### *Lepr*-p.Y985F&Y1077F&Y1138F cas9-ki Mouse Model Strategy -CRISPR/Cas9 technology

**Designer: Zihe Cui** 

**Reviewer: Yanhua Shen** 

**Design Date: 2021-9-8** 

# **Project Overview**





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

025-5864 1534

# Strategy



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



# **Technical Description**



- According to Ensembl data, the mouse *Lepr* gene has 7 transcripts.
- According to the structure of *Lepr* gene and requirements of customer, this project produced *Lepr*-p.Y985F&Y1077F&Y1138F point mutations on exon19 of the transcript of *Lepr*-201(ENSMUST0000037552.10, NM\_146146.3 $\rightarrow$ NP\_666258.2). The 985th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAT to TTC. The 1077th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAT to TTC. The 1138th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAT to TTC. The 1138th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAT to TTC.
- The mouse *Lepr*-201 transcript contains 19 exons. The translation initiation site ATG is located at exon2, and the translation termination site TAA is located at exon19, encoding 1162aa.
- In this project, *Lepr* 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, homozygous of this model is female and male infertility.
- ➤ The 3' gRNA will be located on about 600bp downstream of *Lepr*-201.
- ➢ One or two synonymous mutations of amino acids may be intronduced on exon19 of Lepr-201.
- > Two or four base mutations may be intronduced on downstream of *Lepr*-201.
- The effect of Lepr-202, Lepr-203 and Lepr-205 transcripts is unknown in this strategy. Lepr-204, Lepr-206 and Lepr-207 transcripts may not be affected.
- Mouse Lepr gene is located on Chr4. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr4, 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.

# Reference





Fig. 1. Abrogation of hypothalamic STAT3 activation in Y3F and Y123F mice. (A) Schematic diagram showing the strategy of homologous gene targeting to replace exon 18 of the leptin receptor gene with the mutant exon 18 (Y123F) harboring phenylalanine (F) substitutions for all of the 3 tyrosine residues at positions 985, 1077, and 1138. The selection marker (LNL) cassette was subsequently removed by *cre* excision. The Y3F knockin mice were likewise generated. (*B*) Chimeric

Tyrosine-dependent and -independent actions of leptin receptor in control of energy balance and glucose homeostasis. Proc Natl Acad Sci U S A. 2008 Nov 25;105(47):18619-24.

### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

# **Mutation Site**



### **Before mutation**

GTGACCAGTGTAACAGTGCTAACTTCTCTGGGTCTCAGAGCACCCAGGTAACCTGTGAGGATGAGTGTCAGAGACCACCCTCAGTTAAATATGCAACTCTGGTCAGCAACGATAAACTAG



- 6	6	1	- 11	110	17	1	12	8	115	1	- 11 - 11 - 11 - 11 - 11 - 11 - 11 - 1		17	120	12	6	6	- N	125	- Q	17	1	12	130	1.0	- 1 C	- 11 - 11 - 11 - 11 - 11 - 11 - 11 - 1	- 11 C	135	1 12	12	10	- G	140	- 14 - I	- Q	12	18	145
V	E	т	D	E	E	Q	G	F	I	Н	S	Ρ	V	S	Ν	С	Ι	S	S	Ν	Н	S	Р	L	R	Q	S	F	S	S	S	S	W	E	Т	E	Α	Q	Т
																			Ex	con19	)																		

TTTTCCTTTTATCAGACCAGCAACCCACCATGATTTCACCACAACTTTCATTCTCGGGGGTTGGATGAGCTTTTGGAACTGGAGGGAAGTTTTCCTGAAGAAAATCACAGGGGAGAAGTCTG

12	81	1	14	150		ĩ.		12	155	1	13	ii.	T.	160	1	18	31	- A -	165	1	1	- îč	12	170	Si	1		Ĩ.	175	- Î.	- í	12	Si	180	1	10	T.	Ĩ.	185
F	F	L	L	S	D	Q	Q	Ρ	Т	М	Ι	S	Ρ	Q	L	S	F	S	G	L	D	E	L	L	E	L	E	G	S	F	Ρ	E	E	N	Н	R	E	К	S
-																			Ex	on19	)																		

12	31	<u>.</u>	3	190		- Ê	11	18	195	1	3	1		200	11		3	1	205	1	1	18	11	210	3	0	3		215	Ĕ	10	. 6	3	220	1	1	1	ii.	225
V	С	Y	La	G	V	Т	S	V	N	R	R	E	S	G	V	L	L	Т	G	E	Α	G	Ι	L	С	Т	F	Р	A	Q	С	L	F	S	D	Ι	R	Ι	L
																			Ex	on19	<u>k</u> :																		

1077 mutation

AGGAGAGATGCTCACACTTTGTAGAAAATAATTTGAGTTTAGGGACCTCTGGTGAGAACTTTGTACCTTACATGCCCCAATTTCAAACCTGTTCCACGCACAGTCACAAGATAATGGAGA



1138 mutation

The red region represents the mutation site.

# **Mutation Site**



025-5864 1534



GTGACCAGTGTAACAGTGCTAACTTCTCTGGGTCTCAGAGCACCCAGGTAACCTGTGAGGATGAGTGTCAGAGACCAACCCTCAGTTAAATTCGCAACTCTGGTCAGGACCACCGATAAACTAG



-				110		1.5			115	- I-	1	1.		120					125	S 15		1.5		130				15	135	1.1			-	140		1		1.2	145
V	E	Т	D	E	E	Q	G	F	I	н	S	Ρ	V	S	N	С	I	S	S	N	Н	S	Ρ	L	R	Q	S	F	S	S	S	S	W	E	Т	E	A	Q	Т
																			E	xon1	9																		

Ϋ́	3		i i	150	12	- P	19	14	155		1	E.	12	160	19	14	3	1	165	12	12	E.	6	170	3		- F	12	175	1	- 6	14	3	180	¥	i.	12	1	185
F	F	L	L	S	D	Q	Q	Ρ	Т	М	I	S	Ρ	Q	L	S	F	S	G	L	D	E	L	L	Е	L	E	G	S	F	Ρ	E	E	N	Н	R	E	К	S
																			Ex	on19																			

÷.	- G	<u>.</u>	7	190	12	Ê		1	195	1	12	1	12	200	6	- N	1	1	205	1	- il	8		210	1	- Q	$\overline{T}$	1	215	E.		1	1	220	1	Ĩ.	12	12	225
V	С	F	L	G	V	Т	S	V	N	R	R	E	S	G	V	L	L	Т	G	Е	Α	G	Ι	L	С	Т	F	Ρ	Α	Q	С	L	F	S	D	Ι	R	Ι	L
																			Ex	on19																			

1077 mutation

AGGAGAGATGCTCACACTTTGTAGAAAATAATTTGAGTTTAGGGACCTCTGGTGAGAACTTTGTACCT**TTC**ATGCCCCCAATTTCAAACCTGTTCCACGCACAGTCACAAGATAATGGAGA TCCTCTCTACGAGTGTGAAACATCTTTTATTAAACTCAAATCCCTGGAGACCACTCTTGAAACATGGAAAGTACGGGGTTAAAGTTTGGACAAGGTGCGTGTCAGTGTTCTATTACCTCT

i i	17	n.	230	1	ĩ	13	- 23	235	1	1	1	E.	240	15	- 20	i i	3	245	1	T.	ĩ	12	250	1	3	a.	T	255	Ē.	6	80	1	260	1		E.	ĩ	265	
E	R	С	S	Н	F	V	E	N	N	L	S	L	G	Т	S	G	Е	N	F	V	Ρ	F	М	Ρ	Q	F	Q	Т	С	S	Т	H	S	Н	К	I	М	E	
																		E	con 19	2																			

The red region represents the mutation site.

1138 mutation

# Gene name and location(NCBI)



Gene ID: 16847, updated on 31-Aug-2021



集萃

**±** Download Datasets

# **Transcript information (Ensembl)**

### 集萃药康 GemPharmatech

### The gene has 7 transcripts, and all transcripts are shown below:

Name 🔺	Transcript ID 💧	bp 🖕	Protein 🖕	Biotype 🝦	CCDS 🍦	UniProt Match	Flags 🔶
Lepr-201	ENSMUST0000037552.10	4127	<u>1162aa</u>	Protein coding	CCDS51240	P48356-1	GENCODE basic APPRIS ALT2 TSL:1
Lepr-202	ENSMUST00000102777.10	3410	<u>892aa</u>	Protein coding	CCDS18397	P48356-3	GENCODE basic APPRIS P3 TSL:1
Lepr-203	ENSMUST00000106921.9	5542	<u>894aa</u>	Protein coding	<u>CCDS51239</u> 률	P48356-2	GENCODE basic APPRIS ALT2 TSL:1
Lepr-204	ENSMUST00000128948.8	787	No protein	Processed transcript	-	-	TSL:3
Lepr-205	ENSMUST00000145024.2	208	<u>30aa</u>	Protein coding	100 B <u>a</u> nn	A2AV66	TSL:5 CDS 3' incomplete
Lepr-206	ENSMUST00000151733.2	415	No protein	Processed transcript	100	20	TSL:3
Lepr-207	ENSMUST00000156402.2	889	No protein	Processed transcript	22	1000	TSL:2

The strategy is based on the *Lepr*-201 transcript, the transcription is shown below:



# **Genomic location distribution**





### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 025-5864 1534

# **Protein domain**



### **Protein domains for ENSMUSP00000037385.4**

ENSMUSP000000373 Transmembrane heli Low complexity (Seg) Superfamily SMART Pfam PROSITE profiles	-	Leptin	Hbronec Hbrone receptor, immunoglobuli Hbron	tin type III superfami ectin type III Immun in-like domain ectin type III	ly oglobulin C2-set-like,	, ligand-binding		-		_		-	
PROSITE patterns				Short hematop	poietin receptor, fam	ily 1, conserved site		Long he	matopoietin receptor,	Gp130 family 2, con	served site		
PANTHER	PTHR23036												
Gene3D CDD	Leptin recepto	or Immuno	globulin-like told	ectio type III			_		_				
All sequence SNPs/in	Sequence varia	ants (dbSNP and	all other sources)		1 T	31 1 11	1.11.01.00.1	1.11	11	<u>10</u>	1.1 11	0.000	
Variant Legend	inframe in	sertion			missen	ise variant	1400		synonym	ous variant			- Circ
Scale bar	δ	100	200	300	400	500	600	700	800	006	1000		1162

### Mouse phenotype description(MGI)



URL link is as follows: http://www.informatics.jax.org/marker/MGI:104993



Homozygous mutants are hyperphagic, low-activity, poorly cold-adapted, sterile and have enhanced fat conversion. They are obese, hyperinsulinemic and, on certain strains, severely hyperglycemic. Heterozygotes are normal but resistant to prolonged fasting.

### 江苏集萃药康生物科技股份有限公司

### GemPharmatech Co., Ltd.

### If you have any questions, please feel free to contact us. Tel: 025-5864 1534





