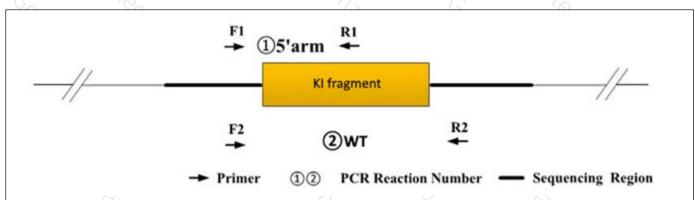
Genotyping Report

Strain ID	T052792	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tianjiao Wang	Gene Name	5.4%	Kdr-iCre	8

1. Strategy of Genotyping



Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.

Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.

Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.

Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the target band, it is likely to produce dominant amplification, the reaction is only used to judge whether there is a WT allele.

2. Primer Information

PCR No.	Primer No.	Sequence		Band Size	
①5'arm	T052792-F1	CATACCGCCTCTGTGACTTCTTTG		WT:0bp	
	T052792-R1 AGCATCTTCCAGGTGTGTTCAG		Targeted:248bp		
②WT	T052792-F2	AAGGAGTCTGTGCCTGAGAACTG	30	WT:287bp Targeted:0bp	
29/2	T052792-R2	TGGCTGAGTCATTTCGGAAGAG		Targeted:00p	

3. Gel Image & Conclusion



Note: P:Heterozygous samples; 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

			<u> </u>	
PCR Reaction Comp	ponent		- 20,	
Seg.	reaction co	Volume (μl)		
1 6	2 × Rapid Taq Master Mix (Vazym	apid Taq Master Mix (Vazyme P222)		
2 70/2	ddH2O	9,/	9.5	
9/2	Primer A(10pmol/μl)	, \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	P ₂	
· _ ?	Primer B(10pmol/μl)	1 7		
5 %	Template(20~80ng/μl)	1		
PCR program I pr	iority selection	3/x	75, C	
Seg.	Temp.	Time	Cycle	
C. 73	95℃	5min	J. J. J. J. C. J.	
· ^>	98℃	30s	20×	
732	65°C* (-0.5°C/cycle)	30s	3 S	
72.	72℃	45s*	7/3, 3/4/	
	98℃	30s	15×	
	55℃*	30s	3	
, G	72°C	45s*	3,	
172	72℃	5min	70/	
	10°C	hold	79/2	
CR program II th	e second choice	(C)	, Jax	
Seg.	Temp.	Time	Cycle	
1/2/2	95℃	5min	7% (S.)	



2	1/2/2	98℃ 🦴	1900	30s	70	35×	(C)
3	9/2	58℃*		30s	· ,		3,4%
4	9/	72 ℃	, C.	45s*			, Ó,
5	S/2 ~	72℃	C.	5min	602	3	
6	7/2	10℃	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	hold	7%		6

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