

**STRAIN: EM01645 (Br1F) MIX A**

**PRIMER INT 4 F 5'-TAT/CAC/CAC/TGA/ATC/TCT/ACC/G -3'**

**PRIMER INT 4 REV 5'-GAC/CTC/AAA/CTC/TGA/GAT/CCA/C -3'**

PRIMER INT 4 F 15pm / total volume 30ul Tm: 64°C

PRIMER INT 4 REV 15pm / total volume 30ul Tm: 66°C

**PCR MIX**

DNTPS (2mM) 3ul

PCR BUFFER 10x 3ul

PRIMER INT 4 F (0.5pm/ul)

PRIMER INT 4 REV (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) 62°C x 0'45''
- 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: 550 BP**

**WILD TYPE: 400 BP**

**STRAIN: EM:01645 (Br1F) MIX B**

**PRIMER INT 13 F 5'-TAT/TCT/TAC/TTC/GTG/GCA/CAT/C-3'**

**PRIMER INT 13 REV 5'-TCC/ATA/GCA/TCT/CCT/TCT/AAA/C-3'**

PRIMER INT 13 F 15pm / total volume 30ul Tm: 62°C

PRIMER INT 13 REV 15pm / total volume 30ul Tm: 62°C

**PCR MIX**

DNTPS (2mM) 3ul

PCR BUFFER 10x 3ul

PRIMER INT 13 F (0.5pm/ul)

PRIMER INT 13 REV (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) 60°C x 0'45''
- 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: 600 BP WILD TYPE: 520 BP**