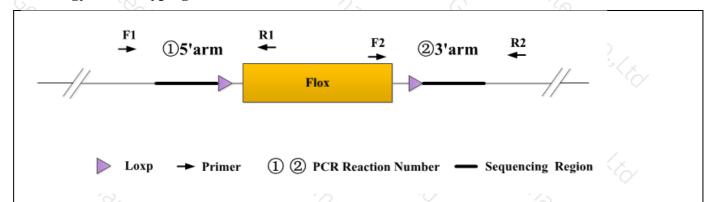
Genotyping Report

Strain ID	T008741	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chao Zhao	Gene Name	-3 </td <td>Hmgb1</td> <th>0)</th>	Hmgb1	0)

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.

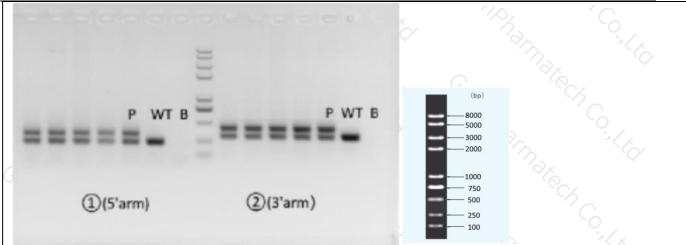
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm) GC:63.8%	T008741(P3A)-F1	AGCCAGCCCCATTTCGAGC	WT: 240bp Targeted:345bp
	T008741(P3A)-R1	GTTTGTCTCCCAAACCGGGCC	
②(3'arm)	T008741(P3)-F2	CACCAAGTATAGCTCTGGTGCAGTG	
	T008741(P3)-R2 TGTACTTAGGGATGATGTGGCCAC		Targeted:392bp

3. Gel Image & Conclusion



Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH2O); 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

			^	
nponent	i G	0	~~~	
reaction	reaction component		Volume (μl)	
2 × Rapid Taq Master Mix (Vaz	2 × Rapid Taq Master Mix (Vazyme P222)		3/2	
ddH2O	~ C	9.5		
Primer A(10pmol/μl)	6	1	3	
Primer B(10pmol/μl)	, 'Y	1	6	
Template(≈100ng/μl)	Template(≈100ng/μl)			
priority selection	1/2 /	26	7	
Temp.	Time	Cycle		
95°C	5min	70/ _{3/}	6.7×	
98℃	30s	20×	(4)	
65℃* (-0.5℃/cycle)	30s	1 CC		
72°C	45s*			
98℃	30s	20×	3/x,	
55℃*	30s	~/ ₁		
72℃	45s*	(9/2)		
72℃	5min	C.	, X	
10℃	hold	~ C/2/	3	
the second choice	7. K	72.	0	
	reactio 2 × Rapid Taq Master Mix (Vaz ddH2O Primer A(10pmol/μl) Primer B(10pmol/μl) Template(≈100ng/μl) priority selection Temp. 95°C 98°C 65°C* (-0.5°C/cycle) 72°C 98°C 55°C* 72°C 10°C	reaction component $2 \times \text{Rapid Taq Master Mix (Vazyme P222)}$ $ddH2O$ Primer A(10pmol/μl) Primer B(10pmol/μl) Template(≈100ng/μl) priority selection Temp. 5min 95 °C 30s $65 ^{\circ}$ C* (-0.5 °C/cycle) 30s $72 ^{\circ}$ C 45s* $98 ^{\circ}$ C 30s $55 ^{\circ}$ C* 30s $72 ^{\circ}$ C 45s* $72 ^{\circ}$ C 5min $10 ^{\circ}$ C hold	reaction component Volume (μ 2 × Rapid Taq Master Mix (Vazyme P222) 12.5 ddH2O 9.5 Primer A(10pmol/μl) 1 primer B(10pmol/μl) 1 Template(≈100ng/μl) 1 priority selection 5min 98 °C 30s 65 °C * (-0.5 °C/cycle) 30s 72 °C 45s* 98 °C 30s 55 °C * 30s 72 °C 45s* 72 °C 5min 10 °C hold	



Seg.	Temp.	Time	Cycle
1 3/7	95℃	5min C	973 3/50
2 (98℃	30s	35×
3	58°C*	30s	3
4	72℃	45s*	3/
5 7 _{0×}	72℃	5min	(2) (A)
6	10℃	hold	~ C

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