

## Genotyping Report

Strain ID	T008741	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chao Zhao	Gene Name	<i>Hmgb1</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.

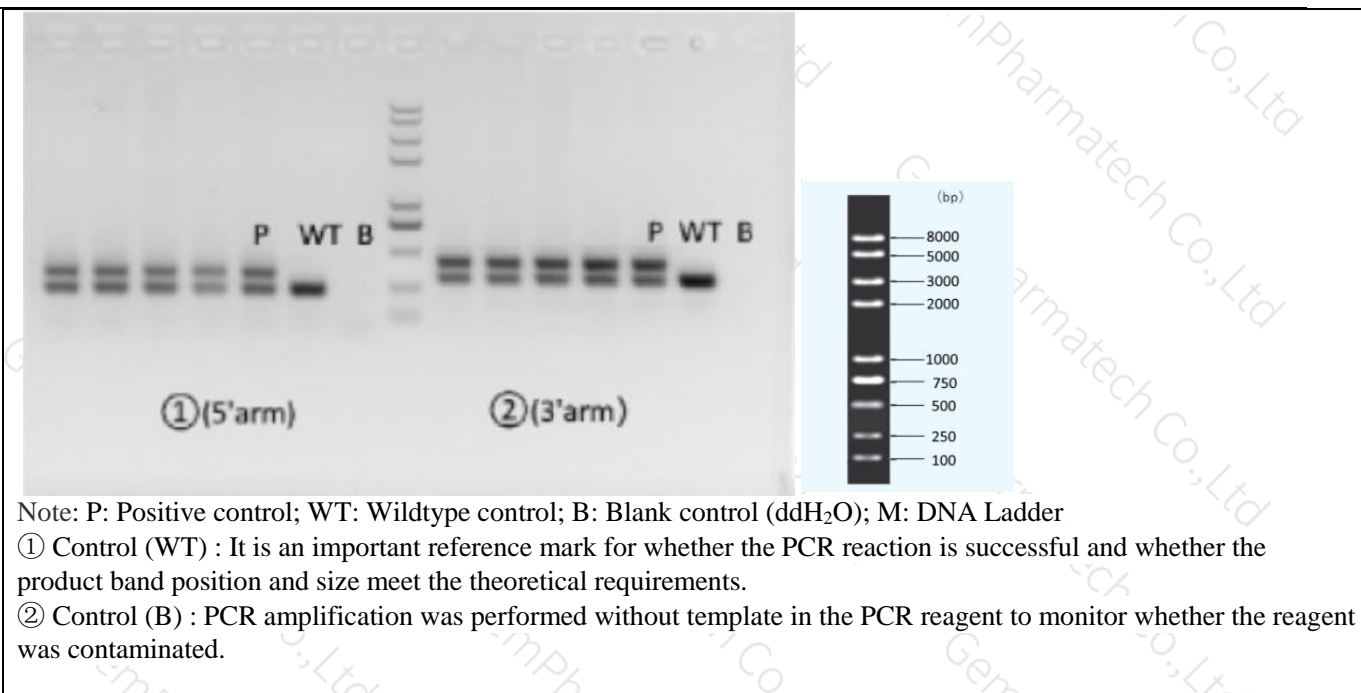
Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm) GC:63.8%	T008741(P3A)-F1	AGCCAGCCCCCATTTTCGAGC	WT: 240bp Targeted:345bp
	T008741(P3A)-R1	GTTTGTCTCCCAAACCGGGCC	
②(3'arm)	T008741(P3)-F2	CACCAAGTATAGCTCTGGTGCAGTG	WT: 291bp Targeted:392bp
	T008741(P3)-R2	TGTACTTAGGGATGATGTGGCCAC	

### 3. Gel Image & Conclusion

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#### 4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Rapid Taq Master Mix (Vazyme P222)		12.5
2	ddH2O		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℃	5min	20×
2	98℃	30s	
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	20×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program ② the second choice			

Seg.	Temp.	Time	Cycle
1	95℃	5min	
2	98℃	30s	35×
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.