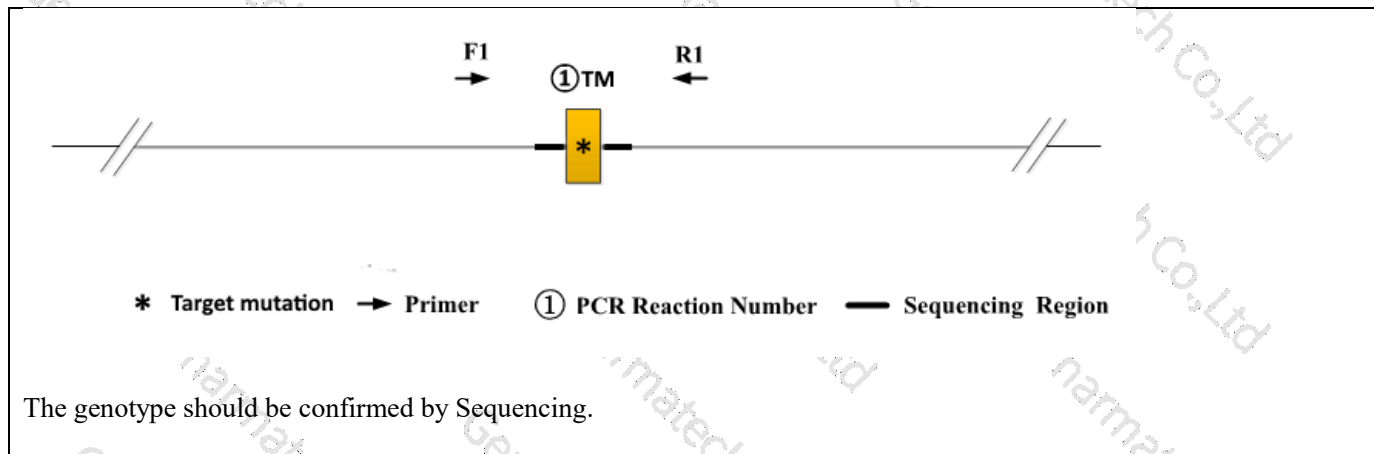


## Genotyping Report

|           |               |             |                   |                    |             |
|-----------|---------------|-------------|-------------------|--------------------|-------------|
| Strain ID | T055207       | Strain Type | KI(Cas9)          | Genetic Background | C57BL/6JGpt |
| Designer  | Tianjiao Wang | Gene Name   | <i>Ptk7-P690R</i> |                    |             |

### 1. Strategy of Genotyping



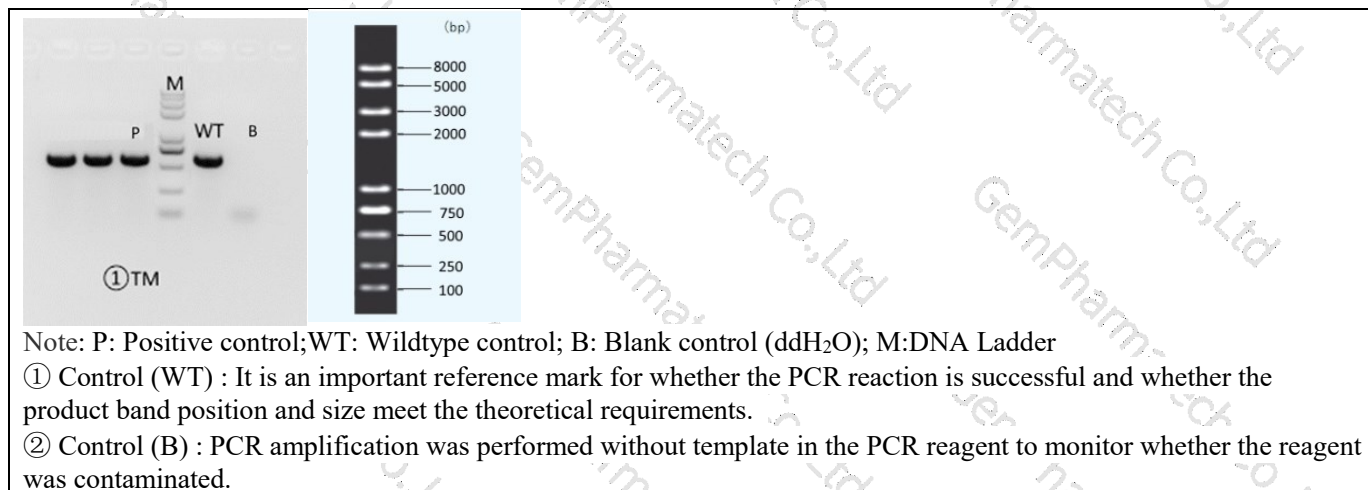
### 2. Primer Information

| PCR No. | Primer No. | Sequence                  | Band Size                  |
|---------|------------|---------------------------|----------------------------|
| ①TM     | T055207-F1 | CTTAGCTGGGATGTTAAGCCAGAAG | WT:538bp<br>Targeted:538bp |
|         | T055207-R1 | AGCAACTCTAGGTTTGCAGCCAG   |                            |

#### Sequencing:

| Primer Name | Sequence                  |     |
|-------------|---------------------------|-----|
| T055207-F1  | CTTAGCTGGGATGTTAAGCCAGAAG | ①TM |

### 3. Gel Image



#### 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 | 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 |             |
| PCR program ② the second choice  |                                        |      |             |
| Seg.                             | Temp.                                  | Time | Cycle       |
| 1                                | 95℃                                    | 5min | 35×         |
| 2                                | 98℃                                    | 30s  |             |
| 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.