

STRAIN: EM00002 A (129TgH (GS-T1) Brl)

MIX A

PRIMER T1/ST2 5'-AAA/ACT/AAA/ATT/CTA/TGA/TGG/GCG-3'

PRIMER T1/ST2 5'-GGA/TGT/GCT/GCA/AGG/CG-3'

PRIMER T1/ST2 15pm / total volume 30ul Tm: 64°C

PRIMER T1/ST2 15pm / total volume 30ul Tm: 56°C

PCR MIX

dNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
PRIMER T1/ST2 (0.5pm/ul)	
PRIMER T1/ST2 (0.5pm/ul)	
H2O	
TAQ POLYMERASE (5U/ul)	0.16ul
DNA (200ng/ul)	1ul
TOTAL	30ul

PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 54°C x 0'30''
- 4) 72°C x 2'30''
- 5) step 2 to step 5 x 40 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

MUTATION: 1300 BP

STRAIN: EM00002 B (129TgH (GS-T1) Brl)

MIX B

PRIMER T1/ST2 5'-AAA/ACT/AAA/ATT/CTA/TGA/TGG/GCG-3'

PRIMER T1/ST2 5'-GGA/AGT/GTC/AAT/TGC/CA-3'

PRIMER T1/ST2 15pm / total volume 30ul Tm: 64°C

PRIMER T1/ST2 15pm / total volume 30ul Tm: 50°C

PCR MIX

dNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
PRIMER T1/ST2 (0.5pm/ul)	
PRIMER T1/ST2 (0.5pm/ul)	
H2O	
TAQ POLYMERASE (5U/ul)	0.16ul
DNA (200ng/ul)	1ul
TOTAL	30ul

PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 48°C x 0'30''
- 4) 72°C x 3'00''
- 5) step 2 to step 5 x 40 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

WILD TYPE: 1100 Bp

NOTE: ASPECIFIC BAND OFTEN SEEN AT 350 Bp