Original Paper

Eur J Hum Genet 1993;1:64–71

Physical Mapping of Xq24-25 around Loci Closely Linked to the X-Linked Lymphoproliferative Syndrome Locus: An Overlapping YAC Map and Linkage between DXS12, DXS42, and DXS37

Qing Wang[a]
Yumiko Ishikawa-Brush[b]
Anthony P. Monaco[b]
David L. Nelson[c]
C. Thomas Caskey[c,d]
Susanne P. Pauly[a]
Gilbert M. Lenoir[a]
Bakary S. Sylla[a]

[a] International Agency for Research on Cancer, Lyon, France;
[b] Human Genetics Laboratory, Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, UK;
[c] Institute for Molecular Genetics and
[d] Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Tex., USA

Key Words
XLP
YAC
Mapping

Abstract
We have localized several markers in the Xq24-25 region containing DXS12, DXS42 and DXS37 which are closely linked to the X-linked lymphoproliferative syndrome (XLP) locus. A 850-kb restriction map has been established by mapping overlapping YACs and showed that DXS12 and DXS42 are physically linked within about 50 kb. DXS37 is separated from these two loci at a maximum distance of 3,700 kb. Several new probes have been generated which will contribute to further physical mapping of this region.

Introduction

The X-linked lymphoproliferative syndrome (XLP) is an X-linked recessive genetic disorder characterized by fatal mononucleosis, hypogammaglobulinemia or malignant lymphoma following infection by Epstein-Barr virus (EBV) [1]. Genetic linkage studies have localized the XLP locus to the Xq24-25 region. The DXS42 and DXS37 markers were shown to be respectively at about 1–2% ($Z = 17.5$) and 3% ($Z = 11.8$) recombination proximal to the XLP locus [2–5]. A third marker in the Xq25 region, DXS12, has also been reported to be linked to the XLP locus, but no recombination was detected with this marker ($Z = 7.5$) [4, 5]. An interstitial deletion involving a portion of the Xq25 region has recently been detected in an XLP family [6]. This deletion should be useful in physical mapping and the isolation of candidate genes in this region.

Received: March 23, 1992
Final revision received: June 23, 1992
Accepted: July 27, 1992

Bakary S. Sylla
International Agency for Research on Cancer
150, cours Albert-Thomas
F-69372 Lyon Cedex 08 (France)

1018–4813/93/
0011–0064$2.75/0

Establishing physical map around the DXS12 and DXS42 markers that are closely linked to the XLP locus is an important step towards isolating the susceptibility gene. In this paper, we report the assignment of several DNA markers to the Xq24-25 region that contains loci closely linked to the XLP locus, and the mapping of overlapping YACs covering a distance of about 850 kb around DXS42. We also show that DXS12 and DXS42 are situated with a maximum distance of about 50 kb, and that DXS37 is separated from them by a maximum distance of about 3,700 kb.

Materials and Methods

Cell Lines

A human lymphoblastoid cell line GM1202, which contains four X chromosomes per cell, was obtained from J.L. Mandel (Strasbourg, France). The lymphoblastoid cell line IARC745 was established in our laboratory from a female individual (XX).

Two human-mouse hybrid fibroblast cell lines were obtained from H. Ropers (Nijmegen, The Netherlands): 494×393, with translocation (X;13) (q22;q32), retaining human Xq22-qter; and 790×175, with translocation (X;19) (q24;q13), retaining human Xq24-qter [7]. The rodent-human hybrid cell line CH63R, with translocation (X;17) (q26;p12) retaining human Xq26-qter, was obtained from J.L. Mandel [8]. The human-mouse hybrid cell line CY2, with translocation (X;16) (q26;q24) retaining human Xp-q26, was obtained from G. Sutherland (North Adelaide, Australia) [9].

C12D is a human-hamster cell hybrid containing a single human X chromosome and A23N is a parental hamster cell line of C12D; both of these cell lines were obtained from P. Goodfellow (ICRF, London, UK).

DNA Probes

The preparation of probes 36B-2 (DXS10), p22-33 (DXS11), pL2 (DXS12), 30RIB (DXS37), 7F1 (DXS42), St16 (DXS53), St1 (DXS86), pX58C (DXS99), pX45h (DXS100), and p2aB5 (DXS138), has been described elsewhere [10]. The left- and right-end probes of YAC vector were obtained by cutting plasmid pBR322 with BamHI and PvuII: the 2.7-kb fragment was used as the left-end probe and the 1.6 kb fragment was used as the right-end probe.

We prepared the following probes: R3-5 was a 1.8 kb EcoRI/HindIII fragment in pUC18, isolated from YAC137. 63R5, 63L3 were subclones of YAC4563 and contained respectively 2- and 4.5-kb EcoRI/HindIII fragments in pUC18. 64–22 was a 1-kb EcoRI fragment in pUC18 and p64L8 was a EcoRI/HindIII fragment in pUC18; both of these were obtained from YAC4564. Y63.3 was a 1.8-kb BamHI fragment in pBluescript, obtained by the AluPCR technique [11] from YAC4563.

Screening of YAC Libraries

Two independently constructed YAC libraries were screened. One (from the Institute of Molecular Genetics and Howard Hughes Medical Institute) was constructed from the hybrid cell line X3000-11.1, which contains the human X chromosome region Xq24-qter [11]. The second (ICRF YAC library) was constructed from human lymphoblastoid cell line GM1416B which contains four X chromosomes [12]. Both libraries were made by partially digesting DNA with EcoRI and cloning it in pYAC4 vector. The YAC library made from X3000-11.1 was screened as described in Nelson et al. [11]. The ICRF YAC library was screened by hybridization as described in Larin et al. [12] using high- density filters from the ICRF reference library system [13].

DNA Preparation in Agarose Plugs and PFGE Analysis

For preparation of yeast DNA plugs, we used a modified protocol of McCormick et al. [14]. 50 ml of AHC medium cultured (OD_{600} = 2) yeast cells were harvested by centrifugation then suspended in 1.5 ml of 10 m*M* Tris pH 7.5/1 m*M* EDTA (TE) buffer and mixed with 2.5 ml of 1% LMP agarose (InCert) with the addition of 400 µg of Zymolyase 20T (ICN Biomedicals). Each plug was made from 85 µl of this mixture. The plugs were then incubated in ET solution (0.5 *M* EDTA pH 8.0/10 m*M* Tris) at 37 °C for 8–10 h, then in ESP solution (0.5 *M* EDTA/1% N-lauroylsarcosine and 20 mg/ml proteinase K) at 50 °C for about 12 h. After incubation, the plugs were washed with excess TE three times at room temperature, twice for 30 min with 4 µg/ml phenylmethylsulfonyl fluoride (PMSF) solution at 50 °C, followed by storage in +4 °C. Preparation of genomic DNA plugs was performed as described by Nguyen et al. [15].

We used a Bio-Rad CHEF-II system for pulsed-field gel electrophoresis analysis. DNA fragments were routinely separated on 0.8% agarose gels in 0.5 × TBE buffer (1 ×: 90 m*M* Tris-borate/2 m*M* EDTA) at about 15 °C. The migration conditions are described

Table 1. Analysis from mapping Xq24-27 markers with somatic cell hybrids

Probe	Locus	GM1202 XXXX	CL2D X	494×393 Xq22-qter	790×175 Xq24-qter	CH63R Xq26-qter	CY2 Xp-q26	A23 hamster
p22-33	DXS11	++	+	+	+	–	+	–
pL2	DXS12	++	+	+	+	–	+	–
30RIb	DXS37	++	+	+	+	–	+	–
7F1	DXS42	++	+	+	+	–	+	–
pX45h	DXS100	++	+	+	+	–	+	–
p2aB5	DXS138	++	+	+	+	–	+	–
St16	DXS53	++	+	+	+	+	+	–
36B2	DXS10	++	+	+	+	+	–	–
St1	DXS86	++	+	+	+	+	–	–
pX58C	DXS99	++	+	+	+	+	–	–

+ = Presence; – = absence. The hybridization signals were proportionally abundant in GM1202, with four X chromosomes per cell, indicated as ++.

Table 2. Approximate sizes (kb) of rare-cutter restriction fragments detected with Xq24-25 probes

Locus	Probes	XhoI	SmaI	SalI	XhoI/SalI	XhoI/SmaI	NotI	NruI
DXS11	p22-33	120	55	500	40	60	4,000	4,500
		50	40			40	3,500	
			<30					
DXS42	7F1	**90**	**130**	**350**	**90**	**80**	**3,700**	**4,000**
			80	**270**		**75**		
DXS12	pL2	**90**	**130**	**350**	**90**	**80**	**3,700**	**4,000**
			80	**270**		**75**		
			55					
DXS37	30RIb	280	240	180	70	50	**3,700**	**4,000**
			80					
DXS100	pX45h	350	50	250	70	40	3,000	2,800
				40				
				<30				
DXS138	p2aB5	NT	NT	NT	NT	NT	<2,000	3,000

The same filters were used to hybridize subsequently with different probes. The fragments shared by different probes are boldface type. NT = Not tested.

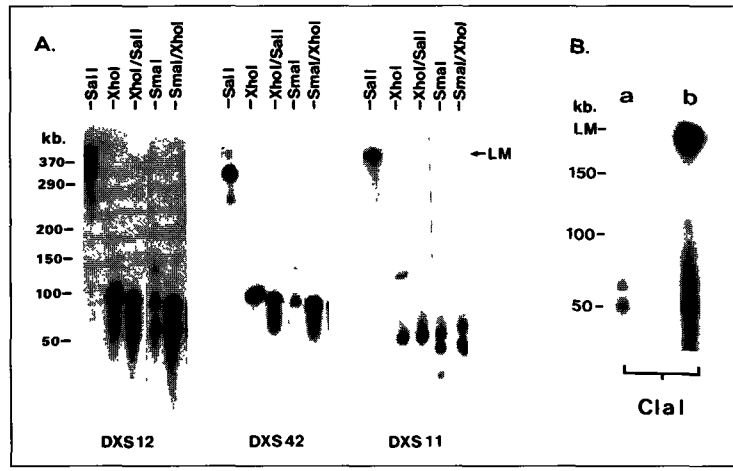

Fig. 1. PFGE analysis of Xq24-25 region probes. GM1202 cell line DNA was digested with different rare-cutter enzymes and separated by a ramping pulse time of 1–10 s for 20 h migration at 200 V. After transfer, the same filters were used for subsequent hybridizations. **A** Hybridizations to DXS12, DXS42 and DXS11 with digestion of SmaI, XhoI, SalI or a combination of them. **B** Hybridizations to DXS42 (**a**) and DXS12 (**b**) with ClaI digestion. Lambda concatamers was used as size standard. LM = Limit of mobility.

in the figure legends. After electrophoresis, DNA was blotted on to Hybond-N^+ membrane in 0.5 *M* NaOH/1.5 *M* NaCl solution preceded by treatment with 0.25 *N* HCl solution for 15 min. The hybridization was carried out in CHURCH solution: 0.5 *M* phosphate buffer pH 7.2/7% sodium dodecyl sulfate (SDS)/1% bovine serum albumin (BSA) at 65 °C and stringent washing conditions were used, i.e. 2 × SSC (1 ×: 0.15 *M* NaCl/1.5 m*M* sodium citrate)/0.1% SDS twice followed by 0.1 × SSC/0.1% SDS once at 55–65 °C. Genomic DNA extraction and conventional Southern blotting were carried out as described elsewhere [16].

Results

Localization of Several Markers in the Xq24-25 Region

Table 1 presents the analysis of the localization of several DNA markers previously assigned to the Xq24-27 region [10], related to a set of somatic hybrids with different breakpoints (see Materials and Methods). On the basis of these results, DXS11, DXS12, DXS37, DXS42, DXS100 and DXS138 were assigned to the Xq24-25 region, DXS53 to Xq26, and DXS10, DXS86, DXS99 to Xq26-qter. These data are consistent with the observation of Reilly et al. [17], who localized several DNA markers from the Xq24-26 region using a different somatic hybrid panel and proposed the following order: Xcen-(DXS42, DXS37, DXS100)-DXS53-HPRT-(DXS10, DXS86, DXS177)-Xqter. DXS10 and DXS86 have been shown to be physically linked [17–19, Wang et al., unpubl. results].

PFGE Analysis and Physical Linkage between DXS12, DXS42 and DXS37

The results presented in table 1 suggested that the loci DXS11, DXS12, DXS37, DXS42, DXS100 and DXS138 are located in the same subregion of the X chromosome at Xq24-25. We therefore attempted to see if they are linked physically by using PFGE. DNA from the cell line GM1202 was digested completely with rare-cutter enzymes XhoI, SmaI, SalI, NotI, NruI and various combination of these, and hybridized with Xq24-25 probes (summarized in table 2). Figure 1 presents the hybridization results with probes for DXS12, DXS42 and DXS11. No evidence of physical linkage between DXS11, DXS100, DXS138 and either DXS12, or DXS37, or DXS42 was detected. However, we observed that the probes for DXS12 and DXS42 gave the same hybridization bands with all of the rare-cutter enzymes used

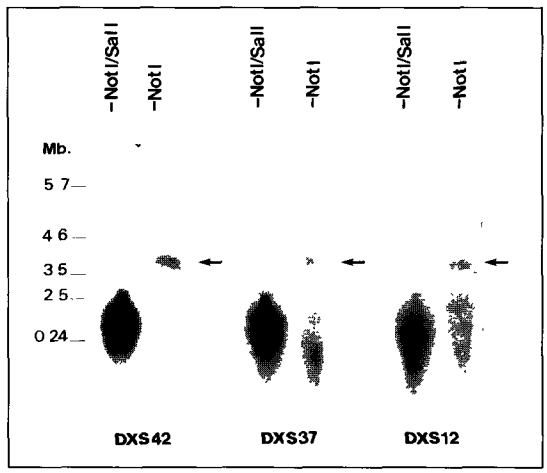

Fig. 2. PFGE analysis of DXS42, DXS12 and DXS37 with NotI digestion and NotI/SalI. The gel was run at 60 V with a pulse time of 35 min for 108 h, in 1 × TAE buffer (0.04 *M* Tris-acetate/1 m*M* EDTA). The same filter was hybridized subsequently with three probes. *S. pombe* and *S. cerevisiae* YNN295 were used as size standard. The fragment of 3,700 kb is indicated.

(fig. 1A, table 2), the smallest being 50 kb with ClaI (fig. 1B). Another observation was that the DXS37 probe hybridized a 3,700-kb NotI fragment, and this fragment was shared by DXS12 and DXS42 (fig. 2). Furthermore, DXS12, DXS42 and DXS37 revealed the same 4000 kb NruI fragment (table 2). The NotI band disappeared after double digestion with NotI and SalI (fig. 2). The cohybridization of these probes could not be an artifact due to incomplete probe stripping, as during the intervals between these hybridizations, the filter was also used with other probes which revealed different-sized fragments.

Mapping of Overlapping YACs around DXS12 and DXS42

The fact that most PFGE fragments for DXS12, DXS42 were very small and that only a limited number of DNA markers were available made it difficult to establish a long-range restriction map directly with genomic DNA. However, the development of the YAC cloning technique provided us a very helpful approach to establishing an extended map of the region around DXS12 and DXS42.

The DXS42 probe was used to screen two independently constructed YAC libraries (see Materials and Methods), by colony hybridization. Three positive clones were isolated: YAC137 (200 kb) from the library made from cell line X3000-11.1; YAC4563 (ICRF code: ICRFy900F0981) and YAC4564 (ICRF code: ICRFy900A0816) from the library constructed form GM1416, 780 and 800 kb in size, respectively.

The restriction map of each YAC was constructed by indirect end-label mapping [20]. DNA plugs of these YACs were digested with various concentrations of rare-cutter enzymes. After electrophoresis and transfer, filters were hybridized successively with vector left- and right-end probes. On the basis of the individual restriction map of each YAC, we were able to orient and align these overlapping YACs to obtain a restriction map shown in figure 3B. As described above, DXS12 and DXS42 are closely linked, both being situated in the 90-kb XhoI fragment of YAC137 (fig. 3), but they could not be oriented further.

In theory, the restriction site pattern in an overlap region between YACs should be consistent. This was the case for the majority of the enzymes used. In order to verify the fidelity of this map, several probes were isolated by subcloning YAC DNA in lambda phage vectors or by the AluPCR technique [11] (fig. 3). Each probe was checked by hybridization on somatic hybrids, to confirm its localization on the same subregion in the X chromosome as DXS12 and DXS42. The restriction site patterns around the DXS42 probe, R3-5 and 64-22 are consistent in three YACs. For instance, R3-5 and 64-22 detected the

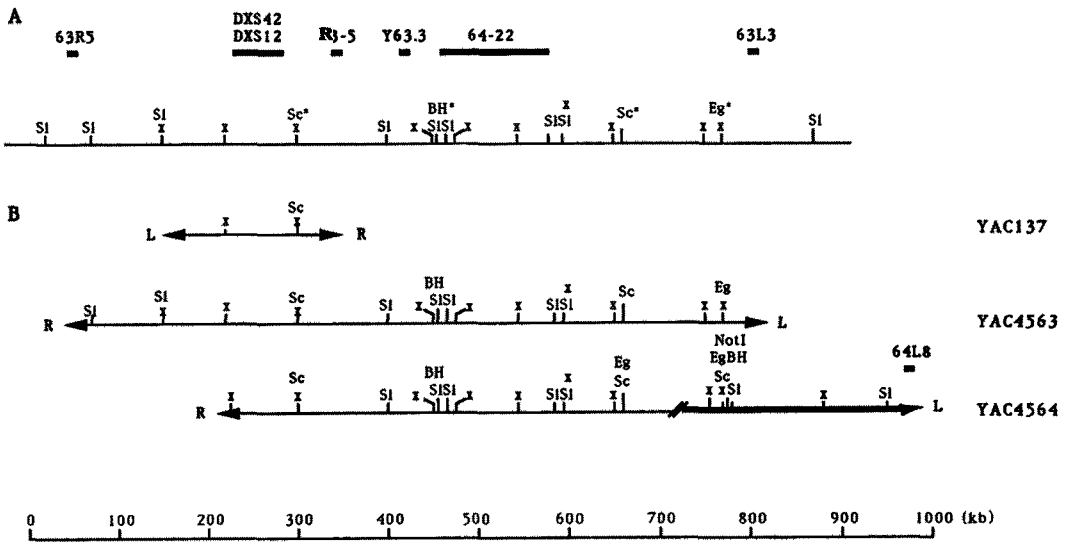

Fig. 3. Physical map of overlapping YACs around DXS12 and DXS42. **A** Restriction map at the genomic DNA level, illustrating the localization of loci DXS12, DXS42 and the probes generated from the YACs. The methylated restriction sites are indicated by *. **B** Three YACs: YAC137 (200 kb), YAC4563 (780 kb) and YAC4564 (800 kb) were mapped and aligned by partial rare-cutter enzyme digestion and PFGE analysis. X = XhoI; Sl = SalI, Sc = SacII; BH = BssHII; Eg = EagI. The cocloned region at the left extremity of YAC4564 is indicated by a black box. L = Left end; R = right end.

same 350-kb SacII fragment in both YAC4563 and YAC4564. However, an important discrepancy between YAC4563 and YAC4564 was noticed in the left extremity. In fact, the probe 64L8 (left-end probe of YAC5464) was found to be located in the Xq22-23 region. Furthermore, the probe 63L3 (left-end probe of YAC4563), which overlaps with YAC4564, did not recognize any YAC4564 sequence (data not shown). Taken together these results indicated that a cocloning event had taken place at the left extremity of YAC4564 (fig. 3).

The other markers in the Xq24-25 region (DXS11, DXS37, DXS100 and DXS138) have been tested but none of them is contained in these overlapping YACs.

The Restriction Map from YACs Related to X Chromosome Genomic DNA Structure

The probes 63R5, R3-5, 64-22 and 63L3 were hybridized to genomic DNA, and all recognized the same 3,700 kb NotI fragment as DXS12, DXS42 and DXS37. This indicated that the 850-kb map is included in this 3,700 NotI fragment, but the relative position of DXS37 could not be determined at this stage.

To verify that the map from the YACs reflects the correct structure of restriction sites around DXS42 in the human X chromosome, DNA from YACs and cell lines GM1202, IARC745 and C12D were digested with various restriction enzymes and fractionated by

classical or PFG electrophoresis. Using classical Southern blotting, all the probes tested hybridized to the same fragments in YACs and in genomic DNA. However, on PFGE, a small difference in fragment migration between YAC and genomic DNA was noticed with all enzymes and probes used, which may be due to the difference in DNA concentration between yeast and genomic DNA plugs. Sites for some rare-cutter enzymes such as SacII, BssHII, and EagI were not cleaved in genomic DNA (data not shown), in contrast to YAC DNA. Taking into account the fact that these enzymes are all sensitive to 5-methylcytosine in the CpG sequence, and that no methylation of cytosine has been observed in yeast [21], we assume that these sites in genomic DNA are methylated.

Discussion

We have used a combined approach to construct a physical map around DXS12 and DXS42 loci, which are genetically linked to the XLP gene. The results from localization of DNA markers with somatic hybrids are consistent with those obtained by other investigators [17, 18, 22]. We confirmed the localization of DXS11, DXS37, DXS42 and DXS100, previously assigned to the Xq24-25 region, and we localized two other loci (DXS12 and DXS138) to the same sub-region of the X chromosome. The results from PFGE and YACs indicate that DXS12 and DXS42 are tightly linked, within 50 kb, and DXS37 is separated from DXS12 and DXS42 by a maximum of 3,700 kb, but we do not know their positions relative to the centromere and telomere.

The YAC cloning technique proved its value for large-scale physical mapping, especially in this region where few DNA markers were available. However, caution must be exercised, as up to 20% of YACs could be chimeric [23], maybe particularly with larger inserts. In our case, YAC4564 was shown to be a cocloned one, with a large insert from Xq24-25 and a small insert probably from Xq23.

The methylation of CpG sequence in genomic DNA complicates the verification of the YAC contig map at genomic DNA level, as described by other authors [24]. However, the fidelity of our YAC map was largely confirmed in an overlapping region where the restriction patterns among three (or two) YACs are identical. Furthermore, internal probes were used and detected the same sized fragment in both YAC and genomic DNA.

In conclusion, although the X chromosome has been much studied, little was known hitherto about the Xq24-25 region, principally because of the lack of DNA markers in this region, which hampered PFGE mapping and isolation of YAC contigs. The work presented in this paper has provided us with insight into this region containing the XLP gene. The probes generated will contribute to the further long-range physical mapping, and the detection of polymorphism of these probes will be helpful in precisely localizing the XLP locus and isolating the gene.

Acknowledgments

We thank Drs J. Skare, J.L. Mandel, B. White, H. Roper, G. Sutherland, K. Paulson and P.N. Goodfellow for kindly providing the probes and cell lines. We thank Drs M. Ross, S. Meier-Ewert and H. Lehrach for high-density filters of the ICRF YAC library and G. Zehetner and C. Douglas for YAC clone acquisition through the ICRF reference library database. We thank Dr. J. Feunteun for helpful suggestions, Drs R. Montesano and I. Oberlé for their critical reading of the manuscript and Dr. J. Cheney for editing the manuscript. We also thank C. Bonnardel for material collection and A. Trochard for preparing the manuscript. Q.W. is a recipient of a grant from Comité Départemental de l'Ain, Ligue Nationale Française contre le Cancer.

References

1 Purtilo DT, Yang JP, Cassel CK, Harper P, Stephenson SR, Landin BH, Vawter GF: X-linked recessive progressive conbined variable immunodeficiency (Duncan's disease). Lancet 1975;i:935–940.

2 Skare JC, Grierson HL, Sullivan JL, Nussbaum RL, Purtilo DT, Sylla BS, Lenoir GM, Reilly DS, White BN, Milunsky A: Linkage analysis of seven kindreds with the X-linked lymphoproliferative syndrome (XLP) confirms that the XLP locus is near DXS42 and DXS37. Hum Genet 1989;82:354–358.

3 Sylla BS, Wang Q, Hayoz D, Lathrop GM, Lenoir GM: Multipoint linkage mapping of the Xq25-26 region in a family affected by the X-linked lymphoproliferative syndrome. Clin Genet 1989;36:459–462.

4 Skare JC, Grierson H, Wyandt H, Sanger W, Milunsky J, Purtilo D, Sullivan J, Milunsky A. Genetics of the X-linked lymphoproliferative syndrome. Am J Hum Genet 1989; 45:A161 (0630).

5 Purtilo DT, Grierson HL: Methods of detection of new families with X-linked lymphoproliferative disease. Cancer Genet Cytogenet 1991;51: 143–153.

6 Sanger WG, Grierson HL, Skare J, Wyandt H, Pirruccello S, Fordyce R, Purtilo DT: Partial Xq25 deletion in a family with the X-linked lymphoproliferative disease (XLP). Cancer Genet Cytogenet 1990;47: 163–169.

7 Wieacker P, Davis KE, Cooke HJ, Pearson PL, Williamson R, Bhattacharya S, Zimmer J, Ropers HH: Toward a complete linkage map of the human X chromosome: regional assignment of 16 cloned single-copy DNA sequences employing a panel fo somatic cell hybrids. Am J Hum Genet 1984;36:265–276.

8 Oberlé I, Camerino G, Kloepfer C, Moisan JP, Grzeschik KH, Hellkuhl B, Hors-Cayla MC, Van Cong N, Weil D, Mandel JL: Characterization of a set of X-linked sequences and of a panel of somatic cell hybrids useful for the regional mapping of the human X chromosome. Hum Genet 1986;72:43–49.

9 Callen DF: A mouse-human hybrid cell panel for mapping human chromosome 16. Ann Genet 1986;29: 235–239.

10 Tenth International Workshop on Human Gene Mapping. New Haven Conference. Cytogenet Cell Genet 1989;51:1–1148.

11 Nelson DL, Ballabio A, Victoria MF, Pieretti M, Bies RD, Gibbs RA, Maley JA, Chinault AC, Webster TD, Caskey CT: Alu-primed polymerase chain reaction for regional assignment of 110 yeast artificial chromosome clones from the human X chromosome: Identification of clones associated with a disease locus. Proc Natl Acad Sci USA 1991;88:6157–6161.

12 Larin Z, Monaco AP, Lehrach H: Yeast artificial chromosome libraries containing large inserts from mouse and human DNA. Proc Natl Acad Sci USA 1991;88:4123–4127.

13 Lehrach H, Drmanac R, Hoheisel J, Larin Z, Lennon G, Nizetic D, Monaco AP, Zehetner G, Poustka A: Hybridization fingerprinting in genome mapping and sequencing; in Davies DE, Tilghman SM (eds): Genome Analysis. Cold Spring Harbor, Cold Spring Harbor Library Press. 1990, vol 1, pp 39–81.

14 McCormick MK, Shero JH, Connelly CL, Antonarakis SE, Hieter PA: Methods for cloning large DNA segments as artificial chromosomes in *S. cerevisiae* Technique 1990;2: 65–71.

15 Nguyen C, Pontarotti P, Birnbaum D, Chimini G, Rey JA, Mattei JF, Jordan BR: Large scale physical mapping in the q27 region of the human X chromosome: The coagulation factor IX gene and the mcf.2 transforming sequence are separated by at most 270 kilobase pairs and are surrounded by several 'HTF islands'. EMBO J 1987;6:3285–3289.

16 Sambrook J, Fritsch EF, Maniatis T: Molecular cloning. Cold Spring Harbor Laboratory Press, 1989.

17 Reilly DS, Lewis RA, Nussbaum RL: Genetic and physical mapping of Xq24-26 markers flanking the Lowe oculocerebrorenal syndrome. Genomics 1990;8:62–72.

18 Nicklas JA, Hunter TC, O'Neill JP, Albertini RJ: Fine stucture mapping of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene region of the human X chromosome (Xq26). Am J Hum Genet 1991;49: 267–278.

19 Zucchi I, Schlessinger D: Distribution of moderately repetitive sequences pTR5 and LF1 in human Xq24-q28 and their use in assembling YAC contigs. Genomics 1992; 12:264–275.

20 Burke DT, Carle GF, Olson MV: Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 1987;236:806–812.

21 Proffitt JH, Davie JR, Swinton D, Hattman S: 5-Methylcytosine is not detectable in *Saccharomyces cerevisiae.* Mol Cell Biol 1984;4:985–988.

22 Oberlé I, Camerino G, Wrogemann K, Arveiler B, Hanauer A, Raimondi E, Mandel JL: Multipoint genetic mapping of the Xq26-28 region in families with fragile X mental retardation and in normal families reveals tight linkage of markers in q26-27. Hum Genet 1987;77:60–65.

23 Schlessinger D, Little RD, Freije D, Abidi F, Zucchi I, Porta G, Pilia G, Nagaraja R, Johnson SK; Yoon JY, Srivastava A, Kere J, Palmieri G, Ciccodicola AC, Montanaro V, Romano G, Casamassimi A, D'Urso M: Yeast artificial chromosome-based genomic mapping: Some lessons from Xq24-28. Genomics 1991;11:783–793.

24 Butler R, Ogilvie DJ, Elvin P, Riley JH, Finniear RS, Slynn G, Morten JEN, Markham AF, Anand R: Walking, cloning, and mapping with yeast artificial chromosomes: A contig encompassing D21S13 and D21S16. Genomics 1992;12:42–51.