

**STRAIN: EM09574**

**PRIMER** FRT 24 REV                      **5'** CCTTCCTCCTACATAGTTGGCAGTG **3'**

**PRIMER** chga for                              **5'** CCTTGGAGAACTACCTTACGAGTG **3'**

**PRIMER** FRT 24 REV                      15pm / total volume 30ul                      Tm: 72°C

**PRIMER** chga for                              15pm / total volume 30ul                      Tm: 72°C

**PCR MIX**

DNTPS (2mM)                                      3ul

PCR BUFFER 10x                                      3ul

**PRIMER** FRT 24 REV                      (0.5pm/ul)

**PRIMER** chga for                              (0.5pm/ul)

H2O

TAQ POLYMERASE (5U/ul)                      0.3ul

DNA    2ul

**TOTAL**    30ul

**PCR PROGRAM:**

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 58°C x 0'30''
- 4) 72°C x 1'00''
- 5) step 2 to step 5 x 50 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

**MUTATION:** 208 bp