Nodal Cas9-CKO Strategy

Designer: Jiayu

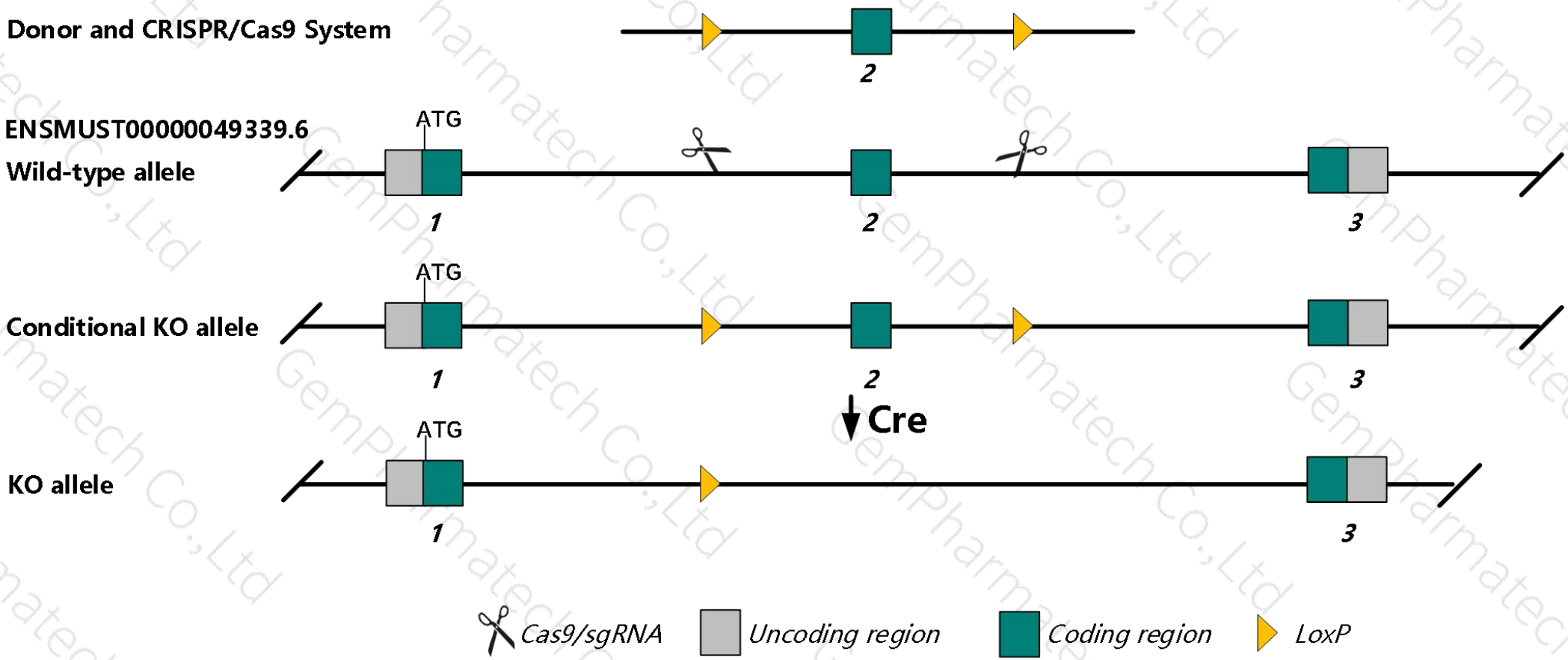
Project Overview

Project Name	***Nodal***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

集萃药康 GemPharmatech

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



Technical routes

- The *Nodal* gene has 1 transcript. According to the structure of *Nodal* gene, exon2 of *Nodal-201* (ENSMUST00000049339.6) transcript is recommended as the knockout region.The region contains 716bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nodal* 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.

Notice

➢ According to the existing MGI data, Homozygous null mutants fail to form a primitive streak, show placental defects and die at gastrulation. Hypomorphic mutants are defective in anterior-posterior, anterior-midline, and left-right body patterning, resulting in multiple organ defects.

➢ The *Nodal* gene is located on the Chr10. 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.

Gene information （NCBI）

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Nodal nodal [*Mus musculus* (house mouse)]

Gene ID: 18119, updated on 2-Jul-2019

Summary

Official Symbol	Nodal provided by MGI
Official Full Name	nodal provided by MGI
Primary source	MGI:MGI:97359
See related	Ensembl:ENSMUSG00000037171
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Tg.413d
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate the mature protein, which regulates early embryonic development. Homozygous knockout mice for this gene exhibit early embryonic lethality, while expression of a hypomorphic allele results in defects in anteroposterior and left-right patterning. [provided by RefSeq, Aug 2016]
Expression	Low expression observed in reference dataset See more
Orthologs	human all

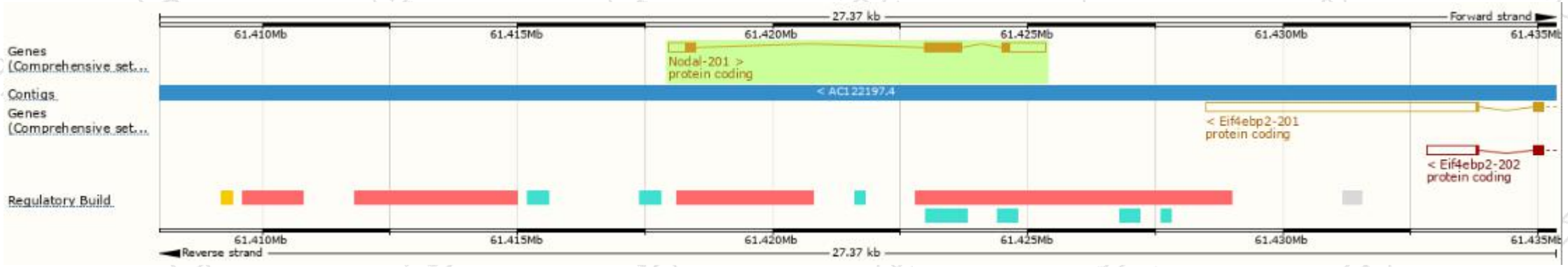
Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Nodal-201	ENSMUST00000049339.6	2095	354aa	Protein coding	CCDS23878	P43021	TSL:1	GENCODE basic	APPRIS P1

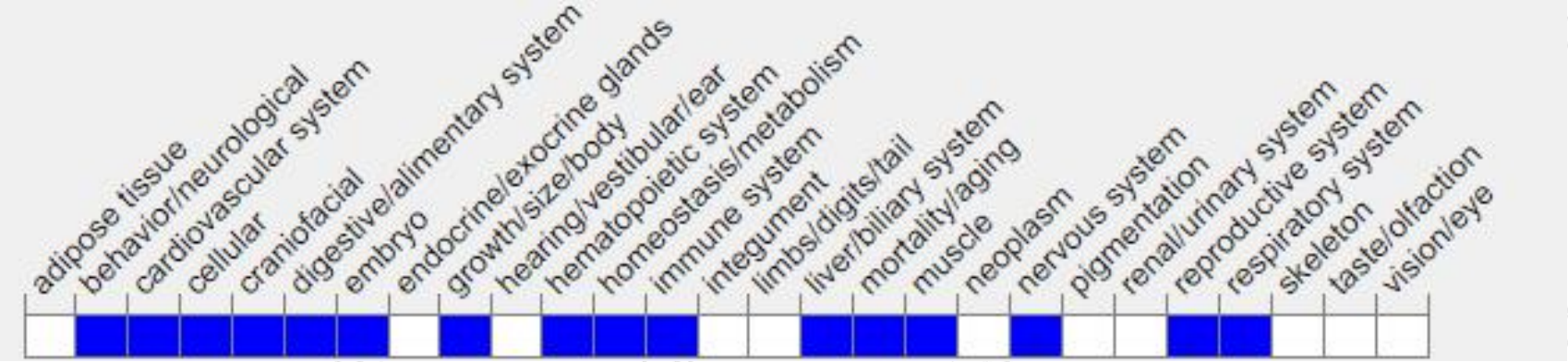
The strategy is based on the design of *Nodal-201* transcript,The transcription is shown below

Genomic location distribution



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 null mutants fail to form a primitive streak, show placental defects and die at gastrulation. Hypomorphic mutants are defective in anterior-posterior, anterior-midline, and left-right body patterning, resulting in multiple organ defects.

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GemPharmatech

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd