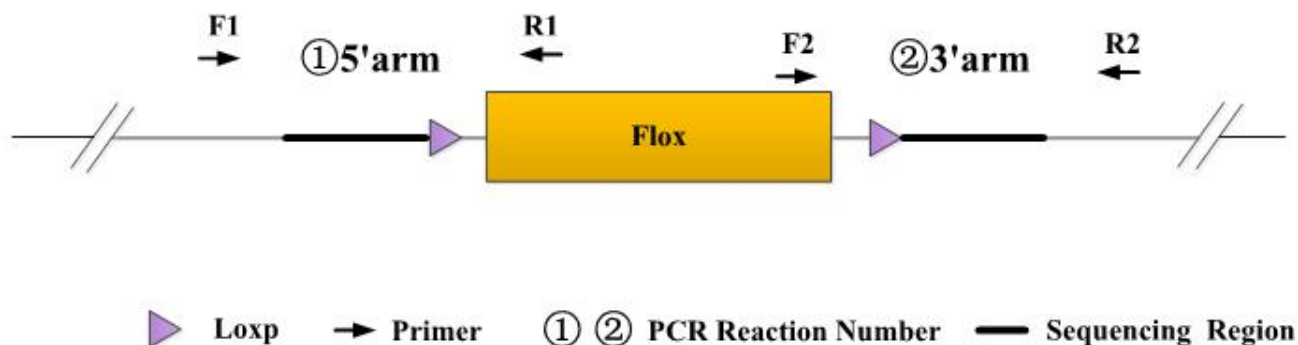


Genotyping Report

Strain ID	T020320	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Cd300a</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.

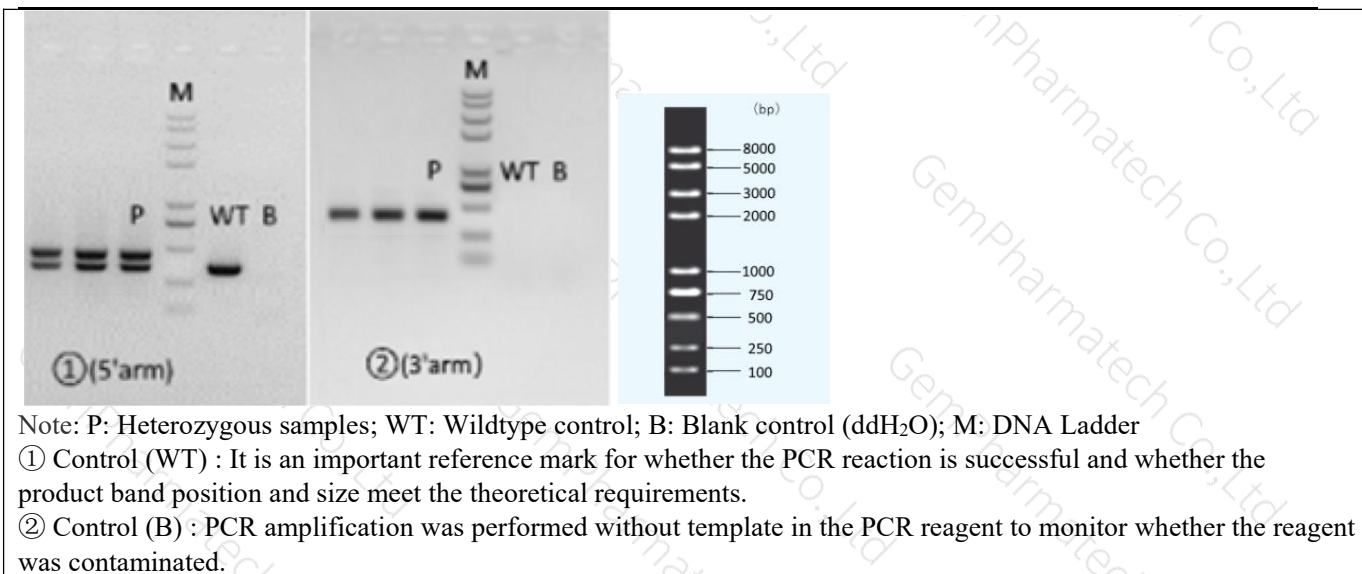
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	JS20614-Cd300a-5wt-tF1	GCCTTGAGGCAGATACTGAGCAA	WT: 322bp Targeted: 426bp
	JS20614-Cd300a-5wt-tR1	GTTTCAATCCAGACATGGCATTGC	
②(3'arm)	NEO-3F	TCTGAGGCGGAAAGAACCAG	WT: 0bp Targeted: 368bp
	JS20614-Cd300a-3wt-tR1	TCTGTGGGTGATGACTTCAGTGAAC	

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(20~80ng/μl)		1
PCR program I 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	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program II 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.