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E.coli host strains significantly affect the quality of small scale plasmid DNA preparations used for sequencing

Robin G.Taylor, David C.Walker[+] and Roderick R.McInnes*

Department of Molecular and Medical Genetics, University of Toronto and Department of Genetics, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada

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Several methods have been reported for the small scale preparation of plasmid DNA for sequencing (1–9), but little attention has been given to the effect of the *E.coli* host strain on the quality of the sequence obtained from double-stranded DNA. It has been observed that endonuclease A (*endA*$^+$) *E.coli* strains may produce nicked plasmid DNA resulting in 'shadow' bands on sequencing gels (1, 8), but no systematic evaluation of this phenomenon has been performed. We have found that of ten host strains tested, DH5α, JM109 and SURE cells consistently produced the highest yields of good quality plasmid DNA for double-stranded sequencing, whereas two of four *endA*$^+$ strains tested (NM522 and TG-1) exhibited significantly increased background on sequencing gels.

Plasmid DNA was prepared from *E.coli* host strains DH5α and DH5αF′ (Bethesda Research Laboratories), HB101 (10), JM101 (11), JM105 (11), JM109 (11), NM522 (12), SURE (Stratagene), TG1 (Amersham), and XL1-Blue (Stratagene) transformed with pBluescriptII KS(+) (Stratagene). DNA was isolated from 1.5 ml of the overnight cultures by the alkaline lysis mini-preparation method of Sambrook *et al.* (6) with and without the optional phenol:chloroform:isoamyl alcohol (24:24:1) extraction. Dideoxy sequencing reactions were performed on alkali-denatured plasmid with 10 ng of either T7 or T3 oligonucleotide primer (Stratagene) and Sequenase v.2 (US Biochemical) as described in the Sequenase protocol. Gel electrophoresis of sequencing reactions was performed as described previously (13). All plasmids were isolated and sequenced three times with similar results (data submitted but not shown).

We initiated these studies to determine which commonly used *E.coli* host strains produce the highest yields of plasmid DNA for good quality sequencing. DNA from all strains prepared with

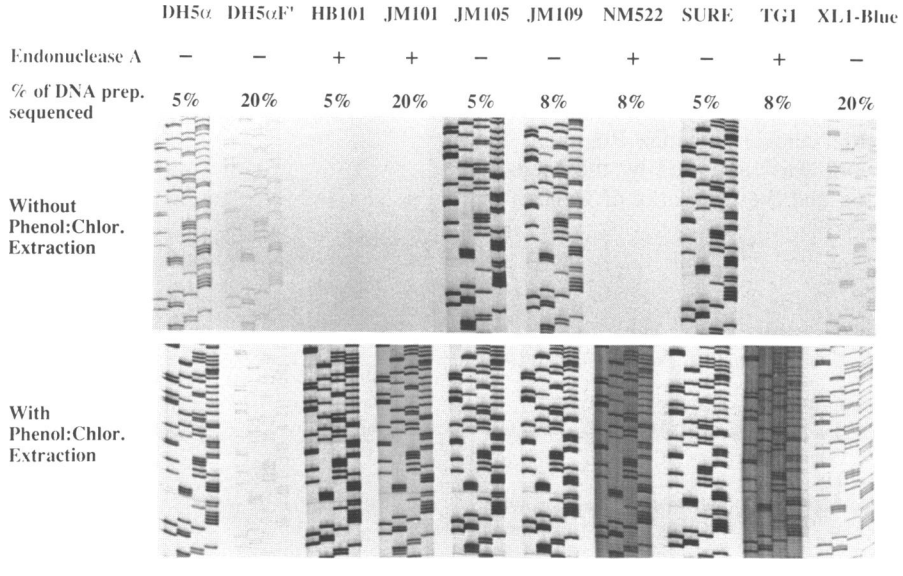

Figure 1. Assessment of DNA quality for sequencing. Dideoxy sequencing of pBluescriptII KS(+) plasmid DNA with T7 oligonucleotide primer (Stratagene) and Sequenase v.2. (U.S. Biochemical). DNA sequenced in the top set of lanes was prepared without phenol:chloroform extraction, whereas DNA sequenced in the bottom set of lanes was prepared with phenol:chloroform extraction. The *E.coli* host strains from which plasmids were isolated, the presence of endonuclease A, and the amount of DNA sequenced (% of total DNA in the preparation) are shown above each lane. Lanes are loaded in the order ACGT.

*To whom correspondence should be addressed

[+]Present address: Department of Cancer Biology, NNI-13, Research Institute, The Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA

the optional phenol:chloroform step appeared intact on agarose gels. However, when this step was omitted, DNA from *endA*$^+$ strains was degraded. To assess the quality of the DNA for double-stranded dideoxy sequencing, approximately equal amounts of plasmid DNA, prepared with or without phenol:chloroform:isoamyl alcohol extraction, were sequenced. The quantity of DNA used for sequencing (expressed as a percent of the total amount of DNA in the preparation) is shown above each lane in Figure 1. Two of the *endA*$^+$ strains, NM522 and TG1, consistently produced DNA that exhibited high background on acrylamide gels following sequencing (Figure 1). However, DNA prepared from the other two *endA*$^+$ strains, HB101 and JM101, did not have increased background compared to the *endA*$^-$ strains. This suggests that factors other than or in addition to *endA* affect nicking of the DNA.

Phenol:chloroform:isoamyl alcohol extraction of plasmid DNA from the *endA*$^-$ strains (DH5α, DH5αF′, JM105, JM109, SURE, and XL1-Blue) did not affect the yield or sequencing quality of the DNA produced (Figure 1). However, DH5αF′, JM101, and XL1-Blue consistently produced lower yields of plasmid DNA, necessitating the use of larger proportions of the plasmid mini-preparations, thus reducing the number of sequencing reactions that could be performed from a single preparation.

We conclude that the *E.coli* host strain has a significant effect on both the yield and quality of plasmid DNA used for sequencing. DNA isolated from *endA*$^+$ strains is highly susceptible to degradation if care is not taken to remove the endonuclease by phenol:chloroform extraction. All of the strains examined, except NM522 and TG1, produced DNA that gave good quality sequence. However, when both yield and quality of DNA were considered, DH5α, JM109 and SURE cells consistently gave the best results. These data suggest that if problems are encountered in sequencing denatured plasmid DNA, the cause may be the *E.coli* host strain rather than the method of DNA preparation.

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