

Runx1t1 Cas9-CKO Strategy

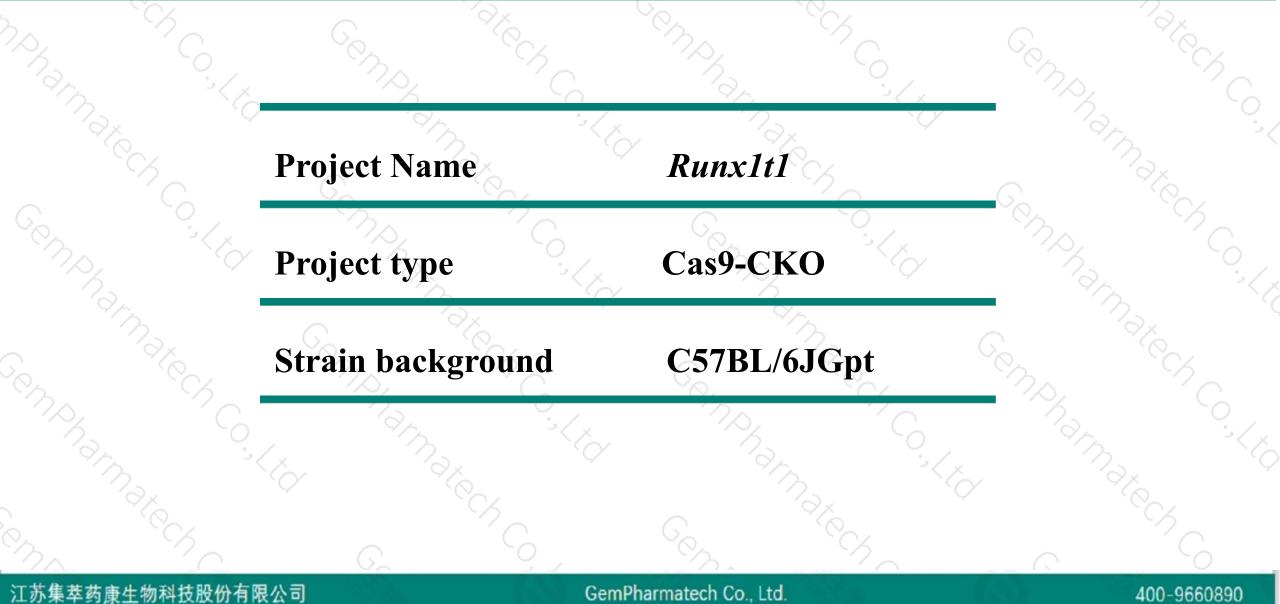
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-9-25

Project Overview



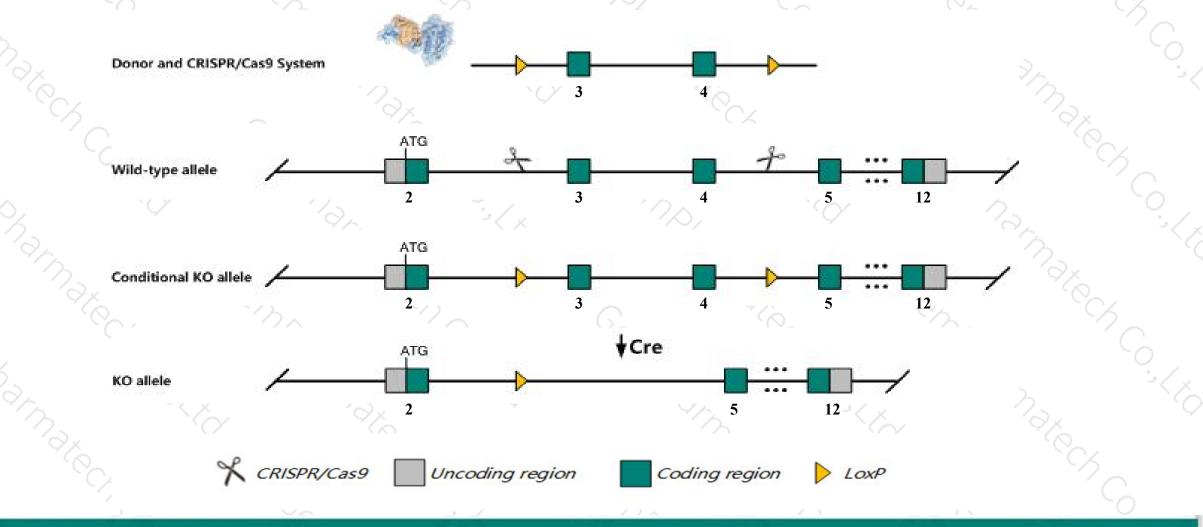


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Runx1t1 gene. The schematic diagram is as follows:



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The Runx1t1 gene has 6 transcripts. According to the structure of Runx1t1 gene, exon3-exon4 of Runx1t1-204 (ENSMUST00000105566.8) transcript is recommended as the knockout region. The region contains 380bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Runx1t1* 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, Homozygous disruption of this gene results in increased perinatal lethality and surviving animals show severe growth retardation. The midgut is absent in 25% of mutant animals which could explain increased perinatal mortality. Surviving animals display thinned intestinal walls and dilated lumens.
- The Runx1t1 gene is located on the Chr4. 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)



\$?

Runx1t1 RUNX1 translocation partner 1 [Mus musculus (house mouse)]

Gene ID: 12395, updated on 31-Jan-2019

Summary

| Official Symbol | Runx1t1 provided by MGI |
|----------------------|--|
| Official Full Name | RUNX1 translocation partner 1 provided by MGI |
| Primary source | MGI:MGI:104793 |
| See related | Ensembl:ENSMUSG0000006586 |
| 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 | Cbfa2t1h, ETO, MTG8 |
| Expression | Broad expression in whole brain E14.5 (RPKM 6.0), CNS E18 (RPKM 4.8) and 15 other tissues See more |
| Orthologs | human all |

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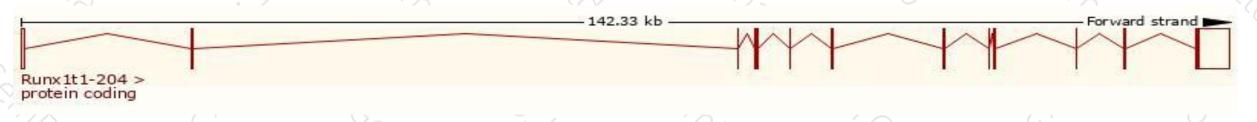
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-------------|----------------------|------|--------------|----------------|-----------|---------------|---------------------------------|
| Runx1t1-204 | ENSMUST00000105566.8 | 6090 | <u>604aa</u> | Protein coding | CCDS51125 | B1AXH8 | TSL:5 GENCODE basic APPRIS ALT2 |
| Runx1t1-201 | ENSMUST0000006761.9 | 5783 | <u>584aa</u> | Protein coding | CCDS51124 | <u>Q3UQX8</u> | TSL:1 GENCODE basic APPRIS ALT2 |
| Runx1t1-202 | ENSMUST0000098256.3 | 3317 | <u>577aa</u> | Protein coding | CCDS17978 | B1AXH9 Q61909 | TSL:1 GENCODE basic APPRIS P3 |
| Runx1t1-203 | ENSMUST0000098257.9 | 2332 | <u>604aa</u> | Protein coding | CCDS51125 | B1AXH8 | TSL:1 GENCODE basic APPRIS ALT2 |
| Runx1t1-206 | ENSMUST00000150583.7 | 2062 | No protein | IncRNA | 5 | 7 | TSL:1 |
| Runx1t1-205 | ENSMUST00000139736.1 | 405 | No protein | IncRNA | | - | TSL:3 |

The strategy is based on the design of Runx1t1-204 transcript, The transcription is shown below

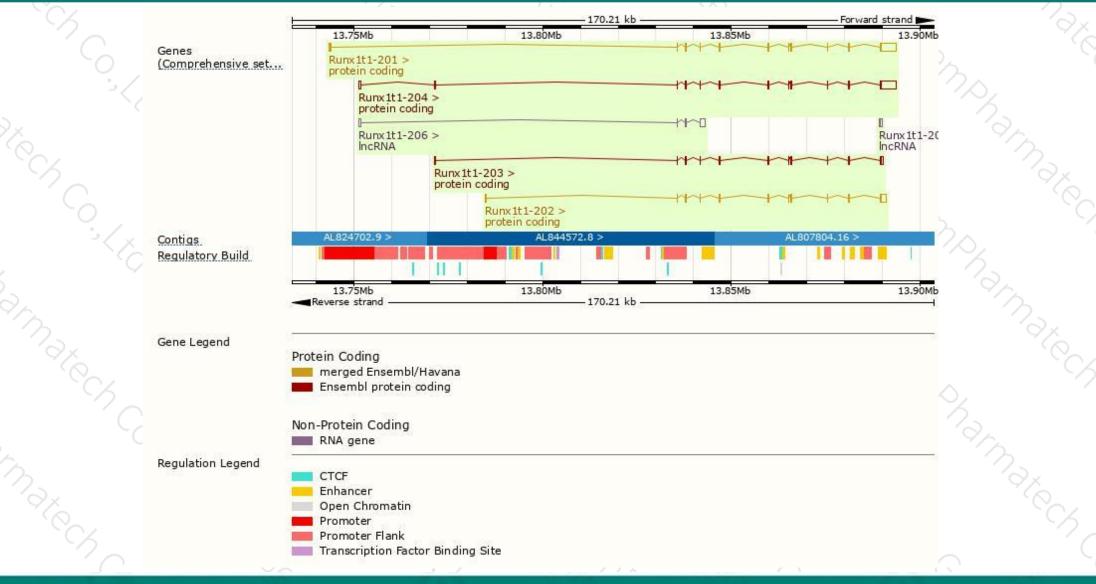


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Genomic location distribution



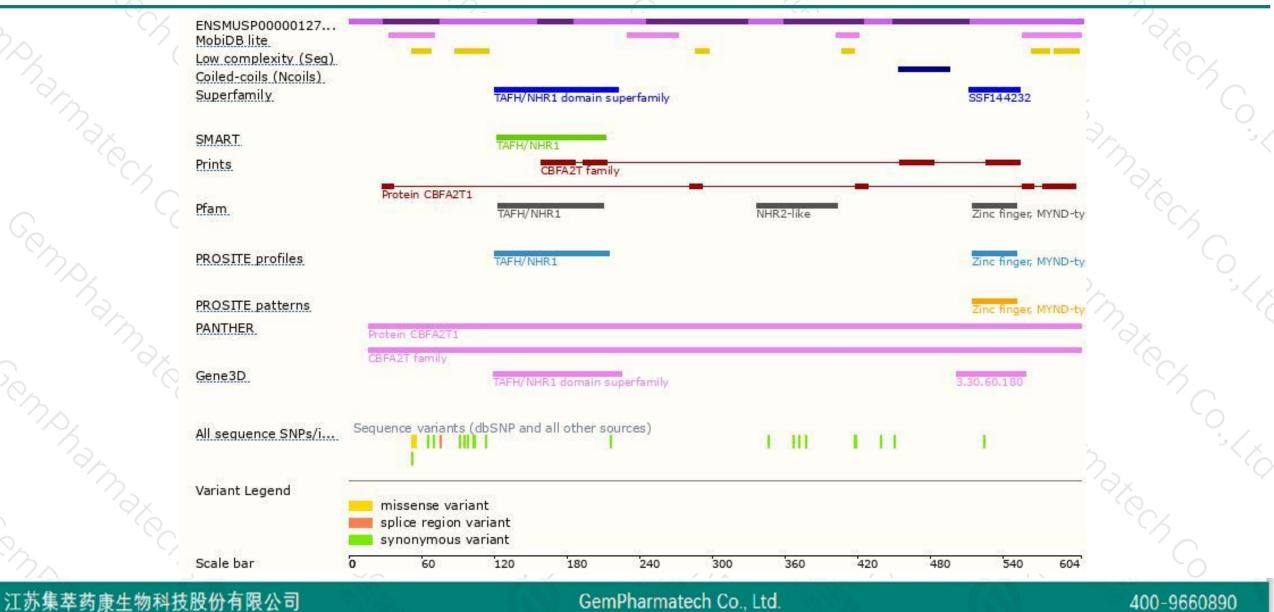


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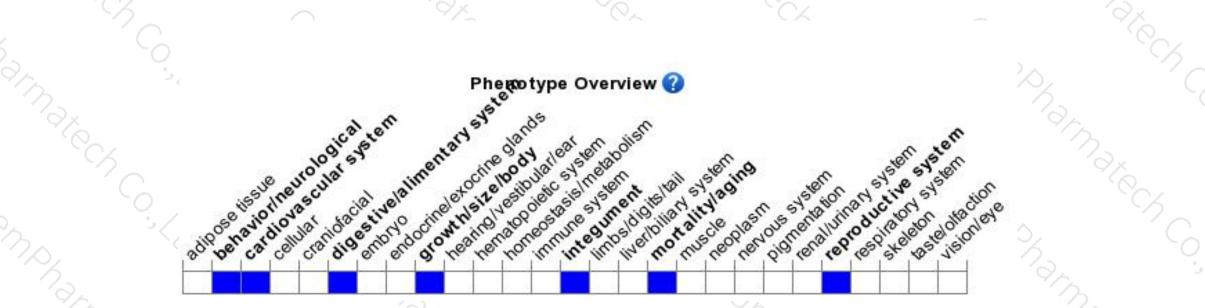
Protein domain





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 disruption of this gene results in increased perinatal lethality and surviving animals show severe growth retardation. The midgut is absent in 25% of mutant animals which could explain increased perinatal mortality. Surviving animals display thinned intestinal walls and dilated lumens.

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



