American Journal of Medical Genetics 9999:1–13 (2003)

Sequence-Based, In Situ Detection of Chromosomal Abnormalities at High Resolution

Joan H.M. Knoll* and Peter K. Rogan
Children's Mercy Hospital and Clinics, University of Missouri-Kansas City School of Medicine, Kansas City, Missouri

We developed single copy probes from the draft genome sequence for fluorescence in situ hybridization (scFISH) which precisely delineate chromosome abnormalities at a resolution equivalent to genomic Southern analysis. This study illustrates how scFISH probes detect cryptic and subtle abnormalities and localize the sites of chromosome rearrangements. scFISH probes are substantially shorter than conventional recombinant DNA-derived probes, and $C_o t1$ DNA is not required to suppress repetitive sequence hybridization. In this study, 74 single copy sequence probes (>1,500 bp) have been developed from ≥100 kb genomic intervals associated with either constitutional or acquired disorders. Applications of these probes include detection of congenital microdeletion syndromes on chromosomes 1, 4, 7, 15, 17, 22 and submicroscopic deletions involving the imprinting center on chromosome 15q11.2q13. We demonstrate how hybridization with multiple combinations of probes derived from the Smith-Magenis syndrome interval on chromosome 17 identified a patient with an atypical, proximal deletion breakpoint. A similar multi-probe hybridization strategy has also been used to delineate the translocation breakpoint region on chromosome 9 in chronic myelogenous leukemia. Probes have also been designed to hybridize to multiple cis paralogs, both enhancing the chromosomal target size and detecting chromosome rearrangements, for example, by splitting and separating a family of related sequences flanking an inversion breakpoint on chromosome 16 in acute myelogenous leukemia. These novel strategies for rapid and precise characterization of cytogenetic abnormalities are feasible because of the sequence-defined properties and dense euchromatic organization of single copy probes.

KEY WORDS: fluorescence in situ hybridization; single copy DNA probes; cytogenetic abnormalities; chromosomal breakpoints; DNA sequence analysis; genomic rearrangement

INTRODUCTION

The diversity of chromosomal abnormalities responsible for acquired and inherited human genetic disorders far exceeds the catalog of available commercial DNA probes for chromosomal fluorescence in situ hybridization (FISH). Chromosomal FISH depends on the availability of cloned DNA probes for hybridization to fixed, denatured chromosomes, nuclei or DNA fibers immobilized on microscope slides [Lichter et al., 1988; Pinkel et al., 1988; Florijn et al., 1995]. Commercially available probes detect the most common genomic rearrangements and are generally unsequenced. These probes often consist of long recombinant genomic sequences cloned into cosmids, BACs, PACs, or YACs which are comprised of interspersed single copy and repetitive sequences or repetitive sequences that localize to distinct chromosome structures such as specific centromeres, telomeres, or heterochromatin [Trask et al., 1993; Korenberg et al., 1999].

Specific hybridization of single copy sequences to chromosomal targets in large probes is achieved by enriching for single copy sequences [Fuscoe et al., 1989; Craig et al., 1997], or by suppressing the repetitive sequences by preannealing with excess, unlabeled, repetitive DNA [Sealey et al., 1985; Lichter et al., 1988; Pinkel et al., 1988]. The sequences of these clones are

This work was presented in part at the American Society of Human Genetics Meetings (2000, 2001) and the 10th International Congress of Human Genetics (2001).

Grant sponsor: Hall Foundation; Grant sponsor: Public Health Service; Grant number: 1 R21 CA095167-01; Grant sponsor: Katherine B. Richardson Trust; Grant sponsor: Paul Patton Charitable Trust.

*Correspondence to: Joan H.M. Knoll, Ph.D., Children's Mercy Hospital, 2401 Gillham Road, Kansas City, MO 64108.
E-mail: jknoll@cmh.edu

Received 5 November 2002; Accepted 3 February 2003

DOI 10.1002/ajmg.a.20123

Published online 00 Month 2003 in Wiley InterScience (www.interscience.wiley.com)

often not precisely determined, and their considerable size precludes detection of small deletions, duplications, or cryptic abnormalities within genomic sequences encompassed by the probe. Nevertheless, these probes have been useful in demonstrating both heterogeneous breakpoints and shortest regions of overlap (SRO) in disease states, and have led to the assignment of chromosomal intervals to specific clinical phenotypes. Probes designed specifically to recognize such critical genetic loci may lead to more precise definitions and efficient characterizations of chromosomal disorders.

We have previously described a procedure to design and produce single copy DNA probes for fluorescence in situ hybridization that is based upon the draft human genome reference sequence [scFISH; Rogan et al., 2001; patent pending]. In this study, we demonstrate that it is feasible to design and produce single- or low-copy number probes from many different chromosomal regions for the detection of both common and rare chromosomal abnormalities. We also demonstrate the high resolution capabilities of these sequence-defined probes for detecting regions of breakage in structural rearrangements.

MATERIALS AND METHODS

Probe Design, Development, and In Situ Hybridization

scFISH probes for chromosomal regions associated with inherited or acquired clinical abnormalities were selected for probe design and development. Since the method of producing these probes was first described [Rogan et al., 2001], we have found that probe yields could be increased for some longer intervals (>6 kb) by generating multiple sets of shorter, overlapping internal PCR products. Subsequently, higher yields for larger products have been obtained using Pfx DNA polymerase (Invitrogen, Carsbad, CA). For intervals that failed to generate amplified products, the quality of the draft sequence was assessed, and in some instances primers were redesigned to amplify alternative target sequences within the same intervals.

The purified amplification products were labeled by nick translation with modified nucleotides such as digoxigenin-dUTP or biotin-dUTP (Roche Molecular Biochemicals, Indianapolis, IN) and were subsequently denatured and hybridized to chromosomal preparations fixed to microscope slides [Knoll and Lichter, 1994]. An important difference between scFISH and conventional FISH is that single copy probes do not require preannealing with repetitive DNA (such as $C_o t1$ DNA) since they are devoid of repetitive sequences. Hybridizations of individual probes and multiple probes from neighboring genomic intervals were readily visible at the microscope. Hybridization efficiency of 80% or greater on metaphases was required to proceed with a probe, otherwise labeling was further optimized. The goal was to demonstrate and validate probe development of a wide variety of probes at acceptable levels of specificity and sensitivity not to establish maximally efficient conditions for every probe.

Probe development was generally fast and robust, due to custom software developed to automatically identify single copy intervals and select primer sequences for PCR. A Unix script, integrated_scFISH, manages the process. The user is requested to provide the version of the human genome draft sequence from which probes are designed, the coordinates of the chromosomal region (<350 kb in length) and the minimum length of the single copy interval. The script retrieves the sequence of the entire chromosomal region from the appropriate assembly at the University of California-Santa Cruz website (http://genome.ucsc.edu). A Perl program, findirepeatmask.pl, then computes the coordinates of adequately-sized single copy intervals from the output of the RepeatMasker program (Smit A and Green P; http://ftp.genome.washington.edu/RM/RepeatMasker.html), which itself requires a comprehensive database of human repetitive sequences (http://www.girinst.org). The Delila program, xyplo (http://www.lecb.ncifcrf.gov/~toms) displays a scatterplot indicating the locations of the intervals (e.g., Fig. 1). The integrated_scFISH script then calls a series of sequence analysis programs (Wisconsin package; http://www.accelrys.com), first extracting sequences of each single copy subinterval from the larger sequence (with the fromfasta and assemble programs), and then selecting oligonucleotide primer sequences optimized for long PCR for each subinterval (with the prime program). Primer selection is performed with a Perl script (primwrapper.pl which executes the Wisconsin program prime) by dynamically decrementing primer annealing temperature, product G/C composition and interval length beginning with the most stringent conditions, as we have previously described [Rogan et al., 2001]. Design of a set of probes in each genomic region usually required <30 min on a 300 MHz Sun Unix workstation.

Uniform hybridization and post hybridization wash ($4 \times$ SSC) conditions were employed for all probes. Multiple probe sets with similar amplification protocols (e.g., common T_m and extension times) were prepared and analyzed simultaneously. Microscopy could often be performed within a few days of probe design.

Chromosome Preparations

Residual fixed cell preparations from patient and control peripheral blood specimens were obtained from the clinical cytogenetics laboratory following completion of routine cytogenetic analysis and/or conventional FISH with commercially available probes (Vysis, Inc., Downers Grove, IL). Institutional Review Board exemption was obtained for the use of these cell preparations. Test specimens were from individuals with submicroscopic deletions of chromosomes 7q11.23 (Williams Syndrome), 15q11.2 (Angelman/Prader-Willi Syndromes), 17p11.2 (Smith-Magenis Syndrome) and 22q11.2 (DiGeorge/Velo-Cardio-Facial Syndromes); an imprinting center deletion within 15q11.2 [PWS-U in Saitoh et al., 1996], microscopic deletions of chromosomes 1p36 (Monosomy 1p36 Syndrome) and 4p (Wolf-Hirschorn Syndrome), inversions of chromosome 16 (Acute Myelogenous Leukemia-type M4) and reciprocal

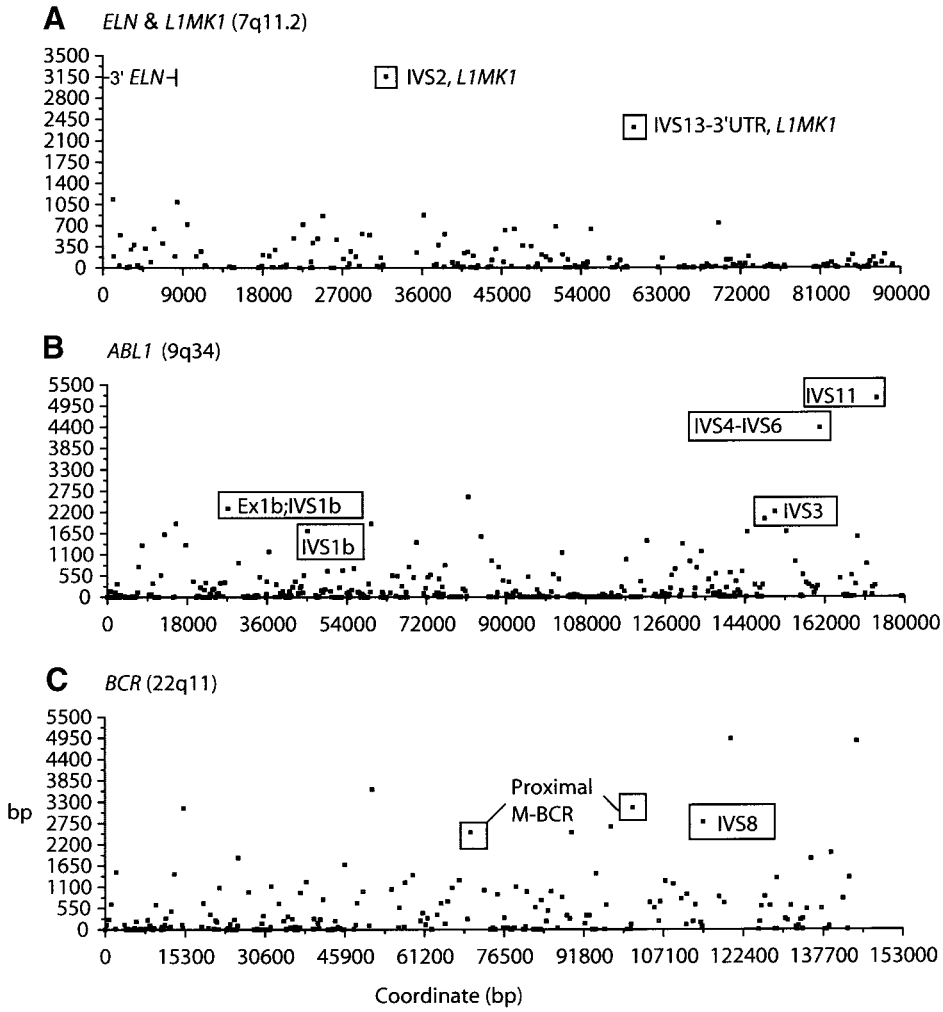

Fig. 1. Scatterplots showing positions and lengths of single copy genomic intervals used in design of probes to detect deletions and translocations. Each point represents a discrete single copy interval, with the ordinate corresponding to the size of the interval in base pairs (bp), and the abscissa showing the location of the initial coordinate. Boxed intervals indicate the probes developed and validated in this study (Table I). A: Probes from the Williams-Beuren syndrome (WS) deletion interval containing LIMK1 and a portion of the ELN on chromosome 7q11.23. B: Probes from both sides of the common breakage region in ABL1 on chromosome 9q34 in chronic myelogenous leukemia (CML). C: Probes to detect breakage at the major (M) breakpoint region in the BCR gene on chromosome 22q11.2 in CML.

translocations between chromosomes 9 and 22 (Chronic Myelogenous Leukemia). Control specimens were from chromosomally normal males and females.

RESULTS

Chromosomal Regions Selected for Probe Design and Synthesis

Probes were developed from the sequences of minimal (or critical) disease intervals obtained from the literature (see table and references in supplementary material see Table S-I, available at www.interscience.wiley.com). Single copy sequences from within these intervals were identified within the GenBank accession ID sequences. Probes were developed for all of the desired intervals, including some with large gaps in the draft genome sequence (e.g., chromosome 16, see Fig. 2A), and for most intervals more than one probe was developed. The probes are listed in Table I. Each probe is defined by the corresponding GenBank Accession number, location within the gene (which is hyperlinked to the ContigViewer at http://www.ensembl.org), PCR primer sets and the beginning and ending sequence coordinates. The probes range in length from approximately 1.2 to 5.2 kb, and are derived from promoter regions, introns and exons of genes and from sequences further upstream and downstream of genes or mapped transcripts. Most probes contain at least one unexpressed or untranslated sequence. Their G + C and CpG contents are representative of the overall euchromatic genome, ranging from 35.1 to 66.9% and 0.3 to 9.1%, respectively.

The distribution and sizes of all single copy sequence intervals for three different gene regions for which probes were developed are shown in Figure 1. Panel A shows single copy intervals (represented by dots) in chromosome 7q11.23 (ELN and LIMK1 genes, for detection of Williams-Beuren syndrome), and panels B

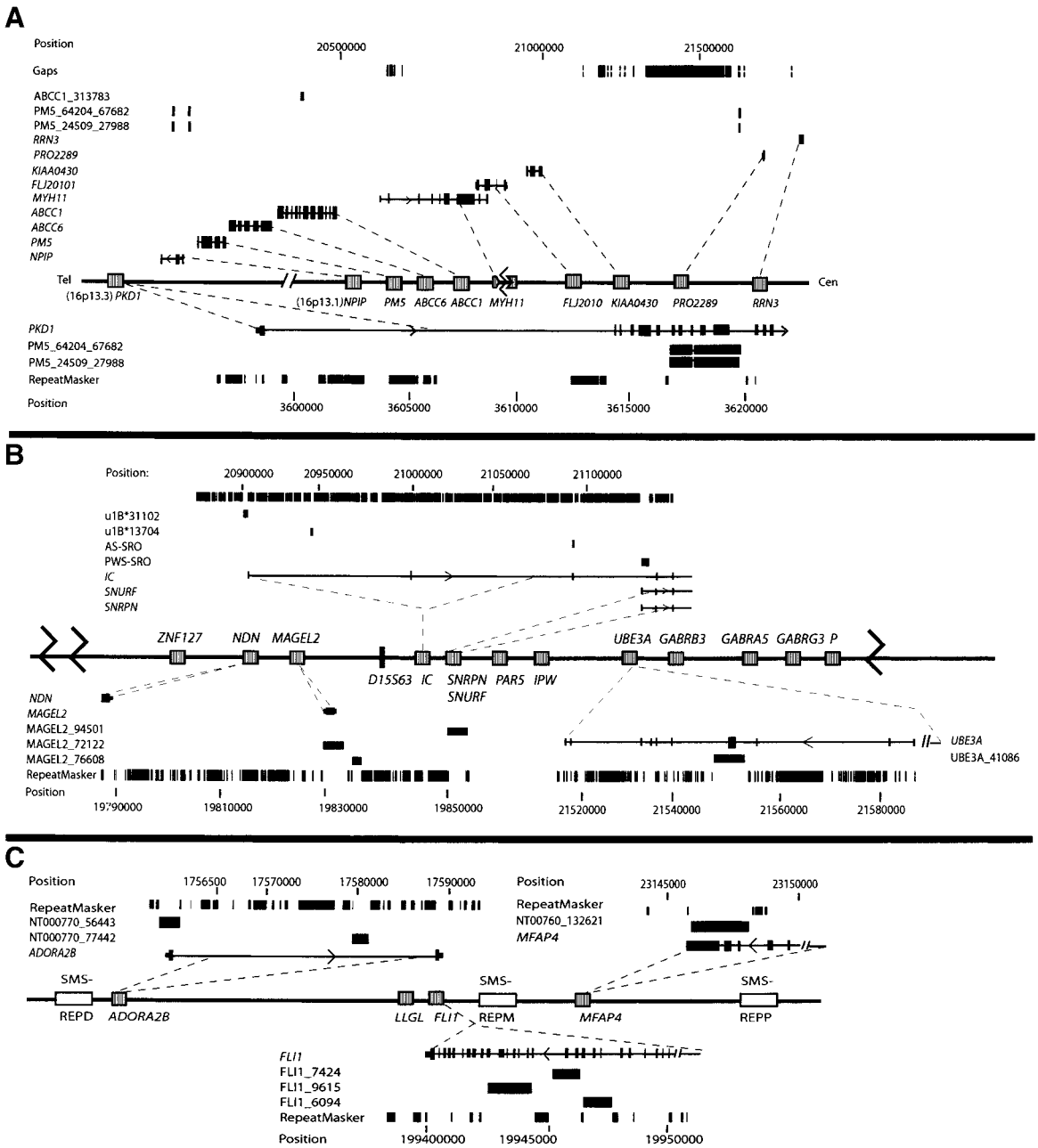

Fig. 2. Locations and context of scFISH probes in the April 2001 genome draft sequence. In each panel, the major genomic features are indicated on the solid horizontal line and are not drawn to scale. Detailed probe-containing regions are magnified to indicate the locations of probes relative to genes, sequence gaps, RepeatMasker track and genomic nucleotide coordinates. The filled boxes in the Repeat Masker track denote the locations of repetitive sequences within each interval. A: Localization of probes from the PM5 (PM5_64204_67682 and PM5_24509_27988) and ABCC1 (ABCC1_313783) genes used to detect the chromosome 16 inversion in AML-M4 leukemia. The scFISH probes derived from sequences 30 and 60 kb downstream of the PM5 gene are related to nearly-identical paralogs on 16p that are found both centromeric (close to PRO2289) and telomeric (within PKD1) of MYH11, the gene interrupted in patients with AML-M4 leukemia and inv(16) (p13.1q22). B: Prader-Willi/Angelman (PWS/AS) Syndrome region extending from MAGEL2 through UBE3A. Multiple scFISH probes were developed for the MAGEL2, SNRPN and UBE3A gene loci as well as from the PWS/AS imprinting center (u1B*31102, u1B*13704, AS-SRO, and PWS-SRO). The AS-SRO and PWS-SRO probes are derived from intervals defining the shortest region of overlaps (SRO) of IC (imprinting center) microdeletions in AS/PWS patients [Ohta et al., 1999]. The single copy probes range from 1.8 to 4.1 kb in length. The PWS-SRO probe was also validated in a PWS family with an IC microdeletion. C: Probes were developed from the ADORA2B (NT000770_56443 and NT000770_77442), FLII (FLI1_7424, FLI1_9615, FLI1_6094) and MFAP4 (NT00760_132621) loci which are in the common deletion interval (SMS-REPD through SMS-REPP) in Smith-Magenis Syndrome (SMS) [Chen et al., 1997]. These single copy probes range from 1.2 to 2.1 kb in length. Smaller SMS deletions defining the minimal Smith-Magenis critical region have been reported recently [Bi et al., 2002 and this study].

and C indicate intervals in chromosome 9q34 (ABL1) and 22q11.2 (BCR) genes for detection of chronic myelogenous leukemia (CML). Many single copy intervals in each region are suitable as potential probes, and the sequences are distributed relatively uniformly across each region. The intervals used for hybridization in this study are indicated by boxes in Figure 1. For example, probes of 3,049 and 2,264 bp were developed specifically for the LIMK1 gene, a gene proposed to be associated with the cognitive deficit in patients with Williams-Beuren syndrome [Osborne et al., 1996]. By contrast, the commercially available probe for Williams-Beuren syndrome is ~180 kb in length, and contains both the LIMK1 and ELN genes (Vysis, Inc.). Thus, scFISH probes, based on their smaller size and specificity for individual genes, may be useful in relating the extent of deletions to clinical phenotypes.

In Table I, all probes except those from chromosomal regions 16p13.1 and 21q22.2 were verified as unique in the genome sequence by BLAT analysis [Kent, 2002; http://www.genome.ucsc.edu/cgi-bin/hgBlat?command=start] and human genome BLAST analysis [Altschul et al., 1990]. Probes from 16p13.1 and 21q22.2 were related to paralogous sequences clustered within the same or neighboring chromosomal intervals on their respective chromosomes.

Nearly all of the probes designed from the April, 2001 genome draft assembly produced homogeneous-sized PCR reaction products of the length predicted from the sequence. We found that successful probe development was related to the accuracy of the specific genomic sequence, as intervals of lower sequence quality (i.e., especially those containing or adjacent to large sequence gaps) produced fewer suitable scFISH probes and more often failed amplification. We found several intervals in which we could not optimize amplification conditions and obtain products. They included intervals on chromosomes 4 (NT000102: positions 264229–271113), 5 (AF119117: positions 34418–40332), 8 (NT002886; positions 271087–274978), 10 (AC011167: positions 149350–153967, 179006–182149, 189958–192784), 15 (U41384: positions 821–4658), 16 (000671: positions 58683–63884 and 87492–93045), 17 (AL035367.4: positions 1194–5365), 21 (AP000057: positions 92651–103709), 22 (U07000: positions 120075–124792), and Y (X96421: positions 11243–13374). Redesign of primers for alternative single copy sequence intervals within the chromosome 4, 8, and 16 regions also failed to generate amplification products.

Verification of Single Copy Probe Locations by FISH

The probes in Table I were hybridized individually to metaphase chromosomes of normal individuals, and all mapped to the predicted chromosomal locations. Whenever possible, adjacent probes were hybridized in combination, and as expected, they localized to the same chromosomal band. Multiple probe hybridizations from adjacent intervals often yielded larger fluorescent signals. Probe localization to a number of disease intervals was also performed by hybridization to cells of patients with known abnormalities. Examples of hybridized probes from disease intervals for chromosomes 1, 7, 9, 15, 16, and 17 are presented in Figure 3. Many other chromosomal abnormalities can also be detected using scFISH probes and respective ISCN designations are included in Table I.

The probes listed in Table I from chromosome 16p13.1 and 21q22.2, which map to the acute myelogenous leukemia-M4 breakpoint region and the Down syndrome critical region (DSCR) respectively, are related to low copy, intrachromosomal paralogs. The quality of the draft sequence and the presence of other duplicons close to the 16p13.1 breakpoint precluded development of single copy probes immediately adjacent to these sequences (Fig. 2A). We therefore developed a set of probes from higher quality sequences (adjacent to the PM5 gene) that have paralogous targets both proximal and distal to MYH11. These probes were also designed to detect multiple chromosomal targets which split when an inversion is present (Fig. 3F). Furthermore, hybridization of a single probe to multiple targets resulted in a brighter fluorescent signal than a probe of comparable size that detects a unique genomic target. Motivated by this observation, we also developed paralogous probes from the Down Syndrome critical region on chromosome 21. Three DSCR4 probes, designed to hybridize to a pair of paralogous targets separated by 1.1 Mb on chromosome 21q22.3 (GenBank accession nos.: AP001417 and AP000160), produced a discrete, larger hybridization signal at high stringency (results not shown).

In addition to chromosomal target size, other factors such as probe sequence composition and labeling method also contributed to the intensity of the hybridization signal. Occasionally, differences in hybridization intensity were observed between homologs in a subset of cells from the same preparation, but these differences could not be attributed to chromosome morphology, probe characteristics or any other single factor.

High-Resolution Applications of scFISH

scFISH can be used to more precisely delineate the regions of breakage in structural abnormalities and detect smaller rearrangements not detectable by conventional FISH, in addition to identifying chromosomal abnormalities. We illustrate four applications of the high-resolution capabilities which leverage the sequence-defined characteristics of scFISH probes.

(1) Detection of imprinting center (IC) microdeletions in Prader-Willi syndrome. We examined the feasibility of detecting chromosome 15 imprinting center (IC) microdeletions with scFISH probes. Overlapping microdeletions in different individuals with either PWS or AS define a 4.3-kb shortest region of overlap (SRO) of the PWS IC [Ohta et al., 1999] and an upstream 0.88 kb SRO in AS [Buiting et al., 1999]. Single-copy genomic intervals and probes containing only these SRO sequences are presented in Figure 2B, and their genomic coordinates are defined in Table I. This schematic also indicates other scFISH probes from neighboring imprinted loci (UBE3A, MAGEL2) within the common PWS/AS deletion interval. Each of these

TABLE I. scFISH Probes Used to Detect Cytogenetic Abnormalities

Chromosome/disorder	GenBank accession	Gene	Interval*	Forward PCR primer coordinates (beginning/end)	Reverse PCR primer coordinates (beginning/end)	C + G (%)	CpG (%)	Cytogenetic nomenclature
1/Monosomy 1p36 Sx	AL031282	CDC2L1	IVS 11-3′ UTR	9137/9167	13960/13931	65.4	3.9	ish del(1)(p36.3)(CDC2L1−)
	AL031282	CDC2L1	3′ UTR	13028/13057	17752/17720	63.4	4.5	idem
4/Wolf-Hirschorn Sx	NT_000102	HD	Exon 67−0.2 kb downstream	267614/267643	271120/271091	55.6	2.4	ish del(4)(p16.3)(HD−)
5/Cri-du-Chat Sx	NT_000149	CTNND2	IVS 17	169655/169685	171976/171945	38.6	0.6	ish del(5)(p15.2)(CTNND2−)
	NT_000149	CTNND2	IVS 14	199168/199202	203507/203473	42.9	1.1	idem
	NT_000149	CTNND2	IVS 13	212490/212519	216569/216536	40.1	0.3	idem
	NT_000147	SEMA5A	IVS 3	14716/14748	17787/17753	41.6	0.7	ish del(5)(p15.31)(SEMA5A−)
	NT_000147	SEMA5A	IVS 3	23905/23935	27710/27676	38.1	0.7	idem
	NT_000147	SEMA5A	IVS 3	30757/30790	33241/33209	41.5	0.8	idem
	AF119117	SLC6A3	IVS 3	28206/28239	31894/31860	62	2.7	ish del(5)(p15.33)(SLC6A3−)[a]
7/Williams Sx	NT_000398	LIMK1	IVS 2	31966/31993	35015/34989	62.6	2.6	ish del(7)(q11.23q11.23)(LIMK1−)
	NT_000398	LIMK1	IVS 13-3′UTR	59947/59976	62211/62187	61.6	3.3	idem
8/Langer-Giedeon Sx	NT_002886	TRPS1	IVS 1	267731/267760	270758/270724	37.8	1.6	ish del(8)(q23.3q24.1)(TRPS1−)
	NT_002886	TRPS1	IVS 1	271242/271271	274437/274404	38.6	0.8	idem
9/CML (chronic myelogenous leukemia)	U07561	ABL1	Exon 1b-IVS 1b	27182/27213	29388/ 29357	56.3	5.6	ish t(9;22)(q34;q11.2)(ABL st)
	U07562	ABL1	IVS 1b	9193/9222	11035/11004	46.8	1.7	idem
	U07563	ABL1	IVS 3	53570/53604	55489/55455	49.8	2.3	ish t(9;22)(q34;q11.2)(ABL mv)
	U07563	ABL1	IVS 3	55807/55836	58077/58046	45.9	1.8	idem
	U07563	ABL1	IVS 4-IVS 6	65951/65985	70266/70237	47.5	1.9	ish t(9;22)(q34;q11.2)(ABL mv)
	U07563	ABL1	Exon 11-IVS 11	78862/78891	83813/83784	59.5	3.9	idem
12/ALL (acute lymphocytic leukemia)	NT_000601	TEL/ETV6	IVS 2	38216/38245	40091/40062	40.0	1.0	ish t(12;21)(p13;q22)(TEL sp)
	NT_000601	TEL/ETV6	IVS 3	72543/72564	74385/74361	42.5	1.0	idem
	NT_000601	TEL/ETV6	IVS 5-IVS 6	95456/95480	97283/97260	43.0	0.8	idem
13/Aneuploidy	AL355338	ZIC2	∼5.8 kb downstream	111114/111145	116046/116012	43.9	1.5	ish del(13)(q32)(ZIC2−) or
	AL355338	ZIC2	∼2 kb upstream	128595/128627	133039/133006	41.8	1.0	ish 13q32(ZIC2x3)
15/Prader-Willi, Angelman & Duplication Sx	AC004600	UBE3A	IVS 8-IVS 9	41085/41119	45354/45325	35.1	0.6	ish del(15)(q11.2q11.2)(UBE3A−)
	AC004737	IC/SNRPN	IVS 3′ to Exon u1B[b]	13740/13769	15414/15387	43.9	3.4	ish del(15)(q11.2q11.2)(IC/ SNRPN−)
	U41384.1	SNRPN	Promoter−IVS 1	13906/13930	16116/16086	37.5	0.9	ish dup(15)(q11.2q13)(UBE3A++,
	AC004737	IC/SNRPN	IVS 5′-Exon u1B[b]-IVS 3′	31102/31128	33347/33323	38	1.2	IC/SNRPN++), and ish dic(15q11.2q13) (UBE3A++,IC/SNRPN++)
	AC004737	IC/SNRPN	IVS 5′-Exon u1B	47792/47821	49470/49441	35.7	0.3	idem
	AC006596	MAGEL2	CDS-3′UTR-2 kb downstream	72122/72146	75658/75638	38.9	2.0	ish del(15)(q11.1q11.2)(MAGEL2−) or
	AC006596	MAGEL2	∼4 kb downstream	76610/76641	78900/78871	39.9	1.2	ish dup(15)(q11.2q11.2)(MAGEL2++)
	AC006596	MAGEL2	∼22 kb downstream	94501/94535	98601/98567	43.6	1.0	idem

16/AML-M4 (acute myelogenous leukemia-M4)	NT_000691	PM5[c]	~20 kb downstream	24509/24538	27988/27958	66.8	5.2	ish inv(16)(p13q22)(PM5 sp)
	NT_000691	PM5[c]	~60 kb downstream	64204/64233	67682/67652	67.1	5.2	idem
	NT_000691	PLA2G10[c] PKD PM5	IVS 3; IVS 12-Exon 15; ~100 kb upstream	68271/68300	71986/71957	66.9	6.4	ish inv(16)(p13q22)(PLA2G10 mv, PKD mv, PM5 sp)
	NT_000691	PLA2G10[c] PKD PM5	IVS 3; Exon 15-IVS 20; ~100 kb upstream, & ~300 kb downstream	71957/71986	75481/75452	66.1	6.0	idem
	NT_025903	ABCC1/MDR1	IVS 6	313783/313812	315675/315645	51.0	2.0	ish inv (16)(p13q22)(ABCC1 st)
16/Rubenstein-Taybi Sx	NT_000671	CREBBP	IVS 19-IVS 20	58653/58685	63854/63823	39.5	1.2	ish del(16)(p13.3)(CREBBP−)
	NT_000671	CREBBP	IVS 19	58833/58862	63347/63318	39.2	1.2	idem
17/Smith-Magenis Sx	NT_000770	ADORA2B[d]	Promoter-IVS 1	56443/56472	58524/58491	67.7	9.1	ish del(17)(p11.2p11.2)(ADORA2B−)
	NT_000770	ADORA2B[d]	IVS 1	77442/77475	79222/79189	51.8	1.6	idem
	U80184	FLI1	IVS 12-IVS 14	7424/7453	8742/8708	59.1	3.2	ish del(17)(p11.2p11.2)(FLI1−)
	U80184	FLI1	IVS 15-Exon 21	9615/9647	11738/11704	60	3.3	idem
	NT_000760	MFAP4	IVS 2-3′ UTR	132621/132654	134663/134634	58.3	1.5	ish del(17)(p11.2p11.2)(MFAP4−)
	AL035367	ZNF179-PAIP1; LLGL/HUGL; SHMT1	Between ZNF179 PAIP1; IVS7-Exon 13; IVS 4	9818/9850	12272/12241	51.5	1.5	ish del(17)(p11.2p11.2) (ZNF179/PAIP1/ SHMT1−)
	AL035367	LLGL/HUGL	Promoter-Exon 1/ promoter-IVS1	1320/1349	5411/5378	57.7	1.9	ish del(17)(p11.2p11.2)(LGLL/HUGL−)
17/Charcot-Marie-Tooth 1A	AC005703	PMP22	Promoter (~5 kb upstream)	153173/153202	155027/154994	48.3	1.1	ish dup(17)(p11.2p11.2)(PMP22++)
	AC005703	PMP22	IVS 3	176746/176778	179073/179044	46.1	1.0	idem
	AC005703	PMP22[d]	IVS 3	184666/184700	186035/186006	39.3	0.5	idem
17/Miller-Dieker Sx	NT_000774	PAFAH1B1 EIF-3[e]	~5 kb downstream IVS 24-IVS 27	63645/63679	66603/66573	54.3	3.9	ish del(17)(p13.3)(PAFAH1B1/EIF-3−)
	NT_000774	PAFAH1B1 EIF-3[e]	~7-8 kb downstream IVS 15-IVS 19	68841/68870	71195/71163	49.8	1.0	idem
	NT_000774	PAFAH1B1 EIF-3[e]	~13 kb downstream IVS 5-IVS 11	75328/75362	78122/78093	43	1.2	idem
20/Alagille Sx	AL035456.24	JAG1	IVS 2-IVS 3	144875/144904	147028/146995	38.2	0.7	ish del(20)(p12.3p12.3)(JAG1−)
	AL035456.24	JAG1	IVS 5-IVS 8	153935/153966	157675/157642	44.2	1.7	idem
21/Down Sx	AP000160	DSCR4	~39 kb upstream	31007/31041	32999/32965	45	0.8	ish (21)(q22.2q22.3)(DSCR4x3)
	AP000160	DSCR4	~30 kb upstream	40725/40754	43078/43045	39.2	1.1	idem
	AP000160	DSCR4	~20 kb upstream	49973/50006	52409/52376	37.1	0.5	idem
21/ALL	AP000057	AML1/RUNX	Promoter-Exon 1	98712/98741	102903/102872	47.5	1.4	ish t(12;21)(p13;q22)(AML1 st)
22/DiGeorge Sx	NT_001039	HIRA	IVS 21-IVS 24	819901/819933	823592/823559	53.0	1.4	ish del(22)(q11.2)(HIRA−)
	NT_001039	HIRA	IVS 13-IVS 15	843602/843631	846946/846915	52.2	1.1	idem
	NT_001039	HIRA	IVS 12-IVS 13	853946/853975	859116/859085	49.3	1.0	idem
	NT_001039	HIRA	IVS 2-IVS 4	875226/875257	878074/878042	46.7	1.2	idem

(Continued)

TABLE I. (Continued)

Chromosome/disorder	GenBank accession	Gene	Interval*	Forward PCR primer coordinates (beginning/end)	Reverse PCR primer coordinates (beginning/end)	C+G (%)	CpG (%)	Cytogenetic nomenclature
22/CML; ALL	U07000	BCR	Proximal to CML M-breakpoint	70259/70288	73463/73434	51.6	1.6	ish t(9;22)(q34;q11.2)(BCR st)
	U07000	BCR	Proximal to CML M-breakpoint	96305/96336	99705/99675	55.6	2.2	idem
	U07000	BCR	IVS 8	114946/114978	117457/117426	61.4	3.0	ish t(9;22)(q34;q11.2)(BCR mv)
X/Kallman Sx	NT_ 001457	GS2	Promoter–IVS 2	78970/79000	82994/82960	43.4	2.3	ish del(X)(p22.31)(GS2–)
	AC006062	KAL1	IVS 6-IVS 7	38822/38852	42042/42012	38.3	0.8	ish del(X)(p22.31)(KAL1–)
	AC006062	KAL1	~8 kb downstream	104433/104465	107097/107072	39.0	0.8	idem
X/Turner & Leri-Weill Sx	NT_001151	SHOX	IVS 2-Exon 3	44615/44646	47505/47473	56.9	8.3	ish del(X)(p22.33)(SHOX–)
	NT_001151	SHOX	IVS 4	49637/49669	52251/52217	50.6	3.4	idem
	NT_001151	SHOX	IVS 6	54357/54387	56821/56791	65.3	3.9	idem
	NT_001159	TBL1	IVS 3	175379/175409	179665/179633	42.2	1.7	ish del(X)(p22.22p22.31)(TBL1–)
	NT_001159	TBL1	Exon 15-3′	247264/247293	251290/251257	47.8	2.5	idem

*WWW hyperlinks are localized to probe sequence in ContigView browser (v9.30a.1; 2 December 2002) at http://www.ensembl.org/Homo_sapiens/
[a]Deleted only in Cri-du-Chat patients with terminal deletions, probe is about 2.5 Mb from telomere.
[b]u1B is ~160 kb upstream from the PWS shortest region of overlap and ~85 kb upstream from the AS shortest region of overlap.
[c]PM5 is ~1.3 Mb telomeric of MYH11 gene, which is disrupted at the inv(16p) breakpoint. PLA2G10 is ~200 kb telomeric of PM5.
[d]Probe was hybridized in combination with other probes and not individually.
[e]Probe is downstream and adjacent to PAFAH1B1 gene (formerly known as LIS1). An expressed transcript homologous to EIF-3 is found at these coordinates.

probes was also developed and validated in normal individuals and in PWS patients with the common large deletions. Figure 3E shows two color chromosome 15q11.2 hybridizations of a single 2214 bp PWS-SRO probe (Promoter-IVS1/SNRPN; red) with 3 probes (4100, 2292, 3536 bp; green) from sequences at the MAGEL2 locus. This PWS SRO probe detects an IC microdeletion in cells of a previously reported PWS family [data not shown; family U of Ohta et al., 1999].

Fig. 3. Normal and abnormal metaphase chromosomes hybridized with a variety of scFISH probes. Rearranged chromosomes are indicated by an asterisk or are referred to as a derivative (der) chromosome. Probes were labeled by nick translation with digoxigenin-dUTP or biotin-dUTP and detected with rhodamine-conjugated antibody to digoxigenin or fluorescein-conjugated avidin, respectively. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Hybridized chromosomes were viewed with an epifluorescence microscope (Olympus, Melville, NY) equipped with a motorized multi-excitation fluorochrome filter wheel. Cells were imaged with an 8 bit Cohu camera system (San Diego, CA) and CytoVision ChromoFluor software (Applied Imaging, Santa Clara, CA). Two probe-two color FISH was performed as described previously [Knoll and Lichter, 1994]. A: Metaphase cell from an individual with 1p36.3 terminal deletion hybridized with two overlapping probes from the IVS11-3′UTR of CDC2L1 (4823 and 4724 bp). Both probes co-localize to chromosomal band 1p36.3. Only the normal chromosome is hybridized. B: Metaphase cell from a Williams-Beuren Syndrome individual with an interstitial deletion of chromosome 7q11.23 hybridized with a 2264 bp probe from IVS13-3′ UTR of LIMK1. Deletions of 7q11.23 are detectable by FISH in ~90% of individuals with WS [Francke, 1999] and LIMK1 has been implicated in the cognitive phenotype [Osborne et al., 1996]. Only the normal chromosome is hybridized. C: Metaphase cell from a SMS patient with an interstitial deletion of chromosome 17p11.2 hybridized with a combination of three probes from within the FLII gene. The probes are located in IVS9-IVS12, IVS12–IVS14, and IVS15-exon 21 and range in size from 1206 to 1300 bp. Only the normal chromosome is hybridized. D: Metaphase cell from the same SMS individual hybridized with multiple probes comprising intervals of ADORA2B (exon1-IVS1, IVS1), FLII (as in panel C), and MFAP4 (IVS2-3′UTR). Both chromosome 17s hybridized indicating that the deletion was smaller than the common rearrangement. Hybridization with probes for each gene indicated that at least one ADORA2B probe is intact. See (Fig. 2C) for a map of the region. E: Normal metaphase cell hybridized with four probes from within the PWS/AS chromosomal region including the PWS IC and detected in two colors. Three probes near MAGEL2 (AC00695: 94501–98601; 76608–78900; and 72122–75658) are detected in green and a single 2214 bp kb PWS-SRO probe (U41354: 765–2979) is detected in red. The probes co-localize and either overlap and appear yellow or remain as distinct colors but are closely spaced. Both chromosomes are hybridized in normal individuals but in PWS individuals with an IC deletion, the SRO probe would be absent on metaphase chromosomes. See (Fig. 2B) for a map of the region. F: Detection of chromosome 16 inversion in cell of a patient with AML-M4 leukemia using a single probe derived from the PM5 locus. PM5 sequences are highly related to several chromosome 16p targets. The inverted chromosome [inv(16)] shows hybridization on each end, while the normal chromosome 16 hybridizes only to the short arm. The PLA2G10/PKD/PM5 probe [NT_000691: 71957–75481] is 3.4 kb in length. The hybridization intensity is strong because the probe detects multiple, paralogous loci in chromosome 16p13.1 and at least one locus in 16p13.3. See (Fig. 2A) for a map of the region. G: Translocation (9;22) positive cell from a CML patient hybridized with three ABL1 probes that are distal to the chromosome 9q34 breakpoint. The probes are from within the IVS3, IVS4-6, and IVS11 intervals of the ABL1 oncogene and are 2.2, 4.3, and 4.9 kb in size, respectively. All probes have translocated to the derivative chromosome 22 [der(22)]. The normal chromosome 9 is also hybridized whereas the derivative chromosome 9 [der(9)] is not. H: Metaphase cell from the same CML patient hybridized with five ABL1 probes that span the chromosome 9 translocation site. The proximal probes are from within exon 1b-IVS1b (2.2 kb) and IVS1b (1.8 kb) and the distal probes are the same as in the previous panel (G). The proximal probes remain on the der(9) and the probes distal of the breakpoint (IVS3; IVS4-IVS6; IVS11) translocate to the der(22). Thus, the breakage interval is between IVS1b and IVS3 and can be precisely determined by hybridization with additional sc probes from this interval (see Fig. 1B for additional single copy sequences). The normal chromosome 9 is also hybridized.

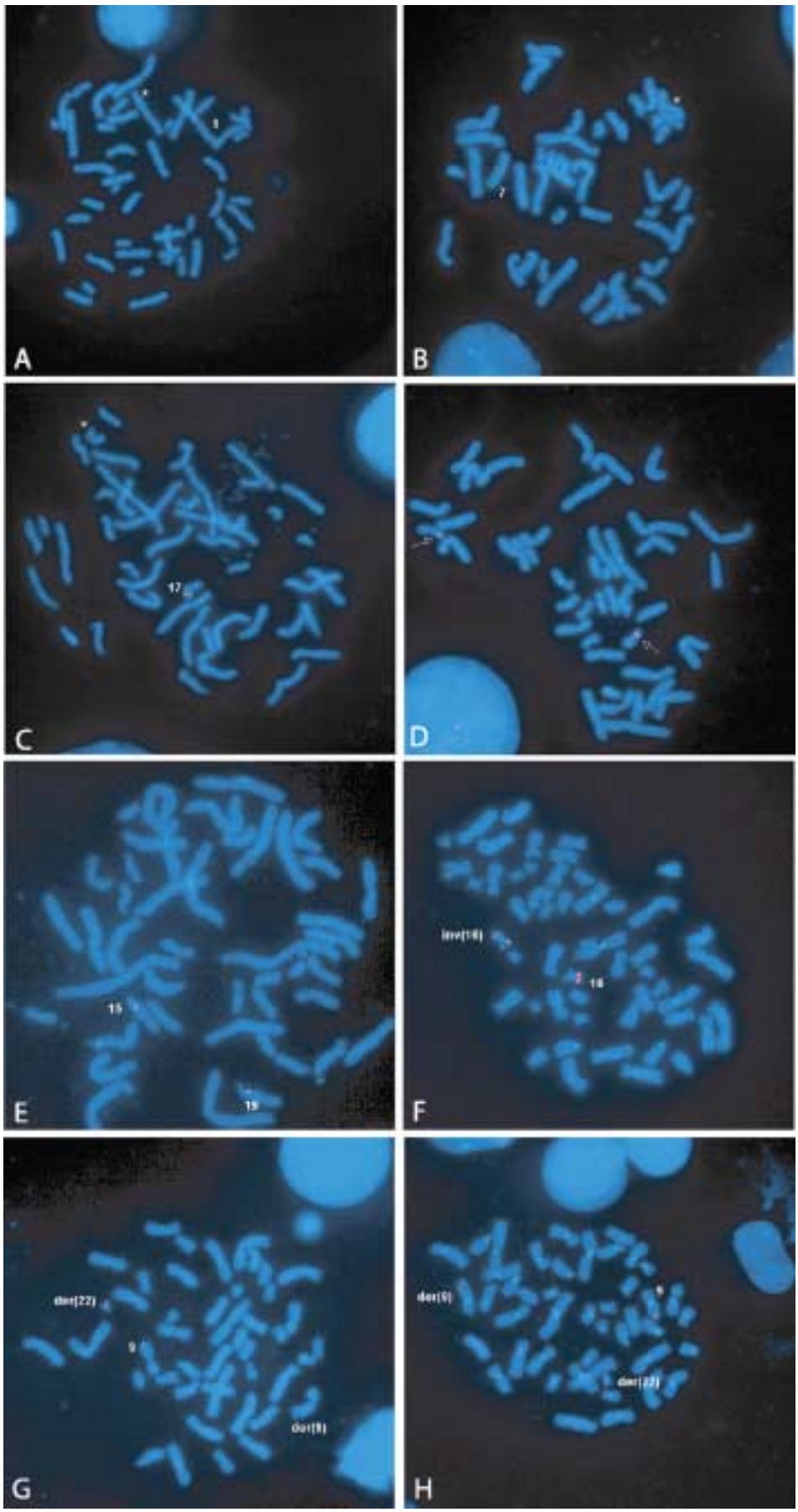

Fig. 3.

(2) Detection of an atypical microdeletion in Smith-Magenis syndrome (SMS). The common chromosome 17p11.2 deletion in patients with SMS extends from SMS-REPD through SMS-REPP and includes numerous genes (Fig. 2C). We have validated 8 probes from single copy intervals within or adjacent to a subset of genes within this region, including ADORA2B, LLGL, FLII, and MFAP4 (shown in the schematic of Fig. 2C; Table I). These probes were tested on cells of a SMS patient with an interstitial deletion of 17p11.2 that was previously confirmed by conventional FISH with a 140 kb probe which spans the SHMT1, FLII, LLGL1 and TOP3 loci (Vysis, Inc.). We confirmed the deletion by hybridization with a mixture of three distinct FLII probes (IVS9-IVS12; IVS12-IVS14; IVS15-exon 21; Fig. 3C) and MFAP4 (IVS2-3′UTR). However, hybridizations with a probe cocktail consisting of two ADORA2B (Promoter-IVS1, IVS1), and the MFAP4 and FLII probes failed to detect a deletion. An additional hybridization using only the ADORA2B probes indicated that this locus was present on both chromosomes (Fig. 3D), and thus that the deletion was smaller than the common SMS one [Chen et al., 1997]. If additional cell pellet had been available, the proximal and distal deletion breakpoints could have been refined by hybridizations with additional probes from single copy intervals between FLI1 and ADORA2B and between MFAP4 and SMS-REPP.

(3) Refinement of a translocation breakage interval, i.e., ABL1 in chronic myelogenous leukemia (CML). We designed and validated several single copy probes in the ABL1 and BCR genes to detect the common breakage sites in t(9;22) leukemia, which results in CML in adults and acute lymphoblastic leukemia (ALL) in children and adults. The positions and lengths of all single-copy sequence intervals within ABL1 and BCR are shown in Figures 1B&C, respectively. The majority of breaks in ABL1 occur within intron 1b between exons 1b and 1a. Breaks in the BCR gene generally occur in exons 12 through 17 in CML (i.e., the major or M-BCR region), and between exons 1 and 2 in ALL (i.e., the minor or m-BCR region). In the ABL1 gene, 10 intervals greater than 2 kb in length were found, 6 of which have been developed into scFISH probes. There are 11 such intervals in BCR, and probes have been developed and validated for 3 of these intervals.

The chromosomal locations of the ABL1 and BCR probes, which hybridize to 9q34 and 22q11.2, respectively, were verified on normal metaphase chromosomes. Subsequently, the probes were hybridized to cells of CML patients with t(9:22). Hybridization with a mixture of three ABL1 probes (IVS11, IVS4-IVS6, IVS3) confirmed that their location was distal of the chromosome 9 break. These probes hybridized to the derivative chromosome 22 [der(22)] but not the derivative chromosome 9 [der(9)] (Fig. 3G). Hybridization with a probe set that included the same three distal probes as well as 2 proximal ones (Exon 1b-IVS1b; IVS1b) revealed hybridization to both the der(22) and der(9) chromosomes (Fig. 3H). Thus, it can be inferred that the breakage is between IVS1b and IVS3. Additional probes from single copy regions can be developed in this interval to delineate this translocation breakpoint by FISH. Figure 1B shows four additional single copy intervals >1.5 kb in length. Shorter probes could also be utilized to further refine the breakpoints using approaches other than FISH (e.g., Southern analysis or long PCR).

(4) Detection of chromosomal rearrangements by separation or removal of paralogous targets in cis, i.e., inversion in acute myelogenous leukemia-type M4. The MYH11 gene is interrupted in 20% of patients with AML-M4 leukemia (with eosinophilia) and an inv(16)(p13q22) [Leblanc and Berger, 1997]. However, probes spanning the MYH11 breakpoint were difficult to design and produce, as this region of the genome has not been accurately or consistently represented in the databases, and its predicted location has changed in successive versions of the genome draft sequence. Probes were developed from the PM5 locus, which is related to a paralogous family of sequences with members both proximal and distal of MYH11. Each PM5 probe (64204–67682; 24509–27988) is highly similar to three other paralogs in 16p13.1 and one in 16p13.3 as demonstrated in the schematic in Figure 2A. The 16p13.1 sequence has 98% identity to the 16p13.3 copy, which spans IVS5–IVS10 of the PKD1 gene. The probe sequence at the 3′ end of PM5 is also found within the adjacent NPIP gene (~40 kb apart), and two copies, respectively 0.7 and 0.85 Mb, are centromeric of the PM5 paralog (close to PR02289). In the April, 2001 version of the genome draft, MYH11 was located between the PM5 and PR02289 paralogs. The PM5 and NPIP paralogs produced a single hybridization signal, consistent with their close proximity on the chromosome. By contrast, the PR02289 and PKD1 paralogs are sufficiently distant (0.7 and 16 Mb, respectively) from the NPIP/PM5 that each would be expected to yield a discrete signal on normal chromosomes. At lower chromosomal resolution (<550 band level), only the PKD1 hybridization is distinguishable from NPIP / PM5 (see the normal chromosome 16 in Fig. 2F), whereas at higher resolution, the PRO2289 paralog is also detectable as a discrete hybridization.

The hybridization signals from these probes split in cells of individuals with AML-M4 and an inv(16)(p13q22). The paralogs distal of MYH11 move adjacent to q arm chromatin (Fig. 2F), but at least one locus (presumably the PRO2289 paralog) is centromeric of MYH11 and remains in its original position on the p arm. The precise locations of these paralogs have been unstable in subsequent versions of the genome draft. Our results are consistent with subsequent genome drafts (August 2001–June 2002) which indicate copies of PM5 and PR02289 both proximal and distal of MYH11. The June 2002 freeze also shows triplication of NPIP and a gene related to PKD1 proximal of MYH11. The centromeric paralog(s) is immediately adjacent to a large sequence gap in the April 2001 sequence that is eliminated from subsequent versions. Since sequences close to large gaps are particularly prone to missassembly [Christian et al., 2002; P. Rogan, unpublished data], this region may not be correctly assembled due to the extensive duplication in this region. Our finding of a

residual signal on 16p in patients with the inversion substantiates the presence of this (and potentially other) paralogous PM5 duplicons proximal of MYH11.

DISCUSSION

Molecular cytogenetic methods can confirm and, in some instances, distinguish between structural chromosomal abnormalities that are indistinguishable by G-banding. We have demonstrated that scFISH probes detect small deletions (e.g., PWS/AS IC) and differentiate chromosomal breakpoints for a variety of genomic rearrangements (e.g., t(9;22) in CML and the atypical deletion in Smith-Magenis syndrome) at a resolution similar to that of genomic Southern analysis. This is feasible because of the dense genomic distribution of single copy intervals and the small size of these probes. Finished and draft genome sequences were of adequate quality to design and produce single copy DNA probes for all of the chromosomal disease regions we analyzed. With scFISH, we can differentiate chromosomal breakpoints among affected individuals that have apparently identical molecular cytogenetic findings using conventional genomic FISH probes (that are longer and generally less densely arrayed across the genome).

Despite their relatively short length, scFISH probes are efficiently detected on the chromosome for several reasons: (1) The probes recognize a contiguous chromosomal target, unlike cDNAs of comparable length which have multiple exons dispersed over a longer, discontinuous target. (2) The labeled single copy sequences in scFISH probes are effectively more concentrated in the hybridization reaction compared to conventional FISH probes, which generally contain numerous interspersed repetitive sequences. The concentration of unique sequences in conventional FISH probes is further reduced by excess $C_o t1$ DNA in the hybridization reaction, since single copy sequences that are contiguous with repetitive DNA are also sequestered in the $C_o t1$ matrix.

scFISH probes are fundamentally different from those used in conventional FISH (Table II). The properties of scFISH probes—shorter probe targets, sequence definition, and the absence of repetitive sequences—enable characterization of clinical cytogenetic abnormalities in unique ways. scFISH can reveal submicroscopic deletions of <10 kb on metaphase chromosomes; such rearrangements are not generally detectable with conventional recombinant DNA probes that span larger chromosomal targets. As an example, we have developed probes to detect imprinting center (IC) deletions in both the Prader-Willi (PWS) and Angelman syndromes [AS; Ohta et al., 1999]. A commercially available SNRPN probe (Vysis, Inc.), which is ~125 kb in length and spans both ICs and the flanking sequences, does not detect IC deletions. By co-hybridizing differentially labeled probes from within and outside of the IC region, different molecular classes of AS and PWS can be detected in a single assay. These classes include IC deletions, the common 3–5 Mb deletions [Nicholls et al., 1989], and uniparental disomy (UPD, based on allele-specific replication timing; White et al., 1996). These classes comprise the etiologies present in most PWS (~99%) and AS individuals (~80%; Cassidy et al., 2000). Such a strategy will obviate the need to perform methylation testing, microsatellite, or Southern hybridization analyses to rule out IC deletions, common deletions, or UPD. Only in the event of a normal finding in an AS individual or a stochastic imprinting defect would it be necessary to perform DNA testing such as sequencing of the UBE3A gene to detect point mutations.

Chromosomal breakpoints can be defined by performing a series of hybridizations using different combinations of ordered arrays of scFISH probes. We have developed three strategies for determining chromosomal breakpoint intervals. (1) Multiple linked probes were combined to localize a translocation interval (e.g., 9;22 translocation in CML). By combining different sets of scFISH probes and scoring the hybridization to the derivative chromosomes, we have localized the chromosome 9 breakage interval within the ABL1 gene in t(9;22). Given the high density of single copy intervals, the boundaries of the translocation interval can be refined by similar hybridizations of multiple scFISH probes closer to the chromosome 9 and 22 breakpoints. Subsequently, it should be feasible to retrieve the corresponding genomic sequences directly from the patient DNA by long PCR across the translocation breakpoint, thereby eliminating the need to isolate recombinant DNA clones spanning these junctions. This strategy is applicable to other types of translocations, however, analysis of complex rearrangements involving both deletions and translocations would require hybridizations of individual probes to verify the deletions. (2) Probes that detected a series of linked, low-copy

TABLE II. Characteristics of Conventional FISH and scFISH Probes

Properties	FISH	scFISH
Size (average)	>100 kb	<10 kb
Composition	Unique and repetitive sequences	Either unique or low copy[a] sequences
$C_o t1$ suppression	Required	None
Source of DNA	Recombinant DNA	Genomic amplification or recombinant DNA
DNA sequence	In some instances determined	Defined
Genome density[b]	153.8 kb to >1 Mb	17.4–20.9 kb
Chromosomal organization	Overlapping; may be heterochromatic or euchromatic	Non-overlapping; generally euchromatic

[a]Low copy probes derived from tightly linked intrachromosomal paralogous sequences.
[b]Average distance between intervals for ≥2 kb scFISH probes [Rogan et al., 2001] and for FISH BAC clones (Cheung et al., 2001).

paralogous sequences were used to detect chromosome breakage between these paralogs (e.g., inversion 16 in AML). Since low-copy paralogous sequence families are apparently common in the genome [Bailey et al., 2002], this may prove to be a general strategy for increasing chromosomal target size and detecting other types of rearrangements by scFISH. (3) Probes were developed that delineate atypical deletion boundaries in contiguous gene syndromes or define specific genes that are associated with specific phenotypes. For SMS, we developed probes and detected a smaller microdeletion than is commonly found [Chen et al., 1997]. For Williams-Beuren syndrome, we developed probes for detection of deletions at the LIMK1 locus which has been associated with impaired visuospatial cognition [Frangiskakis et al., 1996]. Thus, specific scFISH probes can be developed when the contributions of particular genes to clinical phenotypes have been delineated in contiguous gene syndromes.

With this methodology, we were able to develop probes for all of the desired chromosomal regions from the April 2001 draft, but were unable to generate products from certain intervals within these regions. Re-evaluation of these sequences in the June 2002 version by BLAT analysis did not provide an explanation for the majority of failed amplification reactions, since the template sequences are nearly identical to the earlier draft and are apparently comprised exclusively of single copy targets (Accessions NT_000102, positions 264229–271113; NT_002886, positions 271087–274978; NT_000671, positions 58683–63884 and 87492–93045; AP000057, positions 92651–103709; X96421, positions 11243–13374). The failure to amplify a product was due to an inaccurate primer sequence in only one instance (Accession AF119117, positions 34418-40332). The updated draft revealed a lack of primer or primer extension product specificity for a small number of products due to short paralogous regions elsewhere in the genome. Sequences adjacent to primer binding sites were related to non-allelic loci in the more recent genome draft (Accessions U07000, positions 120078–124759; NT_000671, positions 58683–63884) or contained previously undetected repetitive sequences overlapping or adjacent to a primer sequence (U41384, positions 821–4658; AC011167, positions 149350–153967; AL035367.4, positions 1194–5365).

In designing probes, we avoided intervals adjacent to large gaps (>20 kb) whenever possible, especially where placement of adjacent genes varied in successive versions of the draft sequence. Complex paralogous domains have also been shown to produce incorrect assemblies of these regions [Bailey et al., 2002]. These genomic features can lead to unexpected hybridization patterns. The April 2001 genome draft was consistent with the chromosome 16 hybridization pattern of the PM5 probes, which detects paralogs both centromeric and telomeric of MYH11, however, the centromeric loci are not consistently localized in subsequent versions of the draft sequence (through June 2002). We also noticed that a large gap upstream of ABL1 substantially altered the assembly of this region, as the order and distances separating genes and transcripts between ASS and ABL1 changed between April 2001 and June 2002. This suggests that more exhaustive validation (such as probe ordering on interphase cells) is required for probes designed to detect either paralogous sequence families or sequences adjacent to large gaps in the genome sequence.

Detailed molecular cytogenetic analysis of rare or private rearrangements has been traditionally hampered by the ready availability of appropriate commercial probes to detect these abnormalities. The simplicity and speed of single copy probe development now makes this task practical (e.g., monosomy 1p36) and eliminates impediments to rapid probe production using cloned probes (e.g., acquisition or de novo isolation, culturing and purification). Probes incorporating intronic, intergenic, or extragenic sequences can be produced that are distinct from proprietary probes consisting entirely of expressed sequences. Unique, non-expressed sequences, either individually or linked to expressed segments, are contained within every scFISH probe listed in Table I. Software to automate probe design expedited primer selection and probe synthesis, permitting in situ hybridization to be completed approximately 1 week from inception of the experiment. Other aspects of scFISH—including probe synthesis, purification, and labeling—are also amenable to laboratory automation and would further decrease the time required for probe preparation. While this study demonstrates and emphasizes the utility of the approach for characterizing chromosome abnormalities by scFISH, the single copy amplification products will have other applications, for example, as hybridization targets in comparative genomic hybridization and expression array studies.

ACKNOWLEDGMENTS

We thank Patricia Cazcarro for technical assistance and Amy Wolf for expert manuscript preparation.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE. 2002. Recent segmental duplications in the human genome. Science 297:1003–1013.

Bi W, Yan J, Stankiewicz P, Park SS, Walz K, Boerkoel CF, Potocki L, Shaffer LG, Devriendt K, Nowaczyk MJ, Inoue K, Lupski JR. 2002. Genes in a refined Smith-Magenis syndrome critical deletion interval on chromosome 17p11.2 and the syntenic region of the mouse. Genome Res 12(5):713–728.

Buiting K, Lich C, Cottrell S, Barnicoat A, Horsthemke B. 1999. A 5-kb imprinting center deletion in a family with Angelman syndrome reduces the shortest region of deletion overlap to 880 bp. Hum Genet 105:665–666.

Cassidy SB, Dykens E, Williams CA. 2000. Prader-Willi and Angelman syndromes: Sister imprinted disorders. Am J Med Genet 97:136–146.

Chen KS, Manian P, Koeuth T, Potocki L, Zhao Q, Chinault AC, Lee CC, Lupski JR. 1997. Homologous recombination of a flanking repeat gene cluster is a mechanism for a common contiguous gene deletion syndrome. Nat Genet 17:154–163.

Cheung VG, Nowak N, Jang W, Kirsch IR, Zhao S, Chen XN, Furey TS, Kim UJ, Kuo WL, Olivier M, Conroy J, Kasprzyk A, Massa H, Yonescu R, Sait S, Thoreen C, Snijders A, Lemyre E, Bailey JA, Bruzel A, Burrill WD, Clegg SM, Collins S, Dhami P, Friedman C, Han CS, Herrick S, Lee J, Ligon AH, Lowry S, Morley M, Narasimhan S, Osoegawa K, Peng Z, Plajzer-Frick I, Quade BJ, Scott D, Sirotkin K, Thorpe AA, Gray JW,

Hudson J, Pinkel D, Ried T, Rowen L, Shen-Ong GL, Strausberg RL, Birney E, Callen DF, Cheng JF, Cox DR, Doggett NA, Carter NP, Eichler EE, Haussler D, Korenberg JR, Morton CC, Albertson D, Schuler G, de Jong PJ, Trask BJ. 2001. Integration of cytogenetic landmarks into the draft sequence of the human genome. Nature 409(6822):953–958.

Christian SL, McDonough J, Liu Cy CY, Shaikh S, Vlamakis V, Badner JA, Chakravarti A, Gershon ES. 2002. An evaluation of the assembly of an approximately 15-Mb region on human chromosome 13q32-q33 linked to bipolar disorder and schizophrenia. Genomics 79(5):635–656.

Craig JM, Kraus J, Cremer T. 1997. Removal of repetitive sequences from FISH probes using PCR-assisted affinity chromatography. Hum Genet 100:472–476.

Florijn RJ, Bonden LA, Vrolijk H, Wiegant J, Vaandrager JW, Baa F, den Dunnen JT, Tanke JJ, van Ommen GJ, Raap AK. 1995. High-resolution DNA Fiber-FISH for genomic DNA mapping and colour bar-coding of large genes. Hum Mol Genet 4:831–836.

Francke U. 1999. Williams-Beuren syndrome: Genes and mechanisms. Hum Mol Genet 8:1947–1954.

Frangiskakis JM, Ewart AK, Morris CA, Mervis CB, Bertrand J, Robinson BF, Klein BP, Ensing GJ, Everett LA, Green ED, Proschel C, Gutowski NJ, Noble M, Atkinson DL, Odelberg SJ, Keating MT. 1996. LIM-kinase1 hemizygosity implicated in impaired visuospatial constructive cognition. Cell 12:86:59–69.

Fuscoe JC, Collins CC, Pinkel D, Gray JW. 1989. An efficient method for selecting unique-sequence clones from DNA libraries and its application to fluorescent staining of human chromosome 21 using in situ hybridization. Genomics 5:100–109.

Kent WJ. 2002. BLAT—The BLAST-like alignment tool. Genome Res 12: 656–664.

Knoll JHM, Lichter P. 1994. In situ hybridization to metaphase chromosomes and interphase nuclei. In: Dracopoli NC, Haines JL, Korf BR, Moir DT, Morton CC, Seidman CE, Seidman JG, Smith DR, editors. Current protocols in human genetics, Vol 1. New York: Green-Wiley, Unit 4.3.

Knoll JH, Nicholls RD, Magenis RE, Graham JM Jr, Lalande M, Latt SA. 1989. Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 32:285–290.

Korenberg JR, Chen XN, Sun Z, Shi ZY, Ma S, Vataru E, Yimlamai D, Weissenbach JS, Shizuya H, Simon MI. 1999. Human genome anatomy: BACs integrating the genetic and cytogenetic maps for bridging genome and biomedicine. Genome Res 9:994–1001.

Leblanc T, Berger R. 1997. Molecular cytogenetics of childhood acute myelogenous leukemias. Eur J Haematol 59:1–13.

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC. 1988. Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80:224–234.

Nicholls RD, Knoll JH, Glatt K, Hersh JH, Brewster TD, Graham JM Jr, Wurster-Hill D, Wharton R, Latt SA. 1989. Restriction fragment length polymorphisms within proximal 15q and their use in molecular cytogenetics and the Prader-Willi syndrome. Amer J Med Genet 33: 66–77.

Ohta T, Gray TA, Rogan PK, Buiting K, Gabriel JM, Saitoh S, Muralidhar B, Bilienska B, Krajewska-Walasek M, Driscoll DJ, Horsthemke B, Butler MJ, Nicholls RD. 1999. Imprinting-mutation mechanisms in Prader-Willi syndrome. Am J Hum Genet 64:397–413.

Osborne LR, Martindale D, Scherer SW, Shi XM, Huizenga J, Heng HH, Costa T, Pober B, Lew L, Brinkman J, Rommens J, Koop B, Tsui LC. 1996. Identification of genes from a 500-kb region at 7q11.23 that is commonly deleted in Williams syndrome patients. Genomics 36:328–336.

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J. 1988. Fluorescence in situ hybridization with human chromosome-specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85:9138–9142.

Rogan PK, Cazcarro P, Knoll JHM. 2001. Sequence-based desgin of single-copy genomic DNA probes for fluorescence in situ hybridization. Genome Research 11:1086–1094.

Saitoh S, Buiting K, Rogan PK, Buxton JL, Driscoll DJ, Arnemann J, Konig R, Malcolm S, Horsthemke B, Nicholls RD. 1996. Minimal definition of the imprinting center and fixation of chromosome 15q11-q13 epigenotype by imprinting mutations. Proc Natl Acad Sci USA 93: 7811–7815.

Sealey PG, Whittaker PA, Southern EM. 1985. Removal of repeated sequences from hybridization probes. Nucl Acids Res 13:1905–1922.

Trask B, Fertitta A, Christensen M, Youngblom J, Bergmann A, Copeland A, de Jong P, Mohrenweiser H, Olsen A, Carrano A. 1993. Fluorescence in situ hybridization mapping of human chromosome 19: Cytogenetic band location of 540 cosmids and 70 genes or DNA markers. Genomics 15: 133–145.

White LM, Rogan PK, Nicholls RD, Wu BL, Korf B, Knoll JHM. 1996. Allele-specific replication of 15q11-q13 loci: a diagnostic test for detection of uniparental disomy. Am J Hum Genet 59:423–430.