

# *Cdc45* Cas9-CKO Strategy

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# Project Overview

**Project Name**

*Cdc45*

**Project type**

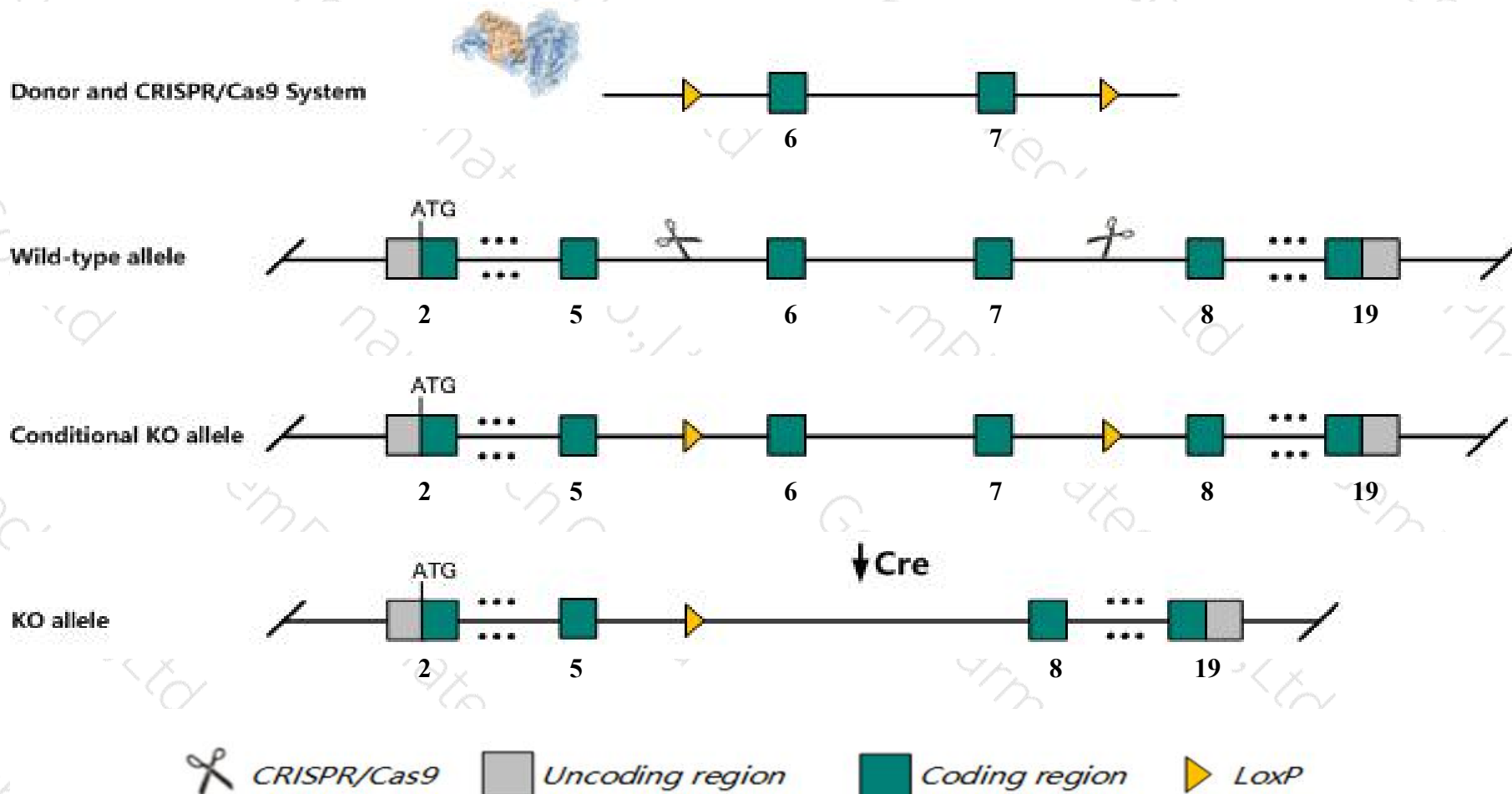
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cdc45* gene. The schematic diagram is as follows:



# Technical routes

- The *Cdc45* gene has 4 transcripts. According to the structure of *Cdc45* gene, exon6-exon7 of *Cdc45-201*(ENSMUST00000000028.13) transcript is recommended as the knockout region. The region contains 200bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cdc45* 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.

- According to the existing MGI data, homozygous mutant embryos do not develop after implantation, resulting in embryonic lethality between E4.5-E5.5. Heterozygous animals appear normal and fertile.
- The *Cdc45* gene is located on the Chr16. 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)

## Cdc45 cell division cycle 45 [Mus musculus (house mouse)]

Gene ID: 12544, updated on 13-Mar-2020

### Summary



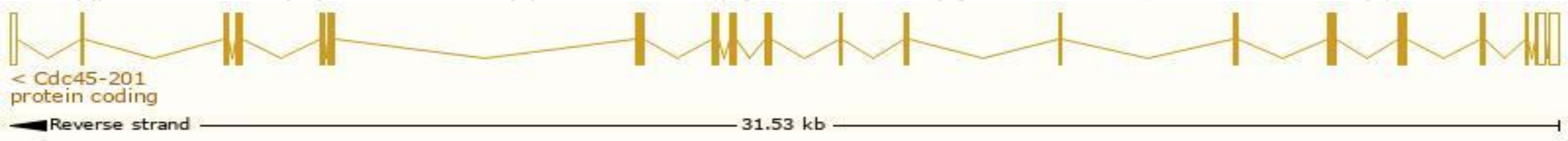
|                    |   |
|--------------------|---|
| Official Symbol    | Cdc45 provided by <a href="#">MGI</a>   |
| Official Full Name | cell division cycle 45 provided by <a href="#">MGI</a>  |
| Primary source     | <a href="#">MGI:MGI:1338073</a>   |
| See related        | <a href="#">Ensembl:ENSMUSG000000000028</a>   |
| Gene type          | protein coding  |
| RefSeq status      | VALIDATED   |
| Organism           | <a href="#">Mus musculus</a>  |
| Lineage            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as      | Cdc45l  |
| Expression         | Broad expression in liver E14.5 (RPKM 24.8), liver E14 (RPKM 24.1) and 20 other tissues <a href="#">See more</a>  |
| Orthologs          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information (Ensembl)

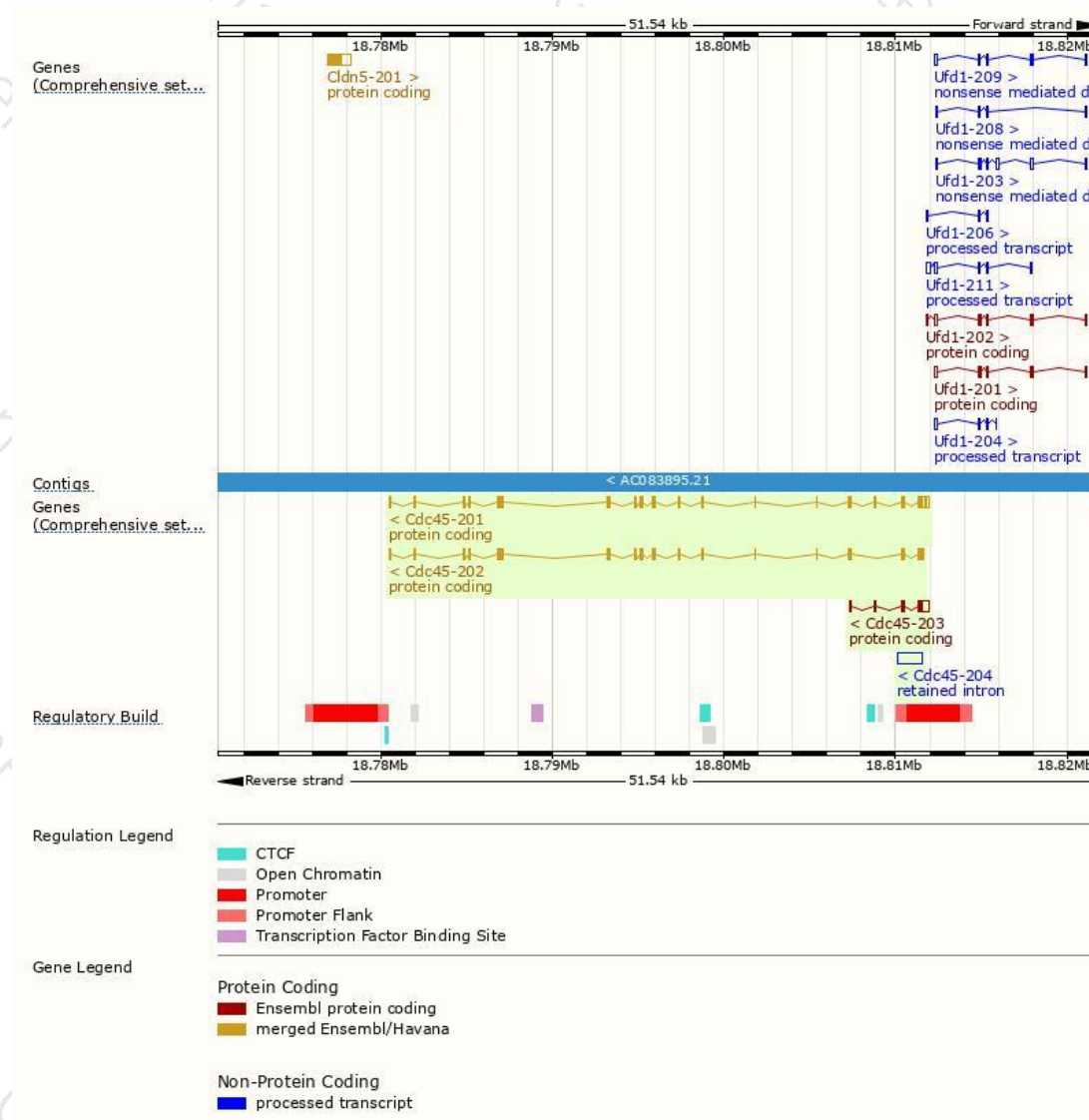
The gene has 4 transcripts,all transcripts are shown below:

| Name      | Transcript ID                         | bp   | Protein               | Biotype         | CCDS                      | UniProt                                       | Flags   |
|-----------|---------------------------------------|------|-----------------------|-----------------|---------------------------|---|---|
| Cdc45-201 | <a href="#">ENSMUST00000000028.13</a> | 2143 | <a href="#">566aa</a> | Protein coding  | <a href="#">CCDS28027</a> | <a href="#">Q3UI99</a> <a href="#">Q9Z1X9</a> | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1 |
| Cdc45-202 | <a href="#">ENSMUST00000096990.9</a>  | 1747 | <a href="#">520aa</a> | Protein coding  | <a href="#">CCDS49788</a> | <a href="#">F8WJ72</a>                        | TSL:1 GENCODE basic   |
| Cdc45-203 | <a href="#">ENSMUST00000115585.1</a>  | 832  | <a href="#">136aa</a> | Protein coding  | -                         | <a href="#">D3Z0L5</a>                        | CDS 3' incomplete TSL:2   |
| Cdc45-204 | <a href="#">ENSMUST00000231819.1</a>  | 1484 | No protein            | Retained intron | -                         | -   |   |

The strategy is based on the design of *Cdc45-201* transcript,the transcription is shown below:

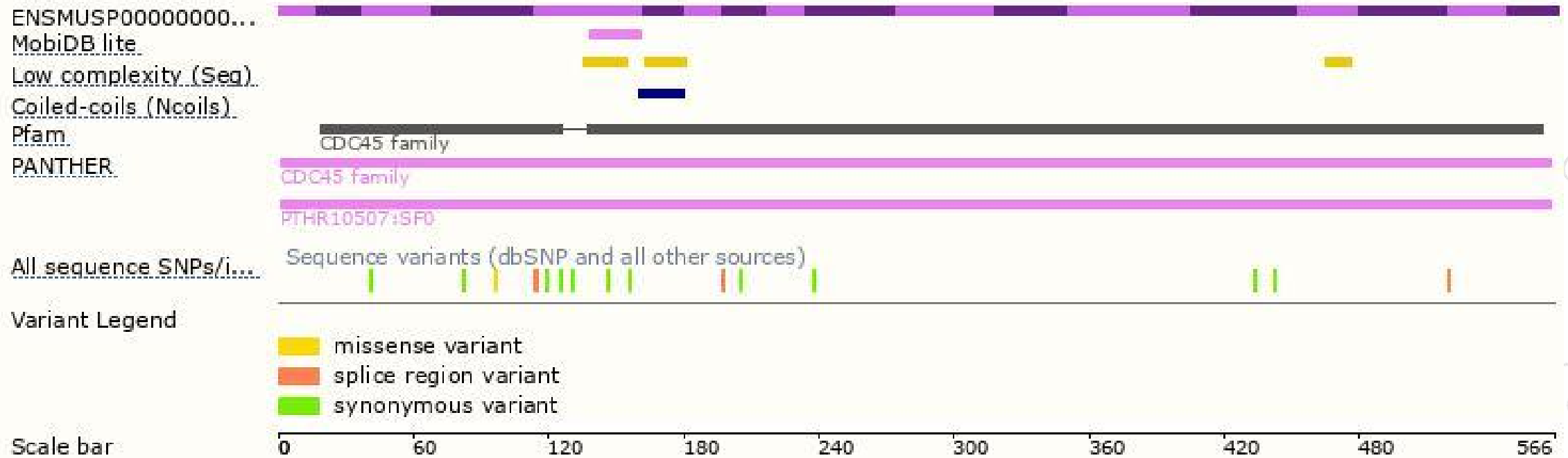


# Genomic location distribution

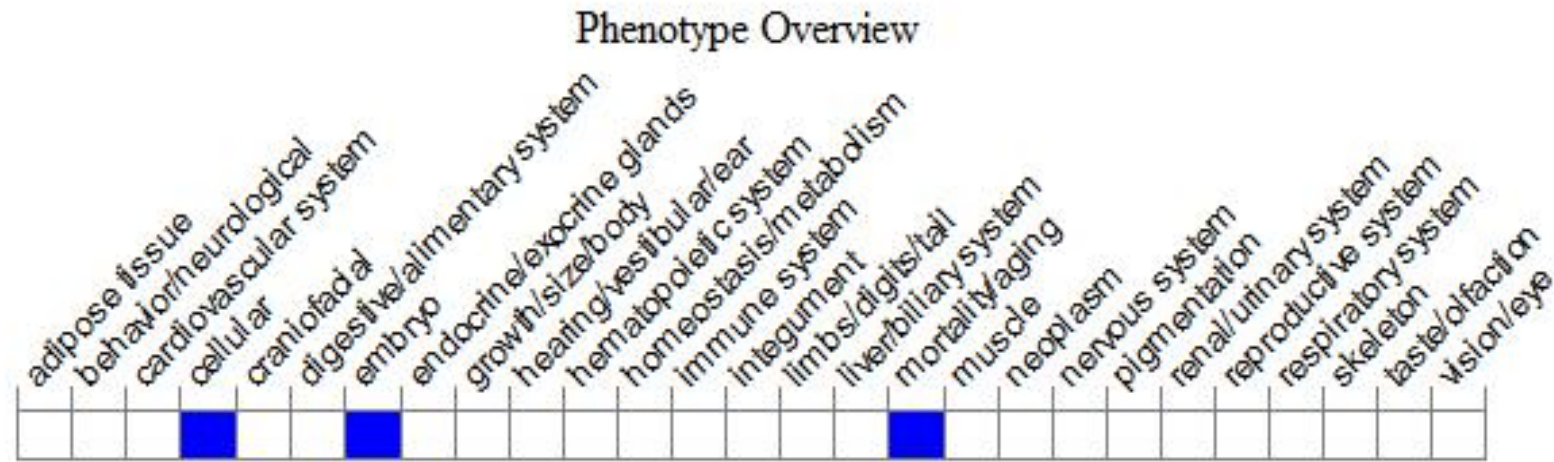




# Protein domain



# 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 mutant embryos do not develop after implantation, resulting in embryonic lethality between E4.5-E5.5. Heterozygous animals appear normal and fertile.

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

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