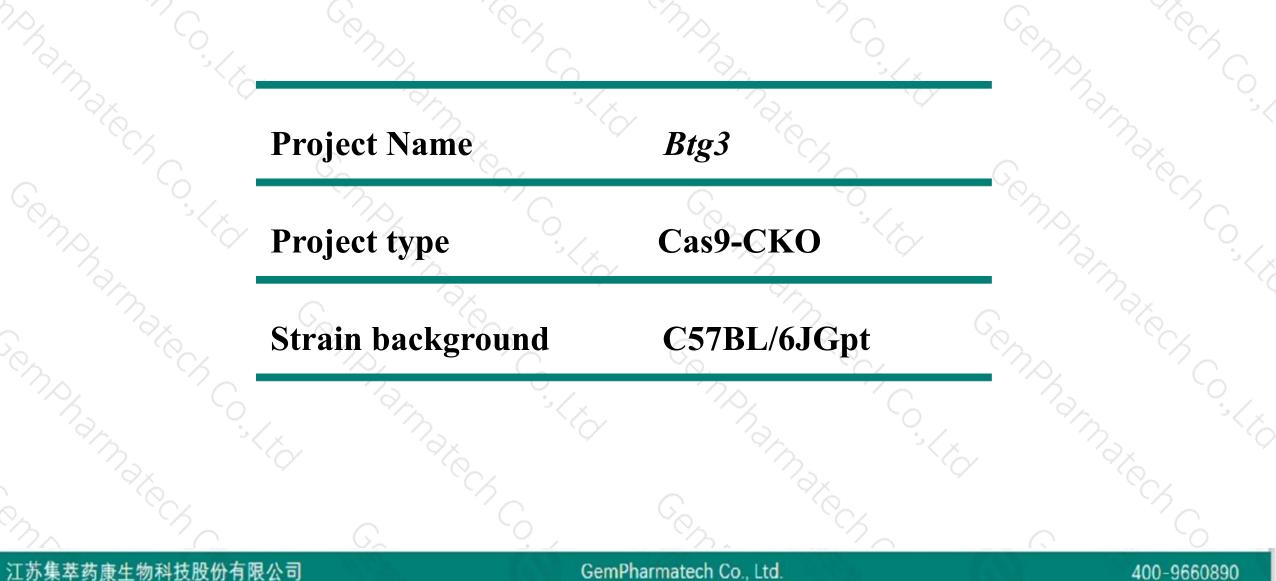


# **Btg3** Cas9-CKO Strategy

Designer:Xueting Zhang Reviwer:Yanhua Shen Date:2020-02-13

# **Project Overview**



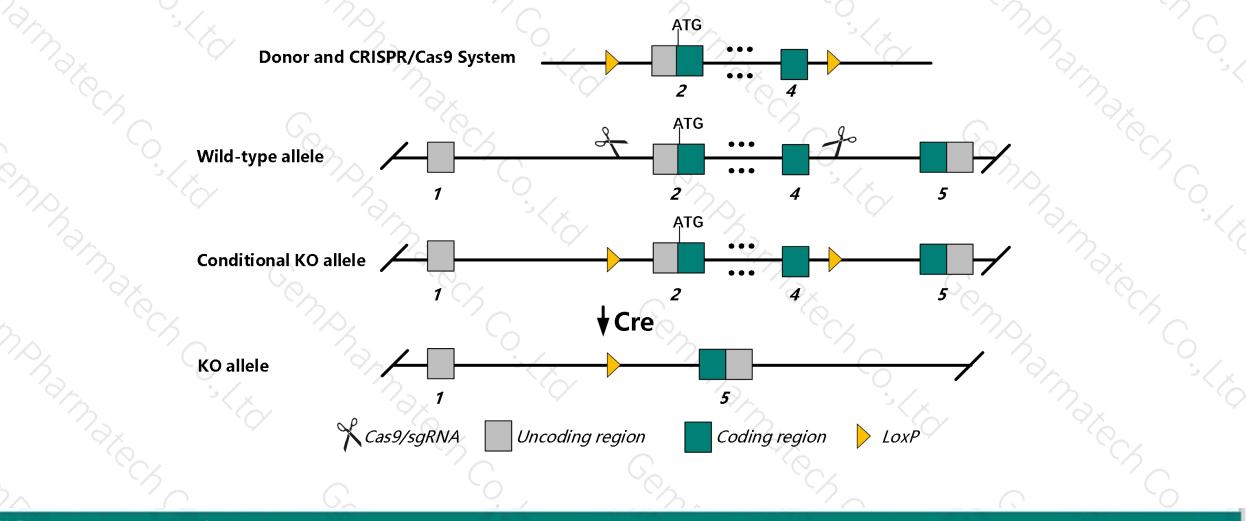


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## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Btg3* gene. The schematic diagram is as follows:



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The *Btg3* gene has 3 transcripts. According to the structure of *Btg3* gene, exon2-exon4 of *Btg3-201* (ENSMUST00000023570.13) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Btg3* 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, Mice homozygous for a knock-out allele exhibit increased incidence of lung tumors.
- > The effect on transcript *Btg3*-202&203 is unknown.
- The *Btg3* 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)

Build 37.2

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previous assembly



14 A.		- 2							
Btg3 BTG anti-pro	liferation factor	3 [ Mus musculus (house mouse) ]							
Gene ID: 12228, updated on	5-Jan-2020								
Summary									
Official Symbol	Btg3 provided by MGI								
	BIG anti-proliferation factor 3 provided by MGI								
	MGI:MGI:109532								
See related									
	protein coding								
RefSeq status									
	Mus musculus								
	Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;								
	Murinae; Mus; Mus								
Also known as	ANA; tob5								
Summary	transcription factors, a transition to suppress	nd a less conserved C-terminal domain. This protein is cell cycle progression. Mice deficient for this gene displ cell translocation gene 3. Alternate splicing results in m	thought to have ay an increase	ily is defined by a conserved N-terminal domain, known to bind we anti-proliferative properties, and may be involved in regulating the G1-S and incidence of lung cancers, and many human lung cancer cells exhibit pt variants. A pseudogene of this gene is found on chromosome 17.					
Expression	Broad expression in ge	enital fat pad adult (RPKM 36.7), placenta adult (RPKM	14.6) and 20 c	other tissues See more					
Orthologs	<u>human</u> all								
Genomic context				8 7					
Location: 16; 16 C3.1 Exon count: 6				See Btg3 in Genome Data Viewe					
Annotation release	Status	Assembly	Chr	Location					
108	current	GRCm38.p6 (GCF_000001635.26)	<mark>1</mark> 6	NC_000082.6 (7835986078377181, complement)					

MGSCv37 (GCF\_000001635.18)

### 400-9660890

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16

NC\_000082.5 (78360105..78377030, complement)

# **Transcript information (Ensembl)**



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

Name 🍦	Transcript ID 🛛 🖕	bp 🖕	Protein 🖕	Biotype 🍦	CCDS 🖕	UniProt 🖕	Flags 🖕	
Btg3-201	ENSMUST0000023570.13	1414	<u>252aa</u>	Protein coding	<u>CCDS28277</u> &	<u>P50615</u> & <u>Q52L83</u> &	TSL:1 GENCODE basic APPRIS P1	
Btg3-203	ENSMUST00000231353.1	3013	<u>152aa</u>	Protein coding	-	<u>A0A338P787</u> &	CDS 5' incomplete	
Btg3-202	ENSMUST00000148124.1	442	<u>137aa</u>	Protein coding	. – .	<u>D3Z1M9</u> &	CDS 3' incomplete TSL:5	

The strategy is based on the design of *Btg3-201* transcript, The transcription is shown below

#### < Btg3-201 protein coding

Reverse strand

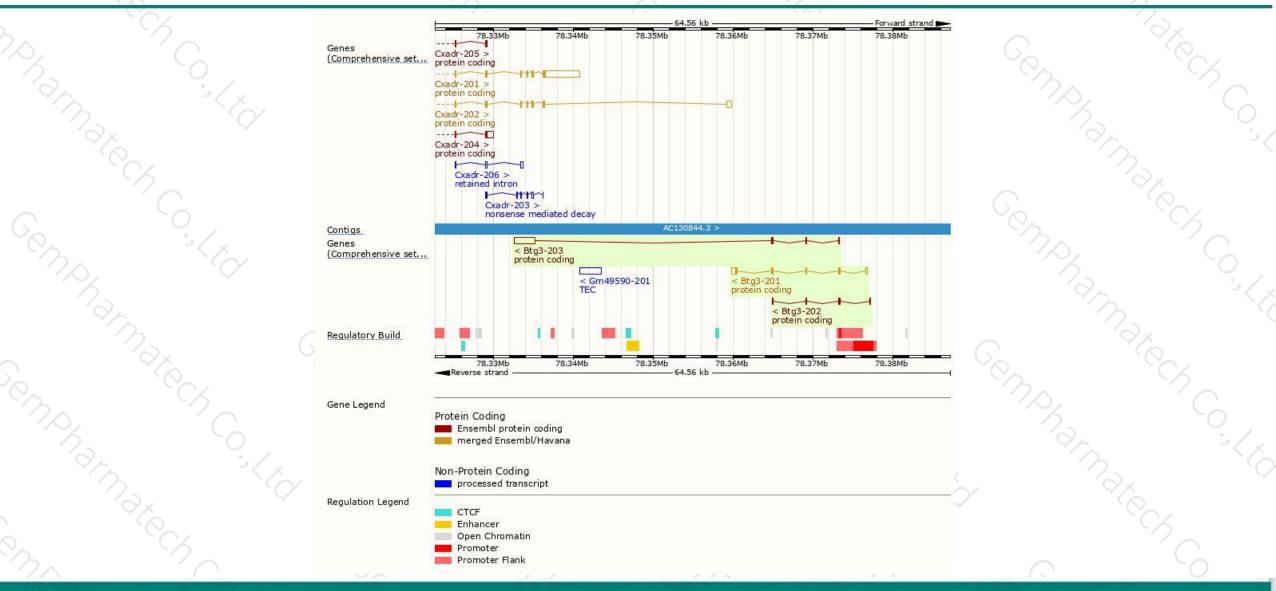
– 16.95 kb –

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### **Genomic location distribution**





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### **Protein domain**

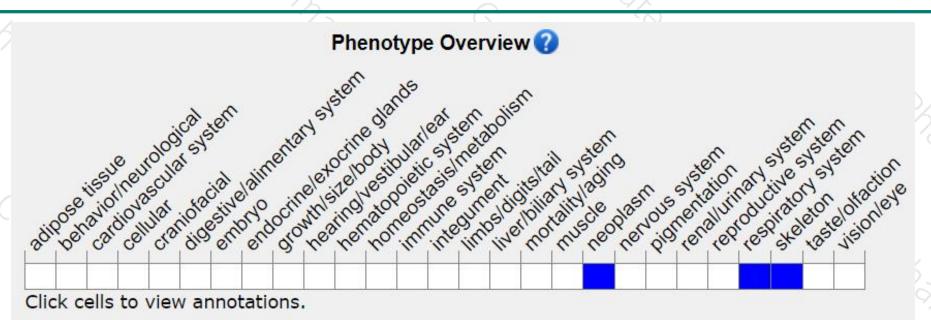


	$\sim$						
50,	ENSMUSP00000023 MobiDB lite Low complexity (Seg)					-	<sup>2</sup> Co
	Superfamily	BTG-like domain supe	erfamily				
	SMART	Anti-proliferative prot	ein				
	Prints	Anti-proliferative p	rotein				
	Pfam.	Anti-proliferative prot	ein				
	PROSITE patterns			Anti-proliferative protein			S
			Anti-proliferative protein				
	PANTHER	PTHR22978					
		PTHR22978:SF6					-
	Gene3D	BTG-like domain supe	erfamily				
32	All sequence SNPs/i	Sequence variants	(dbSNP and all other s	ources)	1.11		6
	Variant Legend						
	vananic Legenu	missense vari					
		synonymous \	variant				
	Scale bar	0 4	10 80	120	160	200	252
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### 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, Mice homozygous for a knock-out allele exhibit increased incidence of lung tumors.

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



