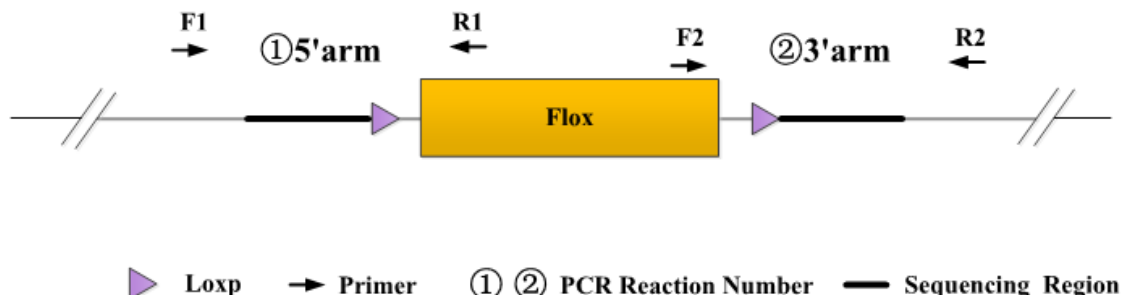


Genotyping Report

Strain ID	T039928	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Ammecr1</i>		

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.

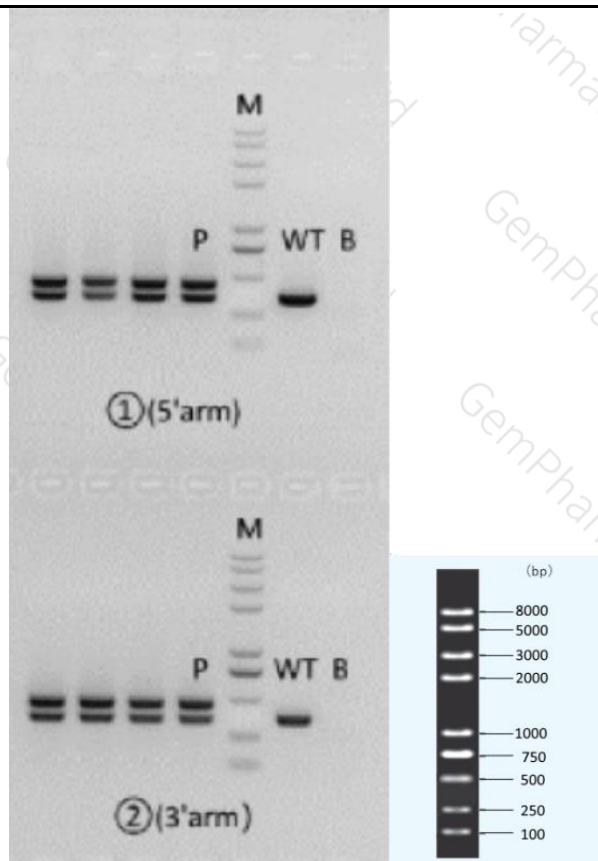
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T039928-F1	GGACGGTACTGTTGATTTAGCTCTTC	WT: 387bp Targeted:492bp
	T039928-R1	AATACAACCTTGCAACGGCTACCAC	
②(3'arm)	T039928-F2	GAAAGAAGTTTGTGACAATGGCAGT	WT: 366bp Targeted:472bp
	T039928-R2	CCCCACACGTTTCTTCATACCTTAT	

3. Gel Image & Conclusion

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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

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Rapid Taq Master Mix (Vazyme P222)		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 ① priority selection			
Seg.	Temp.	Time	Cycle
1	95℃	5min	
2	98℃	30s	20×

3	65°C * (-0.5°C/cycle)	30s	20×
4	72°C	45s*	
5	98°C	30s	
6	55°C *	30s	
7	72°C	45s*	
8	72°C	5min	
9	10°C	hold	
PCR program ② the second choice			
Seg.	Temp.	Time	Cycle
1	95°C	5min	35×
2	98°C	30s	
3	58°C *	30s	
4	72°C	45s*	
5	72°C	5min	
6	10°C	hold	

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