

## Genotyping Report

|           |              |             |               |                    |             |
|-----------|--------------|-------------|---------------|--------------------|-------------|
| Strain ID | T028811      | Strain Type | KO(Cas9)      | Genetic Background | C57BL/6JGpt |
| Designer  | Tiantian Sun | Gene Name   | <i>Plxnb1</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 KO band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.

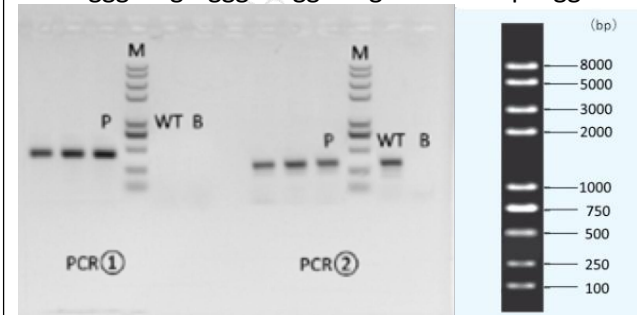
Note: 1)The sizes of WT and Targeted band are shown below.  
2) If the WT band is too large, it may not be possible to obtain a WT band.

### 2. Primer Information

| PCR No. | Primer No. | Primer Name            | Sequence                  | Band Size             |
|---------|------------|------------------------|---------------------------|-----------------------|
| PCR①    | F1         | JS09744-Plxnb1-5wt-tF1 | ACATGGTGGCTCACAACCATCTGT  | WT:6189bp<br>KO:448bp |
|         | R1         | JS09744-Plxnb1-3wt-tR1 | GGTTCTTAGCCAGAAAACAGAAGCC |                       |
| PCR②    | F2         | JS19744-Plxnb1-wt-tF1  | ACAGCTTGTGAAGGTTCTGTGG    | WT:383bp<br>KO:0bp    |
|         | R2         | JS19744-Plxnb1-wt-tR1  | ACCAGCATATAACTGTGACCCTCC  |                       |

### 3. Gel Image

aaaaaggggaacgatgggatttgctcagac---5741bp---ggtaaagagaactcccagaccctctacac



Note: P: Heterozygous samples; 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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC%  $\geq$  60% or GC%  $\leq$  40%, recommend to use Vazyme P515.)

| PCR Reaction Component             |                                                                                        |      |             |
|------------------------------------|----------------------------------------------------------------------------------------|------|-------------|
| Seg.                               | reaction component                                                                     |      | Volume (μl) |
| 1                                  | 2 × Rapid Taq Master Mix(Vazyme P222)<br>or<br>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 | 20×         |
| 2                                  | 98℃                                                                                    | 30s  |             |
| 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 | 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.