Linear amplification DNA sequencing directly from single phage plaques and bacterial colonies

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Quicker ways of sequencing short DNA would facilitate both mutational analyses and long range mapping with sequence tagged sites (STS). The method of sequencing directly from plaques and colonies reported here (Fig. 1.) is a labor-saving modification of the linear amplification method using partially purified DNAs (1,2). Our method is amenable to automation and is more efficient than those requiring prior PCR amplification when only <200 bp of sequence are needed.

To sequence λ, or M13 phage DNAs, a single plaque was suspended in 10 μl water, vortexed for 5 min and incubated at 37°C for 15 min. The suspension was centrifuged at 13,000×g, the supernatant was used for sequencing: 2 pmol of primer was 5′ end-labelled with a 3-fold molar excess of [γ-^{32}P]ATP and 5U of T4 polynucleotide kinase in 10 μl. One-fourth volume was added without purification to tubes containing: (a) 5 μM of each dNTPs, (b) 0.5 mM ddATP, ddCTP or ddTTP, or 0.1 mM ddGTP, (c) 10 mM Tris, pH 8.3, 50 mM KCl and 1.5 mM $MgCl_2$, (d) 2 μl of plaque suspension, and (e) 2.5 U of Taq (Life Technologies) or AmpliTaq (Cetus) Polymerase. The reactions were carried out in a 10 μl final volume in a Thermal Cycler (Perkin-Elmer Cetus) at 95°C, 4 min and then 30 cycles of 95°C, 1 min; 42°C-55°C (depending on primer), 2 min; and 72°C, 2 min. Two μl STOP solution were added to the reaction products which were then preheated (95°C). 4μl were electrophoresed and gels were autoradiographed 12h. To sequence plasmid DNAs, one-tenth of a young colony (~10^5 cells) was suspended in 40 μl distilled water, vortexed for 3 min, and incubated at 37°C for 15 min; 1 μl of this suspension was used in each sequencing reaction, as above.

This protocol generated sequence ladders of >200 bp from λ or M13 plaques or colonies containing pBluescript or pSPORT clones (Fig. 1). Signals from colonies or M13 were more intense than those from λ. Sequence ladders of at most 80 bp were obtained with colonies containing pBR322 (lower copy number). The quality or length of readable sequence was reduced by lowering ddNTP or Taq polymerase 2-fold, omitting KCl using bacteria stored at −20°C or doubling the number of cells. Consistent results as in Fig. 1 were obtained with diverse primers, and with colonies that had been freshly grown or stored for 2 weeks at 4°C. In conclusion, linear amplification sequencing directly from plaques or colonies should be useful in large scale genome mapping and in analyses of gene function.

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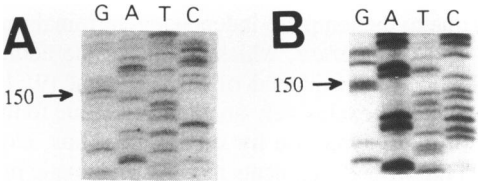

Figure 1. Products of linear amplification sequencing directly from **(A)** a λ plaque, and **(B)** a colony carrying a multicopy plasmid. The segment of the autoradiogram shown is ~150 bp from the 3′ ends of sequencing primers (arrows). The sequence in A extends from a transposon Tn*5supF* insertion in *lacZ* in λ Kohara clone 10A6. The sequence in B extends from the reverse primer in pBluescript SK II+ into a cloned segment of λ. The arrows correspond to *lacZ* nt 2601 in A and λ nt 27887 in B.

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