Isolation of High-Molecular-Length DNA from Human Skin


ABSTRACT

An agarose plug method for isolating high-molecular-length DNA from mammalian tissues has been developed, including from those that are difficult, such as skin. It gives high yields of DNA that contain a minimum of single-strand breaks and is readily digested by restriction and other nucleases. The method requires only simple equipment and is readily adaptable to field or clinical studies.

INTRODUCTION

Isolation of DNA from mammalian tissues for DNA repair determinations or genetic analysis frequently involves small tissue samples that may contain potent nucleases. For example, human skin, the primary tissue damaged by ultraviolet (UV) radiation in sunlight and thus of great importance for assessment of UV-induced DNA damage and repair, is tough and resilient. Chemical or solution methods of DNA isolation result in large losses at solvent-phase interfaces, during transfer among successive tubes, upon chromatography or upon precipitation; they produce DNA of modest molecular length containing many single-strand breaks resulting from shear or adventitious nuclelease action during isolation (2,5,6,8,10–12, 14). We have developed a gentle method based on agarose embedding (13) for isolation of high-molecular-length DNA from mammalian tissues, including difficult samples such as human skin. Our method yields DNA of single-strand molecular length ≥170 kb, containing a minimum of induced single-strand breaks, that can be readily digested with restriction enzymes. The method is not laborious and is suitable for multiple samples. It can easily be adapted to clinical or field studies in which the samples are obtained at one location but DNA is isolated in a remote laboratory.

MATERIALS AND METHODS

All solutions and equipment were sterilized by appropriate means.

Human Skin Samples

Biopsies (3–6-mm diameter) of un-tanned gluteal skin were obtained from healthy adult volunteers who gave informed consent. Lidocaine (0.1 mL; Abbott Pharmaceuticals, N. Chicago, IL, USA) is injected intradermally, and superficial-shave biopsies were obtained from each site. Biopsies were immediately immersed in L buffer (0.1 M EDTA, 20 mM NaCl, 10 mM Tris-HCl, pH 8.3) at 60°C and heated for 30 s. Under an inverted microscope equipped with a red filter, the dermis and epidermis are separated by gentle scraping. Each sample is then transferred to a microcentrifuge tube containing 0.75 mL L buffer at 65°C and heated for 20 min to inactivate cellular nucleases. Samples are washed twice in ice-cold L buffer and transferred to 35-mm suspension culture dishes (Catalog No. 83.1800.002; Sarstedt, Newton, NC, USA) on ice. Tissue samples are minced finely using sterile cuticle scissors, transferred to a pre-weighted microcentrifuge tube and centrifuged (ca. 1 min, Tomy Capsule HF-120; Peninsula, Belmont, CA, USA). The supernatants are removed, and the weight of the tissue is determined. The minced tissue is resuspended in an equal weight of L buffer and mixed with an equal volume of 2% InCert® agarose (FMC BioProducts, Rockland, ME, USA) in double-distilled H₂O. Aliquots (10 µL) of the solution are pipetted onto a 35-mm petri dish on ice, and the buttons are allowed to solidify for 30 min at 4°C. A 5-µL overlay of 1% agarose is pipetted over each chilled button and allowed to solidify for 30 min longer. (The overlay seals the tissue fragments in the agarose button.) The tissue slurry can also be formed into plugs of shape appropriate for the wells of the gel mode anticipated. Buttons or plugs are transferred to 35-mm suspension dishes, and 1.5 mL of freshly prepared lysis solution (L buffer containing 1 mg/mL proteinase K [Boehringer Mannheim, Indianapolis, IN, USA; pre-digested for 1 h at 37°C], 2% sarcosyl) is added to each dish. (It is essential to check each lot of proteinase K for endonuclease activity in the absence and presence of calcium [see below], as lots from some manufacturers introduced substantial levels of breaks into the DNA during the long digestion time required for skin.) The dishes are then sealed with Parafilm®, wrapped with Saran® wrap, placed in a light-proof box and incubated at 45°C with daily changes of proteinase K/lysis solution for approximately 7 days or until the tissue is no longer visible. If tissue is still visible, buttons are soaked in an ice-cold solution of 1 mM CaCl₂, 10 mM Tris-HCl, pH 7.5 (two 20-min changes, 4°C then digested at 45°C for 16–24 h with 1.5 mL proteinase K (1 mg/mL) in a freshly prepared solution of 1 mM CaCl₂, 10 mM Tris-HCl, pH 7.5. The proteinase K solution is removed, buttons washed twice with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5), and TE containing 10 mM ethylene glycol bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA; Sigma Chemical, St. Louis, MO, USA) is added to a final EGTA concentration of 2 mM (to chelate Ca²⁺ ions), and the buttons are soaked at 4°C (two changes, 1 h each).

Preparations are checked for complete removal of interfering proteins by electrophoresis on 0.4% alkaline agarose gels as previously described (1). Samples with residual protein produce DNA that remains at the well-gel interface, whereas samples with adequate removal of cellular proteins contain DNA that electrophoreses readily into an alkaline gel during static field electrophoresis. Samples not meeting this criterion are further digested with proteinase K as described above. When digestion is complete, samples are rinsed twice with ice-cold TE, treated with two changes of 0.22 mM phenylmethylsulfonyl fluoride (PMSF) in a solution of 10 mM Tris-HCl, pH 7.6, 1 mM EDTA at 45°C for 1 h, then rinsed with TE. Plugs are stored at 4°C in L buffer containing 2% sarcosyl. Plugs or buttons are prepared for digestion with restriction or other endonucleases by two 30-min changes of ice-cold buffer suitable for that enzyme.

Probes

Hybridization probes for c-myc
exons 1, 2 and 3 are produced by random priming (4). The exon 3 probe was the 1.7-kb Clal-EcoRI fragment of pMC41-3RC (3); the probe for exons 1 and 2 was the 2.5-kb PstI fragment from a partial PstI digest of pMC41-5PP. Probes for lambda (λ) DNA are prepared by random priming of the entire λ DNA, sheared by passing through a syringe needle. Probes (specific activities >10⁹ cpm/µg) are purified on a 5-mL P-10 column (100–200 mesh; Bio-Rad, Hercules, CA, USA) in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA).

**Electrophoresis**

Alkaline minigel (0.8%) are prepared in a solution of 50 mM NaCl, 1 mM EDTA and soaked for 1 h in alkaline electrophoresis solution (30 mM NaOH, 2 mM EDTA) (9). DNA (10 µL) was denatured by incubation (37°C, 30 min) with 1 µL alkaline stop mixture [one part alkaline dye mixture (0.25% bromocresol green in 0.25 N NaOH, 50% glycerol); one part 6 N NaOH]. Buttons are transferred by use of sterile disposable bacteriological loops as manipulators to the wells of gels soaked in electrophoresis buffer. For determining the single-strand molecular length of the genomic DNA, unidirectional pulsed-field gel electrophoresis (UPFE; Reference 15) is carried out in a Mini-Sub® Cell (Bio-Rad) apparatus (15 V/cm, 0.3-s pulse, 10-s interpulse period, 10°C with buffer recirculation). For resolution of restriction enzyme-digested DNA, samples and molecular length stan-
RESULTS AND DISCUSSION

High-molecular-length DNA, readily digestible with restriction and other endonucleases, can be obtained in good yield from human skin biopsies by proteinase K digestion of tissue in agarose buttons. We found that satisfactory preparations were obtained by heating biopsies to 60°C immediately (which allows separation of dermis from epidermis) then heating at 65°C for 20 min. High-molecular-length DNA could be obtained from such heated biopsies whether they were processed and embedded in agarose immediately or stored on ice for 8 h (simulating the time and conditions required for transporting or shipping samples to the laboratory). However, biopsies collected in buffer on ice until the end of an experiment (ca. 2 h) gave DNA of highly variable size, with significant nicking in some samples. Biopsies could be minced with fine scissors or scalpel. It was important to mince the tissue finely and to mix the resulting slurry as evenly as possible with the agarose to obtain an even distribution of tissue per button. We found it essential to harden the buttons on ice to prevent malformation of the buttons, which led to poor DNA band shapes in subsequent gels.

During the proteinase K digestion, the completeness of digestion of the samples was monitored first by the appearance of the buttons. Samples in which the tissue was clearly visible were not completely digested, and a substantial portion of the DNA remained in the well of an alkaline gel after electrophoresis. Further proteinase K digestion yielded samples with decreasing quantities of DNA that remained in the gel. Figure 1 shows two such incompletely digested samples; lane 3 shows a sample (two-day proteinase K digestion) in which DNA is clearly evident in the well, while lane 2 contains a sample (ten-day digestion) in which digestion is almost complete and almost all the sample entered the gel. Figure 2 (lane 2) shows a completely digested sample. Comparison with the molecular length standards shows that the experimental sample contains DNA at least as large as the largest standard used on this gel, T4 bacteriophage DNA, 170 kb.

We asked whether the human skin DNA isolated in this manner could be digested with restriction enzymes. Figure 3A shows the result of digesting the DNA with EcoRI, electrophoresis on an alkaline agarose gel, neutralization and staining with ethidium bromide. (EcoRI digestion of human DNA produces a 12.5-kb fragment containing all three c-myc exons and introns.) Figure 3B shows an image of the blot resulting from hybridization of the digested DNA to 32P-labeled probes for c-myc. The positions of the molecular length standards (HindIII digest of λ) are also shown. The c-myc band migrates between the 23.1- and 9.4-kb markers, as expected for a molecule of 12.5 kb.

The agarose button digestion method of isolating DNA from human tissue is not laborious and is suitable for multiple samples. The use of proteinase K digestion rather than chemical extraction methods avoids the use of hazardous chemicals. From an average 3–4-mm human skin biopsy, we obtained by chemical extraction, about 300 ng of DNA of average single-strand molecular length 40–50 kb (5,6,14); in contrast, by the agarose button technique, we obtained about 10 buttons.
containing approximately 300 ng high-
molecular-length (> ca. 170 kb) DNA
per button, roughly a tenfold improve-
ment. The agarose button method is
easily adaptable to clinical or field stud-
ies in which samples are obtained at
one location and DNA isolation is car-
died out at another, and it requires mini-
mal equipment (a source of 65°C heat)
at the field location. DNA from skin of
other mammals, e.g., mouse, can be
isolated by simple adaptations of this
procedure. This method provides high-
yield recovery of DNA from samples
available only in small quantities, in-
cluding biopsies of human skin.

Note: R. William Gange passed
away before publication of this article.

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Simultaneous Detection of Microorganisms in Soil Suspension Based on PCR Amplification of Bacterial 16S rRNA Fragments


ABSTRACT
The effect of buffer composition on simultaneous PCR amplification of 16S rRNA gene fragments of five bacterial species was examined using a number of different buffer systems. Tris-based PCR buffers at final concentrations of 10 mM proved unreliable. However, when the final concentration of Tris was increased to 75 mM, all five samples were routinely detected. The use of other buffers, 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO) and 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid (CAPSO), resulted in PCR amplification of five products even at low final concentrations (10 mM). The presence of certain proteins in the amplification reaction could overcome an inhibitory effect seen when soil suspension was present in the reaction, as might occur when testing field samples for the presence of bacteria. Bovine serum albumin was found to be the most effective additive tested in overcoming inhibition.

INTRODUCTION
Detection of bacterial species in environmental samples using conventional microbiological techniques can be laborious and time-consuming. Polymerase chain reaction (PCR) (5) has the potential to allow rapid detection of any bacterial species for which specific amplification primers are available. While PCR is an attractive method for identifying bacteria, there are a number of problems associated with field samples that must be addressed before successful detection can be achieved. Environmental samples can contain a number of different bacterial species, requiring the isolation of a large amount of sample DNA to enable individual species to be detected. Depending on the source of the sample, various