Ncr1-P2A-iCre Cas9-KI Strategy

Designer: Reviewer:

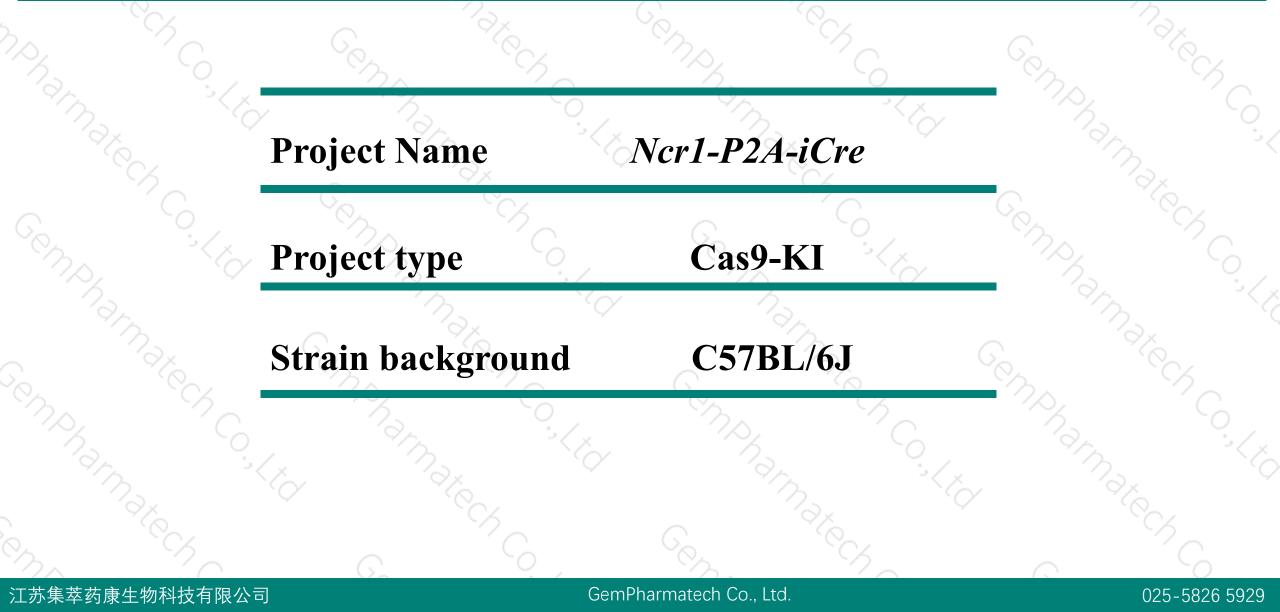
Design Date:

Min Guan

Qiong Zhou 2018-07-25

Project Overview

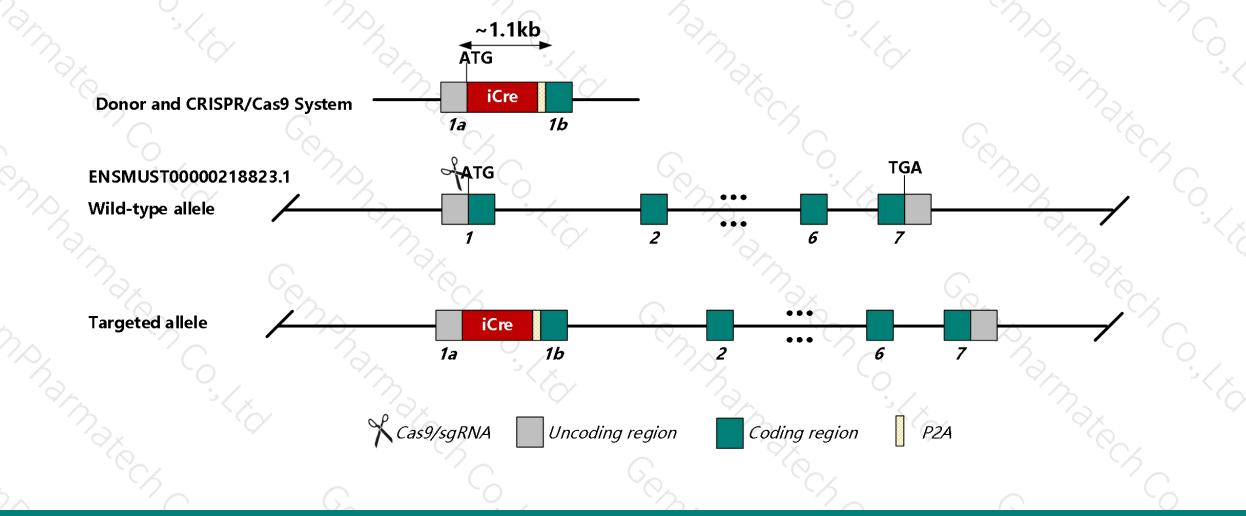




Knockin strategy



This model will use CRISPR/Cas9 technology to edit the Ncr1 gene. The schematic diagram is as follows:





- The Ncr1 gene has 2 transcripts. According to the structure of Ncr1 gene, Ncr1-201(ENSMUST0000006792.5) is selected for presentation of the recommended strategy.
- > Ncr1-201 gene has 7 exons, with the ATG start codon in exon1 and TGA stop codon in exon7.
- We make Ncr1-P2A-iCre knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of Ncr1 gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre after start coding(ATG) of Ncr1 gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

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- According to the existing MGI data, mutations in this gene lead to alterations in susceptibility to viral infection induced morbidity/mortality,
 NK cell number, NK cell cytolysis, and T cell responses.
- Insertion of iCre may affect the regulation of the 5' end of the Ncr1 gene.
- ► Ncr1 gene specific expression site: natural killer (NK) cells.
- > There will be 1 to 2 amino acid synonymous mutation in exon1 of *Ncr1* gene in this strategy.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The Ncr1 gene is located on the Chr7. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Notice

Gene information



2 1

Ncr1 natural cytotoxicity triggering receptor 1 [Mus musculus (house mouse)]

Gene ID: 17086, updated on 12-Aug-2019

Summary

 Official Symbol
 Ncr1 provided by MGI

 Official Full Name
 natural cytotoxicity triggering receptor 1 provided by MGI

 Primary source
 MGI:MGI:1336212

 See related
 Ensembl:ENSMUSG00000062524

 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; Musi Mus

 Also known as
 Ly94; NKp46

 Expression
 Biased expression in spleen adult (RPKM 2.2), lung adult (RPKM 0.8) and 7 other tissues <u>See more</u>

 Orthologs
 human all

(NCBI)

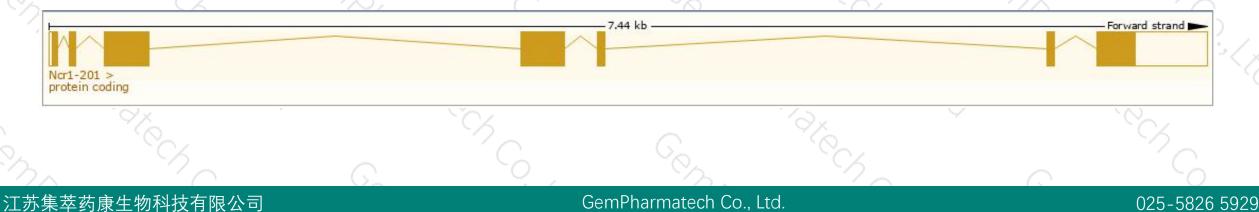
Transcript information (Ensembl)



The gene has 2 transcripts, and the transcript is shown below :

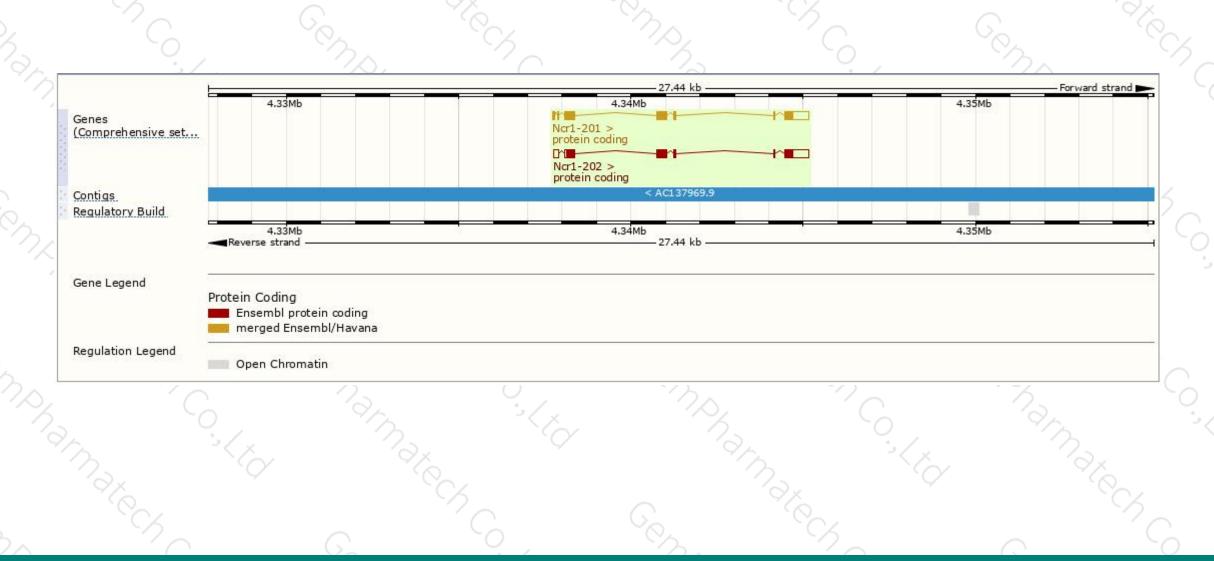
Name 🖕	Transcript ID 💧	bp 💧	Protein	Biotype	CCDS 🖕	UniProt 💧	Flags		
Ncr1-201	ENSMUST0000006792.5	1463	<u>325aa</u>	Protein coding	<u>CCDS20734</u> 교	A0A0R4IZY7	TSL:1	GENCODE basic	APPRIS P2
Ncr1-202	ENSMUST00000126417.2	1495	<u>288aa</u>	Protein coding	8 .	<u>A0A0U1RP63</u> @	TSL:1	GENCODE basic	APPRIS ALT2

The strategy is based on the design of Ncr1-201 transcript. The transcription is shown below



Genomic location distribution





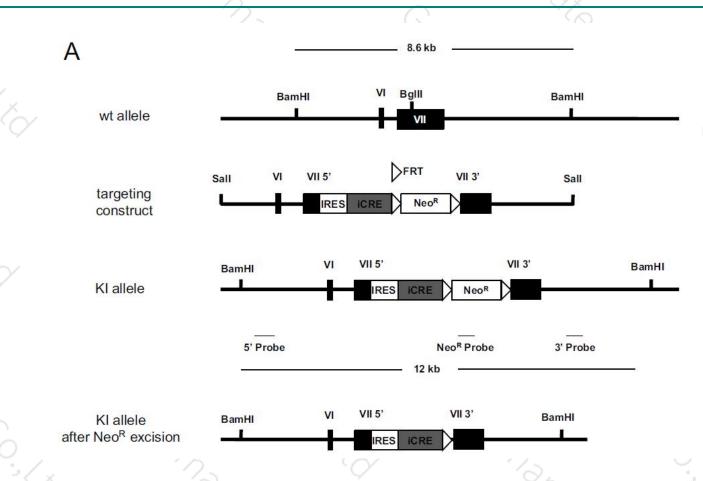
Protein domain



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Reference





 Narni-Mancinelli E, Chaix J, Fenis A, et al. Fate mapping analysis of lymphoid cells expressing the NKp46 cell surface receptor[J]. Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(45):18324-18329.

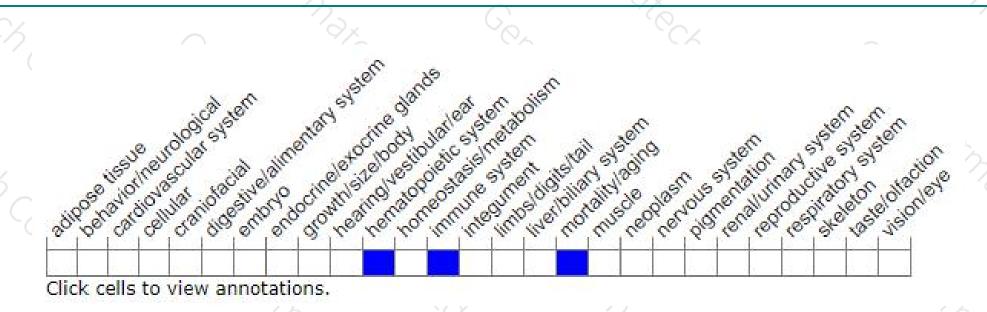
江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

025-5826 5929

Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

Mutations in this gene lead to alterations in susceptibility to viral infection induced morbidity/mortality, NK cell number, NK cell cytolysis, and T cell responses.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



