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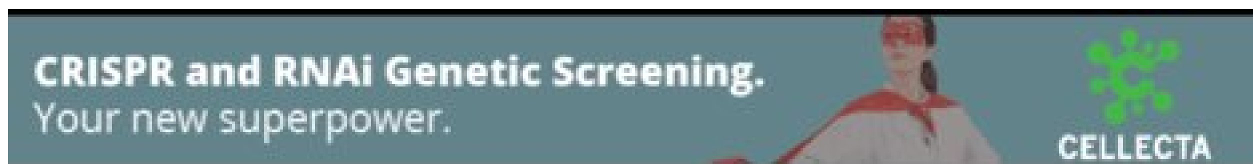

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An Integrated YAC Map of the Human X Chromosome

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The human X chromosome is associated with a large number of disease phenotypes, principally because of its unique mode of inheritance that tends to reveal all recessive disorders in males. With the longer term goal of identifying and characterizing most of these genes, we have adopted a chromosome-wide strategy to establish a YAC contig map. We have performed >3250 inter Alu-PCR product hybridizations to identify overlaps between YAC clones. Positional information associated with many of these YAC clones has been derived from our Reference Library Database and a variety of other public sources. We have constructed a YAC contig map of the X chromosome covering 125 Mb of DNA in 25 contigs and containing 906 YAC clones. These contigs have been verified extensively by FISH and by gel and hybridization fingerprinting techniques. This independently derived map exceeds the coverage of recently reported X chromosome maps built as part of whole-genome YAC maps.

The establishment of clone maps for each human chromosome is a prerequisite for transcript mapping and genomic sequencing. This goal became feasible following the development of the yeast artificial chromosome (YAC) cloning system (Burke et al. 1987). Several human YAC libraries have been made available (Anand et al. 1989; Albertsen et al. 1990; Larin et al. 1991; Chumakov et al. 1995), and YAC maps have now been reported covering most of chromosome Y (Foote et al. 1992), 21 (Chumakov et al. 1992), 22 (Collins et al. 1995), 3 (Gemmill et al. 1995), 12 (Krauter et al. 1995), and 16 (Doggett et al. 1995).

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The X chromosome is one of the most intensively studied of all human chromosomes. A reason for this interest is that males are hemizygous for X chromosome loci, and hence more disease phenotypes have been revealed on the X chromosome than on any autosome (McKusick 1994). The mapping of disease genes on the X chromosome is facilitated by their characteristic phenotypic pattern (female carriers, affected male offspring) and by the manifestation of maternal meiotic recombinations between X chromosomal loci in male offspring.

Three whole X chromosome YAC mapping studies are under way. The first two are part of whole-genome mapping studies at the Centre d'Etudes du Polymorphisme Humain (CEPH) (Chumakov et al. 1995) and Whitehead Institute/

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Massachusetts Institute of Technology (MIT) (Hudson et al. 1995), where the X chromosome is poorly represented compared with autosomes. The third study combines contigs from many groups (R. Nagaraja, S. MacMillan, J. Miao, C. Jones, B. Cho, B. Eble, G. Halley, M. Masisi, J. Terrell, M. Trusgnich, et al., pers. comm.) and was reported at the sixth X chromosome workshop (D.L. Nelson, A. Ballabio, F. Cremers, A.P. Monaco, and D. Schlessinger, pers. comm.). At that time this map, based on sequence-tagged site (STS) content information, was estimated to cover 70% of the chromosome. In addition to these global efforts, numerous YAC contigs have been established in smaller regions defined by genetic mapping or by cytogenetic abnormalities. They have often been a template for the cloning of disease genes by positional cloning [recently HYP (The HYP consortium 1995), OA1 (Bassi et al. 1995)] and sometimes have evolved into physical and transcriptional maps of larger regions (Ferrero et al. 1995). These regional efforts have often used common sets of markers and library clones, and from these it has been possible to establish "consensus" YAC maps over still larger tracts of the chromosome (Nelson et al. 1995).

Our goal is to establish a physical map of the whole X chromosome, and here we report the results of our efforts to establish a map in contiguous YAC clones (Fig. 1). Our efforts have yielded currently 25 contigs covering an estimated 125 Mb (80%) of the X chromosome (Fig. 1). These contigs have been established primarily by direct hybridizations between YAC clones, and are supported by two fingerprinting methods, YAC end mapping and fluorescence in situ hybridization (FISH) localizations (see Fig. 2). The map contains 906 clones known to cover 655 genetic marker loci and a further 192 discrete marker loci (YAC end, cloned inter Alu-PCR product). This result comes at a time when comparisons between autosomes and the X chromosome indicate a shortage of polymorphic STS markers on the latter (Chumakov et al. 1995; Hudson et al. 1995). The present study, based on different techniques, avoids the pitfalls of STSs mapping and draws together global and regional expertise to integrate resources on the X chromosome.

RESULTS

Large-scale Generation of Overlap Information

Identification of X chromosomal YACs and primary YAC overlap information were derived by YAC to YAC hybridization experiments (Fig. 3). Multiple entry points were established along the chromosome by random probe selection from an X chromosome-specific YAC library (Lee et al. 1992) (HHMI hereafter). Probes were derived from individual YACs by inter Alu PCR (Nelson et al. 1989) using a combination of the primers ALE1 and ALE3 (Cole et al. 1991), which recognize the most conserved regions of the human Alu repeat and direct amplification outward from its left and right ends, respectively. Hybridization targets were also inter Alu-PCR products, derived both from the HHMI library and from the whole-

Figure 1 Integrated YAC map of the human X chromosome, slightly modified from the IXDB (acedb version 4.3) map view. The scale is based on a 160-Mb chromosome and each graduation represents 5 Mb. A chromosome ideogram is drawn on the left, and each colored box to the right represents a YAC clone (see color code). The yellow boxes to the right of the clones show the extent of the contigs. These cover ~125 Mb of the chromosome (80%) and include 906 YAC clones. A magnified view of 10 Mb in Xp22 is shown to the right. The color code is an indication of only one of the techniques that contributed to the positioning of a given clone on the map. All clones shown have been either hit or used as probe in a hybridization experiment. White clones have no other evidence for their position. Clear blue clones contain the markers (DXS, genes) indicated to the right of the scale. Pink clones have been mapped by FISH. When the FISH experiment indicates a chimeric clone, the latter is shown in green. An Alu gel fingerprint is stored in IXDB for the dark blue clones and available in a dedicated viewing tool for comparison between clones. However, when a clone has been analyzed by more than one method (e.g., by gel fingerprinting and mapped by FISH), only one technique is indicated by the color code. In IXDB, a single click of the mouse produces a window where the complete set of information attached to a clone is displayed. Small red boxes between the clones and the chromosome bands represent cloned Alu-PCR products identified in hybridization fingerprint experiments. More than one Alu clone in a single position indicates that the order between the clones could not be resolved. A maximum of three clones are shown, and the average number of clones per position is 15. Units are in kilobases, starting from 0 to 160,000 (pter to qter). The scale is only indicative and facilitates the comparison with the X community consensus map.

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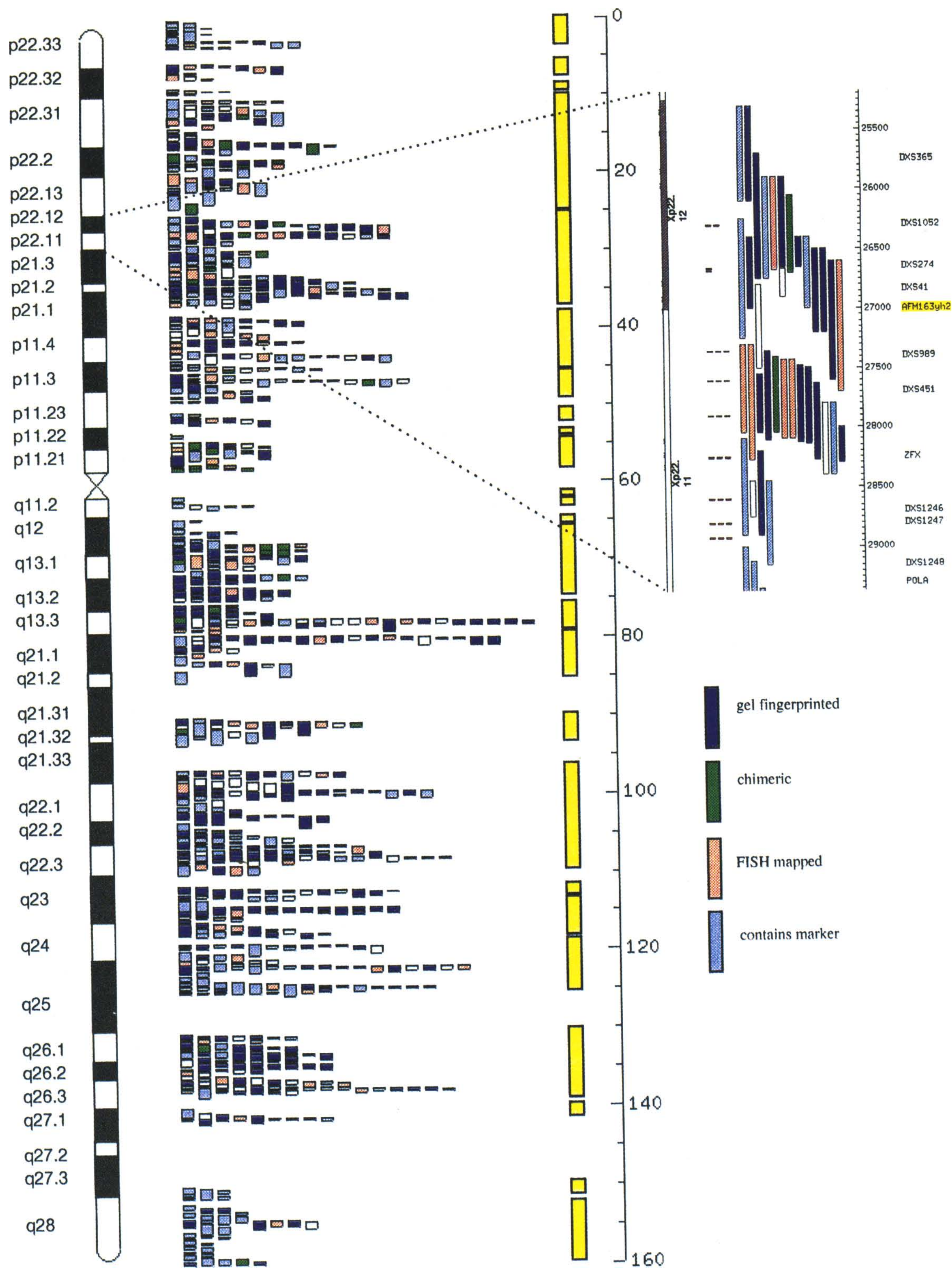

Figure 1 (*See facing page for legend.*)

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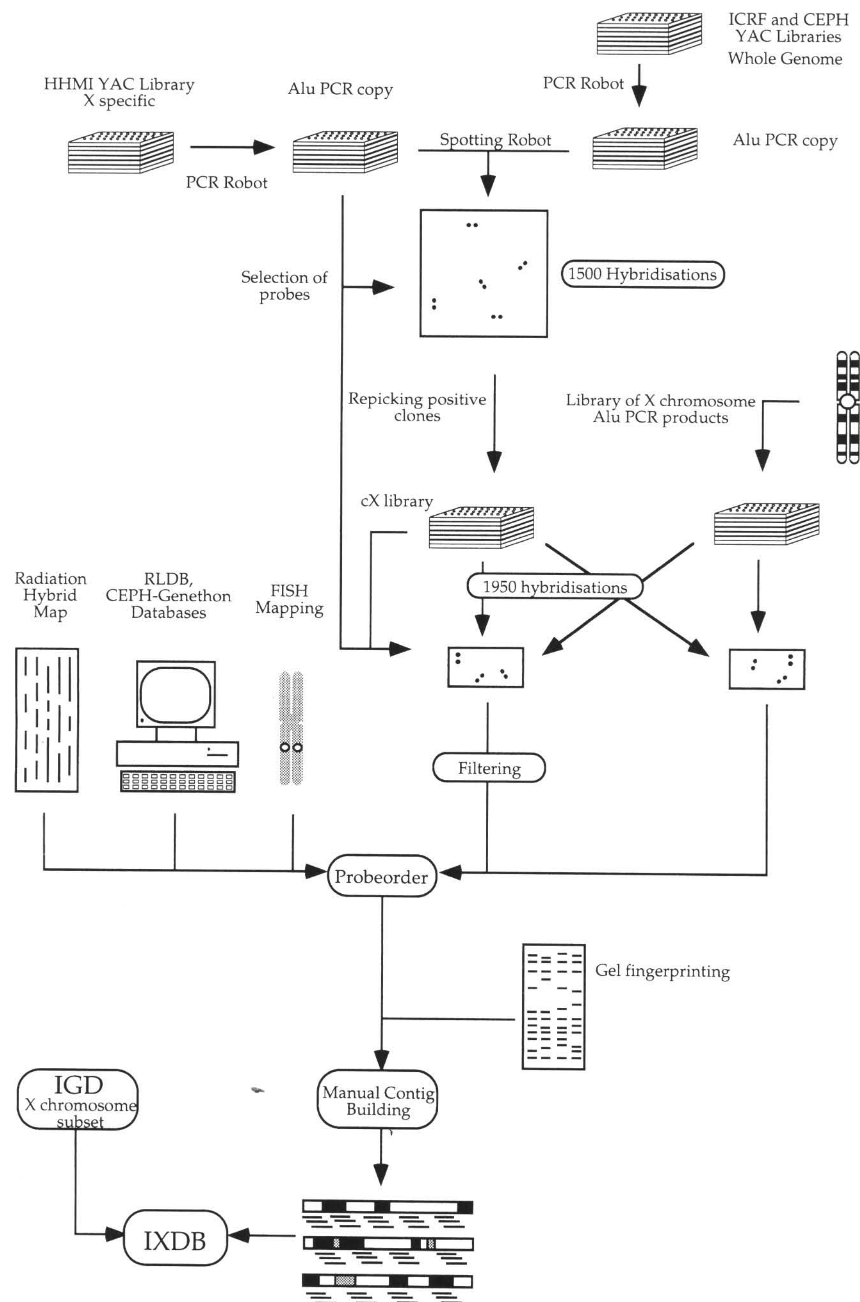

Figure 2 Schema of the strategy used to construct a YAC contig map of the X chromosome. Three YAC Libraries (HHMI, ICRF, and CEPH) were spotted as Alu-PCR products on nylon membranes, and a selection of probes from the X-specific library was used for hybridizations. The positive clones were repicked in a collection of X chromosome YAC clones (cX library), and more hybridizations were carried out with probes from the HHMI, cX, and cloned Alu-PCR product library. After scanning the data to remove cross-contamination and obvious false positives, the experimental data were combined with YAC mapping data from a separate radiation hybrid project, from the RLDB and CEPH-Genethon data bases, and from FISH mapping experiments. This was done using the program Probeorder, which was used to construct YAC clusters and to display all the information in one format. This information was combined with the gel and hybridization fingerprinting data and analyzed manually to build the final contigs. The resulting map and all the experimental data are combined with the X chromosome subset of IGD in IXDB.

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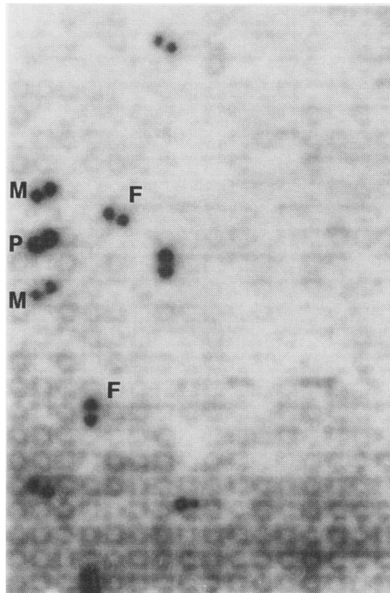

Figure 3 Example hybridization of a YAC Alu-PCR probe to a gridded array of YAC Alu-PCR products. The probe YAC (AA0801) was hybridized to an array of Alu-PCR products from 1536 YAC clones from the cX library (see text), each gridded in duplicate. The YAC hybridizes to itself (P) and to nine other clones. The YAC probe contains the marker loci DXS67 and DXS68 from Xp21.3. The two clones marked M also contain the DXS67 marker locus. The two clones marked F were localized by FISH to Xp22.1-21.3.

genomic Imperial Cancer Research Fund (ICRF) and CEPH "mega" YAC libraries. Together these libraries contain a theoretical 15-fold coverage of the X chromosome. Each of the 50,000 clones was amplified using a microtitre plate PCR robot (Meier-Ewert et al. 1993) and the products were gridded onto nylon membranes in high-density arrays. The inter Alu-PCR products from the YAC probes were radiolabeled and hybridized to the gridded arrays. In a total of 543 successful hybridizations of 764 performed with HHMI YAC probes to these arrays, 3978 different clones were identified (1727 ICRF, 643 CEPH, 1608 HHMI).

The 2370 positive clones from the ICRF and CEPH libraries were repicked into microtitre dishes to create a collection of X chromosomal YAC clones (the cX library). Hybridization filters were generated from the cX and HHMI libraries as described above. Inter Alu-PCR products from 316 clones in the cX library and a further 124 clones in the HHMI library were hybridized to these filter arrays, thus generating additional clone overlap information (Fig. 3).

Anchoring of YACs on the X Chromosome Map

In parallel to the large-scale experimental strategy described above, we collected genetic and physical mapping information on a large number of YAC clones used in this project. We have relied predominantly on preexisting information from two main sources: the Reference Library Database (RLDB; Zehetner and Lehrach 1994) and the CEPH/Genethon data base (Chumakov et al. 1995). The RLDB is a repository of mapping information obtained by the distribution of many types of reference libraries (YAC, cosmid, PAC, cDNA, etc.), including the ICRF and CEPH YAC libraries. We queried the RLDB for all human YAC clones previously mapped to the X chromosome, and retrieved 1723 records, of which 10% had also been confirmed by secondary screening. In addition, 28 RLDB participants provided 42 contigs in candidate regions for disease genes. Although there was some overlap between these data sets, information was collected on the marker content of 1181 clones. From the CEPH-Genethon data base, 711 YAC clones associated with an X chromosome marker and derived from the whole genome map were retrieved.

In total, these two sources provided marker information on 3074 YAC clones. Of these, 1150 were also identified in our hybridization and were used to annotate our contig map with marker information. However, it was not possible to treat all the outside data with the same level of confidence. We based our decisions on two conventions. First, we assumed that contigs provided by RLDB participants were completely correct with regard to the marker content of the YACs, unless a conflict between two or more groups existed in which case the situation that agreed with our data was assumed to be correct. The same applied to confirmed results from the RLDB. Second, nonconfirmed results from the RLDB and marker assignments derived from the CEPH data base were considered as only indicative and never used as sole evidence for the positioning of a clone on the map.

Localization of Unanchored Clones

FISH mapping experiments were performed with YAC clones belonging to unanchored contigs or with clones for which confirmation was needed before placing them on the map. Of 301 clones selected, 212 were assigned to the X chromosome, of which 48 were X-autosome chimerae.

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The 89 non-X clones were mainly in singleton contigs or in very short contigs that could not be linked to other contigs. A radiation hybrid map of the X chromosome constructed in our laboratory was also used for localizing contigs lacking markers relative to each other (Kumlien et al. 1996). The map, comprising 72 hybrids, was constructed using 50 STSs spread evenly along the chromosome. Inter Alu-PCR products from the hybrids were hybridized to filters of the cX library, and, conversely, 450 YACs were hybridized to the hybrid panel. This allowed 971 YACs to be placed with confidence in ~3-Mb intervals (average distance between the STSs used).

Overlap Refinement and Confirmation

As data analysis proceeded (see below) we selected 1149 clones for Alu-PCR-based gel fingerprinting (Coffey et al. 1996). YAC clones were amplified individually by Alu-PCR, and the products separated on polyacrylamide gels. Fingerprints were analyzed automatically by the program contigC (derived from Contig9; Sulston et al. 1988), which yields a probability of overlap based on the number of bands shared between clones. Subsequently, detailed manual comparison of fingerprints was used to confirm potential overlaps and to provide a suggested order of clones based on subsets of shared and nonshared bands. In a second method, Alu-PCR products from 340 YACs were hybridized to a library of cloned Alu-PCR products from the X chromosome (Fig. 4). Shared hybridization patterns between YAC clones suggested YAC overlaps. Results were analyzed by Probeorder, a software successfully applied when constructing a YAC map of the *Schizosaccharomyces pombe* genome (Maier et al. 1992; Mott et al. 1993). When applied to raw hybridization results, Probeorder uses the simulated annealing algorithm to find the optimal order of probes based on their hybridization pattern. In addition, clones are ordered according to the order of the markers they contain. Approximately 2000 cloned inter Alu-PCR products were identified and added to the map. These clones constitute a pool of potential single copy probes, and 350 of them were hybridized to the cX library. An additional set of 100 single-copy probes were developed from the ends of YAC inserts by the vectorette PCR method (Riley et al. 1990), and these were hybridized back to the cX library.

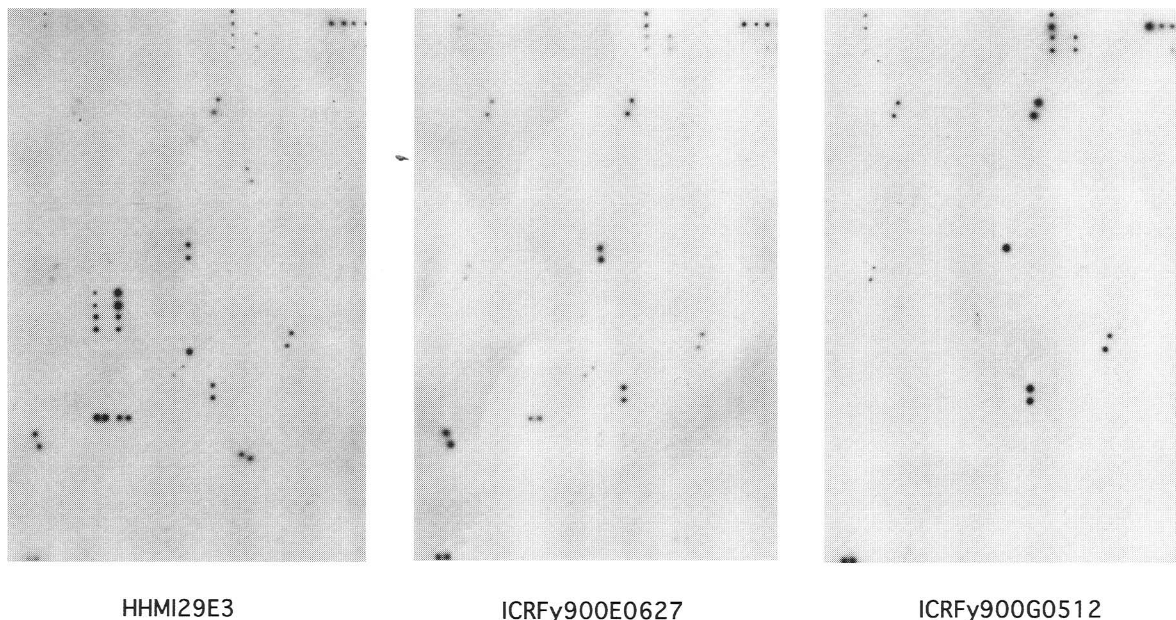

Figure 4 Hybridization results of three overlapping YAC clones on the library of cloned Alu-PRC products of the X chromosome. Each filter measures 7 × 11 cm and contains 9600 DNA spots, each in duplicate. Each clone has been amplified by Alu-PCR before spotting. Clones shared between the three YAC clones are clearly visible, and indicate that the YACs overlap.

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Automatic Data Integration and Map Construction

The YAC to YAC hybridization data and the positional information were integrated using Probeorder. Positions derived from FISH experiments and from the radiation hybrid map were also taken into account at this stage. We used Probeorder to analyze the entire data set (2700 hybridization results) and constructed 113 clusters comprising 4087 clones. Clusters were ordered along the chromosome according to positional information. In 38 cases, clusters contained a single probe (singletons) and, therefore, were not useful for contig construction. The remaining 75 clusters containing 3973 clones were used for constructing contigs manually. This was done by considering the different types of mapping information in order of importance; FISH and marker content information were considered together first, and then fingerprints (gel- and hybridization-based) were compared to confirm the overlaps and determine a relative order of clones within a cluster. In this procedure, reliable contigs were progressively extracted from the Alu-PCR hybridization data set generated by Probeorder. Links between clusters were identified in the output of Probeorder by seeking YAC probes hybridizing consistently to clones present in two different clusters. Identification of such links reduced the total number of contigs from 75 to 25. When available, results generated by RLDB collaborators with the same clones were compared and used to orientate contigs and confirm orders and overlaps between clones. Nonetheless, each single overlap presented on the map can be deduced directly from the experimental data described above, with the exception of 19 clones in the two most telomeric bands (Xp22.33 and Xq28), which were contributed directly by outside groups (Ried et al. 1995 and Rogner et al. 1995, respectively).

Finally, marker/YAC association derived from RLDB supported by FISH results was used to place the contigs on the consensus map of markers, constructed jointly by the X chromosome community. Because all of the RLDB collaborators that indirectly contributed to our project also participated in the establishment of the consensus map, the integration of our contigs with the consensus map was greatly facilitated. The result of this strategy is a YAC contig map of the X chromosome integrating 655 genetic markers with 906 YAC clones, organized into 25 contigs (see Fig. 1). The total coverage is estimated to be 80% of the length of the chromosome, or 125 Mb of DNA. Based on the hybridization fingerprints, 79 intervals could be defined in the YAC contigs, in which 1420 cloned PCR products were placed.

Public Availability

At an early stage in the course of this study, we opted for ACEDB as a graphical data base system, first to store the collection of mapping information derived from their various sources and, subsequently, as a software tool to construct the map in its graphical representation. In the data base called IXDB (Integrated X DataBase), the map presented here is combined with information obtained from the Integrated Genome Database, which uses ACeDB to assemble data from the major genome-related data bases (GDB, OMIM, GenBank, RLDB, etc.). IXDB is available on the World Wide Web at http://www.mpimg-berlin-dahlem.mpg.de/~xteam. In this repository, all the experimental data supporting the map is presented in a user friendly environment. The YAC clusters constructed automatically by Probeorder are also available at the same address.

DISCUSSION

We describe a predominantly hybridization-based experimental approach that has been applied to establish YAC clone contigs covering ~80% (125 Mb) of the human X chromosome in 25 contigs. The map comprises some 750 discrete markers of all types (genetic, vectorette, inter Alu-PCR products). We generated a large experimental data set that was first processed with computer programs to lower its complexity. A stringent manual analysis was then performed on each YAC cluster, using all available information.

We observed that in the Alu-PCR hybridization data, 22% of the probes did not hybridize to themselves. The hybridization data generated by these probes was still considered in the analysis, for the following reasons. The most frequent source of false negative can be ascribed to the absence of the probe DNA on the filter, either because the robot pins did not transfer liquid on this particular spot, or because the YAC did not amplify properly in the waterbath PCR robot. In these cases connections between the probe and the clones it identifies are still correct. Alternatively, it is possible that probes were accidentally

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mixed up, leading to apparent false negatives. These results could be detected easily in the redundant data set, as they present a clear aberrant hybridization pattern in the later analysis. We removed 51 probes from the data set based on this observation (7% of all probes). False-positive hybridization results can introduce false connections between clones. It is impossible to measure accurately the rate of false positives, but based on the number of links between probes that had to be ignored in the manual analysis, we estimate it at 10%–15%. The reason for their presence can be attributable either to human error (typing, scoring, sample handling), which is particularly acute in a large-scale project, or to nonspecific sequence similarities between clones. Mis-scoring was limited by the fact that each X-ray film was scored by two different persons independently. Human error was corrected further by scanning the data set with programs to detect specific patterns, for example, cross-contamination (probes in adjacent wells with identical hybridization patterns) or nonremoval of a probe from a filter (same clones positive in two successive hybridizations). Nonspecific sequence similarities are a well-known problem in mapping large regions of the human genome, and in addition come to the high level of chimerism observed in YAC libraries (30%). Whether attributable to repeat sequences or gene families, this problem can be avoided only by using complementary techniques to help make decisions. We have addressed this problem by complementing the Alu-PCR data with a battery of different types of data (fingerprints, marker content, FISH, end mapping, radiation hybrids) in a stringent manual analysis.

The map covers 80% of the chromosome, with 25 gaps. The depth of coverage is uneven (Fig. 1) with up to a 20-fold difference within 2Mb around the Menkes syndrome locus, for example. Long-range coverage is balanced, however, with 38% of the YACs localized on the short arm (36% of chromosomal length), and the remaining 62% on the long arm. The largest gap in the YAC contig map is in Xq27, where almost the complete band, which measures ~11 megabases, is not represented. Because a complete lack of Alu sequences over such a large region can be excluded, the reason for this underrepresentation must be ascribed to an unfortunate absence of probes mapping to this region in our random selection of HHMI clones. The region is represented at least partially in the target libraries as YAC contig construction has been reported in this cytogenetic band (Zucchi et al. 1996). Nevertheless in some cases we do observe a correlation between large gaps and the presence of a G-dark band. This is consistent with studies showing that these regions are relatively poor in Alu sequences (Korenberg and Rykowski 1988). However, not all G-dark bands are poorly represented (for example, Xp22.2, Xq13.1, and Xq23).

We have compared our map with X chromosome YAC contig maps built as part of whole genome efforts by CEPH (Chumakov et al. 1995) and by the Whitehead/MIT (Hudson et al. 1995) groups. In both cases, the X chromosome stands out because of its poor coverage compared with the average of the autosomes. This is principally attributable to the low representation of the X chromosome in the CEPH library made from a male cell line, which was the only substrate for the construction of the physical maps. Also the human and mouse X chromosomes contain either fewer CA repeats or fewer polymorphic CA repeats (Dietrich et al. 1996). This leads to a lower density of genetic markers available for whole-genome physical maps based on this type of STS. Therefore, an independently derived map of the X chromosome in YAC clones using libraries enriched for X chromosome DNA and independent from CA repeat content is particularly complementary. The approximate coverage of the X chromosome in the CEPH map, calculated with markers in common with the workshop consensus map, is 52 Mb (32%). The marker order between our map and the CEPH map agrees well except in one instance, where a group of markers is clearly misplaced in the CEPH map (XIST is placed in Xp11). Comparison with the Whitehead/MIT map of the X chromosome is more difficult, as the majority of markers have been developed very recently by this group and, therefore, are not placed on our map. It was possible to find 35 DXS markers common to both maps, for which the order broadly agrees, except for the first half of the short arm. In that region, the order of the nine common DXS markers strongly disagrees with the X chromosome community consensus map and with our map, over a 30-Mb region. The Whitehead/MIT order used for comparison is extracted from the radiation hybrid/STS content map. Again, on the basis of the physical distances between common markers in our map and the Whitehead/MIT map, we estimate the coverage of this map at ~50 Mb. This is also sustained by the maximal length of the contigs presented, based on the average length of a

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YAC clone. The consensus map established at the sixth X chromosome workshop (Nelson et al. 1995) reported an 80% coverage of the chromosome in YAC contigs and the presence of 24 gaps in the map. This consensus was derived by collating the contigs from >50 different groups, and concentrates on marker order rather than attempting to present YAC clone organization. Therefore, the estimation of the size and number of gaps and the YAC coverage has to be taken with caution.

We are working on gap closure using two strategies, bypassing the use of inter Alu-PCR and establishing useful landmarks for a cosmid/P1/P1 artificial chromosome (PAC) map of the chromosome. First, we are identifying these *Escherichia coli*-based clones using genetic and physical STS markers developed in the CEPH and Whitehead/MIT mapping efforts, which are likely to be positioned in our gaps. The STSs are amplified from total human DNA and used as hybridization probes. The positive clones in turn are used to screen the genomic YAC libraries to identify clones missed by Alu-PCR YAC probes. Second, we are using a combination of L1 (Line repeat) and Alu primers to amplify YAC clones from the ends of our contigs. These are used to screen the same cosmid and PAC libraries, therefore identifying cosmids and PAC clones at the edges of the gaps. When used to screen against the YAC genomic libraries, these probes can identify new clones extending from the original contig.

Arising out of the X chromosome workshop was a common accord that a repository of all YAC clones known or supposed to map to the X chromosome must be established, in combination with a dedicated data base that would make available all the published mapping information. We have taken on this project and have distributed 15 copies of a 9000-clone collection to genome centers worldwide. Also, we make available high-density gridded YAC colony filters of the collection, and DNA pools for PCR screening. This will increase the value of the X chromosome YAC resources available worldwide, and will allow verification and completion of the existing consensus YAC map. The clone collection includes the cX library reported here and clone sets from groups based at the Sanger Centre, the Baylor College of Medicine, the Washington University School of Medicine, and many others.

Clearly, the mapping of the X chromosome is reaching a stage where increasing efforts will be put into the construction of higher resolution maps in bacterial cloning systems [cosmids, P1, PACs, bacterial artificial chromosomes (BACs)], in which the YAC clone resources and maps will play a central role. These bacterial clones will be essential for the large-scale genomic sequencing and transcriptional mapping of the chromosome. Using the YAC contig map presented here, we have started a systematic identification of PAC, BAC, and X chromosome-specific cosmid clones. This is the next logical step toward a high resolution ("sequence ready") clone and transcript map of the chromosome, itself the consummate template for large-scale sequencing projects.

METHODS

YAC Libraries

The human YAC libraries used were the ICRF whole genomic library (Larin et al. 1991; M.T. Ross, S. Meier-Ewert, and A. Monaco, unpubl.), the CEPH "mega" YAC library (Chumakov et al. 1995) (plates 737–984), and the University of Pennsylvania X chromosome-specific library (Lee et al. 1992; HHMI thereafter). The HHMI library was made from a hybrid cell line (Micro-21D) carrying Xpter-Xq27.3 as its human component. The ICRF library comprises clones derived from the DNA of the cell lines GN1416B (48, XXXX National Institute of General Medical Sciences, Human Genetic Cell Repository), OXEN [49, XYYYY (Bishop et al. 1983) and HD1 (46, XX, homozygous for Huntington disease; Wexler et al. 1987)]. The CEPH library was made from a male lymphoblastoid cell line DNA source. The total coverage of the three libraries combined is estimated to be 14.5 X chromosome equivalents.

Large-scale Inter Alu-PCR of YAC Clones

Whole yeast DNA was extracted by a modification of the procedure of Chumakov et al. (1992) and used as template for large-scale inter Alu-PCR of YAC clones. The three libraries were replicated into 96-well microtitre plates containing 100μl selective medium (Anand et al. 1989) (SD ura, -trp), and cultures were grown for 3 days at 30°C. Cells were pelleted for 10 min at 2000 rpm (Beckman J6-MC), and supernatants were removed by inversion. Cell pellets were washed in 50 μl SCE buffer [1M sorbitol, 0.1M sodium citrate (pH 5.8), 10 mM EDTA], then harvested as before. Yeast cells were converted to spheroplasts by incubation for 1 hr at 37°C in 25 μl SCE containing 4 mg/ml novozym (NovoBiolabs) and 10 mM dithiothreitol. Then, 60 μl 0.14 N NaOH were added to each well and plates were incubated for 7 min at room temperature. DNA extracts were neutralized with 60 μl of 1M Tris-HCl (pH 8.0) and stored at −20°C.

Inter Alu-PCR was carried out in 67 mM Tris-HCl (pH 8.8), 16.7 mM $(NH_4)_2SO_4$, 6.7 mM $MgCl_2$, 0.5 mM each dNTP, 170 μg/ml BSA, 10 mM 2-mercaptoethanol, 1.3 μM primers ALE1 and ALE3 (Cole et al. 1991), and 0.6 units of *Taq* polymerase. A mixture sufficient for ≤10,000 reac-

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tions was dispensed in 50-μl aliquots into the wells of 384-well polypropylene microtitre dishes (Genetix). DNA from individual clones was transferred to the reaction plate with a 96-pin plastic device (Genetix), and plates were heat-sealed with a plastic film. PCR was carried out by use of a large capacity waterbath robot (Meier-Ewert et al. 1993) for 30 cycles of 3 min at 94°C followed by 6 min at 65°C, with an initial denaturation of 10 min and final extension of 10 min.

High-density Gridded Filter Arrays of YAC Clones

A custom-built robotic device (Lehrach et al. 1990) was used to grid either YAC inter Alu-PCR products or live YAC cultures onto nylon membranes for hybridization screening.

For PCR products, a 384-pin plastic gridding tool (Genetix) was used to transfer a small amount (<0.5 μl) of liquid from each well of the reaction plate onto a 22 × 22 cm nylon membrane (Hybond N+, Amersham). By interleaving the gridding patterns, PCR prodcuts of 9000–18,000 clones were gridded in duplicate on a single filter. Therefore, the three libraries were accommodated on three to five filters.

Gridded filters were transferred onto Whatman 3MM paper soaked with 0.5 N NaOH, 1.5 M NaCl for 2 min, then neutralized in 1M Tris-HCl (pH 7.2), 1.5 M NaCl. Filters were air-dried before use in hybridization experiments.

For screening whole YAC clone DNA, high-density colony grids were produced. The primary library plates in 96-well dishes were condensed into 384-well plates containing SD medium. After 2 days at 30°C, these cultures were used to grid onto nylon membranes, which were processed as described previously (Ross et al. 1992)

Hybridization of Inter Alu-PCR Products of Individual YAC Clones to High-density Filter Arrays of Inter Alu-PCR Products

Inter Alu PCR of individual clones to be used as probes was carried out using the reaction mixture described above. Reactions were carried out in 0.5-ml tubes in a MJ-PTC100 PCR machine using the following cycling conditions: 94°C for 5 min, then 30 cycles of 93°C for 1 min, 65°C for 1 min, 72°C for 4 min, then a final extension of 72°C for 5 min. Reaction products were precipitated by the addition of ammonium acetate to 2.5 M and two volumes of absolute ethanol. For 22 × 22 cm filters, 10–20 ng of DNA were labeled by random priming (Feinberg and Vogelstein 1983) in a 40-μl reaction using 5 μCi alpha [^{32}P]dATP. For 7.5 × 11 cm filters, only 1 μCi dATP was used. Probes were pre-reassociated for 1 hr at 65°C in 125 mM sodium phosphate buffer (pH 7.2) containing 0.75 mg/ml human placental DNA. Hybridization occurred overnight at 65°C in Church hybridization buffer [0.5 M sodium phosphate (pH 7.2), 7% SDS, 1% BSA (fraction V), 1 mM EDTA]. Filters were rinsed in 40 mM sodium phosphate buffer (pH 7.2), 0.1% SDS twice at room temperature, then washed twice in the same buffer at 65°C. Autoradiography was carried out using blue-sensitive film (Genetic Research Instrumentation) at −70°C with a single intensifying screen.

Hybridization Fingerprinting of YAC Clones

A library of cloned inter Alu-PCR products of the X chromosome was constructed in the plasmid vector pAMP1 (GIBCO-BRL). Approximately 100 ng of DNA from the hybrid cell line 578 (Wieacker et al. 1984), which contains a single human X chromosome on a hamster background, was used as a template in a PCR reaction using the same conditions as for the YAC amplification above, with 1.5 mM primer ALE3CA (CAUCAUCAUCAUCCACTGCACTC-CAGCCTGGG) and 1.5 mM primer ALE3CU (CUACUACU-ACUACCACTGCACTCCAGCCTGGG). Cycling was as follows: 94°C for 4 min, 30 cycles for 94°C for 30 sec, 68°C for 2 min, and a final extension at 72°C for 4 min. One to 2 μl of the PCR was used directly for the UDG annealing reaction according to the manufacturer's instructions. Electrocompetent DH5a were electroporated with 1–2 μl of the annealing reaction, and the resulting clones were picked, using a robotic device developed by us (Meier-Ewert et al. 1993), into 384-well microtitre plates.

Amplification of plamid inserts was carried out in 75 mM Tris-HCl (pH 9), 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.1% (wt/vol) Tween, 0.2 mM each dNTP, 1.5 μM primer ALE3, and 0.5 units of *Taq* polymerase. Reactions were set up as for YAC inter Alu-PCR. Template DNA was added directly from thawed glycerol stocks of the plasmid library in 384-well plates, with a 384-pin device. PCR was carried out as for large-scale YAC amplification. The PCR products were gridded robotically by use of the system described for YAC inter Alu-PCR products. A higher gridding density allowed the complete library of 4600 clones to be spotted in duplicated on a 7 × 11 cm filter. A regular array of India ink dots was also spotted to facilitate positive identification when using fluorescent detection.

A hybridization fingerprint was generated by hybridizing the inter Alu-PCR products of a YAC to the gridded filters of the cloned Alu-PCR library. Generation of the probes was as described for the YAC to YAC hybridization except that only the primer ALE3 was used, and the precipitation of the final products was omitted. Approximately 100 ng of probe were competed and hybridized as for the YAC to YAC hybridization. Washing and autoradiography of radioactive filters were also identical to the method above. X-ray films were scored by use of semi-automated methods and purpose-built software.

Hybridization of Cloned Inter Alu-PCR Products to High-density Filter Arrays of YAC Inter Alu-PCR Products

In all cases hybridizations were performed by use of digoxigenin labeling of the probes. DIG-11-dUTP was incorporated during PCR amplification of selected cloned inserts by use of primer ALE3 and 19:1 ratio of dTTP:dUTP. The PCR conditions were as described above for the amplification of the whole library before filter gridding, except that reactions were carried out in polycarbonate 96-well plates in an MJ-PTC100 thermocycler. Approximately 100 ng of amplified insert was subjected to competition, as were the YAC inter Alu-PCR probes, and hybridized to high-density arrays of YAC inter Alu-PCR products of the cX library. Washes and detection were carried out as recommeded by the manufacturer, using the Attophos substrate (JBL Scientific).

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Gel Fingerprinting of YAC Clones

The method for gel fingerprinting of YAC clones by comparison of inter Alu-PCR products has been described elsewhere (Coffey et al 1996). Briefly, a single YAC colony was resuspended in 100 μl 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA. Five microliters of the suspension were used as template for the primers ALE1 and ALE3 in a 25-μl primary PCR. Reaction composition and cycling conditions were as described above for inter Alu-PCR of individual YAC probes. An aliquot of the primary PCR was used as template for a secondary PCR containing radiolabeled ALE1 and ALE3 primers. The products of the secondary PCR were electrophoresed on a 4% polyacrylamide, 7M urea gel containing Sau3AI digested and ^{35}S-labeled λ DNA markers. Dried gels were autoradiographed at room temperature for ~65 hr. Autoradiographs were then scanned (Amersham) and the image edited before analysis.

Generation of Vectorette End Probes from YACs

Protocols for total yeast DNA preparation was according to Riley et al. (1990), with essentially the following modifications. Novozym (8 mg/ml) was used instead of Lyticase, DTT (10 mM) was used instead of β-mercaptoethanol, and agarose was 2% before cell resuspension. The isolation of vectorette ends from YAC clones was performed according to Coffey et al. (1992). Prerassociation and hybridization of the probe and washing and autoradiography of the filters were performed as described above.

FISH Mapping of YAC Clones

Whole yeast DNA was used for FISH. Single YAC colonies were grown to saturation in 40 ml SD broth at 30°C. Cells were harvested at 1000*g* for 5 min, then resuspended in 3 ml of 0.9 M sorbitol, 0.1 M EDTA (pH 7.5) containing 50 μl zymolase 20T. Spheroplasting was carried out for 60 min at 37°C, then spheroplasts were pelleted at 200*g* for 5 min and resuspended in 5 ml of 50 mM Tris-HCl (pH 7.4), 20 mM EDTA. Five hundred microliters of 10% SDS were added, and samples were incubated at 65°C for 30 min. Potassium acetate was added to a concentration of 1 M and samples were placed on ice for 60 min. Debris was pelleted at 15,000*g* for 10 min, then DNA was precipitated with two volumes absolute ethanol. DNA was redissolved in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (TE) containing RNase and incubated for 30 min at 37°C. DNA was reprecipitated using 0.1 volumes of 3 M sodium acetate (pH 5.2) and two volumes absolute ethanol, washed in 70% ethanol and redissolved in 200 μl TE. The total yeast DNA was labeled by nick translation with biotin-16-dUTP and hybridized overnight onto metaphase spreads essentially as described (Lichter et al. 1988). Hybridization was visualized using a standard two-layer avidin-FITC protocol (Pinkel et al. 1988).

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