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Construction and Characterization of Plasmid Libraries Enriched in Sequences from Single Human Chromosomes

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Plasmid libraries enriched in sequences from single chromosome types have been constructed for all human chromosomes. This was accomplished by transferring inserts from the Charon 21A phage libraries constructed by the National Laboratory Gene Library Project into Bluescribe plasmids. Insert material freed by complete digestion of the phage libraries with *Hin*dIII or *Eco*RI was cloned into the corresponding sites in Bluescribe plasmids. The sizes of the Bluescribe library inserts determined by gel electrophoresis range from near 0 to ~6 kb. Fluorescence *in situ* hybridization (FISH) with the plasmid libraries showed that all hybridize along both arms of the expected (target) chromosome type with varying intensity. However, the plasmid libraries for chromosomes 1, 4, 9, 11, 16, 18, and 20 hybridize weakly or not at all near the centromeres of the target chromosome types. The libraries for chromosomes 13, 14, 15, 21, and 22 cross-hybridize near the centromeres of all members of this group and hybridize weakly to the short arms of the target chromosomes. FISH with each library allows specific staining of the target chromosome type in metaphase spreads. The signals resulting from FISH with libraries for chromosomes 1, 4, 8, 9, 13, 14, 17, 18, 21, and Y are sufficiently intense to permit analysis in interphase nuclei. Examples of the use of these libraries for translocation detection, marker chromosome characterization, and interphase aneuploidy analysis are presented.

INTRODUCTION

Recombinant DNA libraries enriched in sequences from single chromosome types, originally produced to facilitate selection of probes for molecular genetic studies (Davies *et al.*, 1981; Krumlauf *et al.*, 1982; Latt *et al.*, 1989), also are proving useful as composite probes for *in situ* hybridization. Fluorescence *in situ* hybridization (FISH) with these libraries allows entire chromosomes to be stained both in metaphase spreads and in interphase nuclei (Kuo *et al.*, 1991; Lichter *et al.*, 1988; Pinkel *et al.*, 1988). This facilitates diagnosis of genetic diseases associated with specific aberrations (Fuscoe *et al.*, 1989; Gray *et al.*, 1990), identification of the chromosomal origin of new translocations or marker chromosomes (Evans *et al.*, 1991; Lichter *et al.*, 1988; Pinkel *et al.*, 1988), and rapid measurement of chromosome translocation frequencies to estimate the extent of induced genetic damage (Cremer *et al.*, 1990; Lucas *et al.*, 1991).

Chromosomally enriched recombinant DNA libraries have been constructed in several different vector systems by cloning DNA from chromosomes of a single type purified by fluorescence-activated sorting (reviewed by Gray, 1989). Phage have been used most often because of the relatively high efficiency with which small amounts of DNA can be cloned into these vectors and because of the efficiency with which they can be screened. Charon 21 A phage libraries are now available from the ATCC for all human chromosomes (Van Dilla *et al.*, 1986, 1988). However, these libraries are not ideal for all purposes. We have shown, for example, that plasmid colonies can be screened with higher sensitivity than phage plaques because of the increased amount and density of insert DNA in the plasmid colonies (Fuscoe *et al.*, 1989). They also are not suitable as probes for FISH because ~90% of the DNA is from the vector and may bind nonspecifically during hybridization.

We report here the construction and characterization of a set of chromosomally enriched plasmid libraries produced by subcloning inserts from the Charon 21A phage libraries into Bluescribe plasmids. These libraries are more suitable as hybridization probes because ~50% of the total DNA is human. These plasmid libraries have been characterized by

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gel electrophoresis and by FISH to human metaphase chromosomes. We illustrate the use of FISH with these libraries for detection of numerical and structural chromosome aberrations.

MATERIALS AND METHODS

Vectors and Libraries

Charon 21A phage libraries produced by the National Laboratory Gene Library Project (Van Dilla *et al.*, 1986, 1989) and listed in Table 1 were used as starting material for subcloning. Phage libraries produced at the Lawrence Livermore National Laboratory were constructed by cloning complete, *Hin*dIII digests of sorter-purified chromosomal DNA into the *Hin*dIII site in Charon 21A. Phage libraries produced at the Los Alamos National Laboratory were constructed by cloning into the *Eco*RI site in Charon 21A. Charon 21A is a 40-kb insertion vector (Williams and Blattner, 1980) and the average insert size in the libraries is ~4 kb; the insert to vector ratio is thus ~0.1.

Inserts released from the Charon 21A libraries by complete digestion with *Hin*dIII (or *Eco*RI) were subcloned into the *Hin*dIII (or *Eco*RI) portion of the multicloning site in Bluescribe plasmids (Stratagene, La Jolla, CA). Bluescribe is a small, 2.8-kb pUC-derived plasmid with T3 and T7 transcription promoters flanking the multicloning site.

Phage DNA Preparation

Each Charon 21A library was titered and then amplified at 37°C for 8 h in the *Escherichia coli* host strain LE392 on three 150-mm LB plates at a density of 3×10^5 plaque-forming units per plate. The plaques were not overlapping at this density. Each plate was overlaid with 12 ml SM buffer (0.1 *M* NaCl, 8 m*M* M_gSO_4, 50 m*M* Tris–HCl, 0.01% gelatin, pH 7.8) and phage were allowed to diffuse into the SM buffer overnight. The plate lysates were treated with 5% chloroform and the phage were pelleted by centrifugation in Beckman polyallomer tubes (1 × 3 in.) at 25,000 rpm for 90 min in a SW28 rotor. The resulting pellets were resuspended in 2.5 ml SM buffer and layered onto CsCl step gradients, $\rho = 1.7$ g/ml, $\rho = 1.5$ g/ml, $\rho = 1.45$ g/ml in Beckman ultra clear tubes ($\frac{1}{2}$ × 2 in.). The phage were then banded by centrifugation at 28,000 rpm in a Beckman SW50.1 rotor. Two bands were observed after centrifugation. The lower band contained packaged recombinant DNA and the upper band contained empty phage particles. The lower phage band was recovered from the gradient using a 26-gauge needle. The isolated phage were dialyzed extensively against 10 m*M* NaCl, 20 m*M* Tris–HCl, 10 m*M* $MgCl_2$, pH 7.5. DNA was released from the phage by digestion with 50 μg of proteinase K (in 5 m*M* EDTA, 0.5% SDS, 10 m*M* Tris–HCl) at 65°C for 1 h. The DNA was then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol. The DNA yields ranged from 50 to 75 μg with this procedure. Approximately 15 μg of DNA from each Charon 21A library was digested with 50 units of *Hin*dIII with REACT 2 buffer (or with 30 units of *Eco*RI with REACT 3 buffer) containing 4 m*M* spermadine for 4 h at 37°C. *Hin*dIII, *Eco*RI, and the REACT 2 and REACT 3 buffers were from Bethesda Research Laboratories (BRL, Grand Island, NY). The digested DNA was extracted with phenol:chloroform, twice with chloroform, and precipitated in 0.25 *M* sodium acetate with 2.5 vol of ethanol. The resulting purified DNA was resuspended in TE buffer (10 m*M* Tris–HCl, 1 m*M* EDTA, pH 8.0) at 1 μg/μl.

Plasmid Preparation

Fifty micrograms of the Bluescribe plasmid vector (BRL, Grand Island, NY) was digested with 200 units of *Hin*dIII in REACT 2 buffer (or 40 units of *Eco*RI in REACT 3 buffer) for 4 h at 37°C. The digested plasmid DNA was dephosphorylated by adding of 0.2 unit of calf alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) directly to the restriction digest. This mixture was incubated for 40 min at 37°C. The reaction was terminated by incubation at 60°C for 1 h. The digested, dephosphorylated DNA was then extracted as described above and ethanol precipitated with 7.5 *M* NH_4OAC, pH 7.5. This material was resuspended in TE buffer at 100 ng/μl.

Ligation and Transformation

The *Hin*dIII (or *Eco*RI)-digested, dephosphorylated plasmid DNA was ligated to the *Hin*dIII (or *Eco*RI)-digested Charon 21A DNA in 20 μl ligation buffer (BRL) using 1 μl T4 DNA ligase (400 U/μl; New England Biolabs, Beverly, MA) overnight at 15°C. Ligations were carried out using a variety of Charon 21A to plasmid DNA ratios. Those with 5–10 μg Charon 21A DNA and 100 ng plasmid DNA proved to be most efficient. Ligation reactions were assayed by diluting the reaction mix fivefold with water and transforming MAX Efficiency DH5α competent cells as described by BRL. The transformed cells from two libraries were plated onto separate LB plates containing 50 μg/ml ampicillin and 40 μg/ml Xgal to determine the recombinant fraction of these libraries. The fraction of blue colonies was taken as an estimate of the nonrecombinant fraction. These assays showed the libraries to be at least 90% recombinant.

Library Amplification

The libraries were amplified by spreading a transformation reaction on large LB plates containing 100

μg/ml ampillicin at a colony forming density of 10,000/plate. Sufficient transformants were used in each amplification so that the minimum total representation was always greater than 0.6×. Colonies were grown overnight at 37°C and were counted to determine the complexity of each library. The colonies were then removed by adding 2.5 ml Lauria–Burtani (LB) medium per plate and removing the colonies using a glass cell spreader. The cells from all plates were then pooled, divided into several aliquots containing 20% glycerol, and frozen.

DNA Preparation and Labeling

The bacterial libraries were grown overnight in LB broth with 50 μg/ml ampicillin. Plasmid DNA was prepared using the alkaline lysis procedure, with modifications, of Maniatis *et al.* (1982) and purified by centrifugation through a CsCl/ethidium bromide gradient at 48,000 rpm in a Vti50 rotor for 24 h. The DNA was cut with 0.0005 unit/μl of DNase I at 37°C for 10 min and the DNase inactivated at 70°C for 10 min. DNA size was determined on a neutral agarose gel and the cutting procedure repeated until the double-stranded DNA fragment size ranged from 0.1 to ~4 kb with most of the DNA at ~0.7 kb. The cut DNA was stored at −20°C for future use.

The cut plasmid DNA was labeled with biotin-14-dATP by nick translation. One microgram of DNase-digested DNA was nick translated in a reaction mixture containing 2.5 μl of 0.53 m*M* biotin-14-dATP (BRL), 0.5 μl of 10 U/μl Pol I, 5 μl of solution A1(0.2 m*M* for each of dTTP, dGTP, dCTP, 500 m*M* Tris (pH 8), 50 m*M* $MgCl_2$, 100 m*M* 2-mercaptoethanol, 100 μg BSA) in a total volume of 50 μl. The reaction was incubated at 15°C for 90 min and stopped with 3 μl of 500 m*M* EDTA. Labeled DNA was separated from unincorporated nucleotides with a Sephadex G-50 spin column. The concentration of the labeled probe DNA in the spin column eluent was ~20 ng/μl.

Gel Electrophoresis

DNA from each *Hin*dIII–pBS library was digested with 20 units of *Hin*dIII in a 50-μl reaction mixture containing 10 μg of DNA, 5 μl of REACT 2 buffer, and 2 μl of 0.1 *M* spermadine. These digests were incubated at 37°C for 2 h and then separated by electrophoresis through a 1% agarose gel in 1× TAE (40 m*M* Tris–acetate, 1 m*M* EDTA) containing 0.5 μg/ml ethidium bromide.

Fluorescence in Situ Hybridization

FISH with chromosomally enriched plasmid library probes was accomplished using a modification of previous published protocols (Pinkel *et al.*, 1986, 1988). Slides carrying metaphase spreads from human lymphocytes were prepared from methotrexate-synchronized cultures (Harper *et al.*, 1981) and fixed in methanol:acetic acid (3:1). The DNA in the slide-mounted cells was denatured by immersion in denaturing solution (70% formamide, 2× SSC, pH 7) for 2 min at 70°C, and dehydrated in 70, 85, and 100% ethanol series. Ten microliters of each hybridization probe mixture was denatured at 70°C for 5 min, incubated at 37°C for 1 h, and then applied to a 22-cm^2 area on a dried slide containing denatured target cells. Each 10-μl hybridization mixture contained 20 to 40 ng of biotinylated plasmid library DNA, 0.5 μg of herring sperm DNA, unlabeled human placental DNA (to inhibit hybridization of nonspecific repeated sequences) in 50% formamide, 2× SSC, and 10% dextran sulfate. Both herring sperm and human placental DNA were sonicated to ~200–600 bp. The amount of blocking human placental DNA, m_b, added to each hybridization was calculated as previously described (Pinkel *et al.*, 1988) using the equation $m_b = Qm_p/f_i$, where m_p is the amount of human DNA in the probe (approximately one-half of the total mass), f_i is the fraction of the genome comprised by the target chromosome i, and Q is the ratio of unlabeled to labeled sequences. Q was selected to be approximately 4 in these hybridizations. Thus, m_b ranged from ~0.5 μg for chromosome 1 to ~2.5 μg for chromosome 22. Hybridization was allowed to proceed overnight at 37°C under a sealed coverslip in a moist chamber. The slides were washed in three changes of 50% formamide, 2× SSC, pH 7, and twice in PN buffer (0.1 *M* NaH_2PO_4, 0.1 *M* Na_2HPO_4, and 0.1% Nonidet P-40; pH 8) at 45°C for 5 min each. The slides were then treated with alternating layers of fluoresceinated avidin and biotinylated goat antiavidin, both at 5 μg/ml in PNM buffer (5% nonfat dry milk, 0.1% sodium azide in PN buffer, centrifuged to remove solids) for 20 min each at room temperature until two layers of fluoresceinated avidin were applied. The avidin and goat anti-avidin (Vector Laboratories, Burlingame, CA) treatments were separated by two washes of 3 min each in PN buffer at room temperature. After the final avidin treatment, an antifade solution (Johnson and de C Nogueria Araujo, 1981) containing 2 μg/ml of propidium iodide (PI) was applied to counterstain DNA. The cells were viewed under a fluorescent microscope with a filter set (Omega optics) that allowed the fluorescence from FITC (green) and PI (red) to be visualized simultaneously. In some cases, the chromosomes were counterstained with 0.3 μ*M* DAPI to produce chromosome bands. Observation of these bands allowed determination of the chromosomal location of the hybridization signals. Observation of chromosomes stained in this manner was accomplished using two filter sets (Omega optics). One set allowed observation of the blue DAPI bands and the

TABLE 1

Characterization of pBS Libraries as by Fluorescence in Situ Hybridization

Probe	Phage library	Complexity	Hybridization to target[a]			Nontarget hybridization	Overall quality[b]
			p arm	q arm	Centromere		
pBS-1	LL01NS02	2.0	+; distal p weak	+	−−	ND	3
pBS-2	LL02NS01	1.3	+	+	++	20cen	2
pBS-3	LL03NS01	1.2	+	+	++	Weakly to 10cen	2
pBS-4	LL04NS02	0.8	+	+	−−	ND	3
pBS-5	LA05NS01	1.7	+	+	++	Strongly to centromeres on 1, 12, 16, 19; weakly to 3cen (see notes)	2
pBS-6	LL06NS01	1.4	+	+	+	ND	2
pBS-7	LL07NS01	1.6	+	+	+	ND	2
pBS-8	LL08NS02	1.2	+	+	++	ND	3
pBS-9	LL09NS01	2.5	+	+; ter −−	−−	ND	3
pBS-10	LL10NS01	2.5	+	+	+	ND	2
pBS-11	LL11NS01	2.0	+	+	−−	Weakly to 14	2
pBS-12	LL12NS02	0.7	+	+	++	ND	2
pBS-13	LL13NS02	1.3	+; ter −−	+	+	Weakly to 21cen	3
pBS-14	LL14NS01	1.5	+; ter −−	+	+	Weakly to centromeres on 2, 12, 13, 15, 21, 22	3
pBS-15	LL15NS01	1.5	+; ter −−	+	+	Weakly to centromeres on 13, 14, 21, 22	2
pBS-16	LL16NS03	1.7	+	+	−−	ND	2
pBS-17	LL17NS02	1.0	+	+	++	ND	3
pBS-18	LL18NS01	1.3	+	+	−−	2cen and weaker to 12cen and 20cen	3
pBS-19	LL19NS01	1.2	+	+	+	ND	1
pBS-20	LL20NS01	2.3	+	+	−−	ND	2
pBS-21	LL21NS02	0.6	+; ter −−	+	+	13cen, 14cen, 15cen and weaker to 22cen	3
pBS-22	LL22NS01	1.0	+; ter −−	+	+	Centromeres on 13, 14 15 and 21	2
pBS-X	LL0XNS01	1.3	+	+	+	Yp and proximal Yq	2
pBS-Y	LL0YNS01	1.8	+	+; distal q −−	+	Xp22.3, Xq21 and weakly throughtout X	3

Note. The hybridization of pBS-5 to centromeres on chromosomes 1, 3, 12, 16, and 19 can be suppressed by including Cot1 DNA during hybridization.

[a] Hybridization intensity: ND, no detectable hybridization; −−, light hybridization; +, detectable hybridization; ++, strong hybridization.

[b] Hybridization quality

Grade	Metaphase	Interphase
0	ND	ND
1	Marginal	ND
2	Good	Marginal
3	Excellent	Good

other allowed observation of the FITC hybridization signal.

RESULTS

Library Complexity and Insert Size

The clones included in the plasmid library construction were sufficient to yield libraries of intermediate complexity. Table 1 shows that the number of chromosome equivalents in each library ranges from 0.6 to 2.5 (assuming that all portions of each chromosome are clonable). The complexity and fragment size range of the libraries were investigated by analyzing the distribution of DNA fragment sizes in each *Hind*III library. DNA fragments in *Hind*III-digested samples of each of the pBS libraries were separated by

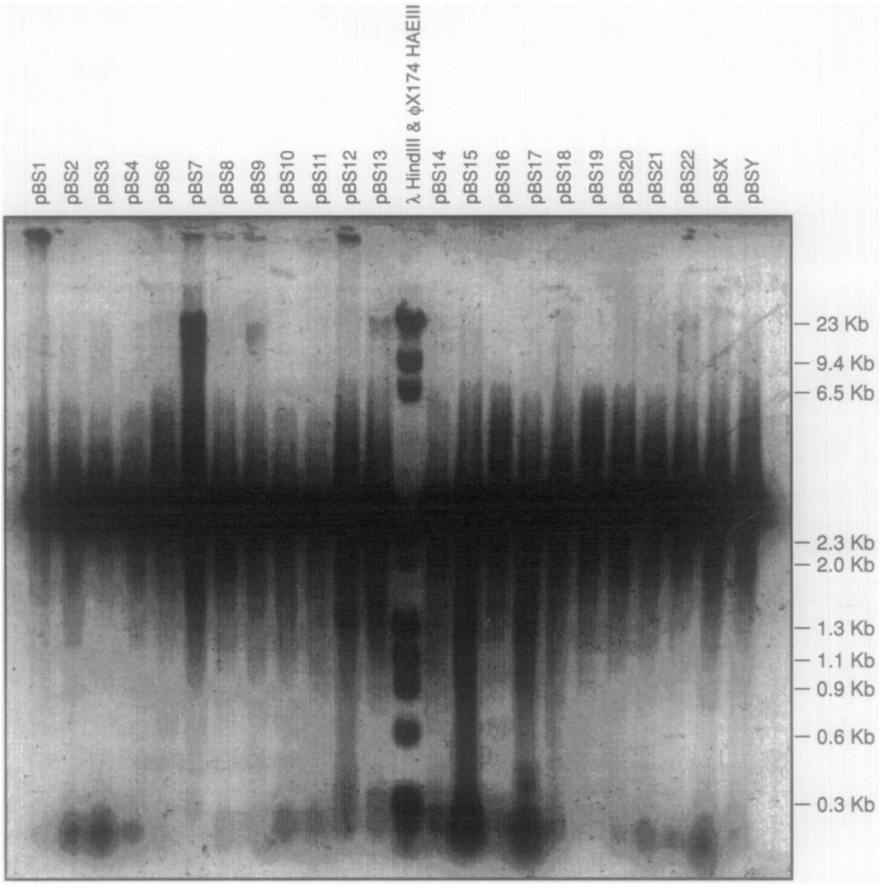

FIG. 1. Gel electrophoresis patterns for the *Hin*dIII–Bluescribe libraries. These comprise the libraries designated pBS-1 through pBS-4, pBS6 through pBS-22, pBS-X, and pBS-Y. The *Eco*RI digest for pBS-5 is not shown. All DNA samples were digested to completion with *Hin*dIII and separated by electrophoresis through 1% agarose gels containing ethidium bromide. The origin of the DNA in each lane is indicated above that lane. DNA size markers composed of a *Hin*dIII digest of λ phage and *Hae*III digest of φX174 DNA were run in a middle lane. The sizes of the various restriction fragments are shown to the right.

gel electrophoresis and stained with ethidium bromide. The gel photograph in Fig. 1 shows that the recombinant DNA fragments in the libraries constitute continua ranging in size from a few hundred base pairs to >6 kb with media around 2–3 kb. Thus, the libraries are not dominated by a few clones and their average insert size to vector size ratios are ~1.

Fluorescence in Situ Hybridization

All libraries were characterized by FISH to metaphase spreads and to interphase nuclei. Hybridization patterns for all of the libraries are shown in Fig. 2. The results determined from analysis of the hybridization patterns on several metaphase spreads and to interphase nuclei for each library are summarized in Table 1. All *Hin*dIII libraries except that for chromosome 5 show hybridization more or less continuously along the target chromosome type. The *Hin*dIII library for chromosome 5 seemed to be heavily contaminated with sequences from chromosome 10, probably as a result of similar contamination in the starting phage library LL05NS01. As a result, the *Eco*RI library for chromosome 5, LA05NS01 was used and is designated pBS-5. Some libraries (pBS-1, pBS-4, pBS-8, pBS-9, pBS-13, pBS-14, pBS-17, pBS-18, pBS-21, and pBS-Y) hybridize more intensely than the others. Hybridizations with these libraries stain the target chromosomes distinctly in metaphase spreads and in interphase nuclei. The remaining libraries hybridize with an intensity sufficient to allow determination of chromosomal identity in metaphase spreads. However, their utility in interphase nuclei is marginal. Several libraries (pBS-1, pBS-4, pBS-9, pBS-11, pBS-16, pBS-18, and pBS-20) fail to hybridize to the centromeres of the target chromosome types. Other libraries (pBS-13, pBS-14, pBS-15, pBS-21, and pBS-22) do not hybridize to the short arms of the target chromosomes but do cross-hybridize near the centromeres of the acrocentric chromosomes. The library pBS-9 does not hybridize to 9qter. Others (pBS-2, pBS-3, pBS-5, pBS-8, pBS-12, and pBS17) hybridize strongly to the centromeres of the target chromosomes. In addition, pBS-5 hybridizes to the centromeres of chromosomes 1, 3, 12, 16, and 19; however, this can be effectively suppressed by includ-

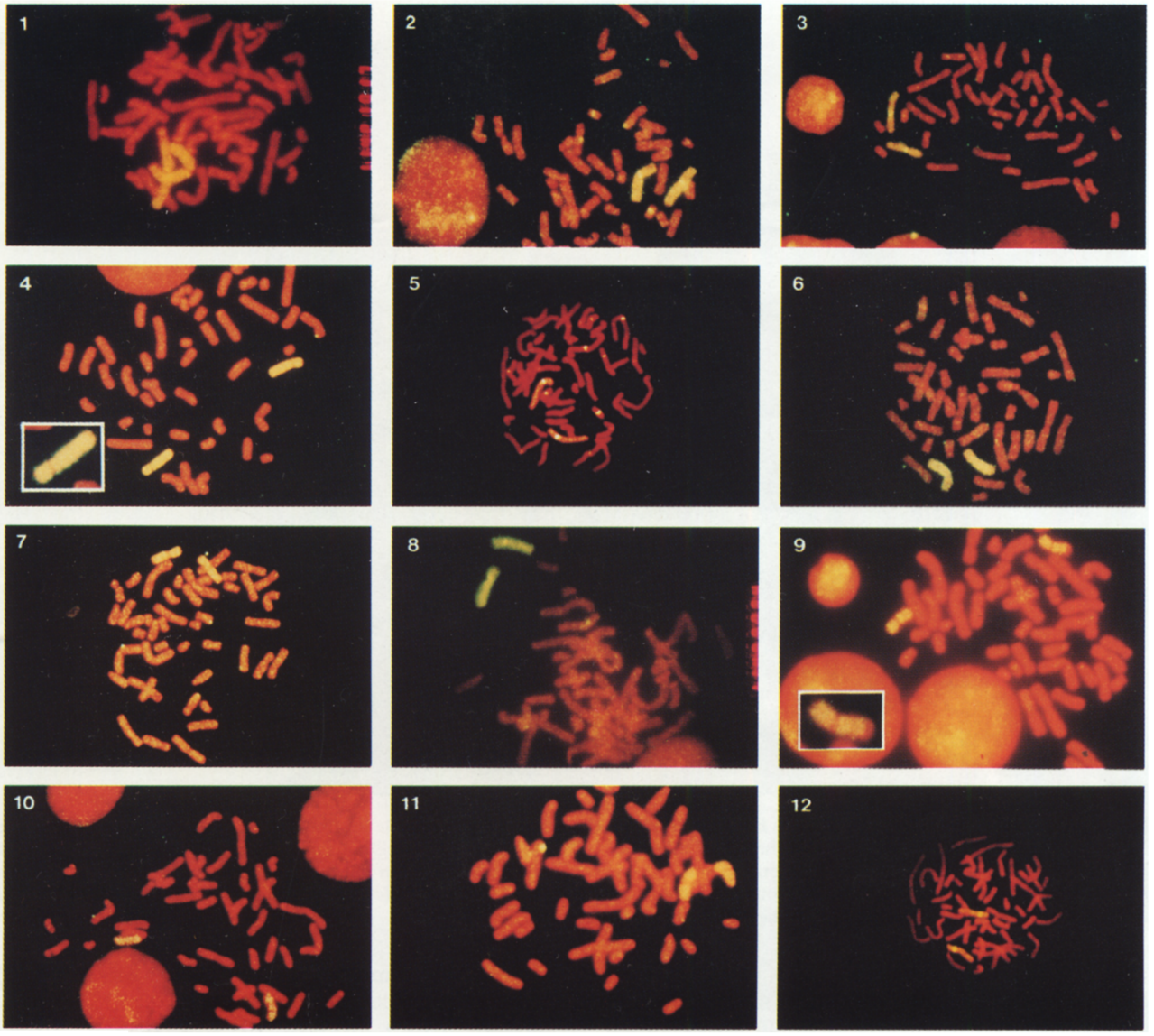

FIG. 2. Fluorescence photomicrographs of human metaphase chromosomes stained by FISH with each of the pBS libraries. The number of the target chromosome type is indicated in the upper left corner of each panel. Enlarged photomicrographs are shown for chromosomes 4, 9, 13, 21, 22, X, and Y so that the hybridization patterns for these libraries can be seen more clearly. The hybridized probe was detected using FITC and appears yellow in the photograph. The spreads were counterstained with propidium iodide so that the chromosomal regions to which the probe does not hybridize appear red.

ing Cot1 DNA (BRL) in the hybridization mixture (data not shown). pBS-18 does not appear to hybridize to the alpha satellite repeat regions known to be at the centromere of chromosome 18 but does hybridize to the centromeric regions of chromosomes 2, 12, and 20. pBS-X hybridizes intensely to the X chromosome as well as to Yp and proximal Yq. pBS-Y hybridizes strongly to Yp, proximal Yq, Xp22.3, and Xq21 and weakly all along chromosome X. It does not hybridize to distal Yq.

Aberration Detection

These libraries facilitate detection of both numerical and structural chromosome aberrations. Figures 3A–3C, for example, show hybridization and DNA images of a metaphase spread prepared from the human fibroblast cell line GM01881 (NIGMS Human Genetic Mutant Cell Repository, Camden NJ). Hybridization was accomplished using digoxigenin-labeled pBS-1 (detected using anti-digoxigenin-FITC; Fig. 3A) and biotin-labeled pBS-21 (detected using avidin-TR; Fig. 3B). The spread was counterstained using DAPI (Fig. 3C). The karyotype for GM01881 is 46,XY,t(1:21). The derivative chromosomes resulting from the translocation stain with both pBS-1 and pBS-21 and thus can be scored without difficulty. Figures 3D and 3E show hybridization and DNA images to a metaphase spread prepared from a human lung

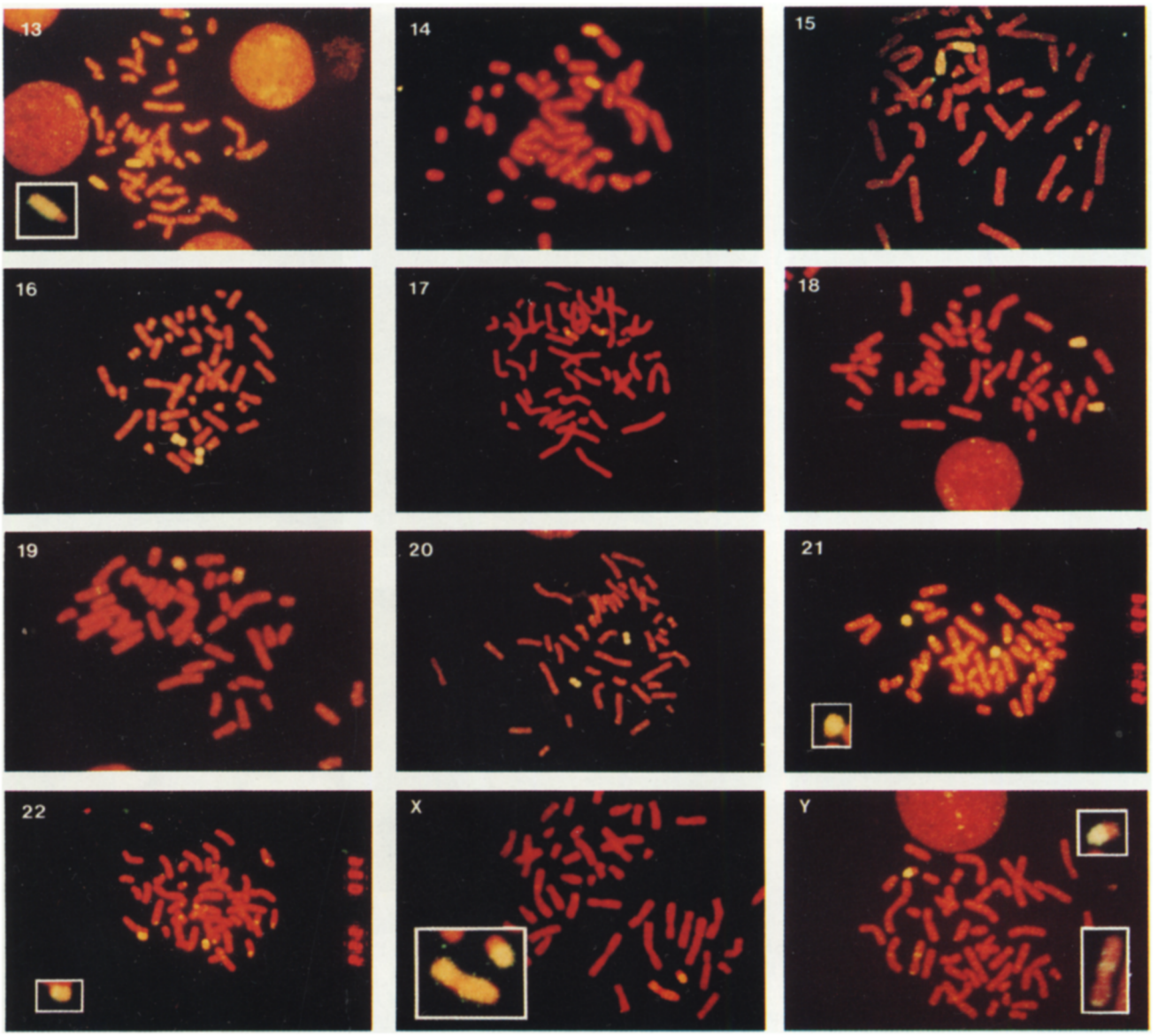

FIG. 2.—*Continued*

cell carrying a marker chromosome. Hybridization was accomplished using biotinylated-pBS-15 (detected using avidin-FITC; Fig. 3D) and DAPI was used as a counterstain (Fig. 3E). This hybridization shows a small marker chromosome that originated from chromosome 15. Figure 3F shows hybridization to an interphase nucleus from a preparation of cultured amniocytes that were trisomic for chromosome 21. Hybridization was with biotinylated pBS-21 (detected using avidin-FITC) and PI was used as the counterstain. Three hybridization domains are clearly visible.

DISCUSSION

Chromosomally enriched *Hin*dIII (or*Eco*RI) recombinant DNA libraries originally produced in the phage vector Charon 21A by the National Laboratory Gene Library Project have been subcloned into Bluescribe plasmids to form a series of libraries designated pBS-1 through pBS-22 plus pBS-X and pBS-Y. The suffix for each library designator indicates the chromosomal origin of the cloned material. These libraries have been characterized by gel electrophoresis and by FISH. Overall, the pBS libraries appear as continua on ethidium bromide-stained gels. In addition, all libraries hybridize more or less continuously along the appropriate chromosomes. This indicates that the libraries (and their Charon 21A progenitors) are complex and substantially enriched in sequences from the target chromosome type.

Several of the libraries do not hybridize to portions of the target chromosome. Libraries for chromosomes

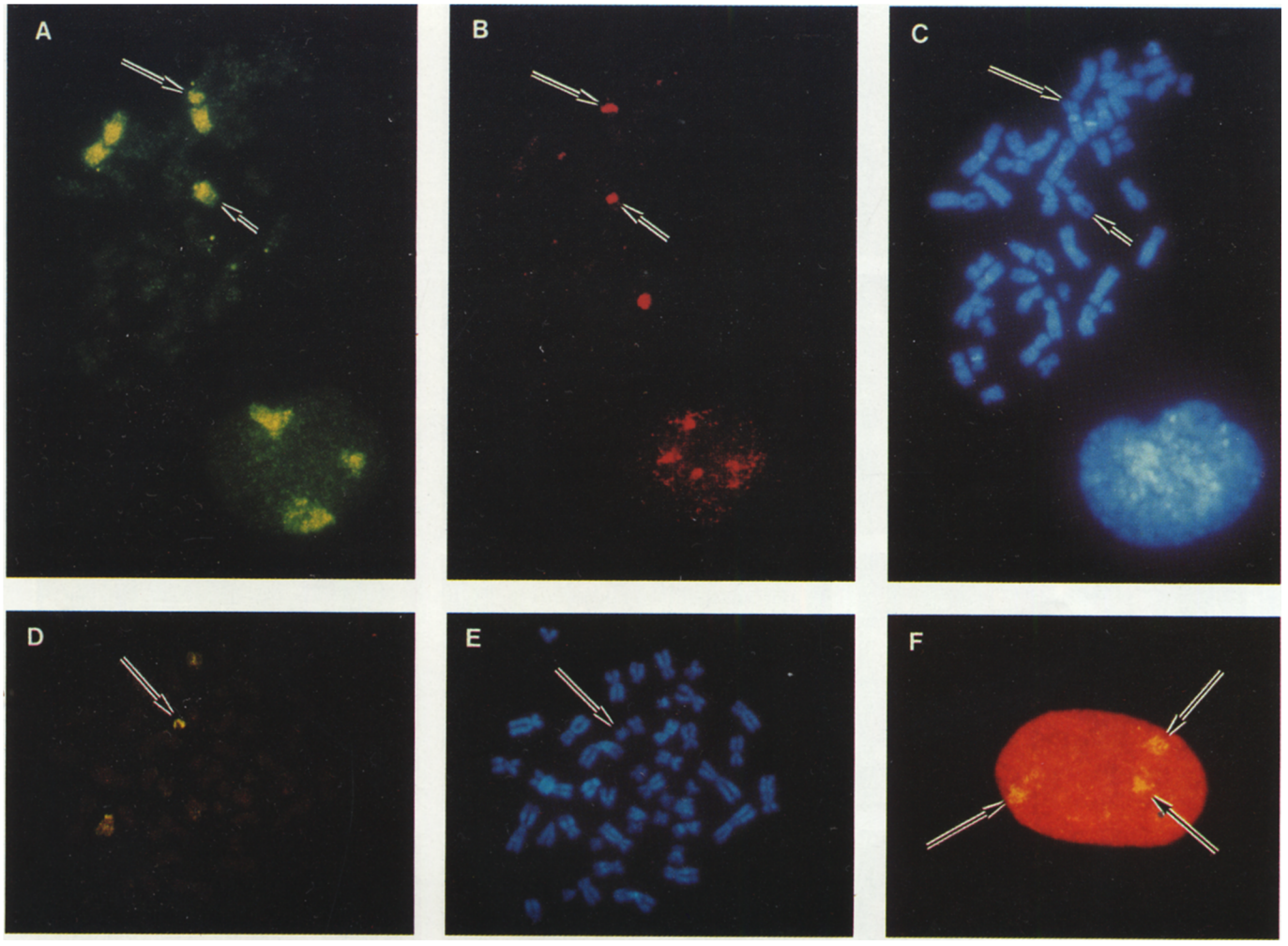

FIG. 3. (A–C) Photomicrographs of metaphase spreads prepared from human fibroblast cell line GM01881 (NIGMS Human Genetic Mutant Cell Repository, Camden, NJ). (**A**) FISH with pBS-1 (detected with FITC) and (**B**) FISH with pBS-21 (detected with Texas red) to the same spread. (**C**) The DAPI image of this spread. The karyotype for GM01881 is 46,XY,t(1:21)(1qter→1p31::21q22→21qter; 21pter→21q22::1p31→1pter). The arrows indicate the derivative chromosomes that result from the t(1;21) translocation. (**D**) FISH with pBS-15 (detected with FITC) to a metaphase spread prepared from a human lung cell carrying a marker chromosome. (**E**) The DAPI image of the same spread. The arrow indicates a marker chromosome. (**F**) FISH with pBS-21 to an interphase nucleus trisomic for chromosome 21. The arrows indicate the three hybridization domains.

1, 4, 9, 11, 16, 18, and 20, for example, do not hybridize near the centromeres. We believe that this is due to the absence of sequences for these chromosome regions in the original complete, *Hin*dIII-digest phage libraries (as opposed to competitive inhibition of the binding of these sequences during FISH). The existence of such deficiencies is not surprising since most human chromosomes carry highly repeated DNA sequences near their centromeres (Bauman *et al.*, 1989, Cooke and Hindley, 1979; Waye and Willard, 1987). The lack of a *Hin*dIII site in the repeated region (often several megabases in extent) would make these regions unclonable in Charon 21A since the maximum cloning capacity of this vector is only ~9 kb. This interpretation is supported by the restriction analysis of a large repeated sequence on chromosome 1 (Cooke and Hindley, 1979). This study showed that the repeated sequence does not contain a *Hin*dIII site but does contain *Eco*RI sites. Thus, hybridization with pBS-1 (made from the complete-digest *Hin*dIII library LL01NS02) would be expected to be weak in this region but hybridization with inserts from the *Eco*RI library LA01NS01 would be expected to be intense. Hybridization in the present study and in that of Lichter *et al.* (1988) shows that this is the case. The libraries pBS-2, pBS-3, pBS-5, pBS-8, pBS-12, and pBS-17, on the other hand, hybridize intensely to the centromere of the target chromosome type. This suggests the presence of a strongly repeated centromeric sequence in each of these libraries. The pBS libraries for chromosomes 13, 14, 15, 21, and 22 hybridize only weakly to the short arms of these chromosome types. These are the sites of the ribosomal DNA sequences that comprise the nucleolar organizer region (Hen-

derson *et al.*, 1972). Restriction mapping of these sequences has shown them to be deficient in *Hin*dIII sites. Thus, their absence in complete, *Hin*dIII-digest libraries is to be expected.

Several libraries hybridize to sequences on chromosomes other than the target chromosome type. pBS-18, for example, hybridizes near the centromeres of chromosomes 2, 12, and 20 but not to the large centromeric repeat known to be present near the centromere of chromosome 18 (Bauman *et al.*, 1989). We speculate that this may be caused by the presence of an element homologous to a weakly repeated sequence on chromosomes 2, 12, and 20 and the lack of an element for the chromosome 18-specific alpha satellite sequence (perhaps because the chromosome 18 repeat element lacks *Hin*dIII sites). pBS-5 hybridizes to the centromeres of chromosomes 1, 3, 12, 16, and 19. However, this can be suppressed by including Cot1 DNA in the hybridization mixture so that it does not interfere with cytogenetic analyses based on FISH with this probe. pBS-13, pBS-14, pBS-15, pBS-21, and pBS-22 cross-hybridize weakly near the centromeres of all acrocentric chromosomes. This may be due to the presence of repeated sequences (other than those for the ribosomal sequences), such as the 724 family (Kurnit *et al.*, 1984, 1986), that are common to all of the acrocentric chromosomes. The hybridization characteristics of pBS-X and pBS-Y are also noteworthy. Both hybridize to Yp and neither hybridizes to distal Yq. This suggests that sequences from this highly repeated region of chromosome Y lack *Hin*dIII sites. In addition, pBS-Y hybridizes more or less continuously along chromosome X (strongly near Xq21 and Xp22.3). These hybridizations indicate that pBS-Y and pBS-X contain sequences from the pseudoautosomal regions of the X and Y chromosomes at Xp22.3 and Yp11.3. In addition, hybridization with pBS-Y suggests the existence of a region of substantial Y homology at Xq21 and indicates moderate Y homology throughout X (Davies *et al.*, 1987).

FISH with these libraries allows selected chromosome types to be stained intensely and specifically both in metaphase spreads and in interphase nuclei. These libraries are preferable to the parent Charon 21A libraries as hybridization probes because their increased insert to vector DNA content ratio decreases the amount of nonspecific hybridization that results from the binding of labeled vector sequences. In addition, amplification and isolation of DNA are easier from plasmid libraries than from phage libraries. FISH to metaphase spreads facilitates detection of numerical and structural aberrations and determination of the chromosomal origin of the involved chromosomes (Gray *et al.*, 1990; Lichter *et al.*, 1988; Pinkel *et al.*, 1988) as long as the aberrations involve regions covered by the hybridization probes. Figure 3, for example, shows that translocations and marker chromosomes can be detected and characterized using FISH with the libraries described in this paper. However, FISH with these libraries may not allow detection of marker chromosomes or subtle rearrangements involving regions not represented or weakly represented in the libraries. For example, FISH with pBS-9 will not detect the chromosome 9 material translocated to chromosome 22 in the t(9;22) rearrangement associated with chronic myelogeneous leukemia (data not shown). Thus, the regions to which these probes do not hybridize (see Table 1) should be understood prior to their use for chromosome aberration analysis. The deficiencies noted in these libraries are now being removed by adding inserts cloned into Bluescribe plasmids from the *Eco*RI–Charon 21A libraries produced at Los Alamos by the National Laboratory Gene Library Project to the pBS libraries. These more complex libraries will be made available as they are produced. The deficiencies in the pBS libraries have proved useful in some cases, however, since the subtle nonuniformities in hybridization patterns may allow discrimination between chromosome types that are similar in size when more than one library is used simultaneously (e.g., discrimination between chromosomes 1 and 2 during hybridization with pBS-1 and pBS-2 can be accomplished using the fact that pBS-1 does not hybridize to 1cen while pBS-2 does hybridize to 2cen). Figure 3 also illustrates that FISH with some of these probes can be used to determine the number of copies of the target chromosome in interphase nuclei of homogeneous cell populations (e.g., human amniocytes or chorionic villus cells taken for prenatal diagnosis of genetic disease; Fuscoe *et al.*, 1989; Kuo *et al.*, 1991; Pinkel *et al.*, 1988). This application is much more demanding than metaphase analysis, however, and we recommend that only libraries with a grade of 3 (Table 1) be considered for this purpose.

The libraries listed in Table 1 also serve as convenient sources for probes that may be useful in genetic linkage analysis or to span rare restriction sites. Users should be aware, however, that some of the Charon 21A libraries from which the pBS libraries were made have been found to be contaminated at low level (~1%) with GC-rich bacterial DNA (Van Dilla; private communication). This has been found to complicate isolation of GC-rich sequences such as *Not*I linking clones.

These libraries will be made available for experimental purposes upon written request.

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