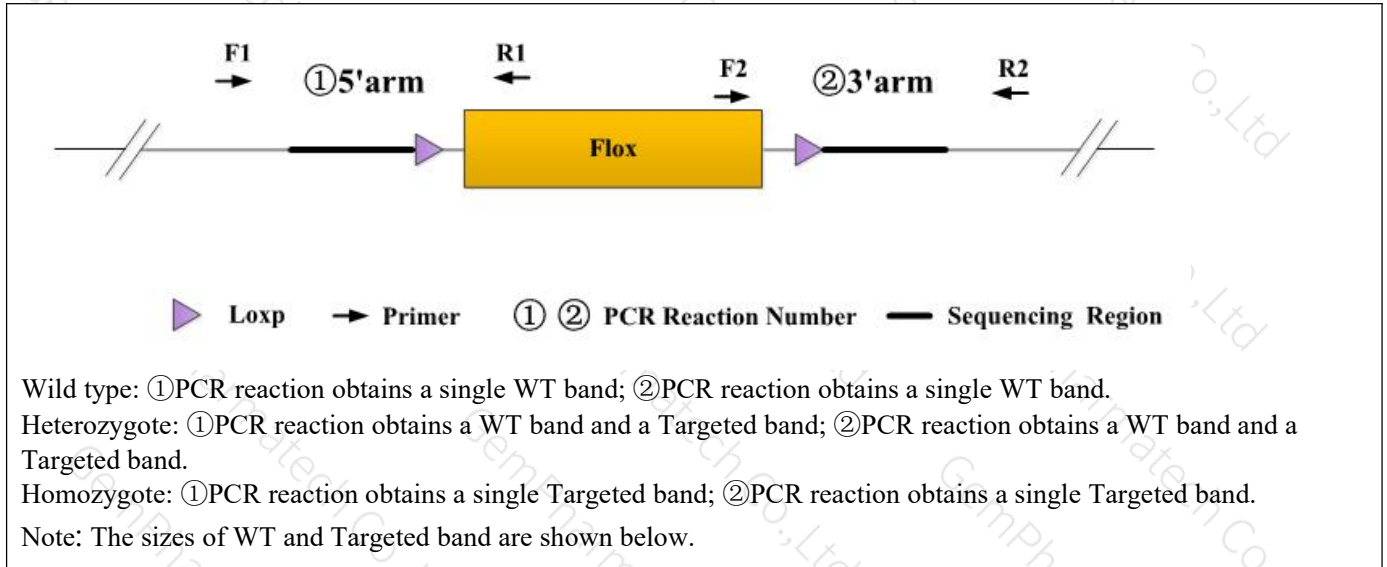


## Genotyping Report

Strain ID	T039483	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	KLHL21		

### 1. Strategy of Genotyping

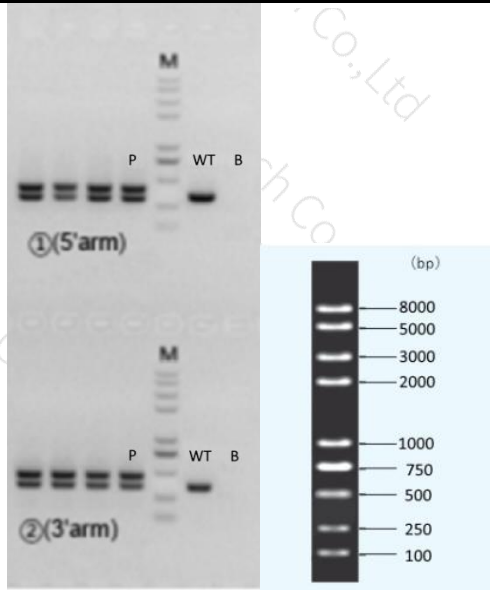


### 2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	T039483(P2)-F1	TAAGACAGGGTTTCTCTGTCTGGA	WT:315bp Targeted:420bp
	R1	T039483(P2)-R1	CAAATCCTAGACTTCCTGGGCACT	
②(3'arm)	F2	T039483(P2)-F2	ATTGGATGCCTATGTCCTGTGTGT	WT:374bp Targeted:480bp
	R2	T039483(P2)-R2	GAAAGTTCAGGGCATTCTCAA	

### 3. Gel Image & Conclusion

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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	20×
2	98°C	30s	
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	
<b>PCR program II the second choice</b>			
<b>Seg.</b>	<b>Temp.</b>	<b>Time</b>	<b>Cycle</b>
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.