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Sensitivity of Multiple Color Spectral Karyotyping in Detecting Small Interchromosomal Rearrangements

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ABSTRACT

Multiple color spectral karyotyping (SKY) has been proven to be a very useful tool for characterization of the complex rearrangements in cancer cells and the *de novo* constitutional structural abnormalities. The sensitivity of SKY in detecting interchromosomal alterations was assessed with 10 constitutional translocations involving subtelomeric regions. Among the 13 small segments tested, 9 were clearly visualized and 8 were unambiguously identified by SKY. Fluorescence in situ hybridizations (FISH) with subtelomeric probes confirmed the reciprocity in three of the four translocations in which a small segment was not detectable by SKY. On the basis of resolution level of G-banding and the information obtained from the FISH analysis, the minimum alteration that SKY can detect is estimated to be 1,000–2,000 kbp in size with the currently available probes. This study has demonstrated the power, but also the limitations, of SKY in detecting small interchromosomal alterations, particularly those in subtelomeric regions.

INTRODUCTION

The applications of fluorescence in situ hybridization (FISH) with unique DNA sequences, whole chromosome painting probes, and the probes generated from particular regions by microdissection, along with the use of comparative genomic hybridization, have enabled us to characterize numerous chromosome abnormalities, and thus have dramatically enhanced the capacity of cytogenetic diagnosis (Cremer *et al.,* 1998; Fan *et al.,* 1990, Guan *et al.,* 1994; Ledbetter and Ballabio, 1995; Levy *et al.,* 1998; Blancato, 1999; Fan *et al.,* 1999a–c]. The recently developed technology of spectral karyotyping (SKY) allows a simultaneous visualization of all human chromosomes by a single hybridization of 24 differentially labeled chromosome painting probes and provides a very useful tool for the characterization of the complex chromosome rearrangements in cancer cells (Garini *et al.,* 1996; Schröck *et al.,* 1996; Coleman *et al.,* 1997; Veldman *et al.,* 1997; Allen *et al.,* 1998; Rao *et al.,* 1998; Sawyer *et al.,* 1998; Zattara-Cannoni *et al.,* 1998; Fleischman *et al.,* 1999; Padilla-Nash *et al.,* 1999; Rogatta *et al.,* 1999; Rowley *et al.,* 1999; Trakhtenbrot *et al.,* 1999) and in *de novo* constitutional structural abnormalities (Schröck *et al.,* 1997; Haddad *et al.,* 1998; Huang *et al.,* 1998; Phelan *et al.,* 1998; Reddy *et al.,* 1999).

The size of chromosome alteration detectable by SKY was initially suggested to be >1,500 kbp based on the analysis of a reciprocal translocation, t(1;11)(q44;p15.3) and a submicroscopic translocation t(16;17) (Schröck *et al.,* 1996). An assessment with a sufficient number of translocations involving small segments appears to be necessary, because the sensitivity of SKY on each individual chromosome may vary depending on the coverage of each particular probe in the hybridization cocktail (Garini *et al.,* 1996; Schröck *et al.,* 1996). We have tested the sensitivity of SKY in detecting small interchromosomal rearrangements in 10 selected cases with previously identified constitutional translocations.

MATERIALS AND METHODS

Patients

The criterion for selecting the cases was that a translocation had involved a single terminal band at the resolution level of

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TABLE 1. TRANSLOCATION IN THE SELECTED CASES

Cases	*Clinical history*	*Karyotype*
1	Multiple miscarriages	46,XX,t(2;19)(p23;q13.4)
2	Familial translocation	46,XX,t(12;14)(q24.31;q32.3)
3	Multiple miscarriages	46,XX,t(2;7)(p23.2;q36.1)
4	Multiple miscarriages	46,XX,t(5;8)(q15;q24.22)
5	Triogonocephaly	46,XX,t(18;22)(q23;q11.2)
6	Multiple miscarriages	46,XX,t(14;18)(q13;q23)
7	Son with der(15)	46,XY,t(6;15)(q25.3;q26.3)
8	Fetus with der(18)	46,XX,t(2;18)(p12;p11.32)
9	Familial translocation	46,XY,t(14;18)(q32.3;p11.23)
10	Multiple miscarriages	46,XX,t(19;21)(q13.1;q22.3)

550 bands or a segment smaller than three terminal bands at the resolution level of 850 bands per haploidy in at least one of the derivative chromosomes. Ten cases were selected for this study on the basis of previous cytogenetic findings (Table 1). The reasons for chromosome study in 9 adult patients included a history of multiple miscarriages (5 cases), a familial translocation (2 cases), a chromosomally abnormal child (1 case), and a pregnancy with an unbalanced karyotype (1 case). In a pediatric patient (case 5), trigonocephaly was the only clinical concern.

High-resolution G-banding

Chromosome slides were prepared from synchronized lymphocyte cultures for most of the cases, and 5–10 prophase or prometaphase cells were analyzed with G-banding at the level of resolution of 550–850 bands per haploidy. The karyotypes were interpreted according to the ISCN 1995.

Sky analysis

To test if the small segments involved in the translocations can be visualized and identified with SKY analysis, chromosome slides were hybridized with the 24-color SkyPaint probes (Applied Spectral Imaging, Carlsbad, CA), according to the protocol recommended by the manufacturer of the probes. Briefly, slides were treated in pepsin solution (10–30 μg/ml in 0.01 *M* HCl) at 37°C for 3–5 min, and washed with phosphate-buffered saline (PBS), PBS/$MgCl_2$ solution and 1% formaldehyde, and then dehydrated through 70%, 80%, and 100% ethanol. Slides were denatured in 70% formamide/2× SSC at 70°C, and hybridized with the denatured SkyPaint mixture at 37°C for 36 hours. Slides were then washed and stained with DAPI. SKY analysis was performed with the SkyVision imaging system (Applied Spectral Imaging, Carlsbad, CA) equipped with a Zeiss Axioskop fluorescence microscope. Each individual chromosome was visualized with both display and classified images. Ten metaphases were analyzed by Sky for each case.

FISH with subtelomeric probes

FISH was performed according to the protocols recommended by the manufacturers of DNA probes (Vysis, Downers Grove, IL). Specific subtelomeric probes for 14q, 18p, and 18q were used in this study. Slides were stained with DAPI and analyzed with the SkyVision imaging system. G-banding converted from DAPI staining after hybridization was also used to facilitate the identification of the derivative chromosomes.

RESULTS

High-resolution G-binding analysis confirmed the karyotypes previously reported (Table 1, Fig. 1). In most of these cases, only one translocated segment could be clearly seen at the early metaphase or prophase stage, although these translocations were assumed to be reciprocal based on the finding of two derivative chromosomes in each case. The results of SKY imaging analysis are listed in Table 2 and shown in Fig. 1. Of 13 small chromosome segments tested, SKY clearly visualized nine segments on the derivative chromosomes and unambiguously identified the structural origins of eight segments.

Case 7 represented a situation when a small rearranged segment was detectable, but not identifiable. The recriprocal translocation in this case involved two small segments, 6q25.3-qter and 15q26.3-qter. Although both were visualized with distinct colors, SKY could only identify the origin of the segment translocated onto derivative 15 from 6q, but not the one translocated onto 6q from 15q with certainty.

In cases 5 and 6, SKY could only detect the large segments, and not the small ones involved in the translocations. In case 8, a very tiny yellow dot was occasionally visualized at the tip of the short arm of der(2) in some cells (see Fig. 1), but the structural origin of this small segment could not be identified. The translocation in case 9 involved two small segments, 14q32.3-qter and 18p11.23-pter. SKY was able to detect the segment 18p11.23-pter on the der(14), but not the segment 14q32.3-qter on the der(18). FISH with subtelomeric probes for 18q, 18p, and 14q confirmed the reciprocity of translocation t(14;18)(q13;q23) in case 6, t(2;18)(p13;p11.32) in case 8, and t(14;18)(q32.3;p11.23) in case 9, respectively. In case 5, however, FISH with the 18q subtelomeric probe showed signals on both the normal chromosome 18 and the der(18) at the breakpoint junction (Fig. 2), but not on the der(22), indicating that the breakpoint in the der(18) is likely to be distal to the subtelomeric sequences, or else no material has been translocated from 18q onto 22q.

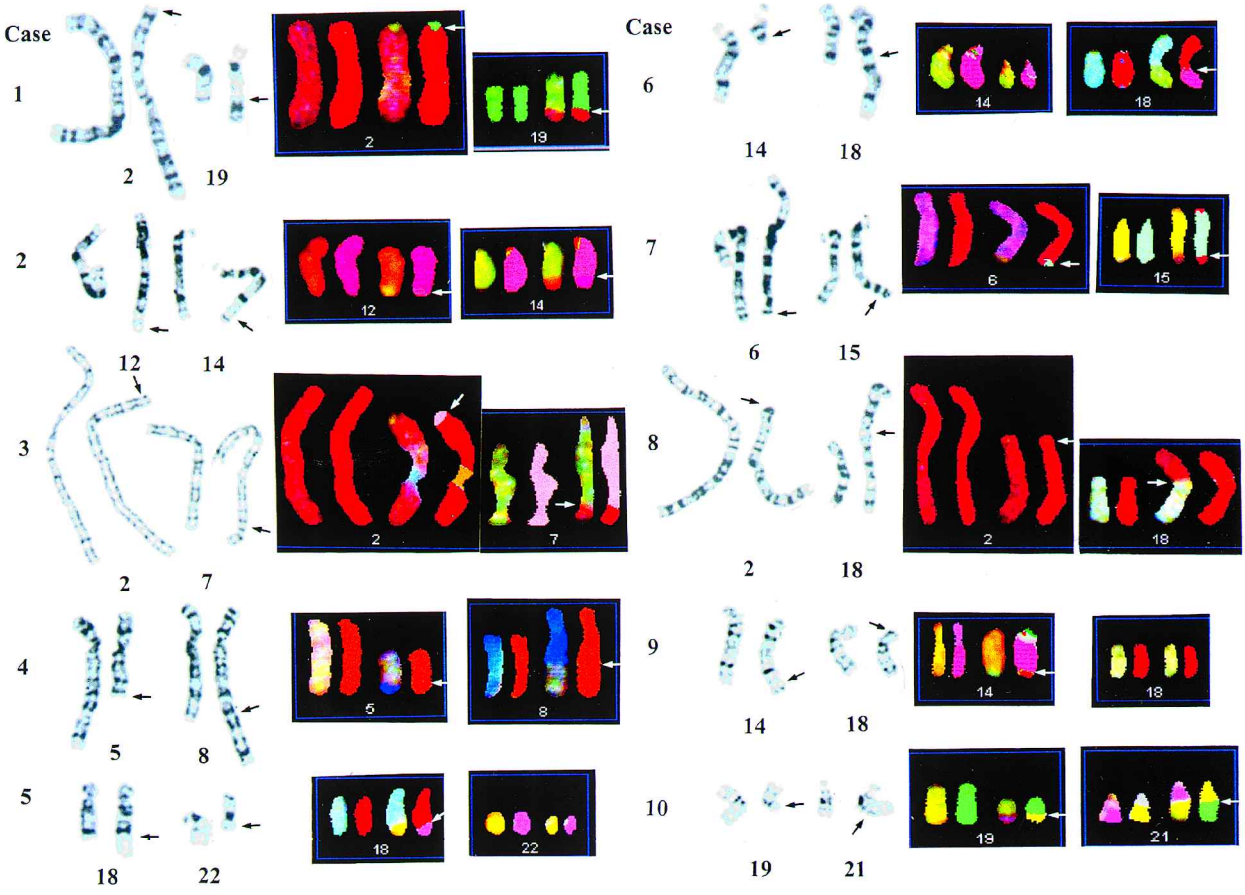

FIG. 1. Partial karyotypes showing the translocations identified by high resolution G-banding and the results of SKY. Arrows indicate the breakpoints of translocations assigned with G-banding analysis and visualized by SKY. Each color insert shows a pair of chromosomes (normal one on the left and abnormal one on the right), and each chromosome is shown with a display color (left) and a classified color (right).

DISCUSSION

We have tested the ability of SKY to detect 13 small telomeric segments involved in 10 reciprocal translocations. Nine small segments were clearly demonstrated and the origins of eight segments were unambiguously identified. These detectable segments originated from 6q, 7q, 8q, 12q, 14q, 15q, 18p, 19q, and 21q, respectively. At the level of resolution of 850 bands, the size of these segments ranged from part of a single band to two and one-half terminal bands. Based on the breakpoints at this level of resolution, the small segments involved in these translocations are approximately in the range of 2,000–9,000 kbp in size (assuming 3,500 kbp/band). The translocated small segment, 15q26.3-qter, in case 7 was clearly visualized, but its structural origin could not be identified. The segment 15q26.3-qter represents only a part of a single terminal band at the resolution level of 850 bands per haploidy, likely with a size of 1,000–2,000 kbp.

Cases 6, 8, and 9 have illustrated the limitations of SKY. Because the reciprocity of the translocations in cases 6, 8, and 9 could be confirmed by FISH with the subtelomeric probes, the small segments involved in these translocations are estimated to be at least larger than 100 kbp in size, given that the subtelomeric DNA probe contains a 60 ~ 65 kbp unique sequence within 100–300 kbp of the chromosome end (Ning *et al.,* 1996).

In case 5, the small segment 18q23-qter translocated onto

TABLE 2. RESULTS OF SKY ANALYSIS

Cases	*Small translocated segments*	*Results of SKY*
1	19q13.4-qter	+
2	12q24.31-qter/ 14q32.3-qter	+/+
3	7q36.1-qter	+
4	8q24.22-qter	+
5	18q23-qter	−
6	18q23-qter	−
7	6q25.3-qter/ 15q26.32-qter	+/+?
8	18p11.32-pter	−
9	14q32.3-qter/ 18p11.23-pter	−/+
10	21q22.3-qter	+

+, Segment detectable with its origin unambiguously identified; +?, detectable with a questionable identification; −, not detectable.

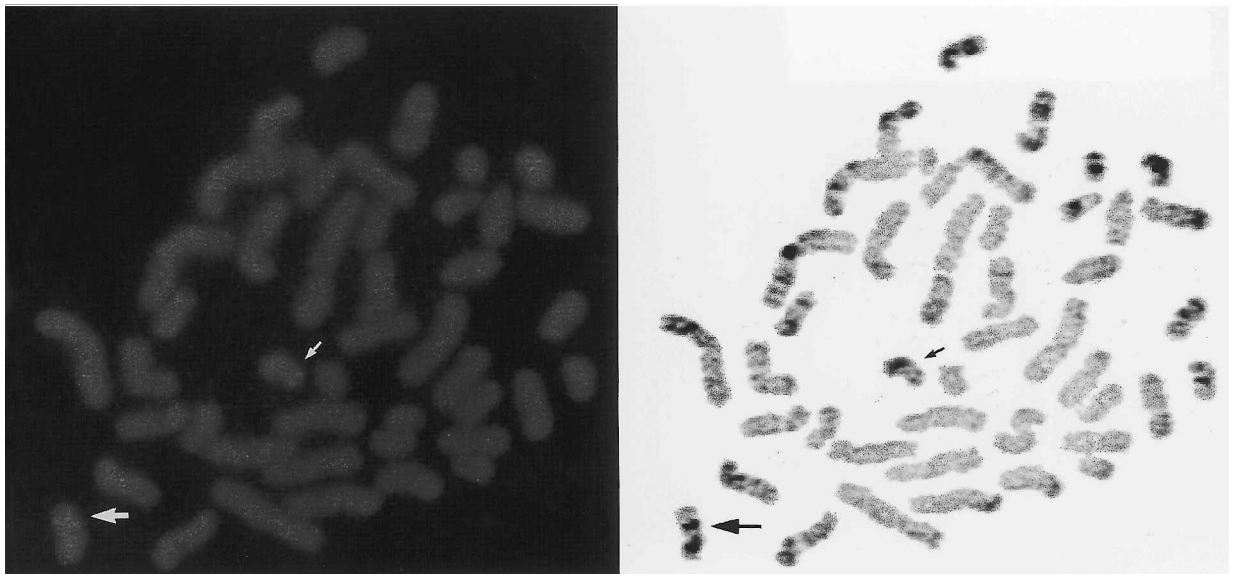

FIG. 2. A metaphase of case 5 with DAPI staining (left) showing hybridization signals of 18q specific subtelomeric probe on the normal chromosome 18 in the telomeric region (small arrow) and on the derivative 18 at the breakpoint junction (large arrow). Chromosomes 18 were identified in the same metaphase with G-banding converted from DAPI staining (right).

22q was not detectable by both SKY and the subtelomeric probe used. The conventional G-banding analysis revealed an 18q+ and a 22q− in the patient, and normal karyotypes in both parents. The father had the same variant staining patterns observed in the child in the short arms of a chromosome 21 and the der(22), indicating that the translocation is a real *de novo* change. The translocation with breakpoints at 18q23 and 22q11.2 was assumed to be reciprocal. SKY could only detect the 22q material on the der(18), but not the 18q material on the der(22). Interestingly, FISH with the 18q subtelomeric probe showed hybridization signals on the normal 18q and the der(18) at the junction of breakpoints instead of on the der(22). The interpretation for this result is that the breakpoint at 18q is distal to the sequences of the subtelomeric probe. Thus, the size of the segment 18q23-qter translocated onto 22q in this case is likely to be smaller than 200 kbp, if the translocation is indeed reciprocal. However, a very subtle deletion in the telomeric region of 18q could not be ruled out.

It was noted that three of the four undetectable small segments originated from chromosome 18. However, this may not be related to the probe used, but determined by the DNA size of the segments translocated. Obviously, the same chromosome segment described in different cases could differ by 1,000–2,000 kbp in size, even though they have the same breakpoint, as exemplified by the segment 18q23-qter in cases 5 and 6.

SKY has already demonstrated its diagnostic value in clinical and cancer cytogenetics. Many complex chromosome rearrangements in leukemia and cancer cells have been characterized with this recently developed technology (Coleman *et al.,* 1997; Veldman *et al.,* 1997; Allen *et al.,* 1998; Rao *et al.,* 1998; Sawyer *et al.,* 1998; Zattara-Cannoni *et al.,* 1998; Fleischman *et al.,* 1999; Padilla-Nash *et al.,* 1999; Rogatta *et al.,* 1999; Rowley *et al.,* 1999; Trakhtenbrot *et al.,* 1999). SKY has also refined the cytogenetic diagnosis for some constitutional chromosomal abnormalities, thereby contributing to the understanding of genotype–phenotype correlations in dysmorphic syndromes (Schröck *et al.,* 1997; Haddad *et al.,* 1998; Huang *et al.,* 1998; Phelan *et al.,* 1998; Reddy *et al.,* 1999). On the basis of the resolution level of G-binding and the information obtained from the FISH studies with subtelomeric probes, we estimated that the minimum alteration that SKY can detect is in the range of 1,000 to 2,000 kbp in size with the currently available probes. We also believe that the quality of chromosome preparation would affect the efficiency of hybridization and, therefore, the sensitivity of SKY. It appears that FISH with chromosome-specific subtelomeric probes is more sensitive in detecting the deletions or translocations in the telomeric regions. For this particular clinical application, FISH using a device containing 41 telomere-specific probes, which allows simultaneous analysis of the subtelomeric regions of every chromosome, has provided another powerful tool for cytogenetic screening or diagnosis (Knight *et al.,* 1997; Anderlid *et al.,* 1999). It is known that comparative genomic hybridization arrays for the telomeric regions are being developed (Lese *et al.,* 1999). While SKY is very useful to identify small interchromosomal alterations, efforts may need to be made to improve further the sensitivity of SKY by increasing the coverage of DNA probes for the whole genome, including the telomeric regions.

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REFERENCES

ALLEN, R.J., SMITH, S.D., MOLDWIN, R.L., LU, M.M., GIORDANO, L., VIGNON, C., SUTO, Y., HARDEN, A., TOMEK, R., VELDMAN, T., REID, T., LARSON, R.A., LE BEAU, M.M., ROWLEY, J.D., and ZELEZNIK-LE, N. (1998). Establishment and characterization of a megakaryoblast cell line with amplication of MLL. Leukemia **12,** 1119–1127.

ANDERLID, B., ANNERÉN, G., BLENNOW, E., and NORDENSKJOLD, M. (1999). Subtelomeric rearrangements detected by FISH in patients with idiopathic mental retardation. Am. J. Hum. Genet. **65** (Suppl.), A67.

BLANCATO, J.K. (1999). Fluorescence in situ hybridization. In *The Principles of Clinical Cytogenetics.* S.L. Gersen and M.B. Keagle (eds.). Humana Press, pp. 445–471.

COLEMAN, A.E., SCHRÖCK, E., WEAVER, Z., DU MANOIR, S., YANG, F., FERGUSON-SMITH, M.A., RIED, T., and JANZ, S. (1997). Previously hidden chromosome aberrations in t(12;15)-positive BALB/c plasmacytoma uncovered by multicolor spectral karyotyping. Cancer Res. **57,** 4585–4592.

CREMER, T., LICHTER, P., BORDEN, J., WARD, D.C., and MANUELIDIS, L. (1998). Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes. Hum. Genet. **80,** 235–246.

FAN, Y.S., DAVIS, L.M., and SHOWS, T.B. (1990). Mapping small DNA sequences by fluorescence in situ hybridization directly on banded metaphase chromosomes. Proc. Natl. Acad. Sci. USA **87,** 6223–6227.

FAN, Y.S., RIZKALLA, K., and BARR, R.M. (1999a). Complex rearrangement of chromosome 1, 7, and 16 in chronic myelocytic leukemia. Cancer Genet. Cytogenet. **112,** 190–192.

FAN, Y.S., RIZKALLA, K., and BARR, R.M. (1999b). A new complex variant Philadelphia chromosome, t(1;9;22)ins(17;22), characterized by fluorescence in situ hybridization in an adult ALL. Leukemia Res. **23,** 1001–1006.

FAN, Y.S., JUNG, J., and HAMILTON, B. (1999c). Small terminal deletion of 1p and duplication of 1q: cytogenetics, FISH studies, and clinical observations at newborn and at age 16 years. Am. J. Med. Genet. **86,** 118–123.

FLEISCHMAN, E.W., RESHMI, S., SOKOVA, O.I., KIRICHENKO, O.P., KONSTANTINOVA, L.N., KULAGINA, O.E., FRENKEL, M.A., and ROWLEY, J.D. (1999). Increased karyotype precision using fluorescence in situ hybridization and spectral karyotyping in patients with myeloid malignancies. Cancer Genet. Cytogenet. **108,** 166–170.

GARINI, Y., MACVILLE, M., DU MANOIR, S., BUCKWALD, R.A., LAVI, M., KATZIR N., WINE, D., BAR-AM, I., SCHRÖCK, E., CABIB, D., and RIED, T. (1996). Spectral karyotyping. Bioimaging **4,** 65–72.

GUAN, X.Y., MELTZER, P., and TRENT, J. (1994). Rapid generation of whole chromosome painting probes (WCPs) by chromosome microdissection. Genomics **22,** 101–107.

HADDAD, B.R., SCHRÖCK, E., MECK, J., COWAN J., YOUNG, H., FERGUSON-SMITH, M.A., DU MANOIR, S., and RIED, T. (1998). Identification of de novo chromosomal markers and derivatives by spectral karyotyping. Hum. Genet. **103,** 619–625.

HUANG, B., NING, Y., LAMB, A.N., SANDLIN, C.J., JAMEHDOR, M., RIED, T., and BARTLEY, J. (1998). Identification of an unusual marker chromosome by spectral karyotyping. Am. J. Med. Genet. **80,** 368–372.

KNIGHT S.J.L., HORSLEY, S.W., REGAN, R., LAWRIE, N.M., MAHER, E.J., CARDY, D.L.N., FLINT, J., and KEARNEY, L. (1997). Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. Eur. J. Hum. Genet. **5,** 1–8.

LEDBETTER, D., and BALLABIO, A. (1995). Molecular cytogenetics of continuous gene syndromes. In *The Metabolic Bases of Inherited Diseases,* 7th ed. C. Scriver, A. Beaudet, W. Sly, and D. Valle (eds.). McGraw Hill, pp. 811–839.

LESE, C.M., ZHANG, X., PINKEL, D., BAO, P., LERMER, N., CHE, D., SHI, J., MÜLER, U., and LEDBETTER, D.H. (1999). Comparative genomic hybridization arrays: towards a "telomere chip." Am. J. Hum. Genet. **65** (Suppl.), A9.

LEVY, B., DUNN, T.M., KAFFE, S., KARDON, N., and HIRSCHHORN, K. (1998). Clinical applications of comparative genomic hybridization. Genet. Med. **1,** 4–12.

NING, Y., ROSCHKE, A., SMITH, A.C.M., MACHA, M., PRECHT, K., REITHMAN, H., LEDBETTER, D., FLINT, J., HORSLEY, S., REGAN, R., KEARNEY, L., KNIGHT, S., KVALOY, K., and BROWN, W.R.A. (1996). A complete set of human telomeric probes and their clinical application. Nature Genet. **14,** 86–89.

PADILLA-NASH, H.M., NASH, W.G., PADILLA, G.M., ROBERSON, K.M., ROBERSON, C.N., MACVILLE, M., SCHRÖCK, E., and RIED, T. (1999). Molecular cytogenetic analysis of the bladder carcinoma cell line BK-10 by spectral karyotyping. Genes Chromosomes Cancer **25,** 53–59.

PHELAN, M.C., BLACKBURN, W., ROGERS, R.C., CRAWFORD, E.C., COOLEY, N.R., JR., SCHRÖCK, E., NING, Y., and RIED, T. (1998). FISH analysis of a complex chromosome rearrangement involving nine breakpoints on chromosomes 6, 12, 14, and 16. Prenat. Diagn. **18,** 1174–1180.

RAO, P.H., CIGUDOSA, J.C., NING, Y., CALASANZ, M.J., IIDA, S., TAGAWA, S., MICHAELI, J., KLEIN, B., DALLA-FAVERA, R., JHANWAR, S.C., RIED, T., and CHAGANTI, R.S.K. (1998). Multicolor spectral karyotyping identifies new recurring breakpoints and translocations in multiple myeloma. Blood **92,** 1743–1748.

REDDY, K.S., SULCOVA, V., YOUNG, H., BLANCATO, J.K., and HADDAD, B.R. (1999). De novo mosaic add(3) characterized to be trisomy 14q31-qter using spectral karyotyping and subtelomeric probes. Am. J. Med. Genet. **82,** 318–321.

ROGATTA, S.R., RAINHO, C.A., ZHANG, Z.M., FIGUEIREDO, F., BARBIERI-NETO, J., GEORGETTO, S.M., and SQUIRE, J.A. (1999). Hemangioendothelioma of bone in a patient with a constitutional supernumerary marker. Cancer Genet. Cytogenet. **110,** 23–27.

ROWLEY, J.D., RESHMI, S., CARLSON, K., and ROULSTON, D. (1999). Spectral karyotype analysis of T-cell acute leukemia. Blood **93,** 2038–2042.

SAWYER, J.R., LUKACS, J.L., MUNSHI, N., DESIKAN, K.R., SINGHAL, S., MEHTA, J., SIEGEL, D., SHAUGHNESSY, J., and BARLOGIE, B. (1998). Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. Blood **92,** 4269–4278.

SCHRÖCK, E., DU MANOIR, S., VELDMAN, T., SCHOELL, B., WIENBERG, J., FERGUSON-SMITH, M.A., NING, Y., LEDBETTER, D.H., BAR-AM, I., SOENKSEN, D., GARINI, Y., and REID, T. (1996). Multicolor spectral karyotyping of human chromosomes. Science **273,** 494–497.

SCHRÖCK, E., VELDMAN, T., PADILLA-NASH, H., NING, Y., SPURBECK, J., JALAL, S., SHAFFER, L.G., PAPENHAUSEN, P., KOZMA, C., PHELAN, M.C., KJELDSEN, E., SCHONBERG, S.A., O'BRIEN, P., BIESECKER, L., DU MANOIR, S., and RIED, T. (1997). Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities abnormalities. Hum. Genet. **101,** 255–262.

TRAKHTENBROT, L., COHEN, N., ROSNER, E., GIPSH, N., BROK-SIMONI, F., MANDEL, M., AMARIGLIO, N., and RECHAVI, G. (1999). Coexistence of several unbalanced translocations in a case of neuroblastoma: the contribution of multicolor spectral karyotyping. Cancer Genet. Cytogenet. **112,** 119–123.

VELDMAN, T., VIGNON, C., SCHRÖCK, E., ROWLEY, J.D., and RIED, T. (1997). Hidden chromosome abnormalities in haematological malignancies detected by multicolor spectral karyotyping. Nature Genet. **15,** 406–410.

ZATTARA-CANNONI, H., DUFOUR, H., LEPIDI, H., CHATEL, C., GRISOLI, F., and VAGNER-CAPODANO, A.M. (1998). Hidden chromosome abnormalities in a primary central nervous system lymphoma detected by multicolor spectral karyotyping. Cancer Genet. Cytogenet. **107,** 98–101.

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