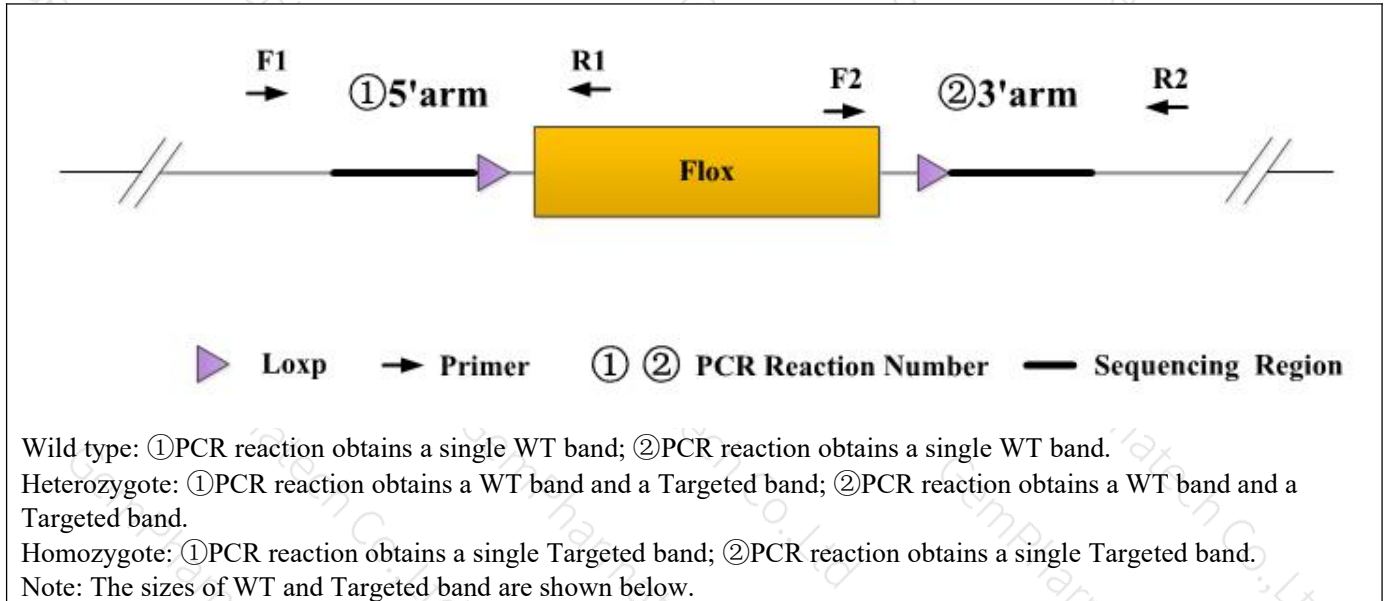


## Genotyping Report

Strain ID	T015183	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Cfb</i>		

### 1. Strategy of Genotyping



### 2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	T015183(P2)-F1	AGAAGGCGGAATGCAGAGGTT	WT: 344bp Targeted: 449bp
	R1	T015183(P2)-R1	CTGAAGATCGTTAAGCTCCTGCC	
②(3'arm)	F2	T015183(P2)-F2	AGACAGCATCGGAAGCAGCAAC	WT: 313bp Targeted: 419bp
	R2	T015183(P2)-R2	AGGAGACCATATCGTGGCCTCA	

### 3. Gel Image & Conclusion



- 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 ( $\mu$ l)
1	2 $\times$ Rapid Taq Master Mix (Vazyme P222)		12.5
2	ddH <sub>2</sub> O		9.5
3	Primer A(10pmol/ $\mu$ l)		1
4	Primer B(10pmol/ $\mu$ l)		1
5	Template(20~80ng/ $\mu$ l)		1
PCR program I priority selection			
Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	20 $\times$
3	65°C* (-0.5°C/cycle)	30s	
4	72°C	45s*	
5	98°C	30s	
6	55°C*	30s	15 $\times$
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 $\times$
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.

