Obsl1-p.F796S cas9-ki(PM) Mouse Model Strategy -CRISPR/Cas9 technology

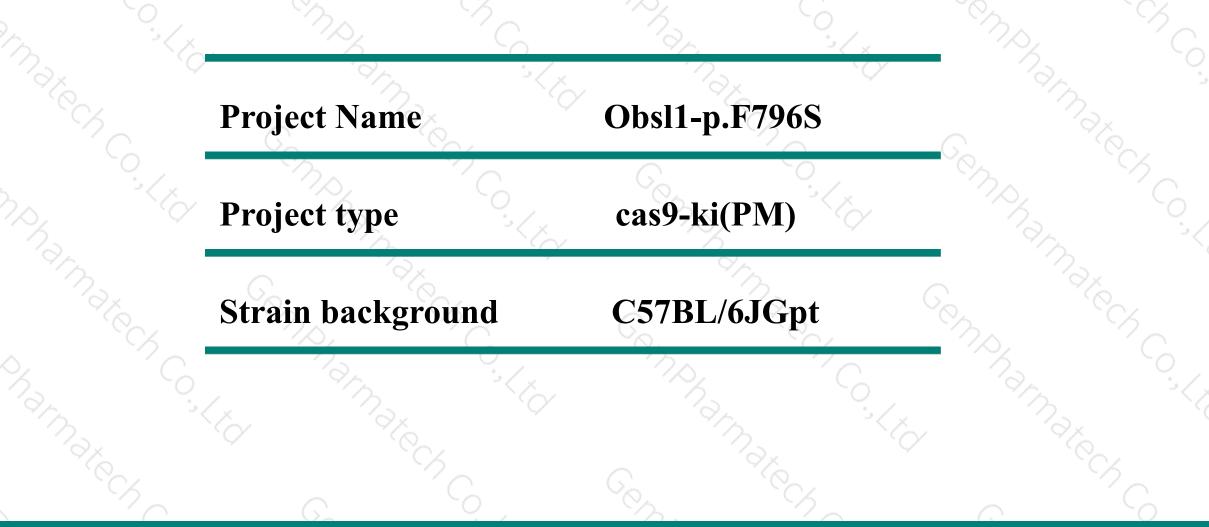
Designer: Xueting Zhang

Reviewer: Yanhua Shen

Design Date: 2021-8-23

Project Overview





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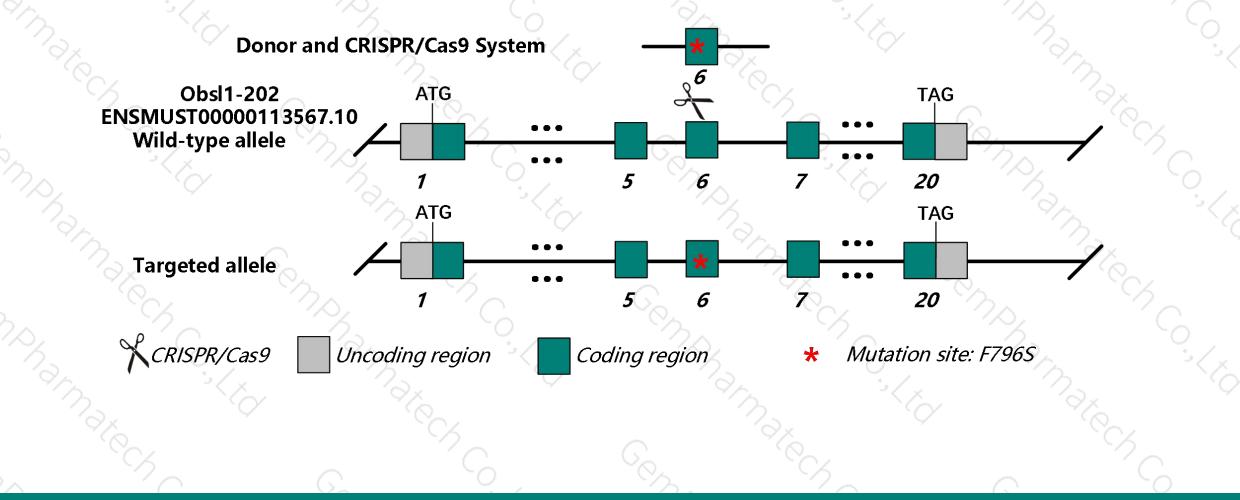
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Strategy



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This model uses CRISPR/Cas9 technology to edit the *Obsl1* gene and the schematic diagram is as follow:



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The mouse Obsl1 gene has 8 transcripts. The human OBSL1 gene has 32 transcripts.

- According to the structure of *Obsl1* gene and requirements of customer, the 796th amino acid(F) of human *OBSL1* gene(NM_015311.3) corresponds to the 796th amino acid(F) of mouse *Obsl1* gene after comparing homology of mouse *Obsl1* gene and human *OBSL1* gene. This project produced *Obsl1*-F796S point mutation on exon6 of the transcript of *Obsl1*-202(ENSMUST00000113567.10,NM_178884.5). The 796th amino acids will be mutated from F to S, and the corresponding codon will be mutated to TCC by the TTC.
- The mouse *Obsl1*-202 transcript contains 20 exons. The translation initiation site ATG is located at exon1, and the translation termination site TAG is located at exon20, encoding 1804aa.
- The human NM_015311.3 transcript contains 21 exons. The translation initiation site ATG is located at exon1, and the translation termination site TAG is located at exon21, encoding 1896aa.
- In this project, *Obsl1* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: CRISPR/Cas9 system and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.



- According to the data of MGI, mice homozygous for a knock-out allele are embryonic lethal.
- ➤ The effect on transcript-205 is unknown.

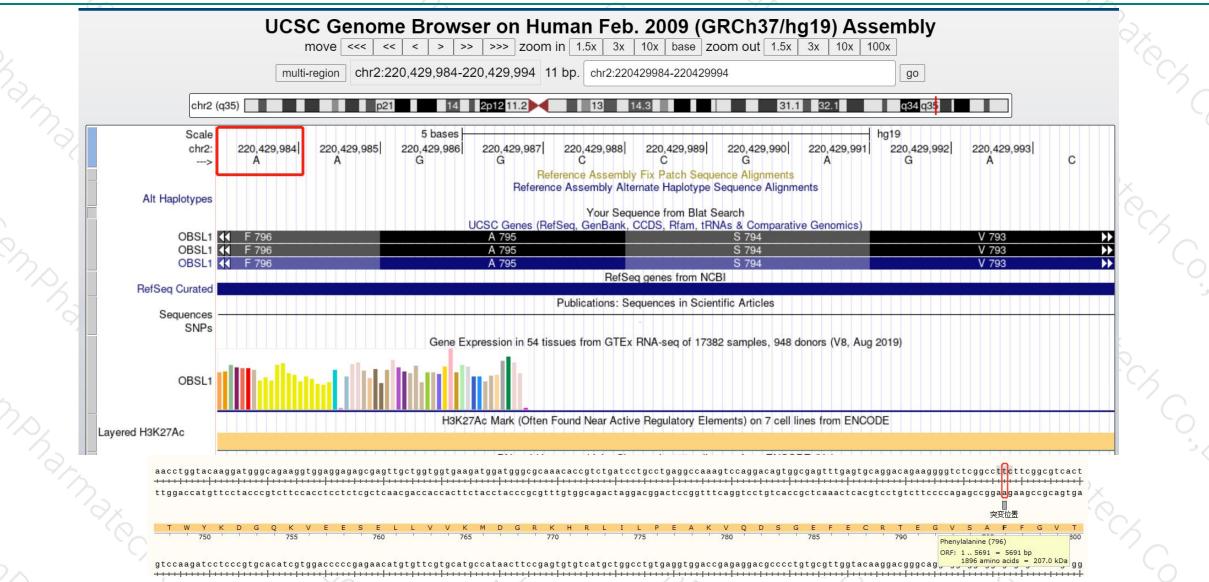
Notice

- > One or Two synonymous mutations of amino acids will be intronduced on exon6 of *Obsl1*.
- Mouse Obsl1 gene is located on Chr2. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr2, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

The information of chr2:220429984:A:G(hg19,OBSL1)



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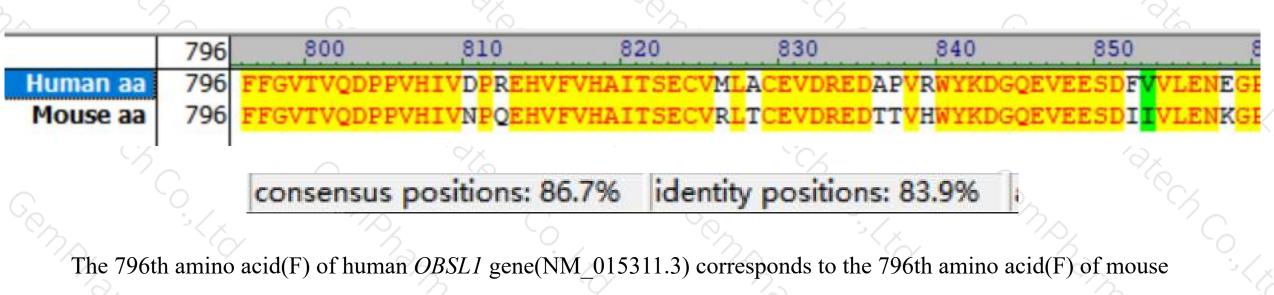


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A comparison of the aa homology of human and mouse Obsl1 gene





Obsl1 gene after comparing homology of mouse Obsl1 gene and human OBSL1 gene.

Targeted Mutation Site

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+2 6801 +2 6901 +2 7001	GCI CGT 7S TC(AG(GG(CC(2F TT(ACG IGC GGG GCA CGI E GAG	CA GT Q AG TC CT C TG	GAC CTG GAC CTG K AGG TCC	T T T T T T T T T T T T T T	GGI K AG TC TC T AC	TCT V GTG CAC E AGG TCC E GGA	TT S TC AG E AG TC	TC AC TG S AG TC G G G G	CG L TG AC CG GC	FAC	GTA F CTI GAA S TCG S CGG	ATZ F TCZ AGI CGZ A		GC GA CT I AT TA S CC	TCCT GGZ AGT TCZ F	S S TCT AGZ V TGZ ACT G G	E GAA K ACTT K AAGA TTCT		AGA		STAL STG CACO GCCC CGG CGG CGG		GAC GAC K AAZ TTT	ICA I I I I I I I I I I I I I I I I I I	CTG GTG CAC F CCG GGC	G C (TCG GCT CGA L CTG GAC	AAA CTC GAC I ATC TAC	AGA S CAA GTT L CCT GGA	AGA R AGG CCC CCC	GG V GT CA P CT GA	TA GGI CC GA CT	CG1 D AT1 TAJ CCC CCC	F F AAA A CTC GAG			CAA GTT AAA BTT GCCG	GTG(CAC(T CCT(GGA(AGA(ACA TGT GTA CAT S AGC TCG	GGI GGI GGI	GAC K AGG TCC E TGA ACT	GAG TC D GAT TA F AT TA	2	5,	2	2

The blue region is coding sequence of exon6 of Obsl1-202, the yellow region represents the targeted mutation site.

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Gene name and location(NCBI)-Obsl1

L Download Datasets

2

Gene ID: 98733, updated on 23-Jun-2021

Summary

Official Symbol	Obsl1 provided by MGI
Official Full Name	obscurin-like 1 provided by MGI
Primary source	MGI:MGI:2138628
See related	Ensembl:ENSMUSG0000026211
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; Musi; Mus
Also known as	AW822216

Expression Biased expression in ovary adult (RPKM 125.4), limb E14.5 (RPKM 44.0) and 9 other tissues See more

Orthologs human all

NEW Try the new <u>Gene table</u> Try the new <u>Transcript table</u>

Obsl1 obscurin-like 1 [Mus musculus (house mouse)]

nnotation release	Status	Assembly	Chr	Location	
09	current	GRCm39 (GCF_000001635.27)	1	NC_000067.7 (7546246975483143, complement)	
08.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (7548582575506486, complement)	
uild 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC_000067.5 (7548240075503027, complement)	
14	2	Chromosome 1 - NC	000067	7	\subseteq

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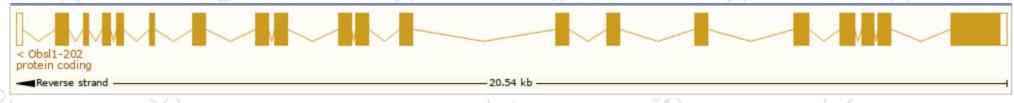
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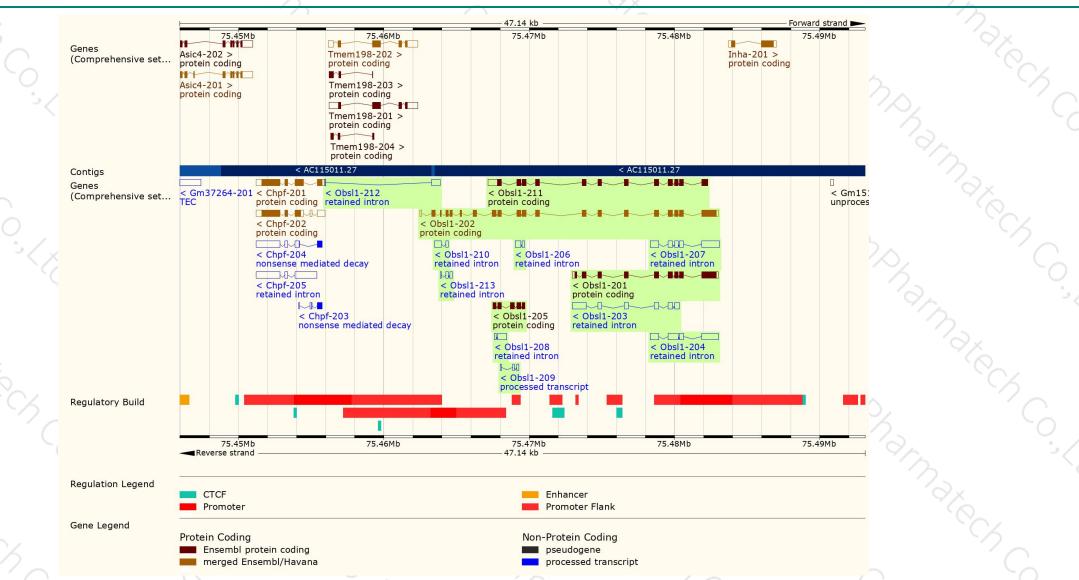
Transcript information(Ensembl)-Obsl1 「集萃药」

The ge	ene has 13 transcr						
Name 🖕	Transcript ID 💧	bp 💧	Protein 🖕	Biotype 💧	CCDS 🖕	UniProt Match 🖕	Flags 🖕
Obsl1-202	ENSMUST00000113567.10	5679	<u>1804aa</u>	Protein coding	<u>CCDS15077</u> &	<u>D3YYU8-1</u> &	GENCODE basic APPRIS P1 TSL:1
Obsl1-201	ENSMUST00000113565.3	3379	<u>1025aa</u>	Protein coding	CCDS78624	<u>A0A0B4J1L4</u> &	GENCODE basic TSL:1
Obsl1-211	ENSMUST00000155084.8	4131	<u>1162aa</u>	Protein coding	-	<u>A0A0A0MQG1</u> &	TSL:2 CDS 5' incomplete
Obsl1-205	ENSMUST00000132252.2	1252	<u>417aa</u>	Protein coding	-	F7AGE9 &	TSL:5 CDS 5' and 3' incomplete
Obsl1-209	ENSMUST00000150293.2	341	No protein	Processed transcript	-	-	TSL:5
Obsl1-204	ENSMUST00000129403.8	2709	No protein	Retained intron	-	1	TSL:1
Obsl1-207	ENSMUST00000145306.2	2649	No protein	Retained intron	-	-	TSL:1
Obsl1-203	ENSMUST00000127507.8	2404	No protein	Retained intron	<u>-</u>	<u>in</u> 1	TSL:1
Obsl1-208	ENSMUST00000145382.2	724	No protein	Retained intron		- 1	TSL:2
Obsl1-212	ENSMUST00000156499.3	656	No protein	Retained intron	-	-	TSL:3
Obsl1-210	ENSMUST00000154636.2	651	No protein	Retained intron	i.e.		TSL:3
Obsl1-206	ENSMUST00000138352.2	571	No protein	Retained intron	-	- 1	TSL:2
Obsl1-213	ENSMUST00000156705.2	440	No protein	Retained intron	-		TSL:3

The strategy is based on the design of *Obsl1*-202 transcript, the transcription is shown below:



Genomic location distribution-*Obsl1*



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Protein domain-Obsl1



		<u>`</u>							∇_{A}		
anna.	ENSMUSP00000109 Low complexity (Seg) Superfamily	-	_	Fibronectin type III s	uperfamily			-			
S.C.	SMART	Immunog <mark>lobulin-like domain s</mark> Immunoglobulin subtype 2	uperfamily						_		
	Pfam.	Immunoglobulin subtype Immunoglobulin I-set						_		-	
Con l	PROSITE profiles	Immunoglobulin-like domain			PF13927						30
Chop	PANTHER Gene3D CDD	PTHR35971 Immunoglobulin-like fold cd00096		Fibronectin type III							
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Mouse phenotype description(MGI)



URL link is as follows: http://www.informatics.jax.org/marker/MGI:2138628 Phenotype Overview 🕜 digestive/aimentary system endocrine exocrine glands homeostasisImetabolist cardiovascular system hematopoletic system heatinglyestibularlear behaviormeurological reproductive system impsidioitsitail system respiratory system renallutinary syste newoussystem mortality/aging adiposetissue tastelofaction pigmentation neoplasm visionleve Click cells to view annotations. Mice homozygous for a knock-out allele are embryonic lethal.

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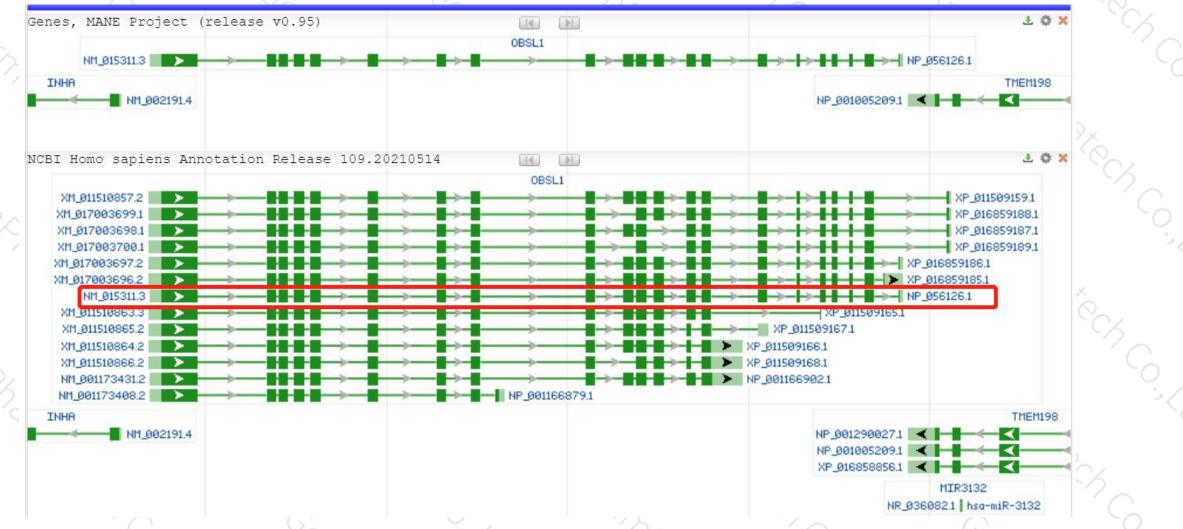
Gene name and location(NCBI)-OBSL1 「 集萃药康

							- / <u>)</u>	
	OBSL1 obscurin l	ike cytoskeletal a	daptor 1 [<i>Homo sapiens</i> (hur	man)]		🗄 Download Datasets	25	
	Gene ID: 23363, updated or	11-Jul-2021						
	Summary					8 1	1^{2}	
and and							<u>`</u> ?`?	
~	Official Symbol	OBSL1 provided by HGN	<u>IC</u>					
12			al adaptor 1 provided by <u>HGNC</u>					
	Primary source							1
		Ensembl:ENSG0000012	<u>24006 MIM:610991</u>					
	RefSeg status	protein coding					·	
	Organism							
	Production of the second		nordata; Craniata; Vertebrata; Euteleostomi	: Mamma	lia: Eutheria: Euarchontoolires: Primat	es: Haplorrhini: Catarrhini:		
		Hominidae; Homo	······································		, <u> </u>		S CZ	
	Summary	Cytoskeletal adaptor pro	oteins function in linking the internal cytoske	eleton of c	cells to the cell membrane. This gene e	encodes a cytoskeletal		
$\langle x \rangle$			s a member of the Unc-89/obscurin family.				22	
			bronectin type 3 domain. Mutations in this			spliced transcript variants	\sim	
	-		ms have been found in this gene. [provideo				25	
	Expression		ary (RPKM 37.4), testis (RPKM 15.3) and 19	9 other tis	sues See more		9 m. 3	
	Orthologs							
	NEW	Try the new Gene table						
		Try the new Transcript	Table					
	Genomic context							
1					\mathcal{L}			
	Location: 2q35				See	OBSL1 in Genome Data View		
	Exon count: 23						<u>р</u> О	
	Annotation release	Status	Assembly	Chr	Location		$[\mathcal{Q}_{L}] = [\mathcal{Q}_{L}]$	
	109.20210514	current	GRCh38.p13 (GCF_000001405.39)	2	NC_000002.12 (21954940821957	71573, complement)		
	105.20201022	previous assembly	GRCh37.p13 (GCF_000001405.25)	2	NC_000002.11 (22041545022043			
-	100.20201022	previous assembly					<u> </u>	
		219538949	Chromosome 2 - NC	_000002	2.12		No.	
		CHPF	MIR3132 -		LOC112806080 -			
			OBSL1 4					
股份有	限公司		GemPharr	natec	h Co., Ltd.		025-5864 15	34

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Transcript information(NCBI)-*OBSL1*

The gene has 13 transcripts, and all the transcripts are shown below, the strategy is based on the design of NM_015311.3 transcript



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集萃药康 GemPharmatech If you have any questions, please feel free to contact us. Tel: 025-5864 1534





