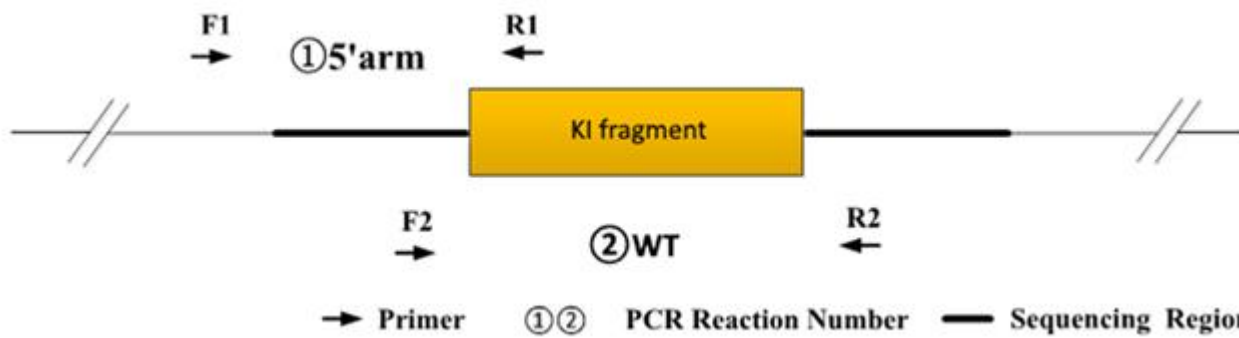


Genotyping Report

Strain ID	T003856	Strain Type	KI(Cas9)	Genetic Background	DBA/1JGpt
Designer	Dongdong Zhang	Gene Name	CAG-tdTomato		

1. Strategy of Genotyping



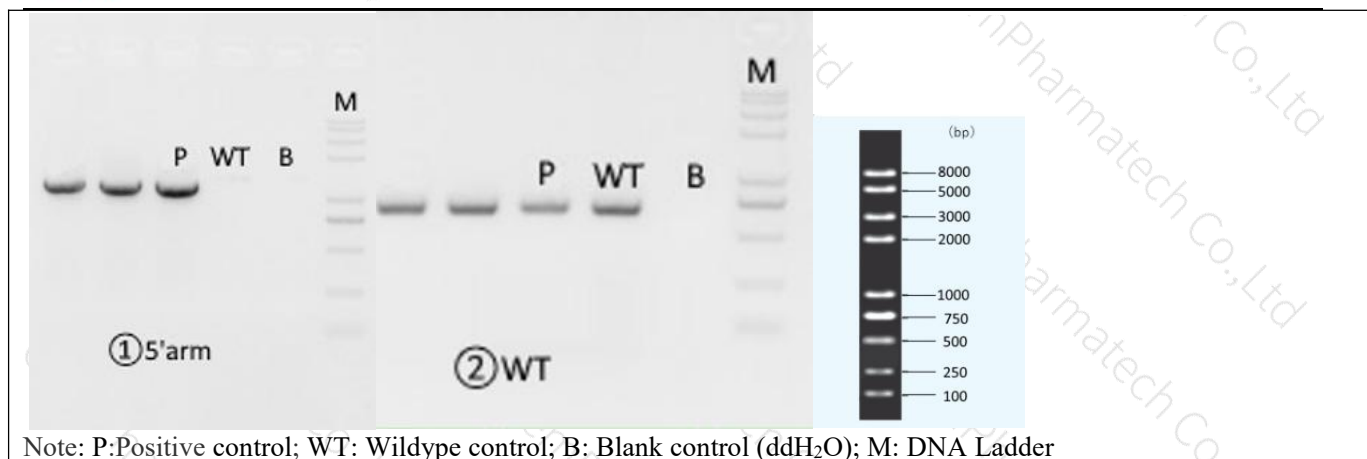
Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
① 5'arm GC%:38%	T003856-F1A	CATTGGAGCACACTATGTGAAGATG	WT:0bp Targeted:1240bp
	T003856-R1A	CGGGCCATTTACCGTAAGTTATG	
② WT	T003856-F2	AACAGTTGAACCTGAGACCCTACT	WT:620bp Targeted:0bp
	T003856-R2	TTTGGAGCAAAACAGTAGTGTAGC	

备注：跨臂短方案，无法更换成初筛方案

3. Gel Image & Conclusion



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	