Low pressure DNA shearing: a method for random DNA sequence analysis

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Several methods have been described for random fragmentation of DNA. These methods, often used for library preparation and subcloning prior to DNA sequence analysis, include sonic treatment (1, 2), partial digestion by restriction endonucleases (3) and treatment with DNase I in the presence of manganese ions (4). While all of these methods have been used successfully to prepare random DNA fragments for further manipulation and analysis, each has difficulties and limitations. In an effort to minimize template DNA preparation tasks and simplify primer- and PCR-directed DNA closure methods after an initial shotgun sequencing approach, we wished to prepare random subclones containing inserts with an average size of 4 to 6 kilobase pairs (kb). As an alternative approach, several different DNA samples were passed through a small French pressure cell at a variety of low to intermediate pressures (Figure 1b). A lever device was constructed to allow controlled application of low to intermediate pressures to the cell (Figure 1a). The results of these initial experiments suggested that low-pressure shearing offered a useful alternative to sonic and enzymatic DNA fragmentation methods. Subsequently, regions of the Caenorhabditis elegans genome cloned in cosmid vectors were sheared using an application of 250 psi. Shearing experiments with three different C. elegans cosmid clones (insert sizes ca. 35–42 kb) all produced essentially the same results (data not shown). The sheared cosmid DNA fragments were made flush with T4 DNA polymerase in the presence of 100 μM dNTPs (2), and DNA fragments of the desired size range were purified by preparative agarose gel electrophoresis and subcloned in the HindII (SalI) site of the phagemid vector pUC118. To check the efficiency of this subcloning method, 109 of these subclones were examined by standard plasmid mini-prep and agarose gel electrophoresis procedures. 101 (92%) subclones contained an insert of the expected 4 to 6 kb size range. 72 subclone DNAs were sequenced using a linear amplification method with fluorescent dye-labeled primers. Identical subclones were not observed in this analysis, and no sequence-specific shearing hot spots were detected. Additional DNA sequencing experiments using subclones produced by the low pressure-shearing method are in progress in order to determine the complete nucleotide sequence of a 100 kb region in the large cluster of C. elegans chromosome III.

REFERENCES

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Figure 1. a) Apparatus for shearing DNA under low to intermediate pressures in a French pressure cell (a detailed protocol and schematic drawings for the device are available from the authors upon request). All experiments used a model number FA-003 French pressure cell (SLM Instruments, Inc., Urbana, IL). The lever device used to apply controlled low to intermediate pressures to the cell was constructed from common building materials. The applied pressure is calculated based on the inside diameter of the pressure cell, and the force delivered to the piston. To deliver a known pressure, for example 250 psi, the required force for a pressure cell with a piston diameter of 0.375 inches is 27.50 pounds (force = desired pressure x piston area). Using the lever arm of the pressure-applying device, a mechanical advantage is gained, and the weight needed to generate the required force may be reduced. Starting with a known weight (w = 8.3 pounds), a lever arm advantage ratio is calculated (lever arm advantage = required force/applied force). With a lever arm ratio of 3.3 (in diagram, the distance x is 3.3 times the distance y) the lever device applies the specified pressure of 250 psi to the sample using an 8.3 pound weight. DNA to be sheared was diluted to a final volume of 1 ml in 0.5 M NaCl, 10 mM Tris-HCl, pH 8.0 and 10 mM EDTA. Best results in subsequent cloning procedures were obtained when 20 to 30 μg of DNA were used. Shearing was performed at 4°C, and the DNA was collected in a clean microcentrifuge tube and precipitated by addition of 0.6 volumes of isopropanol and incubation at room temperature for 15 minutes. For cosmid subfragment cloning, end-repaired DNA was electrophoresed in a low-gelling temperature agarose gel, and sections containing 4 to 6 kb DNA fragments were excised, eluted and ligated into pUC118. The highest subcloning efficiencies were obtained with a 3:1 vector to insert DNA ratio. All ligation reactions were performed overnight at room temperature in the presence of 20% polyethylene glycol-8000 (5). Following transformation of JM101 (6), subclone DNAs were prepared using a small scale alkaline lysis procedure (7), incubated with RNase A (200 μg/ml) for one hour at 37°C to remove RNA, extracted once with phenol (saturated with 1 M Tris-HCl, pH 8.0) and ethanol precipitated. Subclones were sequenced directly using a linear amplification method with fluorescent dye-primers (M.Craxton and J. Sulston, pers. communication). b) Agarose gel electrophoresis of DNA sheared with low to moderate pressure. Lane 1, bacteriophage lambda DNA cleaved with HindIII; lanes 2–6, bacteriophage lambda DNA after: no pressure treatment (lane 2), 100 psi (lane 3), 250 psi (lane 4), 500 psi (lane 5), 1000 psi (lane 6); lanes 7–11, high molecular weight human DNA after: no pressure treatment (lane 7), 100 psi (lane 8), 250 psi (lane 9), 500 psi (lane 10), 1000 psi (lane 11); lane 12, 1 kb DNA ladder.