南模生物
SHANGHAI
MODEL ORGANISMS

SMOC-Report for

Slc6a1 KI mouse model

Focus on Model Organism for Life Science

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1. Background and Objective

1.1 Objective

Generation of the point mutation mice with p.S295L mutation of Slc6a1 gene via CRISPR/Cas9 technology

1.2 Background

Gene Name (MGI Number): *Slc6a1* （95627 ）

Gene URL Link (MGI) : http://www.informatics.jax.org/marker/MGI:95627

Gene URL Link (Ensembl) :

http://www.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000030310;r=6:114282635-114317532

Gene Ensembl ID: *Slc6a1* -201 （ENSMUST00000032454.7）

Mutant locus：p.S295L

2. Abstract

To mutant the p.S295L of gene *Slc6a1* , the project process was as follows: 1) Cas9 mRNA and gRNA were produced by *in vitro* transcription; 2) oligo donor DNA was synthesized; 3) the mixture of Cas9 mRNA、gRNA and donor DNA was microinjected into fertilized eggs (C57BL/6J), then got positive F0 mice that identified by PCR and sequencing; 4) F0 mice crossed with wild type C57BL/6J mice to generate F1 mice, then got six positive F1 mice that identified by PCR and sequencing.

3. Recombinant strategy

3.1 Strategy figure:

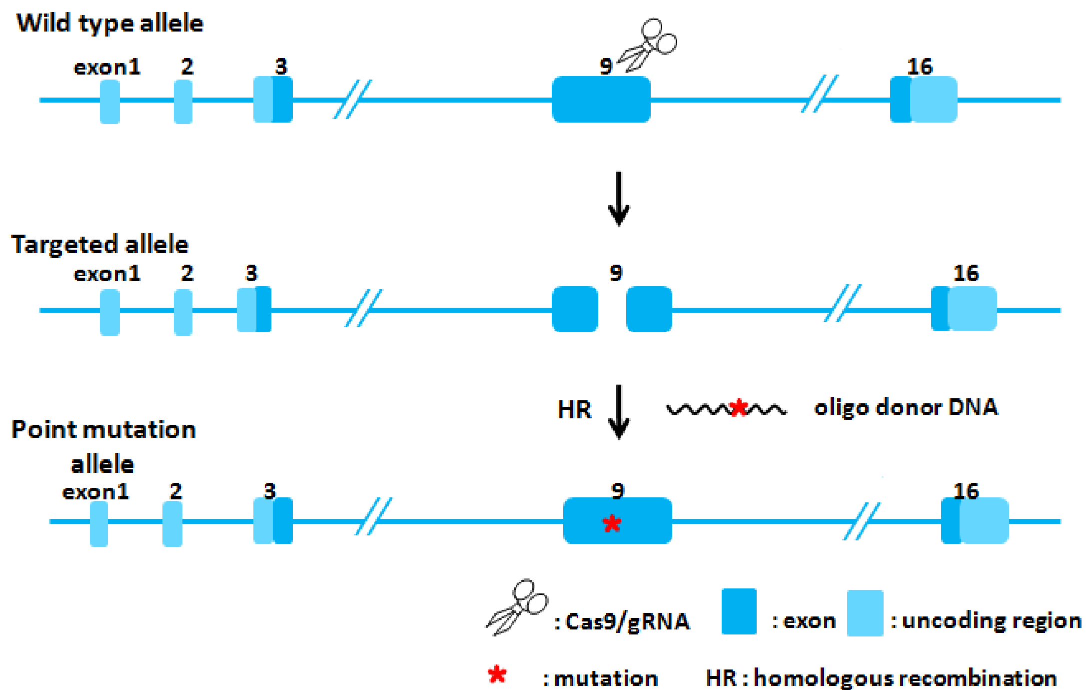

Figure 1: Strategy of Slc6a1-PM mouse model

3.2 Information of Knockin locus

GTGTGGCTTGACGCCGCCACCCAGATCTTCTTCT**CC**TACGGGCTGGGCCTGGGGTCCCTGATTG CTCTGGGAAGCTACAACTCTTTCCACAACAATGTGTACAG

(Bold black indicates the site where the mutation is required ,The underlined letter is Guide RNA target site and the red font is mutant site. The green font is a synonymous mutation site to avoid repeated Cas9 activity.)

3.3 Oligo donor DNA sequence

GTGTGGCTTGACGCCGCCACCCAGATCTTCTTCTTATACGGGCTCGGCCTGGGGTCCCTGATTG CTCTGGGAAGCTACAACTCTTTCCACAACAATGTGTACAG

4.Results

4.1 Result of *in vitro* transcription of Cas9 mRNA and gRNA

gRNA	Sequence(5’--3’)
gRNA1	GATCTTCTTCTCCTACGGGC TGG

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4.2 F0 mice genotyping

F0 mice were produced by microinjection. By PCR identification and sequencing, we confirmed there were two homologous recombinant mice.

4.2.1 Genotyping strategy of F0 mice

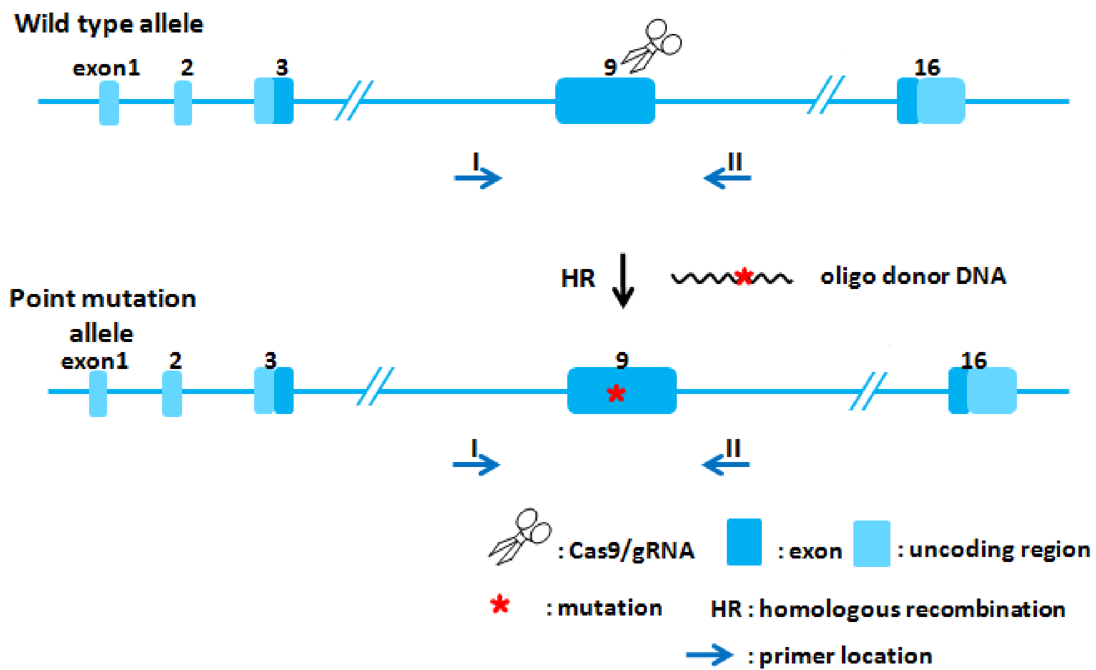

Figure 2: Strategy of F0 mice genotyping

4.2.2 Method for identifying 5’ homologous recombinant F0 mice

Primers for PCR:

	Sequence 5' --> 3'	Primer Type
I	CCAGGAGGAGGAGGAGGAACAGAT	Forward
II	CAAGCCAGGCAGGTAGAGCAGAGA	Reverse

Primers for sequencing:

Primer	Sequence (5’-3’)
III	CCAGGAGGAGGAGGAGGAACAGAT

Reaction system:

Reaction Component	Volume (μl)
ddH2O	13.2
GXL PCR Buffer	2
2.5 mM dNTP	2
Primer I (10pmol/μl)	0.5
Primer II (10pmol/μl)	0.5
GXL DNA Polymerase*	0.8
Tail genomic DNA	1
Total	20

*La Taq（TaKaRa，Code No：RR02MA）

PCR program:

Step #	Temp (℃)	Time	Note
1	94	3 min	-
2	98	15 sec	-
3	57	15 sec	-
4	68	1 min	repeat steps 2-4 for 35cycles
5	68	5 min	-
6	12	-	hold

4.3 F1 mice genotyping

The F0 mice crossed with wild type C57BL/6J to generate F1 mice. By PCR identification and sequencing, we confirmed there were three homologous recombinant F1 mice：NO. 1, 2, 5, 6, 8, 9。

4.3.1 PCR genotyping of homologous recombinant F1 mice

Identification methods refer to 4.2. All positive PCR products were confirmed by sequencing. The alignment of F1 mice sequencing result Vs. object sequence was shown in 4.3.1.1and 4.3.1.2（1# F1 mice as example）.

4.3.1.1 The alignment of F1 mice sequencing result

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Query  597   CTGTCCCGCCCAAATGGATCCCCAGAGTCAAGAGCATGCTACAGGCACTAGGTATCTGAG  656
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  64    CTGTCCCGCCCAAATGGATCCCCAGAGTCAAGAGCATGCTACAGGCACTAGGTATCTGAG  123

Query  657   CAGGTCGGATGGAGCAGATAATCCCTGAGGACTCCCAGGAGGCCGCGGTAGCGATGAAGA  716
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124   CAGGTCGGATGGAGCAGATAATCCCTGAGGACTCCCAGGAGGCCGCGGTAGCGATGAAGA  183

Query  717   TGTCGACTCTGACCTTGATCAGTTCTGAGGTTCTGATCCTCTGTCTGCAGGTGTGGCTTG  776
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184   TGTCGACTCTGACCTTGATCAGTTCTGAGGTTCTGATCCTCTGTCTGCAGGTGTGGCTTG  243
                                    mutation
Query  777   ACGCCGCCACCCAGATCTTCTTCTCCTACGGGCTGGGCCTGGGGTCCCTGATTGCTCTGG  836
             ||||||||||||||||||||||||  ||||||||||||||||||||||||||||||||||
Sbjct  244   ACGCCGCCACCCAGATCTTCTTCTTATACGGGCTGGGCCTGGGGTCCCTGATTGCTCTGG  303

Query  837   GAAGCTACAACTCTTTCCACAACAATGTGTACAGGTGCGAGGGGGCGGGCTTTGAGGCTT  896
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  304   GAAGCTACAACTCTTTCCACAACAATGTGTACAGGTGCGAGGGGGCGGGCTTTGAGGCTT  363

Query  897   CCTTCCTGGCCACGCCCCTAAACATGCCTTCCTGGTGGAGGCACACTGAGGCCACGCCCC  956
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  364   CCTTCCTGGCCACGCCCCTAAACATGCCTTCCTGGTGGAGGCACACTGAGGCCACGCCCC  423

Query  957   AGGATGATTGCCAACCCCTAACCCTGTGCCTACCTCAGTAATACACACTTATCTCCAGCA  1016
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424   AGGATGATTGCCAACCCCTAACCCTGTGCCTACCTCAGTAATACACACTTATCTCCAGCA  483

Query  1017  CTTCTCCCCTGTCACATGCTGGAGGCCATACACGCTCTTCATCTGAGACTACATCCCCTG  1076
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484   CTTCTCCCCTGTCACATGCTGGAGGCCATACACGCTCTTCATCTGAGACTACATCCCCTG  543
```

Sbjct: sequencing result; Query: wildtype sequnce.

4.3.2 Information of homologous recombinant F1 mice

Table2: The information of positive F1 mice

Mice ID	DOB	Gener-ations	Sex	Type	Geno-type	Father	Mother
1	2019/1/14	F1	♂	PM	He	27	WT
2	2019/1/14	F1	♂	PM	He	27	WT
5	2019/1/14	F1	♂	PM	He	27	WT
6	2019/1/14	F1	♀	PM	He	27	WT
8	2019/1/14	F1	♀	PM	He	27	WT
9	2019/1/14	F1	♀	PM	He	27	WT

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5. Advice for the project

5.1 Identification of the delivered mice

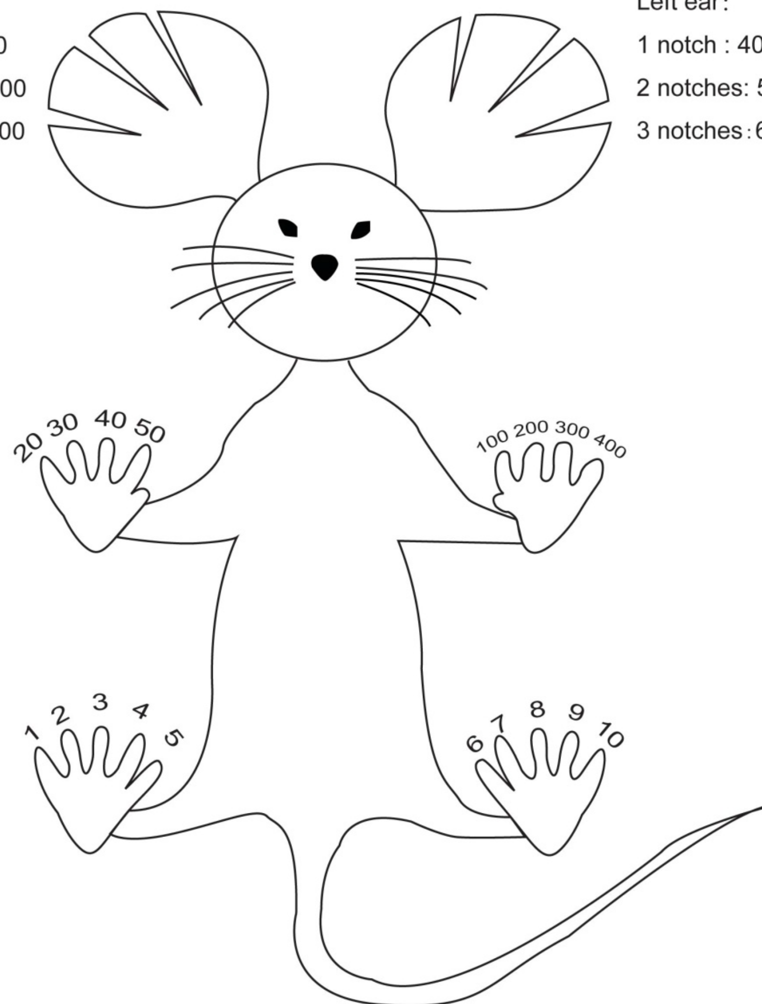
Please identify the delivered mice by genotyping method described in 4.3 and 4.4.

Principles of mouse numbering are described as below:

Mouse Numbering Scheme

Right ear:
1 notch : 1000
2 notches: 2000
3 notches : 3000

Left ear:
1 notch : 4000
2 notches: 5000
3 notches : 6000



Note: The numbers obtained by adding all the numbers is the ID number of the mouse.
The number 10 is not involved in the combination of numbers from 20 to 90.

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5.2 Breeding process

The delivered mice were heterozygous unless there were additionalinformations. Please design an appropriate breeding plan according to the experimental requirements. Based on peculiarity of CRISPR/Cas9 technology, we suggest that:

1) There might be off-targeting via CRISPR/Cas9 technology. We can provide services for detecting potential off-target sites. For eliminating the off-target effect, homologous recombinant mice should be back-crossed with wild type mice before experiment.

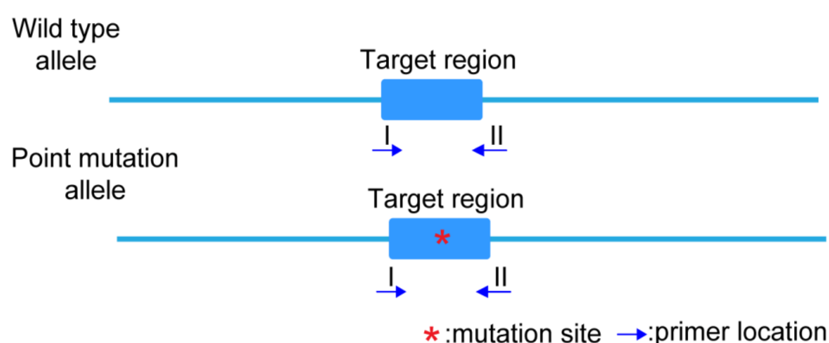
5.3 Genotyping of offspring mice

For genotyping the offspring, PCR is used to identify homozygous, heterozygous and wild type mice. The proposed genotyping method is shown in below. You can also design other primers according to the sequence. Primer design software, e.g. DNASTAR, can be downloaded from the internet.

5.3.1 Genotyping method Ⅰ (for identifying homozygous, heterozygous and wild type mice)

There are several methods for genotype identification. In this report, we provide one of them for reference.

5.3.1.1 Map of genotyping primers locus



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5.3.1.2 PCR reaction parameters

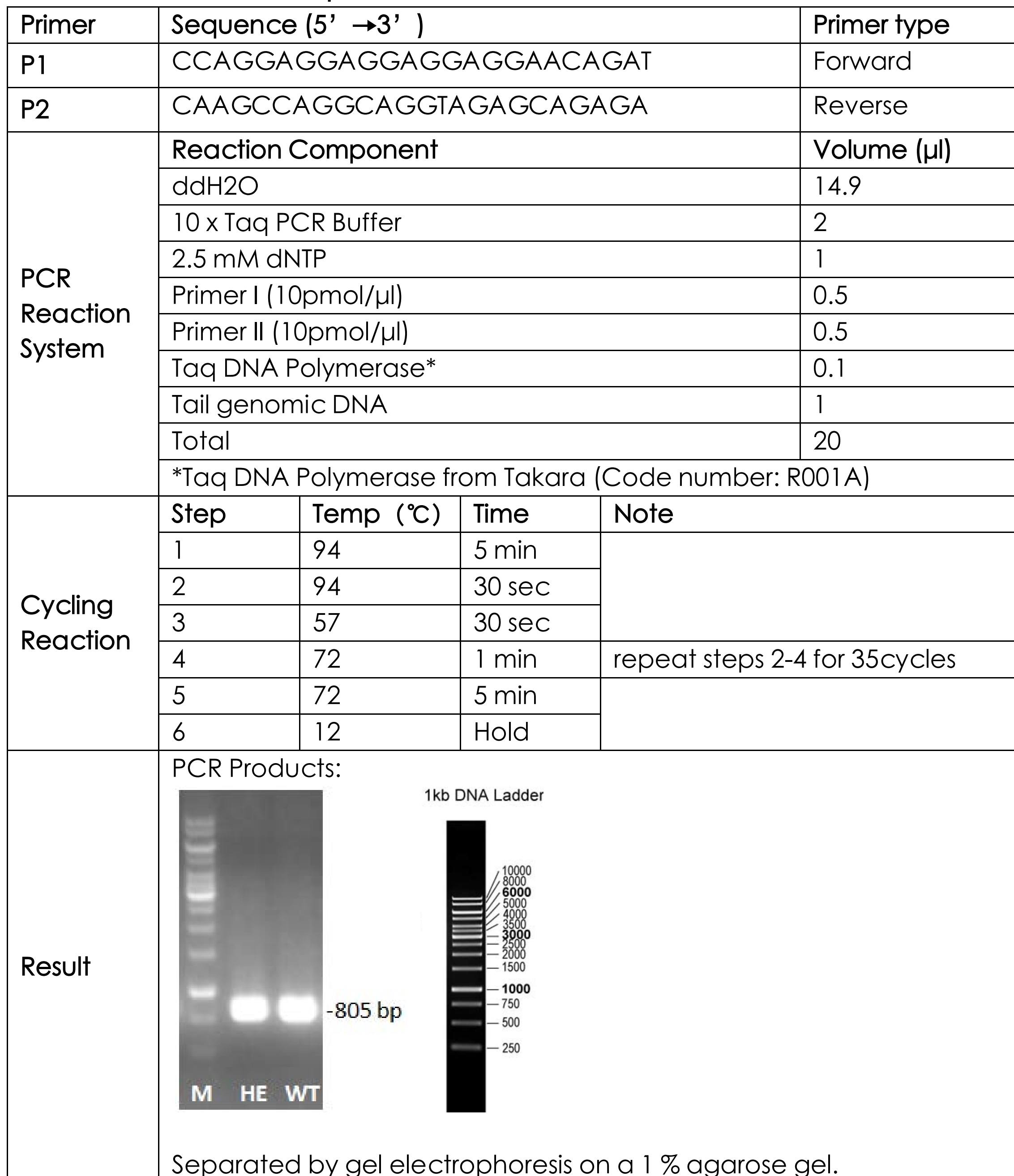
Primer	Sequence (5’ →3’)	Primer type
P1	CCAGGAGGAGGAGGAGGAACAGAT	Forward
P2	CAAGCCAGGCAGGTAGAGCAGAGA	Reverse

PCR Reaction System	Reaction Component	Volume (μl)
	ddH2O	14.9
	10 x Taq PCR Buffer	2
	2.5 mM dNTP	1
	Primer I (10pmol/μl)	0.5
	Primer II (10pmol/μl)	0.5
	Taq DNA Polymerase*	0.1
	Tail genomic DNA	1
	Total	20
	*Taq DNA Polymerase from Takara (Code number: R001A)	

Cycling Reaction	Step	Temp（℃)	Time	Note
	1	94	5 min	
	2	94	30 sec	
	3	57	30 sec	
	4	72	1 min	repeat steps 2-4 for 35cycles
	5	72	5 min	
	6	12	Hold	

Result

PCR Products:



Separated by gel electrophoresis on a 1 % agarose gel.

5.3.1.3 Primers for sequencing:

Primer	Sequence 5' --> 3'	Primer Type
1	CCAGGAGGAGGAGGAGGAACAGAT	Forward

5.3.1.4 Genotyping methods

The PCR product should be sequencing. Sequnencing peak data is for identifying homozygous, heterozygous and wild type mice.

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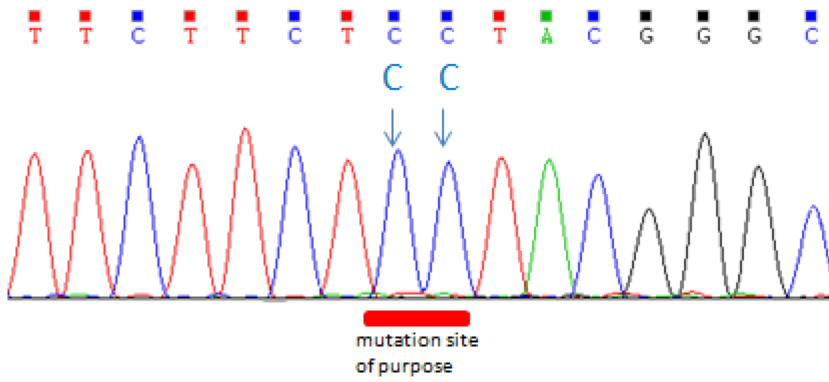
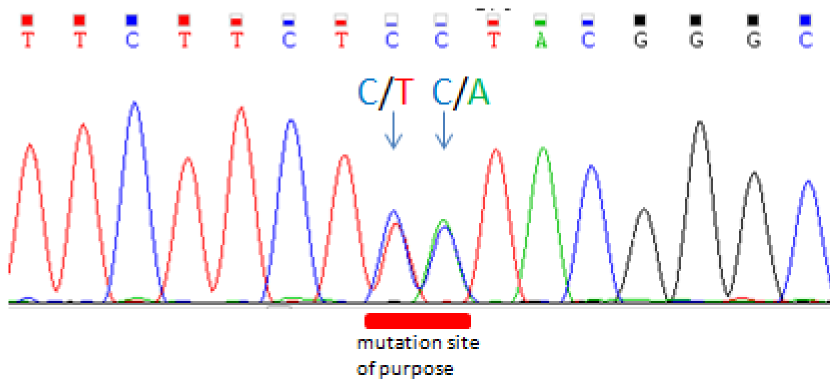
wild type:



Heterozygous (there are two peaks on mutant site) :



6. References

1. Mali P, Yang L, Esvelt KM, Aach J, Guell M, et al. (2013) RNA-guided human genome engineering via Cas9. Science 339: 823-826.
2. Cong L, Ran FA, Cox D, Lin S, Barretto R, et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819-823.
3. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, et al. (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153: 910-918.
4. Shen B, Zhang J, Wu H, Wang J, Ma K, et al. (2013) Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell Res 23: 720-723.