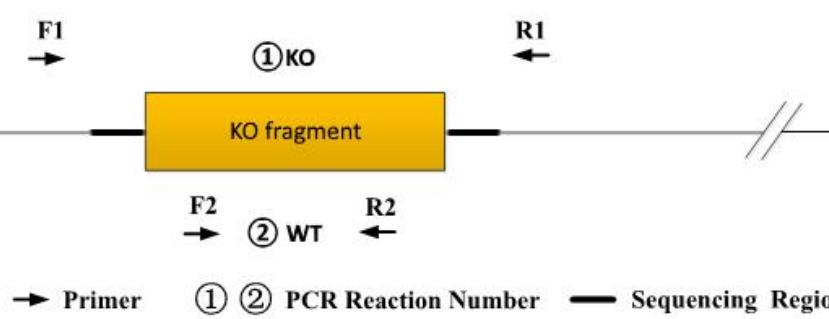




## Genotyping Report

Strain ID	T030050	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name			<i>Recql5</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.

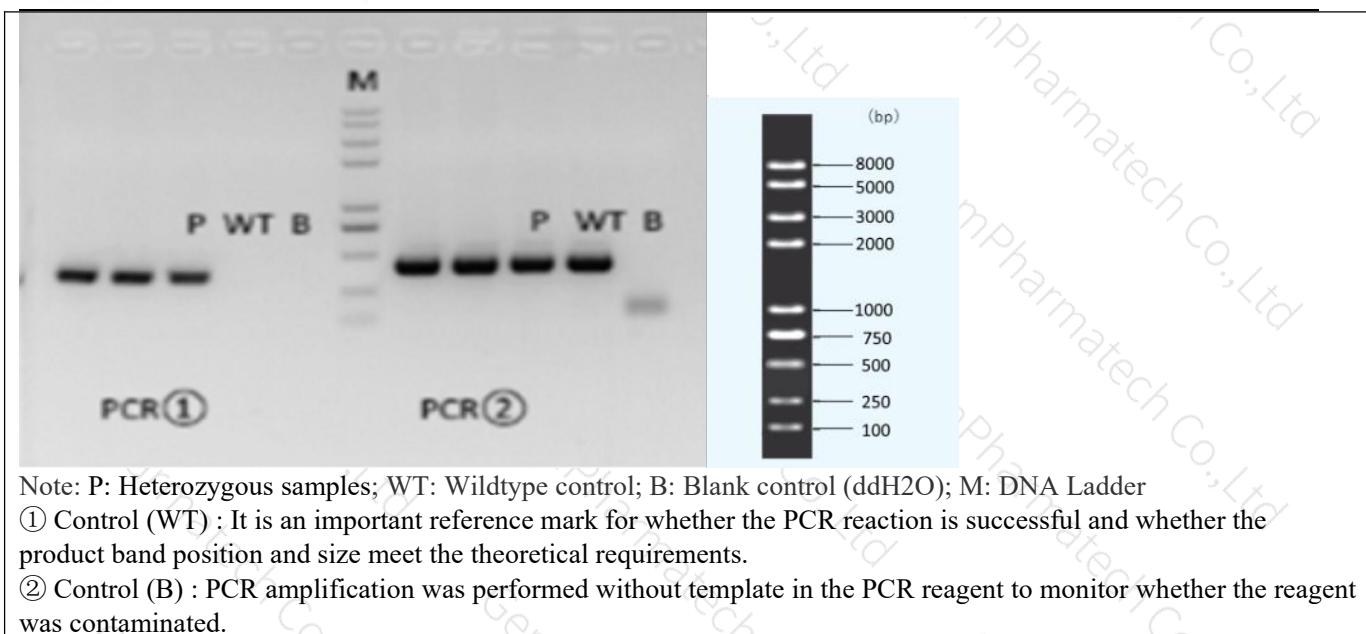
2) If the WT band is too large, it may not be possible to obtain a WT band.

### 2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T030050-F1	TTTGGCATAGTACAGGGATGTGC	WT:2504bp KO:333bp
	T030050-R1	AAATCCCCTGCTTGAGAGCTTC	
PCR②	T030050-F2	TTGAGATGCTGTGCCGATTGC	WT:430bp KO:0bp
	T030050-R2	ACACATCCTTTAACCTGTGGG	

### 3. Gel Image

aagcacaggcacatggctaagagccaccga---2171bp---gagtagacatgttggaaattgtaatgtgg



#### 4. PCR Condition

PCR Reaction Component			
Seg.	reaction component	Volume (μl)	
1	2 × Rapid Taq Master Mix (Vazyme P222)	12.5	
2	ddH <sub>2</sub> O	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 °C	5min	20×
2	98 °C	30s	
3	65 °C * (-0.5 °C/cycle)	30s	
4	72 °C	45s*	
5	98 °C	30s	
6	55 °C *	30s	
7	72 °C	45s*	
8	72 °C	5min	
9	10 °C	hold	

#### PCR program II (the second choice)

Seg.	Temp.	Time	Cycle
1	95 °C	5min	



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2	98°C	30s	35x
3	58°C*	30s	
4	72°C	45s*	
5	72°C	5min	
6	10°C	hold	

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