

# Adcy9-KO Genotyping Protocol



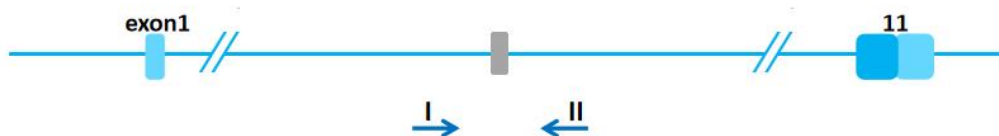
|                         |          |                 |              |
|-------------------------|----------|-----------------|--------------|
| <b>Common Name</b>      | Adcy9-KO | <b>Cat. NO.</b> | NM-KO-201381 |
| <b>Strain of Origin</b> | C57BL/6J | <b>Version</b>  | V1           |

## Genotyping strategy

Wild type allele



Knockout allele

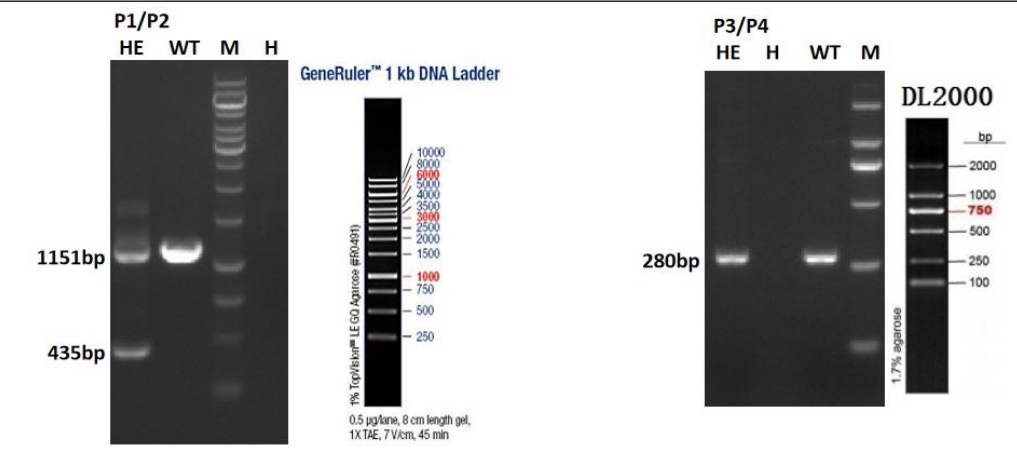


→ : primer location

## Primers

| Primer    | Sequence (5' →3' )   | Primer type |
|-----------|----------------------|-------------|
| <b>P1</b> | GGTCACCCCCACATTGTGAT | Forward     |
| <b>P2</b> | CTCTGTCAGTGTGAGGCCAG | Reverse     |
| <b>P3</b> | GGTCACCCCCACATTGTGAT | Forward     |
| <b>P4</b> | GCAACCTTCGTCTTCCCAGA | Reverse     |

## Expected results

|          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  |  <p><b>P1/P2</b><br/>HE WT M H<br/>1151bp<br/>435bp<br/>GeneRuler™ 1 kb DNA Ladder<br/>10000<br/>8000<br/>6000<br/>5000<br/>4000<br/>3500<br/>3000<br/>2500<br/>2000<br/>1500<br/>1000<br/>750<br/>500<br/>250<br/>0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min</p> <p><b>P3/P4</b><br/>HE H WT M<br/>280bp<br/>DL2000<br/>bp<br/>2000<br/>1000<br/>750<br/>500<br/>250<br/>100<br/>1.7% agarose</p> |
| Genotype | <p>Knockout type: -716 bp</p> <p>Wild type: P1P2 =1151 bp; P3P4 =280 bp</p> <p>Heterozygote: P1P2 =1151 bp and 435 bp; P3P4 =280 bp</p> <p>Homozygote: P1P2 =435 bp</p>                                                                                                                                                                                                                                                                                                                        |

**Note :** In both wild-type and heterozygous mice, whether the P1 and P2 primers can amplify larger bands does not affect the interpretation of the results, because the purpose of designing this pair of primers is to amplify KO band.

## Reaction &Cycling

|                           |                                  |             |
|---------------------------|----------------------------------|-------------|
| PCR<br>Reaction<br>System | Reaction Component               | Volume (µl) |
|                           | ddH2O                            | 8.0         |
|                           | 2 × Rapid Taq Master Mix         | 10.0        |
|                           | P1(10 pmol/µl) or P3(10 pmol/µl) | 0.5         |

|                     |                                                            |      |        |                 |
|---------------------|------------------------------------------------------------|------|--------|-----------------|
|                     | P2(10 pmol/μl) or P4(10 pmol/μl)                           |      |        | 0.5             |
|                     | Genomic DNA                                                |      |        | 1.0             |
|                     | Total                                                      |      |        | 20              |
|                     | 2 × Rapid Taq Master Mix from Vazyme(Code Number: P222-01) |      |        |                 |
| Cycling<br>Reaction | Step                                                       | Temp | Time   | Note            |
|                     | 1                                                          | 95°C | 5 min  |                 |
|                     | 2                                                          | 95°C | 20 sec |                 |
|                     | 3                                                          | 60°C | 20 sec |                 |
|                     | 4                                                          | 72°C | 20 sec | 35 repeats to 2 |
|                     | 5                                                          | 72°C | 5 min  |                 |
|                     | 6                                                          | 12°C | Hold   |                 |