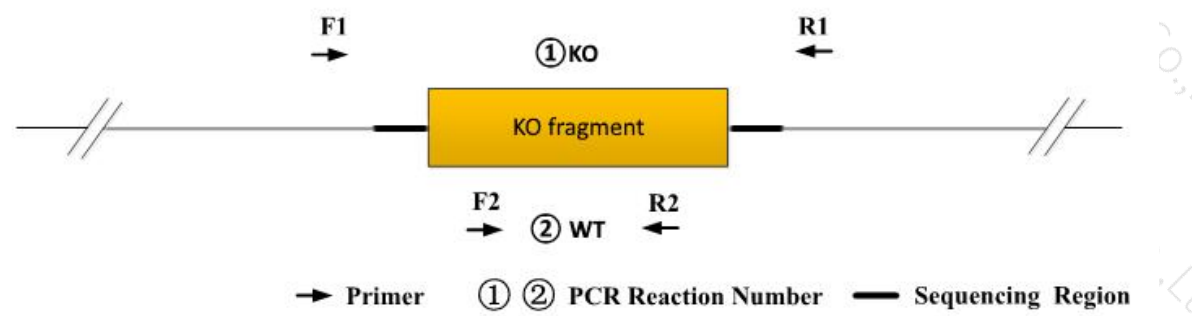


Genotyping Report

Strain ID	T003408	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Sucnr1</i>		

1. Strategy of Genotyping

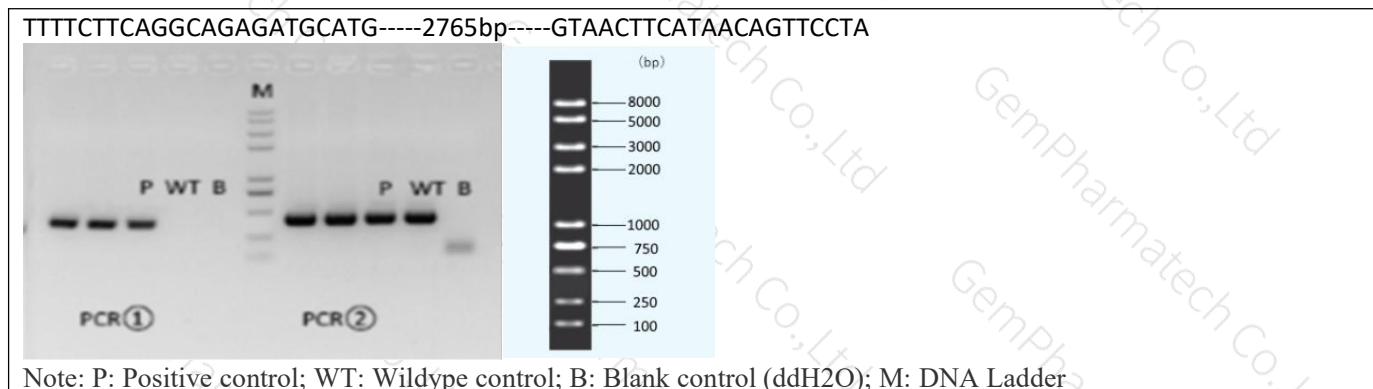


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ②PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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备用	F1	Sucnr1-5in-gtFA	AGAATTTGTAGGGATCTGTGGCTG	WT:3188bp KO: 423bp
	R1	Sucnr1-3in-gtRA	AACATGGGAGCCAGACTCTCTCA	
PCR②备用 GC: 34.9%	F2	Sucnr1-wt-tF1A	AGCCTTCTTCTTCCTTCTACAATGAG	WT:438bp KO:0bp
	R2	Sucnr1-wt-tR1A	GACTTATGTGAGGCCATGAACTATCTC	

3. Gel Image



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(≈100ng/μl)		1
PCR program			
Seg.	Temp.	Time	Cycle
1	95℃	5min	20×
2	98℃	30s	
3	65℃ (-0.5℃/cycle)	30s	
4	72℃	45s	
5	98℃	30s	20×
6	55℃	30s	
7	72℃	45s	
8	72℃	5min	
9	10℃	hold	