


Genotyping Report

Strain ID	T018599	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Gabra3</i>		

1. Strategy of Genotyping



▶ 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.	Sequence	Band Size
①(5'arm)	T018599-F1	TTTATTGGGTAGGGCTGCTTTCCA	WT: 379bp Targeted:484bp
	T018599-R1	GTACTCCAAACCTGAACTCTCCCTT	
②(3'arm)	T018599-F2	TACATTGGACCTAGAAAGGGGAAA	WT: 339bp Targeted:440bp
	T018599-R2	TGAACACTTCTCAGTTGCTGTGCTAG	

3. Gel Image & Conclusion

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4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Taq Master Mix , Dye Plus, (Vazyme P112-03)		12.5
2	ddH ₂ O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(≈100ng/μl)		1
PCR program			
Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	20×
3	65°C (-0.5°C/cycle)	30s	
4	72°C	45s	
5	98°C	30s	
6	55°C	30s	20×
7	72°C	45s	
8	72°C	5min	
9	10°C	hold	

