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Isolation and chromosomal localization of unique DNA sequences from a human genomic library

(recombinant DNA/human chromosomes/synteny analysis/hybrid cell clone panel)

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ABSTRACT **Recombinant bacteriophage λ from a human genomic library were screened to identify human DNA inserts having only unique sequences. Unique human inserts were found in about 1% of the phage screened. One recombinant phage, P3-2, was studied in detail. It contains a human insert of 14.7 kilobases with four internal *Eco*RI cleavage sites. A restriction map was constructed for *Eco*RI and *Bam*HI sites. Hybridization of the ^{32}P-labeled P3-2 probe to a Southern blot of *Eco*RI-digested total human DNA yielded distinct bands at positions corresponding to the human insert fragments contained in P3-2. By using a series of human–Chinese hamster somatic cell hybrids containing unique combinations of human chromosomes, the human DNA segment in phage P3-2 was assigned to human chromosome 22 by blot hybridization and synteny analysis. In addition, another human DNA segment, 11.4 kilobases, in phage P3-10 was assigned to human chromosome 10 by similar procedures. With this approach, more unique DNA sequences can be isolated, assigned to specific human chromosomes, and used as genetic markers for gene mapping and linkage, polymorphism, and other genetic studies in the human genome.**

Advances in recombinant DNA technology have provided a novel system for generating cloned human DNA fragments which can be amplified to any desired extent to serve as genetic markers (1, 2). Presumably, the number of such markers that can be made has no limit and could eventually encompass the entire human genome. These DNA fragments can be mapped to specific human chromosomes and to particular regions of each chromosome (2). Furthermore, DNA polymorphisms can be explored in these markers (3) for use in detecting linkage with other markers such as specific morphological traits and inherited diseases (4).

A procedure for isolating the DNA fragments from a specific human chromosome has been described (2). In this approach, a human–Chinese hamster hybrid cell clone containing a complete Chinese hamster genome plus a single human chromosome, chromosome 11, was used. Recombinant bacteriophage λ were constructed from DNA of these hybrid cells, some of which contained human DNA inserts derived from the specific human chromosome used. Moreover, by using a series of cell hybrids each containing a different specific terminal deletion of human chromosome 11, regional mapping of five human DNA inserts was achieved (2). This approach has been extended to other human chromosomes (5).

In the present paper, we demonstrate an approach for isolating recombinant phage containing human unique DNA inserts of unknown origin but whose chromosomal location can be determined by standard synteny analysis. The chromosomal assignment of a human unique DNA sequence in a recombinant phage is achieved by blot hybridization to test the presence or absence of the human DNA fragment used in a panel of human–Chinese hamster cell hybrids which contain unique combinations of human chromosomes. This approach appears to be general and permits assignment of DNA segments to each of the 24 human chromosomes.

MATERIALS AND METHODS

Growth and Screening of Recombinant Phage from the Maniatis Human Genomic DNA Library. Random samples of recombinant λ phage from the human genomic DNA library (1), kindly supplied by T. Maniatis, were inoculated into 100-mm agar plates containing a confluent lawn of *Escherichia coli* DF50 SupF. Individual phage colonies were picked and reinoculated onto grids of agar plates containing either 50 or 100 phage per plate. Phage plaques were transferred to nitrocellulose filters by the method of Benton and Davis (6), and the filters were hybridized with ^{32}P-labeled total human DNA. The phage colonies that showed no hybridization were isolated and tested twice again by the same procedure to confirm their lack of hybridization to human middle-repetitive sequences. All recombinant DNA experiments involved an EK2 host–vector system and were conducted under P2 containment according to National Institutes of Health guidelines.

Cultured Mammalian Cells. The Chinese hamster ovary cell mutant ade$^-$B (7) and the human fibrosarcoma cell line HT-1080 (8) were grown in F-12 medium supplemented with 8% fetal calf serum.

Human–Chinese Hamster Cell Hybrids. Various auxotrophic mutants derived from Chinese hamster ovary cells CHO-K1 were hybridized to different kinds of human cells, and more than 40 primary clones were isolated. Isozyme analysis was performed in these hybrids to determine the presence or absence of specific isozyme markers for all the human chromosomes except Y. In addition to various numbers of human chromosomes, each hybrid contained one or, more often, two CHO-K1 genomes. The latter tend to retain their human chromosome complements more stably than do the hybrids with only one CHO-K1 genome. Several hybrids have been analyzed karyotypically and the human chromosomes found in these hybrids in general agreed with the results of isozyme analysis. We are aware of the possibility that certain human chromosomes may not be intact in some hybrids through deletions or translocations at levels that may or may not be microscopically detectable.

From this series of hybrid clones, seven were chosen for their unique combinations of human chromosomes capable of discriminating all 24 human chromosomes except Y. These seven hybrids constitute the "clone panel." In addition, 13 other

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Abbreviation: kb, kilobase(s).

hybrids have been selected and used for confirmatory studies of the synteny relationship established by the original clone panel. The hybrids were grown in F-12 medium supplemented with 10% fetal calf serum or in F-12D medium supplemented with the macromolecular fraction of fetal calf serum.

Preparation of DNA from Cultured Mammalian Cell Lines and Cell Hybrids. The human HT-1080, the CHO-K1/ade$^-$B, and the human–Chinese hamster hybrid cells were grown in 150-mm plates and harvested by trypsinization. The procedures for extracting DNA were similar to those described by Gusella *et al.* (9), except that the first phenol extraction was followed by one chloroform and another phenol extraction before the three extractions with chloroform/isoamyl alcohol, 24:1 (vol/vol).

Preparation of DNA from Phage. The Charon 4A phage were propagated in 50-ml cultures of *E. coli* DP50 SupF. After a culture was lysed, chloroform was added together with DNase and RNase, and the lysate was held at 4°C for 1 hr followed by centrifugation at 5900 × *g* for 15 min. The supernatant was removed and centrifuged at 30,900 × *g* for 3 hr to sediment the phage. The pellet was collected, suspended in an Eppendorf tube containing 0.2 ml of buffer (0.1 M Tris, pH 7.9/0.3 M NaCl), and held at 4°C overnight with shaking. The phage DNA was extracted three times with phenol saturated with 1 M NaCl/20 mM Tris, pH 8/1 mM EDTA, and once with phenol/chloroform, 1:1. The aqueous phase was transferred to another Eppendorf tube and extracted three times with ether. The residual ether was evaporated, and the DNA was precipitated with 2 vol of ethanol and kept at −20°C overnight.

Restriction Enzyme Digestion, Agarose Gel Electrophoresis, and Southern Transfer. Restriction endonucleases were obtained from New England BioLabs or Miles. Horizontal electrophoresis on 0.75% agarose (Sigma), used to separate digested DNA fragments, was followed by staining with ethidium bromide (5 μg/ml). For transfer of DNA from gels to nitrocellulose filters, the method of Southern (10) was used with the modifications of Wahl *et al.* (11).

DNA Labeling, Filter Hybridization, and Autoradiography. Phage DNA was labeled with [α-^{32}P]dCTP (New England Nuclear) by nick-translation (12). High specific activity (>10^8 cpm/μg of DNA) of the labeled DNA was routinely obtained. Hybridization of probe to cellular DNA was carried out by using dextran sulfate as described by Wahl *et al.* (11). After hybridization, the filters were exposed to DuPont Cronex-4 x-ray film at −70°C for 16 hr to 2 weeks, with a DuPont Lightning Plus intensifying screen.

RESULTS

Isolation of Recombinant Phage Containing Human Inserts with No Repetitive DNA Sequences. Random phage samples taken from the Maniatis human genomic library (1) were plated on agar plates containing *E. coli* DF50 SupF. Phage plaques developing on these plates were transferred to grids of agar plates (Fig. 1*A*), and nitrocellulose filters were prepared and tested for hybridization to ^{32}P-labeled total human DNA under conditions such that only repetitive sequences form visible aggregates (Fig. 1*B*) Among 700 phage tested, 11 failed to hybridize with total human DNA. When these 11 phage were isolated and retested for hybridization to total human DNA, they again failed to show hybridization. Therefore, these 11 phage contained no detectable repetitive human DNA sequences.

The DNA prepared from these phage was digested with *Eco*RI and fractionated by agarose gel electrophoresis. Of these 11 phage, 4 exhibited *Eco*RI fragment patterns identical to those of the parental λ Charon 4A, indicating that these phage did not contain appreciable amounts of inserted human DNA. With another restriction enzyme, *Hin*dIII, two of the four phage yielded fragments identical to those found in Charon 4A. Although the other two phage exhibited different digestion patterns, the fragment sizes still could be explained as arising from parental λ Charon 4A if the two internal *Eco*RI fragment, 7.8 and 6.9 kilobases (kb), had been ligated in reverse order during the generation of the human genomic library—i.e., instead of the order 19.8–7.8–6.9–10.9 as in the wild-type Charon 4A, the order could have been 19.8–6.9–7.8–10.9.

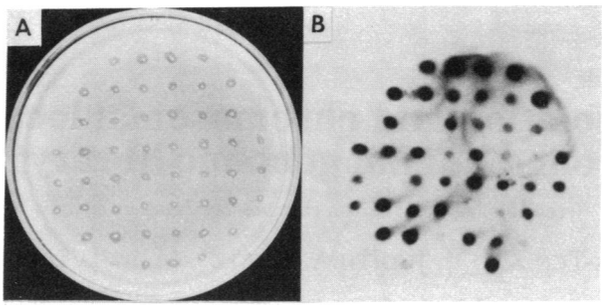

FIG. 1. Isolation of phage that showed no detectable hybridization with the labeled total human DNA. (*A*) Fifty random phage from the human genomic library. (*B*) Nitrocellulose filter made from *A* was exposed to ^{32}P-labeled total human DNA; plaques showed different degrees of hybridization.

All the remaining seven phage yielded the expected *Eco*RI fragments of the Charon 4A arms (19.8 and 10.9 kb and the hybridized 30.7-kb form), plus various human DNA fragments of different sizes characteristic of the human DNA inserts in each recombinant phage. When ^{32}P-labeled probes were prepared from these phage and hybridized to total human DNA blots, distinct bands were found at the same fragment sizes as seen for the human DNA inserts, indicating that these fragments contained unique human DNA sequences. Thus, approximately 1% (7/700) of the recombinant phage in the human genomic library represent human inserts containing only unique DNA sequences. This number is consistent with the estimate of 1–3% reported by Botstein *et al.* (4).

Characterization of Recombinant Phage P3-2. Among the seven recombinant phage identified to contain human unique sequences, one phage, designated P3-2, was studied in detail. *Eco*RI digestion of the λ phage Charon 4A produced five fragments: 30.7, 19.8, 10.9, 7.8, and 6.9 kb (Fig. 2, lane 1). In contrast, the P3-2 digested by *Eco*RI yielded eight fragments: 30.7, 19.8, 10.9, 6.3, 3.9, 2.4, 1.2, and 0.9 kb (Fig. 2, lane 2). The three largest bands are of λ origin; the remaining five bands represent *Eco*RI fragments of the human DNA insert. Thus, the human DNA inserted into the phage P3-2 has a length of 14.7 kb with four internal *Eco*RI sites.

The orientation of the five human fragments in the phage was determined by the analysis of partial digestion products (13). Using similar procedures, we also determined the *Bam*HI restriction sites in the phage P3-2. *Bam*HI cleaved Charon 4A into six fragments: 23.5, 10.5, 5.5, 3.9, 1.5, and 0.5 kb. It cleaved P3-2 into nine fragments. Fig. 3 is a restriction map of P3-2 depicting the *Eco*RI and *Bam*HI cleavage sites. These restriction sites were confirmed by double digestion with both *Eco*RI and *Bam*HI.

Demonstration that the Human Insert in P3-2 Is a Unique Sequence. The ^{32}P-labeled P3-2 probe was hybridized to a Southern blot of total human DNA digested with *Eco*RI (Fig. 2, lane 3). Three prominent bands are visible on the autoradiogram at positions corresponding to 6.3, 3.9, and 2.4 kb, which are the sizes of the three larger *Eco*RI fragments of the human DNA insert in P3-2 (Fig. 2, lane 2). The hybridization bands corresponding to the two smaller *Eco*RI fragments, 1.2 and 0.9

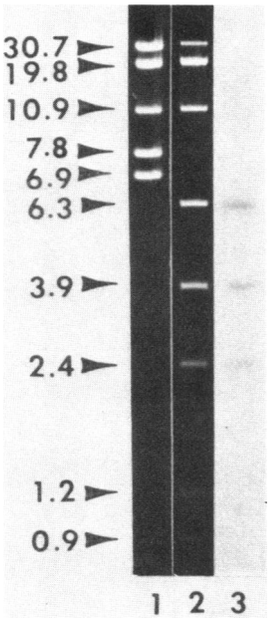

FIG. 2. *Eco*RI-digested DNA fragments from Charon 4A (lane 1) and P3-2 (lane 2), separated by agarose electrophoresis stained with ethidium bromide. Lane 3 is an autoradiogram showing a Southern blot of *Eco*RI-digested human DNA hybridized to the ^{32}P-labeled phage P3-2 probe. *Hin*dIII restriction fragments of phage DNA were run in the same gel as markers (labeled in kb).

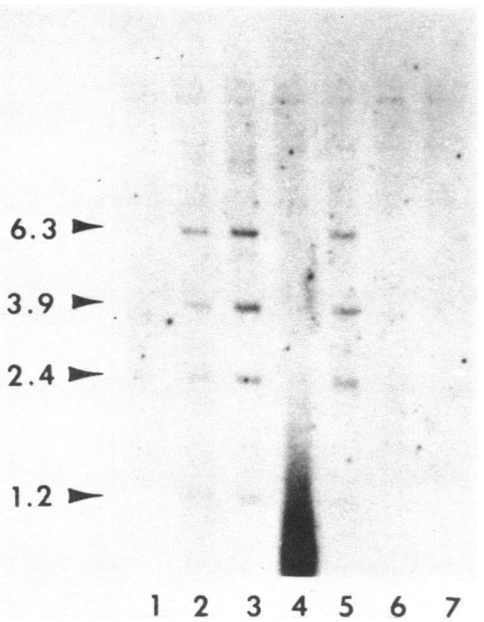

FIG. 4. Blot hybridization pattern of labeled P3-2 hybridized to seven members of the clone panel. Lanes: 1, CP27; 2, CP28; 3, CP29; 4, CP15; 5, CP18; 6, CP26; 7, CP16. Positive hybridization occurred in lanes 2, 3, and 5. The two faint bands at higher molecular weight positions could be Chinese hamster DNA with partial homology to P3-2. The total Chinese hamster DNA (data not shown) also showed no prominent hybridization bands except the two upper faint bands which were present in all somatic cell hybrids.

kb, were faintly visible in some preparations. The formation of these distinct bands in a clear backgound confirms that the human insert in P3-2 contains unique sequences.

Hybridization of Such Probes with the Total DNA from a Human–Chinese Hamster Cell Clone Panel. In order to assign the insert to a specific human chromosome, ^{32}P-labeled P3-2 was used to probe DNA isolated from members of the clone panel (Fig. 4). The only human chromosome that matched the hybridization data was chromosome 22 (Table 1). Thus, the human DNA fragment of 14.7 kb inserted in the phage P3-2 at least provisionally can be assigned to human chromosome 22.

In addition to P3-2, another phage, P3-10, was also analyzed for its human chromosomal assignment. *Eco*RI digestion of P3-10 yielded three fragments from the human insert: 5.9, 5.0, and 0.5 kb (Fig. 5, lane 2). When the ^{32}P-labeled P3-10 probe was hybridized to a Southern blot of total human DNA digested with *Eco*RI, two distinct bands appeared at positions corresponding to 5.9 and 5.0 kb (Fig. 5, lane 3). A ^{32}P-labeled probe prepared from phage P3-10 was also hybridized to blots of the somatic cell hybrid clone panel (Fig. 5, lanes 4 and 5). When the hybridization results are matched with the human chromosome content in the hybrids, the data are consistent only with assignment of the human DNA insert in P3-10 to chromosome 10 (Table 1).

Assay of P3-2 and P3-10 Probes in Other Cell Hybrids. Thirteen additional cell hybrids containing various human chromosomes were assayed for the presence or absence of the two phage probes. From these 20 cell hybrids the highest concordant segregations were obtained between P3-2 and human chromosome 22 (80%) and between P3-10 and chromosome 10 (89%). Further support for the assignment of P3-2 to chromosome 22 was found in two cell hybrids containing small numbers of human chromosomes. Both were positive for the P3-2 probe. One hybrid contained six human chromosomes—4, 5, 7, 14, 20, and 22—and the other contained two human chromosomes—9 and 22. The only human chromosome present in both hybrids was chromosome 22. The concordancies between P3-2 and the other chromosomes are: chromosome 4 (35%), 5 (50%), 7 (37%), 20 (63%), 9 (55%). Thus, these results further support the assignment of P3-2 to chromosome 22.

DISCUSSION

In this report, we describe procedures for the isolation, characterization, and chromosomal assignment of unique DNA segments derived from a human genomic library. Assignment of the two DNA segments to human chromosomes 22 and 10, respectively, has been achieved. By using these procedures, it should be feasible to assign at least one or more DNA fragment markers to each of the 24 human chromosomes.

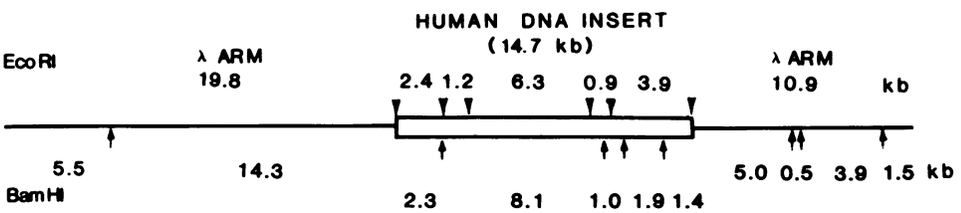

FIG. 3. Restriction map of phage P3-2 showing the *Eco*RI and *Bam*HI cleavage sites.

Table 1. Human chromosome constitution in the seven members of the somatic cell hybrid clone panel and the blot hybridization results of P3-2 and P3-10 with the clone panel

Human chromosome	Isozyme marker*	Clone panel						
		CP27	CP28	CP29	CP15	CP18	CP26	CP16
1	PGD	−	−	−	−	+	+	−
2	MDH1	+	−	+	+	−	−	−
3	GALB1	+	+	+	+	+	−	+
4	PGM2	+	+	+	+	−	+	−
5	ARSB	+	+	−	+	−	+	+
6	SOD2	+	+	−	−	−	+	−
7	GUSB	+	−	−	−	−	−	+
8	GSR	−	−	+	−	+	−	−
9	AK1	−	+	−	+	−	+	−
10	GOT1	−	−	−	−	−	+	−
11	LDHA	−	−	+	+	+	+	+
12	LDHB	+	−	+	+	−	+	−
13	ESD	+	−	−	−	−	+	−
14	NP	+	−	+	+	+	+	+
15	HEXA	−	−	+	+	+	+	−
16	PGP	+	+	−	−	+	−	+
17	GALK	−	−	−	+	+	+	+
18	PEPA	+	+	+	−	+	+	−
19	GPI	−	+	+	+	+	+	+
20	ITPA	−	+	+	−	+	−	+
21	SOD1	+	+	+	−	+	+	+
22	ACONM	−	+	+	−	+	−	−
X	G6PD	+	−	+	−	−	−	+
P3-2†		−	+	+	−	+	−	−
P3-10†		−	−	−	−	−	+	−

Results are shown as presence (+) or absence (−) of the human isozyme marker.

* PGD, phosphogluconate dehydrogenase; MDH1, malate dehydrogenase, NAD, soluble; GALB1, β-galactosidase 1; PGM2, phosphoglucomutase 2; ARSB, arylsulfatase-B; SOD2, superoxide dismutase, mitochondrial; GUSB, β-glucuronidase; GSR, glutathione reductase; AK1, adenylate kinase-1; GOT1, glutamic-oxaloacetic transaminase, soluble; LDHA, lactate dehydrogenase A; LDHB, lactate dehydrogenase B; ESD, esterase D; NP, nucleoside phosphorylase; HEXA, hexosaminidase-A; PGP, phosphoglycollate phosphatase; GALK, galactokinase; PEPA, peptidase A; GPI, glucose phosphate isomerase; ITPA, inosine triphosphatase; SOD1, superoxide dismutase, soluble; ACONM, aconitase, mitochondrial; G6PD, glucose-6-phosphate dehydrogenase.

† These data are presence (+) or absence (−) of hybridization bands corresponding to digested DNA fragments of human origin.

In the present study, the chromosomal assignment of DNA fragments has been facilitated by the use of a clone panel consisting of seven human–Chinese hamster somatic cell hybrids. These seven hybrids contain unique combinations of human chromosomes capable of discriminating all 22 human autosomes and the X chromosome. Subsequent analysis of additional hybrids should provide further evidence to confirm any assignment. The assignment of a DNA segment derived from the Y chromosome can be achieved by demonstrating its presence in DNA from male but not female human cells. This series of hybrids thus provides a highly efficient system for assigning cloned DNA fragments or specific genes to the human genome.

We are aware of the possibility that, in some hybrids, certain human chromosomes may not be intact and some may still be segregating. Thus, when the probes of P3-2 and P3-10 were hybridized to 13 additional hybrid clones, a few discordant clones were observed. However, synteny analysis for all 23 human chromosomes based on 20 hybrids revealed that the highest concordance was again obtained between P3-2 and chromosome 22 and between P3-10 and chromosome 10. Furthermore, the P3-2 probe was positive in two hybrids with small numbers of human chromosomes, and the only human chromosome that is present in both hybrids is chromosome 22. Finally, assays of three additional isozyme markers on chromosome 22 (diaphorase-1, arylsufatase-A, and β-galactosidase 2) showed that the presence of broken chromosome 22 could explain some of the discordant hybrid clones between P3-2 and aconitase 2, an isozyme marker assayed in the clone panel and in all the other hybrids as the marker for chromosome 22.

We therefore have adopted the following strategy for using these cell hybrids for chromosomal assignment of random DNA segments, cloned genes, or other markers: (*i*) Hybridization of the test DNA to blots of seven members of the clone panel to establish initial assignment to one or a few human chromosomes. (*ii*) Hybridization of the test DNA to blots of the additional 13 cell hybrids to establish highest concordance with a specific human chromosome. (*iii*) Hybridization of the test DNA to blots of cell hybrids, if available, with morphologically intact single or a small number of human chromosomes which include the crucial chromosome under study (14–18).

The synteny analysis based on the above data will be considered as provisional assignment for the DNA segment. A confirmed assignment will come from synteny analysis conducted by other laboratories using other series of somatic cell hybrids, preferably involving a species different from Chinese hamster, or using other methods such as chromosome sorting (19) or *in situ* hybridization (20).

Finally, it should be feasible to assign at least one DNA fragment for each human chromosome. From this collection of 24 fragments, one restriction subfragment can be carefully chosen

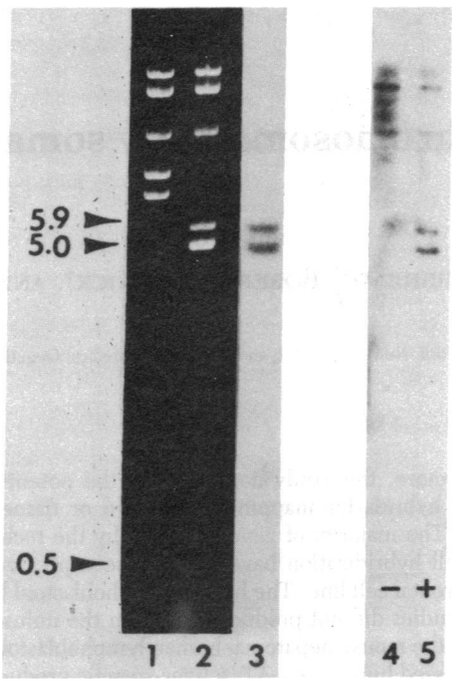

FIG. 5. Characterization of the *Eco*RI-digested phage P3-10. Lanes 1 and 2 are ethidium bromide-stained gels, and lanes 3, 4, and 5 are autoradiograms. Lanes: 1, Charon 4A; 2, P3-10; 3, ^{32}P-labeled P3-10 probe hybridized to total human DNA; 4, somatic cell hybrid (CP18) showing negative hybridization to P3-10 probe; 5, somatic cell hybrid (CP26) showing positive hybridization to P3-10 probe.

from each fragment to represent that human chromosome. These 24 subfragments with different sizes can be used as mixed probes in nick-translation (12). When the labeled mixed probes are hybridized to the digested total human DNA blots, 24 bands are expected to occur, each occupying a position on the blot characteristic of each of the 24 human chromosomes, Thus, with this set of 24 subfragments, the human chromosome composition in each hybrid can be conveniently determined simply by hybridizing the mixed probes with DNA blots made from the somatic cell hybrids, thus eliminating the need to perform 24 isozyme assays as is currently done.

The present paper describes a general procedure for isolating recombinant phage containing unique human sequences from the Maniatis human genomic library and for subsequent assignment to a specific human chromosome. This procedure is simple and can be used to generate large numbers of human unique DNA sequences with known chromosome location for gene mapping and linkage, polymorphism, and other genetic studies in the human genome.

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