

		Genotyp	ing Report		Co.
Strain ID	T024666	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Binjie Jiao	Gene Name		Slc22a17	G
Strategy of (	Genotyping	(Na)	3	C Armak	
	F1 → ①5'a	rm <del>▲</del>	F2	23'arm	22
/		<b>_</b> >-	Flox	_ <b>_</b>	_//_

▶ Loxp → Primer ① ② PCR Reaction Number — Sequencing Region

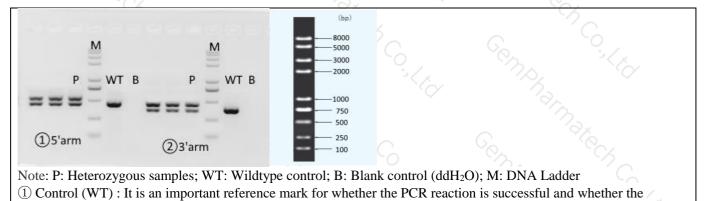
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band. Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.

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.	Primer Name	Sequence	Band Size
(1)(5'arm)	<b>F</b> 1	T024666(P1)-F1	CTGGGCAATCTGAGCAAGACA	WT: 449bp
	R1 🛇	T024666(P1)-R1	CAAGAGTACTGAAGCTATAAAAGATGGG	Targeted: 554bp
	F2	T024666(P1)-F2	GCACCTCCAGACAATTTCTGCTG	WT: 382bp Targeted: 488bp
2(3'arm)	R2	T024666(P1)-R2	TTTACATTCCGTCTCTGACTTGACC	

## 3. Gel Image & Conclusion





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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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as  $GC\% \ge 60\%$  or  $GC\% \le 40\%$ , recommend to use Vazyme P515.)

PCR Reaction Co	omponent			
Seg.		reaction component	Volume (µl)	
1 <sup>73</sup> m	34	2 × Rapid Taq Master Mix(Vazyme P222) or 2 × Phanta Max Master Mix (Vazyme P515)		
2	°°	ddH2O	9.5	
3		Primer A(10pmol/µl)	1	
4		Primer B(10pmol/µl)		
5 6	, , , ,	Template(20~80ng/µl)		
PCR program $ \mathrm{I}^{\mathrm{c}}$	priority selection		12mp	
Seg.	Temp.	Time	Cycle	
	95°C	5min	Charles Charles	
2 72/	98°C	30s	20×	
3	65℃* (-0.5℃/cycle)	30s	The second	
	72℃	45s*	Co YCo	
5 70/	98°C	30s	15×	
5 Pr	55°C*	30s	Dr. Str.	
7	<b>72℃</b>	45s*	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
3 0	<b>72℃</b>	5min		
) ^/	10°C	hold	na C	
PCR program II	the second choice	Contraction of the contraction o	an ite	
Seg.	Temp.	Time	Cycle	
1	95°C	5min	No No	
2 6	98°C	30s	35×	
3 70	58℃*	30s		
1	<b>72℃</b>	45s*	1200	
5	<b>72</b> ℃	5min	n naz	
6	10°C	hold		

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



and amplification enzyme efficiency.

