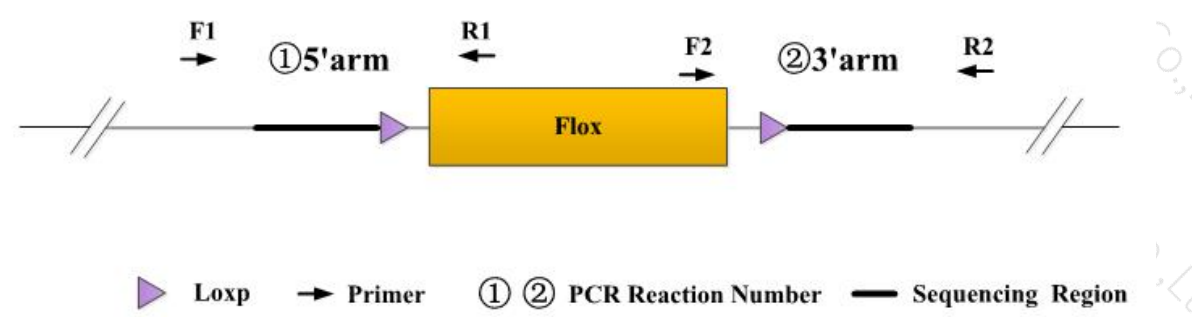


## Genotyping Report

Strain ID	T010125	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Eif3a</i>		

### 1. Strategy of Genotyping



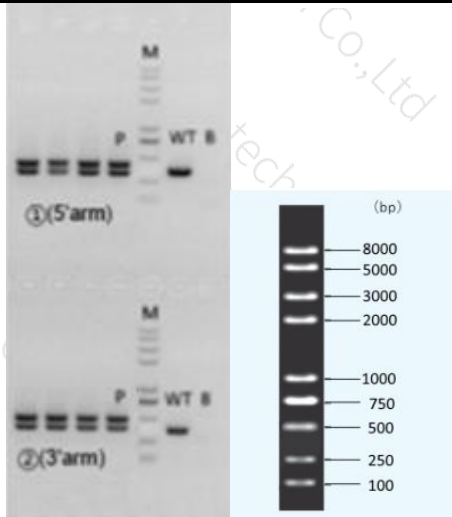
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.  
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.  
 Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T010125-F1	AGGATCTTAAC TTTGTAGCCCTGGA	WT: 320bp Targeted:425bp
	T010125-R1	GATACTGAACCTGCTACCTTATCATGG	
②(3'arm)	T010125-F2	CCGCTGATTACA ACTAAAGACA ACTAGC	WT: 430bp Targeted:536bp
	T010125-R2	CCAATCGATGAGCATTCTTTAAGC	

### 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)	12.5
2	ddH <sub>2</sub> O	9.5
3	Primer A(10pmol/μl)	1
4	Primer B(10pmol/μl)	1
5	Template(≈100ng/μl)	1

##### PCR program ① 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	20×
6	55 °C*	30s	
7	72 °C	45s*	
8	72 °C	5min	

9	10℃	hold	
<b>PCR program ② the second choice</b>			
Seg.	Temp.	Time	Cycle
1	95℃	5min	35×
2	98℃	30s	
3	58℃*	30s	
4	72℃	45s*	
5	72℃	5min	
6	10℃	hold	

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