

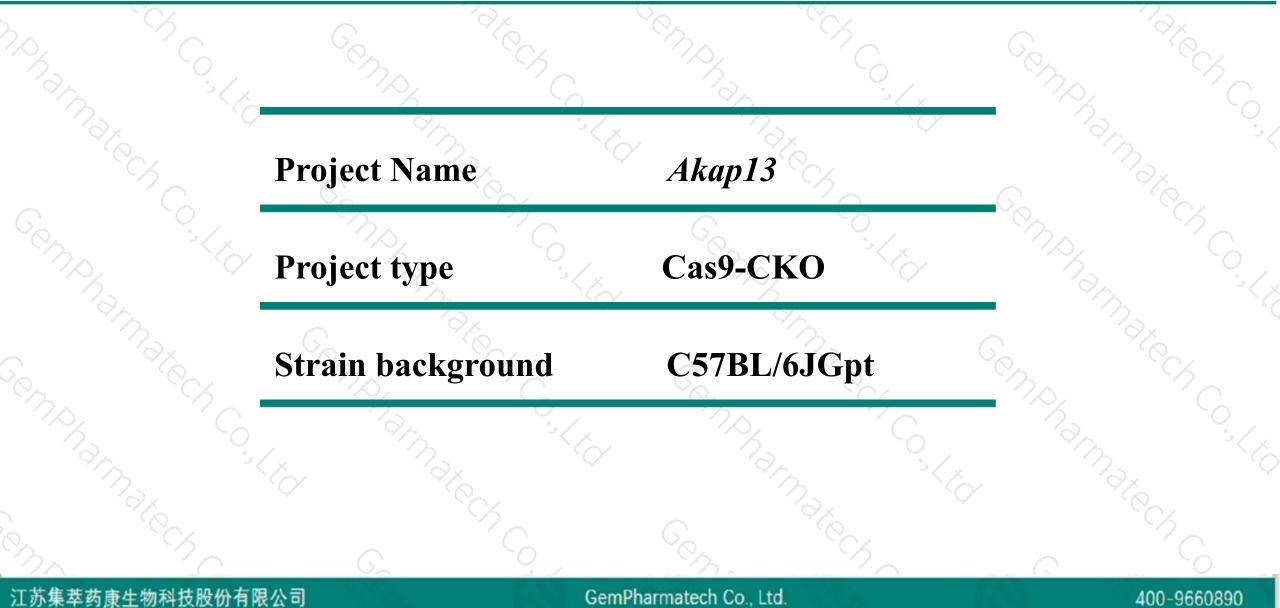
Akap13 Cas9-CKO Strategy

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empharmatect

Project Overview



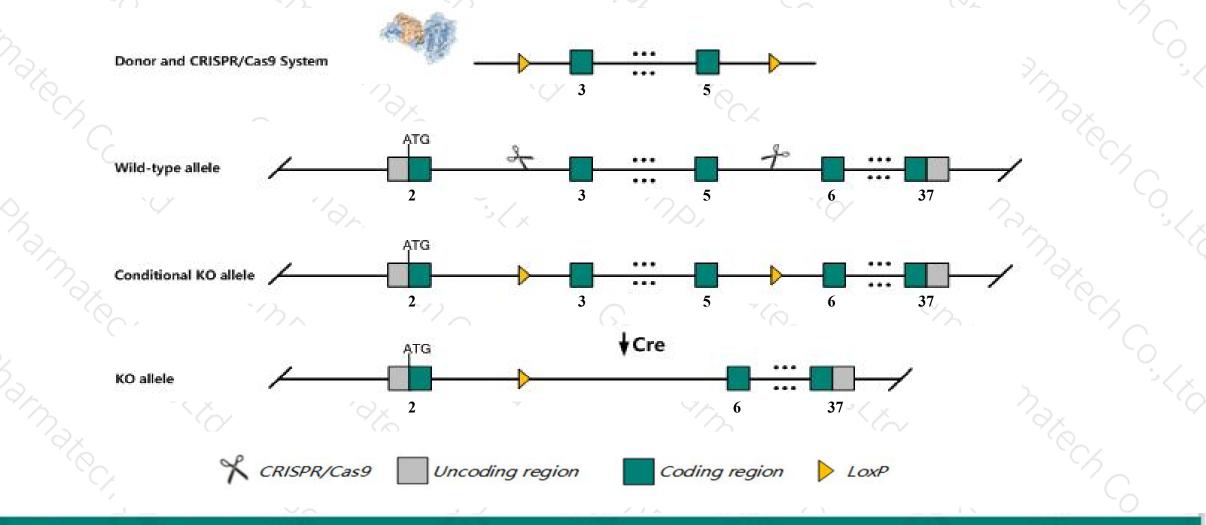


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Akap13 gene. The schematic diagram is as follows:



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The Akap13 gene has 16 transcripts. According to the structure of Akap13 gene, exon3-exon5 of Akap13-201 (ENSMUST00000166315.6) transcript is recommended as the knockout region. The region contains 629bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Akap13* 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 null allele exhibit embryonic lethality during organogenesis, arrested heart development, and forebrain hypoplasia. Heterozygous mice exhibit small spleen, impaired lymphocyte response to osmotic stress, decreased response to glucocorticoid, osteoporosis and impared osteogenesis.
- Transcript Akap13-202&203&204&209&210&211&213&214&216 may not be affected .And the effect on transcript-207&2018&215 is unknown.
- The *Akap13* gene is located on the Chr7. 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)



\$?

Akap13 A kinase (PRKA) anchor protein 13 [Mus musculus (house mouse)]

Gene ID: 75547, updated on 19-Mar-2019

Summary

| Official Symbol | Akap13 provided by MGI |
|----------------------|--|
| | A kinase (PRKA) anchor protein 13 provided by MGI |
| Primary source | MGI:MGI:2676556 |
| 2270 | Ensembl:ENSMUSG0000066406 |
| 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 | 1700026G02Rik, 5730522G15Rik, 5830460E08Rik, AKAP-13, AKAP-Lbc, BRX, Ht31, LBC, PROTO-LB, PROTO-LBC |
| Expression | Ubiquitous expression in spleen adult (RPKM 15.7), lung adult (RPKM 14.2) and 28 other tissues See more |
| Orthologs | human all |

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Transcript information (Ensembl)



The gene has 16 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|------------|----------------------|-------|---------------|----------------------|-----------|------------|---|
| Akap13-201 | ENSMUST00000166315.6 | 12543 | <u>2776aa</u> | Protein coding | CCDS52276 | E9Q394 | TSL:5 GENCODE basic APPRIS P2 |
| Akap13-205 | ENSMUST00000207750.1 | 12597 | <u>2794aa</u> | Protein coding | | A0A140LJJ5 | TSL:5 GENCODE basic APPRIS ALT2 |
| Akap13-207 | ENSMUST00000207923.1 | 2612 | <u>870aa</u> | Protein coding | -3 | A0A140LIX0 | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:1 |
| Akap13-203 | ENSMUST00000207239.1 | 1908 | <u>607aa</u> | Protein coding | 20 | A0A140LHG3 | CDS 3' incomplete TSL:1 |
| Akap13-215 | ENSMUST00000208708.1 | 1705 | <u>569aa</u> | Protein coding | - | A0A140LID7 | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5 |
| Akap13-208 | ENSMUST00000207998.1 | 1269 | <u>423aa</u> | Protein coding | -8 | A0A140LHQ3 | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:1 |
| Akap13-214 | ENSMUST00000208456.1 | 716 | No protein | Processed transcript | -3 | - | TSL:1 |
| Akap13-216 | ENSMUST00000209040.1 | 470 | No protein | Processed transcript | - | 22 | TSL:2 |
| Akap13-213 | ENSMUST00000208248.1 | 3133 | No protein | Retained intron | - | 17 | TSL:NA |
| Akap13-204 | ENSMUST00000207511.1 | 2061 | No protein | Retained intron | -8 | 8 | TSL:NA |
| Akap13-211 | ENSMUST00000208182.1 | 1455 | No protein | Retained intron | 20 | - | TSL:NA |
| Akap13-202 | ENSMUST00000207079.1 | 1406 | No protein | Retained intron | 20 | 2 | TSL1 |
| Akap13-212 | ENSMUST00000208187.1 | 1381 | No protein | Retained intron | 50 | 17 | TSL:2 |
| Akap13-206 | ENSMUST00000207751.1 | 1295 | No protein | Retained intron | - | 8 | TSL:1 |
| Akap13-210 | ENSMUST00000208053.1 | 615 | No protein | Retained intron | 23 | - | TSL:3 |
| Akap13-209 | ENSMUST00000208009.1 | 601 | No protein | Retained intron | 20 | 12 | TSL:1 |

The strategy is based on the design of Akap13-201 transcript, The transcription is shown below

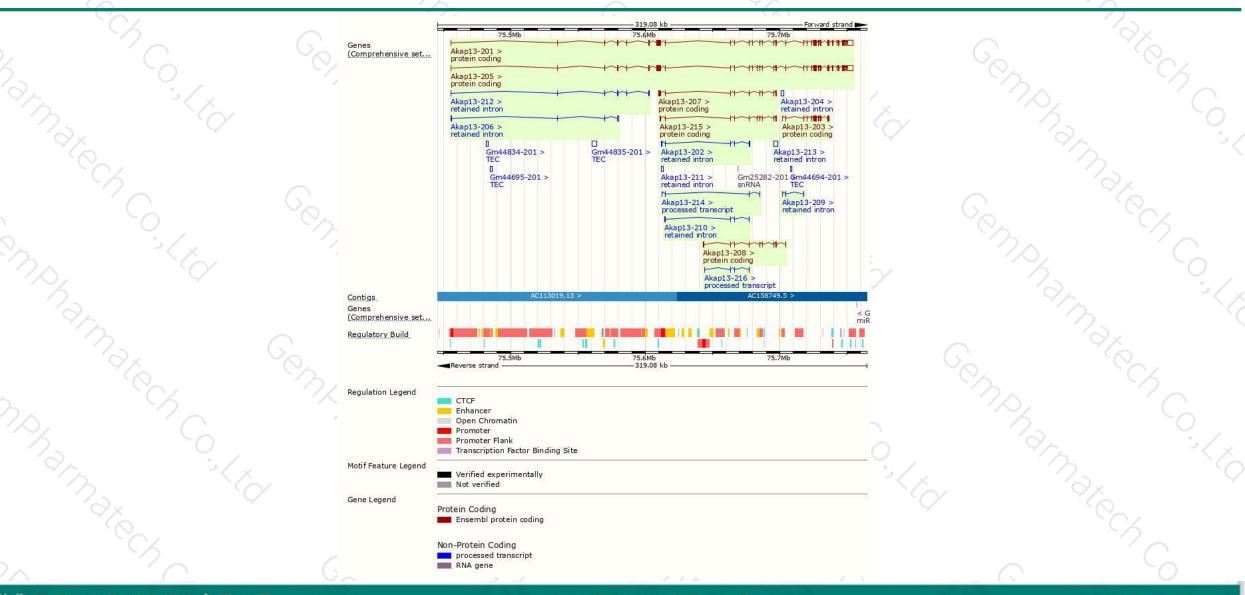


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Genomic location distribution



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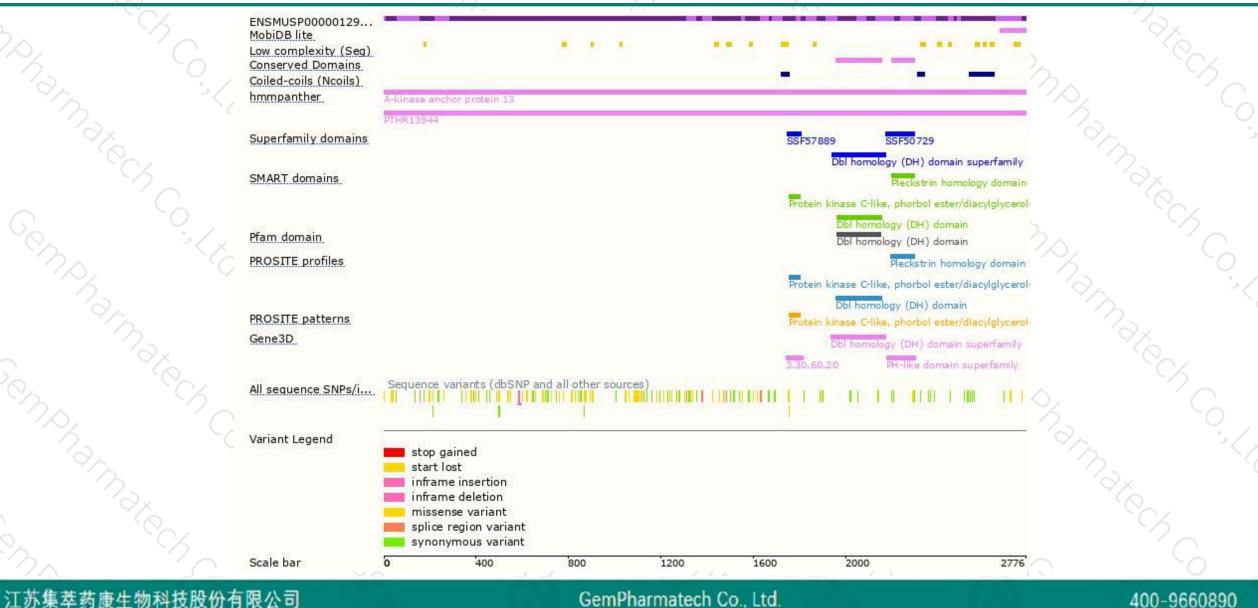
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Protein domain

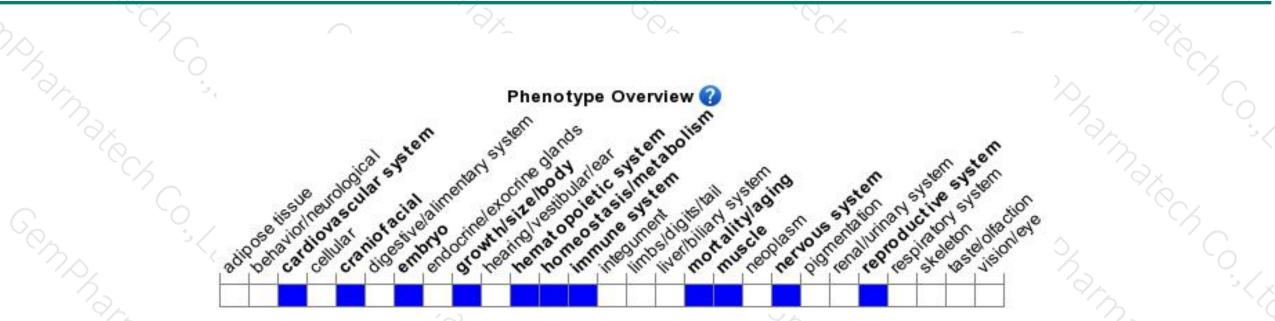




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Mouse phenotype description(MGI)





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 null allele exhibit embryonic lethality during organogenesis, arrested heart development, and forebrain hypoplasia. Heterozygous mice exhibit small spleen, impaired lymp response to osmotic stress, decreased response to glucocorticoid, osteoporosis and impared osteogenesis.

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



