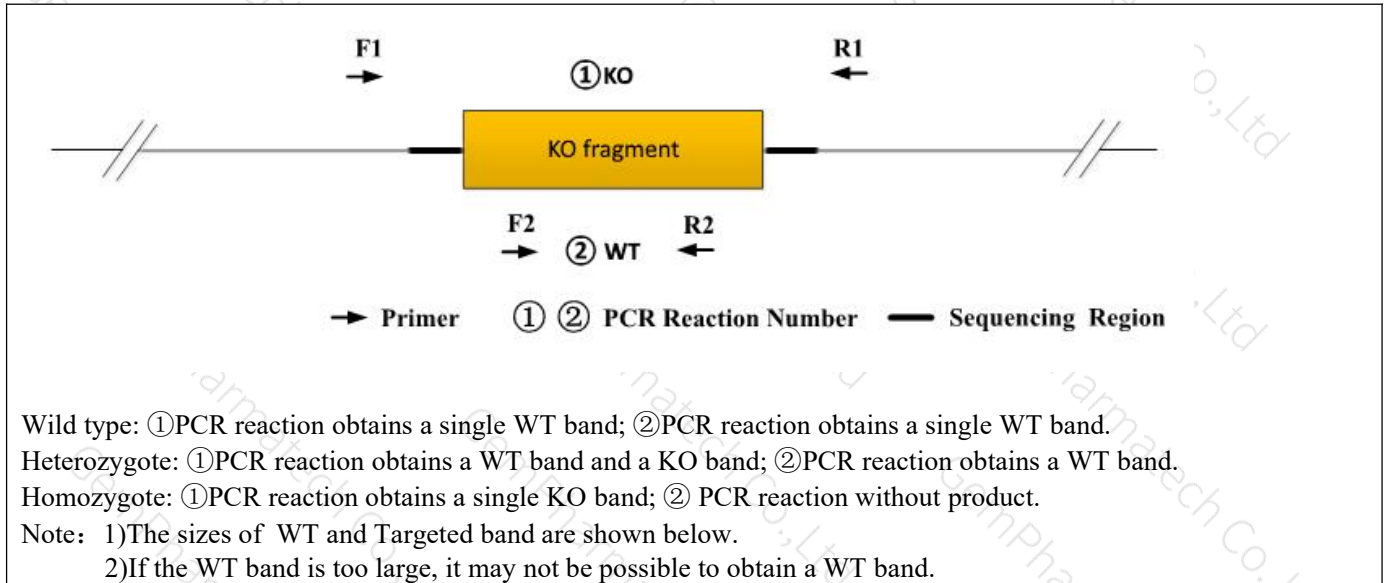


## Genotyping Report

Strain ID	T052863	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Fgfr4</i>		

### 1. Strategy of Genotyping

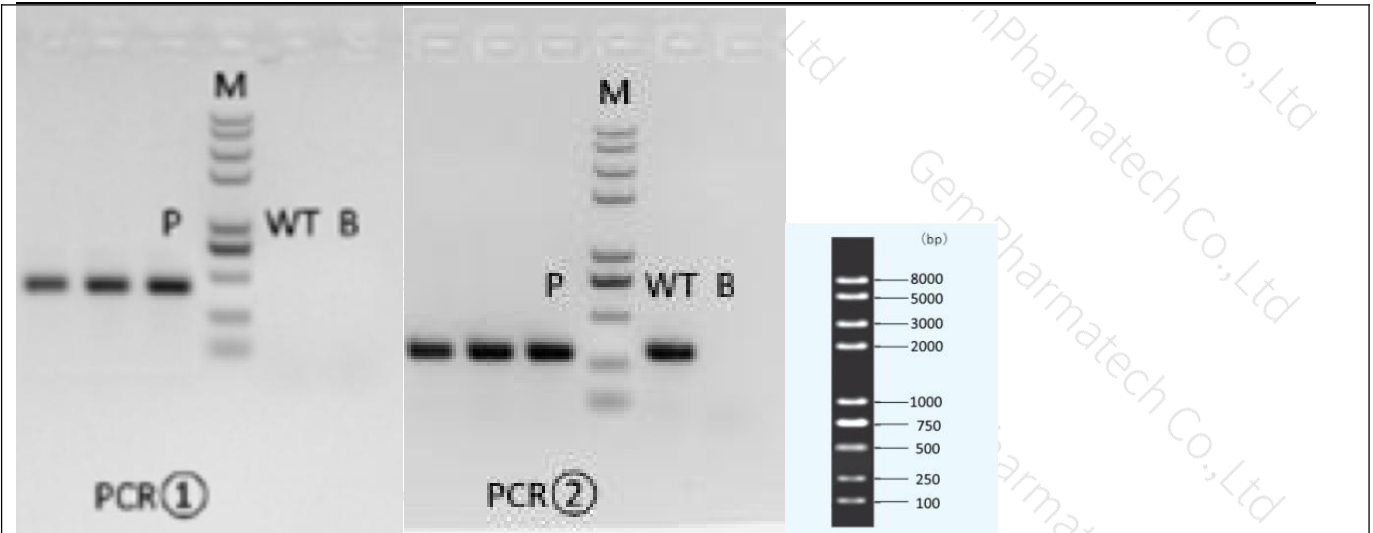


### 2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T052863-F1	CAAGAGAGGATTAGCCTTACAGGG	WT:1967bp KO: 450bp
	T052863-R1	CACTGCCCGGCATCTATATCTA	
PCR②	T052863-F2	TGGTCATGTCTACCCACAGCAAG	WT:295bp KO:0bp
	T052863-R2	GAATGCCTCCAATACGATTCTCC	

### 3. Gel Image

cagcatgtgtgatgatgaaccaag-----**1517bp**-----tggaactcactatgtagacttgct



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH<sub>2</sub>O); 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% ≥ 60% or GC% ≤ 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	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	
2	98°C	30s	20×
3	65°C*(-0.5°C/cycle)	30s	
4	72°C	45s*	
5	98°C	30s	15×
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	
2	98°C	30s	35×
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.