

**STRAIN: EM08963 (Entpd5 tm1)**

**PRIMER FRT24 REV**                      **5'-CCT TCC TCC TAC ATA GTT GGC AGT-3'**

**PRIMER ENTP5GBK FOR**            **5'- CTCCTTCCTAATGCTGCAGCAAAG -3'**

PRIMER FRT24 REV                      15pm / total volume 30ul                      Tm: 72°C

PRIMER ENTP5GBK FOR                15pm / total volume 30ul                      Tm: 72°C

**PCR MIX**

DNTPS (2mM)                              3ul

PCR BUFFER 10x                            3ul

PRIMER FRT24 REV                        (0.5pm/ul)

PRIMER ENTP5GBK 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:** 198 bp