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Fluorescence *in Situ* Hybridization Mapping of Human Chromosome 19: Cytogenetic Band Location of 540 Cosmids and 70 Genes or DNA Markers

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We report here the band location of 540 cosmids mapped to chromosome 19. The cosmids were mapped by fluorescence *in situ* hybridization (FISH) relative to chromosomal bands produced by DAPI/actinomycin staining. The cosmids are distributed throughout the chromosome, with a sampling bias for the q-arm. A detailed analysis of the distribution of three different subtelomeric and 22 pericentromeric chromosome 19 cosmids on other chromosomes is also reported. Colony hybridization identified 142 cosmids that contain sequences representing genes or DNA markers that map to chromosome 19. FISH mapping of these cosmids sublocalizes a total of 70 genes and DNA markers on chromosome 19, revises the previously published map assignments of 2 genes, and narrows the location of over 20 markers.

INTRODUCTION

As a part of an ongoing effort to map human chromosome 19, a cosmid library has been prepared from flow-sorted chromosome 19 DNA (de Jong *et al.*, 1989). This library contains ≈12,000 human-positive cosmids with 35-kb average insert size, representing a sevenfold average coverage of chromosome 19. Cosmids from this library are presently being ordered with respect to each other along chromosome 19 using a number of strategies. These strategies include identification of overlapping sets (contigs) of cosmids by automated restriction enzyme fingerprint analysis (Branscomb *et al.*, 1990; Carrano *et al.*, 1989; Trask *et al.*, 1992), identification of cosmids/contigs containing genes or polymorphic markers known to map to chromosome 19 (e.g., Thompson *et al.*, 1992), cosmid walking using probes generated from contig/cosmid ends (A. Olsen, unpublished results), hybridization of cosmid DNA to yeast artificial chromosomes (YACs) and vice versa (P. de Jong, unpublished results), and ordering in metaphase and interphase chromatin by two-color fluorescence *in situ* hybridization (FISH) (Trask *et al.*, 1991; Trask *et al.*, in preparation). DNA from mapped cosmids in turn is being used to generate STSs for continuing refinement of the genetic and physical map of chromosome 19.

An integral component of the chromosome 19 mapping effort is the localization of cosmids to cytogenetic bands. Such localization provides a link between the developing physical map and the cytogenetic map. Fluorescence *in situ* hybridization is the most direct and efficient way to localize cosmids along a chromosome (reviewed in Lichter *et al.*, 1991; Trask, 1991). Cosmids are labeled with a reporter molecule, such as biotin, and are hybridized to prometaphase chromosome spreads. Unlabeled human genomic DNA is added to the hybridization mixture to suppress annealing of repetitive elements in the labeled probe to the chromosome target (Landegent *et al.*, 1987). A fluorescent signal is produced at the site of probe hybridization by incubation of the slides in fluorescein–avidin, in the case of biotinylated probes. The hybridization site can then be mapped with respect to fluorescent chromosome bands, which can be produced using a number of techniques, such as DAPI/actinomycin staining (Schweizer, 1976).

In this paper, we report the band location of 540 chromosome 19 cosmids. A number (31) of cosmids show homology to the telomeric or centromeric regions of several chromosomes including chromosome 19. Over 140 of the 540 cosmids have been shown to contain sequences representing genes or markers using probes supplied by a large number of investigators. These probes had been mapped previously to chromosome 19 with varying degrees of sublocalization precision by genetic mapping; by

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mapping to somatic cell hybrid panels, deletion panels, or breakpoint panels; or by radioactive *in situ* hybridization. Our FISH mapping of probe-positive cosmids more narrowly defines, and in some cases redefines, the map location of several markers compared to that previously reported in the literature.

MATERIALS AND METHODS

Cosmid library, filters, and DNA preparation. A cosmid library was generated from human chromosome 19 (de Jong *et al.*, 1989) flow-sorted from the Chinese hamster–human hybrid cell line (5HL9-5B). This hybrid contains a chromosome 19 as its only human material (Thompson *et al.*, 1989). The library was constructed in the cosmid vector Lawrist 5 (de Jong *et al.*, 1989) and propagated in two bacterial hosts, ED8767 and DH5αMCR. Individual clones from the cosmid library were arrayed in master microtiter trays, which were used to inoculate replicate microtiter arrays and to prepare colony arrays on hybridization filters (Hybond N or MSI). Hybridization of human genomic DNA to the filters identified the 50–60% clones that contained human DNA inserts. Thus, an approximate sevenfold coverage of chromosome 19 is contained in 12,000 cosmids with human inserts. DNA was isolated from 4-ml cultures by alkaline lysis and purification on Qiagen tip-20 columns (Qiagen, Inc., Chatsworth, CA). The DNA was biotinylated using a commercial nick translation kit [BRL Nick Translation System, spiked with additional DNase (0.04–0.12 μg, BRL lot AFU405)] to give 200- to 400-bp fragments when analyzed on a neutral 1% agarose electrophoresis gel.

Marker/gene assignment to cosmids. Gene- and marker-specific probes in a variety of forms [cDNA, DNA, synthetic oligonucleotide probes, polymerase chain reaction (PCR) products] were kindly made available by a number of external collaborators. The locus and probe names, contributors, and references are listed in Table 1. The probes were radioactively labeled and used to identify cosmids by at least one of several approaches. The contents of 96 wells of each of 214 microtiter arrays were pooled, and the DNA was digested with *Eco*RI, separated on agarose gels, and Southern blotted onto nylon membranes (Tynan *et al.*, 1992). Screening of these pools determined the colony arrays to be further analyzed. Subsequent probing of individual colonies from each of these positive arrays revealed the specific probe-positive clones. In later experiments, probes were hybridized to filters carrying high-density arrays of individual colonies (1536 colonies/8.5 $\times$ 11-cm^2 filters) (A. Olsen *et al.*, manuscript in preparation). DNA from positive colonies was digested with restriction enzymes and transferred to membranes for Southern blotting to confirm the presence of probe-positive fragments of appropriate size. In some cases, positive cosmids were confirmed by PCR amplification using probe-specific primers and subsequent gel electrophoresis to identify unique fragments of appropriate size. An effort was made to confirm gene assignments by Southern blot or PCR analysis or by "blind" FISH mapping of two or more positive cosmids to the same band position. Of the 70 genes/DNA markers mapped, 3 were mapped on the basis of a single cosmid identified by colony hybridization only (FCE2, JUNB, and TYK2).

Fluorescence in situ hybridization and banding. Prometaphase chromosome spreads were prepared from MTX-synchronized peripheral blood lymphocyte cultures of a healthy male donor as described elsewhere (Yunis, 1976). Biotinylated cosmids were hybridized to chromosome spreads in the presence of unlabeled human genomic DNA as described elsewhere (Trask, 1990). The sites to which probe hybridized were labeled green with fluorescein by incubation of the slides in avidin–fluorescein (FITC), biotinylated goat anti-avidin, and avidin–FITC. After hybridization and avidin–FITC treatment, chromosomes were incubated in DAPI and actinomycin as described (Schweizer, 1976; Tucker *et al.*, 1988) to produce a Q-banding pattern similar to that of enhanced QFH-type bands (Verma and Babu, 1989). Slides were mounted in an antifade solution containing 0.5–1.0 μg/ml propidium iodide.

Microscopy. A Zeiss Axiophot microscope was used at 1000-fold overall magnification (100×, 1.3 n.a. objective). Chromosomes were viewed alternately through a Zeiss filter set simultaneously transmitting FITC-labeled probe sites and PI counterstaining (excitation filter: BP450-490, reflector: FT510, emission filter: LP520) or a Zeiss filter set transmitting DAPI fluorescence (BP360-371, FT395, LP397). With the exception of the subtelomeric cosmids (7501, 16716, and 16432), mapping was done at the microscope. For these three cosmids, mapping was done from photomicrograph slides of FITC/PI and corresponding DAPI images, because signals faded before all their chromosomal locations could be identified. Photomicrographs were taken using Scotch 3M 640T film (exposures: $\approx$5 s for FITC/PI and $<$1 s for DAPI). All cosmids were mapped in "blind" fashion, without prior knowledge of previous localization, gene assignment, or membership in overlapping sets of cosmids (contigs). The raw idiograms showing mapping results of each of the 540 cosmids and LLNL's database reporting map locations are accessible to other researchers upon request.

RESULTS

Localization of 540 Cosmids by FISH

A representative example of FISH mapping of a cosmid probe to a metaphase spread of a normal individual is shown in Fig. 1. For mapping, each metaphase spread was alternately viewed, in rapid succession, through two filter combinations. This rapid alternation effectively produced for the viewer two side-by-side images, as shown in Fig. 1. In one image, the hybridization site could be localized along the chromosome's length. The yellow-green hybridization sites appear as tightly localized fluorescent signals on each of the four propidium iodide-stained chromosome 19 chromatids (Fig. 1A). In the alternative view, QFH-type bands, produced by DAPI and actinomycin (DAPI/AMD) staining of the chromosomes after hybridization, are visible (Fig. 1B). Using these views, the positions of the probe sites were mapped relative to DAPI/AMD bands on at least 10 metaphase spreads, selected on the basis of banding quality, chromosomal overlap, and signal intensity. Idiograms showing observed signals were compiled for each cosmid (Fig. 2).

The band locations of 540 cosmids mapped by FISH are shown in Fig. 3. The position where the maximum number of hybridization signals was observed for each cosmid (the peak position) is indicated. In practice, these positions were either at the center of a band or at the border between bands. In addition, a significant number of cosmids (23) were assigned to the proximal or distal regions of q13.1, a relatively long band. Although the signals observed after any given hybridization were confined to a span of 5 to 10% of the chromosome (46 and 54% of experiments, respectively), the confidence intervals for cosmid assignment were estimated from the results of duplicate mapping experiments.

Blind duplicate mappings of the same cosmid or of different cosmids positive for the same gene were conducted to assess the reproducibility of the mapping process. Figure 2 compares duplicate mappings of two cosmids that both contain the JUND sequence (see also below). The cosmids were independently mapped to the

TABLE 1

Loci (Probes) Assigned to Cosmids Mapped by FISH

Locus (probe)	Name	Probe	Contributor	Reference
(Apa813)	Apa813 subtelomeric repeat	apa813	J. Ijdo	Ijdo *et al.*, 1991
APOC2	Apolipoprotein CII	apoc2.480bp	J. Breslow	Jackson *et al.*, 1984
APOE	Apolipoprotein E	apoE.421bp	J. Breslow	Breslow *et al.*, 1982
ATP1A3	ATPase, Na^+K^+ transporting, $\alpha 3$	p101RI	R. Levenson	Harley *et al.*, 1988
BCKDHA	Branch-chain ketoacid dehydrogenase	E1α subunit	G. Hoganson	Tiu *et al.*, 1988
BGP	Biliary glycoprotein	TM-cyto	T. Barnett	Barnett *et al.*, 1989
CD33	Myeloid differentiation antigen CD33	CD33	S. Peiper	Peiper *et al.*, 1988
(C/EBP)	CAAT/enhancer binding protein	c/ebp.675bp	G. Darlington	Hendricks-Taylor *et al.*, 1992
CEA	Carcinoembryonic antigen family member	pCEA1	ATCC	Zimmerman *et al.*, 1987
CGM1	CEA gene family member 1	CGM1-3'utr	J. Thompson	Thompson *et al.*, 1992
CGM2	CEA gene family member 2	CGM2	J. Thompson	Thompson *et al.*, 1992
CGM6	CEA gene family member 6	CGM6-3'ut	J. Thompson	Thompson *et al.*, 1992
CGM7	CEA gene family member 7	MW2	J. Thompson	Thompson *et al.*, 1992
CGM8	CEA gene family member 8	CGM8	J. Thompson	Thompson *et al.*, 1992
CGM9	CEA gene family member 9	Pas-4	J. Thompson	Thompson *et al.*, 1992
CKM	Creatine kinase, muscle	pJN2CK-M	R. Dottin	Nigro *et al.*, 1987
CYP2A, 2B, 2F1	Cytochrome P450 subfamilies IIA, IIB, IIF1	CYP2A3, 2B7, 2F1	F. Gonzalez	Nhamburo *et al.*, 1990; Yamano *et al.*, 1989, 1990
D19S7		p4.1	ATCC	Shutler *et al.*, 1992
D19S9		pIJ2	ATCC	Brook *et al.*, 1984
D19S8		p17.1	ATCC	Shaw *et al.*, 1986
D19S11		p13-1-82	M. Litt	Bufton *et al.*, 1986
D19S20		pJCZ3.1	ATCC	Nakamura *et al.*, 1987
D19S22		pEFD4.2	Y. Nakamura	Nakamura *et al.*, 1987
D19S24		pMCT6	M. Carlson	Brook *et al.*, 1984
D19S51		p134C	K. Johnson	Johnson *et al.*, 1990
D19S63		pD10	D. Shaw	Brook *et al.*, 1991
D19S116		pKE2.1	R. Korneluk	Shutler *et al.*, 1992
D19S117		pKE0.6	R. Korneluk	Shutler *et al.*, 1992
D19S177		mfd120	J. Weber	Ropers and Pericak-Vance, 1991
DNMT	DNA methyltransferase	hmt2.5, TT6	P. Vertino	Yen *et al.*, 1992
EPOR	Erythropoietin receptor	EPO	J. Winkelmann	Winkelman *et al.*, 1990
ERCC1	Excision repair cross-complem. group 1	pLB1.14	M. Siciliano	Thompson *et al.*, 1989
ERCC2	Excision repair cross-complem. group 2	pKer2	C. Weber	Weber *et al.*, 1988
(ETS00080)	Anonymous cDNA	hhcc76	C. Venter	Adams *et al.*, 1991
(ETS00368)	Anonymous cDNA	hhcj80	C. Venter	Adams *et al.*, 1991
FCE2	Fc fragment of IgE receptor	CD23	B. Mach	Wendel-Hanse *et al.*, 1990
(GNA11)	GNA11, G-protein 11	9097A	T. Wilkie	Wilkie *et al.*, 1992
(GNA15)	GNA15, G-protein 15	849J10	T. Wilkie	Wilkie *et al.*, 1992
GPI	Glucosephosphate isomerase	HS10	M. Gurney	Chaput *et al.*, 1988
ICAM1	Intracellular adhesion molecule 1	pHRR2	A. McClelland	Greve *et al.*, 1989
INSR	Insulin receptor	pHIR/P12-1	ATCC	Ullrich *et al.*, 1985
JUNB	jun B proto-oncogene	465.20	ATCC	Ryder *et al.*, 1988
JUND	jun D proto-oncogene	XHJ-12.4	ATCC	Ryder *et al.*, 1988
LDLR	low-density lipoprotein receptor	pLDLR3	ATCC	Sudhof *et al.*, 1985
LIG1	DNA ligase 1	SK-(pHL)	D. Barnes	Barnes *et al.*, 1990
LYL1	Lymphoblastic leukemia-derived sequence 1	LYL1	M. Cleary	Mellentin *et al.*, 1989
MAG	Myelin-associated glycoprotein	pEmbl-Mag	M. Arquint	Barton *et al.*, 1987
NCA	Nonspecific cross-reacting antigen	NCA-3'	J. Thompson	Thompson *et al.*, 1992
(OLFR)	Olfactory receptor gene family member	ORc20722r1	R. Reed	R. Reed, personal communication
(pE670)	Minisatellite	pE670	J. Breslow	Das *et al.*, 1987
PRKCG	Protein kinase C-γ	phPKC-g6	L. Coussens	Coussens *et al.*, 1986
PSG2	Pregnancy-specific glycoprotein 2	SYM2420	S. Hammarstrom	Khan *et al.*, 1992
PSG3	Pregnancy-specific glycoprotein 3	psg3-oligo	H. Mohrenweiser	H. Mohrenweiser, unpublished data
PSG4	Pregnancy-specific glycoprotein 4	SYM2421	S. Hammarstrom	Khan *et al.*, 1992
PSG5	Pregnancy-specific glycoprotein 5	SYM2422	S. Hammarstrom	Khan *et al.*, 1992
PSG8	Pregnancy-specific glycoprotein 8	SYM2184	S. Hammarstrom	Khan *et al.*, 1992
(PSG13)	Pregnancy-specific glycoprotein 13	SYM2179	S. Hammarstrom	Khan *et al.*, 1992
PVS	Poliovirus sensitivity	PVS	E. Wimmer	Mohrenweiser *et al.*, 1991
RAB3A	*ras*-related oncogene	Rab3	A. Tavitian	Rousseau-Merck *et al.*, 1989
RAB8	NK-14-derived transforming oncogene	PEmel600	ATCC	Padua *et al.*, 1984
RYR1	Ryanodine receptor 1 (skeletal)	HRR5	D. McLennan	Zorzato *et al.*, 1990
SNRPA	Small nuclear ribonucleoprotein A	pU1A3	R. Spritz	Surowy *et al.*, 1992
TCF3	Transcription factor 3 (E2A)	pE47-8.5H	M. Cleary	Nigro *et al.*, 1987
TNNT1	Slow troponin T	MSL-366	J. Gilbert	Samson *et al.*, 1990
TYK2	Tyrosine kinase 2	TYK2	J. Krolewski	Krolewski *et al.*, 1990
VAV	c-*vav* oncogene	pSK8	M. Barbacid	Martinerie *et al.*, 1990
XRCC1	X-ray repair complementing gene 1	pH9T3-6,7	K. Brookman	Thompson *et al.*, 1990
(19R1-1)		19R1-1	M. Cleary	M. Cleary, personal communication

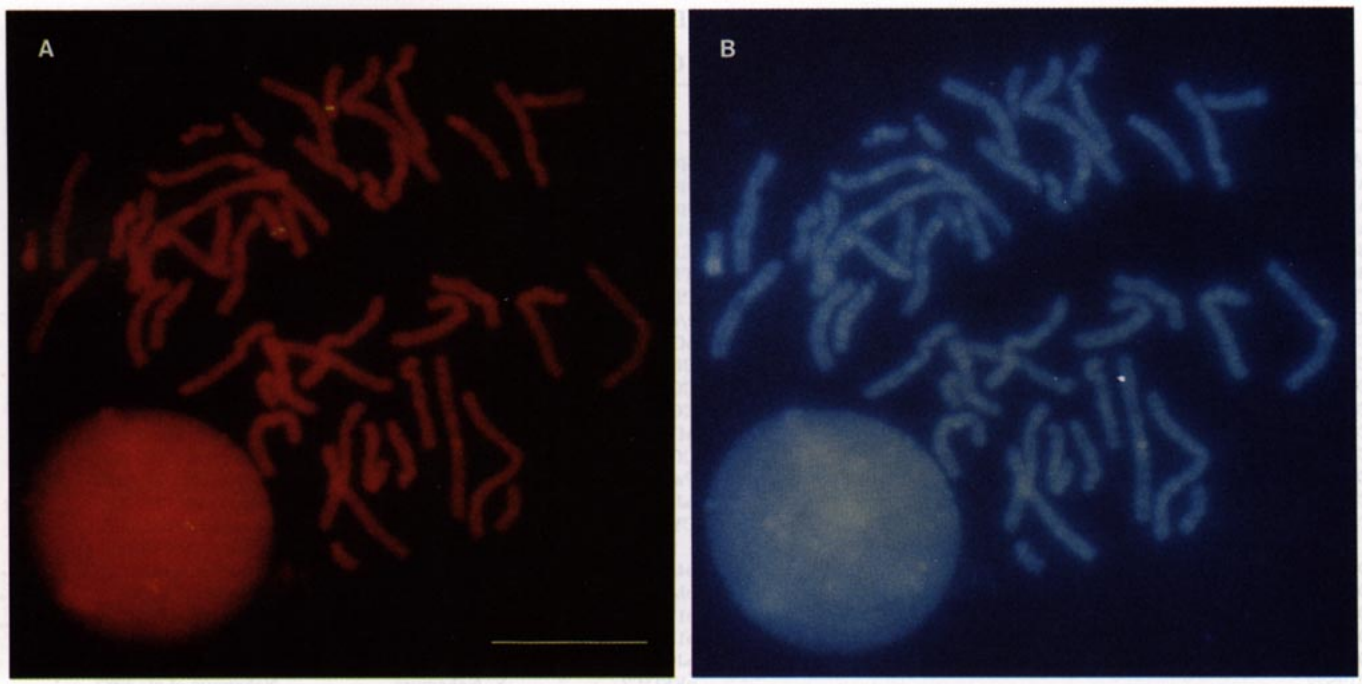


FIG. 1. Representative example of cosmid mapping by FISH. (A) Fluorescein (FITC) signal on a prometaphase spread of a normal male after hybridization of biotinylated cosmid 15929, which probes positive for JUNB, and incubation in avidin–FITC. A discrete yellow-green FITC signal is discernible on all four chromosome 19 chromatids. The chromosomes have been counterstained with propidium iodide (red). The band location of these signals (p13.2) is determined relative to DAPI/actinomycin bands shown in (B). The subtle bands on chromosome 19 are more vivid at the microscope than in this reproduction. Bar = 10 μm.

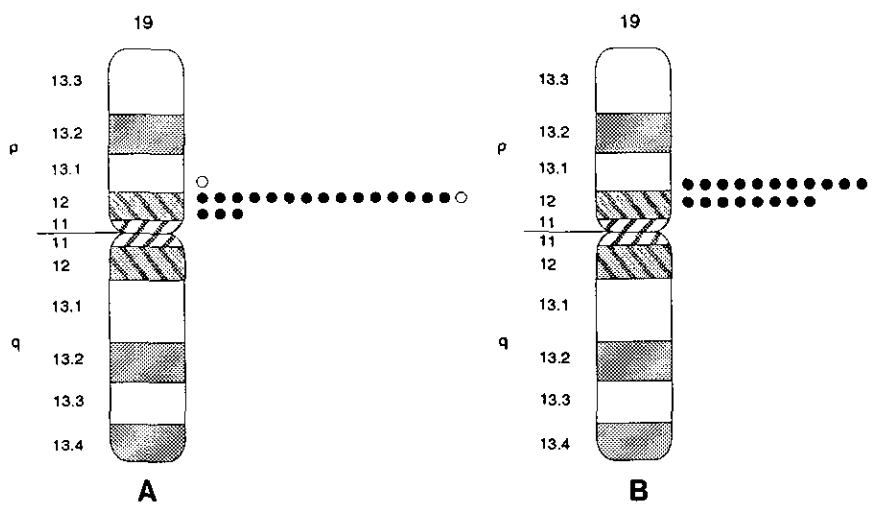
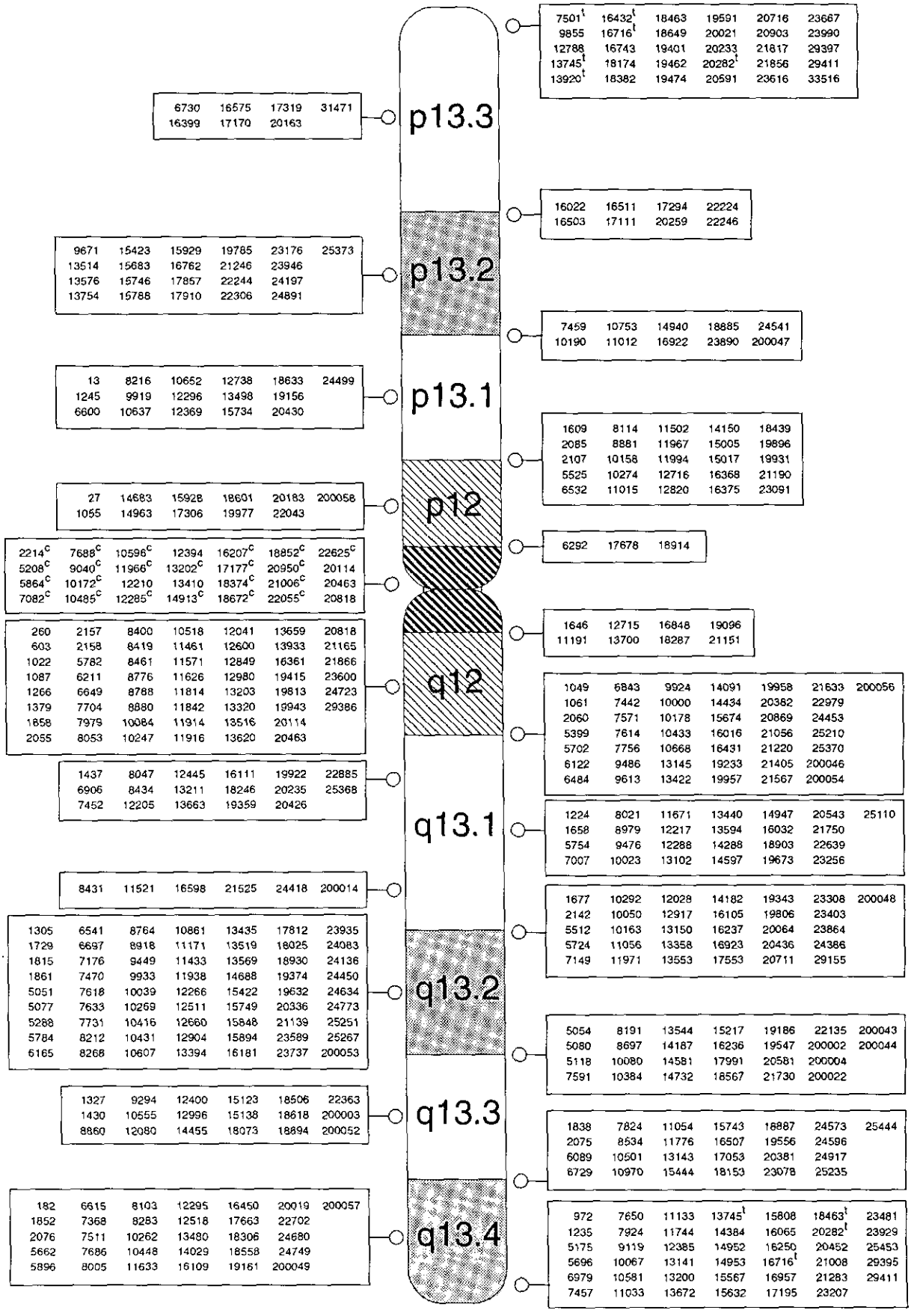


FIG. 2. Idiogram showing the hybridization site location of two different cosmids (19931 and 23091) on 10 metaphases. Both cosmids probe positive for the JUND sequence. No signal was observed on any chromosome type other than 19. (A) Hybridization signals from probe 19931 were observed on both chromatids (solid symbols) on 18 of the 20 scored chromosomes 19 and on only one chromatid (open symbols) on the remaining 2 chromosomes. (B) Hybridization signals from probe 23091 were observed on both chromatids on each of the 20 scored chromosomes 19.

FIG. 3. Map location of 540 chromosome 19 cosmids as determined by FISH mapping relative to DAPI/actinomycin bands. The position where the maximum number of hybridization signals was observed for each cosmid (the peak position) is indicated. The confidence intervals on *map assignments were estimated from the results of duplicate mapping experiments (see Results). The 80% confidence interval for each* cosmid's assignment should be taken as the range centered at the indicated position and extending 0.5 band in each direction (an interval spanning ~10% of the chromosome). The 98% confidence intervals extend an equivalent of one band in one or the other direction. The cosmids labeled with a superscript "t" hybridize to a terminal location on other chromosome types and to some interstitial locations (see Fig. 4). Four of these telomeric cosmids map to both the p and q termini of chromosome 19. A superscript "c" following a cosmid identification number indicates that it hybridizes to the centromeres of other chromosome types (see Fig. 5).


p13.3
p13.2
p13.1
p12
q12
q13.1
q13.2
q13.3
q13.4
6730 16575 17319 31471
16399 17170 20163
9671 15423 15929 19785 23176 25373
13514 15683 16762 21246 23946
13576 15746 17857 22244 24197
13754 15788 17910 22306 24891
13 8216 10652 12738 18633 24499
1245 9919 12296 13498 19156
6600 10637 12369 15734 20430
27 14683 15928 18601 20183 200058
1055 14963 17306 19977 22043
2214C 7688C 10596C 12394 16207C 18852C 22625C
5208C 9040C 11966C 13202C 17177C 20950C 20114
5864C 10172C 12210 13410 18374C 21006C 20463
7082C 10485C 12285C 14913C 18672C 22055C 20818
260 2157 8400 10518 12041 13659 20818
603 2158 8419 11461 12600 13933 21165
1022 5782 8461 11571 12849 16361 21866
1087 6211 8776 11626 12980 19415 23600
1266 6649 8788 11814 13203 19813 24723
1379 7704 8880 11842 13320 19943 29386
1858 7979 10084 11914 13516 20114
2055 8053 10247 11916 13620 20463
1437 8047 12445 16111 19922 22885
6906 8434 13211 18246 20235 25368
7452 12205 13663 19359 20426
8431 11521 16598 21525 24418 200014
1305 6541 8764 10861 13435 17812 23935
1729 6697 8918 11171 13519 18025 24083
1815 7176 9449 11433 13569 18930 24136
1861 7470 9933 11938 14688 19374 24450
5051 7618 10039 12266 15422 19632 24634
5077 7633 10269 12511 15749 20336 24773
5288 7731 10416 12660 15848 21139 25251
5784 8212 10431 12904 15894 23589 25267
6165 8268 10607 13394 16181 23737 200053
1327 9294 12400 15123 18506 22363
1430 10555 12996 15138 18618 200003
8860 12080 14455 18073 18894 200052
182 6615 8103 12295 16450 20019 200057
1852 7368 8283 12518 17663 22702
2076 7511 10262 13480 18306 24680
5662 7686 10448 14029 18558 24749
5896 8005 11633 16109 19161 200049
7501t 16432t 18463 19591 20716 23667
9855 16716t 18649 20021 20903 23990
12788 16743 19401 20233 21817 29397
13745t 18174 19462 20282t 21856 29411
13920t 18382 19474 20591 23616 33516
16022 16511 17294 22224
16503 17111 20259 22246
7459 10753 14940 18885 24541
10190 11012 16922 23890 200047
1609 8114 11502 14150 18439
2085 8881 11967 15005 19896
2107 10158 11994 15017 19931
5525 10274 12716 16368 21190
6532 11015 12820 16375 23091
6292 17678 18914
1646 12715 16848 19096
11191 13700 18287 21151
1049 6843 9924 14091 19958 21633 200056
1061 7442 10000 14434 20382 22979
2060 7571 10178 15674 20869 24453
5399 7614 10433 16016 21056 25210
5702 7756 10668 16431 21220 25370
6122 9486 13145 19233 21405 200046
6484 9613 13422 19957 21567 200054
1224 8021 11671 13440 14947 20543 25110
1658 8979 12217 13594 16032 21750
5754 9476 12288 14288 18903 22639
7007 10023 13102 14597 19673 23256
1677 10292 12028 14182 19343 23308 200048
2142 10050 12917 16105 19806 23403
5512 10163 13150 16237 20064 23864
5724 11056 13358 16923 20436 24386
7149 11971 13553 17553 20711 29155
5054 8191 13544 15217 19186 22135 200043
5080 8697 14187 16236 19547 200002 200044
5118 10080 14581 17991 20581 200004
7591 10384 14732 18567 21730 200022
1838 7824 11054 15743 18887 24573 25444
2075 8534 11776 16507 19556 24596
6089 10501 13143 17053 20381 24917
6729 10970 15444 18153 23078 25235
972 7650 11133 13745t 15808 18463t 23481
1235 7924 11744 14384 16065 20282t 23929
5175 9119 12385 14952 16250 20452 25453
5696 10067 13141 14953 16716t 21008 29395
6979 10581 13200 15567 16957 21283 29411
7457 11033 13672 15632 17195 23207

interval centered at the p12–p13.1 border. In 80% of 137 independent duplicate mappings, the cosmids were assigned to the same position. In 18% of the duplicate mappings, peak positions were displaced 0.5 band from each other (for example, one at the center of a band and another at the border between that band and the next). Two percent were mapped to different locations and required further scrutiny. Thus, the 80% confidence interval for each cosmid's assignment should be taken as the range centered at the position indicated in Fig. 3 and extending 0.5 band in each direction (an interval spanning ~10% of the chromosome). The 98% confidence intervals extend an equivalent of one band in one or the other direction.

The mapped cosmids are distributed throughout chromosome 19, with a sampling bias in favor of the q-arm (see Discussion). Of the cosmids, 23% mapped to the p-arm, 7% to the centromeric region, and 70% to the q-arm. The positions of only 48 cosmids, which are associated with several genes, have been reported previously (Tynan *et al.*, 1992; Wilkie *et al.*, 1992; Samson *et al.*, 1992; Surowy *et al.*, 1993; Barnes *et al.*, 1991; Yen *et al.*, 1992; Hendricks-Taylor *et al.*, 1992).

Telomere-Associated Cosmids

Nine cosmids were observed to hybridize to one or both ends of chromosome 19 as well as to telomeric and interstitial locations on a number of other chromosomes. The identification numbers of these nine cosmids are followed by a superscript "t" in Fig. 3. Two of these cosmids (16716 and 20282) were identified by hybridization to Apa813, a probe detecting a subtelomeric repeat (Ijdo *et al.*, 1991). The remaining cosmids were identified by FISH after random selection from the library. We have made a detailed analysis of the chromosomal distribution of three of these cosmids (16716, 7501, 16432) on metaphase spreads of one male donor (Fig. 4). The chromosomal distributions of the three cosmids are not identical. For example, the terminal site on 15qter appears to be unique to 7501, whereas 3qter and 19pter are labeled by all three cosmids. Cosmid 16716 appears to label the most sites of the three. Sites on 19qter, 10qter, 10cen, 17qter, 21qter, and 22qter are labeled in the majority of metaphases by this probe and not by the other two cosmids. Cosmid 16716 is the only one of three that consistently labels an interstitial site at 2q13–14.1. Cosmid 16432 is unique among the three cosmids in that it labels a site at 4q26–q28 and, to a lesser extent, sites at 12q12–q14 and 12qter. A total of 11 interstitial sites are reproducibly labeled by cosmids 16716 and/or 16432 (1q41–q42, 2q13–q14.1, 4q26–q28, 7p11.2–p12, 7p13–p14, 7q11.2–q21, 10cen, Yq11.2, and, to a lesser extent, 5q, 7q31–q32, and 12q12–q14).

Pericentromeric Cosmids

Twenty-two cosmids hybridized intensely to the centromeric regions of chromosome 19 and various other chromosomes. A superscript "c" identifies these cosmids in Fig. 3. The chromosomal distribution of these cosmids is detailed in Fig. 5. These cosmids contain sequences that are common to various subsets of chromosomes 1, 3, 5, 6, 10, 12, 15, 16, and 20, as well as to 19.

Localization of Cosmids Representing 70 Genes and Anonymous DNA Markers

We have used FISH to sublocalize cosmids representing 70 genes and anonymous DNA markers on chromosome 19. The locations of 142 cosmids representing the 70 loci are summarized in Table 2. These markers had previously been mapped to chromosome 19 with varying degrees of precision by genetic analysis, somatic cell hybrid analysis, or radioactive *in situ* hybridization (summarized in Ropers and Pericak-Vance, 1990, 1991). DNA sequences representing these markers have been obtained from a number of investigators (Table 1). Cosmids identified by hybridization to gene or DNA marker probes were mapped by FISH to cytogenetic bands. FISH mapping was done without prior knowledge of gene location in an effort to confirm previously published assignments.

A large number (403) of cosmids in the library also probed positive for pE670, a chromosome 19-specific middle-repetitive minisatellite (Das *et al.*, 1987). PE670 is associated with at least nine genes or DNA markers, ERCC1, ERCC2, PVS, XRCC1, PRKCG, ATP1A3, APOC2, TNNT1, and D19S22 (K. Tynan, unpublished results; Das *et al.*, 1987). FISH mapping of 33 pE670-positive cosmids confirms previous observations that this repeat is confined to, but distributed throughout, the distal long arm of chromosome 19 (q13.1–q13.4) (Das *et al.*, 1987) (Table 3).

For all mapped genes and markers, assignment to chromosome 19 was confirmed by FISH. Several new genes or markers were sublocalized on chromosome 19 in the course of our mapping effort, and characterization of some of these genes is reported in detail elsewhere: DNA ligase, LIG1 (Barnes *et al.*, 1991); CCAAT enhancer binding protein, C/EBP (Hendricks-Taylor *et al.*, 1992); troponin, TNNTI (Samson *et al.*, 1992); two new carcinoembryonic antigen (CEA) family members, CGM9 and CGM8 (Thompson *et al.*, 1992; Tynan *et al.*, 1992); several pregnancy-specific glycoproteins, PSG2, -3, -5, and -13 (Khan *et al.*, 1992); small nuclear riboprotein A, SNRPA (Surowy *et al.*, 1993); two G-proteins, GNA15 and GNA11 (Wilkie *et al.*, 1992); the DNA methyltransferase gene, DNMT (Yen *et al.*, 1992); members of the olfactory receptor gene family, OLFR (R. Reed, unpublished results); the anonymous cDNAs, ETS00080 and ETS00368 (Adams *et al.*, 1991); D19S116, D19S117, and D19S177, all CA-repeat markers (Shutler *et al.*, 1992; Ropers and Pericak-Vance, 1991); and 19R1-1, a marker closely linked to LYL1 (M. Cleary, unpublished data).

Our FISH mapping results more narrowly define the location of 27 genes/markers over that previously reported [see HGM10.5 and HGM11 (Ropers and Pericak-Vance, 1990, 1991)]. These loci include

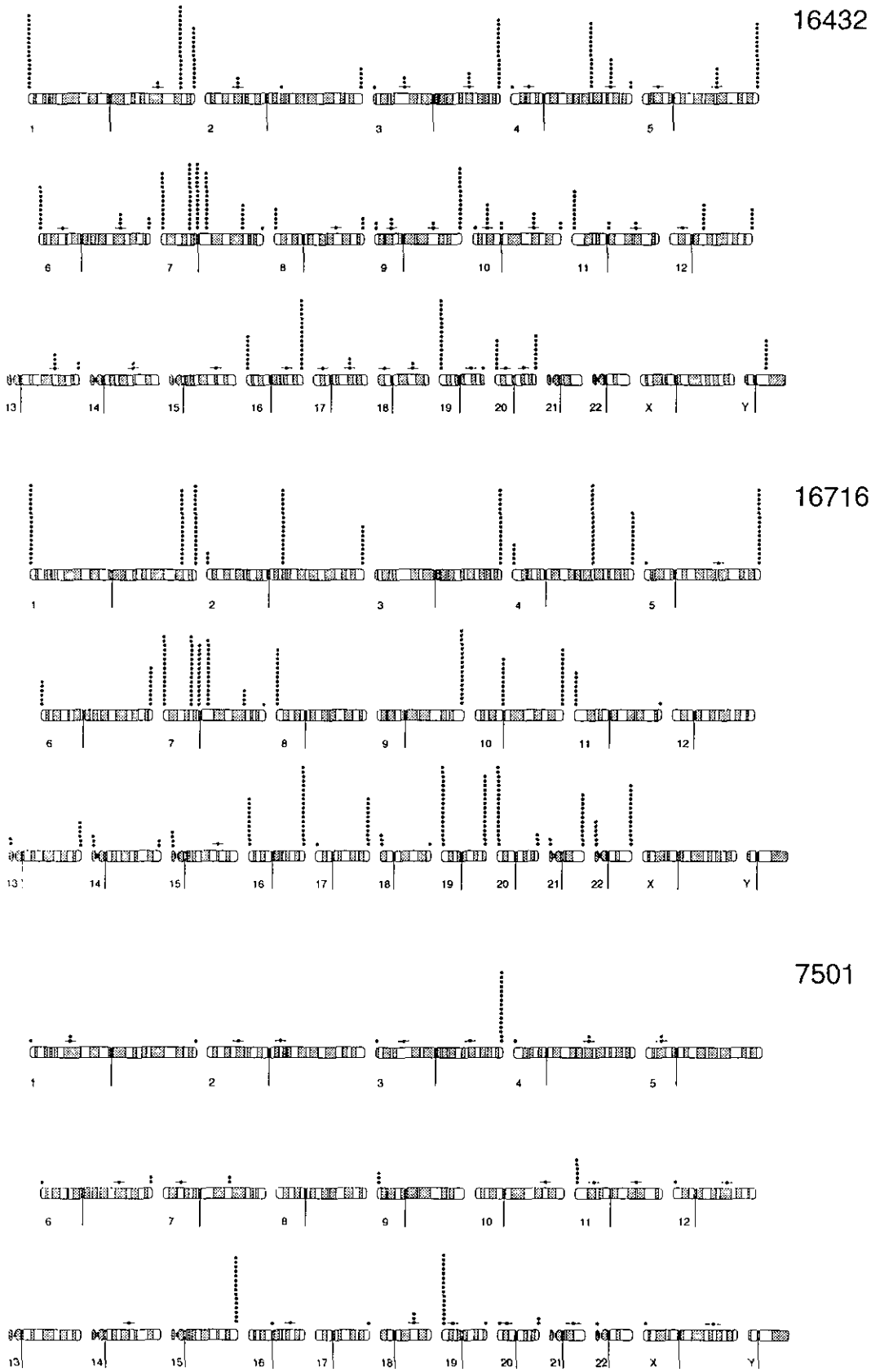


FIG. 4. Chromosomal distribution of three different cosmids isolated from the chromosome 19-enriched cosmid library. These cosmids appear to contain a repeat sequence restricted to terminal locations of a number of chromosomes and several interstitial locations. The idiograms show the hybridization sites observed in 10 metaphase spreads of one male donor. Cells from the same donor were used for each of the three different probes. The symbols indicate sites at which hybridization signals were observed on both chromatids. Symbols crossed by horizontal lines denote hybridization signals that were not mapped precisely to bands, but were interstitial on the indicated chromosomal arm.

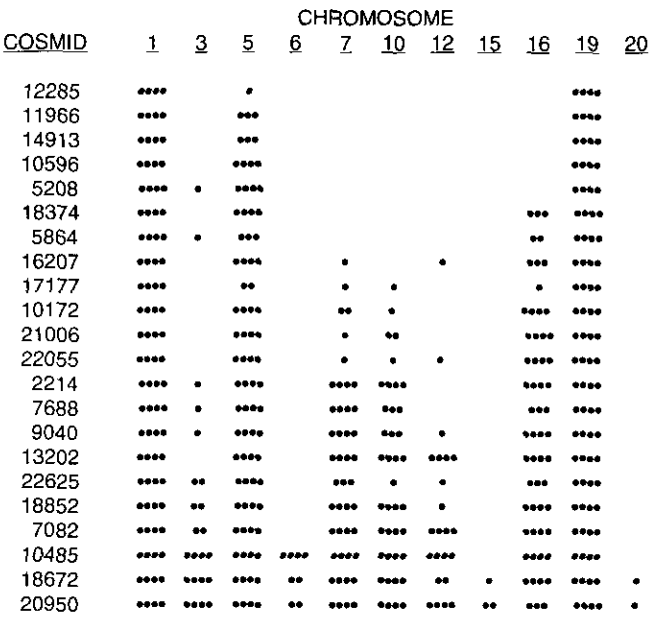


FIG. 5. Centromeres labeled with chromosome 19 cosmids. The number of dots indicates the relative frequency with which label was observed on the indicated chromosome, with the total number of labeled chromosomes 19 set at 100% (1–25% = 1 dot, 26–50% = 2 dots, 51–75% = 3 dots, 76–100% = 4 dots).

D19S9, D19S24, D19S63, D19S177, EPOR, ETS00080, ETS00368, FCE2, ICAM1, LYL1, OLFR, RAB8, TYK1, and 19R1-1. In addition, regional assignments shown in Table 2 for BGP, C/EBP, CGM1, CGM2, CGM6–CGM9, DNMT, GNA11, GNA15, LIG1, and TNNT1 have been recently reported elsewhere (Tynan *et al.*, 1992; Wilkie *et al.*, 1992, Barnes *et al.*, 1991; Surowy *et al.*, 1993; Yen *et al.*, 1992). The location of LDLR is p13.2, rather than p13.3 as was incorrectly reported in the HGM11 report (Mohrenweiser *et al.*, 1991; Ropers and Pericak-Vance, 1991). We find that two loci map by FISH to slightly different locations on chromosome 19 than has been reported previously (Ropers and Pericak-Vance, 1990). These loci are the *jun*-D proto-oncogene (JUND) in p12–p13.1 (Fig. 2) and the *ras*-related oncogene RAB3A in p12–p13.1. These loci had been previously assigned to p13.2 (Mattei *et al.*, 1990) and p13.1–p13.2 (Rousseau-Merck *et al.*, 1989), respectively, on the basis of isotopic *in situ* hybridization.

DISCUSSION

For this report, we used FISH to sublocalize 540 cosmid probes to chromosome 19. FISH is increasingly being used to sublocalize DNA sequences on a number of chromosomes, because of its advantages over other techniques. By the use of FISH on metaphase chromosomes, probes can be more precisely mapped (≤10 Mb in practice) than is possible using radioactive hybridization. This precision is largely due to the confined localization of fluorescent signal at the site of hybridization. Hybridization efficiencies approaching 100% (one signal on each of four chromatids in a normal metaphase) are frequently obtained when cosmids are used for FISH. Technical difficulties in DNA preparation and/or nick translation result in the initial failure of fewer than 5% of cosmids to hybridize efficiently, and the majority of these cosmids are successfully labeled when repeated. Results are obtained more rapidly with FISH (<2 days) than with radioactive hybridization. We have found that a skilled individual can label, hybridize, and regionally localize ≈20 cosmids per week. We have also chosen FISH over somatic cell hybrid panel mapping because hybridization sites on all chromosome types can be determined simultaneously with FISH.

In our study, cosmids were mapped relative to chromosomal bands, so as to directly link location with the cytogenetic map of chromosome 19. We anticipate that several banding procedures that have been described recently will simplify and make more precise the process of mapping (Takahashi *et al.*, 1990; Fan *et al.*, 1990; Cherif *et al.*, 1990). These procedures produce fluorescent banding patterns that can be viewed through the same microscope filter combination as the fluorescein-labeled probe sites, rather than through a separate filter set as is needed with the DAPI banding procedure we have used. Alternatively, hybridization sites can be mapped in terms of the fractional length of the chromosome between the site and a chromosome end (Lichter *et al.*, 1990). This approach allows persons who are not trained in cytogenetics to precisely localize probes along the chromosome, but requires equipment for image capture and analysis.

The observed distribution bias for the q-arm of chromosome 19 may result in part from the nonrandom selection of cosmids for FISH mapping during the course of the Livermore chromosome 19 mapping effort. The overwhelming majority of the cosmids mapped by FISH were selected on the basis of either (1) hybridization to gene or anonymous marker sequences as shown in Table 2 [142 (26%) of the mapped cosmids are probe-positive] or (2) indication of overlap with other cosmids during contig formation by automated fingerprinting [443 (81%) of the mapped cosmids fall in contigs formed by restriction enzyme fingerprinting (Carrano *et al.*, 1989; Trask *et al.*, 1992)]. Since significantly more genes have been assigned to the q-arm than to the p-arm to date (Ropers and Pericak-Vance, 1990), this selection criterion may impose a q-arm bias on the distribution of mapped cosmids. In addition, contigs may be more easily assembled for the q-arm than the p-arm if a cloning bias for the q-arm exists in the original cosmid library. Indeed, the p-arm is less intensely labeled than the q-arm after hybridization with pooled DNA from the portion of the cosmid library grown in host ED8767 (A. Fertitta, unpublished observations).

In the course of this study, we have identified 31 cosmids that contain sequences located at multiple sites in the genome. Nine cosmids identify sequences common to the subtelomeric regions of a number of chromosomes. Here, we have detailed the chromosomal location

TABLE 2

Band Assignments of 70 Genes/Markers Determined by FISH

Locus (probe)	FISH location	Mapped positive cosmids	Locus (probe)	FISH location	Mapped positive cosmids
(Apa813)	19pter, 19qter	16716, 20282	ERCC1	19q13.2–q13.3	8697, 15123
APOC2	19q13.2	19374, 23589, 23737	ERCC2	19q13.2–q13.3	19186, 25251, 200004
APOE	19q13.2	19374, 23589, 23737	(ETS00080)	19cen–p12	17678, 18964, 22043
ATP1A3	19q13.2	24450	(ETS00368)	19q13.1–q13.2	18930, 23864
BCKDHA	19q13.1–q13.2	5051, 7149, 10163	FCE2	19p13.3	20233
BGP	19q13.2	18025	(GNA11)	19p13.3	23990
CD33	19q13.3–q13.4	19556	(GNA15)	19p13.3	18649, 20903
(C/EBP)	19q13.1	6909	GPI	19q13.1	22885
CEA family[a]	19q13.1–q13.3	2142, 7591, 9449, 10050, 10416, 10607, 13150, 13569, 20436, 24083	ICAM1	19p13.2–p13.3	17111, 22306
			INSR	19p13.3	17170
			JUNB	19p13.2	15929
CGM1	19q13.2	7176	JUND	19p12–p13.1	19931, 23091
CGM2	19q13.2	1729	LDLR	19p13.2	13576
CGM6	19q13.2	19632	LIG1	19q13.2–q13.3	22135
CGM7	19q13.1–q13.2	14947, 23308	LYL1	19p13.1–p13.2	16922, 18885
CGM8	19q13.1–q13.2	16105	MAG	19q13.1	19359
CGM9	19q13.2	9933	NCA	19q13.1–q13.2	7176, 11521
CKM	19q13.2–q13.3	14455, 14581	(OLFR)	19p12–p13.1	5525, 19156
CYP2A	19q13.2	15422, 15848		19p13.2	13514
CYP2B	19q13.2	15848	(pE670)	19q	(see Table 2)
CYP2F	19q13.2	15749, 17812	PRKCG	19q13.4	20019
D19S7	19cen–q12	16848, 19096	PSG2	19q13.2	10861, 11433, 11938
D19S8	19q13.2–q13.3	10039, 17991	PSG3	19q13.2	24773
D19S9	19q13.1	13663	PSG4	19q13.2	12904
D19S11	19p12–p13.1	13, 1609, 8216, 11994, 12716, 15005, 15017, 19156	PSG5	19q13.2	12904
			PSG8	19q13.2	1861
			PSG13	19q13.1–q13.2	1677, 10431, 12660
D19S20	19p13.3	18382, 19401	PVS	19q13.2–q13.3	5784, 8212, 14732
D19S22	19q13.3–q13.4	18887	RAB3A	19p12–p13.1	19896
D19S24	19p13.2–p13.3	17294, 22244	RAB8	19p13.1	15734
D19S51	19q13.2–q13.3	12996, 19547	RYR1	19q13.1	8431, 9476
D19S63	19q13.2	7731, 25267	SNRPA	19q13.1–q13.2	5724, 23403
D19S116	19q13.2–q13.3	5118, 8191, 10080, 13544	TCF3	19p13.3	16399, 23667
D19S117	19q13.2–q13.3	5118, 13544	TNNT1	19q13.4	16957
D19S177	19p13.3	20716	TYK2	19p13.2	25373
DNMT	19p13.2–p13.3	15746, 15788, 16503, 16762, 17857, 21246, 23176, 24891	VAV	19p13.2–p13.3	22224, 22246
			XRCC1	19q13.2	5077, 8764
			(19R1-1)	19p13.2	23946
EPOR	19p13.1–p13.2	24541			

Note. This table summarizes the cytogenetic band locations of 142 cosmids representing 70 genes or anonymous DNA markers. Cosmids were mapped in blind fashion, without prior knowledge of gene assignment or prior sublocalizations on chromosome 19. Abbreviations are decoded in Table 1. Probes without official locus names are noted in parentheses. The FISH location is the cumulative location range of all cosmids used to map a given marker. When a single cosmid was mapped for a given marker, the range should be taken as the 80% confidence interval of marker location (see Fig. 3 and text).

[a] Specific gene assignment not made.

of three of these cosmids on a single male individual. It is interesting to note that these cosmids label different subsets of chromosomes than the subtelomeric repeat-containing clones described in previous reports (Riethman *et al.*, 1989; Wells *et al.*, 1990; Brown *et al.*, 1990; Cross *et al.*, 1990; Weber *et al.*, 1991) and may therefore contain different telomere-related sequences. The chromosomal distributions of the three cosmids are not identical, suggesting that there may be sequence differences among the cosmids and among some of the subtelomeric regions. Polymorphism among individuals in the intensity and distribution of subtelomeric repeat probes has been noted by others (Brown *et al.*, 1990; Cross *et al.*, 1990). We will report the results of a study of the variation among individuals in the hybridization intensity and distribution of cosmids 7501, 16716, and 16432 elsewhere (J. Youngblom *et al.*, in preparation).

Interstitial sites are also labeled reproducibly by two of the "subtelomeric" cosmids. Cosmid 16716 labels an interstitial site at 2q13–q14.1. Hybridization of the telomeric repeat sequence and the subtelomeric repeat probe, Apa813, at this site has been previously noted (Ijdo *et al.*, 1991; Wells *et al.*, 1990). This location may be the site of the fusion of two ancestral ape chromosomes (Yunis and Prakash, 1982) and is also associated with a known fragile site, FRA2B (Sutherland and Ledbetter, 1989). Other interstitial sites are also labeled by cosmids 16716 and/or 16432 (1q41–q42, 4q26–q28, 7p11.2–p12,

TABLE 3

Map Location of pE670-Positive Cosmids

Cosmid	Location	Gene association
25110	19q13.1	
25368	19q13.1	
5077	19q13.2	XRCC1
7633	19q13.2	
8268	19q13.2	
8764	19q13.2	XRCC1
13394	19q13.2	
14688	19q13.2	
20336	19q13.2	
23589	19q13.2	APOC2, APOE
23737	19q13.2	APOC2, APOE
24136	19q13.2	
24450	19q13.2	ATP1A3
18567	19q13.2–q13.3	
8860	19q13.3	
12400	19q13.3	
15123	19q13.3	ERCC1
15138	19q13.3	
18560	19q13.3	
15444	19q13.3–q13.4	
17053	19q13.3–q13.4	
18887	19q13.3–q13.4	D19S22
24917	19q13.3–q13.4	
5662	19q13.4	
7368	19q13.4	
8283	19q13.4	
10448	19q13.4	
13480	19q13.4	
16957	19q13.4	TNNT1
19161	19q13.4	
20019	19q13.4	PRKCG
22702	19q13.4	
24680	19q13.4	

7p13–p14, 7q11.2–q21, 10cen, Yq11.2, and, to a lesser extent, 5q, 7q31–q32, and 12q12–q14).

It is interesting that 9 of the 11 interstitial sites labeled with cosmids 16716 and 16432 lie at or near human fragile sites (FRA2B, a rare site at 2q13; FRA1H, a common site at 1q42–q43; FRA7G, a common site at 7q31.2; FRA7J, a common site at 7q11; FRA7A, a rare site at 7p11.2; FRA7D, a common site at 7p13; FRA12A, a rare site at 12q13.1; and two tentative sites, FRA4E, a common site at 4q27, and FRAYA, a site at Y11.2) (Sutherland and Mattei, 1987; Sutherland and Ledbetter, 1989). While this association may be fortuitous given the high density of fragile sites described in human (113/genome), it merits further investigation.

It is tempting to speculate whether some of the interstitial labeled sites represent regions that resided at a telomeric location in the chromosomes of our ancestors. For example, the site on proximal 7q is near the breakpoint of a pericentromeric inversion that distinguishes the banding pattern of human chromosome 7 (and the corresponding chimp and gorilla chromosomes) from its counterpart in the orangutan (Yunis and Prakesh, 1982). This inversion appears to have brought the tip of the short arm of an ancestral chromosome, which was perhaps similar to the orangutan chromosome, to a location in 7q11 of the human, chimp, and gorilla chromosomes. It is at or near this interstitial location where we find hybridization with cosmids 16716 and 16432. Comparison of the banding patterns of human, chimp, orangutan, and gorilla chromosomes does not point to inversions involving telomeres that could easily account for the other observed interstitial sites, however. It is possible that these sites represent ancestral telomeres, resituated as a result of inversions that occurred prior to the divergence of these four species.

Twenty-two cosmids hybridized to a family of chromosomes whose centromeric regions share homology with the centromeric region of chromosome 19. This family includes chromosomes 1, 3, 5, 6, 7, 10, 12, 15, 16, and 20. Chromosomes 1 and 5 are most frequently labeled by cosmids that hybridize to the centromere of chromosome 19 (all of the 23 analyzed cosmids). The alphoid sequences of chromosome 1 and 5 have been previously shown to share sequence homology with those on chromosome 19 (Baldini *et al.*, 1989). Centromeres on chromosomes 16, 7, and 10 are labeled by fewer of the tested cosmids (17, 15, and 14 of the cosmids, respectively). Centromeres on chromosomes 3, 12, 6, 15, and 20 are labeled by fewer than half of the tested cosmids (11, 10, 3, 2, and 2, respectively). The differential labeling among these chromosome types may reflect the degree of divergence in the sequence or repeat structure from the sequence arrangement on chromosome 19, with the sequences on chromosomes 3, 12, 6, 15, and 20 being most diverged.

The chromosome 19 cosmid library forms the basis of the Livermore genome mapping effort. Overlap among these cosmids is being detected using an automated restriction enzyme fingerprinting approach to assemble cosmid contigs. In another paper, we report on the use of FISH to verify contig assembly and to assign the contigs to cytogenetic bands (Trask *et al.*, 1992). Closure of the cosmid map is being conducted with a variety of strategies including cosmid walking and YAC overlap detection. In a third paper, we will report the results of two-color FISH mapping to order cosmids derived from overlapping cytogenetic regions (Trask *et al.*, in preparation).

The cosmids that we have identified as containing gene sequences represent a resource for the molecular analysis of gene structure and expression. The more than 400 regionally localized, but as yet anonymous, cosmids also may be useful in a variety of efforts to produce a contiguous map of chromosome 19.

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