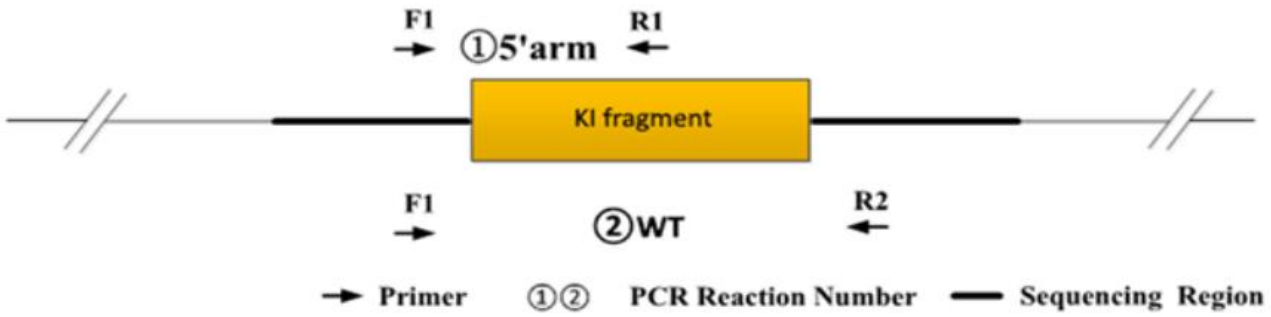
Genotyping Report

Strain ID	T057504	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	*Foxd4-P2A-EGFP*		

1. Strategy of Genotyping

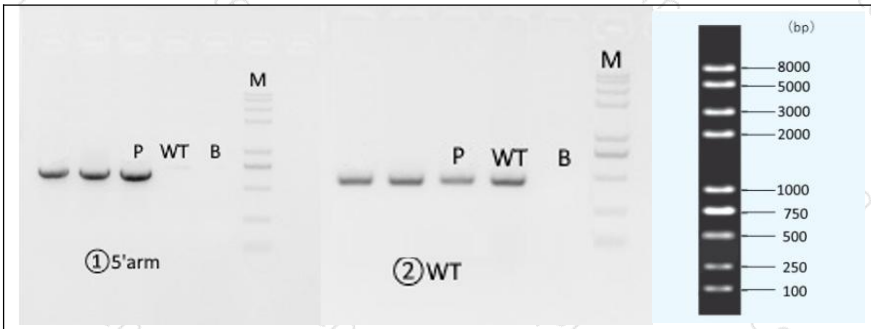


Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the target band, it is likely to produce dominant amplification, the reaction is only used to judge whether there is a WT allele.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①5'arm GC：62.4%	F1	T057504-Foxd4-wt-tF1	CAGCAAGAACAGCATCACTGTGC	WT:0bp Targeted:681bp
	R1	EGFP-5tR1	GCTGTTGTAGTTGTACTCCAGCTTG	
②WT	F1	T057504-Foxd4-wt-tF1	CAGCAAGAACAGCATCACTGTGC	WT:472bp Targeted:1298bp
	R2	T057504-Foxd4-wt-tR1	CACTTTCATAGTCATAGGTCCTCGG	

3. Gel Image & Conclusion



Note: P:Heterozygous samples; WT: Wildtype control; B: Blank control (ddH_2O); 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

Generally recommend to use Vazyme P222;If the sequences contain special structures such as GC% ⩾ 60% or GC% ⩽ 40%,recommend to use Vazyme P515

PCR Reaction Component		
Seg.	**reaction component**	**Volume (μl)**
1	2 × Rapid Taq Master Mix(Vazyme P222) or 2 × Phanta Max Master Mix (Vazyme P515)	12.5
2	ddH2O	9.5
3	Primer A(10pmol/μl)	1
4	Primer B(10pmol/μl)	1
5	Template(20~80ng/μl)	1

PCR program Ⅰ priority selection			
Seg.	**Temp.**	**Time**	**Cycle**
1	95℃	5min	
2	98℃	30s	20×
3	65℃*（-0.5℃/cycle）	30s	
4	72℃	45s*	
5	98℃	30s	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	

PCR program Ⅱ the second choice			
Seg.	**Temp.**	**Time**	**Cycle**
1	95℃	5min	
2	98℃	30s	35×
3	58℃*	30s	
4	72℃	45s*	
5	72℃	5min	
6	10℃	hold	

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