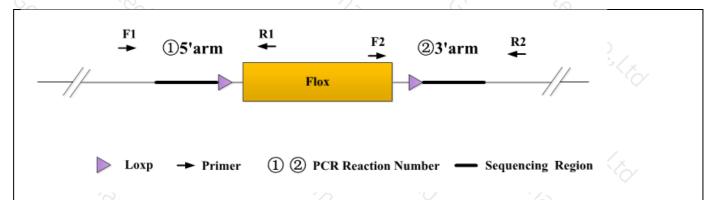
Genotyping Report

Strain ID	T013366	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	3/2	Vav2	G

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

- L			7/1	
PCR No.	Primer No.	Sequence	Band Size	
①(5'arm)	T013366-F1	ATAACCCTGGCTGTCCCG	WT:345bp	
	T013366-R1	TCATTCACATGGCAGCACTT	Targeted:423bp	
②(3'arm)	T013366-F2	CCTTTGGAATGGCTACTGC	WT:290bp	
	T013366-R2	ATCAGAAAGCCCATCCTCA	Targeted:371bp	

3. Gel Image & Conclusion





Note: P: Positive control; WT: Wildype 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

PCR Reaction Comp	onent	2× Co	**C
Seg.	reaction comp	onent	Volume (μl)
1 9/2	2 × Rapid Taq Master Mix (Vazyme P2	12.5	
2 2	ddH2O	34	9.5
3	Primer A(10pmol/μl)	1 %	
4 🕟	Primer B(10pmol/μl)	1	
5	Template(≈100ng/μl)	1 3	
PCR program ① pr	iority selection		**************************************
Seg.	Temp.	Time	Cycle
1 6	95℃	5min	9×
2	98℃	30s	20×
3 8/2	65℃* (-0.5℃/cycle)	30s	19/2 3/
1 0	72 ℃	45s*	Jax (
5	98℃	30s	20×
6	55℃*	30s <	76
7	72℃	45s*	3/2
80.	72℃	5min	79%
9 %	10°C	hold	3
PCR program ② th	e second choice		6
Seg.	Temp.	Time	Cycle
í	95℃	5min	7000 Y
2	98℃	30s	35×
3	58℃*	30s	% 3 <x< td=""></x<>
4	72℃	45s*	· · · · · · · · · · · · · · · · · · ·
5	72℃	5min	9/2
6	10℃	hold	(4)X

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

