GemPharmatech™

Spg21 Cas9-CKO Strategy

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Design Date: 2024-3-12

Overview

Target Gene Name

- Spg21

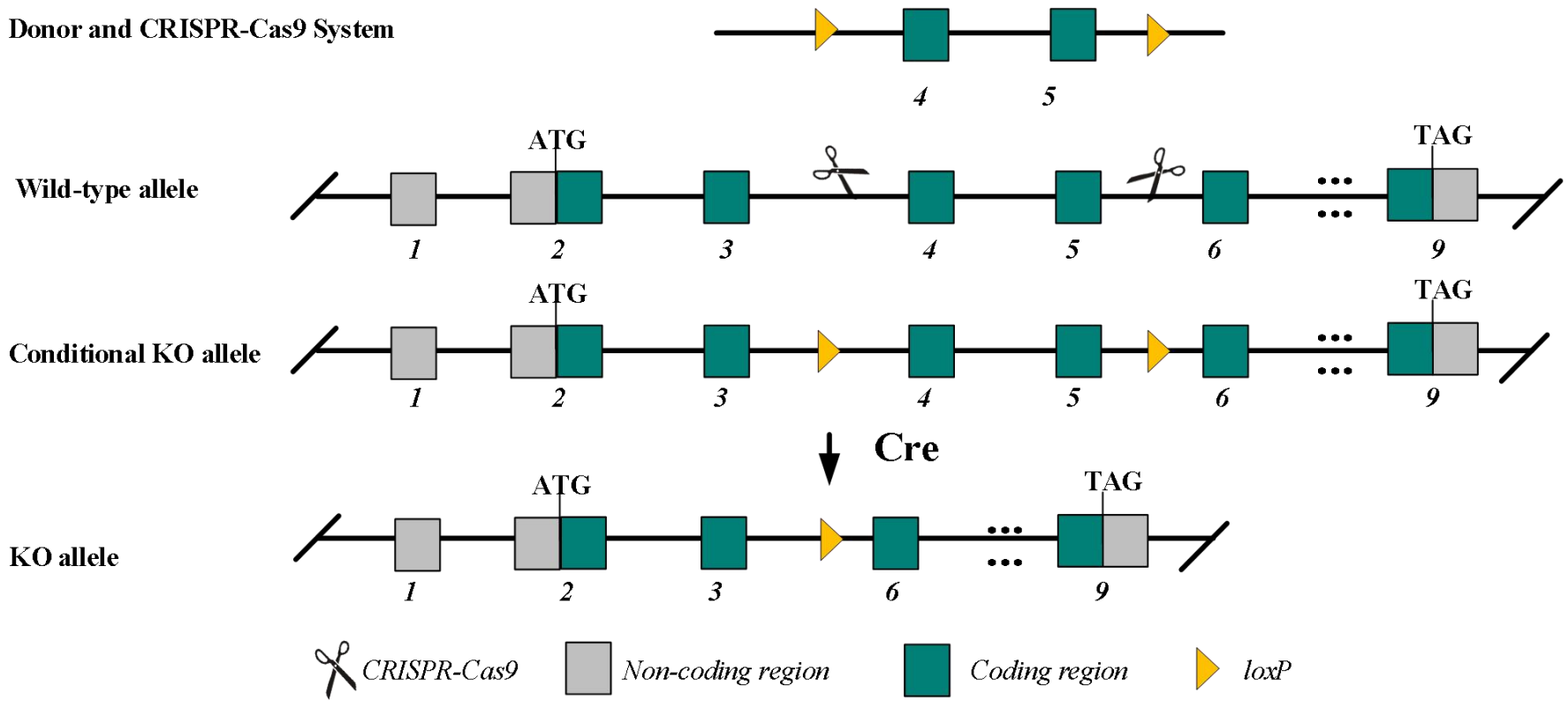
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Spg21* gene.

Technical Information

- The *Spg21* gene has 4 transcripts. According to the structure of *Spg21* gene, exon 4-exon 5 of *Spg21*-201 (ENSMUST00000034955.8) transcript is recommended as the knockout region. The region contains 227 bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Spg21* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Spg21 SPG21, maspardin [Mus musculus (house mouse)]

Gene ID: 27965, updated on 31-May-2023

Summary

Official Symbol	Spg21 provided by MGI
Official Full Name	SPG21, maspardin provided byMGI
Primary source	MGI:MGI:106403
See related	Ensembl:ENSMUSG00000032388
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	ACP33, BM-019, D9Wsu18e, GL010, MAST
Summary	Predicted to enable CD4 receptor binding activity. Predicted to be located in intracellular membrane-bounded organelle. Predicted to be active in cytosol and trans-Golgi network transport vesicle. Is expressed in extraembryonic component; head mesenchyme; tongue; and vertebral axis musculature. Used to study Mast syndrome. Human ortholog(s) of this gene implicated in Mast syndrome and hereditary spastic paraplegia. Orthologous to human SPG21 (SPG21 abhydrolase domain containing, maspardin). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 15.5), limb E14.5 (RPKM 15.0) and 28 other tissuesSee more
Orthologs	human all

Source: https://www.ncbi.nlm.nih.gov/

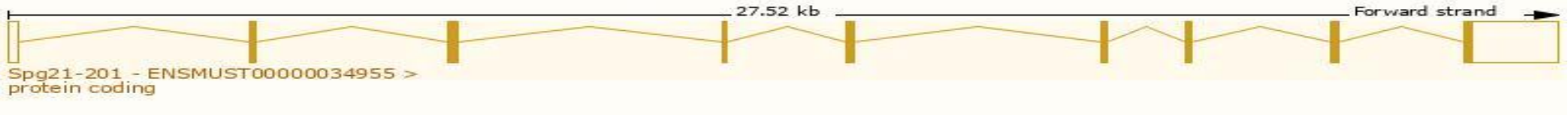
Transcript Information

The gene has 4 transcripts,all transcripts are shown below:

Show/hide columns (1 hidden) Filter

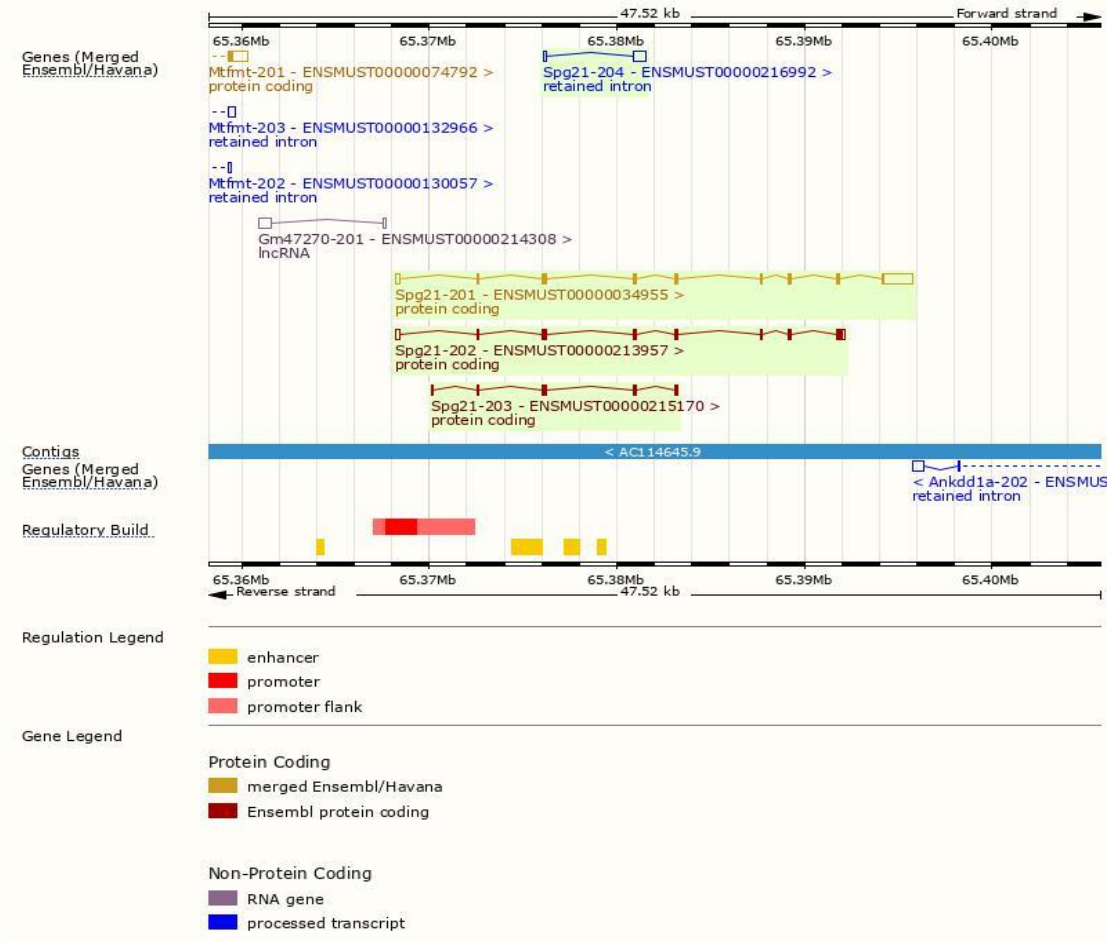
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000034955.8	Spg21-201	2680	308aa	Protein coding	CCDS23293	A2RT57 Q9CQC8	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000213957.2	Spg21-202	1302	306aa	Protein coding		A0A1L1SST5	GENCODE basic TSL:1
ENSMUST00000215170.2	Spg21-203	484	135aa	Protein coding		A0A1L1SSV6	TSL:2 CDS 3' incomplete
ENSMUST00000216992.2	Spg21-204	795	No protein	Retained intron		-	TSL:3

The strategy is based on the design of *Spg21*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information

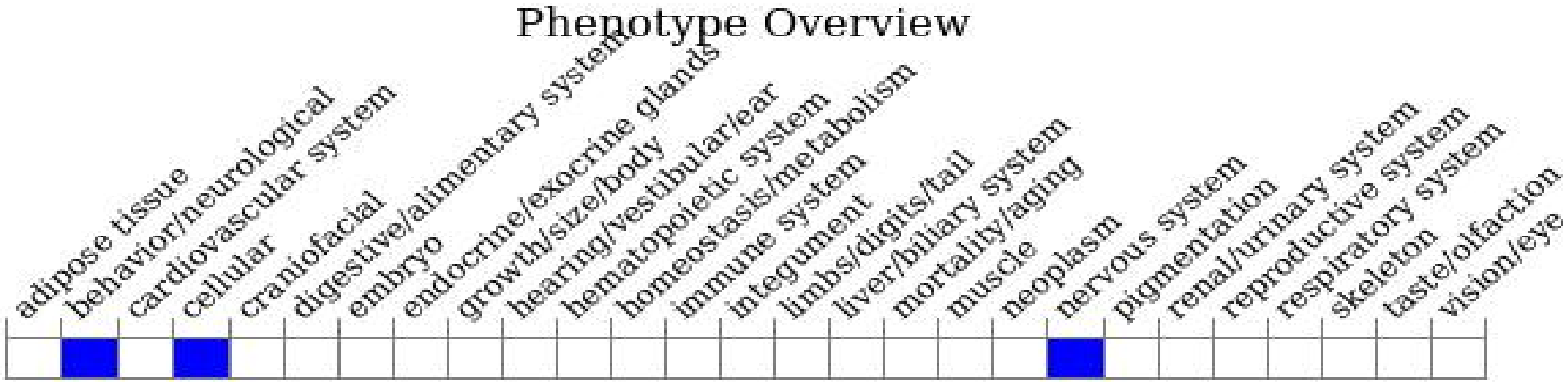


Source: : https://www.ensembl.org

Protein Information

Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a null allele exhibit impaired coordination, dragging of the hind limbs, attenuated growth and maturation of cortical neurons and abnormal cortical neuron axonal branching.

Source: https://www.informatics.jax.org

Important Information

- *Spg21* is located on Chr9. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- The influence of the transcript *Spg21*-203 is unknown.
- After cross cre, 76 amino acids remained at the N-terminus of this strategy, with unknown effects.
- 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 the existing technology level.