Generation of nonradioactive bromodeoxyuridine labeled DNA probes by polymerase chain reaction

S.Tabibzadeh, U.G.Bhat and X.Sun

Department of Pathology, City Hospital Center at Elmhurst, Elmhurst, NY 11373 and the Mount Sinai School of Medicine, New York, NY 10029, USA

Submitted April 9, 1991

The following report describes a method for generating a nonradioactive probe. This was achieved by substituting the dTTP with BrdUTP during polymerase chain reaction (PCR). Hybridization of the PCR amplified bromodeoxyuridine (BrdU) labeled probe to DNA sequences could be detected using an immunoenzymatic procedure.

Methods: Preparation of a probe to human β actin was used as a model. The BamHI fragment of pHF β A-1 cDNA of human β actin (received from Dr. L.Kedes, Dept of Biochemistry, Stanford University School of Medicine, Palo Alto, CA) was used for the preparation of probe by PCR. The sequences of the 5' and 3' primers of human β actin (Clontech, Palo Alto, CA) were respectively: ATGGATGATGATATCGCCGCG;CTAG-AAGCATTTGCGGTGGACGATGGAGGGGCC. These sequences flank a region in cDNA consisting of 1126 base pairs. Briefly, PCR was carried out in 100 μ L volume by addition of the amplitaq (2.5 U), dNTP (200 μ M), primers (1 μ M), 10 μ L reaction buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl₂, 0.01% gelatin), and water. When preparing the BrdU labeled probe, the same reaction mixture was used with the exception that dTTP was replaced by an equimolar concentration of BrdUTP. The thirty cycle amplification profile consisted of denaturation at 95°C for one minute, annealing at 55°C for two minutes, extension at 72°C for two minutes with a final extension at 72°C for 5 minutes. Southern blotting and the immunostaining were performed as described (1, 2). Briefly, immunostaining consisted of incubation with 2% blotto (2hr, 37°C), followed by incubation with a monoclonal antibody to BrdU (Sera lab, England) (2 μ g/ml, 1 hr, 37°C), biotinylated secondary antibody (1 hr, 37°C) and avidin-biotin-peroxidase complex (1 hr, 37°C) and development in diaminobenzidine (10 min, 25°C). Each incubation was followed by a five minute washing in 0.5% blotto.

Comments: Taq polymerase incorporated BrdUTP as efficiently as dTTP into the newly synthesized strands of amplified DNA without a need to alter the concentration of any of the reagents in the PCR mixture or the conditions of the amplification (Figure 1). The incorporation of the biotinylated UTP by Taq polymerase, however, is not efficient and requires special attention to the ratio of the biotinylated dUTP to dTTP (3). The optimal concentration of the probe for the detection of the β actin cDNA was 10–20 ng/ml (Figure 2). Preparation of the BrdU labeled probes by PCR

offers distinct advantages which include ease of preparation of a vector free probe, control over the length of the probe, and the stability of the probe at 4°C.

ACKNOWLEDGEMENT

This work is supported by Public Health Research Grant CA46866.

REFERENCES

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. Tabibzadeh, S.S., Santhanam, U., Sehgal, P.B. and May, L. (1989) *J. Immunol.* **142**, 3134–3139.
3. Dennis, L.Y.-M., Mehal, W.Z. and Fleming, K.A. (1990) Incorporation of biotinylated dUTP. In: *PCR protocols. A guide to methods and applications*. Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. (eds), pp. 113–118.

Figure 1. cDNA insert for human β actin (10 ng) was amplified (small arrowhead) using a human actin primer set that yields an amplified band of 1126 basepairs (large arrowhead). The PCR reaction mixture contained dTTP (200 μ M) (lane 1), and BrdUTP (200 μ M) (lane 2). Φ X174 RF DNA/HaeIII fragments (Lane 3). 1.5% agarose gel electrophoresis.

Figure 2. Southern blot of a cDNA insert of human β actin (about 2000 base pairs), hybridized with the BrdU labeled probe to human β actin (20 ng/ml) and immunostained for BrdU. Lane 1: 10000 pg, Lane 2: 1000 pg, Lane 3: 100 pg, Lane 4: 10 pg.