

## RMRC Genotyping Protocol-PCR

|     |           |             |                            |
|-----|-----------|-------------|----------------------------|
| No. | RMRC13390 | Strain Name | FVB/N-Tg(PGK-Cnr1)Wfs/Narl |
|-----|-----------|-------------|----------------------------|

### A. Primer

|   | Primer name         | Sequence             |
|---|---------------------|----------------------|
| 1 | CNR1 Tg mice 1716-F | AGGAGCAAGGACCTGAGACA |
| 2 | CNR1 Tg mice 1974-R | CAACAGATGGCTGGCAACTA |

### B. Reaction Conditions

| Component        | Concentration                                          | Volume      | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|--------------------------------------------------------|-------------|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | --                                                     | 30 $\mu$ l  | 1    | 94              | 5min      | 1                |
| PCR buffer       | 10x                                                    | 5 $\mu$ l   | 2    | 94              | 15sec     | 30               |
| dNTP             | 2 mM                                                   | 8 $\mu$ l   | 3    | 60              | 20sec     |                  |
| Primer 1         | 10 $\mu$ M( $\mu$ mol/ $\mu$ l)                        | 2 $\mu$ l   | 4    | 72              | 20sec     |                  |
| Primer 2         | 10 $\mu$ M( $\mu$ mol/ $\mu$ l)                        | 2 $\mu$ l   | 5    | 72              | 10min     | 1                |
| DMSO             | --                                                     | 1.5 $\mu$ l | 6    | 4               | 60min     | 1                |
| DNA              | < 0.5 $\mu$ g 取 1 $\mu$ g<br>> 0.5 $\mu$ g 取 2 $\mu$ g | $\mu$ l     |      |                 |           |                  |
| Taq              | 5 U/ $\mu$ l                                           | 1.5 $\mu$ l |      |                 |           |                  |
| Total            |                                                        | 50 $\mu$ l  |      |                 |           |                  |

The brand (廠牌) and product name (品名) of Taq : Q5 High-Fidelity PCR kit NewEngland Biolabs

### C. PCR Product

|   | Primer set |   | Product size | Target region |
|---|------------|---|--------------|---------------|
| 1 | F          | R | 270 bp       |               |

D. Gel Photo

