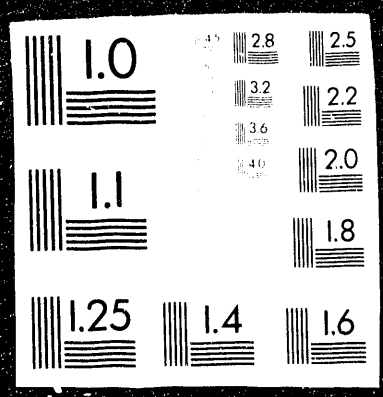

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MOLECULAR MAPPING OF CHROMOSOMES 17 AND X

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PROGRESS REPORT

The basic aims of this project are the construction of high density genetic maps of chromosomes 17 and X and the utilization of these maps for the subsequent isolation of a set of physically overlapping DNA segment clones. The strategy depends on the utilization of chromosome specific libraries of small (1-15 kb) segments from each of the two chromosomes. From these libraries, clones are screened for those detecting genetic polymorphisms. These polymorphic markers are used to construct the genetic maps in linkage reference families. When a genetically well-ordered set of markers has been established where the average genetic distance between the markers corresponds to 1-2 megabase physical distance, we will attempt to bridge the gap between the genetic markers with a set of physically overlapping cloned DNA segments. The availability of human rodent hybrid cell lines containing various subsegments of chromosomes X or 17 will be used to facilitate and confirm the results of the genetic mapping effort. The breakpoints present in these hybrids allow the preliminary localization of candidate genetic markers to a chromosomal subregion. In some cases, the breakpoints may also provide additional information on the relative order of probes which are not orderable by genetic analysis.

Since the time of submission of our previous progress report, we have refined the genetic map of markers which we had previously isolated for chromosome 17. The genetic mapping results for 17p are in good agreement with the recently published physical mapping in this region by our collaborators David Ledbetter and Bert Vogelstein (Ledbetter et al., 1989, Proc. Natl. Acad. Sci. USA 86:5136-5140 and Baker et al., 1989, Science 244:217-221). The results of this genetic analysis have been submitted for publication (Wright et al., submitted) In addition, we have completed our genetic mapping in CEPH reference and NF1 families of 15 markers in the pericentric region of chromosome 17 (Fain et al., Am. J. Hum. Genet. 45:in press). This paper also describes physical mapping results with three probes, which were shown to be in very close genetic proximity to the NF1 gene, with respect to two translocation breakpoints which disrupt the activity of the gene. All three of the probes were found to lie between the centromere and the most proximal translocation breakpoint, providing important genetic markers proximal to the NF1 gene. Close distal markers had been previously identified by genetic mapping of probes isolated in this laboratory (Fain et al., 1989, Am. J. Hum. Genet. 44:68-72). Two of the close distal probes were found to reside on the same pulsed-field gel fragment by Fountain et al. (1989, Am. J. Hum.

Genet. 44:58-67).

An important development in the past year was the detection of genetic linkage of Charcot-Marie-Tooth syndrome type 1a to genetic markers in the pericentric region of chromosome 17. Probes which we had provided to the group at Duke, who made this discovery, were critical to the detection of this linkage. Further collaborative work is in progress to refine the genetic localization of the CMT17 locus.

Since our last progress report, the primary focus of our project has been on the X chromosome. We have isolated an additional 30 polymorphic markers, bringing the total number we have isolated to over 80. We have invested substantial effort in characterizing the polymorphisms at each of these loci and constructed plasmid subclones which reveal the polymorphisms for nearly all of the loci. These subclones are of practical value in that they produce simpler and stronger patterns on human genomic Southern blots, thus improving the efficiency of the genetic mapping experiments. These subclones may also be of value for deriving DNA sequence information at each locus, necessary for establishing polymerase chain reaction (PCR) primers specific for each locus. Such information would allow the use of each locus as a sequence tagged site (STS).

By establishing collaborations with investigators who have isolated or characterized new cytogenetic breakpoints on the X, we have increased the resolution of our physical map from 10 intervals to 25. All of the 80 probes that we have isolated have been localized to one of these 25 intervals. The new intervals are defined by two deletions in the Xq21-q22 region, a set of 4 deletions at or near the terminus of Xp, 5 hybrid cell lines with breakpoints in the Xq26-q28 region, one "pushmi-pullyu" hybrid and a set of 99 radiation hybrids with undefined breakpoints. To date, the radiation hybrids and pushmi-pullyu hybrids have only been characterized with our probes in the Xq21-q25 region. One unique breakpoint was found in the set of radiation hybrids. We expect to find several additional breakpoints when probes from other parts of the X are characterized. The accompanying figure summarizes the results of physical localization of the X chromosome probes.

Many of the new X probes that we have characterized have heterozygosities in excess of 50% and are very useful genetic markers. To date, we have determined genotypes for 30 of the X RFLP markers on part or all of the informative CEPH linkage reference families. Data for the average marker provides information in 83 meioses. The genetic linkage map generated for probes in the Xq13-Xq26 region from these data was consistent with our physical map of this region (Goldgar et al., 1989, Cytogenet. Cell Genet. 51:1006). In addition, we have determined genotypes for about 25 markers in Xq13-Xq26 in two large kindreds with Alport syndrome, one with about 90 and the other with about 110 members. Several genetic breakpoints allowed the refined ordering of probes which had been localized to the same physical

interval. Further analysis of the combined genetic data sets is in progress.

X chromosome polymorphic probes which we have isolated and localized are proving useful to several collaborators who are trying to identify genes for specific X-linked diseases. We have provided probes and/or established collaborations with the following groups: C. Petit and J. Weissman at the Institut Pasteur working on Kallmann syndrome, M. Econs at Duke University mapping X-linked hypophosphatemia, J. Gorski at the University of Michigan identifying a translocation causing incontinentia pigmenti, J. Puck at the Childrens Hospital Philadelphia mapping severe combined immune deficiency, Sau-Ping Kwan at Rush Medical Institute mapping X-linked agammaglobulinemia, J.-L. Mandel at CNRS-LGME Strasbourg mapping fragile X, D.H. Ledbetter at Baylor mapping X-linked mental retardation and S. Warren at Emory University mapping Emery-Dreifuss muscular dystrophy. Several of the probes have been useful in the laboratory of H. Willard, the subcontractor on this grant, for the definition of the X-inactivation center (Brown et al. 1989, Am. J. Hum. Genet. 45:A178). Probes genetically linked to the gene causing Alport syndrome have been identified in our laboratory (Barker et al. 1989, Cytogenet. Cell Genet. 51:957).

SUMMARY OF PLANNED RESEARCH FOR THIRD PROJECT YEAR

BREAKPOINT MAPPING

For both chromosomes 17 and X, the number of RFLP markers that we have isolated, together with other markers available publicly or from friendly collaborators is sufficient to construct maps with an average resolution of 1-2 cM. The focus of our project is now to establish a reliable order of these markers and to define an effective program for integrating them into the effort to construct complete chromosome physical maps and sets of overlapping clones. Our plan is to focus our effort on the X chromosome, because of the availability of useful deletion lines, a radiation hybrid panel and the "pushmi-pullyu" set developed by the grant subcontractor, H. Willard. The opportunity for useful collaborations with the many other labs doing genetic mapping of X-linked diseases and fine-structure physical mapping is also an important advantage.

With a sufficient number of probes available, the ordering task is dependent on the identification of reliable breakpoints. These breaks may be points of genetic crossing over, or actual physical breakpoints. The latter may be clinically significant translocations and deletions or rearrangements isolated in cultured cells. The advantage of the physical breakpoints is that DNA probes may be tested directly on Southern blots or by PCR on a single DNA sample to directly detect their presence or absence. All probes, polymorphic or not, are informative in such a test and all probes tested against the same rearrangement are

informative with respect to each other, allowing the construction of complete, unambiguous maps. The advantage of the genetic approach is that there is an essentially unlimited supply of meiotic breakpoints. However markers must be polymorphic and informative to determine their position with respect to the break. Since not all tested markers will be informative with respect to all breakpoints, the resulting map can not place all of the markers relative to all of the breakpoints. Of course, the ordering of polymorphic markers is essential to the placement of genetic disease loci into the map.

Because there are significant advantages to both of these approaches, we plan to continue using both of them for our mapping project. We will continue to type our polymorphic X and 17 chromosome probes on the CEPH reference linkage families and on other sets of disease families available to us. Our collaborators working on their disease families will be an additional potential source of critical meiotic breakpoints. We will also continue to test DNA probes directly against rearranged genomes with characterized and uncharacterized X and 17 specific breakpoints. The X chromosome radiation hybrid panel available to us through our collaboration with R. Nussbaum at the University of Pennsylvania will be an important component of this approach. Maps made with these two approaches will confirm and supplement each other.

We have also recently established a collaborative effort with D. Ward and P. Lichter at Yale University who have developed a high resolution in situ hybridization technique. This technique can potentially resolve and order probes within 1 megabase or less of each other. We have agreed to provide this group with the probes that we have developed on X. They will attempt to construct a complete ordered map with their in situ approach. The correlation of in situ localization, genetic distance and physical breakpoints may provide additional clues to the genetic structure and function of different regions of the X chromosome.

ISOLATION AND MAPPING OF "LINKING CLONES"

In order to facilitate the correlation of the breakpoint map into the large-scale restriction map of the X chromosome we are planning to isolate and map X-specific clones containing NotI and other rare-cutter sites. The following protocol utilizes the CH35 library LA0XNL01, made from chromosomally sorted X by the Los Alamos group, because of its relatively high purity and coverage. First, the total library is amplified in the host K802recA, to prevent rearrangements or recombination between individual clones. DNA is prepared from the library and allowed to circularize at low concentration. The circles which form by the annealing of the "sticky ends" are covalently closed with T4 ligase. The pool of DNA is then cut with NotI and ligated with a NotI cut derivative of pKSII(+) [Stratagene Inc.] in which the EcoRI site has been destroyed. The important feature of this

step is that the pKSII(+) vector includes the gene for ampicillan resistance. Only those clones which contain a NotI site can become ligated to pKSII(+). Following the ligation, the circular DNA is repackaged into lambda phage. Packaging of circular DNA by the lambda system is an efficient process in vitro, eliminating any need to concatamerize the DNA. The packaged phage will then be used to infect a derivative of K802recA, into which the plasmid pRK248cIts has been introduced. This plasmid carries and expresses a thermosensitive form of the lambda cI repressor gene. In preliminary experiments, we have shown that the phage lambdaZAP (Stratagene Inc) which has the pbluescript (Stratagene Inc) plasmid integrated into it can transduce the K802recA/pRK248cIts host to ampicillan resistance with an efficiency approaching 100%. The structure of the NotI ligated clones should be nearly identical to this model and should be readily recoverable by infection and selection for ampR transductants.

Characterization of the clones will be facilitated by the absence of an EcoRI site from the pkSII(+) vector. DNA prepared from the ampR primary transductants will be cut with EcoRI, ligated at low concentration to favor fragment circularization and transformed into E. coli. Selection for ampR transformants should yield primarily clones which include the previously integrated plasmid and the DNA segments extending from the NotI site to the nearest adjacent EcoRI sites on both sides of the original NotI site. Hybridization of this clone to a Southern blot of human DNAs cut with EcoRI and EcoRI plus NotI should allow a rapid confirmation that the clone represents a contiguous genomic region. The protocol can be extended to additional unique rare cutter sites already present in the pKSII(+) vector (SacII, EagI, ClaI and SalI for example) and by the introduction of additional such sites. We have constructed a derivative vector which is missing the EcoRI site and which contains MluI, BstBI and BspMII as additional rare-cutter sites.

As we isolate these rare-cutter linking clones, we will map them with respect to our physical breakpoint panel. Clones which map to physically small intervals will be used to probe blots of pulsed-field gels and the patterns compared with those obtained with other probes in the interval. The detection of common hybridizing fragments will help establish the restriction map of pulsed-field fragments.

YAC SCREENING WITH PCR

The potential of cloning large fragments in yeast artificial chromosomes has not yet been fully realized due to restrictions on the size of clones which can be transformed efficiently into yeast and retained stably. The use of PCR to efficiently screen pools of YAC clones has been reported by several investigators as a means of simplifying the process of identifying YACs corresponding to specific loci. We plan to sequence portions of DNA probes known to lie in in close genetic proximity to the gene

for Alport syndrome and develop appropriate PCR primers. The primers will be used to isolate corresponding clones from YAC libraries with large average insert sizes which we construct or available from collaborators. We will proceed with the isolation of distal fragments from appropriate YACs to perform walking experiments. We will also search for overlaps between YACs detected with different probes in this region.

RECENT PUBLICATIONS

Vance JM, Nicholson GA, Yamaoka LH, Stajich J, Stewart CS, Speer MC, Hung W-Y, Roses AD, Barker D and Pericak-Vance MA: Linkage of Charcot-Marie-Tooth neuropathy type 1a to chromosome 17. Exp. Neurology 104:186-189 (1989).

Ledbetter DH, Ledbetter SA, vanTuinen P, Summers KM, Robinson TJ, Nakamura Y, Wolff R, White R, Barker DF, Wallace MR, Collins FS and Dobyns WB: Molecular dissection of a contiguous gene syndrome: Frequent submicroscopic deletions, evolutionarily conserved sequences, and a hypomethylated "island" in the Miller-Dieker chromosome region. Proc. Natl. Acad. Sci. 86:5136-5140 (1989).

Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, vanTuinen P, Ledbetter DH, Barker DF, Nakamura Y, White R, and Vogelstein B: Chromosome 17 deletions and p53 mutations in colorectal carcinomas. Science 244:217-221 (1989).

Goldgar DE, Nguyen KN, Fain PR, Skolnick MH and Barker DF: Genetic linkage map of 19 loci on chromosome Xq. Cytogenet. Cell Genet. 51:1006 (1989).

Barker DF, Wright EC, Andersen WL, Willard HF, vanTuinen P and Ledbetter DH: Isolation and Physical localization of chromosome 17 RFLP markers. Cytogenet. Cell Genet. 51:957 (1989).

Barker DF, Dietz-Band JN, Donaldson CW, Andersen WL, Turco AE, Pole AR, Willard HF and Vincent A: Further isolation, characterization and physical localization of X-chromosome RFLP markers, comparing VNTR-directed and random isolation strategies. Cytogenet. Cell Genet. 51:958 (1989)

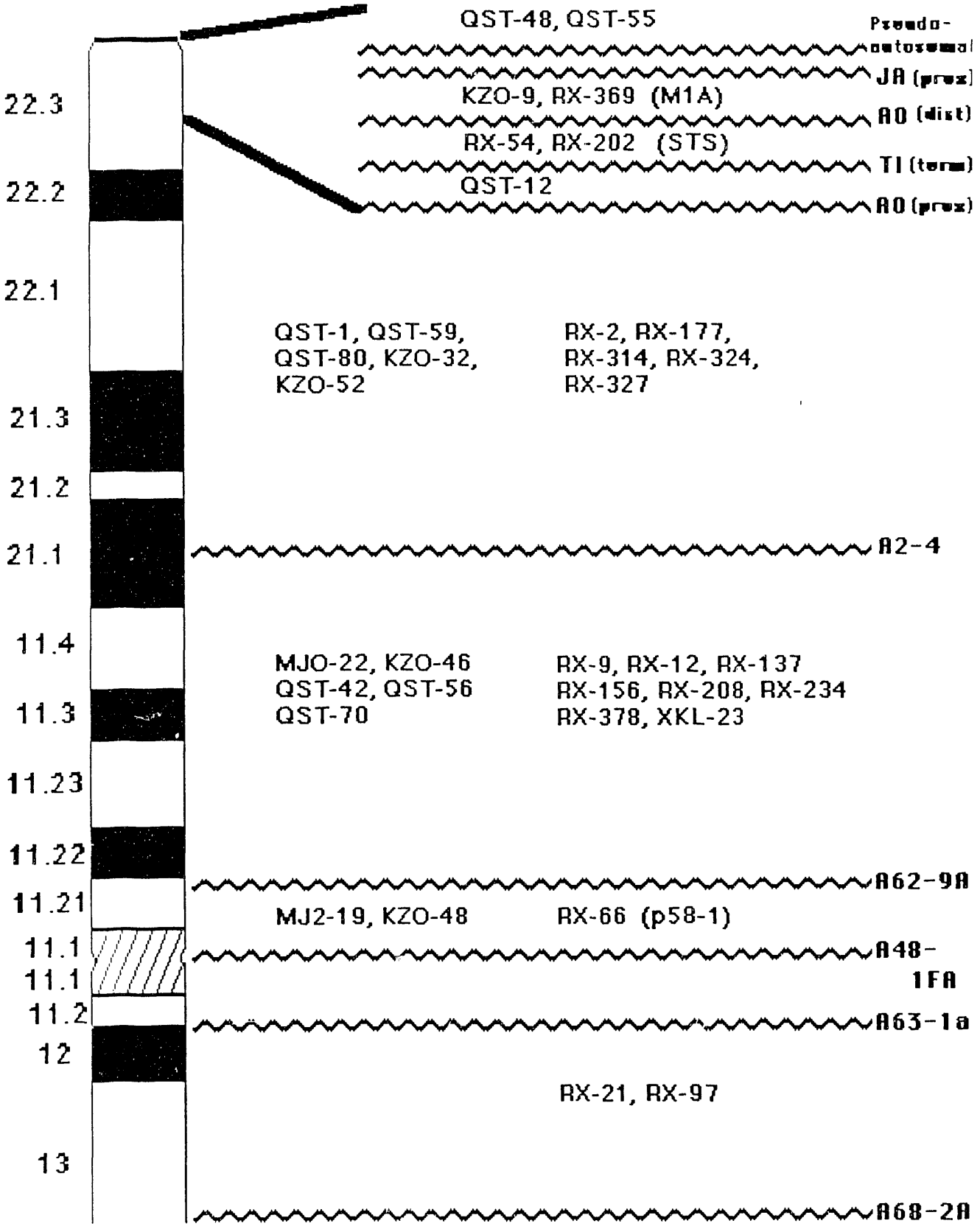
Barker DF, Dietz-Band JN, Goldgar DE, Nguyen KN, Turco AE, Donaldson CW, Willard HF, Skolnick MH and Atkin CL: Mapping of the gene for Alport Syndrome within a physically and genetically mapped cluster of X RFLP markers. Cytogenet. Cell Genet. 51:957 (1989).

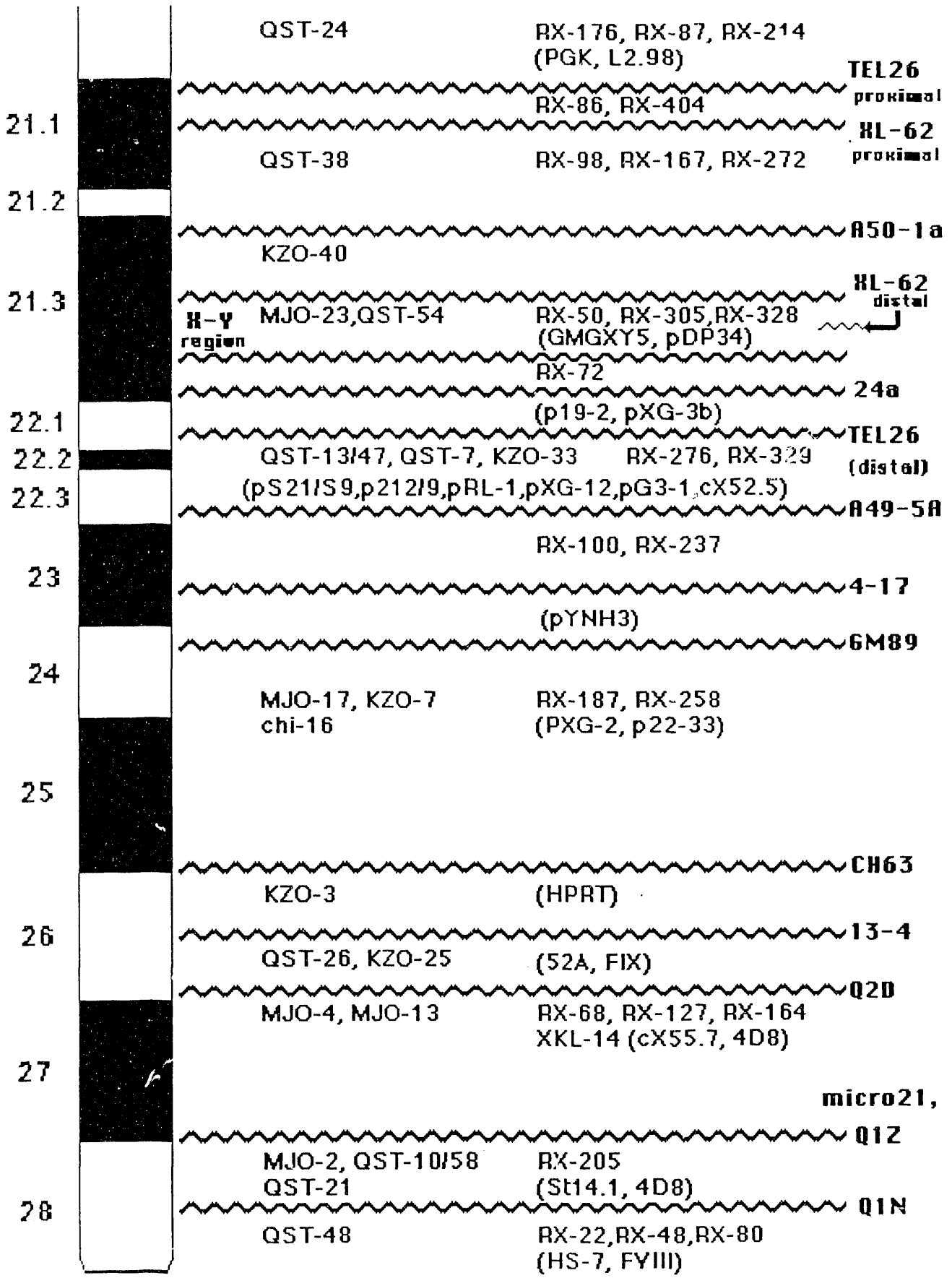
Brown CJ, Barker DF and Willard HF: Studies of X chromosome inactivation in somatic cell hybrids with the tsA1S9 cell line. Am. J. Hum. Genet. 45:A178.

Fain PR, Goldgar DE, Wallace MR, Collins FS, Wright E, Nguyen K and Barker DF: Refined physical and genetic mapping of the NF1 region on chromosome 17. Am. J. Hum. Genet. 45:in press (1989).

Wright EC, Barker DF, Fain PR, Skolnick MH and Goldgar DE: A genetic map of 17 markers on chromosome 17p. submitted (1989)

Location of X polymorphic probes in physically defined intervals.

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RX-86, RX-404
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XL-62
proximal
QST-38
RX-98, RX-167, RX-272
21.2
A50-1a
KZO-40
XL-62
distal
21.3
X-Y
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22.2
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(pYNH3)
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MJO-17, KZO-7
chi-16
RX-187, RX-258
(PXG-2, p22-33)
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CH63
KZO-3
(HPRT)
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13-4
QST-26, KZO-25
(52A, FIX)
Q2D
MJO-4, MJO-13
RX-68, RX-127, RX-164
XKL-14 (cX55.7, 4D8)
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Q1Z
MJO-2, QST-10/58
RX-205
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(St14.1, 4D8)
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Q1N
QST-48
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(HS-7, FVIII)

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