

**STRAIN: EM00404**

**TET RFORW (300)            5'- CCA/TGT/CTA/GAC/TGG/ACA/AGA-3'**  
**REVREV(300)                5'- CTC/CAG/GCC/ACA/TAT/GAT/TAG-3'**

TET RFORW (300)            15pm / total volume 30ul            Tm: 62°C  
REVREV(300)                15pm / total volume 30ul            Tm: 62°C

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
TET RFORW (300) (0,5pm/ul)	
REVREV(300) (0,5pm/ul)	
H2O	
TAQ POLYMERASE (5U/ul)	0.16ul
DNA	1ul
<b>TOTAL</b>	<b>30ul</b>

**PCR PROGRAM:**

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 60°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: 600 BP**