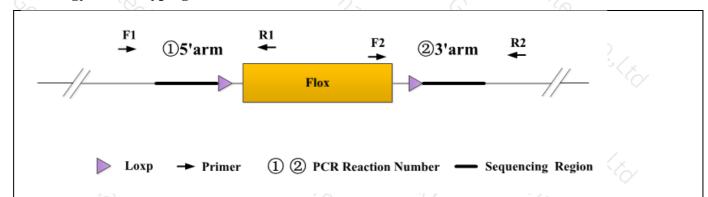
Genotyping Report

Strain ID	T023210	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	3/2	Dnaaf3	C

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.

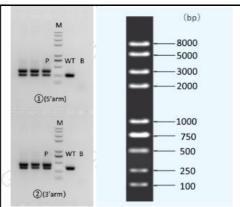
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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

2. Primer Information

/ /			7/1	
PCR No.	Primer No.	Sequence	Band Size	
(7/5)	T023210-F1 AAATCAGGTCTCCAGGAGTGGTG T023210-R1 CCCTGTTTTTCTATTGTGAGGC		WT: 221bp	
①(5'arm)			Targeted:326bp	
	T023210-F2	GTTTCAAAGCGTTCAAGGCCAG	WT: 357bp	
②(3'arm)	T023210-R2	CTTATAGTCCCAGAGATTAAGTCCGG	Targeted:463bp	

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

4. I CK COI		> ` </th <th>25</th>	25
PCR Reaction	Component	19X	4/h
Seg.	reaction	component	Volume (μl)
1	2 × Rapid Taq Master Mix(Vazy	rme P222)	12.5
2	ddH2O		9.5
3	Primer A(10pmol/μl)	7×	17
4	Primer B(10pmol/μl)	30	1 %
5	Template(≈100ng/μl)		1 7
PCR program	① priority selection	· · · · · · · · · · · · · · · · · · ·	72.
Seg.	Temp.	Time	Cycle
100	95℃	5min	2
2	98℃	30s	20×
3	65℃*(-0.5℃/cycle)	30s	75 34x
4	72 ℃	45s*	79%
5	98℃	30s	20×
6	55℃*	30s	G G
7	72°C	45s*	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8	72℃	5min	722
9	10℃	hold	73,
PCR program	② the second choice	~7 _C	G. 100
Seg.	Temp.	Time	Cycle



1	12/2	95℃	(a/2)	5min	70	5 6
2	· 12.	98℃	97.	30s		35×
3 (S	58℃*	5	30s	G.	A COL
4	70/2/	72℃	Go L	45s*	Ϋ́,	7°C
5	79/2	72℃	700	5min	2	24 3/x
6	, Jax	10℃	, 9 ¹ / ₂	hold		(y) (A)

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