

Cecr2 Cas9-CKO Strategy

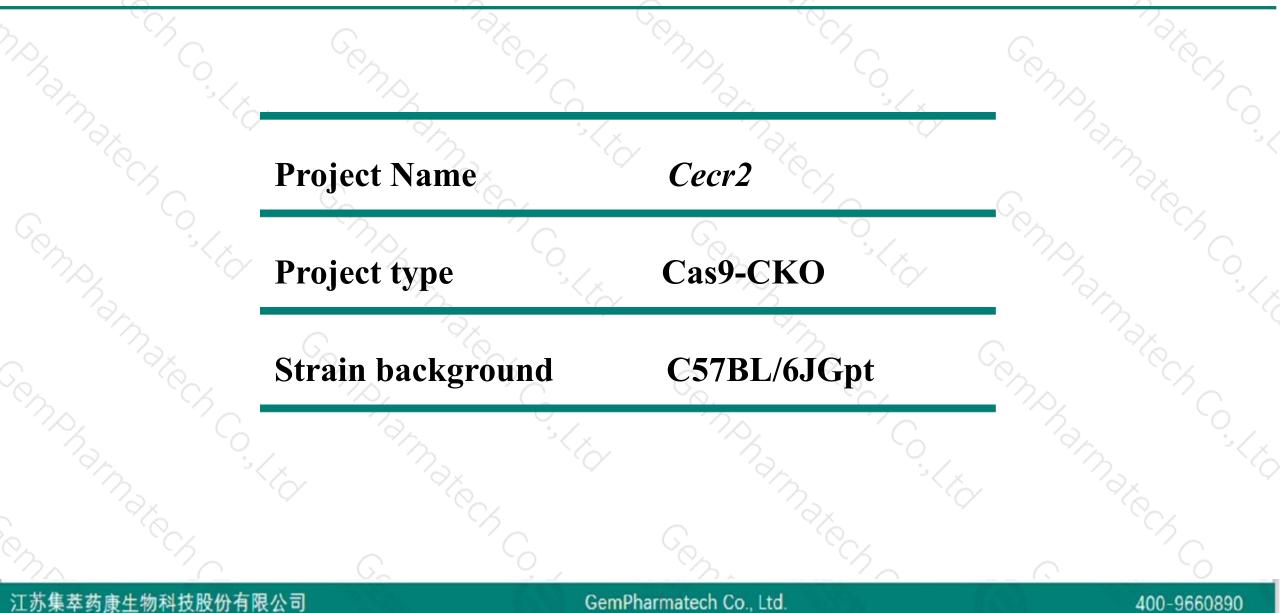
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2019-12-25

Project Overview



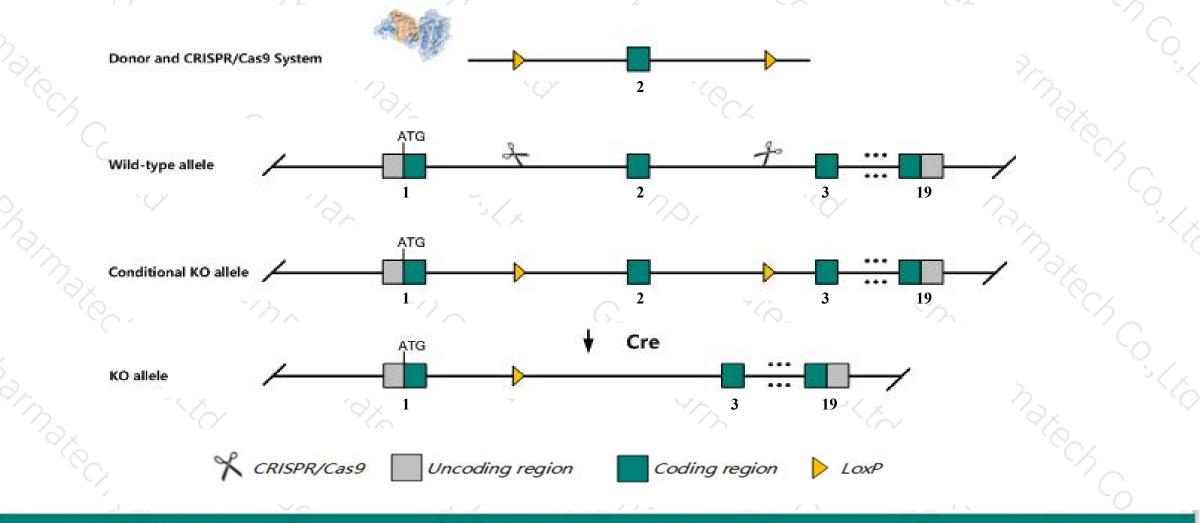


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Cecr2 gene. The schematic diagram is as follows:



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The Cecr2 gene has 8 transcripts. According to the structure of Cecr2 gene, exon2 of Cecr2-202 (ENSMUST00000112686.7) transcript is recommended as the knockout region. The region contains 95bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cecr2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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, Homozygous mutant mice display varied penetrance of exencephaly depending on genetic background.
- Transcript *Cecr2-204,206* may not be affected.
- The Cecr2 gene is located on the Chr6. 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.

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Gene information (NCBI)



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Cecr2 CECR2, histone acetyl-lysine reader [Mus musculus (house mouse)]

Gene ID: 330409, updated on 21-Feb-2019

Summary

| Official Symbol | Cecr2 provided by MGI |
|--------------------|--|
| Official Full Name | CECR2, histone acetyl-lysine reader provided by MGI |
| Primary source | MGI:MGI:1923799 |
| See related | Ensembl:ENSMUSG00000071226 |
| 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 | 2610101016Rik, 2810409N01Rik, Gtl4 |
| Expression | Ubiquitous expression in testis adult (RPKM 4.5), CNS E11.5 (RPKM 4.0) and 23 other tissues See more |
| Orthologs | human all |

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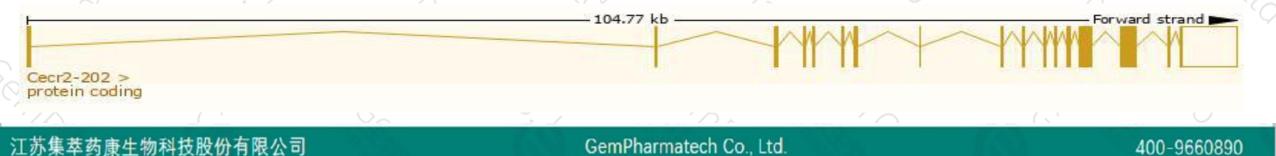
Transcript information (Ensembl)



The gene has 8 transcripts, all transcripts are shown below:

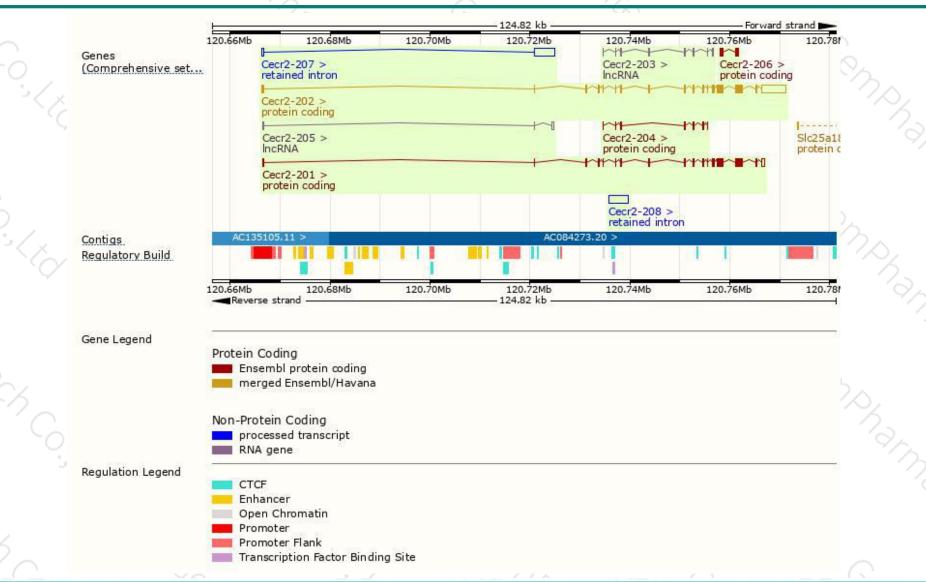
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-----------|----------------------|------|---------------|-----------------|-----------|---------|---|
| Cecr2-202 | ENSMUST00000112686.7 | 9212 | <u>1425aa</u> | Protein coding | CCDS51890 | E9Q2Z1 | TSL:5 GENCODE basic APPRIS P2 |
| Cecr2-201 | ENSMUST00000100993.8 | 4849 | <u>1453aa</u> | Protein coding | -5 | E9Q2Z1 | TSL:5 GENCODE basic APPRIS ALT2 |
| Cecr2-206 | ENSMUST00000143563.1 | 1071 | <u>357aa</u> | Protein coding | 2 | - | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:2 |
| Cecr2-204 | ENSMUST00000129803.1 | 704 | <u>234aa</u> | Protein coding | 2 | 24 | 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 |
| Cecr2-207 | ENSMUST00000148346.1 | 4392 | No protein | Retained intron | - | 17 | TSL:1 |
| Cecr2-208 | ENSMUST00000204732.1 | 3996 | No protein | Retained intron | - | 8 | TSL:NA |
| Cecr2-203 | ENSMUST00000124634.1 | 946 | No protein | IncRNA | - | - | TSL:5 |
| Cecr2-205 | ENSMUST00000135109.1 | 641 | No protein | IncRNA | 2 | 14 | TSL:3 |

The strategy is based on the design of Cecr2-202 transcript, The transcription is shown below



Genomic location distribution





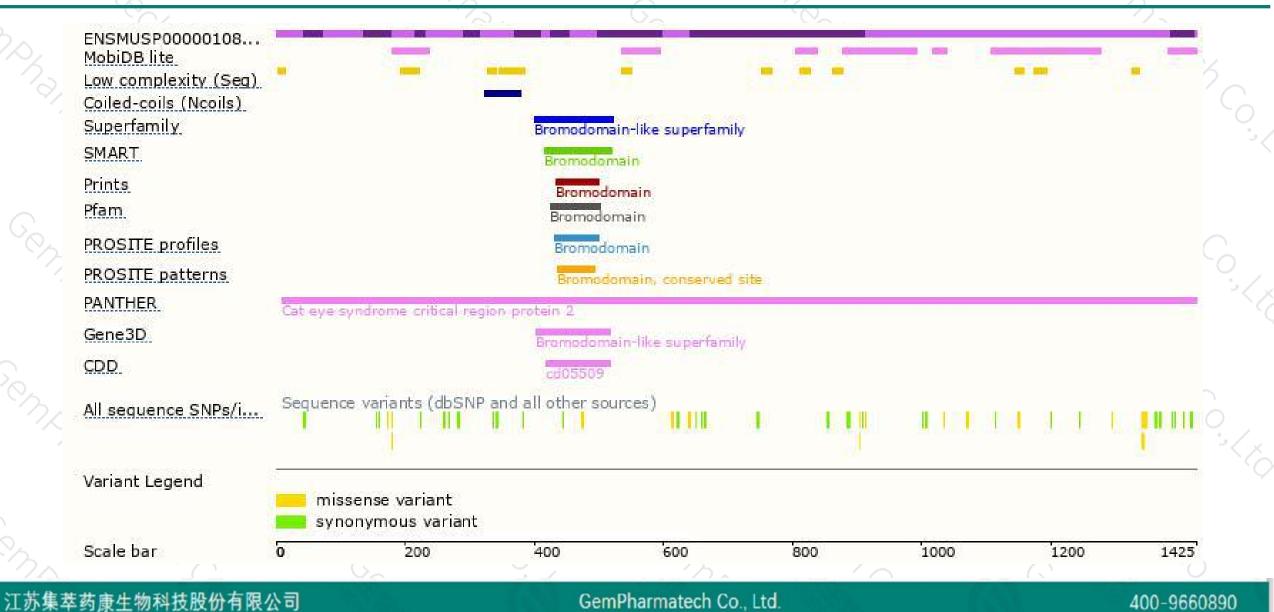
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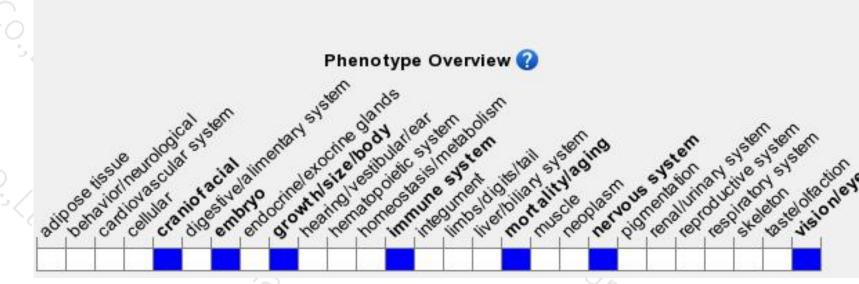
Protein domain





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, Homozygous mutant mice display varied penetrance of exencephaly depending on geneti background.



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



