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Progress Report

for

"MOLECULAR MAPPING OF CHROMOSOMES 17 AND X"

by

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PROGRESS REPORT

MOLECULAR MAPPING OF CHROMOSOMES 17 AND X

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Grant #DE-FG02-88ER60689

OVERVIEW

The basic results of this project have been in two areas. First, progress toward the construction of high density genetic maps of chromosomes 17 and X has been made by isolating and characterizing a relatively large set of polymorphic probes for each chromosome and using these probes to construct genetic maps. Second, we have mapped the same polymorphic probes against a series of chromosome breakpoints on X and 17. The probes could be assigned to over 30 physical intervals on the X chromosome and 7 intervals on 17. In many cases, this process resulted in improved characterization of the relative locations of the breakpoints with respect to each other and the definition of new physical intervals.

The strategy for isolation of the polymorphic clones utilized chromosome specific libraries of small (1-15 kb) segments from each of the two chromosomes. From these libraries, clones were screened for those detecting restriction fragment length polymorphisms (RFLPs). The markers were further characterized, the chromosomal assignments confirmed and in most cases segments of the original probes were subcloned into plasmids to produce probes with improved signal to noise ratios for use in the genetic marker studies. The linkage studies utilize the CEPH reference families and other well-characterized families in our collection which have been used for genetic disease linkage work. The general linkage mapping is an ongoing activity; preliminary maps and maps of portions of specific regions of 17 or X have been the subject of a variety of publications.

CHROMOSOME 17

Work conducted in the early stages of this grant was focused on chromosome 17 based on research momentum established by our earlier genetic studies of the NF1 locus. We isolated 70 polymorphic chromosomsome 17 specific markers from a variety of flow-sorted libraries, including two constructed under the auspices of the National Laboratory Gene Library Project. Figures 1a-1d summarize the results of characterizing and mapping these markers with respect to a series of chromosome 17 deletion and translocation breakpoints. DNAs from the hybrids were provided by our collaborators Dr. Hunt Willard and Dr. David Ledbetter. The application of these markers for various genetic studies has been pursued by Dr. Pam Fain.

The most recently published genetic map included most of the markers that we have isolated on chromosome 17p (Wright et al. 1990). The 17p map was in good agreement with the physical mapping data for this region produced by our collaborators David

Ledbetter and Bert Vogelstein (Ledbetter et al. 1989, Ledbetter et al. 1990 and Baker et al. 1989).

Previously, we had completed our genetic mapping in CEPH reference and NF1 families of 15 markers in the pericentric region of chromosome 17 (Fain et al. 1989b). This paper also describes physical mapping results with three probes, which had been shown to be in very close genetic proximity to the NF1 gene. All three of the probes were found to lie between the centromere and the most proximal of two translocation breakpoints which disrupt the gene, 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. 1989a). Two of the close distal probes were found to reside on the same pulsed-field gel fragment by Fountain et al. (1989). A group of 10 probes which flank the NF1 locus were deposited with the ATCC and many of these had been distributed earlier to the members of the NF1 linkage and gene cloning consortium.

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 (Vance et al. 1989) Further collaborative work with the Duke group refined the genetic localization of the CMT17 locus to proximal 17p. We have now provided probes useful for the detection of RFLP loci in the pericentric region of chromosome 17 to nine different laboratories who are participating in an international consortium that is cooperating to further refine the gene linkage and isolate the CMT1a gene. This set of 12 probes was simultaneously deposited in the ATCC. We are in the process of depositing all of the polymorphic probes which we have isolated for chromosome 17 into the ATCC.

CHROMOSOME X

For nearly the past two years, the primary focus of our laboratory has been the X chromosome. We have now isolated a total of over 80 X-specific polymorphic markers (see Table I). A report of part of this work is in press (Dietz-Band et al.) 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 and increasing their utility to investigators trying to map diseases. 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 cytogenetic breakpoints on the X, we have been able to establish a map of 35 physical intervals. In the past year and a half, we have increased the resolution of our physical map from 10 intervals to about 35. Essentially all of the 80 probes that we have isolated have been localized to one of these defined intervals. Several other X probes which are generally available have also been localized as reference points. Figures 2a and 2b summarize the breakpoint mapping results. It should be noted that the average genetic length of a segment of a 35 interval map is about 6 cM, assuming a 200 cM length for the whole of X.

As part of the project of defining new breakpoints for the X, we have participated in the characterization of two sets of hybrids which were isolated in other laboratories. One is the set of "pushmi-pullyu" hybrids generated in Dr. Hunt Willard's laboratory and the second is a set of 99 radiation hybrids isolated in the laboratory of Dr. Robert L. Nussbaum. To date, the radiation hybrids and pushmi-pullyu hybrids have been characterized with our probes in the Xq21-q25 region where we have focused substantial effort because of our interest in Alport syndrome. In a set of "pushmi-pullyu" hybrids selected for breakpoints in this region, we found one (24a) with a unique breakpoint distinguishable with the available probe set. Another unique breakpoint (4-17) was also found in the set of radiation hybrids. We would expect to find additional breakpoints in the radiation hybrid set when probes from other parts of the X are characterized. A problem that we encountered with both of these types of hybrids was the often poor representation of the segment of the human chromosome in the hybrid cells, leading to problems with probe detection by conventional Southern analysis. Although this is a problem common to most hybrids, it appeared to be particularly acute with these and resulted in considerable ambiguity in the interpretation of hybridization results with DNA from all but a fraction of the lines. It is possible that the utility of these hybrid sets would be much increased with respect to the localization of PCR-based loci, since the sensitivity of detection would be improved.

Some of the breakpoints that are included in our map have been characterized cytogenetically and assigned to a single visible band, although, for relatively large bands, this implies rather poor resolution. Other breakpoints were not studied, can not (as for radiation hybrids) be directly determined, or arrived in our hands with assignments that could be shown to be inconsistent with their hybridization patterns with well-known and/or reliably assigned probes. An independent and more accurate method for the correlation of localization based on breakpoint analysis with physical position on the chromosome is therefore needed. High-resolution in situ hybridization, which has been developed in the laboratory of Dr. David Ward (Lichter et al. 1990) provides a method with the appropriate resolution. In the initial stage of collaboration with Dr. Ward's group, we provided a set of 17 X-specific markers from our map for in situ hybridization. The results are indicated on Figs. 2a,2b and 3. In each figure, the bars to the left of the chromosome idiogram indicate a probe localization based on in situ hybridization. Continuing this process with additional probes will eventually permit the assignment of each breakpoint to a well-defined interval. Application of methods for refined relative localization of probes (Lichter et al. 1990) will supplement multipoint genetic mapping for determination of probe orders, a process which could be of particular value in the vicinity of disease loci.

Many of the new X probes that we have characterized have heterozygosities in excess of 50% and are very useful genetic markers, relative to others which are currently generally available. To date, we have determined genotypes for 48 of the X RFLP markers on part or all of the informative CEPH linkage reference families. Data for the average marker provides information in over 100 meioses. The genetic linkage map generated for probes in the Xq13-Xq26 region from part of these data (Goldgar et al. 1989) was consistent with our physical map of this region. In addition, we have determined genotypes for 22 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. Fig. 3 shows an example of these results, with the genetic ordering of probes within one interval indicated by the ">" symbol. We have also determined genotypes for 16 markers from outside of Xq13-Xq26 in the Alport families. And additional genetic data for probes in Xp22-p23 has been provided by our collaborator M. Econs at Duke. Further analysis of the combined genetic data sets is in progress. The genetic mapping is not described in detail here, as it is carried out separately by Dr. Pam Fain, supported by NIH grant GM-40877.

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 and other diseases in the sub-pseudoautosomal region, M. Econs at Duke University mapping X-linked hypophosphatemia, J. Gorski at the University of Michigan who is building a map that includes translocation breakpoints associated with 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, Jon Zonana at Oregon Health Sciences University mapping hypohydrotic ectodermal dysplasia, U. Muller at Childrens Hospital Boston mapping X-linked torsion dystonia, C. Schwartz at the Greenwood Genetic Center mapping one form of X-linked mental retardation, E. McCabe at Baylor searching for the glycerol kinase deficiency and adrenal hypoplasia congenita genes 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). Probes genetically linked to the gene causing Alport syndrome have been identified in our laboratory (Barker et al. 1989).

ALPORT SYNDROME

Although our work on Alport syndrome is nominally not supported by DOE funding, it has benefited substantially by the polymorphism identification and characterization work and the mapping of probes with respect to breakpoints. A detailed physical and genetic map of the Alport region, which will include information depicted in Fig. 3, will soon be submitted for publication. The Alport gene was recently identified by our group (Barker et al. 1990), working in collaboration with a group in Finland which isolated a candidate collagen gene, COL4A5, (Hostikka et al. 1990) and mapped it to the position where Alport had been mapped genetically. We identified three distinct mutations in the COL4A5 gene in three out of 18 Alport families tested, including an intragenic deletion and a PstI site variant which was associated with a mutation in a highly conserved cysteine residue of the noncollagenous domain of the gene (Zhou et al. submitted). Our current work on Alport is directed towards identifying mutations in the remaining uncharacterized families.

Mycoplasma BIT MAPPING

As an aid in making complete coverage physical maps of megabase DNA fragments we have been exploring variations of the bit mapping technique proposed by Hans Lehrach (Poustka et al. 1986, Michiels et al. 1987, Craig et al. 1990). We are interested in this method for detailed analysis of single large YAC clones. We have nearly completed a map of the 1 megabase *Mycoplasma arthritidis* genome by applying these techniques to a lambda phage library of its genome. We have found bit mapping to be an efficient means to organize a contiguous set of overlapping clones from a larger genome.

A lambda phage library was constructed in the vector EMBL3A and dot blots were made from 500 plaques. In order to identify clones which share segments of DNA, a string of binary information for each clone has been generated by sequence specific probings of the dot blots. As a source of probe, DNA from the *M. arthritidis* genome was cleaved by a restriction endonuclease and separated into three sized fractions by agarose gel electrophoresis. Each of these fractions was radio-labeled and used to probe the library. Twenty different restriction enzymes have been used to generate the 60 bits of binary data required to position each clone with respect to the others.

Computer programs have been written, based on mathematical techniques used in solving the travelling salesman problem, which order clones from this binary information. The bit data from the sequence specific probings of all clones is used to calculate a similarity score for each pair of clones. Two clones having a high similarity score are likely overlapping or spaced only a small distance apart. The clone pair distances are stored in a matrix as a source for further analysis. The program then chooses very efficiently from the many possible orders those which are likely candidates for a tour which maximizes overall similarity. The clone order which shows a maximum similarity between all adjacent clones is considered optimal. A portion of the resulting M. arthritidis map is shown in Figure 4.

Using restricted and size fractionated probes in combination with bit mapping analysis compares favorably with the cost of fingerprinting (Kohara et al. 1987, Olson et al. 1986, Coulson et al. 1986) and "multiplex analysis" (Evans et al. 1989). These techniques are especially appropriate when the number of clones is large (500 and greater) and the genome to be mapped is of moderate size (1 to 10 megabases) as will occur for YAC contigs. This process of mapping will be directly applicable to other organisms which contain small genomes. Other Mycoplasma species, bacteria, and yeast or yeast artificial chromosomes could all be mapped using these methods.

GENERAL TECHNIQUES

Although not reflected in any particular publication, substantial progress has been made in the introduction of a number of techniques which are important to the area of physical mapping. We have successfully used field inversion and CHEF gel electrophoresis techniques for the analysis of several sample YAC clones which were provided to us by David Burke. This procedure involved the growth of the yeast clones and the isolation of the intact chromosomal DNA in agarose blocks. We have also analyzed Southern transfers of high molecular weight human DNAs digested with rare-cutting restriction enzymes. The various sized YAC clones were used as size controls in these experiments. The Southern analysis technique was used in an unsuccessful search for deletions involving the Alport gene.

As part of our search for mutations in the Alport syndrome gene, we are working on development of many of the techniques for detecting microheterogeneity in DNA, small deletions and insertions, basepair changes and differences in the numbers of short tandem repeats. These techniques include denaturing gradient gel electrophoresis, single strand conformation polymorphism detection and other PCR based methods including direct sequencing of genomic PCR products. This work will complement the activities proposed in this application.

Legend to Figure 1.

Figure 1a shows idiograms of the portions of chromosome 17 which are retained in a series of hybrids used for the regional mapping of polymorphic probes. DNAs from the hybrids were provided by H. Willard and D. Ledbetter.

Figure 1b summarizes the results of the localization of 70 polymorphic chromosome 17 probes. All of the probes were localized with respect to 6 breakpoints defining 7 regions. Some of the probes in the "100" region were also mapped against the MH41 breakpoint, which further splits this region.

Figure 1c indicates the genetic informativeness of the markers in each of the defined regions. The scale at the bottom is empirically determined heterozygosity and each bar represents a single polymorphic probe. The bars are arranged such that the probes with the highest heterozygosities appear at the center of each region simply to provide better definition of the limits of each region.

Figure 1d shows the enzymes which were used to detect RFLPs and the number of probes found to be polymorphic with each enzyme.

Legend to Table 1

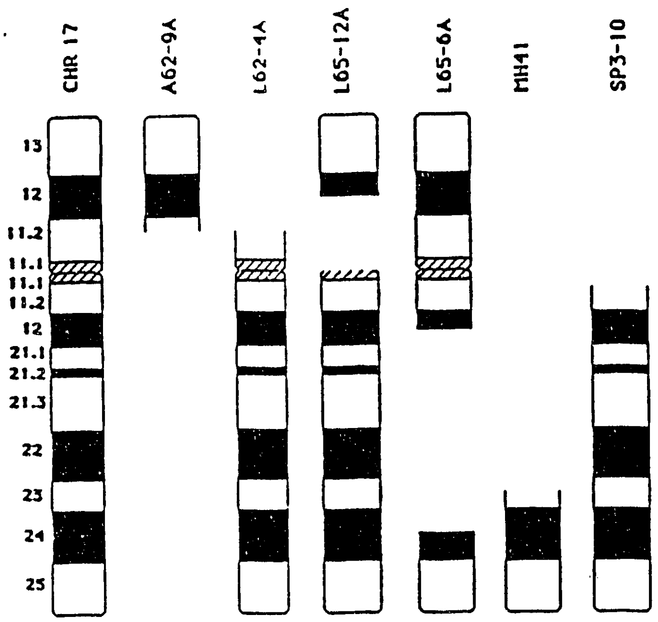
Properties of the polymorphic X-specific probes isolated as part of this project. The locations shown are approximate and are refined by information presented in Figure 2.

Legend to Figure 2.

Figures 2a and 2b summarize the results of mapping the polymorphic probes listed in Table 1, against the set of over 35 hybrid breakpoints , DNA from which has been provided by various collaborators including H. Willard of Stanford University, C. Petit and J. Weissenbach at the Institut Pasteur, E. McCabe at Baylor, G. Gorski at Univ. of Michigan, Robert Nussbaum at Univ. of Pennsylvania, J.-L. Mandel at CNRS-LGME Strasbourg and others. Each breakpoint is indicated by a wavy line. The pseudoautosomal region and the region of "X-Y" homology in Xq21 are assumed to be single contiguous regions. Probes in parentheses are reference probes which have been provided by various investigators or obtained from the ATCC. Probes in lower case letters have not been localized with respect to all relevant breakpoints. The location of RX-127 is ambiguous because it may fall above or below the interval defined by the "LC" deletion. The probe MJ2-19 is superimposed on the A62-9A break, because it hybridizes to a repeat family with members on both sides of this breakpoint. Bars to the left of the idiogram indicate the regions to which the indicated probes have been mapped by *in situ* hybridization in the laboratory of David Ward.

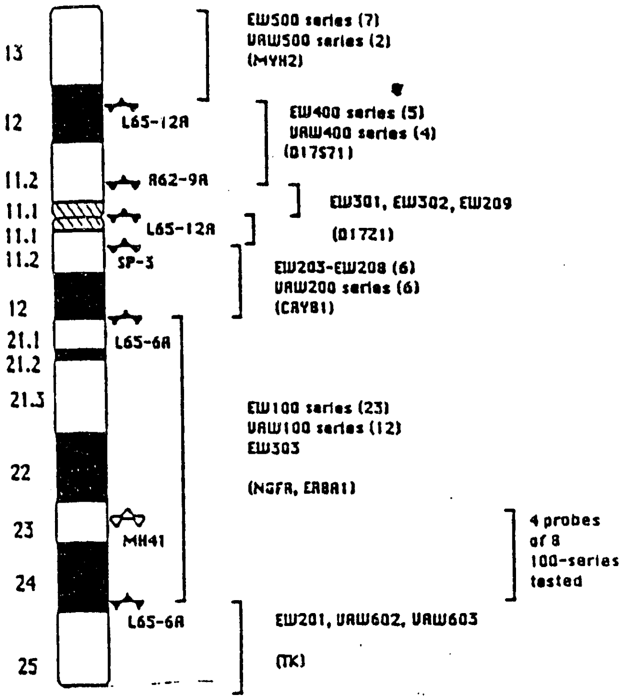
Legend to Figure 3

Detailed map of the regions surrounding the location of the Alport syndrome gene. An example of the genetic mapping results are shown in one of the intervals. The probes RX329, RX276, cX52.5 and S21/S9 have been demonstrated to be in the order shown by the detection of meiotic breakpoints between them and following the segregation of flanking probes. Other probes in this interval could not be mapped with respect to these meiotic breakpoints since they were uninformative. Bars to the left of the idiogram indicate the current limits of localization of probes by *ih situ* hybridization in the laboratory of David Ward.



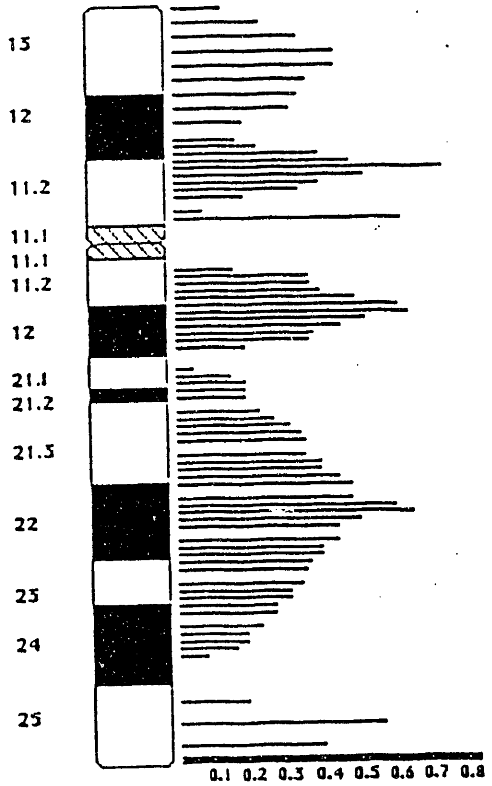
DELETION AND TRANSLOCATION CHROMOSOMES IN HYBRIDS UTILIZED FOR POLYMORPHIC PROBE LOCALIZATION

Figure 1a



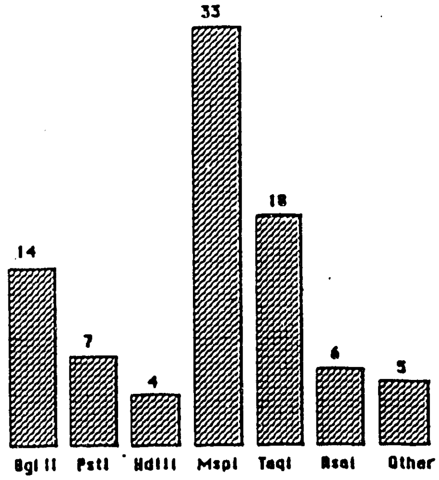
Regional localization of polymorphic chromosome 17 probes. Names in parentheses are reference probes.

Figure 1b



Distribution of chromosome 17 probes by location and heterozygosity

Figure 1c



Numbers of chromosome 17 specific polymorphisms detected by enzymes used for variant screening.

Figure 1d

Figure 2a

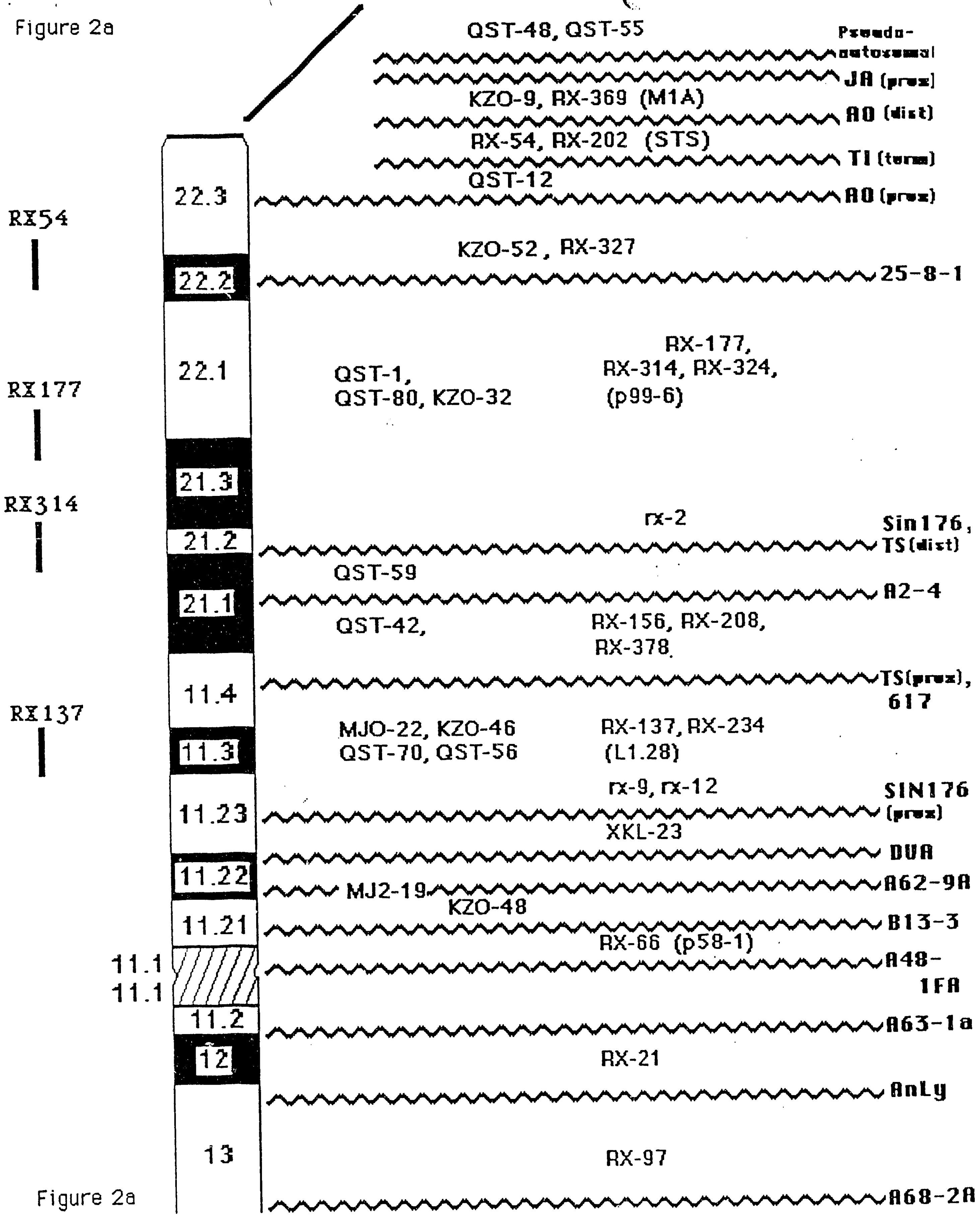


Figure 2a

Figure 2b

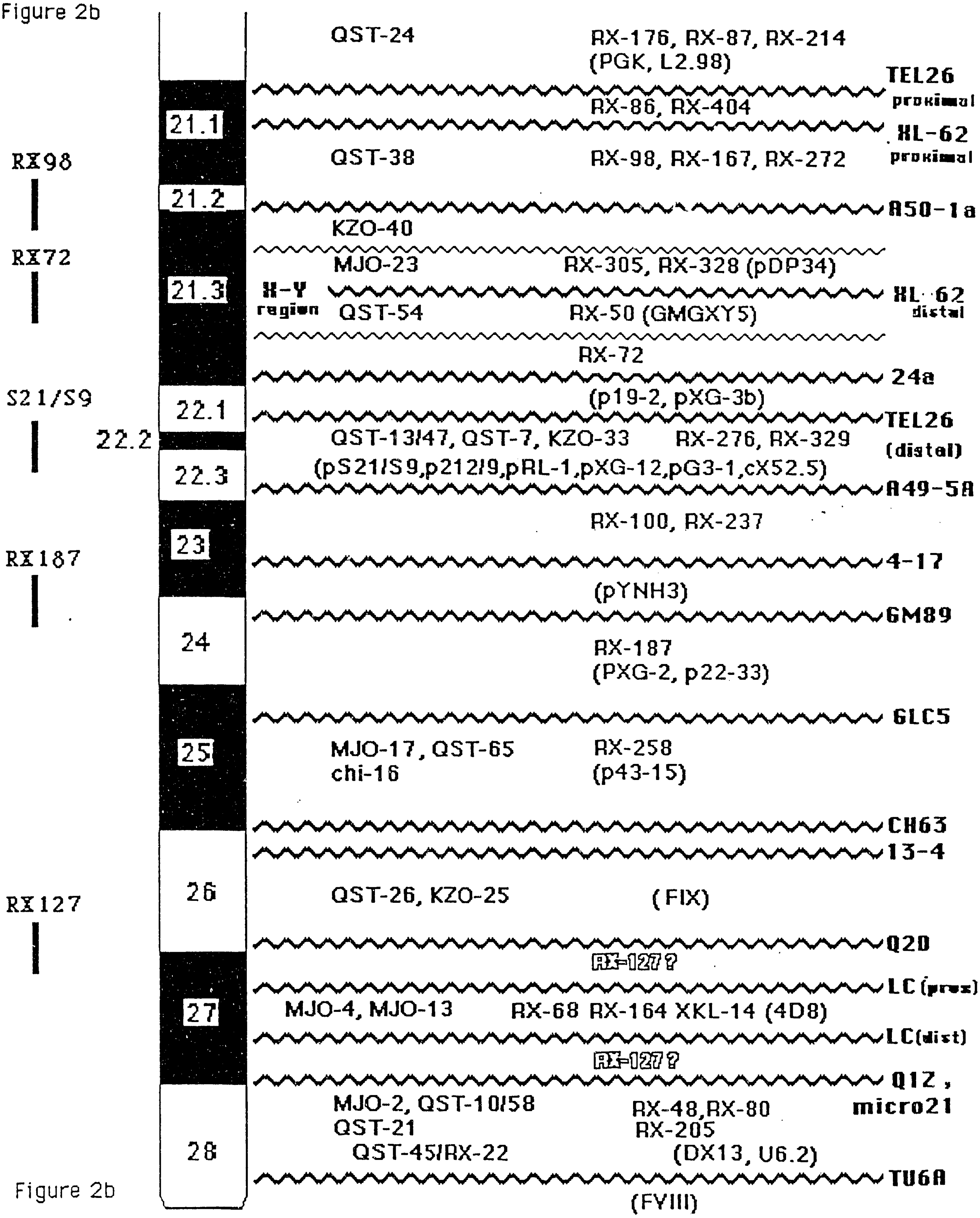


Figure 2b

Figure 3

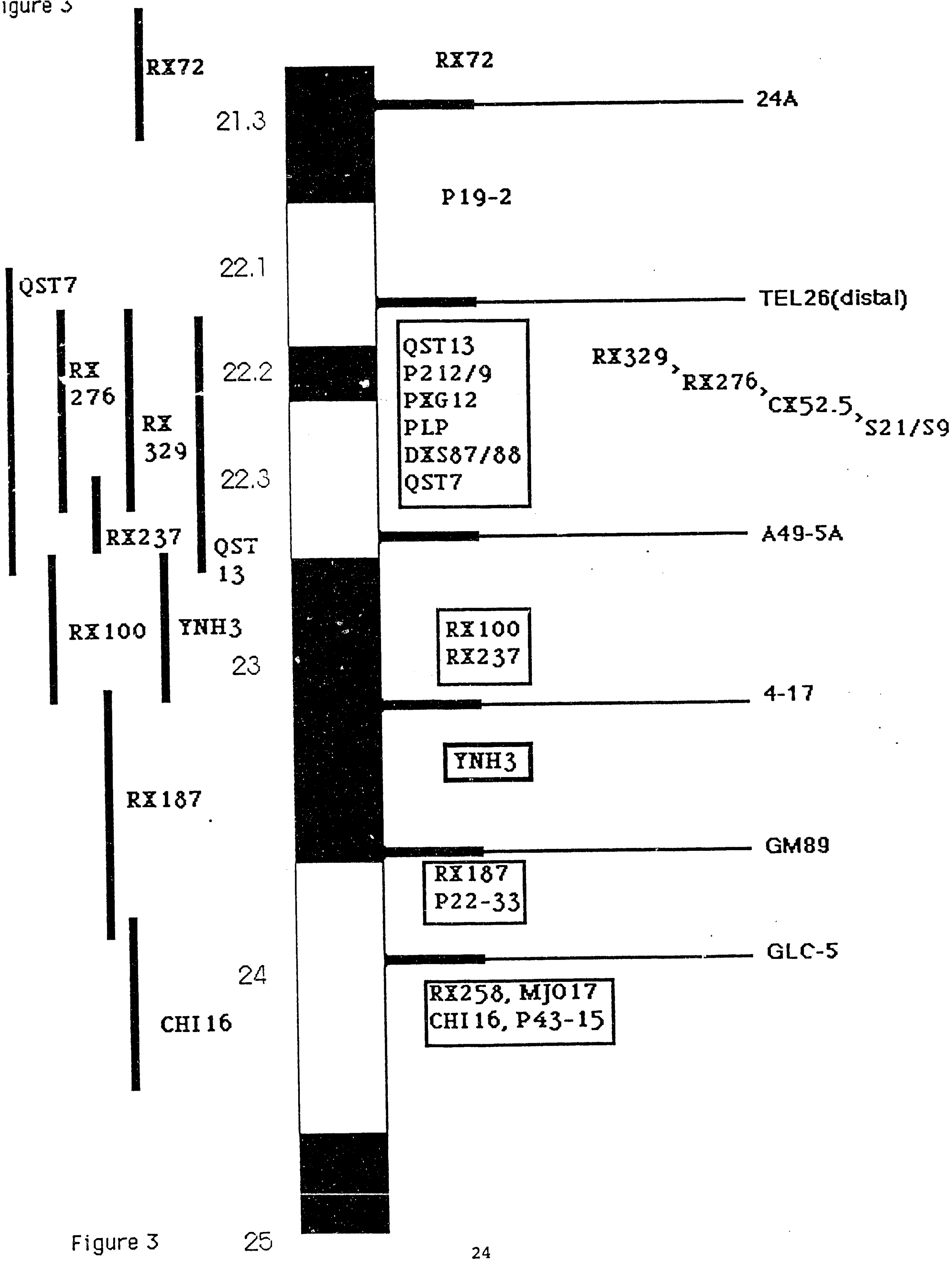


Figure 3

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      A                        B                                          C      D      E                 F                 G

56  1745,100101111101010110111101101101011011101011001101000010  594    290     1  1013  594+  2  1027  445   3  1727  392
57  1013,100101111100010000011111101111011010101011001100100010  464    353     1  1745  594+  2  1727  501   3  1514  464+
58  1514,100100100100010110001111101110001011100010101100110010  570    405     1  1041  570+  2  1808  527   3  1727  496
59  1041,100001110000010010011101101000000010100010101100110010  489    472     1  1514  570+  2  1727  514   3  2337  489+
60  2337,100000110000000000010000101110001011100010000100010010  575    514     1  1727  575+  2  1738  540   3  1808  531
61  1727,100000010100010000011101101110000011101011001100010010  654    531  #  1  1808  654+  2  2337  575+  3  1041  514
62  1808,000000010100010101001000101110000011100011101100010010  509    393     1  1727  654+  2  2337  531   3  1514  527
63  2103,010000010000010000001000001010000000000001001110001000 0 651    280     1  1512  663   2  2322  651+  3  1812  647
64  2322,110000010000010001000000000100001000011010011100010001  726    324  #  1  1848  726+  2  2103  651+  3  1919  585
65  1843,100000000000001001100100000100001000011010010101010001  741    585  #  1  1919  741+  2  2322  726+  3  1415  717
66  1919,100000001000000001100000000100001001011110000001010001  803    621     1  1912  809   2  1920  803+  3  1848  741+
67  1920,100000011000000000100000000100001011011110010001010001  777    716  #  1  1919  803+  2  1905  777+  3  1912  699
68  1905,100000011000000000100000000100000001001110010001000001  815    665     1  1912  849   2  1147  815+  3  2325  785
69  1147,100000001000000000100000010101000001001110010001000001  828    849  #  1  1912  828+  2  1905  815+  3  2325  764
70  1912,100000001000000000100000000100000001001110000001000001  799    764     1  1905  849   2  1147  828+  3  1919  809
71  2325,000000001000000000101000000100000001000110010001000001  693    755     1  1912  799+  2  1905  785   3  1147  764
72  1047,001000^^1000001000100000000100000000101110000001000001  777    735     1  1534  777+  2  1912  755   3  2325  693+
73  1534,101000101000001000101000000100000001101110010001000001  687    563     1  1047  777+  2  2325  735   3  1912  722
74  1148,101001011000101000101000010100000001001110010101000001  561    453     1  1534  687+  2  1905  663   3  1147  628
75  1036,101101010010001001101000010000000001111111110101000001  615    520     1  1909  615+  2  1529  611   3  1841  599
76  1909,100101011000001000101000110100000001111111110101100000  624    580     1  1712  637   2  2204  624+  3  1036  615+
77  2204,100100011000001000101000000101000001111111010101000001  674    554     1  1516  684   2  1139  674+  3  1544  660
78  1139,100001011100000000101000010001000001111111010101000001  691    684  #  1  1516  691+  2  2204  674+  3  1529  623
79  1516,101001001100001000101000000111000001111111010101000001  601    499     1  1139  691+  2  2204  684   3  1534  647
80  2133,100100001000001000101000110110000001111011010101001001  677    550     1  1712  677+  2  2204  647   3  1529  608
81  1712,100101001000001000101000010100000000111011010100100001  676    567  #  1  2133  677+  2  1704  676+  3  1909  637
82  1704,100000000000001000101000000000000000111101101000010000 1 682    478     1  1544  731   2  1529  686   3  1210  682+
83  1210,100000000000000000101000000000001000111110100000100001  809    605  #  1  1915  809+  2  1704  682+  3  1841  648
84  1915,100000000000000000101000010010001000111111100000100000  656    575  #  1  1210  809+  2  2239  656+  3  1704  605
```

Figure 4. Portion of the preliminary M. arthritidis genomic map.

A. Clone ID Number.
B. Bit Pattern of the 54 bits generated for each clone.
C. Similarity score for directly adjacent clones. Clones 1013-1514 have a similarity score of 464.
D. Similarity score for clones separated by one. Clones 1745-1514 have a similarity score of 353.
E.F.G. Clones with the first, second and third highest similarity scores for this clone.

TABLE 1
CHARACTERISTICS OF X CHROMOSOME POLYMORPHIC PROBES

Marker	HGM#	Location	Enzyme	Heterozygosity
MJO-2	DXS258	Xq26-qter	MspI	.04
			HindIII	.17
MJO-4	DXS266	Xq26-qter	TaqI	.46
MJO-13	DXS259	Xq26-qter	TaqI	.22
MJO-17	DXS261	Xq24-q26	TaqI	.39
MJO-22	DXS260	Xp11.2-p21.1	BamHI	.20
MJ2-16	DXS267	Xq24-q26	MspI	.56 (2 alleles, ins-del)
			TaqI	
			PstI	
			HindIII	.11
MJ2-19	DXS324	Xcen-p11.2	PstI	.24
			MspI	.32
KZO-3	DXS330	Xq26-qter	MspI	.25
			TaqI	ND
KZO-7	DXS329	Xq24-q26	PstI	ND
			MspI	ND
			HindIII	.22
KZO-9	DXS264	Xp22.3	BglII	.40 (3 alleles, ins-del)
			MspI	
			PstI	
			HindIII	
KZO-25	DXS263	Xq26-qter	TaqI	.17
KZO-32	DXS314	Xp21-p22.3	BamHI	.32
KZO-33	DXS265	Xq21.2-q22	MspI	.48
KZO-40	DXS262	Xq21.2-q22	BglII	.24
KZO-46	DXS320	Xp11.2-p21.1	BamHI	.56 (3 alleles, ins-del)
			BglII	
			PstI	
KZO-48	DXS323	Xcen-p11.2	BamHI	.16
KZO-52	DXS315	Xp21-p22.3	MspI	.58
QST-1	DXS257	Xp21-p22.3	TaqI	.40
QST-7	DXS327	Xq21.2-q22	MspI	.44
QST-10	DXS331	Xq26-qter	TaqI	.12
			PstI	.20
QST-58	DXS335	Xq26-qter	TaqI	.16
			TaqI	(same as QST-10)
QST-12	DXS316	Xp22.3	MspI	.16
QST-13	DXS328	Xq21.2-q22	PstI	.24
			HindIII	.24
QST-47		Xq21.2-q22	BamHI	.32
			PstI	(same as QST-13)

TABLE 1 (cont.)
CHARACTERISTICS OF X CHROMOSOME POLYMORPHIC PROBES

Marker	HGM#	Location	Enzyme	Heterozygosity
QST-21	DXS256	Xq26-qter	MspI	.48
			TaqI	ND
QST-24	DXS325	Xq13-q21.2	RsaI	.08
			BglII	.16
QST-26	DXS333	Xq26-qter	MspI	.44
QST-38	DXS326	Xq13-q21.2	MspI	.60
QST-42	DXS321	Xp11.2-p21.1	BglII	.54
QST-45	DXS334	Xq26-qter	TaqI	.28
			HindIII	.50
QST-48	DXS317	Xp22.3	BamHI	.52
			BglII	.52
QST-54	DXYS67	Xq21.2-q22	PstI	.44
QST-55	DXS318	Xp22.3	TaqI	.44
QST-56	DXS322	Xp11.2-p21.1	MspI	.12
QST-59	DXS319	Xp21-p22.3	MspI	.36
			BglII	.40
QST-70	DXS450	Xp11.21-p21.2	MspI	ND
QST-80	DXS451	Xp21.2-p22.3	TaqI	.44
RX-2	DXS336	Xp21.1-p22.3	TaqI	.16
RX-9	DXS337	Xp11.21-p21.1	TaqI	.20
RX-12	DXS338	Xp11.21-p21.1	MspI	.27
RX-21	DXS339	Xq11.2-q13	PstI	.63
RX-22	DXS340	Xq26-qter	TaqI	
			HindIII	.74 (Hap.)
RX-48	DXS341	Xq26-qter	MspI	
			PstI	.55 (4 alleles)
RX-50	DXYS68	Xq21.2-q22.1	MspI	.35
RX-54	DXS342	Xp22.3	MspI	.50 (5 alleles ins-del)
RX-66	DXS343	Xcen-p11.21	TaqI	.15
RX-68	DXS344	Xq26-qter	TaqI	.30
RX-72	DXS345	Xq21.2-q22.1	BglII	
			TaqI	.37
RX-80	DXS439	Xq26-qter	RsaI	ND
RX-86	DXS346	Xq13-q21.2	BglII	.59
RX-87	DXS347	Xq13-q21.2	BglII	.18

TABLE 1 (cont.)
CHARACTERISTICS OF X CHROMOSOME POLYMORPHIC PROBES

Marker	HGM#	Location	Enzyme	Heterozygosity
RX-97	DXS348	Xq11.2-q13	BglII	.25
RX-98	DXS349	Xq13-q21.2	MspI	.46 (4 alleles)
RX-100	DXS350	Xq22.1-q24	MspI	.33
RX-127	DXS351	Xq26-qter	BglII	.68 (3 alleles)
RX-137	DXS352	Xp11.21-p21.1	BglII	
			TaqI	.50
RX-156	DXS353	Xp11.21-p21.1	HindIII	
			BglII	.43
RX-164	DXS354	Xq26-q24	PstI	.45 (3 alleles)
RX-167	DXS355	Xq13-q21.2	PstI	
			HindIII	.17
RX-176	DXS356	Xq13-q21.2	RsaI	.18
RX-177	DXS357	Xp21.1-p22.3	PstI	.49
RX-187	DXS358	Xq24-qter	TaqI	
			HindIII	.62 (Hap.)
RX-202	DXS359	Xp22.3	BglII	
			TaqI	.56
RX-205	DXS360	Xq26-qter	MspI	
			TaqI	.59
RX-208	DXS440	Xp11.21-p21.1	MspI	.37
RX-214	DXS441	Xq13-q21.2	TaqI	.56
RX-234	DXS361	Xp11.21-p21.1	MspI	
			TaqI	.48 (Hap.)
RX-237	DXS362	Xq21.2-q24	MspI	
			TaqI	.56
RX-258	DXS363	Xq24-q26	TaqI	.56
RX-272	DXS364	Xq13-q21.2	TaqI	.56
			EcoRI	
RX-276	DXS442	Xq21.2-qter	MspI	.42 (3 alleles?)
RX-314	DXS365	Xp21.1-p22.3	MspI	.56
RX-324	DXS443	Xp21.2-p22.3	MspI	.15
RX-327	DXS444	Xp21.2-p22.3	TaqI	ND
RX-329	DXS366	Xq21.2-q22.1	TaqI	.47
			MspI	
RX-369	DXS445	Xp22.3	MspI	.52 (3 alleles)
			MspI	
RX-378	DXS446	Xp11.21-p21.1	PstI	.5
RX-404	DXS447	Xq13-q21.2	BglII	.54
XKL-14	DXS448	Xq26-qter	MspI	.5 (3 alleles)
XKL-23	DXS449	Xp11.21-p21.1	TaqI	.42

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Other literature citations made in this progress report may be found in the main bibliography.

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