

Btrc Cas9-CKO Strategy

Designer:

Qiong Zhou

Design Date:

2018/6/4

Project Overview



Project Name

Btrc

Project type

Cas9-CKO

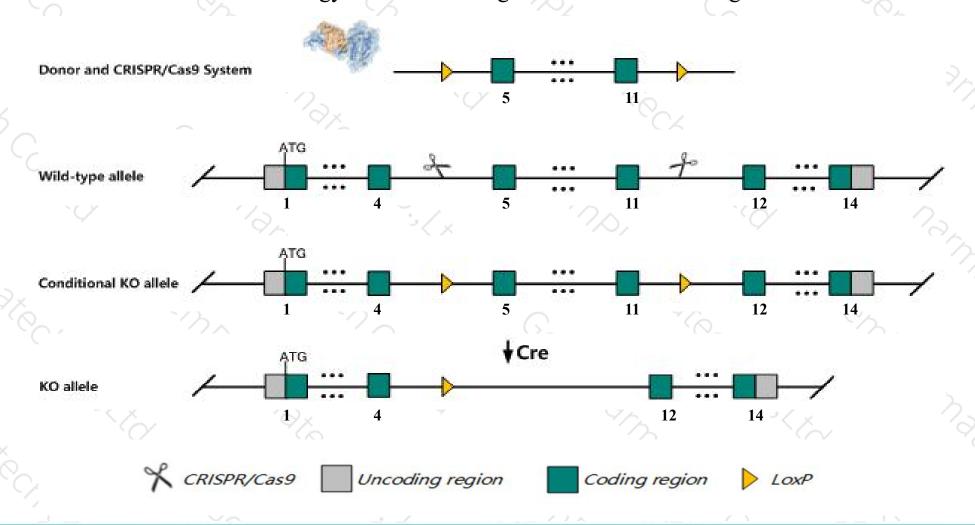
Strain background

C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- ➤ The *Btrc* gene has 11 transcripts. According to the structure of *Btrc* gene, exon5-exon11 of *Btrc-201*(ENSMUST00000065601.12) transcript is recommended as the knockout region. The region contains 1142bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Btrc* 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, Embryonic fibroblasts from homozygotes show an increase in polyploidy and apoptosis and decreased cell proliferation. In a second allele, homozygous mutation results in reduced male fertility and abnormal male meiosis with oligozoospermia.
- > The *Btrc* gene is located on the Chr19. 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)



Btrc beta-transducin repeat containing protein [Mus musculus (house mouse)]

Gene ID: 12234, updated on 12-Feb-2019

Summary

☆ ?

Official Symbol Btrc provided by MGI

Official Full Name beta-transducin repeat containing protein provided by MGI

Primary source MGI:MGI:1338871

See related Ensembl:ENSMUSG00000025217

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 Beta-Trcp1, E3RS-IkappaB, E3RSIkappaB, FWD1, Fbw1a, HOS, SCF b-TRCP, Slimb, b-TrCP, beta-TrCP

Expression Ubiquitous expression in frontal lobe adult (RPKM 10.9), CNS E18 (RPKM 10.0) and 28 other tissuesSee more

Orthologs <u>human</u> all

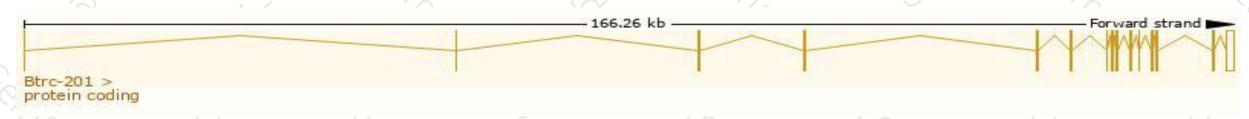
Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

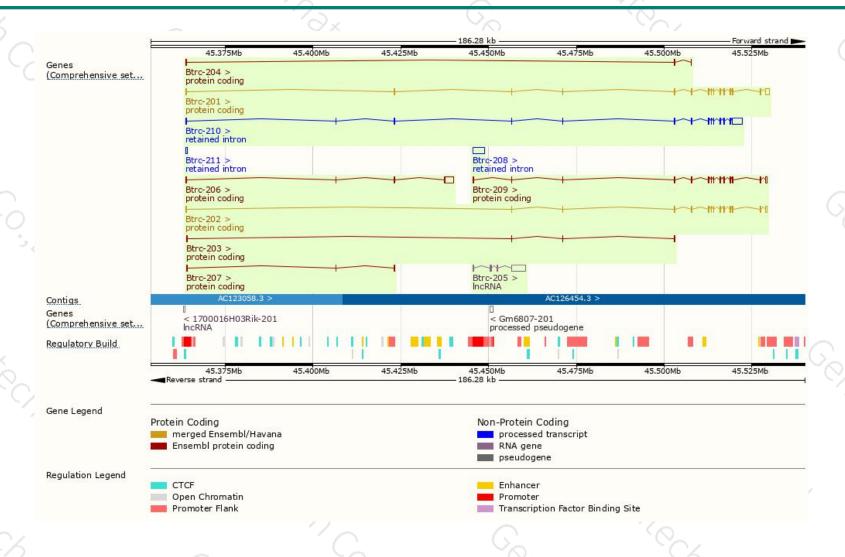
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|-----------------------|------|--------------|----------------------|-----------|------------|--------------------------------|
| Btrc-201 | ENSMUST00000065601.12 | 2978 | 605aa | Protein coding | CCDS29860 | Q3ULA2 | TSL:1 GENCODE basic APPRIS P3 |
| Btrc-202 | ENSMUST00000111936.3 | 2064 | <u>569aa</u> | Protein coding | CCDS38002 | Q3ULA2 | TSL:1 GENCODE basic APPRIS ALT |
| Btrc-206 | ENSMUST00000224102.1 | 3037 | <u>150aa</u> | Protein coding | #8 | A0A286YE08 | GENCODE basic |
| Btrc-209 | ENSMUST00000224478.1 | 2194 | 506aa | Protein coding | 29 | A0A286YDT6 | GENCODE basic |
| Btrc-204 | ENSMUST00000223764.1 | 438 | <u>111aa</u> | Protein coding | 36 | A0A286YCS1 | CDS 3' incomplete |
| Btrc-203 | ENSMUST00000223684.1 | 361 | <u>54aa</u> | Protein coding | | A0A286YCD6 | CDS 3' incomplete |
| Btrc-207 | ENSMUST00000224318.1 | 338 | 34aa | Protein coding | #2 #3 | A0A286YDI3 | CDS 3' incomplete |
| Btrc-205 | ENSMUST00000224053.1 | 4720 | No protein | Processed transcript | 29 | 72.5 | |
| Btrc-210 | ENSMUST00000225662.1 | 4502 | No protein | Retained intron | - | 1.5 | |
| Btrc-208 | ENSMUST00000224439.1 | 3493 | No protein | Retained intron | -8 | 19-3 | |
| Btrc-211 | ENSMUST00000225898.1 | 515 | No protein | Retained intron | 28 | (2) | |
| | -7.17 | | | / | 1 | | 1 V |

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



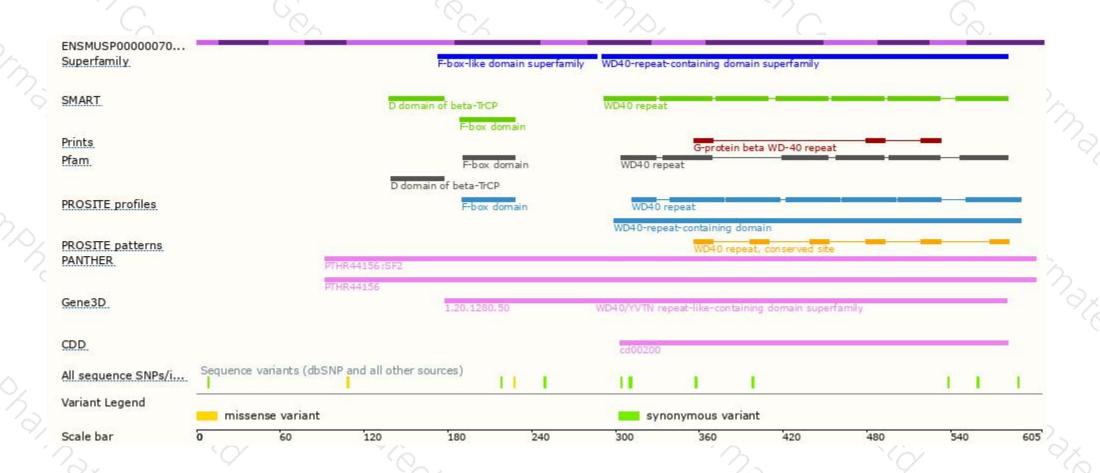
Genomic location distribution





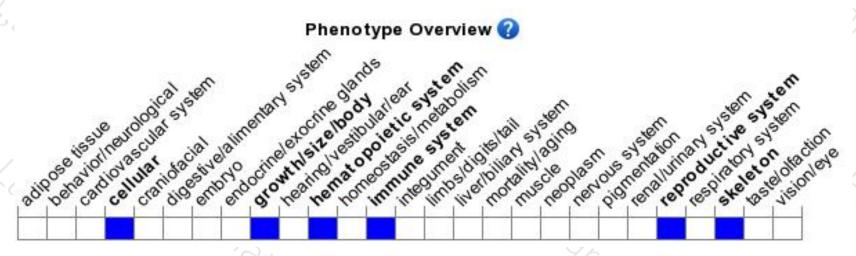
Protein domain





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, Embryonic fibroblasts from homozygotes show an increase in polyploidy and apoptosis and decreased cell proliferation. In a second allele, homozygous mutation results in reduced male fertility and abnormal male meiosis with oligozoospermia.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





