A simple high yield method for the preparation of lambda gt10 DNA suitable for subcloning, amplification and direct sequencing

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This paper describes a simple method for the preparation of high quality λ gt10 DNA utilising double nuclease digestion and PEG precipitation. The use of λ gt10 as a cDNA cloning vector (Huynh *et al.*, 1984) is often limited by difficulties in the preparation of insert DNA once clones of interest have been purified. Most previously described methods give low yields or involve time consuming CsCl step gradients and precipitation by ultracentrifugation (Sambrook *et al.*, 1989). Unlike other liquid lysate methods (Grosberger, 1987), this method is reliable for a wide range of insert sizes, applicable to large numbers of clones, and routinely yields at least 2 mg of high purity DNA per 100 ml cultures.

2 ml plate lysate (of 3 ml taken from 9 cm plates) is added to 100 ml of exponentially growing culture of *E. coli* C600hfl⁻, and grown with shaking until lysis (37°C, 2–3 h). The bacterial cells are removed by centrifugation. DNaseI (100 μ l, 10 mg/ml) and RNaseA (100 μ l, 10 mg/ml) are added to the supernatant and incubated (37°C, 1 h). Following addition of PEG (10 g) and NaCl (4 g), phage is precipitated (1 h, ice/water) and pelleted (1500 g, 10 min). The pellet is resuspended in 5 ml SM buffer and a second DNaseI (50 μ l, 10 mg/ml) and RNaseA (50 μ l, 10 mg/ml) incubation is carried out (37°C, 30 min). The phage is lysed by incubation with SDS (0.2%) and proteinase K (50 μ l, 10 mg/ml, 37°C, 1 h). After phenol/chloroform extraction, the phage DNA is precipitated using 3M sodium acetate pH 5.4 (0.1 volume) and cold ethanol (2.5 volume), centrifuged (1500 g, 10 min), and washed in 70% ethanol.

This method was used to:

1. Prepare DNA from several cDNA clones isolated from independent cDNA libraries containing a range of insert sizes (Fig. 1).

2. Isolate specific genes without library screening. 100,000 plaques from an unamplified fetal kidney cDNA library were used to make library DNA, digested with EcoRI and then subjected to amplification by the polymerase chain reaction (PCR). A specific region of the sodium/hydrogen antiport gene was amplified directly from library DNA without preliminary purification of plaques (Fig. 2).

3. DNA was specifically amplified using PCR from two thyroid peroxidase (TPO) cDNA inserts isolated from a thyroid cDNA library from a patient with Grave's disease (Fig. 2). Two different regions were amplified.

4. Part of a cDNA clone representing interstitial photoreceptor retinoid binding protein (IRBP) was directly sequenced from phage template using primers flanking the EcoRI site of λ gt10 (data not shown).

In conclusion, we have described a quick method for the simple and efficient preparation of large quantities of high purity λ gt10 DNA. The double nuclease digestion step before and after PEG precipitation removes the need for ultracentrifugation. This method has also been used successfully for other lambda vectors such as λ gt11, λ 2001 and EMBL4 (data not shown).

Figure 1. EcoRI digests of λ gt10 clones from different cDNA libraries. The libraries used are given in brackets. 1. IRBP (human retina); 2. S-Antigen (human retina); 3. S-Antigen (human retina); 4. D75 (human fetal kidney); 5. NC2 (N-CAM-like gene from human kidney); 6. NC4 (N-CAM-like gene from human fetal kidney); 7. TPO21 (human thyroid); 8. TPO18 (human thyroid). M. 1 kb ladder (Gibco-BRL).

Figure 2. Amplification from pooled cDNA library and isolated insert using the polymerase chain reaction. Lane 1. 3' region from the sodium/hydrogen antiport gene amplified from DNA prepared from a pooled fetal kidney cDNA library. Lane 2. Regions R2b and R3 from clone TPO18. Lane 3. Regions R2b and R3 from clone TPO21.

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