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M13/pUC Forward Amplification Primers

Cat. No. 18431-015 **Size: 3.5 µg (500 pmol)**
Conc.: 70 µg/ml (10 µM) **Store at -20°C.**

Description:
The M13/pUC Forward Amplification Primer (Figure 1) may be used in conjunction with the M13/pUC Reverse Amplification Primer (Cat. No. 18432-013) for rapid detection and size characterization of inserts cloned into any vector containing *lacZ* (α-peptide) sequences, including inserts cloned into the pAMP vectors (1,2). The two primers are used together in an amplification reaction with *Taq* DNA Polymerase (3,4). Additionally, each primer may be used individually for strand-specific probes (5).

5´ CCC AGT CAC GAC GTT GTA AAA CG 3´

Figure 1. Sequence of the M13/pUC Forward Amplification Primer.

It is recommended that 10 pmol (70 ng) of the M13/pUC Forward Amplification Primer be used in conjunction with 10 pmol (70 ng) of the M13/pUC Reverse Amplification Primer in a standard 50 µl amplification reaction. Based on this recommendation, sufficient primer is supplied for a minimum of 50 amplification reactions.

Storage Buffer:
10 mM Tris-HCl (pH 7.5)
1 mM EDTA

Doc. Rev.: 012802

Protocols:

Insert Detection by Amplification: Inserts cloned into vectors containing *lac*Z sequences (such as pUC vectors, M13 vectors, pSPORT1, and pAMP vectors) may be screened on the basis of size by amplifying the region which lies between the forward and reverse primers on either side of the multiple cloning region, according to the following protocol:

1. To a 0.5-ml microcentrifuge tube, add the following:

   sterile water.....38 µl
   10X amplification buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl] .....5 µl
   $MgCl_2$ (50 mM).....1.5 µl
   10 mM dNTP Mix (10 mM each dNTP) .....1 µl
   M13/pUC Forward Amplification Primer (10 µM) .....1 µl
   M13/pUC Reverse Amplification Primer (10 µM).....1 µl
   Taq DNA Polymerase (5 U/µl; Perkin-Elmer Corporation) .....0.5 µl

2. Add 2 µl of the plasmid of interest (~10^6 copies or 1-10 pg), and amplify using a standard thermocycling profile for 20-30 cycles.
3. Analyze the product by agarose gel analysis followed by ethidium bromide staining and UV fluorescence.

Colony Lysis and Insert Detection: Plasmids containing insert may be detected directly from bacterial colonies on a plate using a colony lysis method followed by amplification (5).

1. Scrape a small portion of a colony from a plate using a sterile pipette tip or toothpick. Transfer the colony to 25 µl of 1X amplification buffer [20 mM Tris-HCl (pH 8.4), 50 mM KCl]. Vortex, and centrifuge briefly.
2. Add one drop of sterile mineral oil.

3. Incubate at 100°C for 10 min using a thermal temperature controller or boiling water bath. Place the reaction tube on ice.
4. Add the remaining components for amplification. Amplify and analyze products according to the preceding protocol.

Probe Preparation by Runoff Synthesis: Strand-specific probes of high specific activity and discrete size may be prepared using a single primer against template DNA (6). DNA of interest is restricted downstream of a primer binding site. The region between the restriction site and primer binding site becomes labeled. Moreover, the method may be employed in labeling with modified nucleotides for nonradioactive detection of nucleic acids. The following protocol may be employed:

1. To a 0.5-ml microcentrifuge tube, add the following:

   sterile water.....31.5 µl
   10X amplification buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl)] .....5 µl
   $MgCl_2$ (50 mM).....1.5 µl
   10 mM dNTP Mix (10 mM each dNTP, except labeled dNTP) .....1 µl
   0.5 mM dNTP ("cold" dNTP corresponding to labeled dNTP) .....1 µl
   Radiolabeled dNTP (≥ 3000 Ci/mmol; 10 µCi/µl) .....2.5 µl
   M13/pUC Forward Amplification Primer (10 µM) .....5 µl
   Taq DNA Polymerase (5 U/µl; Perkin-Elmer Corporation) .....0.5 µl

2. Add 2 µl of template DNA (~1 pmol or 50-100 ng). This mass assumes that the sequence to be amplified is contained within a vector which is 2-4 kb in length. The vector should be linearized appropriately prior to probe preparation.
3. Perform 30-40 repeated cycles of primer extension using a standard thermal profile including denaturation, annealing and extension steps.

Quality Control:
The M13/pUC Forward Amplification Primer is analyzed by 5´-^{32}P end-labeling followed by polyacrylamide gel electrophoresis, and is demonstrated to be functional in standard amplification reactions.

References:

1. Buchman, G.W., Schuster, D.M. and Rashtchian, A. (1992) *Focus® 14*, 41.
2. Rashtchian, A., Buchman, G.W., Schuster, D.M. and Berninger, M. (1992) *Anal. Biochem., 206*, 91.
3. Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. (1985), *Science 230*, 1350.
4. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. (1988) *Science 239*, 487.
5. Young, A. and Blakesley, R (1991) *Focus® 13*, 137.
6. Sturzl, M. and Roth, W.K. (1990) *Anal. Biochem. 185*, 164.