

STRAIN: EM02203 (ufd11) Mix A

PRIMER UFD TAR2R 5'-TGG-GCA-ATT-GTT-TAA-TCT-TCC -3'

PRIMER UFD TAR2F 5'-TCT-TTG-TCA-GCA-GTT-CCC-TTT -3'

PRIMER UFD TAR2R 15pm / total volume 30ul Tm: 58°C

PRIMER UFD TAR2F 15pm / total volume 30ul Tm: 60°C

PCR MIX

DNTPS (2mM) 3ul

PCR BUFFER 10x 3ul

PRIMER UFD TAR2R (0.5pm/ul)

PRIMER UFD TAR2F (0.5pm/ul)

H₂O

TAQ POLYMERASE (5U/ul) 0.2ul

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 1'30''
- 5) step 2 to step 5 x 45 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

MUTATION: 290 BP

STRAIN: EM02203 (ufd11) Mix B

PRIMER UFD TAR2R 5'-TGG-GCA-ATT-GTT-TAA-TCT-TCC -3'

PRIMER UFD WT3 5'-CAG-AGT-TCT-GAC-TTC-TGC-ACA-CTA-A-3'

PRIMER UFD TAR2R 15pm / total volume 30ul Tm: 58°C

PRIMER UFD WT3 15pm / total volume 30ul Tm: 72°C

PCR MIX

DNTPS (2mM) 3ul

PCR BUFFER 10x 3ul

PRIMER UFD TAR2R (0.5pm/ul)

PRIMER UFD WT3 (0.5pm/ul)

H₂O

TAQ POLYMERASE (5U/ul) 0.2ul

DNA 2ul

TOTAL 30ul

PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 56°C x 0'30''
- 4) 72°C x 1'30''
- 5) step 2 to step 5 x 45 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

WILD TYPE:150 BP