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**CHROMOSOME REGION-SPECIFIC LIBRARIES
FOR HUMAN GENOME ANALYSIS**

FINAL PROGRESS REPORT

for Period March 1, 1991 - February 28, 1994

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FINAL REPORT FOR PERIOD MARCH 1, 1991 TO FEBRUARY 28, 1994**ABSTRACT**

The objectives of this grant proposal include (1) development of a chromosome microdissection and PCR-mediated microcloning technology, (2) application of this microtechnology to the construction of region-specific libraries for human genome analysis. During this grant period, we have successfully developed this microtechnology and have applied it to the construction of microdissection libraries for the following chromosome regions: A whole chromosome 21 (21E), 2 region-specific libraries for the long arm of chromosome 2, 2q35-q37 (2Q1) and 2q33-q35 (2Q2), and 4 region-specific libraries for the entire short arm of chromosome 2, 2p23-p25 (2P1), 2p21-p23 (2P2), 2p14-p16 (2P3) and 2p11-p13 (2P4). In addition, 20-40 unique sequence microclones have been isolated and characterized for genomic studies. These region-specific libraries and the single-copy microclones from the library have been used as valuable resources for (1) isolating microsatellite probes in linkage analysis to further refine the disease locus; (2) isolating corresponding clones with large inserts, e.g. YAC, BAC, P1, cosmid and phage, to facilitate construction of contigs for high resolution physical mapping; and (3) isolating region-specific cDNA clones for use as candidate genes. These libraries are being deposited in the American Type Culture Collection (ATCC) for general distribution.

A. INTRODUCTION

This is the final report for the research grant entitled "Chromosome Region-Specific Libraries for Human Genome Analysis", being conducted during the 3-year period starting March 1, 1991 and ending February 28, 1994. As stated in the original grant, the objectives include the development of a novel technology of chromosome microdissection and microcloning, and to apply this technology to the construction of region-specific libraries as useful resources for the genome program, particularly for human chromosome 2 for use by LLNL in their genome studies.

During the past 3 years, the technology was fully developed and applied to the construction of 6 region-specific libraries for chromosome 2. These libraries have been used to isolate other clones with large inserts, like YAC and BAC; to isolate microsatellites for linkage analysis; to paint specific chromosomal regions for refined cytogenetic analysis; and to isolate expressed sequences as candidate genes. We plan to continue to construct additional region-specific libraries for general use in genomic and other genetic studies. In particular, we will complete the remaining 6 libraries on the long arm of chromosome 2 plus a centromeric library, as well as the genomic regions rich in genes. We also plan to develop techniques to construct region-specific cDNA libraries to facilitate genomic mapping of expressed sequences. We believe that the technology is ready for large scale application to many important regions of the human genome to provide valuable resources for genome mapping and sequencing, and the cloning of genes underlying devastating genetic diseases and cancers.

B. PROGRESS REPORT FOR THE ENTIRE GRANT PERIOD STARTING 3/1/91 and ENDING 2/28/94

In this final report, we describe the development of microdissection and PCR-mediated microcloning techniques in the construction of region-specific libraries. In addition, we summarize the application of this methodology to the construction and characterization of microdissection libraries of human chromosome 21, and 6 region-specific libraries of chromosome 2. Finally, we present our progress in the isolation and characterization of cDNA clones from chromosome 21 using the unique sequence microclones from the chromosome 21 microdissection library.

1. Development of microdissection and PCR-assisted microcloning techniques.

During this grant period, we have successfully developed a chromosome microdissection and PCR-assisted microcloning techniques which have been described in detail in several of our publications. In this procedure, 20 fragments from a chromosomal region are microdissected using a glass needle with a fine tip. The dissected fragments were deposited in a microdrop of proteinase K which was covered with a larger drop of buffer-saturated paraffin oil to prevent evaporation. The sample drop was extracted 3x with buffer-saturated phenol using micromanipulation technique aided by a microinjector and a de Fonbrune micromanipulator. After phenol extraction, the purified DNA was cleaved with MboI and ligated to an MboI linker-adaptor. All these steps were carried out in nanoliter volumes under the microscope. After ligation, the sample was transferred to an Eppendorf tube for PCR amplification. The 20-nt sequence of the MboI linker-adaptor was used as primer. The amplified DNA was cleaved with MboI to remove adaptor and cloned into the BamHI site of pUC19.

This procedure has been well established and worked extremely efficiently. We can routinely construct region-specific libraries with as many as 300,000 to 1,000,000 microclones in each library. Due to the successful development of this highly efficient technique for constructing region-specific libraries, we decided not to divert our limited resources to pursue another microcloning technique, i.e. the TdT procedure as originally proposed in the grant.

2. Construction of a microdissection library for human chromosome 21 (designated 21E library).

This is the first microdissection library we constructed using the procedure developed in this lab. In this library, we microdissected 30 pieces of whole chromosome 21 and constructed a large library, 21E, comprising 700,000 recombinant plasmid microclones. Colony hybridization showed that 42% of the clones contained repetitive sequences and 58% contained single or low-copy sequences. The insert sizes generated by complete MboI cleavage ranged from 50 to 1,000 bp, with a mean of 416 bp. Southern blot analysis of microclones from the library confirmed their human origin and chromosome 21 specificity. Over 85% (19/22) of unique sequence microclones were shown to derive from the dissected chromosome. Some of the clones were regionally mapped to specific sites of chromosome 21 by using a regional mapping panel of cell hybrids. In addition, the microclones were also characterized for insert size and were used to identify the corresponding genomic fragments generated by HindIII (2). We further demonstrated that the microclones with short inserts can be efficiently used to identify YAC clones with

large inserts for increased genomic coverage for high-resolution physical mapping. We also used 200 unique sequence microclones to screen a human liver cDNA library and identified two cDNA clones which were regionally assigned to the 21q21.3-q22.1 region. Partial sequencing of these cDNA clones and searching in the GenBank revealed that they are new expressed genes on chromosome 21 not previously reported as searched in the GenBank. Thus, these new gene sequences can be used as candidate genes for Down syndrome and other diseases on chromosome 21. Details have been reported.

3. Importance in rigid control to minimize extraneous DNA during microdissection and PCR/microcloning.

Based on our extensive experience with the construction of microdissection libraries, we have become acutely aware of the importance to exercise highly stringent precautions to remove every possible source of contaminant DNA during microdissection and PCR-mediated microcloning procedures. The following are basic essentials that have been rigorously practiced in order to construct microdissection libraries with good quality, and when these precautions are strictly enforced, non-human microclones of unknown origin will be significantly reduced.

- (a) Use only disposable pipets, flasks, tubes, etc., to avoid common washing facility.
- (b) Wearing gloves in all steps of operation, including microscopic manipulations.
- (c) Autoclave water and all buffers and reagents.
- (d) Exposing to UV (254 nm) the dissecting needles and micropipets and all buffers and reagents.
- (e) Preparing all buffers and reagents in an air-clean hood and storing them in small aliquots for one use only.
- (f) Using each micropipet for one operation only.
- (g) Using "safe" buffer to saturate phenol and paraffin oil.
- (h) Including a control microdrop carried along identically with the sample drop, and if any detectable background PCR products are found in the control drop, discarding the experiment.

4. Construction and characterization of a microdissection library for region 2q35-q37 (designated 2Q1 library). (For details of this library, refer to Publications by Yu et al. Genomics 14, 769-774, 1992)

The first region-specific library we constructed using the microdissection and PCR/microcloning technique was human chromosome 2, region 2q35-q37. Mitotic cells from a normal human lymphoblast cell line GM03714 were harvested and fixed twice, 5-10 min each, in 100% methanol instead of acetic acid to avoid possible DNA damage. For better chromosome spreading, the fixed cells were dropped onto the coverslip and, while they were still wet, a dilute acetic acid mixture (e.g., methanol:acetic acid, 10:1) was rinsed through the cells before complete air-drying with the aid of a fan. The procedure can increase chromosome spreading and yield cytoplasm-free metaphases. The chromosomes were trypsin-Giemsa banded for accurate identification and dissection. The chromosome fragments were dissected using glass needles with fine tips prepared by a de Fonbrune microforge. The dissected fragments were placed in a microdrop (5 nanoliter) of proteinase K covered with a drop of buffer-saturated paraffin oil (Merck 7162). A similar microdrop of proteinase K without dissected chromosome fragments was

placed next to the sample drop as a control and all subsequent steps were performed in an identical fashion for both microdrops.

Twenty fragments were dissected from the 2q35-q37 region. After treatment with proteinase K, phenol and MboI, the sample was ligated to an MboI linker-adaptor and transferred to an Eppendorf tube for PCR. The PCR products were analyzed for DNA contamination and no trace of DNA was found in the control drop, thus the experiment proceeded to transformation as follows. The PCR products were purified, digested with MboI, separated on agarose gel to remove adaptor molecules, purified again, ligated to the BamHI-digested and dephosphorylated pUC19 vector, and transformed to the DH5 α Max Efficiency competent bacterial host cells (BRL). A library comprising 20,000 recombinant microclones was obtained.

A randomly selected sample of 60 microclones from the library were analyzed for insert size, which ranged between 200-700 bp with a mean of 270 bp. Colony hybridization of the microclones with total human DNA showed that about 40% of the microclones contained repetitive sequences and 60% contained unique or very-low-copy sequences. Southern blot analysis of 48 unique sequence microclones randomly selected from the library showed that about 54% (26/48) of the microclones displayed distinct bands when hybridized to human DNA and the DNA from cell hybrid GM10826B containing a single human chromosome 2. In addition, 4 microclones also hybridized to cell hybrid GM11022 containing 2q37, the distal portion of the long arm of chromosome 2, thus further mapping these 4 microclones to a more refined region within the dissected region. Table 1 summarizes the characterization of the 26 unique sequence microclones from the 2Q1 library.

TABLE 1. Analysis of insert size and the hybridizing human genomic fragments cleaved with HindIII in 26 unique sequence microclones isolated from the 2Q1 (q35-q37) microdissection library

Microclone	Insert (bp)	HindIII Bands (kb)	Hybridization				
			CHO	Human	#2	2q37	Mouse
2Q5	530	1.0;1.2	-	+	+	-	-
2Q6	590	5.4;6.2	-	+	+	-	-
2Q9	680	9.6	-	+	+	-	-
2Q17	780	3.0	-	+	+	-	ND
		2.4*	+	-	+	-	ND
2Q19	140	1.2;1.6	-	+	+	-	-
2Q20	260	11.0	-	+	+	+	-
2Q21	630	1.0;1.9	-	+	+	+	-
2Q25	600	12.0	-	+	+	-	-
2Q27	610	10.5	-	+	+	-	-
2Q40	270	14.5	-	+	+	-	ND
2Q58	130	3.3	-	+	+	-	ND
2Q68	750	3.4	-	+	+	-	ND
2Q71	420	5.1	-	+	+	-	ND
2Q75	200	12.3	-	+	+	-	ND
2Q78	290	3.1	-	+	+	-	ND
2Q79	430	5.4	-	+	+	-	ND
2Q102	110	7.4	-	+	+	-	ND
2Q104	120	2.7	-	+	+	-	-
		4.1*	+	-	+	-	-
		5.2**	-	-	-	+	+
2Q113	240	4.1	-	+	+	-	-
2Q130	80	8.2	-	+	+	-	-
2Q131	290	13.5	-	+	+	-	-
2Q135	80	10.5	-	+	+	-	-
2Q139	50	12.0	-	-	+	+	-
		11.5	-	+	-	-	-
2Q209	510	12.5	-	+	+	-	-
2Q220	300	6.8	-	+	+	+	-
2Q244	260	1.3	-	+	+	-	-

*CHO bands

**Mouse bands

ND: Not done

In collaboration with Dr. Teresa Yang-Feng of Yale University, we used the FISH painting technique to demonstrate the 2q35-q37 microdissection library to derive from the dissected chromosome region. The amplified DNA from the entire library was nick-

translated with biotin-11-dUTP and hybridized in situ with metaphase spreads prepared from short-term lymphocyte cultures of a normal individual. For signal detection, chromosomes were blocked with total human DNA using 3% BSA in 4x SSC for 30 min, incubated with fluorescein isothiocyanate-conjugated avidin (Vector Labs) at 37 C for 30 min, washed in 4x SSC/0.1% Tween 20, and counterstained with propidium iodide. A human chromosome 2 centromere-specific probe was included in the hybridization for the identification of the chromosome. From the analysis of 20 cells, the fluorescence signal was found in the distal portion of the long arm of chromosome 2, and no other chromosomal sites exhibited similar hybridization signals. Both homologous chromosomes were labeled in the same region in virtually all the cells analyzed. Details of the construction and characterization of this library have been published.

The entire library of 2q35-q37 in the form of PCR products is freely available for use by the scientific community. In addition, the 26 well-characterized unique sequence microclones from the library are also available for distribution. These unique sequence microclones can be used directly as labeled probes to screen other libraries to isolate corresponding clones with large inserts from the dissected 2q35-q37 region. In addition to the publication in *Genomics*, we also reported to the Chromosome 2 Workshop in October 1992 about the availability of this library for any interested investigators. We have recently sent the library and the unique sequence microclones to Dr. Peiter de Jong formerly of LLNL. He used the probes to screen a BAC (bacterial artificial chromosome) library from Dr. Melvin Simon and was able to isolate corresponding BAC clones from the library, as reported in the DOE Contractor-Grantees Genome Workshop in Santa Fe in February 1993. The material which we sent to Dr. de Jong on November 23, 1992 included the following:

(a) 20 μ l of the entire library prepared as the PCR products amplified from the original library comprising 20,000 microclones. The insert fragments are MboI-cleaved genomic DNA with the linker-adaptor at both ends. The library can be amplified by PCR using the 20-nt adaptor as primer (refer to Kao and Yu, *PNAS* 98, 1844-1848, 1991). Upon purification, the library can be labeled by primer extension or cloned into the BamHI site of pUC19 after cleavage with MboI and ligated to the plasmid vector for transformation.

(b) 20 individual microclones with unique sequence inserts isolated from the library and characterized in detail, as described in Table 1 in *Genomics* 14, 769-774, 1992:

2Q6 (590 bp)	2Q9 (680 bp)	2Q19 (140 bp)	2Q20 (260 bp)
2Q21 (630 bp)	2Q25 (600 bp)	2Q27 (610 bp)	2Q40 (270 bp)
2Q58 (130 bp)	2Q68 (750 bp)	2Q71 (420 bp)	2Q75 (200 bp)
2Q78 (290 bp)	2Q79 (430 bp)	2Q113(240 bp)	2Q130 (80 bp)
2Q131(290 bp)	2Q209 (510 bp)	2Q220(300 bp)	2Q244(260 bp)

These microclones can be labeled by primer extension to high specific activities and yield distinct bands on Southern blots containing total human DNA. The microclones were prepared and sent out in the following two forms:

(i) Bacterial lysates containing microclones. These boiled bacterial lysates, 200 μ l per microclone, can be used directly for PCR using 1-2 μ l sample per PCR reaction, with the pUC19 sequences flanking the cloning site as primer to produce inserts.

(ii) 20 μ l of the PCR products from each microclone. 10 μ l can be gel purified and used for 10-15 labels.

We plan to send out our microdissection libraries and the unique sequence microclones to other investigators as we have done in the above.

5. Construction and characterization of a second region-specific library on the long arm of chromosome 2, region 2q33-q35 (designated 2Q2 library).

Using similar procedure as described above, we microdissected 20 fragments from the 2q33-q35 region and constructed a library comprising 300,000 microclones. The library is much larger than the 2q35-q37 library. Several important improvements have been made since the construction of the 2P1 library, e.g. phenol extraction, ligation, PCR amplification, transformation, etc.. These improvements may have contributed to the large size of the libraries. Some of these improvements are described below. We now can routinely construct large libraries comprising 300,000 or more microclones.

(a) During phenol extraction of the microdrop containing dissected sequences, it is important to remove the last trace of phenol from the microdrop especially during the final phenol extraction, so that the chromosomal DNA is highly purified for maximum efficiency in MboI cleavage and ligation. For this purpose, a micropipet should be constructed with a very small tip and the opening of the tip is sharpened at an angle to allow it to lie flat on the slide so that the pipet tip can reach the last trace of phenol next to the microdrop and to withdraw it completely. The microdrop can be pushed gently to another location and stored overnight with the drop facing down to allow complete evaporation of the residual phenol.

(b) The reagents used in PCR for amplifying the dissected sequences should be prepared with extreme care and precision. The pH measurements and the Mg⁺⁺ concentration should be executed precisely.

(c) For transformation, the reaction was carried out in 17 x 100 mm polypropylene tubes (Falcon 2059) as recommended by the supplier for improving transformation efficiency by 3-10 folds.

For the 2Q2 library, the insert size of the microclones ranged from 50-800 bp, with a mean of 200 bp. We analyzed 35 unique sequence microclones by Southern blot analysis and 63% (22/35) of the clones were shown to hybridize to human and chromosome 2 DNA. Table 2 presents the characterization of the 28 unique sequence microclones isolated from the 2Q2 library.

Table 2. Characterization of 28 unique sequence microclones isolated from the 2Q2 (2q33-q35) library

Microclone	Insert size (bp)	Genomic HindIII band (kb)
2Q2-11	60	4.7
2Q2-13	70	6.0
2Q2-21	110	5.2
2Q2-26	130	7.0
2Q2-33	110	5.1
2Q2-43	140	6.8
2Q2-55	110	4.7
2Q2-69	130	3.1
2Q2-74	90	5.1
2Q2-86	70	4.6
2Q2-88	250	4.1
2Q2-93	80	4.6
2Q2-99	250	4.0
2Q2-101	80	5.0
2Q2-106	120	4.9
2Q2-124	170	8.4
2Q2-131	90	4.5
2Q2-155	90	4.5
2Q2-161	130	6.8
2Q2-162	90	4.2
2Q2-168	330	3.0
2Q2-169	250	2.7
2Q2-171	110	20.0
2Q2-175	110	4.9
2Q2-177	80	4.1
2Q2-178	70	4.3
2Q2-179	300	3.0
2Q2-185	130	4.9

Further characterization of the clones was carried out for 22 of the above microclones using DNAs from the following 3 cell lines, and the results are presented in Table 3.

(i) GM10918 from Cell Repository, Camden, NJ., karyotype 46,XX,del(2)(pter>q34::q36>qter. This patient had an interstitial deletion in the band 2q35 and exhibited anomalies including developmental delay, cleft palate, heart defect, IgG deficiency, hyperextensible joints. Dosage analysis will be used to assign microclones to this deletion region.

(ii) XIX-25A, a rat-human cell hybrid from Dr. Uta Francke of Stanford University. This hybrid contained a translocation chromosome of t(2:6) including 2q33-qter, and the normal copy of chromosome was not present. This hybrid can be used to confirm the microclones to derive from the 2q33-qter region.

(iii) XHB-104, a repair-proficient XRS-6/human cell hybrid containing a small fragment of human chromosome 2, including a part of 2q33-q35, integrated into the XRS-6 genome. This hybrid, provided by Dr. David Chen of LANL, was isolated from a fusion between human cells and a radiation-sensitive mutant of Chinese hamster cells defective in rejoining of radiation-induced DNA double-strand breaks. Using human inter Alu-PCR products amplified from the hybrid DNA as probe to hybridize in situ to normal human metaphase spreads, it clearly showed that a human fragment near the region of 2q33-q35 was integrated in the hamster cell genome. We have obtained a culture of the hybrid XHB-104 from Dr. Chen and have prepared DNA for use in assigning microclones to this region. The microclones mapped to this region will be used by Dr. Chen for his studies in the cloning of the crucial gene.

Table 3. Further characterization of 21 unique sequence microclones from the 2Q2 library hybridized to human cells or cell hybrids containing partial deletions or fragments of the distal portion of the long arm of chromosome 2

Microclone	Ch.2	GM10918	XIX-25A	Rat	XHB-104	CHO
2Q2-13	+	no del	+	-	-	-
2Q2-21	+	no del	+	-	-	-
2Q2-43	+	del	+	-	-	-
2Q2-69	+	del	+	-	-	-
2Q2-74	+	no del	+	-	-	-
2Q2-88	+	del	+	-	-	-
2Q2-93	+	del	+	-	-	-
2Q2-99	+	del	+	-	-	-
2Q2-101	+	del	+	-	-	-
2Q2-106	+	no del	+	-	-	-
2Q2-123	+	no del	+	-	-	-
2Q2-155	+	del	+	-	-	-
2Q2-161	+	no del	+	-	-	-
2Q2-162	+	del	+	-	-	-
2Q2-168	+	del	+	-	-	-
2Q2-169*	+	del	+	_*	+	_*
2Q2-171**	+	del	+	**	-	**
2Q2-175	+	no del	+	-	-	-
2Q2-178	+	del	+	-	-	-
2Q2-179	+	del	+	-	-	-
2Q2-185	+	no del	+	-	-	-

*2Q2-169 also hybridized to rat and CHO at 9.6 kb and 15.0 kb respectively.

**2Q2-171 also hybridized to rat and CHO at 7.4 kb and 10.0 kb respectively.

Of the 22 microclones analyzed so far, 14 clones were mapped to the 2q35 region by the positive hybridization with GM10918. The other 6 clones apparently lie within 2q33-34 but outside the GM10918 deletion. Only one clone, 2Q2-169, was positive in XHB-104, indicating that the portion of 2q33-q35 retained in this hybrid might be very small. All 22 clones hybridized to XIX-25A, confirming that the clones were all derived from 2q33-qter and consistent with the region of dissection from which the clones were derived. As soon as the characterization of other clones is complete, all the clones will be available to investigators interested in using this library as well as all the unique sequence microclones isolated from the library.

6. Construction and characterization of the first region-specific library from the short arm of chromosome 2, region 2p23-p25 (designated 2P1 library). (For details of this library, refer to Publications by Yu et al., Human Genetics, in press)

After microdissection of 20 fragments from the 2p23-p25 region, a large library was constructed comprising 300,000 recombinant microclones. The insert size ranged between 50-600 bp with a mean of 200 bp. Southern blot analysis of 53 unique sequence microclones identified 58% (31/53) of clones hybridizing to human and chromosome 2 DNA. Further characterization of the clones was carried out using GM09216 (Cell Repository, Camden, NJ), karyotype 46,XY, del(2)(pter>p25.1::p23>qter). This individual had an interstitial deletion including 2p23-p25.1, and displayed severe growth and mental retardation, multiple anomalies, heterozygous BA phenotype at ACP-1 locus (5). We prepared DNA from this cell line and used it in dosage analysis to assign microclones to the deleted region of 2p23-p25.1. Of the 31 clones analyzed, 16 clones were apparently in the deletion region and another 15 clones were outside the deletion region. The results are summarized in Table 4. All the 31 clones hybridized to the cell hybrid GM10826B containing a single chromosome 2. The 2P1 library and the 31 unique sequence clones, including those further assigned to 2p23-p25.1, are all available for distribution.

Table 4. Analysis of 31 microclones from the 2P1 (2p23-p25) library for insert size, hybridizing HindIII genomic bands, and the assignment to the 2p23-p25.1 deletion region

Microclone No.	Insert Size (bp)	HindIII Bands (kb)	2p23-p25.1 Deletion
2P1-126	280	2.9	-
2P1-130	540	3.8	-
2P1-168	220	1.5	+
2P1-177	310	16.0	+
2P1-186	310	18.0	-
2P1-282	290	1.2	+
2P1-292	390	6.9	-
2P1-295	240	3.7	-
2P1-296	140	14.0	-
2P1-323	390	4.0	+
2P1-326	390	12.2	+
2P1-328	260	3.5	-
2P1-366	290	12.0	+
2P1-402	510	1.2	-
2P1-405	510	9.5	+
2P1-412	140	8.0?	+
2P1-414	340	11.0	+
2P1-416	240	11.0	+
2P1-421	390	5.0	-
2P1-422	440	2.2	+
2P1-423	110	9.2	-
2P1-425	310	14.0	-
2P1-427	510	5.8	-
2P1-429	190	10.0	+
2P1-430	50	1.2	-
2P1-432	240	1.3	-
2P1-436	250	0.9	+
2P1-441	310	3.0	+
2P1-442	260	14.0	+
2P1-451	350	1.8	+
2P1-453	290	22.0	-

7. Construction and characterization of the second region-specific library for the short arm of chromosome 2, region 2p21-p23 (designated 2P2 library).

For constructing the microdissection library for the region 2p21-p23, 20 fragments were dissected. After MboI cleavage and ligation to MboI linker-adaptor, the sample was amplified by PCR. A sample of 1/20 (5 μ l) of the PCR products from the original amplified DNA was used in ligation to vector. One tenth of the ligation mixture was used in transformation and about 4,500 white colonies were obtained. thus, the size of the whole library was extrapolated to contain approximately 900,000 clones. Individual microclones were isolated and plated on grids for hybridization with labeled total human DNA. About 40-50% of the clones contained repetitive sequences and showed weak and strong hybridization. The rest of the clones displayed very weak or no hybridization, presumably containing single copy or very low copy number sequences.

A total of 60 unique sequence microclones were analyzed by hybridization to a small panel containing DNAs from CHO, human and GM10826B containing a single chromosome 2. Of the 60 unique sequence microclones tested, 56% (34/60) exhibited positive hybridization to both human and chromosome 2 DNA, indicating that these clones were derived from human chromosome 2. The insert size of the microclones ranged between 50-800 bp, with a mean of 250 bp. No significant difference in insert size was found among clones containing unique or repetitive sequences, or clones hybridizing or not hybridizing to human and chromosome 2 DNA. Table 5 summarizes the insert size in the microclones that hybridized to human and chromosome 2 DNA, as well as the size of the human genomic fragments cleaved with HindIII and detected by the microclones.

Table 5. The insert size and hybridizing HindIII genomic bands of 34 unique sequence microclones isolated from the 2P2 (2p21-p23) microdissection library

Microclone	Insert size (bp)	HindIII bands (kb)
2P2-1	260	2.9
2P2-22	390	5.2
2P2-38	150	1.0
2P2-82	460	8.1
2P2-83	410	9.0
2P2-84	210	5.1
2P2-85	130	0.9
2P2-91	260	1.8
2P2-92	380	3.0
2P2-95	110	1.9
2P2-99	450	6.5
2P2-107	200	3.3
2P2-108	100	6.5*
2P2-124	270	2.3
2P2-126	130	3.3
2P2-129	135	6.0
2P2-134	240	5.4
2P2-140	50	9.0
2P2-147	380	8.3
2P2-153	160	2.4
2P2-163	630	3.0
2P2-167	270	6.0
2P2-168	260	5.0
2P2-202	160	10.7
2P2-210	240	8.7
2P2-212	60	1.8**
2P2-213	70	12.0
2P2-227	350	4.7
2P2-230	240	2.4
2P2-239	120	2.1
2P2-240	200	3.7
2P2-249	380	5.1
2P2-261	550	6.4
2P2-263	270	6.7

*Multiple light bands also present

**A light smear in the background

In the dissected region 2p21-p23, a very interesting genetic disease has been localized previously, i.e. holoprosencephaly (HPE). HPE is an embryonic developmental anomaly resulting in incomplete forebrain and midfacial development. The brain malformation may include an abnormally small forebrain mass with complete failure of

formation of the hemispheres, or one single ventricle with rudimentary lobes, or well-formed lobes with communication of the lateral ventricles due to absence of the septum pellucidum. The facial anomalies range from the most severe types with anophthalmia, cyclopia, ethmocephaly or cebocephaly, to less severe forms with hypotelorism, flat nasal bridge, maxillary agenesis, median cleft lip or single upper midline incisor.

The etiology of HPE is heterogeneous, with an incidence of 1 in 16,000 live-born infants and 1 in 250 induced abortions. HPE has been reported as autosomal dominant and recessive forms, as part of syndromes, and as environmentally induced in infants of diabetic mothers. It has also been found in association with chromosome anomalies including del(2)(p21-p22.2), del(18p), del(7)(q36), dup(3)(p24-pter), and del(21)(q22.3). Thus, it is likely that there may be 5-10 genes involved in the normal embryonic development of the forebrain and midfacial structure.

Muenke et al. at the Children's Hospital in Philadelphia reported the presence of an interstitial deletion including 2p21-p22.2 in HPE patients. In collaboration with Dr. Muenke, we obtained the lymphoblast culture from the patient and prepared DNA for dosage analysis using the microclones from the 2p21-p23 library. Preliminary studies identified several clones in the deletion region. We have sent these microclones to Dr. Muenke for further studies, including sequencing, preparing primers for isolating YACs, refined physical mapping of the deletion region, etc. We will also provide him with the whole library for FISH painting analysis of the deletion in the metaphase cells from the patients with or without cytogenetically detectable deletions. We are also isolating cDNA clones from the library to attempt to identify sequences that may yield some clues as candidate genes for HPE, particularly in the search for sequence homologies in those domains or motifs resembling homeo box or other sequences involved in early developmental processes in mouse or Drosophila. It is especially rewarding to be able to provide useful probes in a crucial chromosomal region to facilitate molecular genetic analysis and gene cloning for PHE, because for several years Dr. Muenke could not generate probes in the 2q21-q22.2 region for his molecular studies of this disease.

8. Construction and characterization of the third region-specific library for the short arm of chromosome 2, region 2p14-p16 (designated 2P3 library).

20 fragments from region 2p14-p16 were dissected for microcloning. After PCR amplification, the control drop was clean from any trace of DNA. In addition, the gel was transferred to blot for hybridization with total human DNA to determine the presence of human repetitive DNA. The sample drop exhibited significant signal of human DNA and the control showed no signal, indicating that the library is likely to be of good quality with most of the clones derived from the dissected region. A subset of the PCR products was used in transformation to obtain recombinant microclones for a critical evaluation of the library.

The 2P3 library is very large, comprising approximately 1,000,000 recombinant microclones. About 60% of the clones contained repetitive sequences and 40% contained unique sequences. The insert size ranged from 50-800 bp, with a mean of about 230 bp. Using Southern blot analysis, 83% (39/47) of the clones could be hybridized to chromosome 2. Table 6 summarizes the results on the characterization of the microclones derived from the 2P3 library.

Table 6. Characterization of 39 unique sequence microclones isolated from the 2P3 (2p14-p16) library

Microclone	Insert size (bp)	Genomic HindIII band (kb)
2P3-1	170	7.0
2P3-2	350	1.3
2P3-3	270	6.4
2P3-4	180	6.2
2P3-8	350	12.0
2P3-15	140	3.8
2P3-16	500	3.2
2P3-20	60	4.0
2P3-22	140	4.0
2P3-23	300	2.7
2P3-28	490	8.0
2P3-29	190	5.6
2P3-32	320	9.0
2P3-33	180	2.2
2P3-34	380	3.6
2P3-38	320	5.4
2P3-40	170	6.0
2P3-77	50	2.6
2P3-78	160	3.1
2P3-81	70	5.0
2P3-82	140	9.0
2P3-84	380	3.7
2P3-86	160	8.4
2P3-87	320	14.5
2P3-88	250	14.0
2P3-91	120	3.0
2P3-92	390	7.4
2P3-94	110	5.6
2P3-95	100	4.3
2P3-98	170	8.6
2P3-100	300	1.7
2P3-101	300	5.8
2P3-102	230	5.4
2P3-103	200	7.2
2P3-105	230	8.2
2P3-106	220	7.6
2P3-111	380	6.3
2P3-113	50	3.6
2P3-115	100	11.0*

*Also cross-hybridized to CHO.

Recently, the gene for hereditary colorectal cancer has been mapped to chromosome 2, region 2p15-p16, by Bert Vogelstein, Albert de la Chapelle and their colleagues. Currently, 55,000 Americans die annually from colon cancer, the second leading cause of cancer deaths. This gene apparently confers susceptibility to colon and several other cancers, and 1 in 7 cases (13%) of colon cancer can be attributed to this faulty gene. Moreover, it has been estimated that 1 in every 200 persons carries the defective gene in the Western populations, a frequency more than 10 times higher than that of cystic fibrosis. Thus, this cancer susceptibility gene could be one of the most, if not the most, common causes of inherited disease in humans. For these reasons, the cloning of this crucial gene should not be delayed. Dr. Vogelstein asked us to provide the 2p14-p16 library and microclones for their use in the cloning of this gene. We quickly sent him on May 27, 1993 the library and 30 unique sequence microclones for their urgent need. After testing the unique sequence microclones, Dr. Vogelstein informed us on July 6, 1993 that from 15 clones they had tested so far, 8 clones fell into the critical region 2p15-p16. These clones were used for isolating corresponding YAC clones. Using these and other materials, the colon cancer gene was eventually identified and clones, and published in the Dec. 17, 1993 issue of Cell. This is a clear demonstration of the useful resources provided by the region-specific libraries that we have constructed.

9. Construction and characterization of the last region-specific library for the short arm of chromosome 2, region 2p11-p13 (designated 2P4 library). (For details of this library, refer to Publications by Yu et al., Somat. Cell Mol. Genet. in press)

Similarly, 20 fragments were dissected from the 2p11-p13 region. The PCR products in the control drop was free from any gross contaminant DNA by ethidium bromide staining. By Southern blot hybridization with total human DNA, the sample drop exhibited significant signal while the control drop showed no signal. Thus, the library is considered to be of good quality and transformation was carried out to yield a library comprising 600,000 recombinant clones. The library contains 30-40% unique sequence clones, with a mean insert size of 380 bp. 70% (35/50) of the clones were derived from human and are chromosome 2 specific. 35 unique sequence microclones have been isolated and characterized for use in studies involving this region, as presented in Table 7.

TABLE 7. Characterization of 35 single-copy microclones isolated from the 2p11-p13 (2P4) library for insert size and the hybridizing genomic HindIII bands

Microclone	Insert size	HindIII Bands	Notes
2P4-8	310	6.6	
2P4-9	410	1.3	
2P4-11	370	1.7	
2P4-14	270	11.0	
2P4-19	990	3.2	
2P4-20	220	6.2	
2P4-21	430	2.6	
2P4-24	630	3.8	
2P4-25	280	2.1	
2P4-28	500	2.5	
2P4-29	340	2.2	
2P4-31	230	7.8	
2P4-36	320	20.0	
2P4-38	530	1.5	
2P4-39	480	6.0	
2P4-44	410	20.0;2.1	2 HindIII bands in human and 1 band (20.0 kb) in GM1082B
2P4-47	200	1.9	
2P4-49	450	3.2	
2P4-54	220	2.3	Cross-hybridizing to CHO at 4.4 kb
2P4-57	390	24.0	
2P4-61	380	5.6	
2P4-62	560	3.8	
2P4-66	260	3.5	
2P4-71	320	10.0	
2P4-82	770	2.6	
2P4-84	380	4.0	
2P4-92	330	4.8	
2P4-94	110	5.1	
2P4-96	330	1.5	
2P4-97	500	7.8	
2P4-99	410	14.0	
2P4-102	260	11.0	
2P4-104	300	12.0	
2P4-107	380	9.8;4.2	2 HindIII bands both human and GM10826B
2P4-111	310	2.7	

10. Summary of the construction of region-specific microdissection libraries for human chromosome 2.

Table 8 summarizes our current progress in the construction of region-specific libraries for human chromosome 2. This chromosome contains approximately 240 mb of DNA and can be divided into 10 regions plus the centromere region. Thus, on the average, each region consists of 20-25 mb. Since the libraries are large, generally comprising between 300,000 and 1,000,000 microclones, it is estimated that 10-40 microclones may exist for every kb of genomic DNA (some of which may be duplicates). This should sufficiently cover the dissected region if STSs are prepared from samples of the microclones in the library. If the microclones from the library are used to screen YAC libraries, 20-30 independent YAC clones with inserts of 1-mb or larger may be readily isolated to construct contigs for the entire dissected region. The microclones from the microdissection library should adequately cover most of the exons and can isolate many cDNA clones from the dissected region.

Table 8. Summary of the characteristics of 7 microdissection libraries constructed in this laboratory

Library Designation	Chromosome Region	Library Size	Average Insert Length (bp)	% Clones from Region	Disease Mapped
21E	Ch. 21	700,000	416	85	Down syndrome
2Q1	2q35-q37	20,000	270	54	Developmental anomalies
2Q2	2q33-q35	300,000	200	63	Juvenile amyotrophic lateral sclerosis
2P1	2p23-p25	300,000	200	58	Growth, mental retardation
2P2	2p21-p23	900,000	250	56	Holoprosencephaly
2P3	2p14-p16	1,000,000	250	83	Hereditary colorectal cancer
2P4	2p11-p13	600,000	380	70	

11. Isolation and characterization of cDNA clones from the 21E library.

(a) Screening of a λ gt11 human liver cDNA library

We have conducted a pilot study to use pooled unique sequence microclones for direct screening of a human liver λ gt11 cDNA library. Inserts from unique sequence microclones identified and isolated from our chromosome 21 microdissection library 21E were individually amplified and pooled, 10 or 20 clones in a group, to prepare labeled probes. Phage from the cDNA library were inoculated at a density of 50,000 pfu per

probes. Phage from the cDNA library were inoculated at a density of 50,000 pfu per 150mm dish. One to two dishes were screened for each group of pooled microclones. Duplicate filters were usually prepared from each dish and used in hybridization to confirm positive signals. Labeled probes at a concentration of 2×10^6 cpm/ml were added to each hybridization bag and hybridized at 42°C for 16 h. After hybridization and washing, the X-ray film was exposed for 3-5 days. In the primary screening, positive areas identified on duplicate filters were isolated and plaque purified. Table 9 presents the results of screening a human liver cDNA library with 200 unique sequence microclones and 2 cDNA clones, 21E-C1 and 21E-C2, were isolated.

Table 9. Screening of a human liver cDNA library using 200 unique sequence microclones derived from the chromosome 21 microdissection library.

No. Clones Clone Pooled	No. Positive Clones per Plate	cDNA Identified
10	0,0 ^a	
10	0,0	
10	1,0	21E-C1
10	0,0	
10	0,0	
10	0,0	
10	0,0	
10	0,0	
10	0,0	
10	0,0	
20	0 ^b	
20	0	
20	1	21E-C2
20	0	
20	0	

^a Representing number of positive cDNA clones isolated per plate; 2 plates screened.

^b 1 plate screened.

Preliminary characterization of cDNA clones 21E-C1 and 21E-C2 showed that they contain 1.5 kb and 1.0 kb insert respectively. Regional mapping using a panel of cell hybrids from Dr. Patterson assigned both cDNA clones to 21q22.1. We partially sequenced the clones and searched in the GenBank. No homologous sequences were found in the GenBank, suggesting that the two cDNA clones are likely new expressed gene sequences on chromosome 21. Zoo blot analysis carried out with 21E-C1 showed that it has strong homology with monkey, weak homology with rabbit, rat and Chinese hamster, but no homology with mouse.

(b) Screening of a "normalized" human thymus λ gt10 cDNA library.

We extended the screening using a "normalized" human thymus λ gt10 cDNA library. The library was kindly provided by Drs. Patanjali Sankhavaram and Sherman Weissman (8). Inserts from the microclones of the chromosome 21 microdissection library 21E were amplified and pooled, 20 in a group, to screen 100,000 phage from the cDNA library. After primary screening, positive plaques were isolated and purified. The results are summarized in Table 10.

Table 10. Screening of a "Normalized" Human Thymus λ gt10 cDNA Library (Random Priming) Using Pools of Unique Sequence Microclones from A Human Chromosome 21 Microdissection Library

Group No.	No. Clones Pooled	cDNA Clone Identified	Frequency (per 10 ⁵ phage)	Insert Size
A5	20			
A28	20			
A29	20			
A30	20	21E-C6*	4	370 bp
A31	20			
A32	20			
A34	20			
A35	20			
A38	20			
A39	20	21E-C3	1	350 bp
A40	20			
A41	20			
A44	20			
A47	20			
A50	20			
A52	20	21E-C4	15	610 bp
A53	20			
A54	20			
A55	20	21E-C5	4	1,200 bp
A56	20			
Total	400		4	

*Gene family with 10-15 members in the human genome and possibly 1-3 copies on chromosome 21

After screening with 400 unique sequence microclones, 4 cDNA clones were identified: 21E-C3, 21E-C4, 21E-C5 and 21E-C6. All of the cDNA clones hybridized to chromosome 21 genomic DNA to yield a single HindIII band, except 21E-C6 which

appears to be a member of a gene family with 10-15 copies in the human genome and possibly 1-3 copies on chromosome 21.

We used a refined regional mapping panel from Dr. Patterson to localize these cDNA clones on chromosome 21. This mapping panel comprises 24 cell hybrids and divides the chromosome into 32 subregions (6 subregions tentative: #4, 5, 10, 11, 29, 30): Subregions #1-6 in p13-p11.1; subregions #7-11 in q11.1-q11.2; subregions #12-14 in q21; subregions #15-23 in q22.1; subregions #24-27 in q22.2; and subregions #28-32 in q22.3. Using this mapping panel, we mapped cDNA clones 21E-C3, 21E-C4 and 21E-C5, and also 21E-C1 from the previous study, to the following regions:

(i) 21E-C1: subregion #22, a distal region of 21q22.1, defined by hybrids 6918 and 3x2S.

(ii) 21E-C3: subregion #25, a proximal region of 21q22.2, defined by hybrids 3x2S, 1x18 and 8q-.

(iii) 21E-C4: subregion #23, a very distal region of 21q22.1 near the border between 21q22.1 and 21q22.2, defined by hybrids 3x2S, 1x18 and 21q+.

(iv) 21E-C5: subregion #30, midway in band 21q22.3, defined by hybrids 4x1 and 21:22.

Zoo blot analysis showed that 21E-C3 cross-hybridized strongly to monkey, less strongly to horse, weakly to bovine and sheep, and no hybridization to rabbit, rat, Chinese hamster and mouse. 21E-C4 showed strong cross-hybridization with monkey, and weak hybridization with bovine and sheep, and no hybridization with horse, rabbit, rat, Chinese hamster and mouse.

21E-C6 showed strong cross-hybridization with monkey, less strong hybridization with Chinese hamster and mouse, weak hybridization with bovine, sheep, rabbit and rat. This cDNA clones even cross hybridized to *Drosophila* and yeast.

(c) Screening of a composite cDNA library pooled from 8 different cDNA libraries.

We extended the screening of cDNA libraries using 400 additional unique sequence microclones from chromosome 21 microdissection library. In addition, we used a composite cDNA library pooled from 8 different cDNA libraries constructed from various human tissues and in different developmental stages. These libraries were prepared by Dr. Patanjali Sankhavaram in Dr. Weissman's lab at Yale University. These libraries include total fetus (9 weeks), fetal brain and spinal cord (11-12 weeks), adult brain (cerebellum), adult testes, thyroid, adrenal, thymus and liver. All the libraries were made in λ gt10 vector. The libraries contain short fragments of cDNAs because the corresponding poly A+ RNA was primed with random hexanucleotides (Pharmacia). The advantages for the short fragment cDNA libraries are two-fold: (a) cDNA fragments for any given mRNA would arise from all along the length of the RNA, and (b) the average length of these fragments is 300 to 2,000 bp, an ideal size range for PCR amplifications. The libraries were made with at least 3×10^6 pfu while brain and fetal libraries were made with $7-10 \times 10^6$ pfu.

We obtained these 8 cDNA libraries (1 ml each) from Dr. Sankhavaram in the following actual titers: adult brain 1.5×10^9 pfu/ml, adult thyroid 5.8×10^{10} pfu/ml, adult adrenal 2.0×10^{10} pfu/ml, adult testes 4.2×10^{10} pfu/ml, adult liver 1.0×10^{10} pfu/ml, adult thymus 1.1×10^{11} pfu/ml, fetal brain 8.0×10^8 pfu/ml, total fetus 6.0×10^{10} pfu/ml. For use in our screening, we pooled 1.0×10^8 pfu from each library and inoculated 100,000 phage in 150 mm dish, 2 dishes per screening.

We used additional 400 unique sequence microclones from the 21E library and the inserts from the clones were individually amplified and pooled, 20 in a group, to prepare labeled probes for screening. Table 11 presents the results.

Table 11. Screening of a pool of 8 cDNA libraries using groups of unique sequence microclones from a human chromosome 21 microdissection library

Group N.	No. Clones in a Group	cDNA Clone identified	Frequency (per 2×10^5 phage)	Insert Size
M11	20	21E-C7	1	1,350 bp
M12	20			
M14	20			
M15	20			
M19	20			
M20	20			
M21	20			
M24	20			
M34	20			
M46	20			
M61	20	21E-C8	5	280 bp
		21E-C9*	1	600 bp
		21E-C10	1	180 bp
M62	20			
M72	20			
M74	20			
M81	20			
M94	20			
M97	20			
M10	20			
M11	20			
M11	20			
Total	400		4	

*Gene family with 10-15 members in the human genome and one copy on chromosome 21

After screening with 400 microclones, 4 cDNA clones were isolated, 21E-C7, 21E-C8, 21E-C9 and 21E-C10. All these cDNA clones hybridized to DNAs from human and chromosome 21 and yielded a single HindIII band, except 21E-C9 which appears to be

After screening with 400 microclones, 4 cDNA clones were isolated, 21E-C7, 21E-C8, 21E-C9 and 21E-C10. All these cDNA clones hybridized to DNAs from human and chromosome 21 and yielded a single HindIII band, except 21E-C9 which appears to be a member of a gene family with 10-15 copies in the human genome and 1 copy on chromosome 21. Zoo blot analysis was performed for 21E-C7. It cross-hybridized to all 8 species tested: strong signal for monkey, less strong signal to bovine, sheep, horse, rabbit, rat, Chinese hamster and mouse.

Regional mapping using the above refined mapping panel assigned 21E-C7 to subregion 22, 21E-C8 to subregion 2, 21E-C10 to subregion 27. The refined regional mapping of the cDNA clones is particularly useful as candidate genes for the various component phenotypes of Down syndrome. By using partial trisomy of chromosome 21, the complex Down syndrome can be subdivided into various component phenotypes corresponding to a segment of the chromosome. Thus, the cDNA clones assigned to a refined region can be analyzed specifically for the phenotypic anomaly residing in that region.

Currently, the cDNA clones are being studied for their tissue expression specificity using Northern blots containing RNAs from various human tissues. The clones are also sequenced for search in the GenBank to identify new expressed genes and for possible homologies with known functional domains. For a dissected region of 10 mb, there may be 30-50 expressed genes present in various cDNA libraries. Using the above direct screening procedure, it may be feasible to isolate 30-50 cDNA clones from a microdissection library of the region. However, we are also developing another procedure to improve the yield and efficiency of cDNA clones from the library, as described below.

C. Distribution of the libraries and unique sequence microclones derived from each library.

All the region-specific libraries and unique sequence microclones derived from each library are freely available to investigators involved in genome, genetic or any other biomedical research. The library is stored in the form of PCR products amplified from the original library. Upon receiving the DNA, the investigator can use it to construct a plasmid library of any size he/she desires. In general, only a subset of the library is needed for many studies, e.g. in screening for microsatellites or for YAC clones. Moreover, all the inserts in the library are MboI-cleaved genomic DNA with the MboI linker-adaptor at both ends and can be amplified by PCR using the 20-nt adaptor sequence as primer (refer to Kao and Yu, PNAS 98, 1844-1848, 1991). After purification, the inserts can be labeled by primer extension or cloned into the BamHI site of pUC19 after cleavage with MboI and ligated to the plasmid vector for transformation. The library can also be amplified to large quantities for use in chromosome painting by FISH.

In addition, we have 20-40 unique sequence microclones isolated from each library which have been well characterized for immediate use, such as screening in YAC or cosmid libraries for larger inserts. The microclones are usually stored in the following forms and can be sent to investigators according to their needs:

(a) Bacterial lysates containing microclones ready for increase or for sequencing.

(b) Inserts amplified from microclones ready for labeling by primer extension.

We have sent the 2q35-q37 library and 20 microclones to Dr. Peiter de Jong, formerly LLNL, as prepared in the above protocol. We have also sent microclones from the 2p21-p23 library to Dr. Max Muenke, Children's Hospital in Philadelphia, in both bacterial lysates and pure inserts. More recently, we have sent the 2p14-p16 library to Dr. Bert Vogelstein, John Hopkins University, for his studies with hereditary colorectal cancer. They have used this library to screen for microsatellite probes from the 2p14-p16 region in order to narrow the linkage to a smaller region to facilitate the gene cloning. We have also sent to Dr. Vogelstein 60 of our well-characterized unique sequence microclones from the 2p14-p16 library for his immediate use in screening YAC libraries to isolate corresponding YAC clones with large inserts for genomic mapping and contig construction. We have sent to Dr. Anthony Carrano, Director, LLNL Genome Center, a summary of our recent progress on the construction and characterization of all the region-specific libraries on chromosome 2, and indicated that all these libraries and the unique sequence microclones from each library are available for their use in the genome center at LLNL.

We have begun to deposit our microdissection libraries to the American Type Culture Collection (ATCC) for general distribution. Dr. William Nierman, Head, Department of Molecular Biology at ATCC, has expressed interest in maintaining and distributing our region-specific microdissection libraries. As a start, we have sent the 2q35-q37 library for their use. If this arrangement works satisfactorily to the scientific community, we will deposit all of our microdissection libraries in the ATCC for maintenance and distribution. In this way, it will not only relieve us from the demands of maintaining and sending out libraries to investigators, but also increase the public aware of the availability of such libraries since they will be printed in the ATCC catalogs. However, we will still maintain and distribute individual microclones to interested investigators for their special use, unless ATCC is also interested in maintaining and distributing these clones.

In order to inform the scientific community about the availability of these libraries, we have pursued the following routes:

(a) Publication in journals.

(b) Report to national and international meetings and publish as abstracts.

(c) Report to individual chromosome meetings for the libraries constructed in that chromosome. For chromosome 2 libraries, we have attended the first and second international workshops for chromosome 2 to report the construction and characterization of several region-specific libraries for this chromosome and they are available for distribution.

A good example is the availability of our 2p14-p16 library for use in cloning the gene for hereditary colorectal cancer. Right after the discovery of the location of this gene on the short arm of chromosome 2, region 2p15-p16, Dr. Bert Vogelstein contacted us about the use of this library and we were able to supply the library and the characterized

microclones from the library for his use. This has greatly facilitated the efforts in cloning of this devastating disease gene.

D. Future plans

During the past 3 years, the technology has been fully developed and has been applied to the construction of 6 region-specific libraries for chromosome 2. Now, we believe that the technology is ready for large scale application to provide region-specific libraries for many important parts of the human genome as valuable resources for genome mapping and sequencing, and to facilitate the cloning of genes underlying devastating diseases and specific forms of cancer.

We have applied for renewal of the present grant and have received approval for additional 3 years, starting 3/1/94 to 2/28/97. In the renewal application, we propose to complete the construction of all 11 region-specific libraries for the entire chromosome 2. This chromosome is one of the under-studied human chromosomes with many large gaps to fill in, particularly for the high resolution physical mapping purposes. We also propose to construct region-specific libraries for 20 unique regions of the human genome highly enriched with genes and displaying high transcriptional and recombinational activities. These libraries should contain large portions of human genes and can be used for mapping and studying human expressed sequences. In addition, we propose to construct as many libraries as possible for the regions where specific diseases have been assigned. These libraries will facilitate cloning of genes for many devastating diseases. Finally, we propose to develop methodology to construct region-specific cDNA libraries and to apply it to the regions mentioned above. These cDNA libraries should provide valuable candidate genes for cloning disease-related genes assigned to the region.

We believe that the microdissection/microcloning technology is highly suitable for achieving these objectives. Although well-studied regions or chromosomes in the human genome may not need this technology, there remain regions in the human genome where large gaps exist and no relevant cell hybrids are available. Microdissection aiming at these regions is ideally suited because it can be targeted anywhere in the genome and no selectable marker is needed. This is particularly significant when an important disease is mapped to a chromosomal region deficient with probes, and microsatellite markers are urgently needed to narrow down the linkage to facilitate cloning. One good recent example of our microdissection effort has been the quick response to the urgent call for probes in the 2p14-p16 region for linkage analysis and the cloning of the gene for the hereditary colorectal cancer. This disease phenotype has recently been localized to the 2p15-p16 region by Drs. Bert Vogelstein and Albert de la Chapelle and their collaborators, and reported in the 7 May 1993 issue of Science (Vol. 260, pp. 810-812 and 812-816, 1993). However, this region needs more polymorphic markers to further narrow the linkage and additional useful probes to isolate YAC clones from the critical region for cloning the gene. Dr. Vogelstein contacted us and we immediately sent him our 2p14-p16 library for his use in isolating microsatellite markers for linkage analysis. In addition, we sent him 60 of our well-characterized unique sequence microclones from the library for his immediate use in screening YAC libraries to isolate corresponding YAC clones with large inserts for high resolution physical mapping, contig construction, and the eventual cloning of the gene.

Our effort, through the DOE support, has been very gratifying because we feel that we have made an important contribution to facilitate the cloning of the gene responsible for hereditary colorectal cancer, a disease which is one of the most, if not the most, common hereditary diseases in humans, causing 55,000 deaths annually in this country alone. One in every 200 persons carries this defective gene, more than 10 times higher than the incidence of cystic fibrosis. We would like to provide more of our region-specific libraries in situations like the colon cancer case as we progress in the coming years.

All of the region-specific libraries for chromosome 2 are available to LLNL for their genome, mapping and sequencing studies for this chromosome (refer Appendix letter to Dr. Anthony Carrano). In a pilot study, Dr. Peiter de Jong (formerly of LLNL) used unique sequence microclones which we isolated from the 2Q1 (2q35-q37) library, to screen a BAC (bacterial artificial chromosome) library from Dr. Melvin Simon and was able to isolate corresponding BAC clones from the library. The results were reported in the recent DOE Genome Workshop in Santa Fe in February 1993 .

E. Publications during the entire grant period (including research articles* and abstracts to national and international meetings)

- 1.* Kao, F.T. and Yu, J.W. (1991) Chromosome microdissection and cloning in human genome and genetic disease analysis. Proc. Natl. Acad. Sci. USA 88, 1844-1848.
2. Kao, F.T. and Yu, J.W. (1991) Chromosome region-specific libraries for human genome analysis. Abstract presented in the DOE Human Genome Workshop for Grantees and Contractors, Feb. 17-19, 1991, Santa Fe, N.M.
3. Mendez, M.J., Yu, J., Kao, F.T., Klapholz, S., Brownstein, B.H. and Gemmill, R.M. (1991) Rapid screening of a YAC library by pulsed field gel Southern blot analysis of pooled YAC clones. Abstract presented in the Cold Spring Harbor Meeting on "Genome Mapping and Sequencing", May 8-12, 1991, Cold Spring Harbor Laboratory, Long Island, N.Y.
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CHROMOSOME REGION-SPECIFIC LIBRARIES FOR HUMAN GENOME ANALYSIS

Fa-Ten Kao and Jing-Wei Yu

Eleanor Roosevelt Institute for Cancer Research, and Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80206 (303) 333-4515, FTS (303) 333-8423

Molecular analysis and fine structure mapping of the human genome require isolation of large numbers of DNA probes from defined regions of the chromosomes. A direct approach is to use chromosome microdissection to remove physically the chromosomal region of interest and to clone the minute dissected material by microcloning. We propose to develop expertise in this microtechnology and to apply it to the construction of region-specific libraries for human genome analysis.

In our initial studies, 30 chromosomes of human chromosome 21 were microdissected using de Fonbrune micromanipulator. The dissected chromosomes were collected in a microdrop, treated with proteinase K, extracted with phenol, cleaved with MboI, and ligated to MboI linker-adaptor. All these steps were performed in nanoliter volumes under the microscope. The ligated material was amplified by PCR and cloned into pUC19. Highly stringent precautions were enforced to minimize extraneous DNA unintentionally introduced into these procedures. After cloning, 700,000 recombinant microclones were obtained. Colony hybridization showed that 42% of the clones contained repetitive sequences and 58% contained unique sequences. The insert sizes ranged from 50 to 1100 bp (mean 416 bp). Southern blot analysis confirmed that these microclones are of human origin and chromosome 21 specific. In addition, we used 4 microclones containing unique sequences to screen the human YAC library from Washington U. and identified 5 positive YAC clones with inserts ranging between 50-360 kb. This indicates that it is feasible to use microclones with very small inserts to expand to a large genomic region for molecular genome analysis and for contig construction. Furthermore, the small insert size in the microclones is convenient for sequencing to identify STS as genomic landmarks. Finally, we used unique sequence microclones to screen a human liver cDNA library and isolated at least 4 expressed gene sequences among 100 microclones so far tested.

Physical mapping and sequencing of the human genome requires development of many new molecular approaches and methodologies. The chromosome microtechnology described here appears to offer an additional powerful methodology to achieve these goals. We will further develop expertise in this technology and to apply it to human chromosomes 2 and 5 to construct various region-specific libraries for use in physical mapping by the DOE National Laboratories. If it proves promising, applications to other specific chromosomes will be warranted.

Abstract presented in the DOE Human Genome Workshop for Grantees and Contractors, Feb. 17-19, 1991, Santa Fe, NM

RAPID SCREENING OF A YAC LIBRARY BY PULSED FIELD GEL SOUTHERN BLOT ANALYSIS OF POOLED YAC CLONES

M. J. Mendez¹, J. Yu¹, F. T. Kao¹, S. Klapholz², B. H. Brownstein³ and R. M. Gemmill¹. ¹Eleanor Roosevelt Institute, Denver, CO; ²Cell Genesys, Inc., Foster City, CA; ³Center for Genetics in Medicine, Washington Univ. School of Medicine, St. Louis, MO.

We describe an alternative method for YAC library screening that is both rapid and low-cost. Individual YACs were pooled into groups of 384 clones and prepared as samples suitable for pulsed field gel electrophoresis. A five-hit human YAC library containing approximately 60,000 clones was condensed into 150 such pools. Chromosomal DNAs in each sample were separated on three pulsed field gels containing 50 samples each. Resulting Southern blots were hybridized with probes of interest to identify pools containing homologous YACs. Further purification was performed using standard colony hybridization procedures. Twenty four probes used thus far have identified 60 positive pools and corresponding YACs have been purified from 36 of these. Isolated YACs ranged from 50 to over 1300 kb in size. Useful probes derived from plasmid and phage clones or PCR amplified sequences ranged from under 500 bp to over 5 kb. Moreover, probes derived from microdissection have been pooled into small groups and used to rapidly identify collections of YACs derived from one chromosomal region. Some significant advantages of this method include avoidance of DNA sequence analysis and primer generation prior to YAC screening and the ability to handle the entire library on three filters. PFG separation provides an array of additional information, such as size estimation, and allows targeted isolation. The screening approach described here permits rapid isolation of YACs corresponding to un-sequenced loci and will accelerate establishment of YAC contigs for large chromosomal segments.

Abstract presented in the Cold Spring Harbor Meeting on "Genome Mapping and Sequencing", May 8-12, 1991, Cold Spring Harbor Laboratory, Long Island, NY.

ISOLATION AND MAPPING OF MICRODISSECTION CLONES AND EXPRESSED GENE SEQUENCES FROM HUMAN CHROMOSOME 21

F. Kao, J. Hartz, Y. Xu, D. Patterson, J. Yu
Eleanor Roosevelt Institute for Cancer Research
1899 Gaylord Street, Denver, Colorado 80206 USA

Human genome analysis and search for genetic disease related genes require efficient methods for isolation of large numbers of probes from defined regions of the chromosomes. A direct approach is to physically remove the chromosomal region of interest by microdissection and clone the dissected material using microcloning. Universal amplification of the minute dissected sequences before cloning has proved invaluable in this approach. In order to study Down syndrome, we constructed a microdissection library using microdissected whole human chromosome 21 amplified by the MboI linker-adaptor procedure. The dissected chromosomes were treated with proteinase K, extracted with phenol, cleaved with MboI, ligated to a linker-adaptor, amplified by PCR, and cloned into pUC19. A library comprising 700,000 microclones was constructed (1). About 60% of the microclones in the library contained unique sequence inserts with a mean length of 416 bp. The microclones were confirmed of human origin and chromosome 21 specific. Regional mapping using a hybrid mapping panel of chromosome 21 assigned 5 microclones to the following locations: 1 clone containing 600 bp insert to 21q21.3; 3 clones containing inserts of 280 bp, 300 bp and 380 bp respectively, to 21q22.1-q22.2; 1 clone containing 560 bp insert to 21q22.3. Regional assignment of additional microclones from the library is in progress to determine the general distribution of unique sequence microclones on chromosome 21. We are also constructing region-specific libraries of chromosome 21 to facilitate isolation of clones from defined regions of the chromosome.

In addition, we used unique sequence clones from the microdissection library to screen a human liver cDNA library for isolating expressed sequences from chromosome 21. The insert from each microclone was individually amplified and 10 amplified inserts were pooled to prepare labeled probes for blot hybridization. After screening the cDNA library with 100 unique sequence clones, at least 3 cDNA clones were identified. One cDNA clone, 21E-C1, containing an insert of 1.5 kb was regionally mapped close to 21q21.3-q22.1 boundary, a region in which the beta-amyloid precursor protein gene was assigned. This cDNA clone is likely representing a relatively rare message in the library because only one positive clone was identified in 100,000 cDNA clones screened. The other two cDNA clones, 21E-C2 and 21E-C3, presumably represent more abundant cDNA species since 10 and 28 positive clones were respectively found in a similar screening. Further isolation, characterization, regional mapping, as well as sequencing of cDNA clones from chromosome 21 are in progress. Since the number of genes assigned to chromosome 21 is still limited, it is important to isolate as many cDNA clones as possible from this chromosome for an efficient search for the genes responsible for Down syndrome.

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Abstract presented in the Eleventh International Workshop on Human Gene Mapping (HGM11), August 18-22, 1991, London, UK.

ISOLATION AND MAPPING OF EXPRESSED SEQUENCES OF HUMAN CHROMOSOME 21 USING MICRODISSECTION CLONES

Fa-Ten Kao

Eleanor Roosevelt Institute for Cancer Research, and Department of Biochemistry, Biophysics and Genetics, University of Colorado Health sciences Center, Denver, CO 80206

Human chromosome 21 is important because it carries genes responsible for Down syndrome. We constructed a large library of chromosome 21 comprising 700,000 recombinant plasmid clones using microdissection and PCR microcloning procedures (ref. PNAS 88, 1844-1848, 1991). These microclones contain an average insert size of 400 bp and about 60% of the microclones are devoid of repetitive sequences. Thus, large numbers of unique sequence microclones can be readily isolated for use in direct screening of cDNA libraries.

In our screening procedure, the unique sequence inserts in the microclones were amplified by PCR and pooled, 10 or 20 in a group, to prepare labeled probes. After screening 100,000 phage from a human liver λ gt11 cDNA library using 200 unique sequence microclones, 5 different cDNA clones were identified and plaque purified. One of the cDNA clones, 21E-C1, was shown to be derived from microclone 21E-181. This cDNA clone contains an insert of 1.5 kb and was regionally localized to 21q21.3.

In addition, we used the same 200 unique sequence microclones to screen a normalized human thymus cDNA library, kindly provided by Drs. Patanjali and Weissman (PNAS 88, 1943-1947, 1991). In these screens, we identified the same 5 cDNA clones as found in the previous library, but occurring at higher frequencies. We also identified 2 new cDNA clones not found in the previous library.

The direct screening of cDNA libraries using unique sequence microclones appears to be simple and efficient. We estimate that for a microdissected region of 10 mb, it is entirely practical to use this procedure to isolate most of the cDNA clones from the dissected region that are present in the cDNA library. It also appears feasible to use this approach to isolate a majority of the cDNA clones even for the entire chromosome 21.

Abstract presented in the International Workshop on "Identification of Transcribed Sequences in the Human Genome", October 4-5, 1991, Bethesda, Maryland.

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Chromosome microdissection and PCR microcloning in human genetic disease analysis. J. Yu*, J. Hartz, Y. Xu and F. Kao. Eleanor Roosevelt Institute for Cancer Research and University of Colorado Health Sciences Center, Denver, CO

Efficient isolation of genes underlying genetic diseases requires large numbers of DNA probes from defined regions of the chromosomes. A direct method is to physically remove the chromosomal region of interest by microdissection and to clone the dissected material by microcloning. We have developed a procedure for chromosome microdissection and microcloning and applied to human chromosomes 21 and 12. Microdissected chromosome fragments were cleaved with either MboI or Sau3A, ligated to a linker-adaptor for universal amplification, and cloned into plasmid pUC19. Large libraries comprising tens of thousands of recombinant microclones were obtained. The clones were shown to originate from the dissected region. For study of Down syndrome, we used 100 unique sequence microclones from a microdissected whole chromosome 21 library to screen a human liver cDNA library and identified 4 cDNA clones. Similar strategy and techniques can be applied to other chromosomal regions to which genetic disease loci have been assigned.

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ABSTRACT

**ISOLATION AND REGIONAL MAPPING OF CHROMOSOME 21
MICRODISSECTION CLONES AND cDNA CLONES**

Kao, FT, Hartz J, Xu Y, Gemmill RM, *Korenberg JR, Patterson D, Yu J.
Eleanor Roosevelt Institute for Cancer Research, & University of Colorado Health Sciences
Center, Denver, CO; *Cedars-Sinai Medical Center, UCLA, Los Angeles, CA.

Thirty-four unique sequence microclones were isolated from a previously described microdissection library of human Chromosome 21 (Kao FT & Yu J, PNAS 88:1844, 1991). The microclones have been regionally mapped using a cell hybrid mapping panel which consists of 6 cell hybrids and divides Chromosome 21 into 8 regions. These hybrids include 153E7b (21q), R50-3 (t(6;21)), 21q+ (t(8;21)), GA9-3 (t(4;21)), 1881C-13b (t(1;21)), and JC-6. The mapping results showed that the microclones were unevenly distributed along Chromosome 21, with the majority of microclones located in the distal half of the long arm between 21q21.3 and 21qter. The number of unique sequence clones began to decrease significantly from 21q21.2 to centromere and extending to the short arm. Nearly 90% (30 out of 34) of the clones were clustered in approximately 60% of the long arm. This finding is consistent with those reported in other Chromosome 21 libraries. Thus, it may be inferred that the proximal portion of the long arm of Chromosome 21 contains higher proportions of repetitive sequences than unique sequences or genes. However, not the entire Giemsa dark band 21q21 is less represented by unique sequences. A significant portion of this dark band, including 21q21.3, appears to contain unique sequences as abundant as the Giemsa light band region 21q22. Thus, one should not be tempted to generalize the composition of any Giemsa dark band in the human genome as being a region enriched with repetitive sequences. The microclones have been characterized for insert size and used to identify the corresponding genomic fragments generated by HindIII. In addition, we have demonstrated that the microclones with short inserts can be efficiently used to screen YAC libraries to identify YAC clones with large inserts for increased genomic coverage. We have also used 200 unique sequence microclones to screen a human liver cDNA library and identified two cDNA clones which have been regionally assigned to 21q21.3-q22.1. Partial sequencing of the cDNAs revealed that they bear no homology with any reported sequences in the GenBank, indicating that they are likely new cDNA clones in Chromosome 21. Thus, the microdissection libraries are useful in generating large numbers of unique sequence microclones for regional mapping on Chromosome 21 and for isolating region-specific YAC clones and cDNA clones for high resolution physical mapping and for efficient search of genes responsible for the diseases on Chromosome 21.

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CHROMOSOME MICRODISSECTION AND PCR MICROCLONING IN GENETIC DISEASE DISSECTION. Fa-Tan Kao, Eleanor Roosevelt Institute for Cancer Research, and Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80206

The human genome contains approximately 50,000-100,000 genes of which over 3,000 have been identified. The rapid progress in human gene mapping has assigned more than 2,300 genes and 7,000 DNA fragments to specific regions of the human chromosomes. Due to the effective use of polymorphic DNA markers in genetic linkage analysis, more and more genetic diseases and specific forms of cancer have been mapped to defined regions of the genome. The next major task is to isolate the crucial genes underlying these diseases for better understanding, diagnosis, prevention and treatment of the disease. A direct approach to get access to the specific regions of the chromosome and to isolate large numbers of probes from the region is by physical dissection of the chromosomal region of interest followed by a microcloning technique to clone the dissected DNA sequences into phage or plasmid vectors. Recently, we have developed an efficient method for microdissection and microcloning of human chromosome fragments to construct large genomic libraries for molecular genetic and genome analysis (PNAS 88, 1844-1848, 1991). Generally, 20-30 chromosome fragments are dissected from metaphase spreads and collected in a microdrop. The fragments are treated with proteinase K, extracted with phenol, cleaved with MboI and ligated to an MboI linker-adaptor. All these steps are performed under the microscope in nanoliter volumes. After ligation, the sample is transferred to Eppendorf tube for PCR amplification and the PCR products are cloned into pUC19. The libraries usually contain tens of thousands of microclones and have been shown to derive from the dissected chromosome region. We have constructed microdissection libraries for human chromosome 21 and the proximal long arm of chromosome 21 for the molecular analysis of Down syndrome. In addition, we constructed a library for chromosome 12q12-q15 to facilitate molecular studies of several soft tissue liposarcomas mapped to this region. We also constructed a library for chromosome 2q35-q37 for the analysis of Waardenburg syndrome residing in the region. These microclones can be efficiently converted to YAC clones with large inserts for fine structure physical mapping and contig construction of the dissected region. We have also used the microclones containing unique sequence inserts to isolate region-specific cDNA clones as candidate genes for the diseases assigned to the region. This microtechnology appears powerful in isolating large numbers of molecular probes from any defined regions of the genome to facilitate high resolution physical mapping and for the search of genes responsible for specific genetic diseases.

Abstract presented in the Symposium of the 4th International Symposium and Workshop of the Society of Chinese Bioscientists in America (SCBA), June 14-19, 1992, National University of Singapore, Singapore

CHROMOSOME MICRODISSECTION AND MICROCLONING IN HUMAN MOLECULAR GENETICS

Fa-Ten Kao

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High resolution physical mapping of specific chromosomal regions and cloning of disease-related genes from a genomic region require large numbers of DNA probes from the region of interest. A direct approach is to use a microdissection technique to physically remove the crucial chromosomal region followed by microcloning of the dissected DNA sequences. We have developed an efficient method for microdissection and PCR-mediated microcloning of human chromosome fragments to construct large region- or band-specific genomic libraries for molecular genetic and genome analysis (PNAS 88, 1844-1848, 1991). In this procedure, 20 chromosome fragments are dissected, digested with proteinase K, extracted with phenol, cleaved with MboI and ligated to an MboI linker-adaptor. All these steps are performed under the microscope in nanoliter volumes. After ligation, the sample is transferred to Eppendorf tube for PCR amplification and the PCR products are cloned into pUC19. The libraries usually contain tens of thousands of microclones and have been shown to derive from the dissected region. We have constructed region-specific microdissection libraries to facilitate molecular genetic studies of the diseases assigned to these regions: (a) Chromosome 21 and 21q22 for studying Down syndrome. (b) 12q12-q14 for studying several soft tissue liposarcomas. (c) 2q35-q37 for studying alveolar rhabdomyosarcoma. (d) 2p21-p23 for studying holoprosencephaly, an abnormal embryonic development in forebrain and midface. (e) 17q21 for studying familial breast and ovarian cancer. As we showed previously (Am. J. Hum. Genet. 51, 263-272, 1992), the microclones can be used efficiently as probes to isolate (i) corresponding YAC clones with large inserts for fine structure physical mapping and contig construction of the dissected region, and (ii) region-specific cDNA clones as candidate genes for diseases assigned to the region. This microtechnology appears powerful in isolating large numbers of molecular probes from any defined regions of the genome for fine structure physical mapping and for the cloning of genes responsible for genetic diseases or specific forms of cancer.

(Abstract presented as Symposium Speaker in the International Conference on Molecular Biology of Genetic Diseases, October 4-7, 1992, Shanghai, People's Republic of China)

CONSTRUCTION AND CHARACTERIZATION OF A REGION-SPECIFIC LIBRARY FROM HUMAN CHROMOSOME 2q35-q37 USING MICRODISSECTION AND MICROCLONING TECHNIQUES

F. T. KAO, S. TONG, T. L. YANG-FENG* AND J. YU
ELEANOR ROOSEVELT INSTITUTE FOR CANCER RESEARCH, 1899 GAYLORD STREET, DENVER, CO 80206, AND *YALE UNIVERSITY, NEW HAVEN, CT

A region-specific library from human chromosome 2q35-q37 has been constructed using a previously described microdissection and PCR-mediated linker-adaptor microcloning method (Kao & Yu, PNAS 88, 1844-1848, 1991). Twenty fragments were microdissected from the region and the fragments were digested with proteinase K, extracted with phenol, cleaved with MboI, ligated to a linker-adaptor, amplified by PCR, and cloned into pUC19. A library containing 20,000 recombinant microclones was obtained and characterized in detail. The insert size in the microclones ranged between 50-800 bp, with a mean of 270 bp. About 50-60% of the microclones contained unique sequences. The library was demonstrated to derive from the region of dissection by chromosome painting using the fluorescent in situ hybridization (FISH) technique. Southern blot analysis of the unique sequence microclones from the library showed that 54% (26/48) of the clones are of human origin and chromosome 2 specific, displaying positive hybridization with cell hybrid GM10826B containing a single human chromosome 2. Four of these clones were further mapped to the 2q37 region by using cell hybrid GM11022 (Cell Repository, Camden, NJ) which contained only 2q37. The unique sequence microclones were characterized for insert size, and their hybridizing genomic fragments cleaved with HindIII were also determined. As we showed previously with the human chromosome 21 microdissection library (Yu et al., Am. J. Hum. Genet. 51, 263-272, 1992), large numbers of unique sequence microclones can be easily isolated from the library and used in (a) isolating corresponding yeast artificial chromosome (YAC) clones with large inserts for high resolution physical mapping, and (b) screening cDNA libraries to isolate region-specific cDNA clones as candidate genes of genetic diseases assigned to the region.

Abstract presented in the Second Human Chromosome 2 Workshop in San Francisco, California, November 6-8, 1992.

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Construction and characterization of region-specific microdissection libraries of human chromosome 2. F.T. Kao^{1,2}, S. Tong¹, T.L. Yang-Feng³ and J. Yu¹. ¹Eleanor Roosevelt Institute for Cancer Research; ²University of Colorado Health Sciences Center, Denver, CO; ³Yale University, New Haven, CT.

Four region-specific libraries from human chromosome 2 have been constructed using a previously described microdissection and PCR-assisted linker-adaptor microcloning method (Kao & Yu, PNAS 88, 1844-1848, 1991). These regions include 2q35-q37, 2q33-q35, 2p23-p25 and 2p21-p23. Twenty fragments were microdissected from each region and the dissected sequences were purified, cleaved with MboI, ligated to a linker-adaptor, amplified by PCR and cloned into pUC19. Libraries each containing 20,000-40,000 recombinant microclones were constructed. The 2q35-q37 library was characterized in detail. The insert size in the microclones ranged between 50-800 bp, with a mean of 273 bp. About 50-60% of the microclones contained unique sequences. The library was demonstrated to derive from the region of dissection by chromosome painting using the fluorescent in situ hybridization (FISH) technique. Southern blot analysis of 26 unique sequence microclones from the library confirmed their human origin and chromosome 2 specificity. Four of these microclones were further mapped to the 2q37 region using cell hybrid GM11022 (Cell Repository, Camden, NJ) which contained only 2q37. The unique sequence microclones were also characterized for their insert size and for the hybridizing genomic fragments cleaved with HindIII. As we showed previously with the human chromosome 21 microdissection library (Am. J. Hum. Genet. in press), large numbers of unique sequence microclones can be easily isolated from the library and used in (i) isolating corresponding yeast artificial chromosome (YAC) clones with large inserts for high resolution physical mapping, and (ii) screening cDNA libraries to isolate region-specific cDNA clones as candidate genes to facilitate search for the crucial genes underlying genetic diseases and specific forms of cancer assigned to the region. (Supported by DOE grant DE-FG02-91ER61139 and NIH grant HD17449)

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CHROMOSOME MICRODISSECTION AND PCR-MEDIATED MICROCLONING IN HUMAN GENOME AND GENETIC DISEASE ANALYSIS

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High resolution physical mapping and cloning of disease-related genes from specific chromosomal regions require large numbers of DNA probes from the region of interest. Among different strategies, a direct method is to use microdissection to physically remove the crucial chromosomal region, followed by microcloning of the dissected DNA sequences to construct a genomic library, as demonstrated first in *Drosophila* polytene chromosomes. We developed an efficient method for microdissection of human chromosomes followed by PCR amplification of the dissected sequences using MboI linker-adaptor (PNAS 88, 1844-1848, 1991). In this procedure, 20 fragments are dissected, digested with proteinase K, extracted with phenol, and ligated to an MboI linker-adaptor. All these steps are performed in nanoliter volumes under the microscope. The ligated sequences are transferred to an Eppendorf tube for PCR and the PCR products are cloned into pUC19. Large microdissection libraries have been constructed with tens to hundreds of thousands of microclones in each library. The inserts in the microclones averaged 300-400 bp and 50-60% of the clones contained unique sequences. The libraries have been shown to derive from the dissected regions by using FISH in chromosome painting.

We have constructed microdissection libraries for human chromosome 21, 21q21 and 21q22 for physical mapping and molecular analysis of Down syndrome. In addition, we have constructed 4 region-specific libraries for human chromosome 2, including 2q35-q37, 2q33-q35, 2p23-p25, and 2p21-p23 (Genomics, in press). Characterization of these libraries by Southern blot hybridization and the FISH analysis have shown that they are of good quality and can be used efficiently not only for fine structure physical mapping of these regions, but for the analysis and cloning of disease-related genes mapped to these regions, e.g. Alveolar rhabdomyosarcoma (a pediatric soft tissue tumor mapped to 2q35), aniridia-1 (a dominant disorder of the ocular abnormality mapped to 2p25), holoprosencephaly (an abnormal forebrain and midface development mapped to 2p21-p22), and others.

As we have demonstrated previously (Am. J. Hum. Genet. 51, 263-272, 1992), the microclones can be used efficiently as probes to isolate (i) corresponding YAC clones with large inserts for physical mapping and contig construction of the dissected region, and (ii) region-specific cDNA clones as candidate genes for diseases assigned to the region. This chromosome microtechnology will become even more important as the more accessible areas of the genome are characterized and leave some large gaps, and no relevant cell hybrids are available for those gap regions. Microdissection is ideally suited because it can be targeted anywhere in the genome and no selectable marker is needed.

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4^{ème} Réunion Internationale sur le Chromosome 21
4th International Workshop on Chromosome 21
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ABSTRACT

ISOLATION AND REGIONAL MAPPING OF 4 cDNA CLONES USING A CHROMOSOME 21 MICRODISSECTION LIBRARY AND A REFINED MAPPING PANEL

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Previously, we used 200 pUC19 recombinant microclones containing only single copy sequences from a chromosome 21 microdissection library to screen a λ gt11 human liver cDNA library and isolated 2 cDNA clones, 21E-C1 and 21E-C2. They were partially sequenced and shown to be new expressed genes on chromosome 21. Regional mapping using 6 cell hybrids assigned both clones to 21q22.1 (Yu J et al. Am. J. Hum. Genet. 51: 263, 1992). We now extended this work by using 400 additional unique copy microclones to screen a "normalized" λ gt10 thymus cDNA library (kindly provided by Drs. Patanjali Sankhavaram and Sherman Weissman). Inserts from the microclones were amplified and pooled, 20 in a group, to screen 100,000 phage from the cDNA library. After primary screening, positive plaques were isolated and purified. The following 4 cDNA clones were identified, 21E-C3, 21E-C4, 21E-C5 and 21E-C6, all of which hybridized to chromosome 21 DNA to yield a single HindIII band, except 21E-C6 which appears to be a member of a gene family with 10-15 copies in the human genome and possibly 3 copies on chromosome 21.

A refined regional mapping panel was used to localize these cDNA clones on chromosome 21. This mapping panel comprises 24 cell hybrids and divides the chromosome into 32 regions (6 regions tentative: #4,5,10,11,29,30): Regions #1-6 in p13-p11.1; regions #7-11 in q11.1-q11.2; regions #12-14 in q21; regions #15-23 in q22.1; regions #24-27 in q22.2; and regions #28-32 in q22.3. Using this mapping panel, the cDNAs were mapped to the following regions: (1) 21E-C1 to region #22, a distal region of 21q22.1, defined by hybrids 6918 and 3x2S. (2) 21E-C3 to region #25, a proximal region of 21q22.2, defined by hybrids 3x2S, 1x18 and 8q-. (3) 21E-C4 to region #23, a very distal region of 21q22.1 close to the border between 21q22.1 and 21q22.2, defined by hybrids 3x2S, 1x18 and 21q+. (4) 21E-C5 to region #30, midway in band 21q22.3, defined by hybrids 4x1 and 21;22. "Zoo" blot analysis showed that these cDNA clones displayed evolutionary conservation in some or all of the following species: monkey, bovine, sheep, horse, rabbit, rat, mouse and Chinese hamster. Northern analysis and sequencing of the cDNA clones are currently in progress. The continuing isolation, characterization and refined regional mapping of cDNA clones from chromosome 21 should provide additional needed candidate genes for the analysis of the various component phenotypes of Down syndrome and other diseases mapped on this chromosome.

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 ___ PFGE ___ disorders ___ mouse map ___ linkage ___ mutations

Isolation and refined regional mapping of cDNA clones using a chromosome 21 microdissection library and a hybrid mapping panel. ((F.T. Kao, J. Yu, S. Tong, J. Qi, D. Donaldson, S. Patanjali¹ and D. Patterson.)) Eleanor Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center, Denver, CO and ¹Yale University, New Haven, CT.

We used 400 pUC19 recombinant microclones containing single copy inserts from a chromosome 21 microdissection library to screen a "normalized" λ gt10 thymus cDNA library. Inserts from the microclones were amplified and pooled, 20 in a group, to screen 10^5 phage and 4 cDNA clones were identified, 21E-C3, 21E-C4, 21E-C5 and 21E-C6, all of which hybridized to chromosome 21 DNA to yield a single HindIII band, except 21E-C6 which is a member of a gene family. We extended this screening by using additional 400 unique copy microclones to screen a composite cDNA library pooled from 8 different cDNA libraries constructed from total fetus, fetal brain, adult brain, testes, thyroid, adrenal, thymus and liver. Another 4 cDNA clones were isolated: 21E-C7, 21E-C8, 21E-C9 and 21E-C10, of which 21E-C8 and 21E-C9 are members of gene families. A refined regional mapping panel was used to localize these cDNA clones on chromosome 21. This mapping panel comprises 24 cell hybrids and divides the chromosome into 32 regions. The cDNAs were mapped to the following regions: 21E-C3 very proximal 21q22.2; 21E-C4 very distal 21q22.1; 21E-C5 middle 21q22.3; 21E-C7 distal 21q22.1; 21E-C10 middle 21q22.2; and 21E-C1, a cDNA clone previously isolated from a human liver cDNA library using a similar procedure, to distal 21q22.1. The continuing isolation, characterization and refined regional mapping of cDNA clones from chromosome 21 should provide needed candidate genes for the analysis of the various component phenotypes of Down syndrome and other diseases mapped on this chromosome.

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