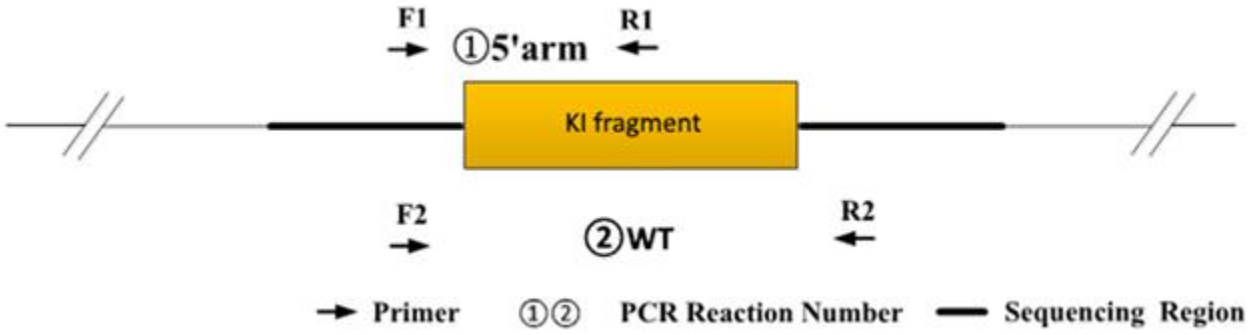
# Genotyping Report

| Strain ID | T055231 | Strain Type | KI(Cas9) | Genetic Background | C57BL/6JGpt |
|---|---|---|---|---|---|
| Designer | Tianjiao Wang | Gene Name | *CAG-loxp-stop-loxP-Cfd-polyA* | | |

## 1. Strategy of Genotyping

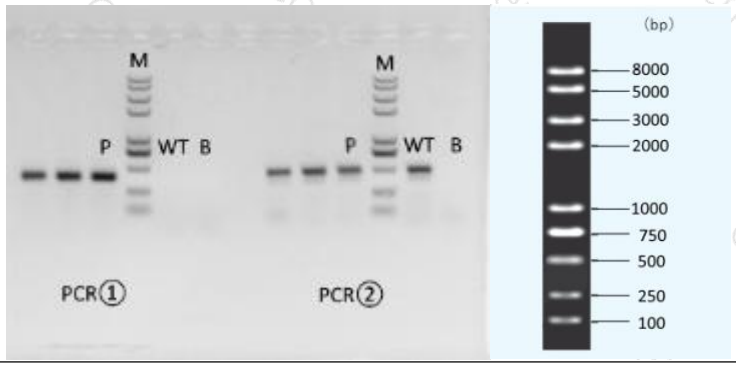



Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. 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. | Sequence | Band Size |
|---|---|---|---|
| ①5'arm | T055231-F1 | CCCAAAGTCGCTCTGAGTTGTTA | WT:0bp<br>Targeted:393bp |
| | T055231-R1 | TCAATGGAAAGTCCCTATTGGCGT | |
| ②WT | T055231-F2 | CCCAAAGTCGCTCTGAGTTGTTA | WT:479bp<br>Targeted:4865bp |
| | T055231-R2 | TCGGGTGAGCATGTCTTTAATCT | |

## 3. Gel Image & Conclusion



Note: P:Positive control; 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

| **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℃*（-0.5℃/cycle） | 30s | |
| 4 | 72℃ | 45s* | |
| 5 | 98℃ | 30s | 20× |
| 6 | 55℃* | 30s | |
| 7 | 72℃ | 45s* | |
| 8 | 72℃ | 5min | |
| 9 | 10℃ | hold | |
| **PCR program ② 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.