Tlr9 Cas9-KO Strategy

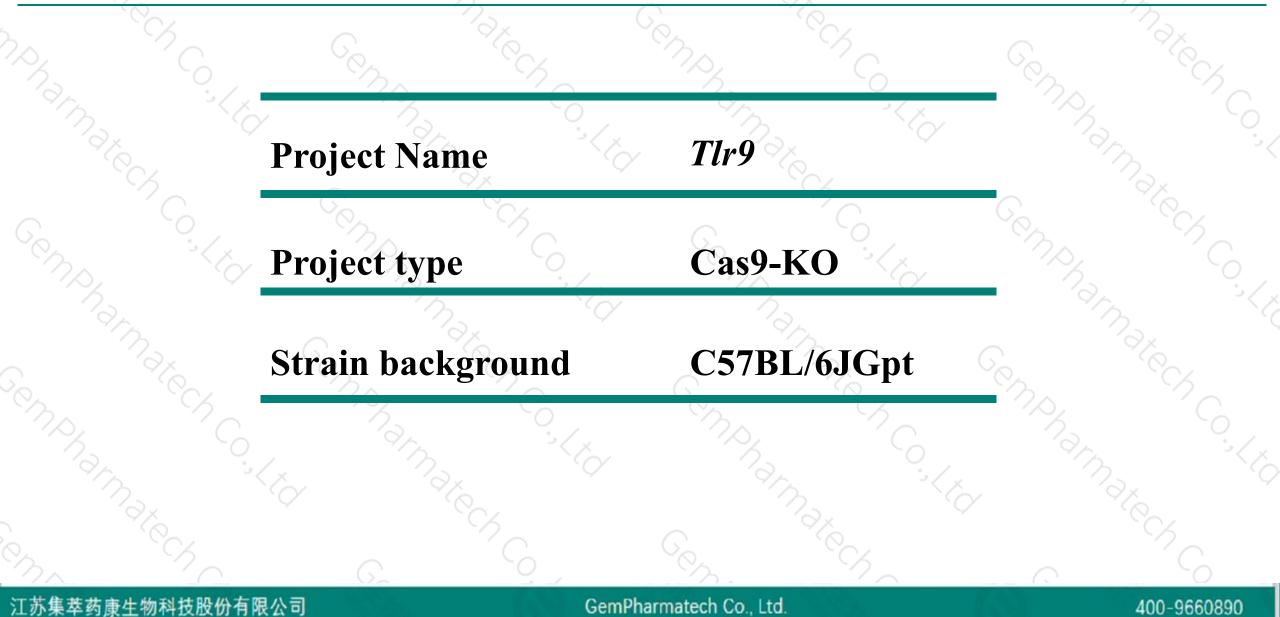
Designer: Design Date:

Jinling Wang 2019-7-22

harman

Project Overview

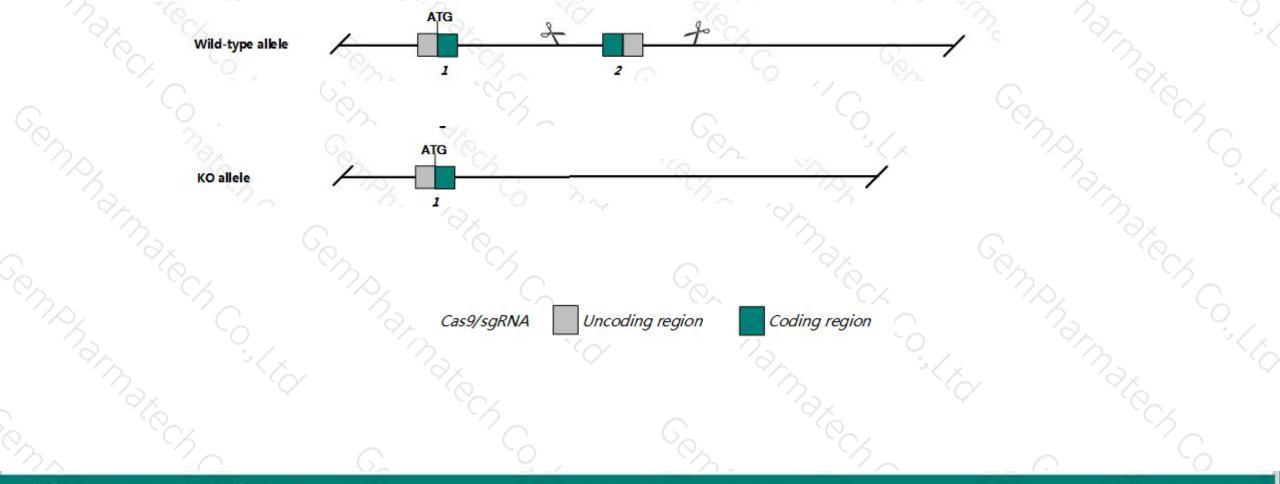




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tlr9* gene. The schematic diagram is as follows:



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- The *Tlr9* gene has 1 transcript .According to the structure of *Tlr9* gene exon2 of *Tlr9*-201 (ENSMUST0000062241.10) transcript is recommended as the knockout region. The region contains the most coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tlr9* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice



- According to the existing MGI data : Nullizygous mice exhibit impaired immune responses to CpG DNA and altered susceptibility to EAE and parasitic infection. ENU-induced mutants may exhibit altered susceptibility to viral infection or induced colitis and impaired immune response to unmethylated CpG oligonucleotides.
- The *Thr9* gene is located on the Chr9. 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)



2 1

Tir9 toll-like receptor 9 [Mus musculus (house mouse)]

Gene ID: 81897, updated on 17-Oct-2018

Summary

- Official Symbol TIr9 provided by MGI Official Full Name toll-like receptor 9 provided by MGI Primary source MGI:MGI:1932389 See related Ensembl:ENSMUSG00000045322 Vega:OTTMUSG00000049422 Gene type protein coding RefSeg status VALIDATED Organism Mus musculus
 - Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha: Muroidea; Muridae; Murinae; Mus; Mus

Expression

Biased expression in spleen adult (RPKM 37.8), mammary gland adult (RPKM 11.5) and 5 other tissues See more Orthologs human all

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Transcript information (Ensembl)



400-9660890

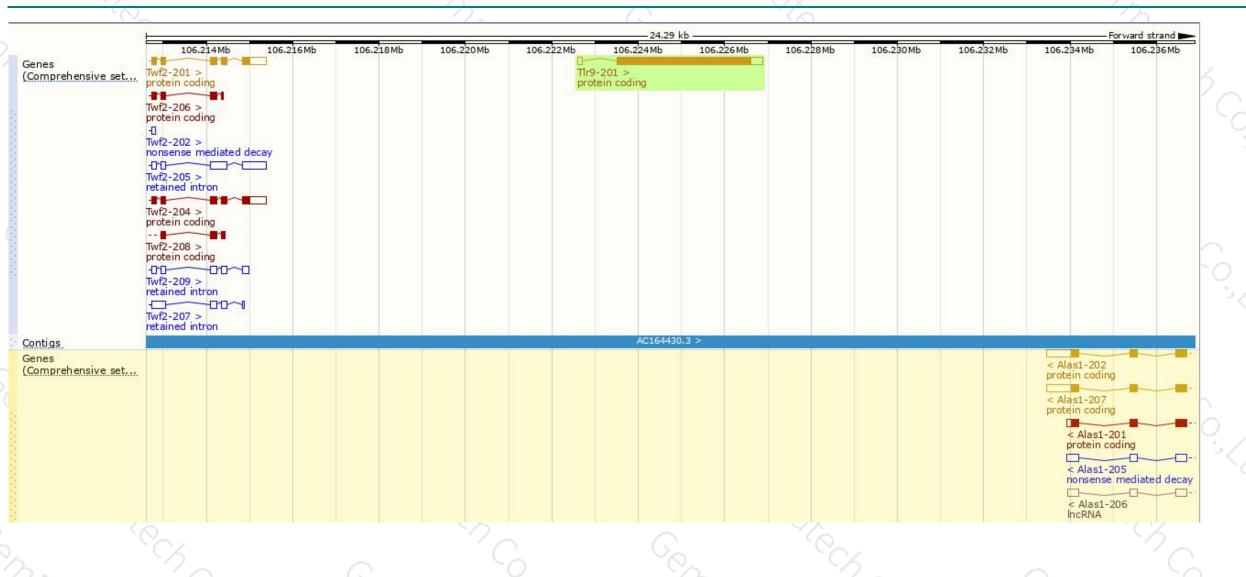
The gene has 1 transcript, and all transcripts are shown below:

| Show/hide columns (1 hidden) | | | | | | | | | | Filter | a | |
|------------------------------|---------------------|----|------|---------------|----------------|---------|--------------|-----------------|----------------------------------|--------|---------------|-----------|
| Name 🍦 | Transcript ID | \$ | bp 👌 | Protein 🖕 | Biotype 🍦 | CCDS | ÷ | UniProt 🖕 | RefSeq 💧 | _ | Flags | 4 |
| TIr9-201 | ENSMUST0000062241.1 | 10 | 3478 | <u>1032aa</u> | Protein coding | CCDS407 | <u>′55</u> @ | <u>Q9EQU3</u> & | <u>NM_031178</u> 교 NP 112455교 | TSL:1 | GENCODE basic | APPRIS P1 |

The strategy is based on the design of *Tlr9*-201 transcript, The transcription is shown below

| | | 4.29 kb | | Forward strand For |
|------------------------------|---|---------|--------|---------------------------|
| Tlr9-201 > protein coding | 197 ₆ 01 | ~ | ALM S. | 1'2× |
| °C/ | G ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ | George | | |

Genomic location distribution



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Protein domain



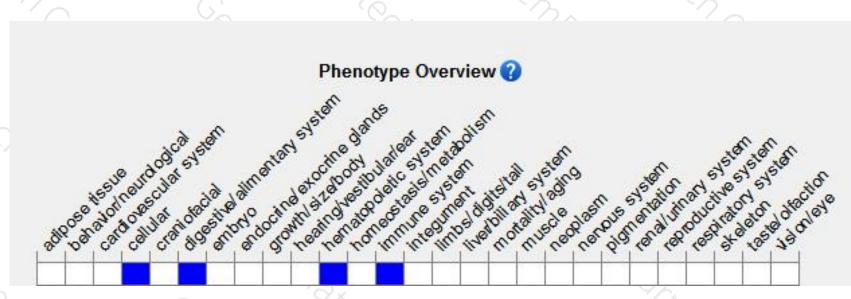
| | | | $-N_{\perp}$ | | |
|---|---|--------------------------------|----------------|--------------------|--|
| ENSMUSP0000082 Low complexity (Seg) Cleavage site (Sign hmmpanther | Toll-like receptor 9 | | | 8 | |
| Superfamily domains | PTHR24373 SSF52047 | | SSF52058 | | Toll/interleukin-1 receptor homology (TIR) dom |
| MART domains | SM00365 | | | | Toll/interleukin-1 receptor homology (TIR) da |
| Prints domain Pfam domain | Leucine-rich repeat, typical subtype | PR00019 Leucine-rich repeat | | | Toll/interleukin-1 receptor homology (TIR) d |
| PROSITE profiles Gene3D | Leucine-rich repeat | | | | Toll/interleukin-1 receptor homology (TIR) dc Toll/interleukin-1 receptor homology (TIR) dc |
| All sequence SNPs/i | Sequence variants (dbSNP and all other sources) | 1111.0.1.10.1 | ա գորում | 1 1 11 11 | |
| /ariant Legend | frameshift variant | missense variant | | synonymous variant | |
| cale bar | 0 100 200 | 300 400 | 500 600 | 700 800 | 900 1032 |
| noparts. | | North Contraction | Cemphan Man | | |
| | | C C C | - Co. 732 | | A C |

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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/).

Nullizygous mice exhibit impaired immune responses to CpG DNA and altered susceptibility to EAE and parasitic infection. ENU-induced mutants may exhibit altered susceptibility to viral infection or induced colitis and impaired immune response to unmethylated CpG oligonucleotides.

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



