Bfar Cas9-CKO Strategy

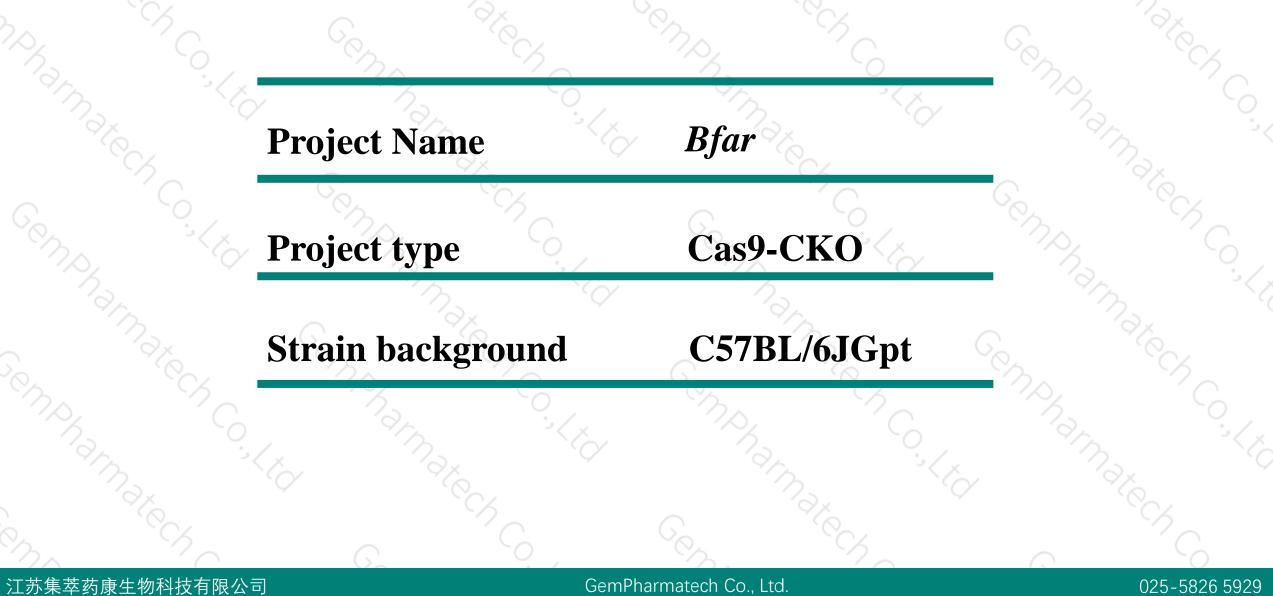
Designer: Reviewer :

Design Date:

Daohua Xu Huimin Su 2019-12-17

Project Overview





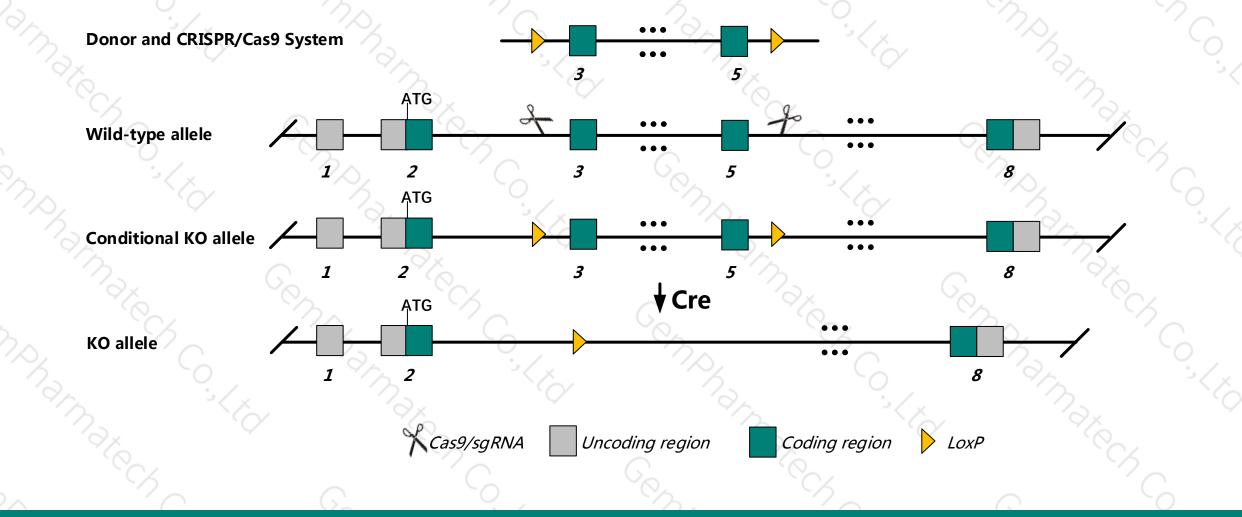
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Conditional Knockout strategy



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



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- The *Bfar* gene has 8 transcripts. According to the structure of *Bfar* gene, exon3-exon5 of *Bfar*-201 (ENSMUST0000023365.12) transcript is recommended as the knockout region. The region contains 520bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Bfar* 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 or cell types.



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

Gene information (NCBI)



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Bfar bifunctional apoptosis regulator [Mus musculus (house mouse)]

Gene ID: 67118, updated on 12-Aug-2019

Summary

Official SymbolBfar provided by MGIOfficial Full Namebifunctional apoptosis regulator provided by MGIPrimary sourceMGI:MGI:1914368See relatedEnsembl:ENSMUSG0000022684 Ensembl:ENSMUSG0000079737Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asBar; Rnf47; Al666707; AW107665; 301001A07Rik; 311001122RikExpressionUbiquitous expression in bladder adult (RPKM 14.9), subcutaneous fat pad adult (RPKM 12.4) and 28 other tissues See more
human all

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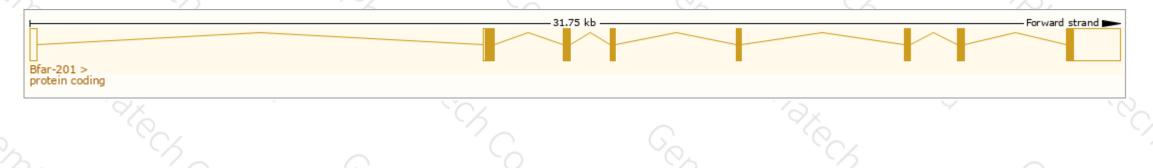
Transcript information (Ensembl)



The gene has 8 transcripts, and all transcripts are shown below:

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ic APPRIS P1		
DE basic		

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



Genomic location distribution





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Protein domain



3.	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	G		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		$^{\sim}$ C/ $_{\sim}$		0	9	100
- Aarr	ENSMUSP0000023 Transmembrane heli MobiDB lite Superfamily	SSF57850		Sterile alpha motif/p	ointed domain superfa	amily		_		36
,	SMART.	Zinc finger, RING-type		Sterile alpha mot	if domain					, c^
	Pfam.	PF15227		Sterile alpha mo	tif domain					-
George	PROSITE profiles	Zinc finger, RING-type		Sterile alpha mo	otif domain					5
102	PROSITE patterns		6-type, conserved site							~~ <u>~</u>
	PANTHER	PTHR15898:SF1 PTHR15898								
	Gene3D	Zinc finger, RING/FYVE/PHD-type		Sterile alpha motif/poin	ited domain superfam	ily				
500	CDD	cd16497		cd09513						
	All sequence SNPs/i	Sequence variants (dbSNP and a	ll other sources)	1.1	1.11.1	1.1	1.1	1.1		
	Variant Legend	missense variant			synonymou	s variant				
	Scale bar	0 40 80	120	160 200	240	280	320	360	400 4	50

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



