Rnf128 Cas9-CKO *Strategy*

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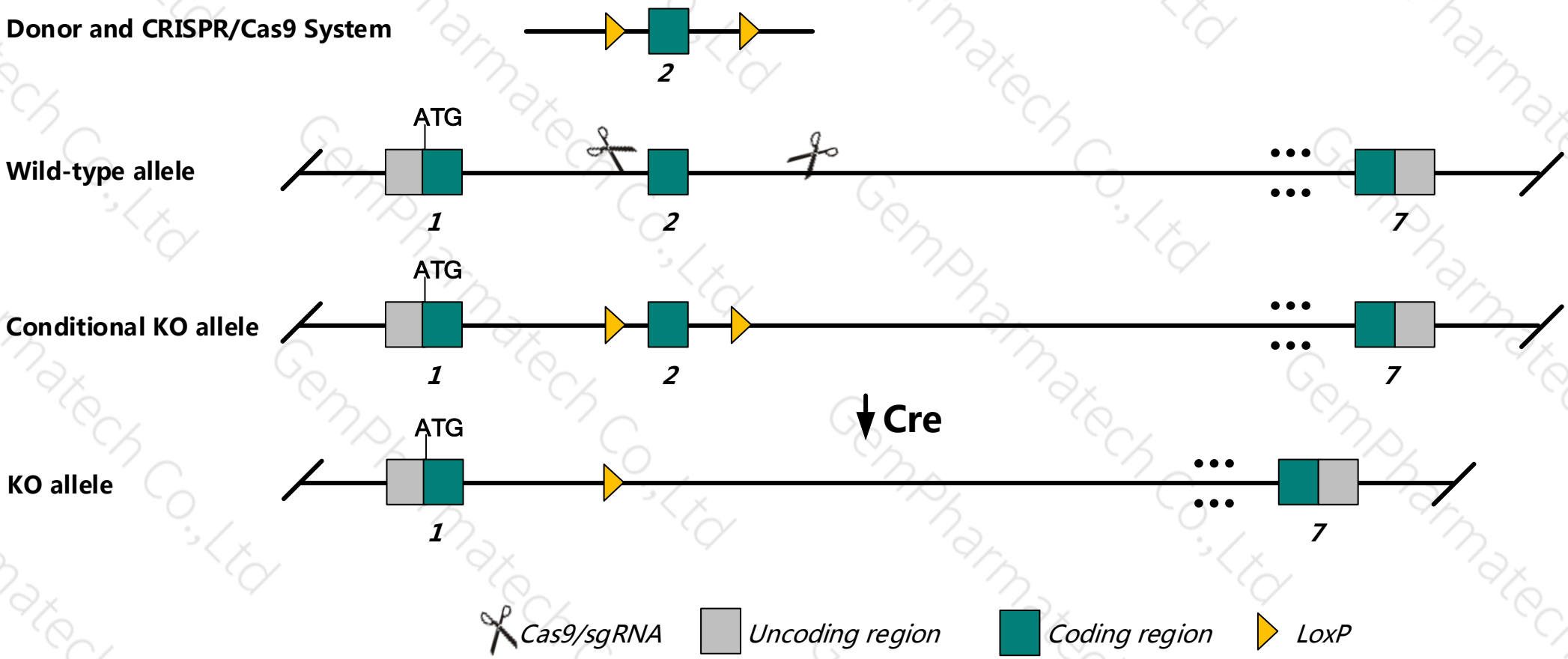
Project Overview

Project Name	***Rnf128***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnf128* gene. The schematic diagram is as follows:



Technical routes

- The *Rnf128* gene has 3 transcripts.According to the structure of *Rnf128* gene, exon2 of *Rnf128*-201 (
- ENSMUST00000113026.1) transcript is recommended as the knockout region.The region contains 248bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rnf128* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

Notice

- According to the existing MGI data，Mice homozygous for a null allele dsiplay defects in naive, helper and anergic T cell states affecting survival, proliferation and cytokine secretion. Homozygotes for another null allele show impaired T cell tolerance and regulatory T cell function and increased susceptibility to autoimmune disease.
- The *Rnf128* gene is located on the ChrX. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Rnf128 ring finger protein 128 [*Mus musculus* (house mouse)]

Gene ID: 66889, updated on 11-May-2019

Summary

Official Symbol	Rnf128 provided by MGI
Official Full Name	ring finger protein 128 provided by MGI
Primary source	MGI:MGI:1914139
See related	Ensembl:ENSMUSG00000031438
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	GRAIL; Greul1; AI987883; 1300002C13Rik
Expression	Broad expression in placenta adult (RPKM 42.6), small intestine adult (RPKM 42.3) and 17 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 3 transcripts, and all transcripts are shown below:

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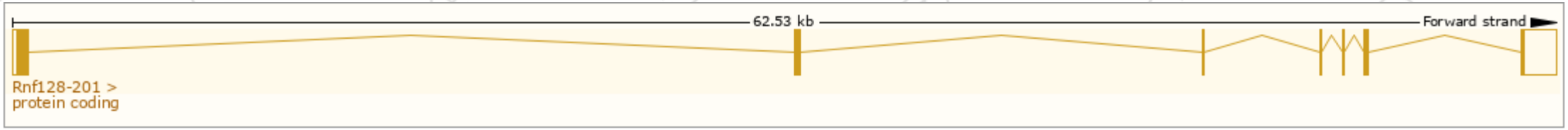
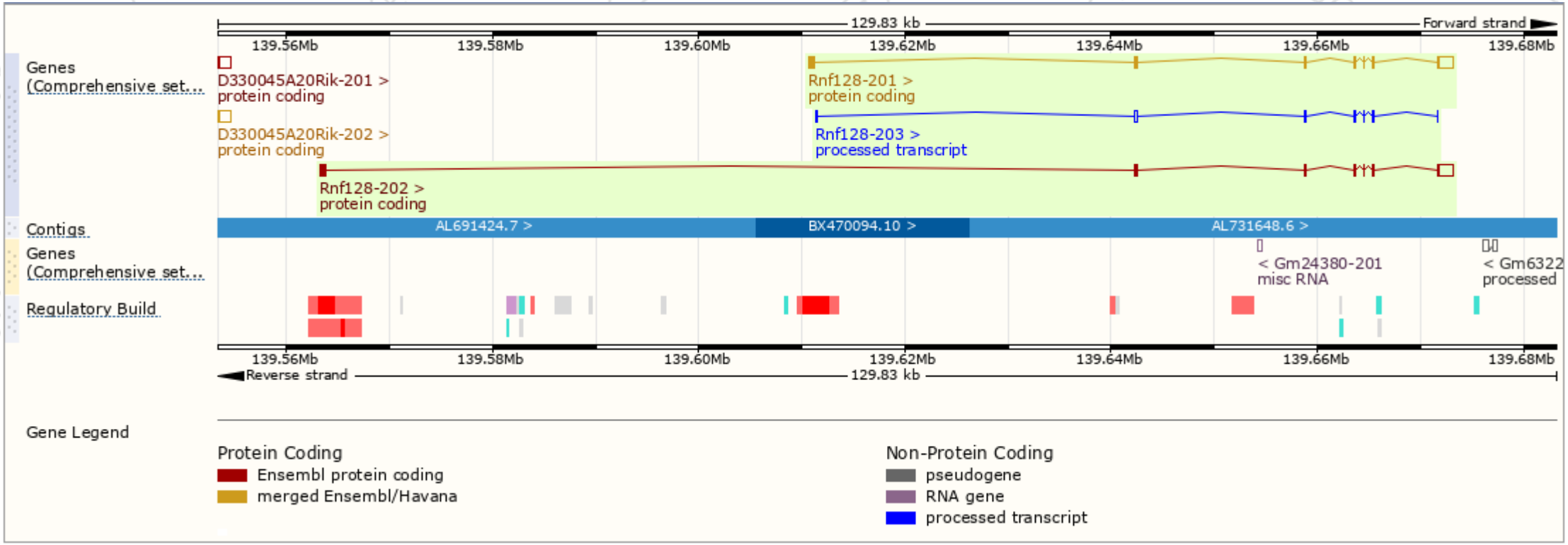
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnf128-201	ENSMUST00000113026.1	2750	428aa	Protein coding	CCDS30435	Q9D304	TSL:1 GENCODE basic APPRIS P1
Rnf128-202	ENSMUST00000113027.7	2641	402aa	Protein coding	CCDS57776	A2AGL8	TSL:5 GENCODE basic
Rnf128-203	ENSMUST00000113029.3	821	No protein	Processed transcript	-	-	TSL:5

The strategy is based on the design of *Rnf128*-201 transcript,The transcription is shown below

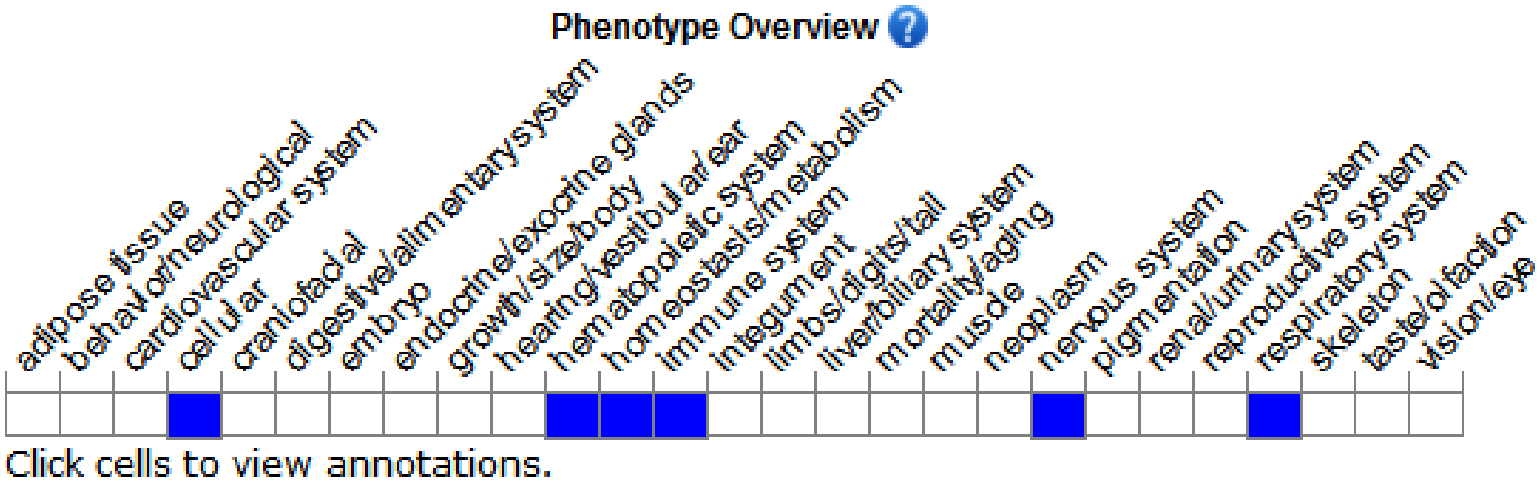


Genomic location distribution



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/) .

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

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