

RBRC 09539

Strain name C57BL/6-Cdc42ep4<tm1.1Ksh>

| Primer | primer_name | primer_seq                  | length |           |
|--------|-------------|-----------------------------|--------|-----------|
| 1      | int1_Fw     | GAAATGACGAAGTTAGGAATTCAGTGC | 27     | RIKEN BRC |
| 2      | ex2_Rv      | CGAGGCTCTTAGCTGCACCTAC      | 22     | RIKEN BRC |
|        |             |                             |        |           |
|        |             |                             |        |           |
|        |             |                             |        |           |
|        |             |                             |        |           |
|        |             |                             |        |           |
|        |             |                             |        |           |
|        |             |                             |        |           |
|        |             |                             |        |           |

**Reaction Components**

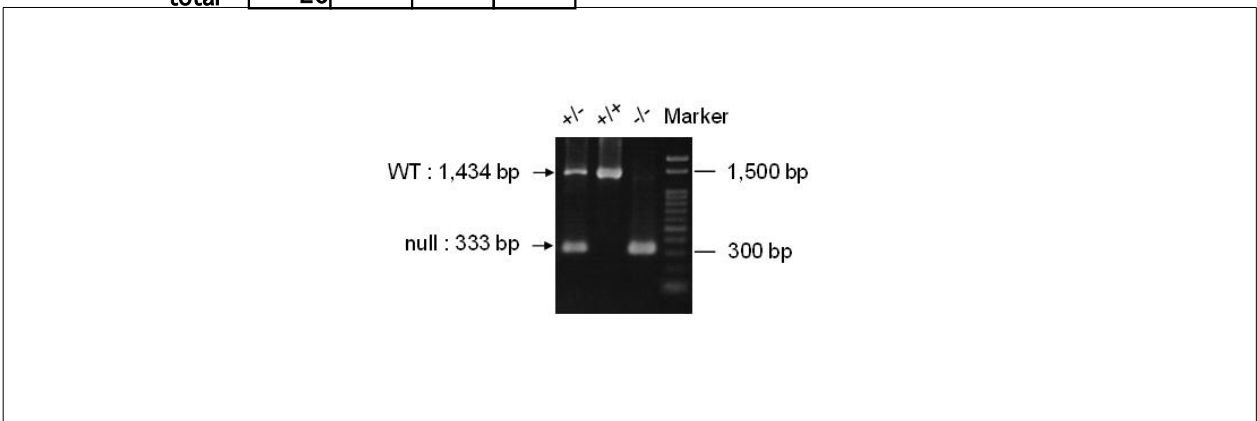
**Enzyme** TOYOBO KOD FX (Code.No : KFX-101)

**Reaction Conditions**

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 120        |                       |
| 2    | 98        | 10*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 68        | 180*       | *Repeat for 30 cycles |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

|              | Conc             | Run 1     | Run 2 | Run 3 | Run 4 |
|--------------|------------------|-----------|-------|-------|-------|
| H2O          |                  | 2.8       |       |       |       |
| PCR buffer   | 2 X              | 10.0      |       |       |       |
| dNTP         | 2.0 mM           | 4.0       |       |       |       |
| primer 1     | 10 pmol/ $\mu$ l | 1.0       |       |       |       |
| primer 2     | 10 pmol/ $\mu$ l | 1.0       |       |       |       |
| DNA          |                  | 1.0       |       |       |       |
| Taq          | 1 U/ $\mu$ l     | 0.2       |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
| <b>total</b> |                  | <b>20</b> |       |       |       |

Standard PCR



**Marker**  
Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**  
2% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

| primer_1 | primer_2 | product_size | product |
|----------|----------|--------------|---------|
| primer 1 | primer 2 | 1,434 bp     | WT      |
| primer 1 | primer 2 | 333 bp       | null    |
|          |          |              |         |
|          |          |              |         |
|          |          |              |         |

**Comment** Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.