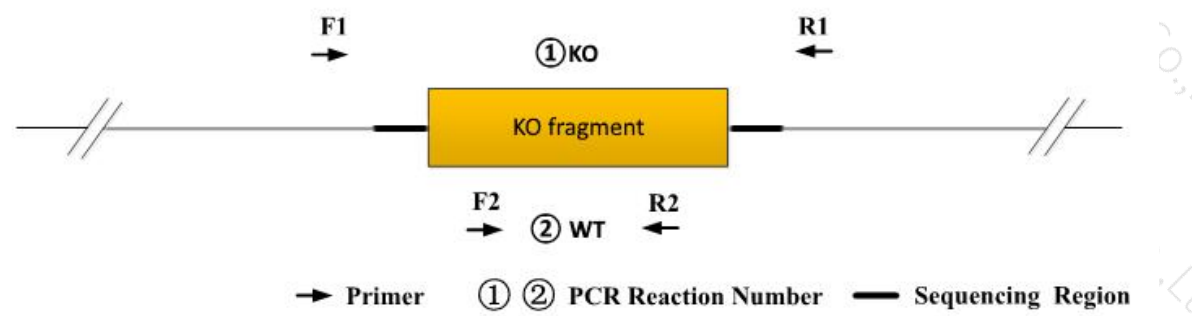


Genotyping Report

Strain ID	T017020	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chen Chen	Gene Name	<i>Kiss1r</i>		

1. Strategy of Genotyping



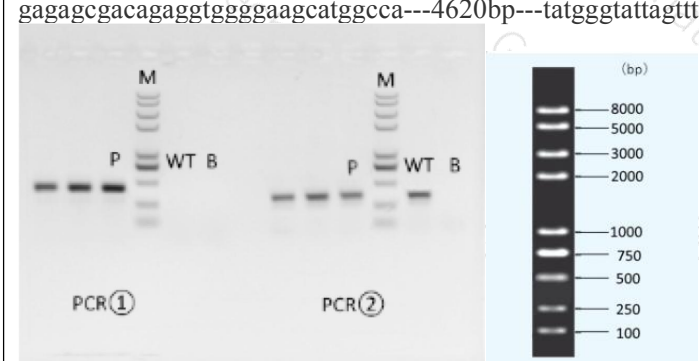
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note: 1)The sizes of WT and Targeted band are shown below.
2) If the WT band is too large, it may not be possible to obtain a WT band.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	T017020(P1)-F1	TACTACGCAGGGAGACGCGAA	WT: 5042bp KO: 422bp
	R1	T017020(P1)-R1	TTCCATAGCGAGATGGAGCCAG	
PCR②	F2	T017020(P1)-F2	TTGCGCTGGTGGGTGGTTAAT	WT: 355bp KO: 0bp
	R2	T017020(P1)-R2	GTCATGGCAGTAGGATCAGAGTCAG	

3. Gel Image

gagagcgacagaggtggggaagcatggcca---4620bp---tatgggtattagtttgagataggggtcca



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH2O); M: DNA Ladder

- ① Control (WT) : It is an important reference mark for whether the PCR reaction is successful and whether the product band position and size meet the theoretical requirements.
- ② Control (B) : PCR amplification was performed without template in the PCR reagent to monitor whether the reagent was contaminated.

4. PCR Condition

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC% $\geq$ 60% or GC% $\leq$ 40%, recommend to use Vazyme P515.)

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Rapid Taq Master Mix(Vazyme P222) or 2 × Phanta Max Master Mix (Vazyme P515)		12.5
2	ddH2O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(20~80ng/μl)		1
PCR program I (priority selection)			
Seg.	Temp.	Time	Cycle
1	95℃	5min	20×
2	98℃	30s	
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
1	95℃	5min	35×
2	98℃	30s	
3	58℃*	30s	
4	72℃	45s*	
5	72℃	5min	
6	10℃	hold	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.