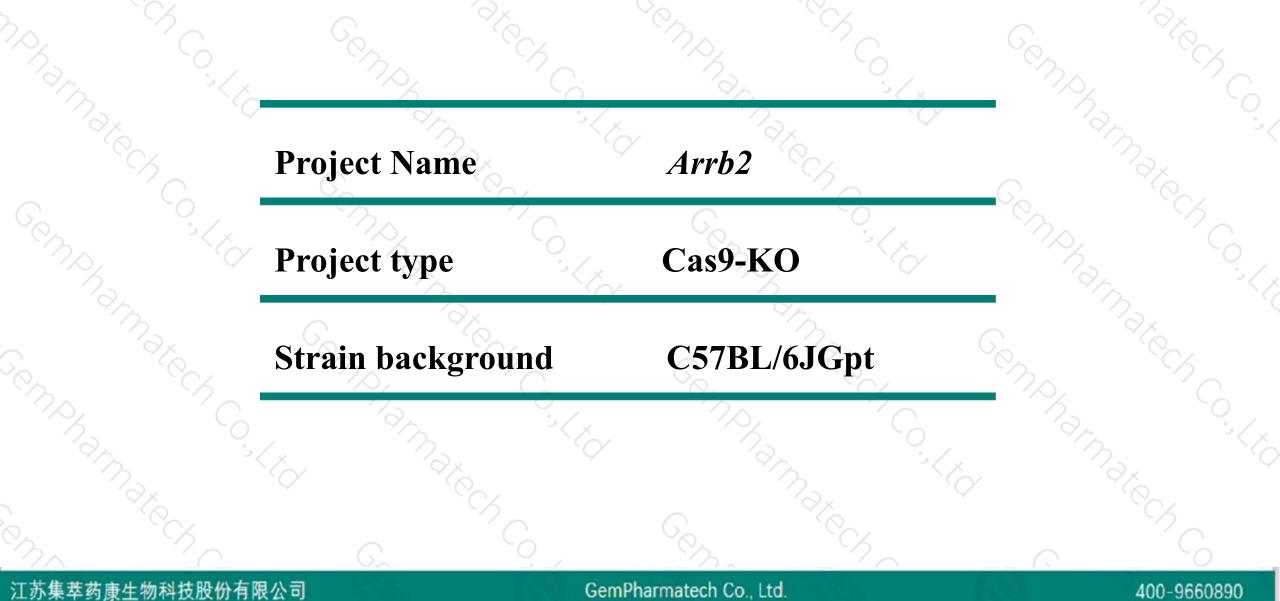
Designer: Daohua Xu

Reviewer: Huimin Su

Design Date: 2020-8-5

Project Overview

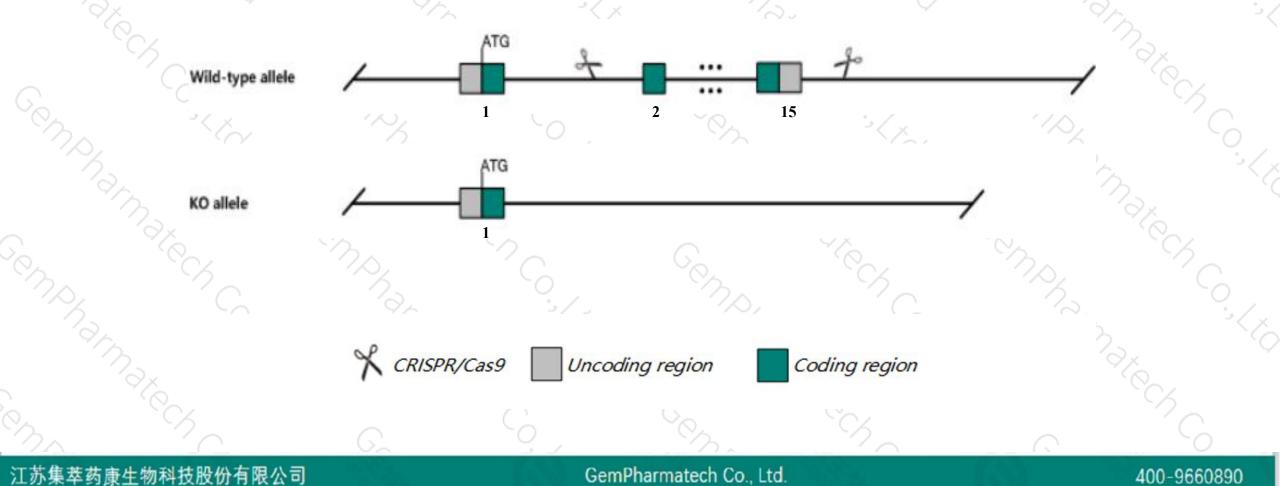




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Arrb2 gene. The schematic diagram is as follows:





> The *Arrb2* gene has 13 transcripts. According to the structure of *Arrb2* gene, exon2-exon15 of *Arrb2*-205(ENSMUST00000108568.9) transcript is recommended as the knockout region. The region contains 1243bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Arrb2* gene. The brief process is as follows: CRISPR/Cas9 system 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.



- > According to the existing MGI data,mice homozygous for a knock-out allele exhibit enhanced morphine analgesia, an enhanced inflammatory response and reduced threshold to lethal endotoxin challenge, and impaired T and B lymphocyte chemotaxis.
- > The KO region contains functional region of the *Mir7115* gene.Knockout the region may affect the function of *Mir7115* gene.
- The *Arrb2* gene is located on the Chr11. 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)



\$?

Arrb2 arrestin, beta 2 [Mus musculus (house mouse)]

Gene ID: 216869, updated on 13-Mar-2020

Summary

Official Symbol	Arrb2 provided by MGI
Official Full Name	arrestin, beta 2 provided by MGI
Primary source	MGI:MGI:99474
See related	Ensembl:ENSMUSG0000060216
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	AI326910, AW122872, Arr3
Expression	Ubiquitous expression in spleen adult (RPKM 25.2), thymus adult (RPKM 24.8) and 28 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 13 transcripts, all transcripts are shown below:

No. No.							
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arrb2-204	ENSMUST00000102564.10	1848	<u>410aa</u>	Protein coding	CCD524946	A0A158SIT9 Q91YI4	TSL:1 GENCODE basic APPRIS P1
Arrb2-205	ENSMUST00000108568.9	1777	<u>421aa</u>	Protein coding	CCDS70226	<u>J3JS97 Q91YI4</u>	TSL:1 GENCODE basic
Arrb2-201	ENSMUST00000079056.8	1443	<u>421aa</u>	Protein coding	CCDS70226	<u>J3JS97 Q91YI4</u>	TSL:1 GENCODE basic
Arrb2-203	ENSMUST00000102563.1	1352	<u>410aa</u>	Protein coding	CCD524946	A0A1585IT9 Q91YI4	TSL:1 GENCODE basic APPRIS P1
Arrb2-202	ENSMUST0000084954.12	1732	<u>406aa</u>	Protein coding	846	Q5F2D9	TSL:5 GENCODE basic
Arrb2-207	ENSMUST00000124943.7	662	<u>83aa</u>	Protein coding	653	J3QNV6	CDS 3' incomplete TSL:3
Arrb2-213	ENSMUST00000150076.7	370	<u>27aa</u>	Protein coding	(-)	<u>J3QN53</u>	CDS 3' incomplete TSL:3
Arrb2-209	ENSMUST00000128748.7	957	<u>37aa</u>	Nonsense mediated decay	-	G3UZ54	TSL:5
Arrb2-210	ENSMUST00000138006.7	1073	No protein	Retained intron	15	7.5	TSL:5
Arrb2-208	ENSMUST00000125441.7	756	No protein	Retained intron	19-21	-	TSL:3
Arrb2-211	ENSMUST00000143232.1	603	No protein	Retained intron	12	<u></u>	TSL:5
Arrb2-206	ENSMUST00000124112.1	574	No protein	Retained intron	2.00	-	TSL:5
Arrb2-212	ENSMUST00000144454.1	488	No protein	Retained intron	343	-	TSL:2
4					/ 1		

The strategy is based on the design of Arrb2-205 transcript, the transcription is shown below:

Arrb2-205 > protein coding

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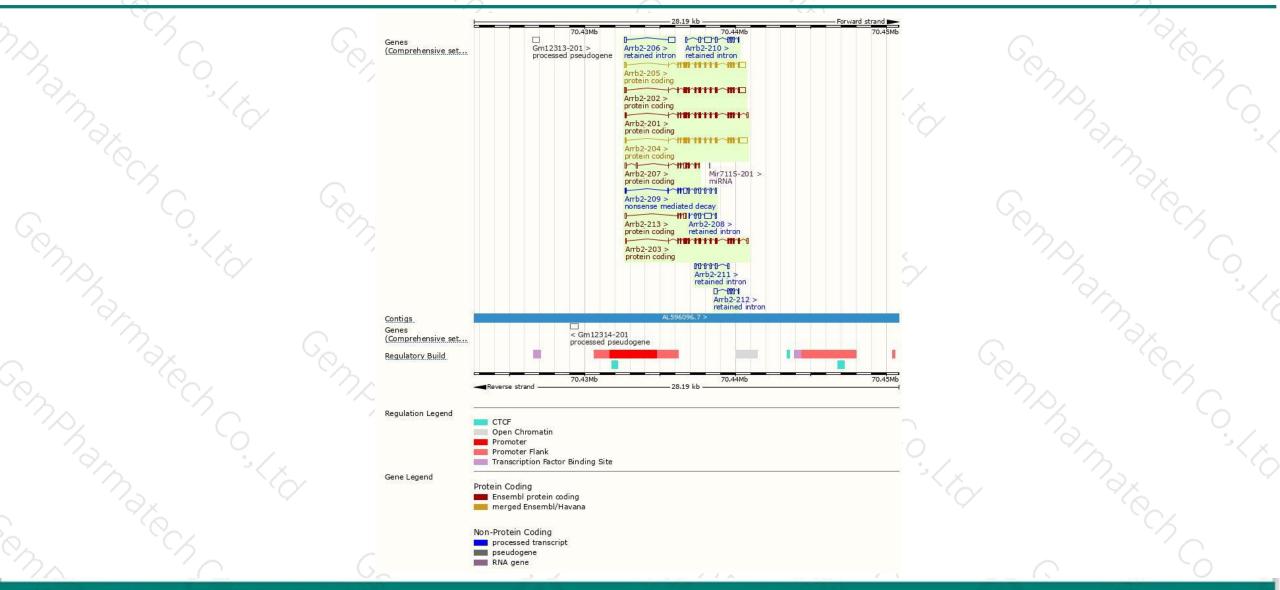
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Forward strand

Genomic location distribution





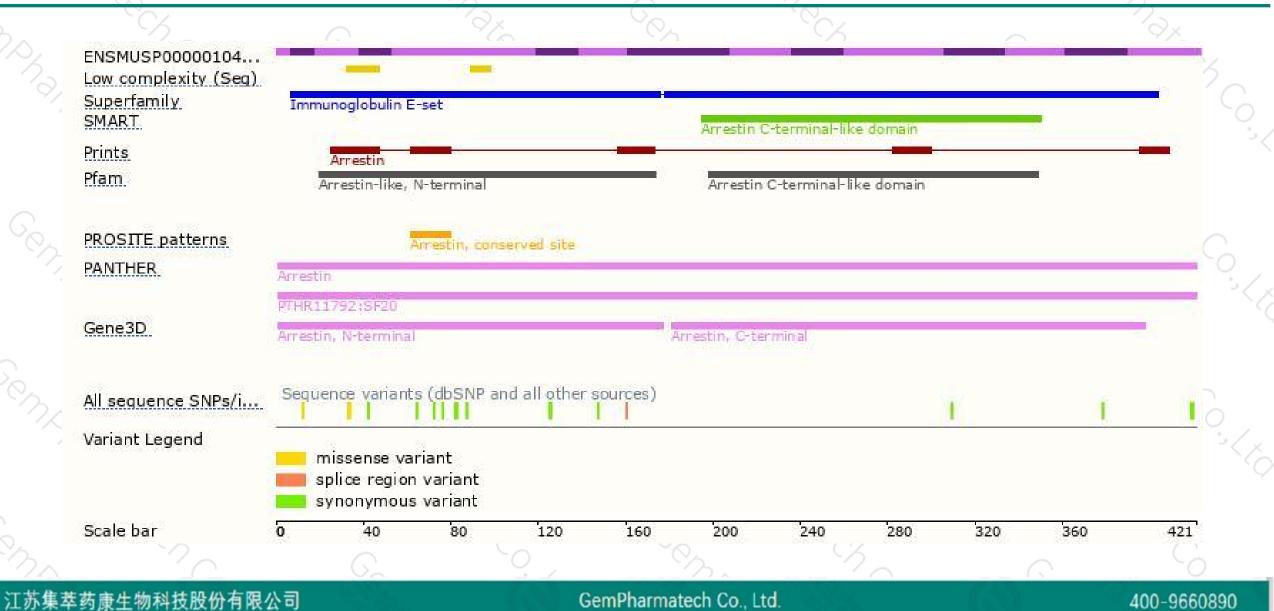
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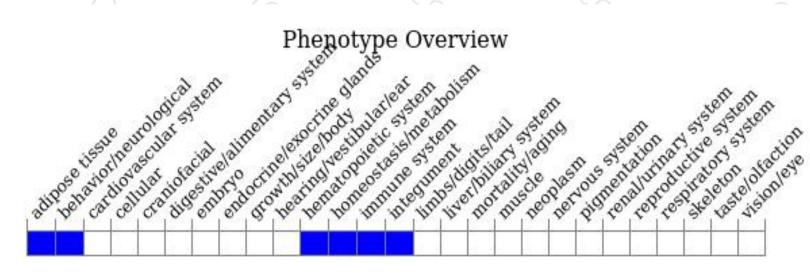
Protein domain





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,mice homozygous for a knock-out allele exhibit enhanced morphine analgesia, an enhanced inflammatory response and reduced threshold to lethal endotoxin challenge, and impaired T and B lymphocyte chemotaxis.

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



