Rapid plasmid library screening using RecA-coated biotinylated probes

(nucleic acid isolation/avidin/affinity chromatography/cosmids)

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Contributed by Sherman M. Weissman, August 25, 1986

ABSTRACT A method for the rapid physical isolation of recombinant plasmids of interest from a mixture of plasmids such as a plasmid cDNA library is presented. This method utilizes (i) the ability of RecA protein to form stable complexes between linear single-stranded and circular double-stranded DNA molecules sharing sequence homology, and (ii) procedures allowing isolation of biotinylated nucleic acid. Biotinylated linear DNA probes coated with RecA have been used to screen reconstituted plasmid libraries consisting of two plasmid species, one homologous and the other heterologous to the probe. When the link between biotin and the nucleotide base could be cleaved by reducing agents, the complex was purified by streptavidin-agarose chromatography and the recovered plasmid was propagated in *Escherichia coli*. When the link was not cleavable the complex was bound to avidin in solution and purified by cupric iminodiacetic acid-agarose chromatography. The complex was then dissociated and the plasmids were propagated in *E. coli*. With either protocol, homologous plasmid recovery was between 10% and 20%, and enrichment was between 10⁴- and 10⁵-fold. Potential applications and extensions of this method, such as plasmid, cosmid, and phage library screening and facilitation of physical mapping of macroregions of mammalian genomes are presented and discussed.

The isolation of recombinant plasmids carrying DNA sequences of interest from a cDNA library by *in situ* colony hybridization (ref. 1, p. 312) is a tedious process that may require the screening of up to 10⁶ colonies (2). Dense plating of colonies to expedite the process makes isolation of individual positive clones impossible, necessitating secondary and even tertiary screenings; decreasing the colony density, while facilitating single-colony isolation, obviously lengthens the screening process.

Recent technical and conceptual advances have placed within reach the complete physical mapping and cloning of large segments of mammalian genomes (3, 4). Approaches that involve serial techniques such as chromosome walking and chromosome jumping require multiple screening steps of libraries containing several genomic equivalents. There are, therefore, substantial applications for a screening method that expedites recombinant clone isolation and allows parallel screening of one or more amplified libraries with several probes.

We have developed a method for the rapid physical isolation of recombinant plasmids of interest. Here we present both the principle and the experimental protocol of this method as it has been applied to reconstructed libraries to demonstrate and assess quantitative aspects of its features. The method makes use of (i) the ability of RecA, an *Escherichia coli* protein involved in genetic recombination,

to catalyze the rapid uptake of homologous single-stranded fragments by duplex DNA, thereby forming a triple-stranded complex (5), and (ii) the ability to selectively isolate nucleic acids bearing biotinylated nucleotides by their interaction with avidin (6). Ordinarily, RecA exchanges a new strand for an old one at the expense of ATP hydrolysis. It has been reported that, under certain sets of conditions, substitution of adenosine 5'-[γ -thio]triphosphate (ATP[γ S]), a nonhydrolyzable ATP analog, for ATP arrests the RecA reaction at the triple-stranded stage, blocking strand exchange (7, 8). For the purposes of this method we have devised conditions favoring the formation of stable triple-stranded complexes.

PRINCIPLE OF THE METHOD

As schematized in Fig. 1, a DNA probe bearing biotinylated nucleotides is converted to single-stranded (ss) form and RecA is allowed to polymerize on it. The RecA-probe nucleoprotein filaments are then allowed to react in solution with double-stranded (ds) plasmid DNA extracted from several library equivalents. The probe pairs with plasmids containing sequences homologous to itself and forms complexes.

Depending on the structure of the linker arm connecting the biotin to the nucleotide base, one of two purification procedures can be followed. If the linker contains a disulfide bond (9) the reaction mixture can be passed over a column of agarose to which streptavidin is covalently bound. The probe-plasmid complex and uncomplexed biotinylated probe are selectively retained on the gel; uncomplexed homologous and heterologous plasmids are eluted in the flow-through fraction. Probe-plasmid complexes (and uncomplexed probe) are released from the resin by elution with buffers containing reducing agents, such as dithiothreitol, which cleave the disulfide bond. The recovered plasmid is then used to transform competent *E. coli* bacteria. The alternative procedure is followed when the nucleotide linker is not cleavable and optionally when it is cleavable. Avidin is added to the reaction solution to bind the biotinylated probe, both free and complexed with plasmid. The reaction mixture is chromatographed over cupric iminodiacetic acid agarose beads (10). Avidin-probe and avidin-probe-plasmid complexes are selectively retained; uncomplexed homologous and heterologous plasmids are eliminated in the flow-through fraction. Retained DNA-avidin complexes are recovered by elution with buffers containing chelators such as EDTA. Since preliminary evidence suggests that avidin reduces the efficiency of

Abbreviations: ATP[γ S], adenosine 5'-[γ -thio]triphosphate; ss, single-stranded; ds, double-stranded; Bio-11-dUTP, biotin bound to the uracil of dUTP by an 11-atom linker; Bio-19-SS-dUTP, biotin bound to the uracil of dUTP by a 19-atom linker that includes a disulfide bond; kb, kilobase(s).

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FIG. 1. Plasmid isolation procedure. The RecA-coated single-stranded (ss) biotinylated probe is allowed to react in solution with the plasmids to be screened. Triple-stranded complexes are formed only with the plasmids bearing sequences homologous to the probe (thicker lines). They are purified by passage over either a streptavidin-agarose column when the linker joining the biotin to the base is chemically cleavable (left; DTT, dithiothreitol) or a cupric iminodiacetic acid column (right); uncomplexed plasmids are eliminated by both columns. The recovered plasmid is propagated in *E. coli*.

transformation, the recovered plasmid is dissociated from the probe by heating in a low ionic strength environment and further purified by extraction with phenol and dialysis prior to bacterial transformation.

METHODS

Biotinylation of Probe. The probe was biotinylated as described below. The biotinylated nucleotides used were Bio-11-dUTP (11) which had an 11-atom linker arm separating the biotin and the pyrimidine base, and Bio-19-SS-dUTP (9) with a 19-atom linker containing a disulfide bond. ^{32}P -labeled dNTPs were included when monitoring of the various steps of the method was desirable.

(i) **Nick-translation.** A typical reaction mixture, 60 μl final volume, contained 1 μg of DNA in 50 mM Tris-HCl at pH 7.5, 10 mM MgSO_4 , 0.1 mM dithiothreitol, 100 μM each of dATP, dGTP, and Bio-11-dUTP or Bio-19-SS-dUTP (gift from T. Herman), 120 μCi (1 Ci = 37 GBq) of $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ (Amersham, ≈ 3000 Ci/mmol), 30 units of DNA polymerase I (New England Biolabs), and DNase I (Sigma) at 27 pg/ml. The reaction mixture was incubated at 14°C for 1 hr, stopped by addition of EDTA to 10 mM, and heated at 68°C for 5 min. Labeled DNA was recovered by chromatography over Sephadex G-50 (Pharmacia) equilibrated and eluted with 10 mM Tris-HCl, pH 7.5/1 mM EDTA (TE buffer).

(ii) **Tailing by terminal transferase.** This was used only for DNA molecules having 3' protruding ends. The reaction mixture consisted of 1 μg of DNA in 100 mM potassium cacodylate at pH 7.2, 2 mM CoCl_2 , 0.2 mM dithiothreitol, 100 μM Bio-11-dUTP, 50 μCi of $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$, and 20 units of terminal deoxynucleotidyl transferase, added last. After incubation at 37°C for 45 min, an additional 20 units of

enzyme was added and the incubation was repeated. The reaction was terminated by EDTA added to 10 mM, and the DNA was recovered as described above, precipitated with ethanol, washed with 70% (vol/vol) ethanol, and resuspended in 50 μl of TE buffer.

(iii) **Labeling by T4 DNA polymerase replacement reaction.** The reaction mixture contained 1 μg of DNA in 33 mM Tris-HOAc at pH 7.9, 66 mM NaOAc, 10 mM $\text{Mg}(\text{OAc})_2$, 0.5 mM dithiothreitol, bovine serum albumin at 0.1 mg/ml, and 0.5 unit of T4 DNA polymerase (P-L Biochemicals). After incubation at 37°C for 7 min, dATP, dGTP, and Bio-11-dUTP were added to final concentrations of 150 μM each, dCTP was added to 10 μM , 50 μCi of $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ (3000 Ci/mmol was added), and Tris-HOAc, NaOAc, $\text{Mg}(\text{OAc})_2$, bovine serum albumin, and dithiothreitol were added to maintain previous concentrations. This reaction mixture was incubated at 37°C for 30 min, then dCTP was added to a concentration of 150 μM and the incubation was continued for an extra 60 min at 37°C. The reaction was stopped by addition of EDTA to 10 mM, and the mixture was heated at 65°C for 10 min, chromatographed, and processed as described before.

(iv) **Klenow fill-in reaction.** The reaction catalyzed by the Klenow fraction of *E. coli* DNA polymerase I was carried out by following standard protocols (ref. 1, p. 380); incubation was at room temperature for 15 min.

RecA Reaction. The appropriate volume of probe solution was put into an Eppendorf tube, along with 2 μl of nuclease-free bovine serum albumin (5 $\mu\text{g}/\mu\text{l}$ in TE buffer, pH 8.0), and the final volume was adjusted to 90 μl with 10 mM TE buffer, pH 8.0. After boiling for 5 min the solution was chilled on ice for 5 min and centrifuged for a few seconds. Then 10 μl of freshly made 10 $\times$ buffer (20 mM CoCl_2 /16 mM ATP γS /80 mM MgCl_2 /300 mM Tris-HCl, pH 8) and the appropriate amount of RecA (Pharmacia or Bethesda Research Laboratories) were added, and the mixture was swirled lightly in a Vortex mixer and incubated at 37°C for 10 min. Plasmid DNA was then added, and the tube was swirled lightly and incubated for 10 min. When the probe was radiolabeled, complex formation was monitored by gel electrophoresis. A 10- μl sample of the final reaction mixture was added to prechilled Eppendorf tubes containing 10 μl of 1 $\times$ TAE buffer (ref. 1, p. 156)/0.2% NaDodSO $_4$, and after Vortex mixing the solution was electrophoresed in a 0.8% agarose gel, which was subsequently autoradiographed.

Agarose-Streptavidin Chromatography. Buffers: buffer A, 10 mM Tris-HCl, pH 7.5/1 mM EDTA/0.3 M NaCl/salmon sperm DNA (phenol extracted and sonicated) at 10 $\mu\text{g}/\text{ml}$; buffer B, 10 mM Tris-HCl, pH 7.5/1 mM EDTA/50 mM NaCl; buffer C, 30 mM Tris-HCl, pH 8.8/0.1 mM EDTA/50 mM dithiothreitol (freshly made). A silane-treated 1-ml disposable syringe plugged with silane-treated glass wool was packed with 0.3 ml of streptavidin-agarose (Bethesda Research Laboratories) and washed with buffer A. After addition of proteinase K and NaDodSO $_4$ to the RecA reaction mixture to final concentrations of 0.2 mg/ml and 0.2%, respectively, it was incubated for 7 min at 37°C and immediately loaded into the column, which was then washed in sequence with buffer A (ten 1-ml fractions) and buffer B (two 1-ml fractions) and eluted with buffer C (one 5-ml fraction). The final 5-ml fraction was concentrated by extraction with butanol to about 400 μl , precipitated with ethanol, washed with 70% ethanol, reconstituted in 50 μl of autoclaved distilled H $_2\text{O}$, and used for *E. coli* transformation (HB101 competent cells, Bethesda Research Laboratories).

Cupric Iminodiacetic Acid Column. Buffers: buffer A, 20 mM NaHCO_3 /1 M NaCl/salmon sperm DNA (phenol extracted and sonicated) at 10 $\mu\text{g}/\text{ml}$; buffer B, 20 mM NaHCO_3 /50 mM NaCl; buffer C, 50 mM EDTA, pH 7.5/50 mM NaCl. All buffers were freshly prepared. Prior to chromatography avidin-DN (Vector Laboratories, Burling-

ame, CA) and 5 M NaCl were added to the RecA reaction mixture to final concentrations of 0.22 mg/ml and 1 M, respectively, and incubated at room temperature for 1–2 hr with gentle shaking every 10–20 min. Longer incubations were required when only one or two biotinylated nucleotides were present per probe molecule. Avidin concentrations between 0.02 and 0.36 mg/ml gave maximal complex retention by the column. A silane-treated 1-ml syringe was packed with 0.3 ml of iminodiacetic acid-agarose (Pierce) and washed in sequence with 10 column volumes of autoclaved distilled H₂O, 0.16 ml of CuSO₄ (5 mg/ml in distilled H₂O, filter sterilized), and 10 column volumes of buffer A. The sample was loaded and the column was eluted in sequence with buffer A (ten 1-ml fractions), buffer B (two 1-ml fractions), and buffer C (two 1-ml fractions). The last two fractions (eluted with buffer C) were diluted 6-fold with autoclaved distilled H₂O, heated at 68°C for 10 min, extracted with phenol once by gently mixing the two phases, concentrated by extraction with butanol to 0.2–1 ml, and dialyzed overnight at 4°C against 1 mM Tris-HCl, pH 7.5/0.1 mM EDTA/5 mM NaCl. The sample was then concentrated under negative pressure (Speed Vac) to about 100 μ l and used for *E. coli* transformation.

RESULTS

The RecA Reaction and Its Parameters. Fig. 2 demonstrates the formation of a complex between ss ³²P-labeled pBR328 fragments and ds circular pBR328 (lane A1). Of note are the following features: (i) the reaction is highly specific—no reaction is detected between the probe and heterologous plasmid (lane A4); (ii) there is no detectable complex formation in the absence of RecA (lane A3); (iii) complex formation appears to involve all topological forms of the ds ring (homologous plasmid) as detected by ethidium bromide staining (lane B1), and the extent of complex formation, judged by the intensity of the autoradiographic signal, ap-

FIG. 2. The RecA reaction. (A) Ten femtomoles of pBR328 was ³²P-labeled by nick-translation, producing fragments of median length 1.6 kilobases (kb) (ref. 1, p. 171), and used in reactions with ds circular DNA as indicated below. The RecA concentration was 1 μ M. Lane 1, 0.16 nM ds pBR328; lane 2, probe alone; lane 3, no RecA, 0.16 nM ds pBR328; lane 4, 2 nM ds M13 derivative, totally heterologous to the probe. The probe was electrophoresed out of the gel. (B) Topological forms and different electrophoretic mobilities of the ds DNA used in A; ethidium bromide staining. Lane 1, pBR328; lane 2, M13 derivative. (C) RecA reactions performed as in A except that the probe was both biotinylated (Bio-11-dUTP) and ³²P-labeled. Lane 1, ds pBR328; lane 2, probe alone. (D) The probe was 21 fmol of LN11A, a 6.5-kb pseudogene from the major histocompatibility complex, ³²P-labeled and biotinylated (Bio-19-SS-dUTP) by nick-translation. Lane 1, 0.11 nM cos 6 (a 38-kb recombinant cosmid containing LN11A as part of its insert) and 3.9 μ M RecA; lane 2, probe alone. The arrow indicates the probe-cosmid complex.

pears roughly the same for each form; (iv) biotinylated probes also carry out the RecA reaction (lanes C1 and C2); (v) the complex, kept in the reaction mixture at –20°C, is stable for at least 48 hr (data not shown); and (vi) dextran blue, used occasionally as a size marker during DNA chromatographic separation, completely inhibits the RecA reaction (data not shown). Fig. 2 also demonstrates complex formation between a biotinylated probe homologous to a portion of a 38-kb cosmid (lanes D1 and D2); this is, however, accomplished at a RecA concentration higher than that used for plasmids (see below).

Fig. 3 documents the (expected) dependence of the reaction on homologous plasmid concentration (A); a biphasic response to RecA concentrations in which complex formation increases up to a critical RecA concentration but decreases thereafter (12) (B); the dependence of the reaction on probe concentration, which appears to reach saturating levels (C); and the very rapid rate of complex formation, with the reaction being complete within the first minute (D).

Fig. 4 clearly demonstrates that the amount of RecA required to form complexes depends on the total DNA mass present in the reaction mixture. Severely diminished complex formation is noted in the presence of large excesses of heterologous plasmid (compare lane 1 with lane 2; and unpublished data), and formation is restored by increased RecA concentration (see lanes 3–6). There is a convenient range of RecA concentrations allowing maximal complex

FIG. 3. Four parameters of the RecA reaction. Reactions were performed as described in the text. Arrows indicate the superhelical forms. (A) Dependence on homologous ds plasmid concentration. pBR328 (8 fmol), ³²P-labeled and biotinylated (Bio-11-dUTP) by nick-translation, was used as probe; 1 μ M RecA; ds pBR328 concentrations were, from lane 1 to lane 5, 0.0032 nM, 0.016 nM, 0.032 nM, 0.1 nM, and 0.32 nM. (B) Dependence on RecA concentration. Probe as in A; 0.32 nM ds pBR328; and RecA concentration, from lane 1 to lane 7, 0.08 μ M, 0.16 μ M, 0.32 μ M, 0.64 μ M, 1.28 μ M, 2.56 μ M, and 5.12 μ M. (C) Dependence on probe concentration. RecA was 1 μ M; 0.032 nM ds pBR328; and probe ³²P-labeled and biotinylated as in A, at 0.14, 0.71, 1.4, 2.9, 4.3, and 5.7 fmol (lanes 1–6). (D) Time dependence of complex formation. Probe (7.2 fmol) as in A; 1 μ M RecA; samples were taken at 1, 2, 3, 5, and 10 min (lanes 1–5) after addition of ds pBR328 (0.32 nM).

FIG. 4. The RecA reaction in the presence of heterologous plasmid. All RecA reaction mixtures included 19 fmol of LN11A, ^{32} P-labeled and biotinylated (Bio-19-SS-dUTP) by nick-translation, as the probe; 0.12 nM ds pLN11A (pBR328 + LN11A) as the homologous target; 227-fold molar excess of heterologous ds plasmid (27.2 nM pMK102) as indicated below; and RecA at various concentrations. Lane 1, homologous target alone; lanes 2–6, both homologous and heterologous plasmids; RecA concentration from lanes 1 through 6; 1.6 μ M, 1.6 μ M, 2.6 μ M, 3.9 μ M, 4.7 μ M, 5.5 μ M.

formation (here between 2.6 and 5.5 μ M). This range has to be optimized for each RecA preparation and for the DNA concentration to be screened.

Performance of Chromatographic Methods. Fig. 5 illustrates the fate of both homologous and heterologous plasmids participating in RecA reactions, after streptavidin-agarose chromatography. Over 99% of the heterologous plasmid is eliminated in the first three fractions, while only traces are present in fractions 13–16, which contain the recovered homologous plasmid. Two-thirds of the homologous plasmid is found in fraction 1, along with about 10% of the probe, the latter being assessed by the distribution of its radioactivity. The remaining one-third is recovered from the column after treatment with dithiothreitol (fractions 13–16; these fractions also contained 80% of the probe). Similar fractionation profiles are obtained with cupric iminodiacetic acid columns (data not shown). It is clear, therefore, that both methods achieve purification by the combination of quantitative elimination of the heterologous plasmid in the early flow-through fractions and selective retention and subsequent release of the homologous plasmid-probe complexes.

Screening of Reconstructed Plasmid Libraries. The reconstructed libraries used in these experiments consist of (i) pLN11A (tetracycline-resistant, kanamycin-sensitive), a recombinant plasmid of pBR328 and LN11A (13), a 6.5-kb major histocompatibility complex pseudogene, and (ii) pMK102 (tetracycline-sensitive, kanamycin-resistant), consisting of pBR322, a short stretch of simian virus 40 sequences, and the kanamycin gene. The molar ratio of pLN11A to pMK102 is 1:219. This model library offers two advantages: first, the fate of each plasmid during screening can be monitored biologically by transformation owing to their differential antibiotic sensitivities; and, second, pMK102 and LN11A, being totally heterologous, do not form base-pair complexes with each other. As summarized in Table 1, this plasmid mixture was screened by using LN11A nick-translated with [α - ^{32} P]dCTP and Bio-19-SS-dUTP. The average recovery of the homologous plasmid is 14.3% after purification by streptavidin-agarose chromatography, with an average enrichment of $10^{4.6}$ -fold. Enrichments of 10^4 -fold to 10^5 -fold are obtained routinely by using this protocol and, on occasion, no detectable heterologous DNA is carried through.

When the probe is uniformly biotinylated by nick-translation with Bio-11-dUTP (bearing a noncleavable linker) and

FIG. 5. Chromatographic purification of biotinylated probe-plasmid complexes. Linear LN11A was biotinylated (Bio-19-SS-dUTP) but not radiolabeled by nick-translation, converted to ss form, and allowed to react in the presence of RecA with 0.12 nM pLN11A (pBR328 + LN11A) and 24 nM simian virus 40 and DNA or, in a second reaction mixture, with 27 nM pMK102 (heterologous to LN11A). The products of each reaction were chromatographed on a streptavidin-agarose column. Control reactions with radiolabeled probe documented complex formation. Portions of each column fraction were dot-blotted on nitrocellulose filters and plasmid DNA was quantitated by hybridization to ^{32} P-labeled pBR328 (no hybridization to either simian virus 40 DNA or LN11A). Data are expressed as percent of total plasmid DNA participating in each reaction. ●, pLN11A; ○, pMK102. Buffers A, B, and C are described in *Agarose-Streptavidin Chromatography*; buffer C cleaved the disulfide bond; 80% of the total radioactivity was present in the last five fractions of the control and 3.7% was retained by the gel.

the complex is purified by cupric iminodiacetic acid chromatography, no transformable homologous plasmid is recovered. Apparently, the multiple biotins in the probe bind several avidin molecules irreversibly or one avidin binds more than one biotin, thereby "locking" the plasmid-probe-avidin complex and producing a topologically unreleasable plasmid. Release of the nick-translated probe-plasmid complex from avidin by reduction of the disulfide bond of Bio-19-SS-dUTP gave variable results and was not pursued rigorously. In contrast, when the probe is end-biotinylated (as in Fig. 6) the target plasmid is released efficiently from the probe and produces *E. coli* transformants. In the experiment

Table 1. Reconstituted plasmid library screening

Recovery, %		
Homologous plasmid (pLN11A)	Heterologous plasmid (pMK102)	Enrichment
14.3	0.00028	$10^{4.6}$
(8.6–18.4)	(0–0.00054)	($10^{3.9}$ – ∞)

A mixture of 0.0124 pmol of pLN11A (pBR328 containing LN11A; tetracycline-resistant, kanamycin-sensitive) and 2.72 pmol of pMK102 (tetracycline-sensitive, kanamycin-resistant; totally heterologous to LN11A) (molar ratio 1:219) was screened with LN11A (a 6.5-kb major histocompatibility complex pseudogene) biotinylated by nick-translation. The LN11A-pLN11A complex was purified on a streptavidin-agarose column. The recovered plasmid(s) was used to transform *E. coli*. Half of the transformation mixture was plated on tetracycline plates and the other half on kanamycin plates. Recoveries were calculated on the basis of the number of colonies grown. Enrichment refers to the ratio of the values of pLN11A to pMK102 at the beginning and the end of the screening. Results are means of six experiments, with the ranges of obtained values in parentheses.

FIG. 6. Probe-plasmid complex purification by cupric iminodiacetic acid chromatography. A reconstituted plasmid library consisting of pLN11A and pMK102 in 200-fold molar excess was screened with LN11A (homologous to a portion of pLN11A). Protruding 5' ends of LN11A were reconstituted by a Klenow fill-in reaction using both [α - ^{32} P]dATP and Bio-11-dUTP. The RecA reaction and chromatographic purification were performed as described in *Cupric Iminodiacetic Acid Column*, except that 5 instead of 10 fractions were collected during elution with the high-salt buffer A. Radioactivity of each fraction was determined by Cherenkov counting. Fractions 1–5 were desalted by passage over a Sephadex G-50 column and then processed as the other three were. Portions of each fraction were used for *E. coli* transformation. Half of each transformation mixture was plated on a kanamycin plate and the other half on a tetracycline plate. Appropriate transformation controls were run in parallel. Homologous plasmid enrichment was almost 10^5 -fold.

of Fig. 6, the fate of both the homologous and heterologous plasmids is monitored simultaneously by transformation throughout the chromatographic separation, demonstrating the rapid elimination of the heterologous plasmid as well as an almost 10^5 -fold enrichment of the homologous plasmid by one application of the method.

DISCUSSION

Rapid methods for selective recombinant DNA isolation are useful for routine plasmid and cosmid library screening. Their significant application will be in expediting the complete physical mapping of large sections of mammalian genomes or moving from a linked gene to a distal gene of interest, approaches that require multiple library screenings utilizing several probes. The method diagrammed in Fig. 1 shortens substantially the actual process of library screening and, in addition, because of the brevity of the experimental protocol, permits multiple samples to be processed in parallel by a single investigator.

The work presented here demonstrates the applicability of the method to plasmid library screening. The data also suggest the feasibility of the method for screening cosmid libraries since the first essential step, "capturing" the target cosmid by the probe in the presence of RecA, has been accomplished. It should be emphasized that a technical aspect critical to the successful application of the RecA reaction is the selection of a RecA concentration appropriate for the level of total plasmid DNA present in a reaction mixture; the biochemical basis for this requires further investigation. The reconstructed library was designed so that at the final step the two plasmid species could be distinguished by their different antibiotic sensitivities. At this stage in the development of the method, the minimal but frequent

contamination of the recovered target DNA with heterologous plasmid necessitates a single *in situ* colony hybridization on a sparse plate to confirm the identity of clones isolated from a library.

The enrichment routinely obtained by application of this method is between 10^4 - and 10^5 -fold; occasionally, absolute purification of the target plasmid has been achieved. This high degree of enrichment is due to both the extraordinary specificity of the pairing reaction and the selectivity of the chromatographic procedures. This is demonstrated by electrophoresis of the RecA reaction mixture, chromatographic fractionation profiles, and *E. coli* transformation. Although recycling of the plasmids recovered from one application has not been attempted, it is plausible that further purification can be achieved in this way.

The biological recovery of homologous plasmid ranged between 10% and 20%. The first elution fraction contains practically all of the nonrecovered target plasmid but only 10% of the probe, suggesting either less than maximal complex formation or rapid release of some of the complexed plasmid. Although the data cannot distinguish between these two possibilities, we suspect that some topological forms of the complex (if they indeed form under our experimental conditions) may be more dissociable (paranemics) than others (plectonemics) (14). This is an area where the method could potentially be improved, especially for the development of alternative applications such as subtractive selections. However, for the purpose of a library screening the current range of recoveries is sufficient. In both theory and practice the starting material is not a limiting quantity, and multiple samples can be processed in parallel. It should be noted that the RecA reaction of Table 1, involving only 9 μ g of plasmid DNA, is equivalent to screening 2×10^{11} colonies.

In conclusion, we have developed a method for the rapid and efficient physical isolation of recombinant plasmids of interest from plasmid mixtures such as plasmid cDNA libraries; it is very likely that it can be applied to cosmids and perhaps phages as well. The method is expected to contribute to current efforts to characterize in detail macroregions of mammalian genomes.

Note Added in Proof. This method has been successfully employed for plasmid isolation using as a probe a synthetic oligonucleotide (43-mer).

We thank Drs. P. Lengyel and A. Weiner for critically reading the manuscript and C. M. Radding for helpful discussions.

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