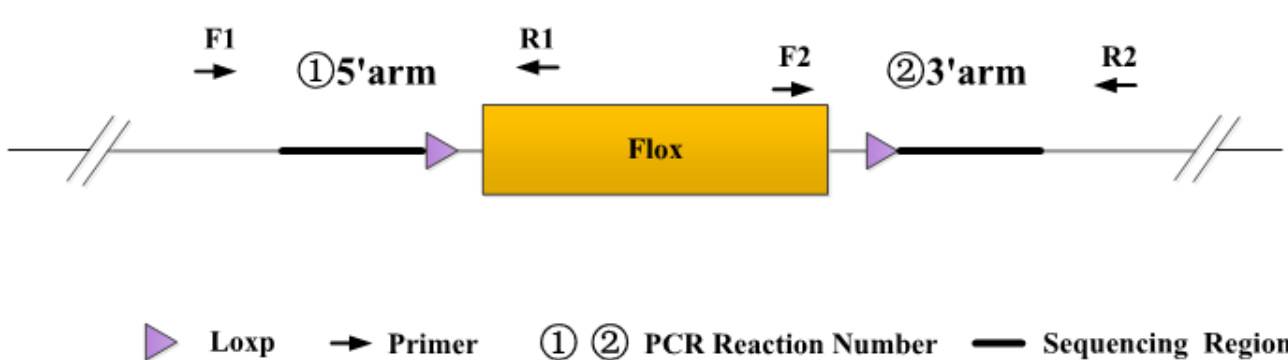


## Genotyping Report

Strain ID	T018842	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Binjie Jiao	Gene Name	<i>Abcd3</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 Targeted band; ②PCR reaction obtains a WT band and a Targeted band.  
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.  
 Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	JS09136-5S1-tF1A	TGTCCTGCTGTGTCTGAGCTATGG	WT:437bp
	R1	JS09136-5S1-tR1A	TTGCTCTTCCAGAGGTCCTGAGTT	Targeted: 542bp
②(3'arm)	F2	JS09136-3S1-tF1A	CCCTAAGGATTGATACTGGCAACT	WT: 421bp
	R2	JS09136-3S1-tR1A	GAGGACAGCTCTACTCTCATAGTGTGG	Targeted:527bp

### 3. Gel Image & Conclusion



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

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	
2	98°C	30s	20×
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	
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.