Am. J. Hum. Genet. 40:312–328, 1987

Efficient Isolation of X Chromosome–specific Single-Copy Probes from a Cosmid Library of a Human X/Hamster Hybrid-Cell Line: Mapping of New Probes Close to the Locus for X-linked Mental Retardation

M. H. HOFKER,* A. A. B. BERGEN,* M. I. SKRAASTAD,* N. J. CARPENTER,† H. VEENEMA,* J. M. CONNOR,‡ E. BAKKER,* G. J. B. VAN OMMEN,* AND P. L. PEARSON*

*Department of Human Genetics, Sylvius Laboratories, University of Leiden, 2333 AL Leiden, The Netherlands; †Chapman Institute of Medical Genetics, Children's Medical Centre, Tulsa, OK; and ‡Duncan Guthrie Institute of Medical Genetics, University of Glasgow

SUMMARY

We isolated X-chromosomal DNA probes from a cosmid library constructed from a single human X/hamster hybrid-cell line (Cl2D). One hundred human clones were isolated and used to construct a pool of X-chromosomal DNA. This DNA was digested into 0.15–2-kb fragments and subcloned into plasmids allowing the rapid characterization of new single-copy probes. These were regionally mapped and used for the detection of restriction-site polymorphisms. Together with a series of subcloned probes from individually isolated cosmids, we found seven polymorphic probes among 53 tested. Thirty-one of the probes were physically localized to different regions of the X chromosome. Four polymorphic probes map to Xq27-Xq28: DXS102 (cX38.1), DXS105(cX55.7), DXS107(cpX234), and DXS134(cpX67). These were genetically mapped by multipoint analysis relative to previously characterized loci, a mapping that resulted in the following order: DXYS1, DXS107, DXS51/DXS102, F9, DXS105, Fra-X, F8/DXS52, DXS15, DXS134. The mapping of DXS105 between F9 and Fra-X makes this probe useful for Fra-X analysis. For the linkage between FraX and DXS105, a maximum lod score of 5.01 at 4 cMorgans has been obtained in one large Dutch pedigree.

Received September 29, 1986; revision received December 16, 1986.

Address for correspondence and reprints: Dr. M. H. Hofker, Instituut voor Anthropogenetica, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

INTRODUCTION

It has recently become clear that the application of restriction-fragment-length polymorphisms (RFLPs) in linkage analysis should lead to a linkage map of the human genome in the near future. The number of RFLPs detected by randomly cloned DNA sequences is virtually unlimited in the human genome (Willard et al. 1985). The most efficient strategy for obtaining an evenly spaced set of marker loci on a particular chromosome requires the use of chromosome-specific sources for the generation of single-copy probes (Lange and Boehnke 1982). Two main approaches exist. First, flow-sorted chromosomes can be used as a highly enriched DNA source to construct chromosome-specific libraries. For a number of chromosomes a purity $\geq 50\%$ can be obtained for the desired chromosomal origin of the cloned fragments (Davies et al. 1981; Kunkel et al. 1982). Most libraries consist of *Eco*RI- or *Hin*dIII-digested DNA fragments of 2–10 kb cloned in a suitable phage vector such as charon 21A or λgtWES. The single-copy fragments are obtained by eliminating, with a Benton and Davis screen, the repeat containing clones. This negative first screening is a major drawback of the method, since it selects for any type of nonhybridizing clone and thus leads to enrichment of undesired contaminants, to empty phages, and to cloning artifacts (Hofker et al. 1985).

The second approach is to use rodent-cell hybrids containing only one (or a very few) human chromosomes to construct libraries. Subsequently, the human clones are identified on the basis of hybridization to human repetitive DNA (Gusella et al. 1980). The chromosome specificity of the hybrid means that the chromosomal origin of the human material is known. Further, such cell lines permit the isolation of high-molecular-weight DNA, suitable for the generation of 40-kb fragments, which can be used for cosmid cloning.

For an initial characterization of the probes, hybrid-cell panels, such as those described for the X chromosome (Wieacker et al. 1984; Oberle et al. 1986*a*), permit a high-resolution mapping of the probes by dividing the chromosomes into several different regions. These panels are efficient tools for mapping of probes in terms of speed and reliability. The obtained resolution is comparable to quantitative hybridization methods employing cell lines with unbalanced chromosomal abnormalities and, in some cases, to in situ hybridization, techniques that are often more time consuming and require more experimental sophistication.

The analytical power of random probes is illustrated by the analysis of the short arm of the X chromosome, around the DMD locus. A large number of probes have been isolated, flanking DMD at genetic distances of 5–20 cMorgans (Goodfellow et al. 1985). DMD-associated translocations in Xp21 have allowed mapping of probes relative to the DMD locus. Deletions of the entire Xp21 band (de Martinville et al. 1985; Ingle et al. 1985; Old et al. 1985), as well as deletions of parts of Xp21 (Francke et al. 1985; Patil et al. 1985; Wieringa et al. 1985), provided mapping tools to a higher resolution. Particularly the latter deletions are highly informative, since their estimated size is $\sim 5 \times 10^6$ kb. The mapping order of the probes so obtained can be confirmed and in some cases

improved by multipoint analysis of recombinants in DMD families (Goodfellow et al. 1985; Bakker et al., 1986). The rapid progress in probe development has allowed prenatal diagnosis and carrier detection with a reliability of >99% (Wieacker et al. 1983; Bakker et al. 1986) and is generally expected to lead to the cloning of the DMD gene itself, without any prior knowledge of its function.

The aim of this study was to enlarge the current set of X probes for extending the overall linkage map of the X chromosome. At least 100 X-linked disease loci have been described, and a further saturation of the chromosome with probes should allow the mapping of many of these loci in a fashion comparable to that used for those present in the Xp21 region. Currently, much attention is focused on the loci for fragile X-linked mental retardation, retinitis pigmentosa, and several other X-linked disorders in which the underlying genetic defect is unknown (Goodfellow et al. 1985).

We have developed a protocol, starting with a cosmid library prepared from a hamster/hybrid-cell line containing one human X chromosome. The handling of a large series of subcloning experiments in parallel was avoided by first screening for human clones and then pooling the DNA of 100 such human clones. This is equivalent to the generation of an almost entirely X-specific cosmid library containing 4,000 kb or 2% of the X chromosome. Subsequently this DNA was subcloned. Thus, the advantages of rapid identification of single-copy clones is combined with having a high fraction (>99%) of X-specific clones.

MATERIAL AND METHODS

Genomic DNA

A test panel for polymorphism screening was prepared from randomly obtained Dutch placentas. Clone 2D (C12D) is a human X/hamster hybrid-cell line provided by Dr. S. Goss (Dunn School of pathology, Oxford). The cell line GM1202 (49,XXXXY) was obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). The hybrid-cell mapping panel, consisting of somatic-cell hybrids containing different fragments of the X chromosome, is a gift of Dr. H. H. Ropers (University of Nijmegen) and is described in detail elsewhere (Wieacker et al. 1984).

Southern Blot Analysis

Genomic DNA isolations were performed as described by Hofker et al. (1985). The DNA was digested with various restriction enzymes obtained from Promega Biotec or Boehringer Mannheim. *Bgl*II was prepared in our own laboratory. Incubations were carried out with 3 units/μg in medium salt buffer (Maniatis et al. 1982), except for *Bgl*II (10 mM Tris-HCl, 25 mM $MgCl_2$, 60 mM NaCl, 1 mM β-mercaptoethanol, pH 9.0) and *Taq*I (100 mM NaCl, 50 mM Tris-Cl pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol). Seven micrograms of the DNA were electrophoresed overnight at 1 V/cm through 0.7% agarose in TEA buffer (Maniatis et al. 1982). Gels were denatured for 2 × 15 min in 0.4 M NaOH/1.5 M NaCl and transferred in the same solution to Genescreen plus (New England

Nuclear). Probes were labeled with α-^{32}PdCTP to a specific activity of 5×10^8 dpm/μg using a nick-translation kit (Amersham). The hybridization and washing conditions were as described elsewhere (van Ommen et al. 1983).

Isolation of Human X-chromosomal Cosmids

A cosmid library was prepared from Cl2D DNA following procedures described by van Ommen et al. (1983). Cl2D DNA was cloned in pJBF, a derivative of pJB8 (Ish-Horowicz and Burke 1981), provided by Dr. F. Grosveld (MRC, Mill Hill, London). The efficiency of cosmid transfection into *E. coli* 1046 was 2×10^6 colonies/μg of size-fractionated, partially *Mbo*I-digested DNA. To screen for human clones, 200 colonies/90-mm dish were plated and replica filters were prepared on nitrocellulose (Sartorius; 0.1-μM pore size), essentially as described by Hanahan and Meselson (1980). The filters were hybridized with nick-translated human DNA (10^9 cpm/μg) at standard stringency (but with a low [0.5 ng/ml] probe concentration) and washed under normal conditions. All clones with a positive signal were purified and rescreened with human and hamster DNA, and those giving hybridization only with human DNA were further used.

Isolation of Single-Copy Probes

Subclones of the cosmids were prepared by using two different procedures as follows: First, small-scale DNA isolations of the cosmids were performed (Birnboim and Doly 1979). DNA of the individual cosmids was digested with *Eco*RI or with *Eco*RI and *Pst*I and then cloned in pAT153. Recombinants were recovered by selection on tetracycline, which allows only pAT153—but not religated pJBF clones—to grow. In a later stage 100 human cosmids were grown as separate cultures overnight for 16 h. A sample of each culture was stored in glycerol, the remainder of all cultures was pooled together, and one large-scale DNA isolation was carried out. This DNA was cleaved with *Eco*RI and *Pst*I or *Sau*IIIA and cloned in pAT153 or pUC12, respectively. The pJBF vector of each clone, although conferring ampicillin resistance as well, is digested to such an extent in this procedure that it cannot be religated and form colonies as well.

Recombinant plasmids were recovered by a rapid minipreparation method (Holmes and Quigley 1981). Cultures were lysed by boiling, and the debris was removed by centrifugation. The supernatant was stored frozen. A 10-μl sample was examined by electrophoresis, blotting, and hybridization with human DNA or *E. coli* DNA. Recombinants remaining negative were further purified by triple extraction with phenol/chloroform and precipitation (0.1 vol 2M NaAc, 1 vol isopropanol). The pellet was resuspended in 50 μl 10 mM Tris-Cl, 0.1 mM EDTA; nick-translated and hybridized to filters containing hamster DNA and human DNA; and digested with four different enzymes.

A single band in at least one of the lanes with human DNA and no hybridization signal in the lane with hamster DNA was accepted as identification of a single-copy human X-specific clone. Probes with multiple bands were further characterized with dosage hybridization to male, female, and 4X DNA. Final

proof of the X-chromosomal origin of the probes was obtained by the observation of (1) an expected segregation pattern in the hybrid-cell panel and (2) a difference in intensity reflecting a difference in copy number between males and females.

RESULTS AND DISCUSSION

Cosmid Isolation

Human cosmids were obtained from the Cl2D library by hybridization with labeled human DNA (fig. 1). The use of an extremely low (0.5 ng/ml) probe concentration resulted in a clear signal of the human clones, while hamster clones did not cross-hybridize under these conditions. To investigate whether human clones are overlooked under these critical conditions, a filter containing 200 clones of a human cosmid library was hybridized in parallel. Every clone of the human library yielded a clear signal, indicating that, in principle, all the human clones from the plating of the human/hamster cosmid library were likely to be isolated. Complete recovery of the human clones is important when a series of cosmids randomly distributed over the chromosome is required. After rescreening the isolated clones with labeled human and hamster DNA, 150 human cosmids were selected. On the basis of an average insert size of 45 kb, this amounts to as much as 6,700 kb of DNA or 3% of the X chromosome.

Single-Copy Probe Isolation

Initially, 23 cosmids were digested with *Eco*RI and subcloned into pAT153. Approximately 12–24 subclones per cosmid were examined. Sixteen cosmids yielded at least one single-copy subclone, as judged by hybridization to filters with human DNA.

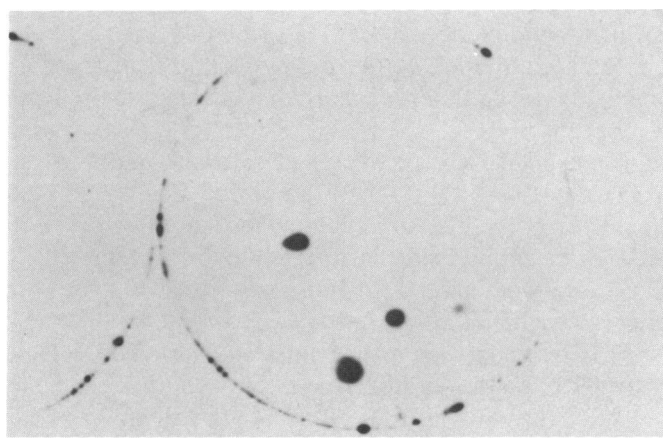


FIG. 1.—Autoradiograph of a hybridization of the Cl2D cosmid library with labeled human DNA. Owing to the low (0.5 ng/ml) probe concentration, only the human clones yield a signal.

In further experiments, DNA of 100 cosmids was pooled to reduce the number of subcloning experiments. A schematic representation of the isolation procedure for human single-copy X probes is shown (fig. 2). In order to obtain representative amounts of DNA of each cosmid in this pool, 10-ml cultures of 100 different cosmids were grown separately. These were all harvested in the stationary-growth phase to counteract differences in growth rate. Pilot experiments have shown that only a minority (<20%) of the clones gives a lower yield. This degree of underrepresentation does not seriously affect the randomness of the pool, so no further steps were undertaken to correct for growth differences. Aliquots of the cultures were stored, the cultures were mixed, and DNA was extracted. The pooled cosmid DNA was either cleaved with *Eco*RI and *Pst*I and subcloned into pAT153 or cleaved with *Sau*3A and cloned into pUC12. After hybridization to human DNA, 50 single-copy subclones were identified (see Material and Methods for details). The majority of these probes was further used in RFLP screening and regional mapping.

The use of either a combination of *Eco*RI and *Pst*I or of *Sau*IIIA individually results in the generation of 100–1,500-bp fragments. The subcloning of such small DNA fragments increases the yield of single-copy probes randomly from all the cosmids. When *Eco*RI fragments with an average size of 3–6 kb are cloned, only 60%–70% of the cosmids yield a single-copy fragment. Subcloning of other cosmids isolated in this laboratory—those obtained, for example, by screening with DMD-related probes—followed by *Sau*3A digestion, yielded an average of four to five different single-copy subclones from each cosmid (Hofker et al. 1986*a*, 1986*c*). This implies that the pool of 100 cosmids should deliver ±400 unique sequences, of which 12.5% have been isolated.

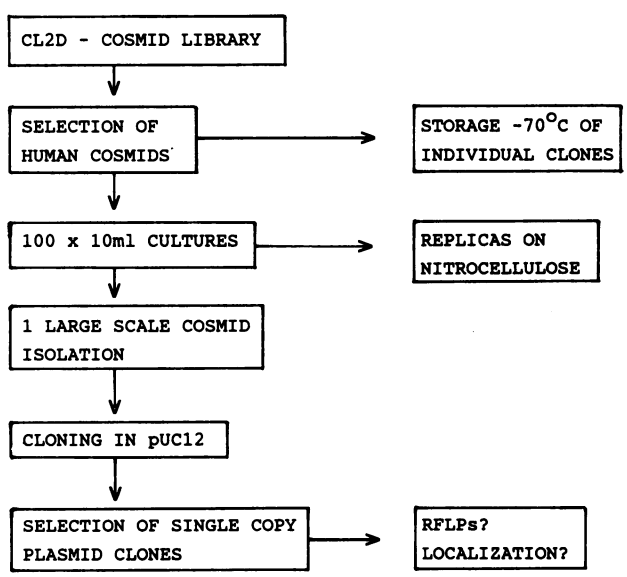


FIG. 2.—Isolation strategy for single-copy X probes.

RFLPs

Six enzymes—*Taq*I, *Msp*I, *Eco*RI, *Hin*dIII, *Pst*I, and *Bgl*II—were used to screen for RFLPs in a test panel of 17 unrelated X chromosomes. With the 53 probes tested, 13 polymorphic sites were found. Two probes had a low minor-allele frequency. The seven probes with relatively high minor-allele frequencies are specified in table 1 and figure 3. Per haploid genome, 4,120 sites were examined, resulting in 1 RFLP/320 bp. This is three times lower than the ratio found for autosomal probes (Hofker et al. 1986*b*).

Probe cX38.1 gives a much higher allele frequency among the Scottish population than among the Dutch (Connor et al. 1987), emphasizing that the allele frequencies of polymorphic probes should be determined independently for different populations.

Regional Localization

A series of 31 single-copy probes was regionally localized with the hybrid-cell panel described by Wieacker et al. (1984). The localization of probe cX50.5 to Xp21-Xp22.3, probe cX37.1 to Xp11-Xq21.3, probe cX32.3 to Xq26-Xq27, and probe cX44.1 to Xq27-Xq28 is shown (fig. 4). The other results are summarized in figure 5.

The mapping of the probes with the hybrid-cell panel is rapid and reliable when identical gels are prepared from a series of batch digestions and blotted on a reusable membrane. A set of identical filters has been made. The integrity of the filters was verified with probes hybridizing in Xq27-Xq28, which is present in all nine hybrids.

One probe, cX52.5, is a cloned *Eco*RI fragment of 3.6 kb yielding five non-polymorphic *Eco*RI bands with human DNA (fig. 6). The size of these bands ranges from 3.6 kb up to 9.5 kb, which is much larger than the insert itself. This indicates that this sequence occurs at least five times in the human genome. Remarkably, all of these bands map to the Xq21.3-Xq22 region of the X chromosome. Moreover, homology is observed in mouse DNA as well as hamster DNA. This indicates that this sequence is highly conserved and therefore most likely is part of an as yet unidentified gene family.

A second probe, cX22.7, has at least two copies, one mapping to Xp21-Xp22.3 and the other to one of the autosomes (not shown).

In the case of probes mapping to interesting regions, the availability of the parental cosmids allows the rapid isolation of additional clones to continue the search for RFLPs. This is demonstrated by the use of the cosmid of cX5.4, mapping into an Xp21 deletion (Seattle deletion or "BB" [Francke et al. 1985]). Further subcloning of cX5 has led to the isolation of cX5.7, which detects an RFLP with *Msp*I (Hofker et al. 1986*c*).

Genetic Mapping

The polymorphic probes in the region Xq27-Xq28 were further mapped in family studies. Probes cpX234 (DXS107) and cX38.1 (DXS102) were mapped relative to pDP34 (DXYS1) (Page et al. 1982), F9 (Choo et al. 1982), 52A

TABLE 1

POLYMORPHIC X PROBES

Probe (HGM8 no.)	Regional Assignment	Vector	Cloning Site	Insert Size (kb)	Polymorphic Enzyme	Allele	Frequency (no. of chromosomes tested)	Mendelian Inheritance[a]
cpX203 (DXS106)	Xp11-Xq21.3	pUCl2	*Bam*HI	0.1	*Bgl*II	1.0	0.64 (75)	+
						5.8	0.36	
cX52.5 (DXS101)	Xq21.3-Xq22	pAT153	*Eco*RI	4.0	*Msp*I	7.5	0.65 (17)	−
						7.7	0.35	
cX38.1 (DXS102)	Xq27-Xq28	pAT153	*Eco*RI	2.6	*Taq*I	10	0.90 (20)	+
						1.2	0.10	
						3.5	sporadic	
					*Msp*I	12	0.80 (31)	−
						1.2	0.20	
cX55.7 (DXS105)	Xq27-Xq28	pAT153	*Eco*RI	2.4	*Eco*RI	10	0.99 (94)	−
						2.5	0.01	
					*Taq*I	3.2	0.84 (94)	+
						4.5	0.16	
cpX67 (DXS134)	Xq27-Xq28	pAT153	*Eco*RI/*Pst*I	0.6	*Msp*I	3.4	0.64 (17)	+
						3.7	0.30	
						1.4	0.06	
cpX234 (DXS107)	Xq27-Xq28	pUCl2	*Bam*HI	0.15	*Taq*I	1.1	0.83 (17)	+
						3.2	0.17	
cpX237 (DXS108)	X	pUCl2	*Bam*HI	0.15	*Eco*RI	12	0.60 (17)	+
						10	0.40	

[a] + = Mendelian inheritance observed; − = Mendelian inheritance not tested.

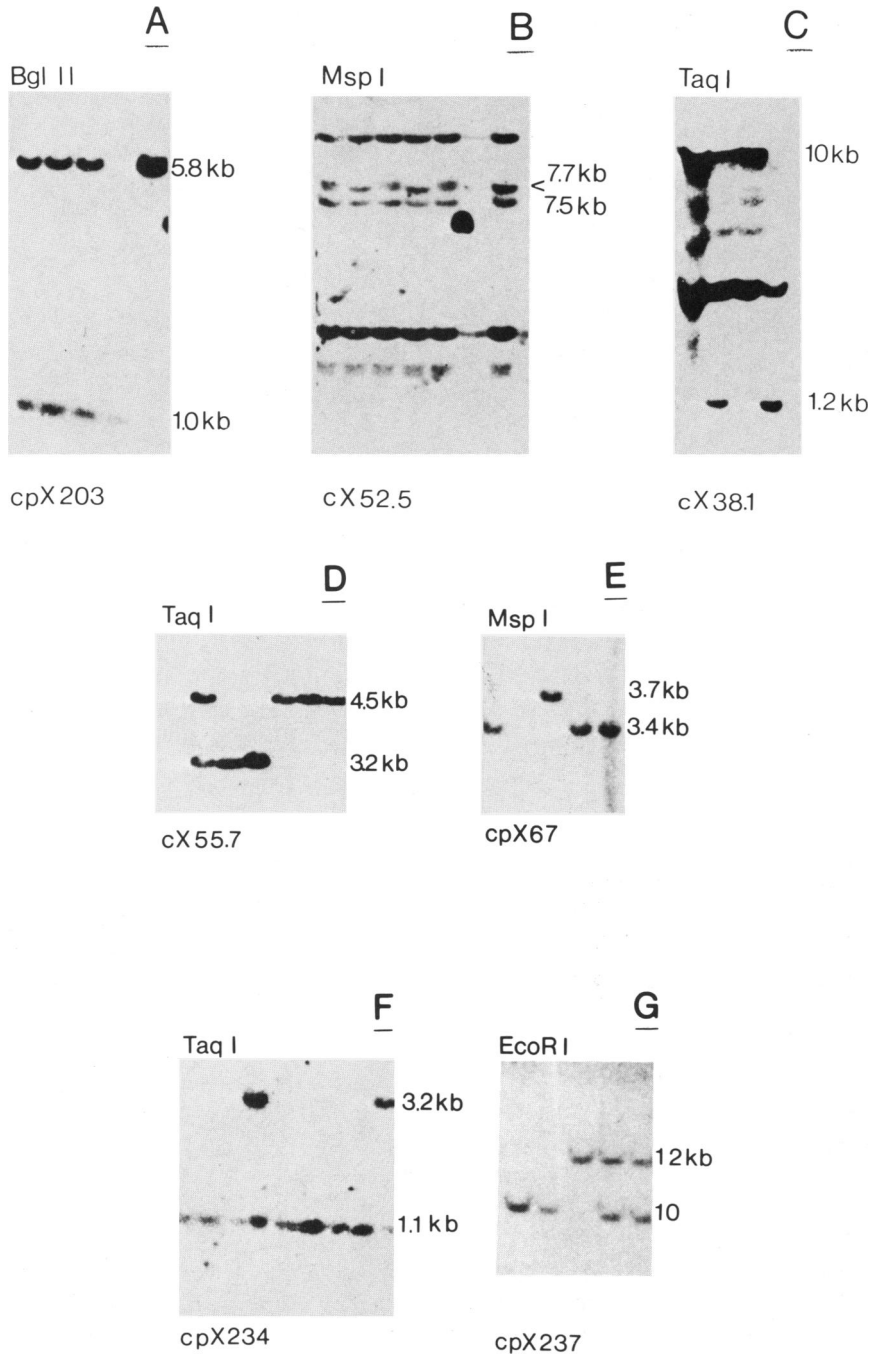


FIG. 3.—Autoradiographs showing the RFLPs of probes cpX203 (panel *A*), cX52.5 (panel *B*), cX38.1 (panel *C*), cX55.7 (panel *D*), cpX67 (panel *E*), cpX234 (panel *F*), and CpX237 (panel *G*).

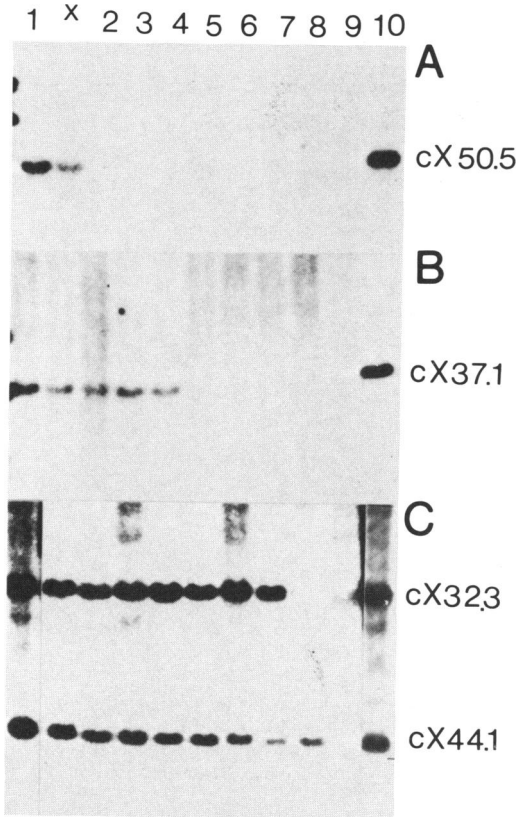
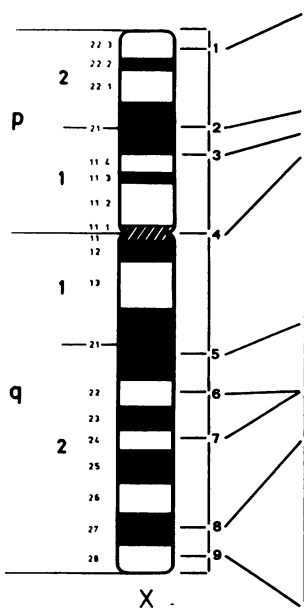


FIG. 4.—Mapping results of the probes cX50.5 (panel *A*), cX37.1 (panel *B*), cX32.3 (panel *C*), and cX44.1 (panel *C*), hybridized to the *Eco*RI-digested DNA of the hybrid-cell mapping panel (Wieacker et al. 1983). The numbered lanes correspond to the following cell lines containing a fraction of the X chromosome from the translocation breakpoint (as indicated between brackets) to Xqter: lane 1, 445x393Kl (p22.3); lane 2, 697x175k27 (p21: Duchenne muscular dystrophy–related breakpoint); lane 3, 617x347k6 (p21.1 [Kunkel et al. 1985]); lane 4, 422k6 (p11); lane 5, 676x175k37 (q21.3); lane 6, 494x393k6 (q22); lane 7, 790x175k6 (q24); lane 8, 795x175k14 (q27); and lane 9, 367 (q28). Lane 10 represents human DNA.

(DXS51) (Drayna et al. 1984), and Fra-X by studying phase-known meiosis in 26 families (Connor et al. 1987). An example of multipoint cross technique is shown in figure 7. The following order was obtained: DXYS1, DXS107, DXS102/DXS51, F9, Fra-X.

The probes cpX67 (DXS134) and cX55.7 (DXS105) were mapped relative to F9, Fra-X, DX13 (DXS15) (Drayna et al. 1984), and St14 (DXS52) (Oberle et al. 1986*b*) in a large Dutch pedigree showing segregation of the Fra-X locus, described in detail elsewhere (Veenema et al. 1987). This family contains a nonpenetrant male, a phenomenon first described by Camerino et al. (1983). From haplotype analysis the following order has been obtained: F9, DXS105, Fra-X, DXS52, DXS15, DXS134. Part of this pedigree is shown (fig. 8). Between F9 and Fra-X three recombinants were observed. In two cases the



CLONE*)	HGM8 NUMBER	INSERT SIZE (KB)	REGIONAL ASSIGNMENT	VECTOR	CLONING SITE
cX22.7	DXS149	1.8	p22.1-p22.3	pAT153	EcoRI
cX42.6	DXS154	3.7	p21 -p22.3	pAT153	EcoRI
cX50.5	DXS158	3.0	p21 -p22.3	pAT153	EcoRI
cX51.12	DXS126	3.2	p21 -p22.3	pAT153	EcoRI
cX5.4	DXS148	3.6	p21	pAT153	EcoRI
cX24.6	DXS150	2.0	p11 -p21	pAT153	EcoRI
cX37.1	DXS153	3.3	p11 -q21.3	pAT153	EcoRI
cX61.1	DXS127	1.2	p11 -q21.3	pAT153	EcoRI/PstI
cX65.3	DXS128	0.7	p11 -q21.3	pAT153	EcoRI/PstI
cpX21	DXS131	1.5	p11 -q21.3	pAT153	EcoRI/PstI
cpX23	DXS132	1.0	p11 -q21.3	pAT153	EcoRI/PstI
cpX73	DXS159	0.5	p11 -q21.3	pAT153	EcoRI/PstI
cpX93	DXS135	1.3	p11 -q21.3	pAT153	EcoRI/PstI
cpX30	DXS133	1.0	p11 -q22	pAT153	EcoRI/PstI
cX4.4	DXS147	3.6	q21.3-q22	pAT153	EcoRI
cX47.4	DXS156	3.6	q21.3-q22	pAT153	EcoRI
cX32.3	DXS151	1.2	q24 -q27	pAT153	EcoRI
cpX1	DXS129	0.7	q24 -q27	pAT153	EcoRI/PstI
cX33.2	DXS152	4.0	q27 -q28	pAT153	EcoRI
cX44.1	DXS155	1.7	q27 -q28	pAT153	EcoRI
cX48.7	DXS157	1.5	q27 -q28	pAT153	EcoRI
cpx6	DXS130	1.0	q27 -q28	pAT153	EcoRI/PstI
cpX206	DXS160	0.1	q27 -q28	pUC12	BamHI
cpX281	DXS161	0.7	q27 -q28	pUC12	BamHI
cpX289	DXS162	0.3	q27 -q28	pUC12	BamHI

FIG. 5.—Regional assignment of the nonpolymorphic X-specific probes. The numbers at the right-hand side of the chromosome correspond to the translocation breakpoints, present in the cell lines as shown in the legend of fig. 6. The prefix cX has been used in case of individual subcloned cosmids; cpX represents single-copy clones isolated from the pool of 100 cosmids.

recombination occurred between DXS105 and F9. Only one recombination was found between Fra-X and DXS105, giving a peak lod score of 5.01 at 4 cMorgans.

A reliable detection of the segregation of a disease locus can be performed in a three-point segregation analysis, as shown for the Duchenne locus (Wieacker et al. 1983; Bakker et al. 1986). In the case of the Fra-X locus, two distal markers—DXS15 and DXS52—are highly informative and closely linked (Oberle et al. 1986*b*). However, at the proximal side, only one marker, the F9 gene, is linked to Fra-X. The finding of an additional probe, DXS105, will be a useful supplement.

Conclusions

This paper describes a probe-isolation strategy that uses a cosmid library of a hybrid-cell line containing only a human X chromosome. This starting material has the advantage that the human clones can be identified positively on the basis of hybridization to human repetitive DNA. In agreement with the cytological characterization of the cell line, all the human single-copy probes were derived from the X chromosome, as judged from the segregation in the hybrid-cell panel and from family studies with the polymorphic probes.

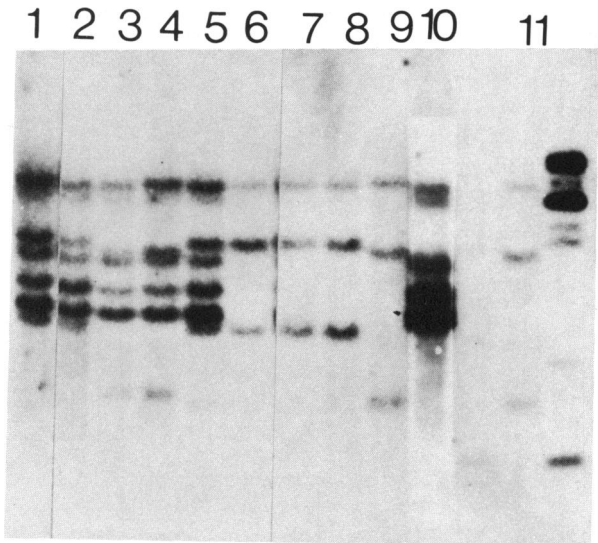


FIG. 6.—Hybridization of probe cX52.5 to the mapping panel (see fig. 4 legend for lanes 1–10). Lane 11 represents A3 (hamster) DNA, yielding three additional bands in lanes 3, 4, and 9. Mouse homology is visible in lanes 1, 2, and 5–8. The human banding pattern is present in lanes 1–5; thus all copies of this probe map to the interval Xq21.3-Xq22.

Because individual subcloning of large series of cosmids is laborious, protocols have been designed by other groups to allow the hybridization of complete cosmid probes to genomic blots after prehybridization of the labeled cosmid to an excess of total human DNA (Sealey et al. 1985; Buroker et al. 1986). In principle these methods allow the examination of a large number of sites per hybridization for the RFLP screening. However, we have found that its applicability depends strongly on the nature of the repetitive sequences in each individual cosmid.

As an alternative approach we have used a pool of 100 human cosmids as a source for single-copy X probes. In the present studies only one DNA isolation and subcloning step was necessary to construct a partial plasmid library of pooled cosmid DNA containing 2% of the X chromosome. The cloning in a plasmid vector allowed rapid handling of the probes, and the size of the inserts allowed the easy selection of single-copy probes. The efficiency of RFLP detection can be further increased when a number of probes are combined in the RFLP screening. Especially when hybrid-cell lines—which contain only one human chromosome or a part of it—are available, this strategy is the most efficient of all methods that we have tested thus far to develop a set of randomly chromosome-specific genetic markers.

Several of our polymorphic probes map to Xq27-Xq28. The region proximal to the Fra-X locus is of particular interest, since it has been suggested that the genetic distance between F9 and Fra-X may be significantly larger in families with penetrant males than in families with nonpenetrant males (Brown et al.

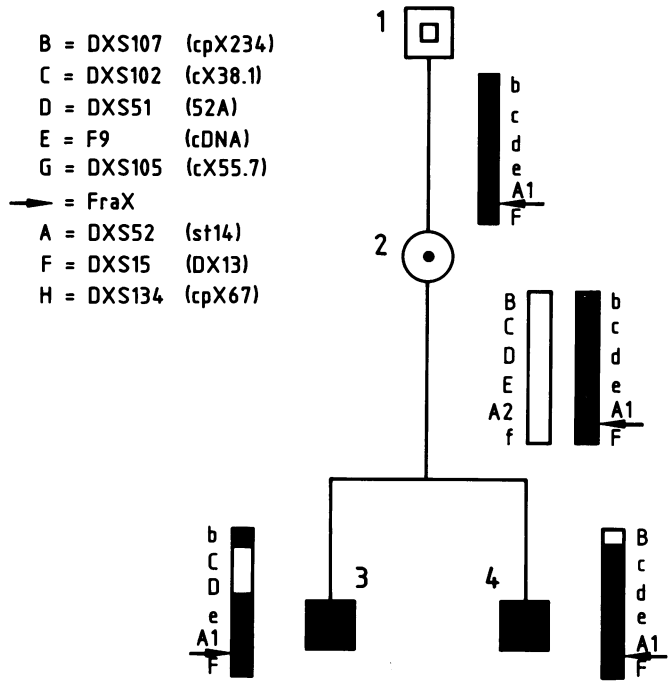


FIG. 7.—This family is segregating for hemophilia B. Each letter stands for a separate probe as indicated. Uppercase letters indicate the more common alleles, lowercase letters the rarer alleles. St14 (DXS52) reveal multiple alleles, which have been given numbers. □ = A healthy male; and ○ = a healthy female; ■ = affected males; ⊡ = nonpenetrant males; and ⊙ = female carriers. In the first grandson two recombinants are evident (between cpX234 and cX38.1 and between 52A and F9), whereas in the second grandson only a single recombination is seen (between cpX234 and cX38.1).

1985). The new marker between F9 and Fra-X should make it easier to test whether the claimed heterogeneity is caused by variation in recombination within the whole region or is restricted to a small subregion. An alternative explanation is the occurrence of an inversion in the region of the fragile site in families with nonpenetrant males, a circumstance that should suppress recombination (Brown et al. 1985). To elaborate this further, data originating from such a family should be compared with data from normal families and from Fra-X families with penetrant males.

In addition, a large number of as yet nonpolymorphic cosmid-derived probes map to the distal part of Xq, a result that may be very useful when applied in a deletion screening, a procedure that itself has been very successful in the mapping of gene loci in the DMD region. Also, the availability of their flanking sequences permits the extension of the RFLP screening in this region with many more probes.

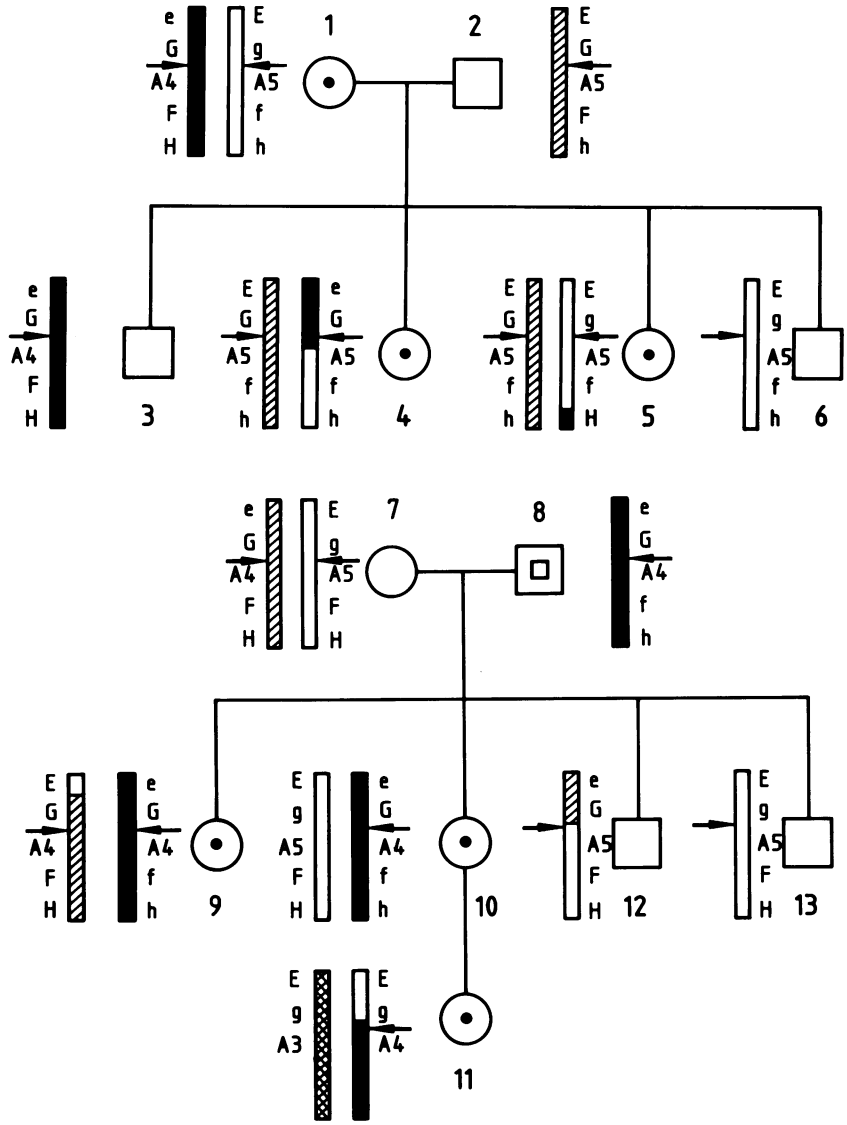


FIG. 8.—Two parts from a large Dutch pedigree segregating for Fra-X. See fig. 6 legend for explanation of symbols. Recombinations are shown between St14 and Fra-X (individual 4), DX13 and cpX67 (individual 5), cX55.7 and F9 (individual 9), cX55.7 and Fra-X (individual 11), and St14 and cX55.7 (individual 12).

ACKNOWLEDGMENTS

We would like to thank Dr. A. van Haeringen for computing the linkage data and Drs. M. H. Breuning and E. C. Klasen for their critical reading of this manuscript and their stimulating discussions. We are indebted to Dr. H. H. Ropers for making available the hybrid-cell panel and to G. G. Brownlee, Dr. D. Page, Dr. K. Davies, Dr. J. L. Mandel, and Dr. D. Drayna for the gift of the DNA probes used in the family studies. This project was financially supported by the Foundation for Medical Research (FUNGO) grant 13-23-47, which is subsidized by The Netherlands Organization of Pure Scientific Research (ZWO), The Netherlands Prevention Fund grant 28-878, and the Wellcome Trust.

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