

# *Gtf2h4* Cas9-CKO Strategy

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# Project Overview



**Project Name**

***Gtf2h4***

**Project type**

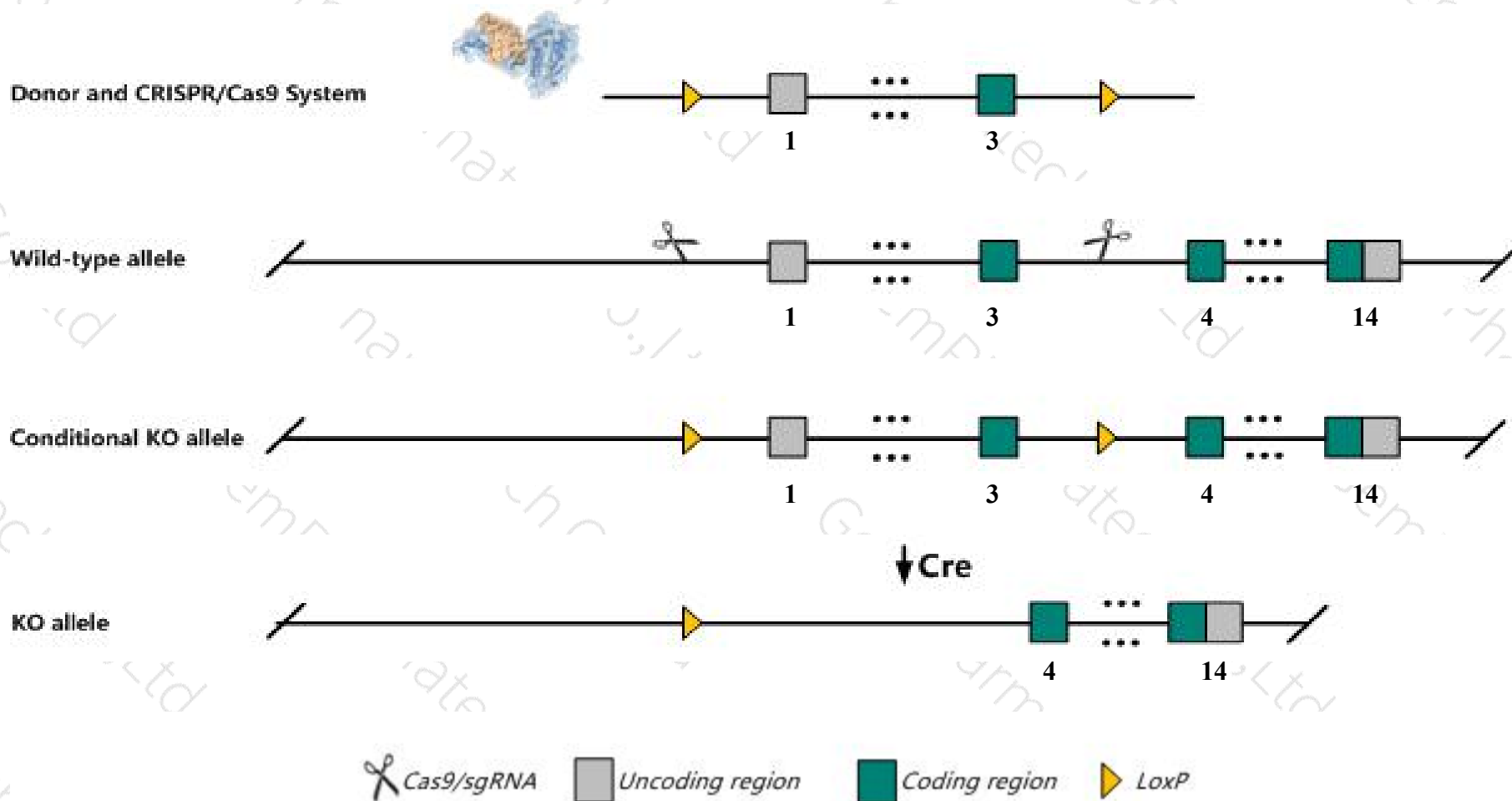
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gtf2h4* gene. The schematic diagram is as follows:



# Technical routes

- The *Gtf2h4* gene has 12 transcripts. According to the structure of *Gtf2h4* gene, exon1-exon3 of *Gtf2h4-201*(ENSMUST00000001565.14) transcript is recommended as the knockout region. The region contains 245bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gtf2h4* 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 and cell types.

# Notice

- The *Gtf2h4* gene is located on the Chr17. 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)

## Gtf2h4 general transcription factor II H, polypeptide 4 [Mus musculus (house mouse)]

Gene ID: 14885, updated on 13-Mar-2020

### Summary



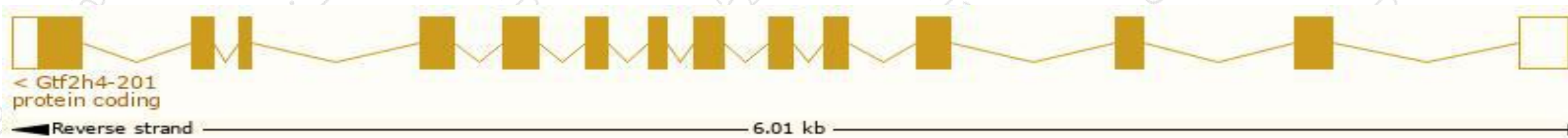
|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Gtf2h4 provided by <a href="#">MGI</a>  |
| <b>Official Full Name</b> | general transcription factor II H, polypeptide 4 provided by <a href="#">MGI</a>  |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1338799</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000001524</a>  |
| <b>Gene type</b>          | protein coding  |
| <b>RefSeq status</b>      | REVIEWED  |
| <b>Organism</b>           | <a href="#">Mus musculus</a>  |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus                       |
| <b>Also known as</b>      | AW545633, BTF2 p52, p52   |
| <b>Summary</b>            | This gene encodes a subunit of the general transcription factor multiprotein complex that plays roles in basal transcription, DNA repair and cell cycle control. [provided by RefSeq, Dec 2014] |
| <b>Expression</b>         | Ubiquitous expression in thymus adult (RPKM 68.7), limb E14.5 (RPKM 11.4) and 28 other tissues <a href="#">See more</a>   |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information (Ensembl)

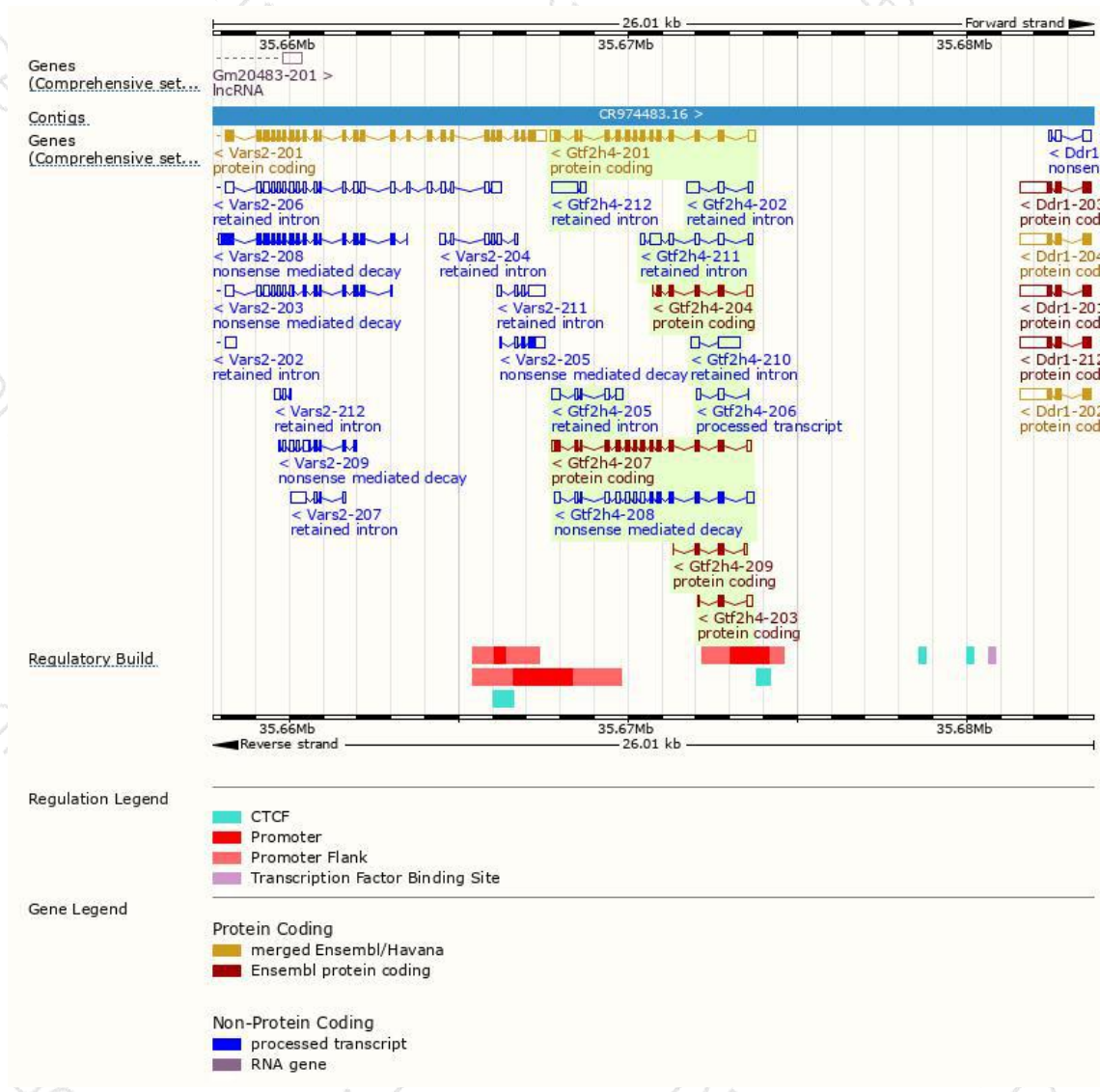
The gene has 12 transcripts, all transcripts are shown below:

| Name       | Transcript ID                        | bp   | Protein               | Biotype                 | CCDS                      | UniProt                       | Flags                         |
|------------|--------------------------------------|------|-----------------------|-------------------------|---------------------------|-------------------------------|-------------------------------|
| Gtf2h4-201 | <a href="#">ENSMUST0000001565.14</a> | 1679 | <a href="#">463aa</a> | Protein coding          | <a href="#">CCDS28702</a> | <a href="#">O70422 Q542U3</a> | TSL:1 GENCODE basic APPRIS P1 |
| Gtf2h4-207 | <a href="#">ENSMUST00000160734.7</a> | 1569 | <a href="#">463aa</a> | Protein coding          | <a href="#">CCDS28702</a> | <a href="#">O70422 Q542U3</a> | TSL:5 GENCODE basic APPRIS P1 |
| Gtf2h4-204 | <a href="#">ENSMUST00000160039.7</a> | 660  | <a href="#">164aa</a> | Protein coding          | -                         | <a href="#">E0CXV5</a>        | CDS 3' incomplete TSL:3       |
| Gtf2h4-209 | <a href="#">ENSMUST00000162266.7</a> | 367  | <a href="#">91aa</a>  | Protein coding          | -                         | <a href="#">E0CYK8</a>        | CDS 3' incomplete TSL:3       |
| Gtf2h4-203 | <a href="#">ENSMUST00000159852.1</a> | 331  | <a href="#">61aa</a>  | Protein coding          | -                         | <a href="#">E0CYM3</a>        | CDS 3' incomplete TSL:3       |
| Gtf2h4-208 | <a href="#">ENSMUST00000160752.8</a> | 1569 | <a href="#">184aa</a> | Nonsense mediated decay | -                         | <a href="#">E0CY64</a>        | TSL:5                         |
| Gtf2h4-206 | <a href="#">ENSMUST00000160711.1</a> | 271  | No protein            | Processed transcript    | -                         | -                             | TSL:5                         |
| Gtf2h4-211 | <a href="#">ENSMUST00000162894.7</a> | 925  | No protein            | Retained intron         | -                         | -                             | TSL:5                         |
| Gtf2h4-212 | <a href="#">ENSMUST00000162927.1</a> | 920  | No protein            | Retained intron         | -                         | -                             | TSL:2                         |
| Gtf2h4-210 | <a href="#">ENSMUST00000162604.1</a> | 827  | No protein            | Retained intron         | -                         | -                             | TSL:2                         |
| Gtf2h4-205 | <a href="#">ENSMUST00000160535.7</a> | 731  | No protein            | Retained intron         | -                         | -                             | TSL:2                         |
| Gtf2h4-202 | <a href="#">ENSMUST00000159671.7</a> | 620  | No protein            | Retained intron         | -                         | -                             | TSL:2                         |

The strategy is based on the design of *Gtf2h4-201* transcript, the transcription is shown below:

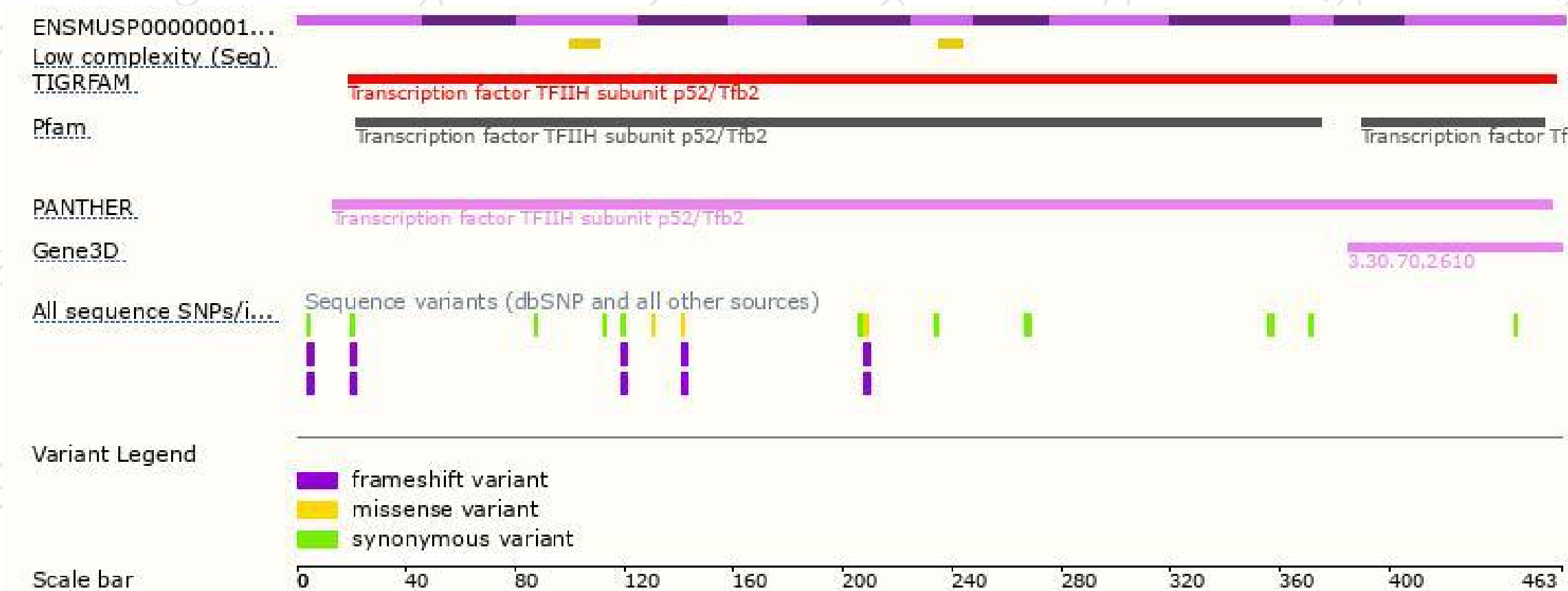


# Genomic location distribution





# Protein domain



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

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