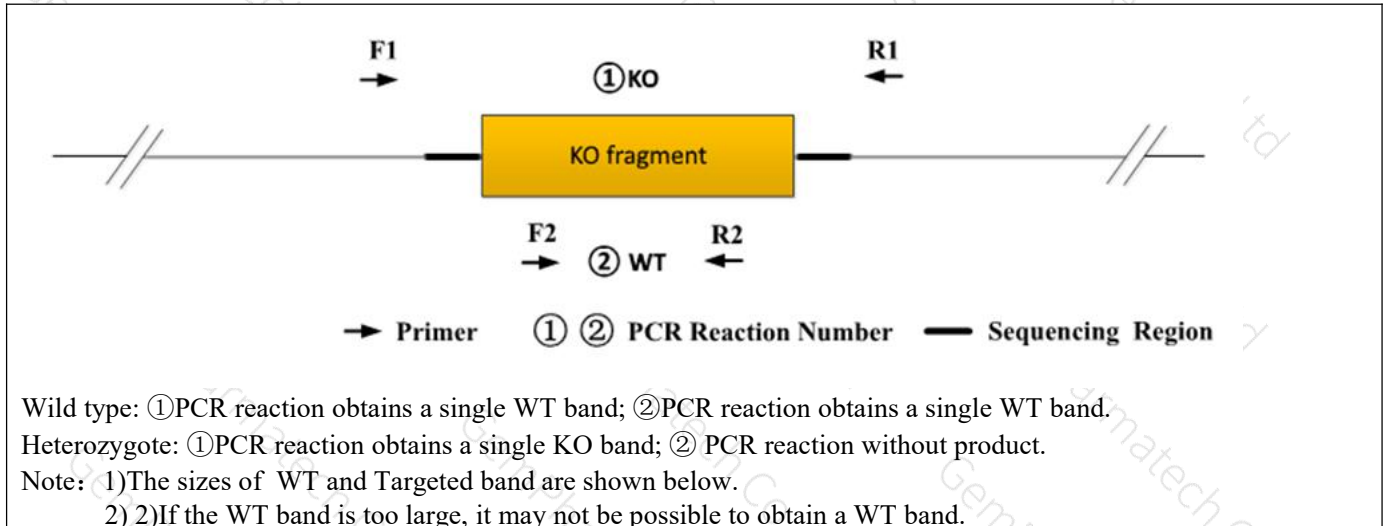


## Genotyping Report

Strain ID	T067549	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Itgax</i>		

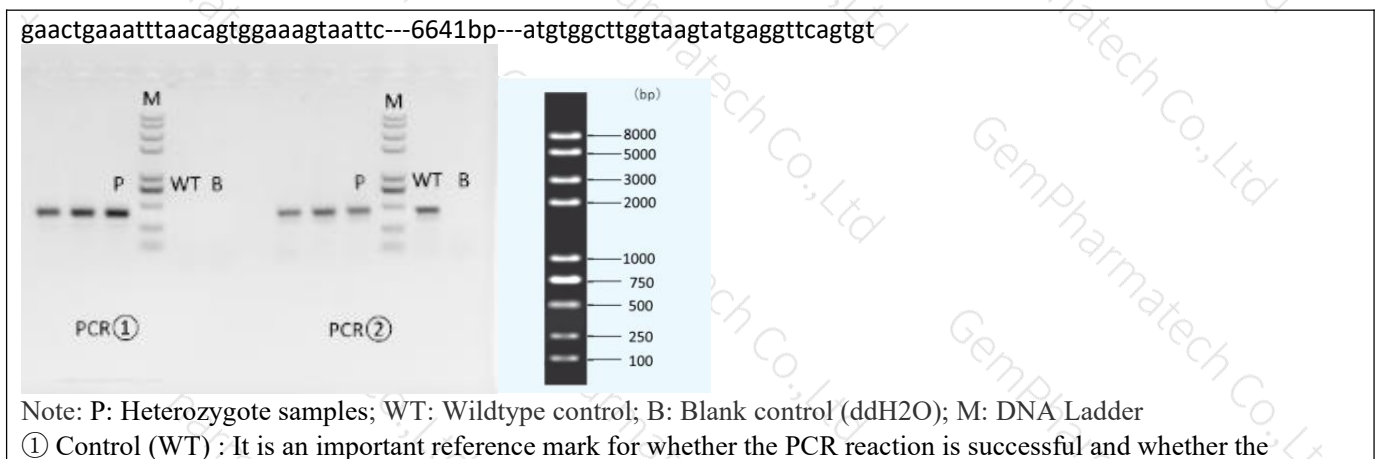
### 1. Strategy of Genotyping



### 2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	T067549(P1)-F1	TTGGCCTTGACAACCCACAGAT	WT:7023bp KO:382bp
	R1	T067549(P1)-R1	GGATCATCGAATGCAGGAATGG	
PCR②	F2	T067549(P1)-F2	CAGTAAGGACAGGGACTGGCCA	WT:452bp KO:0bp
	R2	T067549(P1)-R2	GCTACCCGAGCCATCAATCAGG	

### 3. Gel Image



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) or 2 $\times$ Phanta Max Master Mix (Vazyme P515)		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 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	20 $\times$
3	65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	15 $\times$
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	
PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	35 $\times$
3	58 $^{\circ}$ C*	30s	
4	72 $^{\circ}$ C	45s*	
5	72 $^{\circ}$ C	5min	
6	10 $^{\circ}$ C	hold	

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