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The Region Surrounding The *PKD1* Gene: A 700-kb PI Contig from a YAC-deficient Interval

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As part of an effort to identify the gene responsible for the predominant form of polycystic kidney disease (*PKD1*), we used a gridded human PI library for contig assembly. The interval of interest, a 700-kb segment on chromosome 16p13.3, can be physically delineated by the genetic markers DI6SI25 and DI6S84 and chromosomally characterized as a GC-rich isochore enriched for CpG islands, genes, and Alu-like repeats. Our attempts to recover CEPH YACs that encode this region of chromosome 16 were unsuccessful. However, we screened an arrayed PI library using 15 distinct probes from the DI6SI25–DI6S84 interval and identified 56 independent PI clones. Only one probe from the interval was unsuccessful in identifying a PI clone. Forty-four PI clones were determined to be unique based on restriction enzyme analysis, and 42 of these were found to originate from chromosome 16p13.3, based on FISH to metaphase chromosomes. The 700-kb interval could be defined by a single sequence-ready contig comprised of 12 PI clones and 1 cosmid clone. Our studies support the use of multiple libraries to generate the requisite physical reagents for positional cloning and encourage the use of *Escherichia coli*-based large-insert cloning systems to recover clones from YAC-deficient chromosomal intervals.

The assembly of contiguous cloned genomic reagents is a necessary step in the process of disease-gene identification using a positional cloning approach. The rapid development of high-density genetic maps based on polymorphic simple sequence repeats has facilitated contig assembly using sequence-tagged site (STS) content mapping. Most contig construction efforts have relied on yeast artificial chromosomes (YACs), because their large insert size uses the current STS map density more advantageously than bacterially hosted systems. This approach has been validated for multiple human chromosomes with YAC coverage ranging from 65%–95% for many chromosomes and contigs of 11–36 Mb being described (Chumakov et al. 1995; Doggett et al. 1995; Gemmill et al. 1995; Krauter et al. 1995; Shimizu et al. 1995; van-Heyningen et al. 1995).

Even with numerous successes, the YAC cloning system is not a panacea for cloning the entire genome of complex organisms because of intrinsic limitations that result in substantial proportions of chimeric clones (Green et al. 1991; Bel-lanne-Chantelot et al. 1992; Nagaraja et al. 1994), as well as clones that are rearranged, deleted, or unstable (Neil et al. 1990; Wada et al. 1990; Zuo et al. 1992; Szepetowski et al. 1995). At least some of these cloned artifacts are a product of the recombinational machinery of yeast acting on the various types of repetitive elements in mammalian DNA (Neil et al. 1990; Green et al. 1991; Schlessinger et al. 1991; Ling et al. 1993; Koup-rina et al. 1994; Larionov et al. 1994). Alternative cloning systems must be used in concert with YAC-based approaches to complement localized YAC cloning deficiencies, to enhance the resolution of the physical map, and to provide a sequence-ready resource for genome-wide DNA sequencing.

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To meet this demand, several *Escherichia coli*-based cloning systems have been developed using vectors based on P1 phage (Sternberg 1990) and F-factor, termed bacterial artificial chromosomes or BACs (Shizuya et al. 1992). In each case, these cloning systems permit larger average insert size when compared with traditional genomic approaches using λ phage replacement vectors (15–22 kb) and cosmid vectors (30–45 kb). For example, the “head-full” packaging mechanism of P1 phage has been used to generate a three-genome equivalent human library with an average insert size of ~80 kb (Shepherd et al. 1994). The F-factor system has achieved even larger insert sizes. Using electroporation to introduce the products of ligation into *E. coli* allows generation of clones ≤ 300 kb (Shizuya et al. 1992). Similar results have been reported using the pCyPAC vector, a modified P1 phage vector, which selects for clones with inserts by inactivation of *SacBII* activity and relies on electroporation rather than transduction for introduction into bacteria (Ioannou et al. 1994).

Whereas clones with large inserts are beneficial, clone integrity and stability are paramount in generating an accurate high-resolution physical map and as a resource for genome-wide sequencing. Preliminary data suggest that of all large insert cloning vectors, these requirements have been satisfied most completely by both the P1- and F-factor-based genomic cloning systems. Their physical stability does not depend solely on insert size, as inserts of P1 recombinants (P1s) and F-factor recombinants (BACs) are intermediate to those from cosmids and YACs. This feature must be attributable in part to genes encoded by P1 and F-factor vectors that regulate and maintain one to two episomal copies per cell. Cosmid-derived inserts were shown to be more stably maintained in F-factor based vectors than cosmid vectors (Kim et al. 1992).

As part of an effort to identify the gene responsible for the predominant form of polycystic kidney disease (*PKD1*), we evaluated several human libraries for contig assembly including a P1 library. This 700-kb chromosomal segment serves as a challenging test for large-insert cloning systems in *E. coli* and yeast because it resides in a GC-rich isochores (Saccone et al. 1992) with an abundance of CpG islands (Harris et al. 1990; Germino et al. 1992), genes (Germino et al. 1993), and Alu repetitive sequences (Korenberg et al. 1988). Chromosome 16 also contains more low-copy repeats than other chromosomes with

almost 25% of its cosmid contigs hybridizing to more than one chromosomal location when analyzed by fluorescence in situ hybridization (FISH) (Okumura et al. 1994). These low-abundance repeats, several of which reside in the interval described in this paper, present a challenge to hybridization-based methods of contig construction because they are not blocked by pre-annealing with Cot1 DNA. Furthermore, low-copy repeats may lead to instability of this interval in bacteria, yeast, and higher eukaryotes. Consistent with this prediction is the finding that most cases of severe infantile PDK1 are attributable to chromosomal deletions of this interval that minimally eliminate both *PKD1* and the adjoining tuberous sclerosis type 2 (*TSC2*) gene (Brook-Carter et al. 1994).

We describe our experience using a gridded P1 library to clone a 700-kb interval on chromosome 16p13.3. This interval was not recovered from the Centre d'Etude du Polymorphisme Humain (CEPH) YAC library by STS content mapping (Mark I, Mark II, and Mega-YAC) or hybridization-based screening (Mark II and Mega-YAC). Our analysis of the P1 library and the resulting P1 clones includes library screening efficiency for both multiple probes and multiple rounds of chromosome walking, chimerism rate based on FISH and insert size determination.

RESULTS

As a first step in assembling a transcriptional map of the region around *PKD1*, we attempted to clone the interval by STS screening of the CEPH YAC library. Twelve of the 13 STSs used in the initial screening were derived from the 700-kb region defined by the closest flanking proximal and distal disease markers at that time, D16S125 (26-6) and D16S84 (CMM65), respectively (Fig. 1). An additional STS, D16S276, was also included in the screening set and is located ~250 kb telomeric of D16S84. The set of STSs used included nine genetic markers (D16S84, D16S125, D16S283, D16S291, D16S662, D16S663, D16S664, D16S665, and D16S3252), one gene (D16S666E), and STSs developed from cosmids 16-140N (D16S276), 302H7 (D16S1103), 325A11 (D16S139), and 16-198N (D16S280). Some putative positives were identified in both the Research Genetics and Los Alamos National Laboratory (LANL) primary CEPH pools, but none of these resulted in the identification of individual YACs encoding the desired STS. Following the

A 700-kb P1 CONTIG CONTAINING THE *PKD1* GENE

Figure 1 STS and clone resources used for genomic library screening. The upper portion displays an ~1.5-Mb genomic interval on chromosome 16p13.3 defined by the respective breakpoints within the hybrids CY23HA and CY14 (blue). An expanded view of a 700-kb segment of this map is presented in the middle portion. DNA markers are listed above both horizontal map lines. The closest flanking genetic markers at the time of library screening were D16S125 (proximal) and D16S84 (distal). Individual cosmids and their locations are depicted immediately below each horizontal map line. DNA markers used for STS-based screening of the CEPH YAC library are shown in red in the upper portion. Cosmid-derived IRS products used to screen IRS products from the CEPH YAC pools are displayed in green. In the expanded map view (*middle*), the *NotI* sites are shown as thin vertical bars below the map line. The same panel also shows the location of two gaps (Δ) in a previously described cosmid/phage contig of this interval (Germino et al. 1992). The lower portion lists the ordered minimum tiling set of walking clones (labeled in purple). The set is comprised of 12 P1 clones and a single cosmid clone, 413C12. (○) The T7 end of each cosmid and P1 clone.

discovery of the *TSC2* gene (Chromosome 16 Tuberous Sclerosis Consortium 1993) and the *PKD1* gene (European Polycystic Kidney Consortium 1994), the LANL CEPH primary pools were unsuccessfully screened for the presence of these gene-specific STSs.

Subsequently, two different approaches were taken. First, interspersed-repetitive sequence (IRS) probes were generated by PCR using the previous set of cosmids and two additional cosmids, 16-166N and 16-191N, as templates (Fig. 1). The cosmid-derived IRS probes were used to screen filter grids containing IRS products derived from the CEPH YAC library. The gridded DNA targets consisted of IRS products derived from complex pools of YAC clones (Liu et al. 1995). All YAC-derived products were present in duplicate on the filter grids to ensure hybridization specificity. From the Mark II and Mega-YAC libraries, six

YACs were identified that contained inter-Alu PCR products complementary to those from individual cosmids: 716H6, 723A1, 730C6, 731F2, 818E9, and 889A9. Cosmids from two loci that flank *PKD1* and are separated by ~1 Mb, *ATP6C* and D16S276, yielded inter-Alu products that were complementary to sequences present in the YAC 731F2. YAC sizing data available from CEPH indicated that two of the YACs, 730C6 (240 kb) and 731F2 (330 kb), were too small to contain the entire *PKD1* interval. Similar analyses using STS content mapping results (unambiguous and disambiguated hits only) for each of the YACs in question indicated chimerism for YACs 716H6, 723A1, 731F2, and 889A9. These data were confirmed by FISH analysis of metaphase chromosomes using biotinylated IRS products from the five largest YACs as probes, excluding 730C6. The FISH localizations showed the YACs to be either

chimeric (716H6, 723A1, and 731F2), derived from a chromosome other than chromosome 16 (818E9, 889A9) or to be derived from an interval proximal to chromosome 16p13.3 (716H6 and 731F2). A portion of clone 716H6 appeared to originate from chromosome 16q, whereas clone 731F2 localized to both the long (chromosome 16q23) and short arm (chromosome 16p13.1–p13.2) of chromosome 16. These results, coupled with our success screening the gridded P1 library (see below), prompted us to abandon the chimeric YAC reagents at this stage.

We began to survey a gridded total human P1 library for homologous clones from the *PKD1* region while the YAC library screening was in progress. The P1 library was chosen instead of the gridded chromosome 16 cosmid library for two reasons: First, the larger insert size of P1s (70–95 kb) would minimize the number of walks required; and second, the low-copy number of P1s would enhance the stability of the genomic inserts relative to cosmids and YACs. The P1 library was comprised of ~12,000 pools of 10–12 P1s per pool. The total complexity of the library was estimated to be ~3 genome equivalents (Shepherd et al. 1994). Initial chromosome walking was performed unidirectionally from the ends of the interval (*ATP6C*, *D16S84*) and bidirectionally from several internal loci (*D16S291*, *D16S139*, and *D16S3252*). With one exception, multiple probes were used simultaneously to screen the high-density grids. As new walking clones were identified, they were assigned to developing contigs using multiple criteria (see below). Sets of unique, nonoverlapping ends of each new walking clone were used to rescreen the P1 filter grids. Walking probes, derived from the appropriate cosmid or P1 end, were of two types, labeled transcripts or bubble-PCR products (Riley et al. 1990).

Five screenings were performed using 15 probes (Table 1). One probe, 30.1F T7, was used in two screenings. A total of 66 pools were positive, with an average of 4.1 positive pools per probe. The range of positive pools was two to eight pools per probe, with 80% of the independent screenings showing two to four positive pools per probe. Fifty-six clones (~85% of the primary positives) were recovered after secondary screening; 44 clones were unique. On average, 3.5 P1s were recovered per probe, which is consistent with the complexity of the library. Fourteen of 15 probes were used successfully to recover P1 clones. None of the pools contained P1 clones complementary to the probe cKG8 T7.

However, this failure had little impact on the cloning of the interval (see below).

The chromosomal origin of all walking clones was determined by FISH to metaphase chromosomes. Forty-two of 44 walking clones colocalized with cAJ42 or cCMM65 to chromosome 16p13.3. Two clones did not hybridize detectably to chromosome 16 but, instead, localized to chromosome 1q. On the basis of the FISH results, these two clones were most likely mispicks during colony purification. Clones 91.8B and 97.10G hybridized to chromosome 16p13.3 as well as to a more proximal chromosome 16p locus. Similarly, the overlapping cosmid 2H2 also localized to the same two loci on chromosome 16. This dual localization is consistent with a chromosome 16 low-copy-number repeat described previously (Germino et al. 1992; European Polycystic Kidney Disease Consortium 1994).

The resulting P1 walking clones were characterized by restriction enzyme analysis and Southern blot hybridization. Size determinations for the 42 P1s from the *PKD1* locus were based on summing each clone's *EcoRI* fragments in the range of 2–48 kb. The clone sizes ranged from 48.4 to 116.8 kb with a mean of 85.4 kb and a median of 88.2 kb. The vector component of each P1 clone is 16.8 kb.

Multiple types of information were used to assemble the developing contigs. A *NotI* restriction map of the *D16S125*–*D16S84* interval physically anchored the cosmids used for the initial walks and provided abundant, spatially defined landmarks to assess the extent of each walk (Germino et al. 1992). Comparisons between the *EcoRI* fragment patterns of each walking clone and parental clone were used as a qualitative measure of the overlap between clones. T7 and SP6-derived end probes from walking clones were used as hybridization probes to confirm sequence overlap with the parent clone and to identify which end of the walking clone insert overlapped with the parent clone. Quantitative estimates of overlap and the extent of each walk relied on *NotI* fragment pattern comparisons. Contig closure was established when the nonoverlapping end of a walking clone hybridized to another developing contig.

The entire *D16S125*–*D16S84* interval could be minimally defined by 12 P1 clones but contained a single gap located between 79.2A and 30.1F. This gap was eliminated with the identification of cosmid 413C12 by chromosome walking in a gridded cosmid library. The gap in the P1

A 700-kb P1 CONTIG CONTAINING THE *PKD1* GENE**Table 1. Five screenings with 15 probes**

Probes in pool	Primary Positives (pooled probes)	Primary Positives/Probe	Binned Secondary Positives	Secondary Positives/Probe
4	20	5	17	4.2
cAJ42 T7	1.8F, 6.12E, 9.6E, 19.10E, 22.3B, 24.4F,		cAJ42 T7: 6.12E, 53.8B, 110.1F, 125.9D	4
325A11 T3	28.7D, 29.3F, 43.1E, 47.2H, 53.8B,		325A11 T3: 1.8F, 9.6A, 19.10E, 19.9F,	4
cMM65-2 T3	73.8E, 90.4G, 97.10G, 98.1H, 103.3D,		325A11 T3: 90.4G, 97.10G, 98.1H, 108.11D	4
cMM65-2 T7	108.11D, 109.8C, 110.1F, 125.9D		cMM65-2 T3: 47.2H, 73.8E, 103.3D, 109.8C	4
			cMM65-2 T7: 47.2H	1
1.8F T7	1.8F, 22.7D, 30.1F,			
c291-8A T7	96.2D, 96.4B, 64.12C		1.8F T7: 1.8F, 96.2D, 96.4B	3
c291-13.1 T7			c291-8A T7: 1.8F	1
3	6	2	c291-13 T7: 64.12C, 96.2D, 96.4B	3
			7	2.3
cKG8 T3	2.4G, 27.10G, 43.1E,		cKG8 T3: 91.8B, 109.9G	2
cKG8 T7	44.11C, 45.1B, 45.3D,		cKG8 T7: no positives	0
53.8B SP6	53.8B, 49.10D, 62.6F,		53.8B SP6: 53.8B, 79.2A	2
110.1F SP6	77.3D, 94.10H, 94.11A,		109.8C SP6: 49.10G, 77.3D, 45.3D, 62.6F, 44.11C, 109.8C	6
109.8C SP6	109.8C, 110.1F		110.1F SP6: 45.1B, 64.12A, 94.10G, 94.11A, 110.1F	5
5	14	2.8	15	3
30.1F T7	4.7D, 16.5H, 16.10B, 17.10B, 30.1E,		30.1F T7: 30.1F	1
79.2A SP6	30.1F, 31.3E, 31.3F, 37.6E, 38.8C,		79.2A SP6: 4.7D, 16.5H, 79.2A, 93.11H, 124.6H	5
94.10H T7	46.3E, 46.3F, 48.8C, 60.1B, 79.2A,		94.10H T7: 17.10B, 30.1E, 31.3E, 31.3F, 83.11G,	9
	83.11G, 86.7G, 93.11H, 94.3F, 94.10H		94.10H T7: 86.7G, 94.3F, 94.10H, 123.11A	
3	24	8	15	15
30.1F T7	30.1F, 30.1G		30.1 F T7: 30.1F, 30.1G	2
1	2	2	2	2

contig was small because end probes from 30.1F and 79.2A hybridized to the same 4-kb *EcoRI* fragment in 413C12. A previously described cosmid/ λ phage contig over the same interval (Germino et al. 1992) contained two small gaps shown in Figure 1. The distal gap in the cosmid/ λ phage contig (~20 kb) is in a similar location as the gap in our P1-only contig. Their proximal gap (~60 kb) is at least partially contained within the most centromeric P1 of our contig, 94.10H.

DISCUSSION

We have assembled a set of overlapping cloned genomic reagents to construct a transcriptional map of the minimum genetic interval that encodes *PKD1*. A 700-kb DNA contig was assembled from a set of 42 unique, chromosome 16-derived P1 clones obtained by screening a three-genome equivalent P1 library (Shepherd et al. 1994) with 15 distinct probes. The D16S125–D16S84 interval could be defined using 12 overlapping P1 clones and 1 cosmid clone. The representation of this interval in the P1 library is consistent with the library's predicted complexity. This observation suggests that this interval's preponderance of repetitive sequences (particularly Alu repeats), genes and associated GC-richness had little impact on clone maintenance during construction of the primary library and the subsequent propagation and distribution of secondary library pools of 10–12 clones per well.

We were unable to recover this segment from the CEPH YAC library using either STS screening (Mark I, Mark II, and Mega-YAC) or locus-specific probes from IRS-PCR screening (Mark II and Mega-YAC). Similar results were described at the Third Chromosome 16 Workshop with only 1 Mega-YAC recovered from the distal 3-Mb portion of chromosome 16p13.3 (Doggett and Callen 1995), which includes the *PKD1* locus, whereas the proximal portion of chromosome 16p13.3 is comprehensively covered. Other reports have described chromosomal regions that also appear to be underrepresented in YAC libraries, including the subtelomeric interval of chromosome Xq28 (Willard et al. 1994), and chromosome 4p (Myers et al. 1994), as well as several regions of chromosome 22 (Scambler 1994).

We have found that the P1 and cosmid clones in this interval of chromosome 16 have a lower frequency of *EcoRI* sites than expected for the human genome (data not shown). The reduced occurrence of *EcoRI* sites is consistent with

the interval being a member of the human H3 family, the most GC-rich isochore with an average GC content of 55% (Saccone et al. 1992). Sample sequencing of the interval including the *PKD1* gene (American *PKD1* Consortium 1995) and segments revealed by exon trapping (Burn et al., this issue) indicate >60% GC based on ~75 kb of sequence. The reduced frequency of *EcoRI* sites could decrease the representation of this region in the CEPH (and other) YAC libraries, because *EcoRI* partial digestion is used to generate insert DNA for cloning into pYAC4. Compounding the *EcoRI* effect, is the possibility that the yeast replication machinery, accustomed to a genome of 38.3% GC, cannot function over sustained intervals of high GC content.

The abundance or particular distribution of repetitive sequences within the interval may also have reduced the likelihood of recovering mega-YACs derived from this interval given that mammalian repetitive sequences, particularly the most abundant middle repeats like the Alu class, are believed to facilitate recombination (Green et al. 1991; Schlessinger et al. 1991; Ling et al. 1993; Kouprina et al. 1994; Larionov et al. 1994). As an example of repetitive sequence abundance, we have determined the presence of ~34 Alu repeats in a 63-kb interval that encompasses the *PKD1* gene (American *PKD1* Consortium 1995). Twenty-seven of these elements were located in a 30-kb segment. This high Alu density, if maintained throughout the entire 700-kb interval, may have provided the yeast recombinational machinery with a preferred DNA substrate and prevented the formation of stable YACs with the desired sequence integrity. Similar arguments can be extended to include the effects of low-copy tandem repeats on YAC stability (Neil et al. 1990). The region of chromosome 16 that we analyzed, band 16p13.3, contains several loci that are duplicated tandemly and may have caused YAC rearrangement or instability. One example includes 26-6DIS (D16S125), which is duplicated at 26-6PROX (~70 kb away), and in some individuals a third polymorphic copy exists, OX_{dup} (Harris et al. 1994). Another example is described in Burn et al. (this issue), who used exon trapping of the P1 clones to reveal a pair of ABC gene family members present in P1s 79.2A and 30.1F.

The P1/cosmid contig described here has two advantages over the previously described cosmid/ λ phage contig of the same interval (Germino et al. 1992). First, our predominantly P1

contig is predicted to be more stable and less prone to rearrangements and deletions than the cosmid/ λ phage contig (Kim et al. 1992). This feature is particularly crucial as we begin to sequence the interval. Second, our contig eliminates two small gaps of ~20 and ~60 kb, which remained in the cosmid/ λ phage contig and therefore provides a complete and uninterrupted cloned segment for sequence analysis. Curiously, both gaps in the cosmid/ λ phage contig were within regions that are now known to be locally duplicated. The distal gap is near one of two ABC-like genes that we identified recently in the interval by exon trapping (Burn et al., this issue). The proximal gap in the cosmid/ λ phage contig is within the 26-6DIS/26-6PROX duplication and is substantially covered by the proximal P1 clone in our contig 94.10H.

Defining chromosomal segments with cloned reagents requires the use of multiple library resources. As one of the newer resources available, large insert libraries hosted in *E. coli* have the estimated representation and ease of use to complement the existing approaches to clone specific genomic intervals. Our studies support the estimated $3 \times$ complexity of the P1 library of Shepherd and co-workers (Shepherd et al. 1994) and encourage its use, particularly for cloning regions of known instability or underrepresentation, in YAC libraries. The clones described provide a valuable resource for transcriptional mapping and create a set of sequence-ready templates for a gene-rich interval responsible for at least two inheritable diseases.

METHODS

Cosmids

Multiple cosmids were used as reagents to initiate walks in YAC and P1 libraries. Clones 16-166N (D16S277), 16-191N (D16S279), 16-198N (D16S280), and 16-140N (D16S276) were isolated previously from a cosmid library (Lerner et al. 1992). Cosmids cMCM65 (D16S84), c291 (D16S291), cAJ42 (*ATP6C*), and cKG8 were recovered from total human cosmid libraries (made in-house or by Stratagene, La Jolla, CA) using either a cloned insert (cMCM65) or sequence-specific oligonucleotides as probe. The c326 cosmid contig and clone 413C12 originated from a flow-sorted chromosome 16 library (Stallings et al. 1992). The c326 contig was comprised of clones 2H2, 77E8, 325A11, and 325B10. All of the cosmids were ordered relative to each other by interphase FISH using three probes and two colors.

YACs

Primary and secondary pools of the CEPH YAC libraries

A 700-KB P1 CONTIG CONTAINING THE *PKD1* GENE

(Mark I, Mark II, and Mega-YAC) were screened at Research Genetics (Birmingham, AL) for the presence of STSs derived from the following loci: D16S283, D16S139, D16S84, and D16S276. STS screening was performed at LANL on primary pools of plates 613–736 of the CEPH Mark II library and plates 737–984 of the Mega-YAC library for the following loci: D16S291, D16S283, D16S662, D16S663, D16S664, D16S665, D16S666E, D16S1103, and D16S3252. After the *TSC2* (European Chromosome 16 Tuberous Sclerosis Consortium 1993) and *PKD1* genes (European Polycystic Kidney Disease Consortium 1994) were identified, the LANL primary pools were also screened for the presence of several *PKD1* and *TSC2* STSs. Primers for STSs were either as described in the Genome Database (GDB, Johns Hopkins University, Baltimore, MD) or selected using the programs OLIGO (National Biosciences, Inc., Plymouth, MN) or ASTS (Rappaport et al. 1994).

Screening of gridded interspersed-repetitive sequence (IRS) pools from Mark I, Mark II, and Mega-YAC libraries with cosmid-specific IRS probes was as described previously (Liu et al. 1995). IRS probes were made from cosmids 16-166N, 16-191N, cAJ42, 16-198N, 325A11, cMCM65, and 16-140N.

YACs were localized to metaphase chromosomes by FISH. Biotinylated YAC probes were generated by nick-translating complex mixtures of IRS products from each YAC. Mixtures of sufficient complexity were achieved by performing independent DNA amplifications of total yeast DNA using various Alu primers (Lichter et al. 1990) and then combining the appropriate reactions containing the most diverse products. The hybridization and visualization of the pooled PCR products to standard cytogenetic preparations of short-term cultures of peripheral blood lymphocytes was performed as described previously (Klinger et al. 1992).

P1s

Chromosome walking experiments were done using a single set of membranes that contained the gridded P1 library pools (Shepherd et al. 1994). The gridded filters were kindly provided by Dr. Mark Leppert and the Technology Access Section of the Utah Center for Human Genome Research at the University of Utah. The P1 gridded membranes were screened using end probes derived from a set of chromosome 16 cosmids (see above) and P1 clones as they were identified. Both RNA transcripts and bubble-PCR products were used as end probes. Radiolabeled transcripts were generated using restriction enzyme-digested cosmids or P1s (*AluI*, *HaeIII*, *RsaI*, *TaqI*) as template for phage RNA polymerases T3, T7, and SP6. The T3 and T7 promoter elements were present on the cosmid-derived templates, whereas T7 and SP6 promoter sequences were contained on the P1-based templates. Transcription reactions were performed as recommended by the manufacturer (Stratagene, La Jolla, CA) in the presence of [α - 32 P]ATP (Amersham Corporation, Arlington Heights, IL). Transcription of P1 templates by SP6 RNA polymerase was less efficient (~20%) than T7 RNA polymerase-catalyzed reactions on the same templates. The SP6 RNA polymerase promoter present in the P1 vector is the native sequence and is known to be less active than the optimized element present in other vectors used for in vitro transcription (Promega, Madison, WI).

In addition to labeled transcript probes, we also synthesized bubble-PCR products from restriction enzyme-digested P1s (*AluI*, *HaeIII*, *RsaI*, *TaqI*). Bubble adaptors with appropriate overhangs and phosphorylated 5' ends were ligated to digested P1 DNA basically as described for YACs (Riley et al. 1990). The sequence of the universal vectorette primer derived from the bubble adaptor sequence was 5'-GTTCGTACGAGAATCGCT-3' and differed from that of Riley and co-workers with 12 fewer 5' nucleotides. The T_m of the truncated vectorette primer more closely matched that of the paired amplicon from the vector-derived promoter sequence (SP6, T7). The desired bubble-PCR product was gel purified before radiolabeling (Feinberg and Vogelstein 1983, 1984).

The specificity of all end probes was determined before their use on the single set of gridded P1 filter arrays. Radiolabeled probes were preannealed to Cot1 DNA as recommended (LTI, Gaithersburg, MD) and then hybridized to strips of nylon membrane containing assorted DNAs (see below). Hybridizations were performed in CAK solution (5× SSPE, 1% SDS, 5× Denhardt's solution, 100 mg/mL of torula RNA) at 65°C overnight. Individual probes were present at a concentration of 5×10^5 cpm/mL. Hybridized membranes were washed to a final stringency of $0.1 \times \text{SSC}/0.1\% \text{ SDS}$ at 65°C. The hybridization results were visualized by autoradiography. Membrane-bound DNAs (10–20 ng) included the cloned genomic template used to create the probe, one or more unrelated cloned genomic DNAs, cloned vector (no insert), and human genomic DNA. Probes that hybridized robustly to their cloned template, although not hybridizing to unrelated cloned DNAs, vector DNA, or genomic DNA were used to screen the gridded P1 filters.

Hybridization to the arrayed P1 pools was performed as described for membrane strips except that multiple probes were used simultaneously, each at the recommended concentration (see above). P1 pools that hybridized to the radiolabeled probe sets were plated at a density of 200–500 CFU/100-mm plate (LB plus 25 mg/mL of kanamycin), lifted onto 82-mm HATF membranes (Millipore Corporation, Bedford, MA), processed for hybridization (Sambrook et al. 1989), and screened with the complex probe mixture. A master plate was assembled and consisted of a single positive clone from each P1 pool that was probed. To identify which colony-purified P1s resulted from the various individual probes, multiple P1 DNA dot blots were prepared and each hybridized to individual radiolabeled probes.

The chromosomal origin of each walking clone was determined by two-probe/two-color FISH to metaphase chromosomes. All hybridizations contained a chromosome 16p13.3 reference probe, for example, cAJ42, as well as a uniquely labeled P1 DNA probe. P1 DNAs were labeled and visualized as described for cosmid-derived probes (Klinger et al. 1992).

Each family of P1 clones derived from individual probes was characterized by restriction enzyme digestion (*EcoRI*, *NotI*) and Southern blot hybridization using radiolabeled SP6 and T7 oligonucleotides as probes. Digestion products were resolved electrophoretically by field inversion gel electrophoresis (Carle et al. 1986). Molecular size standards included the 8.3- to 48-kb set of high molecular weight markers (LTI, Gaithersburg, MD), *HindIII*-cut λ DNA and *HaeIII*-cut ϕ x174 DNA. The resolving length of the agarose gel was ~20.5 cm, and gels were generally run

until the 2027-bp *HindIII* fragment of λ DNA was near the bottom of the gel.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (DK44853 to KWK) and the Department of Energy (W-7405-ENG-36 to NAD). We thank Lynne Goodwin, Judy Tesmer, and Linda Meincke for their YAC screening efforts. We also thank Drs. Tim Burn and Bruce Birren for their helpful comments.

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Received February 21, 1996; accepted in revised form April 24, 1996.

The region surrounding the PKD1 gene: a 700-kb P1 contig from a YAC-deficient interval.

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Genome Res. 1996 6: 515-524

Access the most recent version at doi:[10.1101/gr.6.6.515](https://doi.org/10.1101/gr.6.6.515)

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