ULTRAFAST MICROPREP PROTOCOL FOR LARGE-NUMBER PREPARATIONS OF RECOMBINANT PLASMIDS AND COSMIDS

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A very fast, reliable, and low-cost procedure is described to purify both recombinant plasmids and cosmids in large numbers, using the microtiter system for cell growth and DNA preparation. Although the DNA obtained is limited in amount and purity, it meets the requirements of characterization by restriction analyses and gel electrophoresises, as well as probe hybridization. Using basic laboratory equipment, the protocol enables a single person to manually isolate the covalently closed circular DNA of up to 6,000 clones within about 8 h.

KEY WORDS

Microprep; Microtiter system; DNA preparation; Plasmid; Cosmid

Access to a large number of individual, recombinant plasmid or cosmid clones is a requirement of several recombinant DNA techniques. The screening of cDNA libraries, for instance, the characterization of clones resulting from genomic "walking" or "jumping" (Poustka et al, 1986), or approaches to map large areas of genomic DNA (Michiels et al, 1987), all require the isolation of DNA in sufficient amounts and purity for restriction analyses or probe hybridizations. Some protocols were published, either accelerating DNA preparation (Holmes and Quigley, 1981) or for parallel isolation of several clones (Gibson and Sulston, 1987). The Microprep protocol described here combines these points using the 96 well arrays of microtiter plates for cell growth and DNA purification and speeding up the actual preparation by a protocol which is simplified to those steps absolutely necessary to isolate plasmid and cosmid DNA. Nevertheless, based on the reliable and frequently used alkaline lysis procedure of Birnboim and Doly (1979), it produces DNA of a quality that meets the requirements of gel electrophoresis, cleavage with restriction enzymes, and oligonucleotide hybridization. Due to its simplicity, a large number of clones can be easily isolated at reasonable costs.

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METHODS AND RESULTS

Both the pure vector and recombinant derivatives of plasmids pUC18 and pTZ18R (Mead et al, 1986) were used for plasmid-preparations. (Actually, pUC18 was constructed by replacing the polylinker of pUC8 [Vieira and Messing, 1982] by that of M13mp18 [Yanisch-Perron et al, 1985].) They were grown in the *Escherichia coli* strain JM83 (Vieira and Messing, 1982). Highly purified plasmid DNA was prepared using a modified alkaline lysis method as described in detail by Pohl et al (1982). The experiments concerning cosmid DNA were performed with clones of a *S. pombe*-library cloned into lawrist4 and grown in *E. Coli* strain C600 pop2135 (Raibaud et al, 1984).

Due to superior accuracy and thereby increased reproducibility, all stock solutions and most other buffers were prepared on a balance using weight ratio percentages (Protocol 1). Only a few were mixed volume ratio or weight per volume for reasons of convenience.

Preparation of covalently closed circular (CCC) DNA was carried out as described in Protocol 2. The procedure took approximately 50 min, the last 15 to 20 min being the RNase-treatment. Since the centrifuge used was able to spin four plates at a time, usually four plates were processed in parallel, with no increase in the time necessary. Solutions I, II, and III were added continuously, starting with the subsequent solution at the first row of the first plate after finishing the fourth plate. By an extension of the RNase-treatment to approximately 30 min, two sets of plates can be handled in an overlapping manner, adding solutions I, II, and III to the second set while incubating the other one. Thereby, the actual preparation time is reduced to about 30 min/set, which means that 64 plates (6,144 wells) can be processed within 8.5 h. With two centrifuges, that output might easily be doubled without a significant increase in time.

PROTOCOL 1

PREPARATION OF BUFFERS AND SOLUTIONS

1. **Stock-solutions:**
   0.5 M EDTA, pH 8: 16.9% (w/w) EDTA (ethylendinitrilotetraacetate disodiumsalt dihydrate), 1.9% (w/w) NaOH-pellets, 81.2% (w/w) H_2O.
   0.5 M glucose: 8.72% (w/w) glucose (water-free), 91.38% (w/w) H_2O.
   1 M magnesium acetate: 20.44% (w/w) magnesium acetate (4 H_2O), 79.56% H_2O.
   2 M potassium acetate: 18.1% (w/w) potassium acetate, 81.9% (w/w) H_2O.
   3 M potassium chloride: 19.77% (w/w) KCl, 80.23% (w/w) H_2O.
   1 M sodium acetate (pH 4.6): 7.9% (w/w) sodium acetate, 7.0% (w/w) acetic acid, 85.1% (w/w) H_2O.
   1 M sodium phosphate (pH 7.2): 6.68% (w/w) Na_2HPO_4, 0.52% (w/w) H_3PO_4, 92.80% (w/w) H_2O.
   1 M NaOH: 4 g NaOH-pellets, 100 g H_2O.
   2 M Tris base: 22.90% (w/w) Trizma base (Sigma, St. Louis, MO), 77.10% (w/w) H_2O.
   1 M Tris-acetate (pH 7.9): 50% (w/w) 2 M Tris base, 3.2% (w/w) acetic acid, 46.8% (w/w) H_2O.
   1 M Tris-HCl (pH 7.5): 2.30% (w/w) Trizma base, 12.37% (w/w) Trizma hydrochloride, 85.33% (w/w) H_2O.
   1 M Tris-HCl (pH 8.0): 5.16% (w/w) Trizma base, 8.64% (w/w) Trizma hydrochloride, 86.20% (w/w) H_2O.
2. **Microprep solutions:**
   2YT growth medium: 1.6% (w/w) Bacto Tryptone (Difco, Detroit, MI), 1% (w/w) Bacto Yeast Extract (Difco), 0.5% (w/w) NaCl, 96.9% (w/w) H_2O.
   Solution I: 10% (v/v) 0.1 M EDTA, 10% (v/v) 0.5 M glucose, 2.5% (v/v) 1 M Tris-HCl (pH 8.0), 77.5% (v/v) H_2O.
   Solution II: 20% (v/v) 1 M NaOH, 5% (v/v) SDS (20% w/w), 75% (v/v) H_2O.
   Solution III: 25.89% (w/w) sodium acetate, 10.55% (w/w) acetic acid, 63.56% (w/w) H_2O.
   RNase (DNase free: Sigma): 4 mg/ml 50 mM sodium acetate (pH 4.6); boil for 15 min followed by slowly cooling to room temperature (in a waterbath) and store at 4°C.
   Gel loading buffer: 25 ml glycerol, 25 ml H_2O, 4 mg bromphenol blue.
3. **Restriction digestion:**
   10× TAK: 33% (v/v) 1 M Tris-acetate (pH 7.9), 32.5% (v/v) 2 M potassium acetate, 10% (v/v) 1 M magnesium acetate, 5% (v/v) 0.1 M dithiothreitol, 19.5% (v/v) H_2O; sterilize by filtration and store in aliquots at −20°C. Store the aliquot in use at 4°C for up to 2 wk.
   25× TAE electrophoresis buffer (pH 8.2): 11.8% (w/w) Trizma base, 3.1% (w/w) acetic acid, 0.8% (w/w) EDTA, 84.3% (w/w) H_2O.
4. **Hybridization:**
   Denaturing solution: 1.87% (w/w) NaOH-pellets, 8.15% (w/w) NaCl, 89.98% (w/w) H_2O.
   ('Church') hybridization buffer: 6.95% (w/w) SDS, 0.20% (w/w) 0.5 M EDTA, 49.75% (w/w) 1 M sodium phosphate (pH 7.2), 43.10% (w/w) H_2O.
   20× SSC: 15.36% (w/w) NaCl, 7.73% (w/w) trisodium citrate, 76.91% (w/w) H_2O; check pH and adjust with concentrated HCl or NaOH, respectively, to pH 7.5.
   4.8 M TMAC: 53.8% (w/w) Tetramethyl-ammonium chloride (Fluka, Buchs, Switzerland), 46.2% (w/w) H_2O; filter through Whatman paper filter 1 (Whatman, Maidstone, UK)

Figure 1 shows all DNAs isolated from a single microtiter plate that was been inoculated with pUC derivatives. The quality of cosmid preparations is shown in Fig 2. To determine the DNA yield resulting from a micropreparation, plasmid and cosmid DNA from several preparations were run in gels (*e.g.*, Fig 2) containing 0.5 μg ethidium bromide per milliliter. The gels were destained in water overnight and then photographed using Polaroid film type 667 (Polaroid, Cambridge, MA), with an excitation wavelength of 366 nm and a cut-off filter. UV illumination was kept brief to avoid any light-induced bleaching of the bands. The full width of each lane was scanned (LKB Ultroscan XL, Bromma, Sweden) and compared with scans of highly purified plasmid DNA, whose concentration had been measured by a fluorescence assay (Morgan et al, 1979) based on the increase in fluorescence of DNA-intercalated ethidium (Perkin-Elmer MPF-3L Fluorescence Spectrophotometer; excitation at 280 to 320 nm, measurement at 580 to 620 nm). Signals from the monomeric, dimeric, and trimeric forms were taken into account. In a range between 10 and 100 ng CCC DNA, the signal intensity increases linearly with the rise of the DNA amount. Using these methods, it was found that a micropreparation yields 250 (±20) ng plasmid DNA or 40 (±10) ng cosmid-DNA.

For restriction digestion (Protocol 3) the DNA had to be precipitated to change the buffer, because restriction enzymes were inhibited by the high salt solution resulting from the preparation. Contaminating DNases did coprecipitate. They unspecifically cut the DNA, if it was dissolved in buffer containing Mg^{++} ions (data not presented). Heating to only 68°C, instead of 85°C, did not entirely inactivate DNases. However, cosmid DNA incubated at 85°C complexed with the chromosomal *E. coli* DNA and did not enter 0.5% (w/w) agarose gels. Therefore, 68°C was used as a compromise for cosmid

PROTOCOL 2

MICROPREPARATION OF CCC DNA

1. Dispense 96 × 200 µl of 2YT-medium supplemented with an appropriate amount of antibiotic (e.g., 100 µg ampicillin/ml for pUC-derivatives or 40 µg kanamycin for lawrist4-derivatives) into the wells of a sterile microtitre plate with lid.
2. Pick single colonies and inoculate them in the plate. Use at least one well as a control of sterility. Incubate overnight at 37°C without any shaking. For back-up, replica-plate the grown bacteria from the microtitre plate to agarplates.
3. Pellet the cells by centrifugation for 5 min at room temperature (all centrifugations 1,100 × *g*, e.g., Beckman GPR centrifuge, GH-3.7 rotor, at 2,400 rpm). Turn the plate upside down and discharge the supernatants with little verve. Drain the plate briefly onto a paper-towel.
4. Use an multichannel pipette with adjustable volume for all pipetting steps. Do not change the tips during the whole procedure but rinse them once with water after each pipetting.
5. Add the following solutions at room temperature in intervals of about 5 min and mix by briefly vortexing: 25 µl of solution I (25 mM Tris-HCl (pH 8.0), 50 mM glucose, 10 mM EDTA), 50 µl of solution II (0,2 M NaOH, 1% (w/w) SDS), and 38 µl of solution III (3 M potassium acetate, 2 M acetic acid). After 5 min, precipitate cell debris by centrifugation for 5 min.
6. Apply 75 µl of each supernatant to a fresh microtiter plate with lid, add 10 µl pancreatic RNase (4 mg/ml), and incubate for 15 min at 37°C. Since the high salt concentration is inhibiting any DNase contamination, longer incubations cause no damage. Add 20 µl gel-loading buffer (50% (v/v) glycerol, 50% (v/v) H_2O, 0.08% (w/v) bromphenol blue) and mix by vortexing. Store the plate at −20°C.
7. For screening the size of plasmid-DNA, load about 13 µl of each sample to an agarose gel and electrophorese in the presence of appropriate standards (Fig 1). 2-3 µl of standard DNA have to be mixed with 10 µl solution taken from the control-wells, in which no bacteria were grown, to ensure similar conductivity in all slots.
8. Cosmids have to be precipitated as described in sections 1 and 2 of Protocol 3 to be visible by ethidium bromide staining (Fig 2).

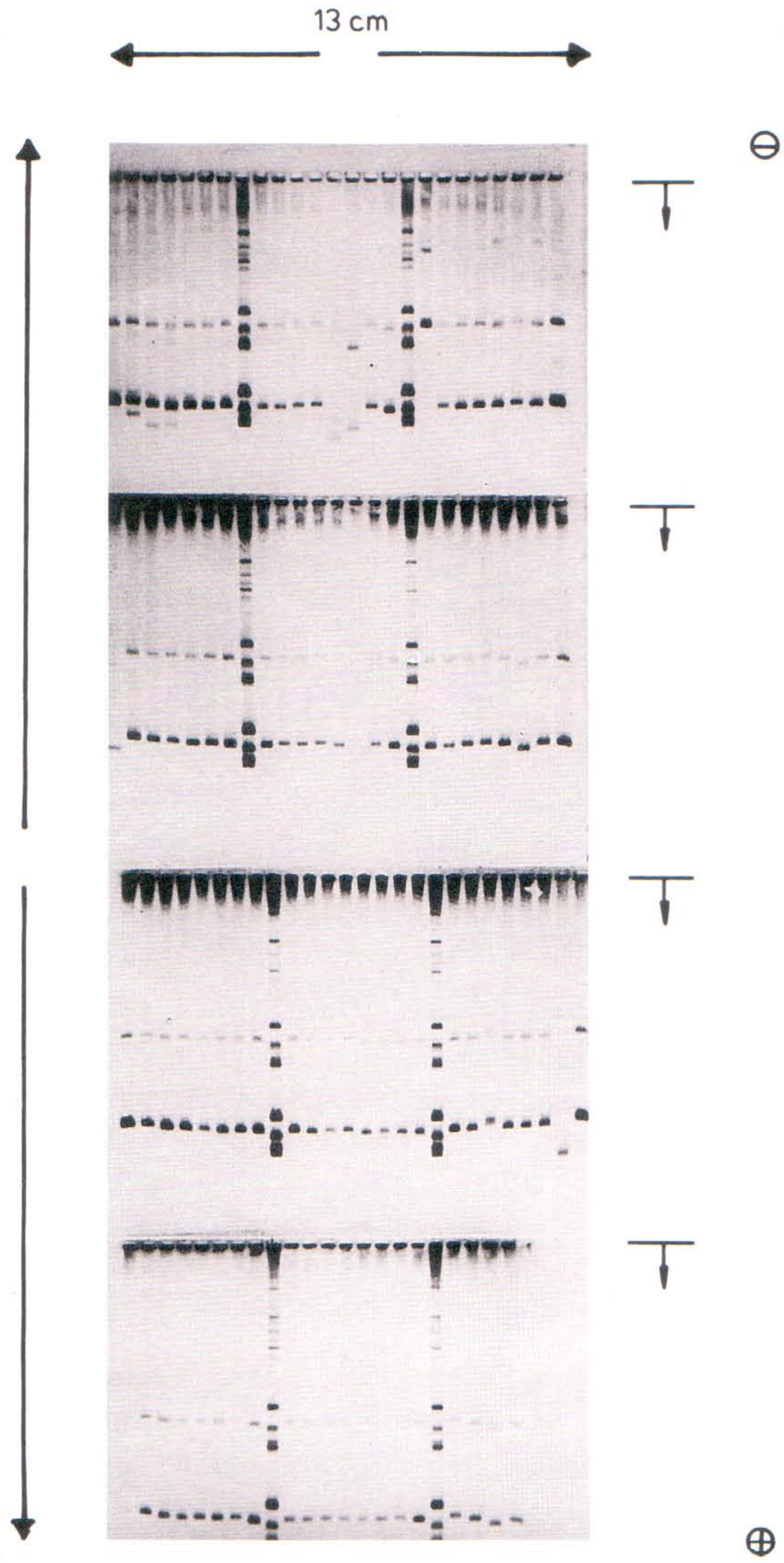

FIG 1. The CCC DNA of 100 (92 + 8) plasmids prepared in one microtiter plate was electrophoresed in an agarose gel. A few wells (first eight lanes) had been simultaneously inoculated with two clones each. Standards are a mixture of highly purified plasmids with 3,465, 2,953, 2,736, and 2,575 base pairs (bp) of their monomeric forms. All plasmids are recombinant pUC18-derivatives unidirectional deleted by exonuclease III (Hoheisel and Pohl, 1986). The gel was run to screen the amount of deletion.

preparations. Figure 3 shows the quality of restriction patterns obtained by ethidium bromide staining in case of plasmid DNA (Fig 3A) or end-labeling and autoradiography with cosmids (Fig 3B).

TAK restriction buffer (33 mM Tris-acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM dithiothreitol) (O'Farrell et al, 1980) was applied because it allows digestion with almost any restriction enzyme. All enzymes listed in Table 1 showed between 70% and more than 100% activity in TAK in comparison to incubations in buffers recommended by the deliverers. Only *Sac*I, an isoschizomer of *Sst*I, was found less active. Since some DNA-modifying enzymes are active in TAK as well, combined use can simplify and accelerate some procedures, e.g., end-labeling of restriction fragments (Protocol 3). The standard of Fig 3B, for instance, was produced by an incubation of 125 ng lambda-DNA (Pharmacia, Uppsala, Sweden) with 5 U *Eco*RI in 9 µl TAK buffer supplemented with dGTP, dCTP, and dTTP (20 µM each) at 37°C for 10 min, followed by a further 5 min after addition of 0.5 µl Klenow enzyme (2 U) and 0.5 µl α^{32}PdATP (10

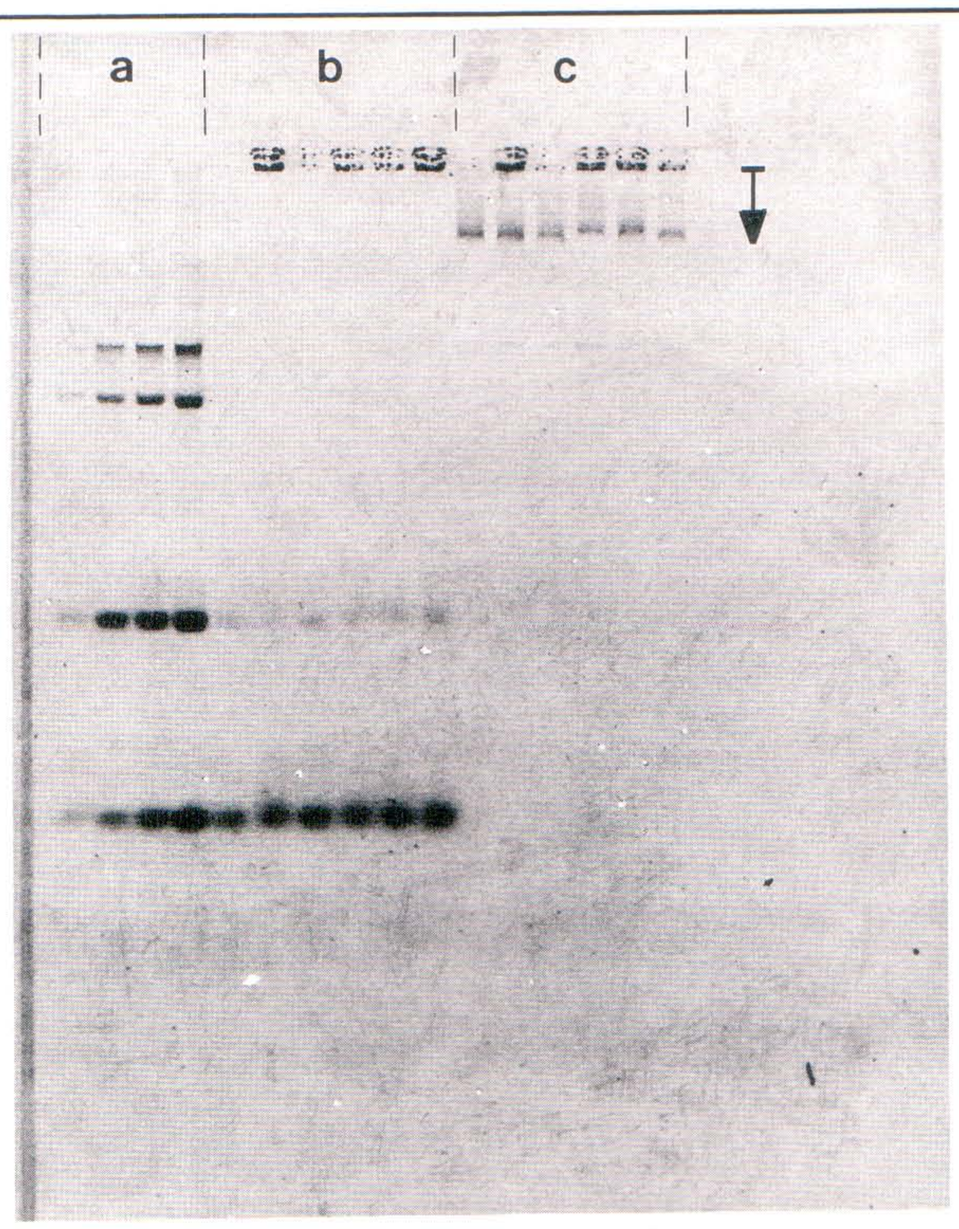

FIG 2. Amount of plasmid and cosmid DNA yielding from the Microprep protocol. (a) 25, 50, 75, and 100 ng of pTZ18R were run together with a sixth of micropreparations made from the same plasmid (b) or half the preparation of lawrist4-derivatives (c), respectively, in a 0.5% (w/w) agarose gel. The lanes were scanned and the amount of DNA was determined in comparison to the four standard-lanes (a) of highly purified plasmid.

mCi/ml, 3,000 Ci/mmol [1 Ci = 37 GBq]; Amersham, UK). With the addition of Klenow enzyme the percentage of glycerol exceeded 5%, inducing star-activity of *Eco*RI.

Although a background of chromosomal *E. coli* DNA is coisolated in micropreparations, hybridizations with 12mer oligonucleotides according to a standard procedure (Protocol 4; Craig and Nizetic, personal communication, August 1988) recognized spotted cosmid DNA with high specifity (Fig 4). Hybridizations to plasmids produced even better results (not shown).

DISCUSSION

The major advantage of the Microprep protocol, to begin with, is its velocity. Not only the parallel treatment of four microtiter plates at a time (a reasonable number, although the limit is set by the capacity of the centrifuge), but also the actual procedure itself increases the turnover considerably. In particular, the number of DNA precipitations, which are time- and work-intensive, is drastically reduced to one, for restriction digestion. If several sets of plates are prepared during the morning, it is possible to analyze the clones by restriction digestion and/or hybridization on the same day. Actually, not the DNA preparation, but the

PROTOCOL 3

RESTRICTION DIGESTION

1. Transfer 50 μl (half the preparation) to a fresh plate (V-formed wells, Greiner, FRG), add 125 μl cold ethanol and mix well. Precipitate the DNA at −70°C for 15 min and spin in a centrifuge (1,100 × g) for 10 min. Turn the plate and remove the supernatants. Put the plate briefly onto a paper-towel to remove remaining liquid. The DNA is clearly visible as a blue precipitate. Note that V-formed wells are preferable to flat bottom wells as the latter give variable amounts of pellet.
2. Dissolve plasmid-DNA in 24 μl, cosmid-DNA in 8 μl TE (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). 8 μl are sufficient for one restriction digestion.
3. For inhibition of contaminating DNase-activity, add 1 μl 10 × TAK buffer (330 mM Tris-acetate (pH 7.9), 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM dithiothreitol;O'Farrell et al, 1980) to 8 μl of the DNA-solution. Cover the plate with Saran-wrap (Dow Chemical Co, USA) and float it about 10 min in water of 68°C for cosmids, or 85°C for plasmids. Collect the liquid by centrifugation for 10 s.
4. Add an excess (1 to 2 U) of enzyme. Mixtures of more than one enzyme can also be used in TAK-buffer (see Table 1). Incubate plasmid-DNA for 30 to 40 min, cosmids for 10 min at the appropriate temperature.
5. For end-labeling DNA-fragments (which is essential for cosmids, because there is insufficient DNA for ethidium bromide staining), add a mixture of 0.5 μl α^{32}P-labeled nucleotide (10 mCi/ml, 3,000 Ci/mmol; 1 Ci = 37 GBq), 0.5 μl of the other three nucleotide triphosphates (440 μM each; final concentration 20 μM), and 0.5 U Klenow-enzyme. Incubate for about 5 min at 37°C.
6. Terminate the reaction by adding 1 μl 0.5 M EDTA.

inoculation by manual transfer of randomly distributed clones from agar-plates into microtiter plates, became a limiting factor, because, in my experience, 5,000 to 6,000 clones seem to be the most a single person is able to manage within a day.

The procedure is simple and robust, resulting in a high degree of reproducibility. Variations of the incubation times in solutions I, II, and III from 3 to 10 min did not influence the amount and quality of isolated DNA, nor an extension of the RNase digestion. The heat-treatment of the RNase (Protocol 1) was carried out routinely, although DNase-free commercial enzyme was used. Nevertheless, in some preparations, obtained from different sources, traces of DNase had been found. Since a high concentration of RNase is used in the preparation, potential contaminations could cause damage in low-salt buffer.

Compared with the approach of Gibson and Sulston (1987), the isolates are obviously of less quality, because the procedure simplifies the preparation to its bones. For many purposes, however, their purity is adequate and the

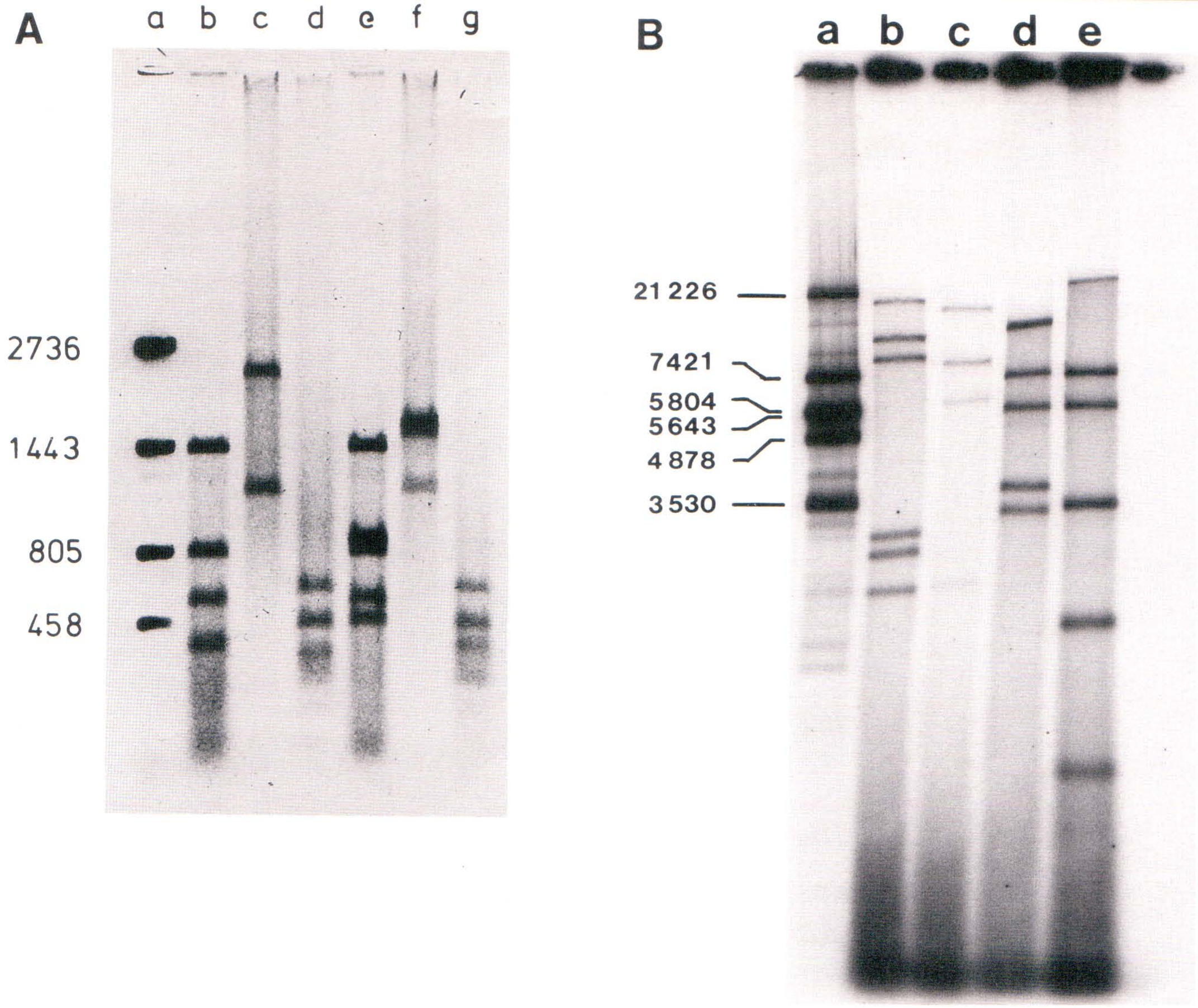

FIG 3. Restriction digestion of DNA derived from micropreparations. (**A**) Two pUC18-derivatives (clone 1: lanes b-d; clone 2: e-g) were cut with the restriction nuclease *Taq*I at 68°C (b, e), overlayered by paraffin to avoid evaporation, or with *Bgl*I (c, f) and *Hae*III (d, g) at 37°C as described in Protocol 3. Electrophoresis was in 1.5% (w/w) low-melting-point agarose. Standard (a) is pUC18-DNA of higher purity linearized with *Eco*RI (2,736 bp) and *Taq*I (1,443 bp, 805 bp, 458 bp). (**B**) Restriction patterns of lawrist4-recombinants cleaved with *Bam*HI as described in Protocol 3. A quarter of yields from micropreparations was loaded to lanes b to e, respectively. Lane a shows a standard of *Eco*RI-cut lambda-DNA. To visualize the bands the 0.65% (w/w) agarose gel was dried and autoradiographed for 1 h using Kodak XAR-5 film (Kodak, Rochester, NY).

velocity allows the handling of much larger clone numbers.

The Microprep protocol seems to be capable for automation. Using microtiter plates with a filter bottom instead of ordinary plates, centrifugation steps may be replaced by filtration, which would make the protocol even more suitable for large-scale application. A few experiments with Millititer-GV plates (Millipore Millititer system, Bedford, USA) showed reasonable results. The major obstacle was cross-contamination on account of the foaming of the SDS during the vacuum transfer of the "supernatants" after addition of solutions I, II, and III. This was circumvent by a previous incubation at −20°C for 15 min. While the high salt concentration prevented freezing, the SDS precipitated and was retarded onto the filter. However, the preincubation equalizes the time advantage of filtration, and the costs of preparation are considerably increased, since filtration plates are much more expensive than ordinary plates. Therefore, only a few preliminary experiments were performed and no claim of highly reproducible performance can be made.

After the ethanol-precipitation, which is necessary for cleavage with restriction enzymes, the DNA was taken up in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA (TE-buffer) first, to allow storage without any risk of unspecific digestion. To heat-inactivate the DNase contamination, Mg^{++} ions are required in the buffer. DNA incubated without Mg^{++} was destroyed at 68°C, as well as 85°C. In an earlier

TABLE 1

ENZYMES CHECKED FOR ACTIVITY IN TAK-BUFFER[a]

Restriction enzymes with 4 bp recognition sites			
*Alu*I (50 mM NaCl)	*Dpn*I	*Hpa*II	*Rsa*I
*Cfo*I	*Hae*III	*Hinf*I	*Sau*3A
*Dde*I	*Hha*I	*Mbo*I	*Taq*I (68°C)
Restriction enzymes with 5 bp recognition sites			
*Ava*II	*Fok*I	*Hga*I	
Restriction enzymes with 6 bp recognition sites			
*Acc*I	*Bgl*II	*Hind*III	*Sal*I (100 mM NaCl)
*Apa*I	*Bss*HII (50°C)	*Kpn*I	*Sca*I
*Ava*I	*Cla*I	*Nae*I	*Sma*I
*Ba*lI	*Eco*RI	*Nde*I (150 mM NaCl)	*Sph*I
*Bam*HI	*Hinc*II	*Pst*I	*Sst*I
*Bgl*I	*Hind*II	*Sac*II	*Xba*I
			*Xho*I
Restriction enzymes with 8 bp recognition sites:			
*Not*I (50 mM NaCl,0.01% (v/v) Triton X-100)			*Sfi*I (50°C)
DNA-modifying enzymes			
Alkaline phosphatase, CIP			
Dam-methylase	(0.08 mM S-adenosylmethionine)		
Klenow enzyme	(0.08 mM dNTPs[c])		
Reverse transcriptase, AMV[b]	(0.08 mM dNTPs)		
T4 DNA-polymerase	(0.08 mM dNTPs)		

[a]Incubation temperatures different from 37°C or supplements to TAK, which are necessary for the activity of particular enzymes, are indicated in brackets.

[b]AMV, avian myeloblastosis virus.

[c]dNTPs, dATP, dGTP, dCTP, dTTP (20 μM each).

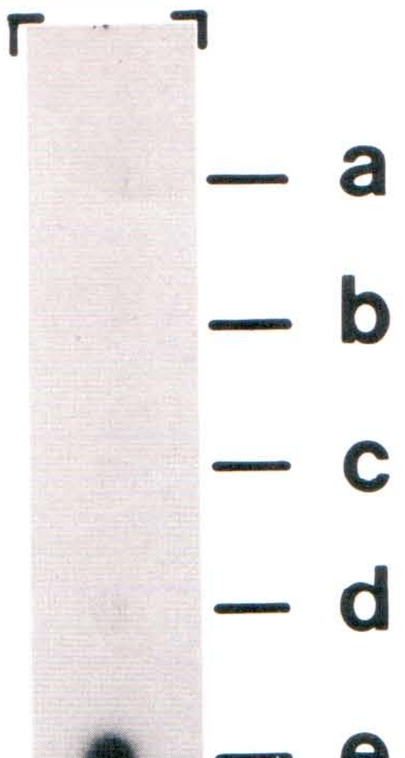

FIG 4. Specific hybridization to Microprep-DNA. Half the micropreparations of pTZ18R (b, d), a tenth of the identical preparations (a, c), and half the DNA-amount resulting from cosmid-clones (e, f) were spotted onto GeneScreen Plus (NEN, Boston, MA; area per spot approximately 10 mm^2). Spots g and h are controls without any DNA. A kinased 12mer oligonucleotide, complementary to lawrist4 vector-DNA (Craig A, personal communication, August 1988), was hybridized in 3 ml solution (Protocol 4). Stringent wash in TMAC (3 M tetramethylammonium chloride, 50 mM Tris-HCl [pH 7.5], 2 mM EDTA, 0.1% [W/W] SDS) was performed at 42°C. After exposition overnight only the cosmid spots could be seen. The autoradiography shown was overexposed for 72 hours to make the filter background visible as well.

PROTOCOL 4

OLIGONUCLEOTIDE HYBRIDIZATION

1. Precipitate the DNA accordingly to sections 1 and 2 of Protocol 3.
2. Spot 4 μl onto GeneScreen Plus (NEN) and put the membrane onto 3MM paper (Whatman) which is soaked with denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 2 to 3 min. Neutralize by immersion in 50 mM sodium phosphate (pH 7.5) for about 5 min. Dry the membrane at 80°C in a vacuum oven and crosslink the DNA by UV illumination (for details see NEN instructions accompanying the membrane).
3. Hybridize the oligonucleotide in 0.5 M sodium phosphate (pH 7.2), 7% (w/w) SDS, 1 mM EDTA (Church and Gilbert, 1984) with 1 Mcpm/ml of hybridization solution overnight at 30°C, after pre-hybridization in the same buffer for about 10 min at room temperature.
4. Rinse twice in 8 × SSC (1.2 M NaCl, 120 mM sodium acetate (pH 7.5)) at room temperature. Wash the filter for 2 × 2 min in TMAC wash solution (3 M tetramethylammonium chloride, 50 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.1% (w/w) SDS [Wood et al, 1985]) and rinse it briefly in 8 × SSC at room temperature.
5. Visualize bound oligonucleotide by autoradiography.

version of the protocol, solution I was supplemented with CDTA (diaminocyclohexan-tetraacetic acid) instead of EDTA. Those preparations could be heated without additional Mg^{++}, because natural Mg^{++} ions attached to the DNA were not chelated during the DNA isolation.

Since micropreparations rely on basic laboratory equipment only, the isolation of a large number of clones can be performed in almost every laboratory and at a reasonable cost. Therefore, the protocol seems to be appropriate for a wide range of applications, from simple size screening of CCC DNAs to mapping efforts at large areas of genomic DNA.

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