

Genotyping Report

Strain ID	T014961	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ying Chen	Gene Name	Crlf2		

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

PCR No.	Primer No.	Sequence	Band Size
① (5'arm) 备用	T014961-F1A	CGAGCAAGAGCTACCAAGTTGCTT	WT:304bp Targeted: 409bp
	T014961-R1A	AATATGCAAAGAAGGAGCGGCC	
② (3'arm) 备用	T014961-F2A	AATGCTATGCTAATGCGGCCAG	WT:252bp Targeted:358bp
	T014961-R2A	TACTCACCACCCGCAGTTCTGT	

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	ddH2O		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℃	5min	20×
2	98℃	30s	
3	65℃（-0.5℃/cycle）	30s	
4	72℃	45s	
5	98℃	30s	20×
6	55℃	30s	
7	72℃	45s	
8	72℃	5min	
9	10℃	hold	