

The ENZYME Company

**PCR-buffers:**

- 1) 10x Y-buffer  
 200mM Tris-HCl, pH8.5 at 25°C  
 160mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
 20mM MgCl<sub>2</sub>
- 2) 10x S-buffer  
 100mM Tris-HCl, pH8.8, at 25°C  
 500mM KCl  
 15mM MgCl<sub>2</sub>

All PCR reactions were performed on the Eppendorf Mastercycler gradient machine at the block control option.

**Tests with human genomic DNA**

Primers:

TNF1 5'-GGTTTCGAAGTGGTGGTCTTG

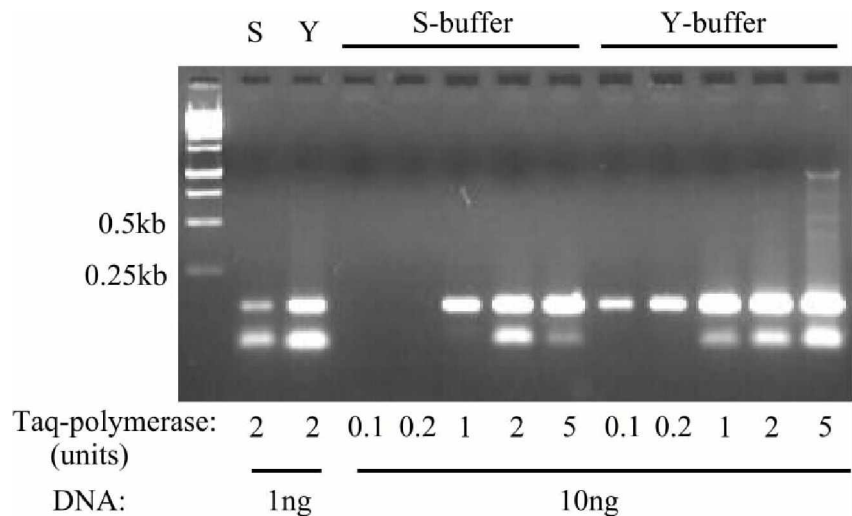
TNF2 5'-CCTGCCCAATCCCTTTATT

**PCR conditions**

- x µl DNA (human)
- 2µl dNTP's (10mM)
- 1µl primer TNF1 (100µM)
- 1µl primer TNF2 (100µM)
- 5µl 10x reaction buffer Y or S
- x µl Taq-polymerase (5u/µl)
- H<sub>2</sub>O up to 50µl

**PCR program**

- 5 min 94°C
- 30 sec 94°C
- 30 sec 55°C
- 30 sec 72°C
- (35 cycles)
- 5 min 72°C
- hold at 10°C



**Tests with lambda DNA**

Primers:

The ENZYME Company

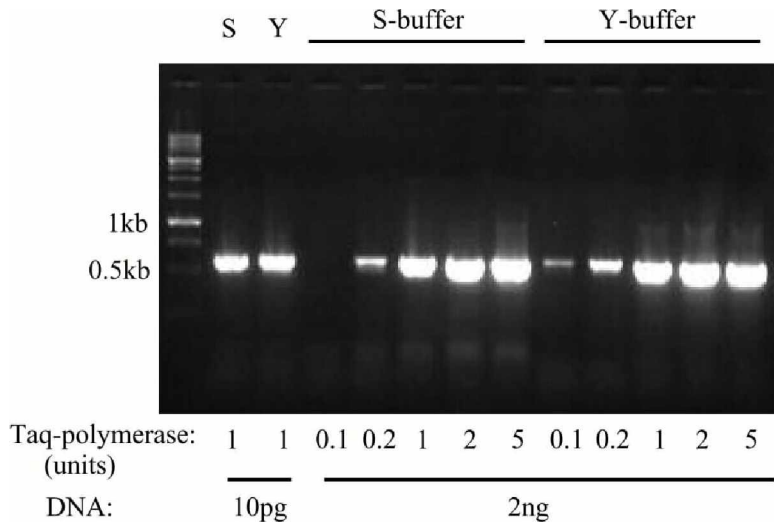
1 5'-CGTATCACCGCACCTGACTG  
 2 5'-CTGATGAGTTCGTGTCCGTACAACCTGGGGTAATC

**PCR conditions**

x µl DNA (lambda)  
 2µl dNTP's (10mM)  
 0.4µl primer 1 (100µM)  
 0.4µl primer 2 (100µM)  
 5µl 10x reaction buffer Y or S  
 x µl Taq-polymerase (5u/µl)  
 H<sub>2</sub>O up to 50µl

**PCR program**

5 min 94°C  
  
 30 sec 94°C  
 30 sec 60°C  
 30 sec 72°C  
 (30 cycles)  
  
 5 min 72°C  
 hold at 10°C



**Shipping and storage test**

In this test we have used vole (*Microtus arvalis*) genomic DNA and primers which amplify 760bp region of the Xist gene.

Primers:

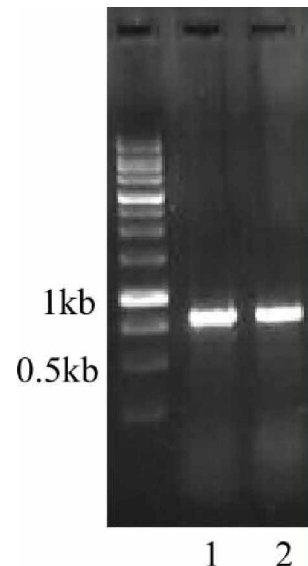
Tsx10 5'-AAGCAGGTATCCATTACC  
 Msx7 5'-TATGTGGCCTTTCCTATAAGC

**PCR conditions**

1µl DNA (10ng/µl)  
 1µl dNTP's (10mM)  
 1µl primer Tsx10 (20µM)  
 1µl primer Msx7 (20µM)  
 2.5 10x Y-buffer  
 1.0µl MgCl<sub>2</sub> (25mM)  
 0.25µl Taq-polymerase (5u/µl)  
 H<sub>2</sub>O up to 25µl

**PCR program**

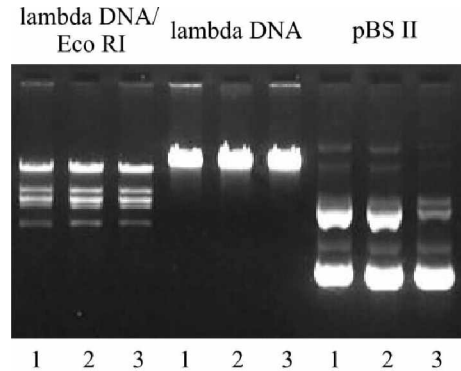
2 min 94°C  
  
 10 sec 94°C  
 20 sec 55°C (1°/sec)  
 1 min 30 sec 72°C (0.5°/sec)  
  
 (30 cycles)  
  
 5 min 72°C



1 – Stored at -20°C  
 2 –Stored 4 days at room temperature

**Endonucleases contamination tests:**

- I. Incubation of 1µg of lambda DNA EcoRI hydrolysate in 1x reaction buffer (Y) with 20 units of Taq-polymerase for 16h at 65°C in 50µl volume.
- II. Incubation of 1µg of lambda DNA in 1x reaction buffer (Y) with 20 units of Taq-polymerase for 16h at 65°C in 50µl volume.
- III. Incubation of 1µg of supercoiled pBS II(SK) DNA in 1x reaction buffer (Y) with 32 units of Taq-polymerase for 16h at 65°C in 50µl volume.



1 - incubation at 65 C with Taq-pol.  
 2 - incubation at 65 C without Taq-pol.  
 3 - without incubation (stored at -20 C)

**Test to minimal amount of template on vole (*Microtus arvalis*) genomic DNA.**

Primers:

Msx7 5'- TATGTGGCCTTTCCTATAAGC

Tsx10 5'- AAGCAGGTATCCATTACC

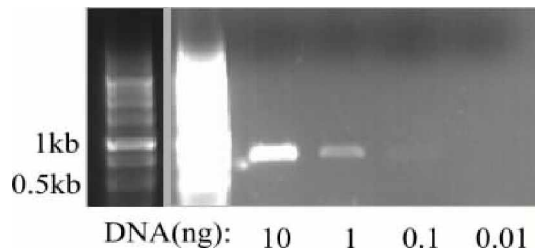
PCR product 803bp

**PCR conditions**

- x µl DNA (vole)
- 1µl dNTP's (8mM)
- 0.5µl primer Msx7 (20µM)
- 0.5µl primer Tsx10 (20µM)
- 2.5µl 10x reaction buffer Y
- 1.2µl MgCl<sub>2</sub> (25mM)
- 0.25µl Taq-polymerase (5u/µl)
- H<sub>2</sub>O up to 25µl

**PCR program**

- 2 min 94°C
- 10 sec 94°C
- 20 sec 55°C (1°C/sec)
- 90 sec 72°C (0.5°C/sec)
- (30 cycles)
- 5 min 72°C
- hold at 10°C



**E.coli DNA contamination test.**

In this test we have used primers from highly conserved region of the bacterial 16S rRNA gene. PCR product: 240 bp.

Primers:

UB-1 5'-GGAGGAAGGTGGGGATGACG

UB-2 5'-ATGGTGTGACGGGCGGTGTG

**PCR conditions**

1 or 0µl E.coli DNA (10ng/µl)

1µl dNTP's (10mM)

1µl primer UB-1 (20µM)

1µl primer UB-2 (20µM)

2.5 10x Y-buffer

0.5µl MgCl<sub>2</sub> (25mM)

0.25 or 0.5 µl Taq-polymerase (5u/µl)

H<sub>2</sub>O up to 25µl

**PCR program**

2 min 95°C

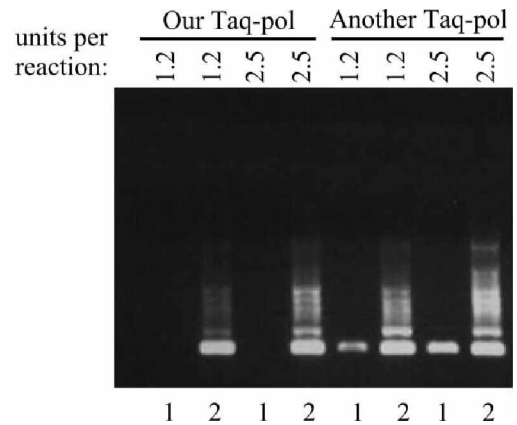
15 sec 94°C

20 sec 53°C

1 min 72°C

(30 cycles)

5 min 72°C



1 – without adding E.coli DNA (Test)  
2 – with adding E.coli DNA (control)