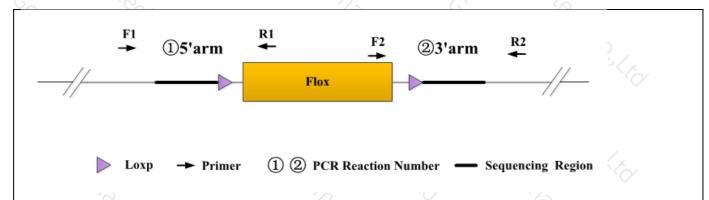
Genotyping Report

Strain ID	T027242	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	3/2	Zxdb	6

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.

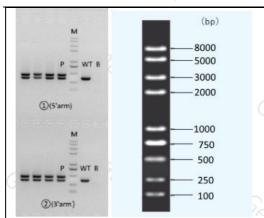
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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

2. Primer Information

			~/	
PCR No.	Primer No.	Sequence	Band Size	
①(5'arm)	T027242-F1 AAACACCCAATGGGGCTGACA		WT: 348bp Targeted:453bp	
	T027242-R1 GTGGACATATGGCTTCTACGCTCTC			
②(3'arm)	T027242-F2	GCTCCTAATCGCTTAACCATCTCTC	WT: 365bp	
	T027242-R2	CTGATAAACACCAGACCCACAAAAG	Targeted:471bp	

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

		72	9/2	
PCR Reaction	Component	7 (S)	· ?>	
Seg.	reactio	reaction component		
1 70	2 × Rapid Taq Master Mix(Va	zyme P222)	12.5	
2	ddH2O), (V	9.5	
3	Primer A(10pmol/μl)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1 7	
4	Primer B(10pmol/μl)	70	1 8	
5	Template(≈100ng/μl)	3/2	701	
PCR program	① priority selection	× ×	79/2 3-4x	
Seg.	Temp.	Time	Cycle	
1 7	95℃	5min	Ch Ch	
2	98℃	30s	20×	
3	65℃* (-0.5℃/cycle)	30s	7	
4	72℃	45s*	19%	
5	98℃	30s	20×	
6	55℃*	30s	G, ,,/x	
7	72 ℃	45s*	70, `¢	
8	72℃	5min	(D)	
9	10℃	hold	0 73%	
PCR program	② the second choice	6	6/2	
Seg.	Temp.	Time	Cycle	



1	10/12/2	95℃	19hh.	5min		£ '6	
2	· 12.	98℃	9/X	30s		35×	N.
3 (S. 78	58℃*	4	30s	0	770	
4	700/	72℃	G _C	45s*	³ 72,	7°C	
5	72/2	72 ℃	700	5min	2	24 34	/ ×
6	, Jax	10℃	, 9 ¹	hold		(Ma)	0

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