# *Ryk* Cas9-CKO Strategy

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**Design Date:** 2019-9-20

# Project Overview

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

# Conditional Knockout strategy

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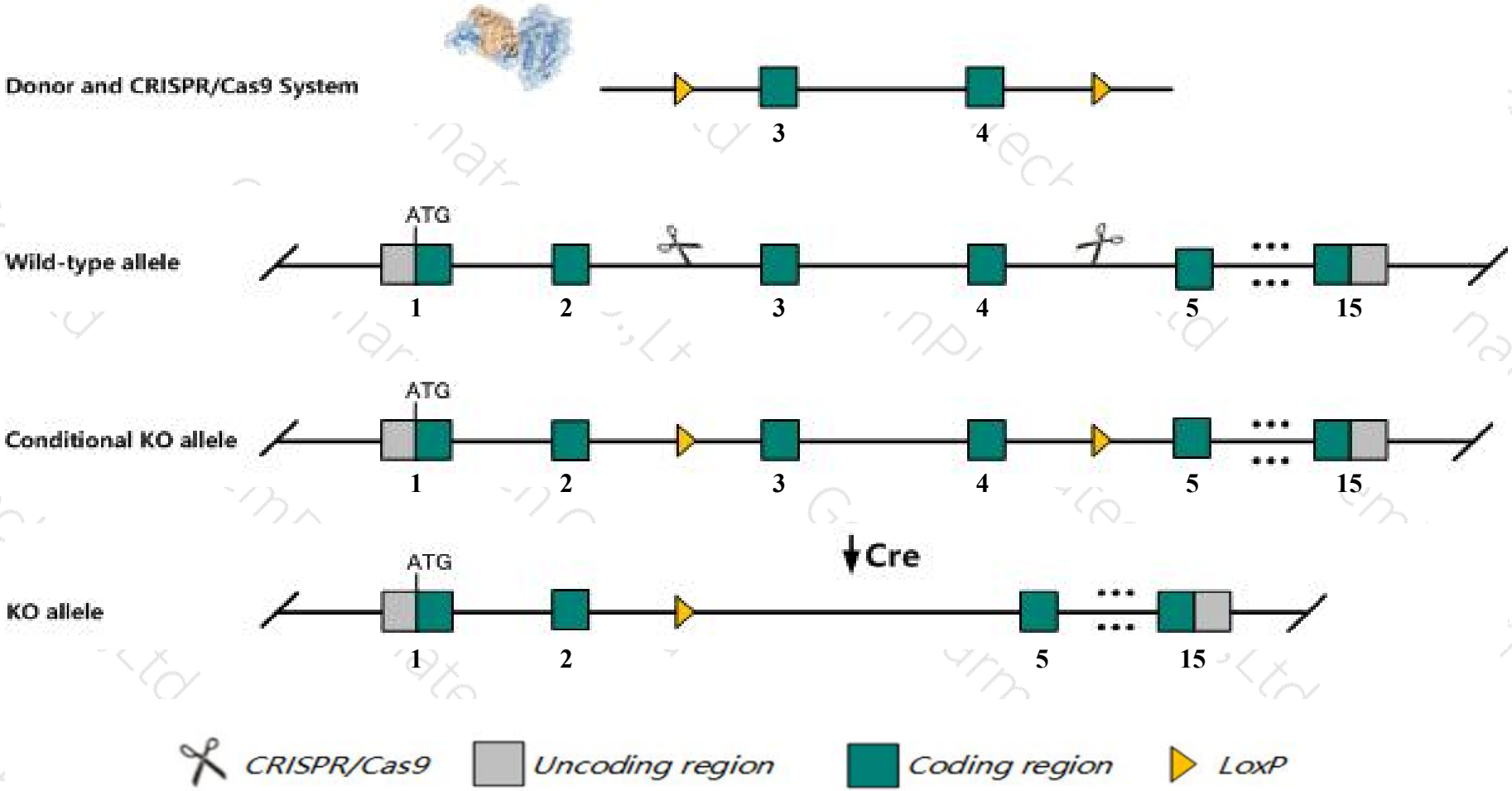
This model will use CRISPR/Cas9 technology to edit the *Ryk* gene. The schematic diagram is as follows:



# Technical routes

- The *Ryk* gene has 6 transcripts. According to the structure of *Ryk* gene, exon3-exon4 of *Ryk-203* (ENSMUST00000175883.7) transcript is recommended as the knockout region.The region contains 235bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ryk* 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 mice have a distinctive craniofacial appearance, shortened limbs and postnatal mortality due to feeding and respiratory complications associated with a complete cleft of the secondary palate.

- The *Ryk* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

# Gene information （NCBI）

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## Ryk receptor-like tyrosine kinase [Mus musculus (house mouse)]

Gene ID: 20187, updated on 9-Feb-2019

### Summary

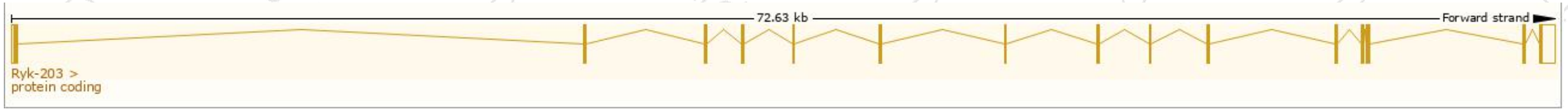
| | |
|---|---|
| **Official Symbol** | Ryk provided by MGI |
| **Official Full Name** | receptor-like tyrosine kinase provided by MGI |
| **Primary source** | MGI:MGI:101766 |
| **See related** | Ensembl:ENSMUSG00000032547 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | AW536699, ERK-3, Vik |
| **Expression** | Ubiquitous expression in bladder adult (RPKM 22.9), lung adult (RPKM 22.2) and 28 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

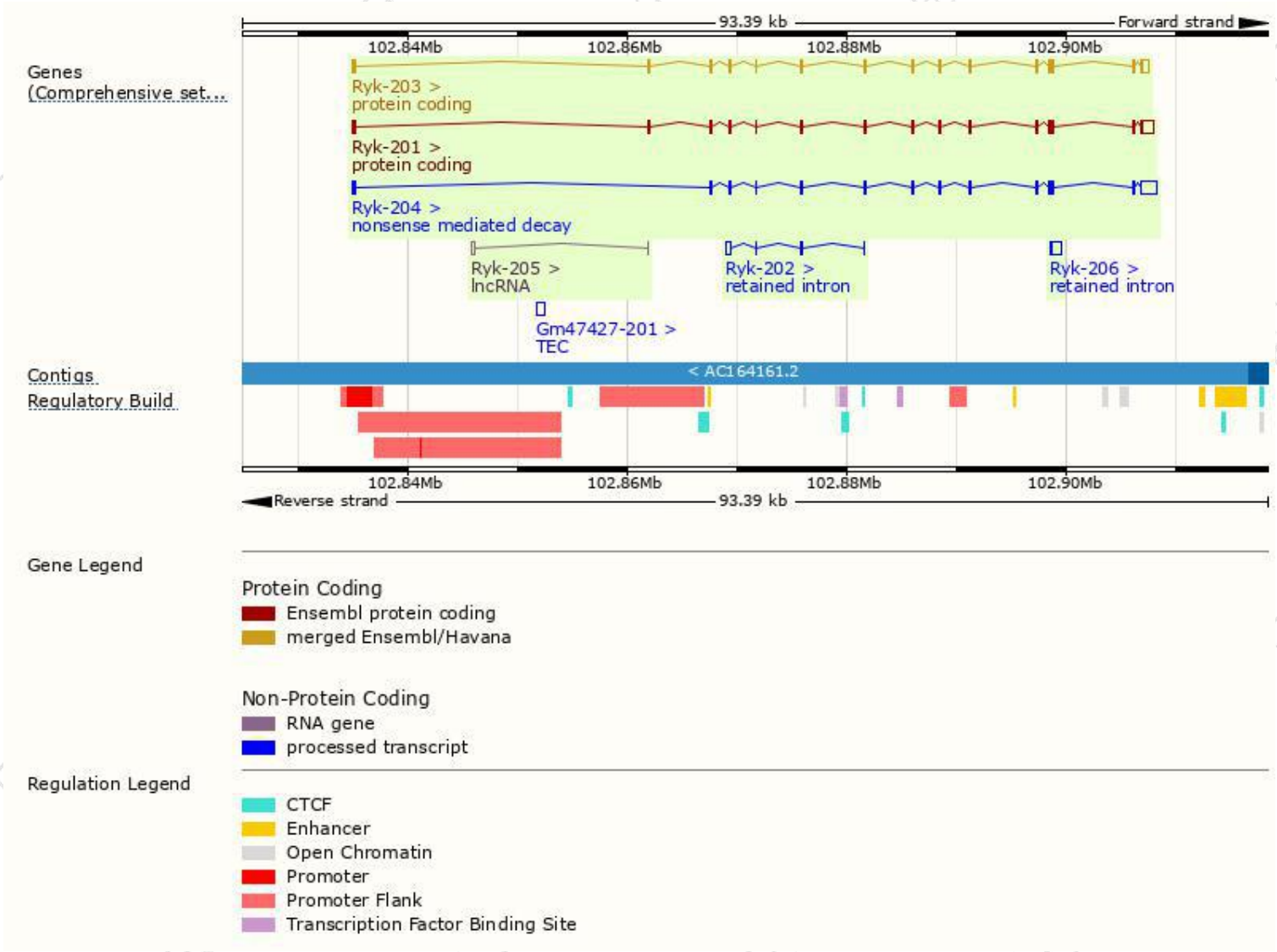
The gene has 6 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Ryk-201 | ENSMUST00000035142.7 | 2852 | 591aa | Protein coding | CCDS57696 | H9H9R6 | TSL:1 GENCODE basic APPRIS ALT2 |
| Ryk-203 | ENSMUST00000175883.7 | 2505 | 594aa | Protein coding | CCDS40747 | Q01887 | TSL:1 GENCODE basic APPRIS P3 |
| Ryk-204 | ENSMUST00000176198.7 | 3072 | 62aa | Nonsense mediated decay | - | H3BKH8 | TSL:1 |
| Ryk-206 | ENSMUST00000177274.2 | 870 | No protein | Retained intron | - | - | TSL:1 |
| Ryk-202 | ENSMUST00000175788.1 | 551 | No protein | Retained intron | - | - | TSL:3 |
| Ryk-205 | ENSMUST00000176573.1 | 386 | No protein | lncRNA | - | - | TSL:5 |

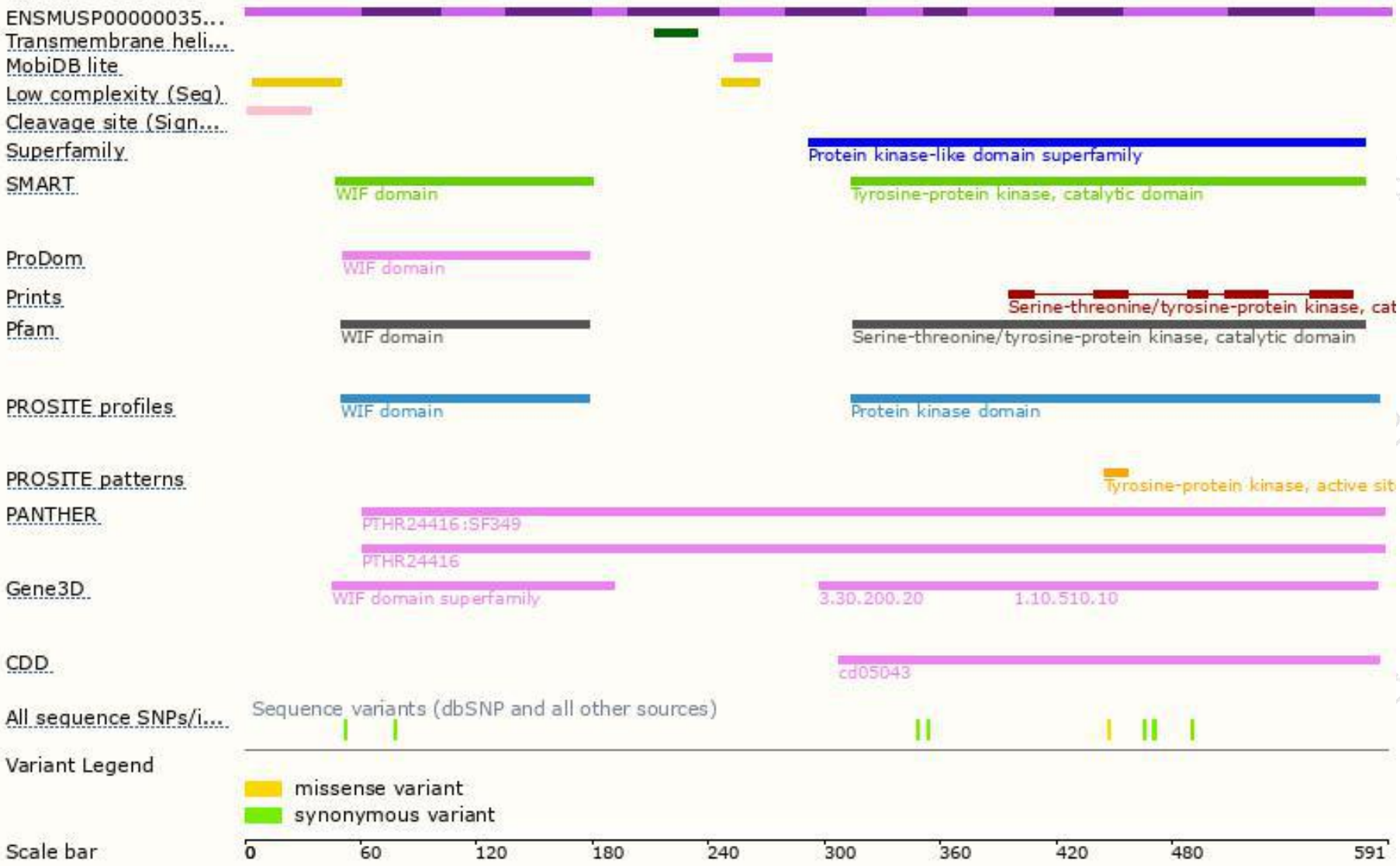
The strategy is based on the design of *Ryk-203* transcript,The transcription is shown below



# 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 mice have a distinctive craniofacial appearance, shortened limbs and postnatal mortality due to feeding and respiratory complications associated with a complete cleft of the secondary palate.

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

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