

# Multicolor banding technique, spectral color banding (SCAN): new development and applications

N. Kakazu<sup>a, b</sup> T. Abe<sup>a</sup>

<sup>a</sup>Department of Molecular-Targeting Cancer Prevention, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto; <sup>b</sup>Department of Environmental and Preventive Medicine, Shimane University School of Medicine, Shimane (Japan)

Manuscript received 17 March 2006; accepted in revised form for publication by T. Liehr, 27 March 2006.

**Abstract.** Conventional banding techniques can characterize chromosomal aberrations associated with tumors and congenital diseases with considerable precision. However, chromosomal aberrations that have been overlooked or are difficult to analyze even by skilled cytogeneticists were also often noted. Following the introduction of multi-color karyotyping such as spectral karyotyping (SKY) and multiplex-fluorescence in situ hybridization (M-FISH), it is possible to identify this kind of cryptic or complex aberration comprehensively by a single analysis. To date, multi-color karyotyping techniques have been established as useful tools for cytogenetic analysis. However, since this technique depends on whole chromosome painting probes, it involves limitations in that the origin of aberrant segments can be identified only in units of chromosomes. To overcome these limitations, we have recently developed spectral color banding (SCAN) as a new multi-color banding technique based on the SKY methodology. This new tech-

nique may be deemed as an ideal chromosome banding technique since it allows representation of a multi-color banding pattern matching the corresponding G-banding pattern. We applied this technique to the analysis of chromosomal aberrations in tumors that had not been fully characterized by G-banding or SKY and found it capable of (1) detecting intrachromosomal aberrations; (2) identifying the origin of aberrant segments in units of bands; and (3) precisely determining the breakpoints of complex rearrangements. We also demonstrated that SCAN is expected to allow cytogenetic analysis with a constant adequate resolution close to the 400-band level regardless of the degree of chromosome condensation. As compared to the conventional SKY analysis, SCAN has remarkably higher accuracy for a particular chromosome, allowing analysis in units of bands instead of in units of chromosomes and is hence promising as a means of cytogenetic analysis.

Copyright © 2006 S. Karger AG, Basel

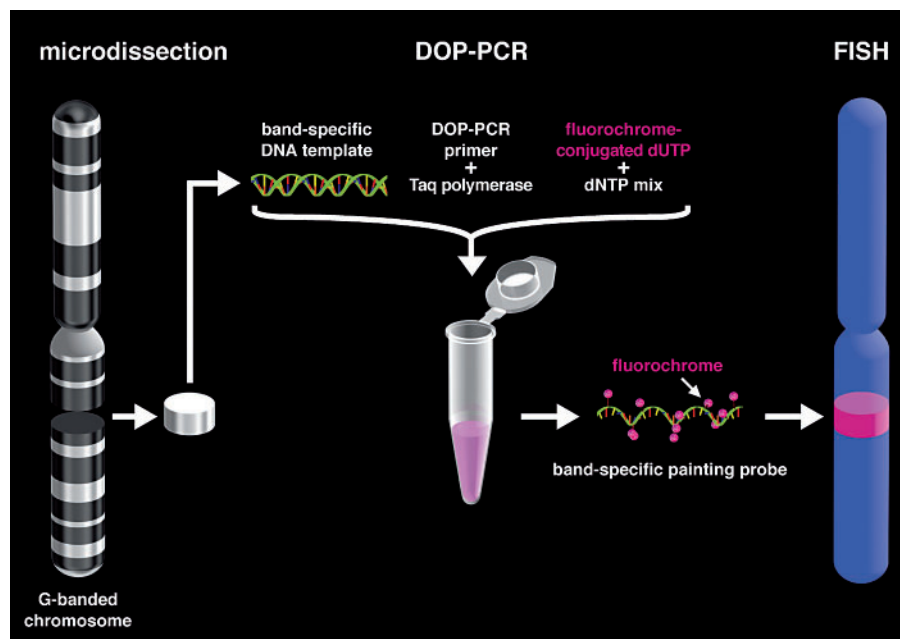
Chromosome banding techniques have been an indispensable tool for the identification of chromosomal aberrations associated with tumors and congenital diseases. Over the past three decades, G-banding has served as a standard

chromosome banding technique. However, since G-banding involves morphological identification of each chromosome based solely on banding patterns of monochrome shade, it has unavoidable limitations in accuracy of karyotyping. Furthermore, long experience and high skill are needed for analyzing chromosomal aberrations with this technique. Still more, it is not uncommon that even experienced cytogeneticists overlook cryptic chromosomal aberrations or are unable to identify complex chromosomal rearrangements completely in G-banding analysis. To overcome these shortcomings of G-banding, new techniques of cytogenetic analysis were developed in 1996; they are multi-color karyotyping techniques such as spectral karyotyping (SKY) (Schröck et al., 1996) and multiplex-fluorescence

This work was supported by Grant-in-Aid for Scientific Research on Priority Areas (C) 'Medical Genome Science' from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Request reprints from Naoki Kakazu, MD, PhD,  
Department of Environmental and Preventive Medicine  
Shimane University School of Medicine  
89-1 Enya-cho, Izumo, Shimane 693-8501 (Japan)  
telephone: +81(853) 20-2166; fax: +81(853) 23-6112  
e-mail: kakazu@med.shimane-u.ac.jp

**Fig. 1.** FISH with a band-specific painting probe generated by microdissection. After a chromosome region corresponding to a particular G-band was cut out physically under a microscope by microdissection and collected into a microtube, the band-specific DNA can be extracted and purified. This DNA was directly labeled with a fluorochrome using DOP-PCR. FISH on a normal metaphase chromosome with this product serving as a band-specific painting probe showed the signal identical to the original cut-out band.

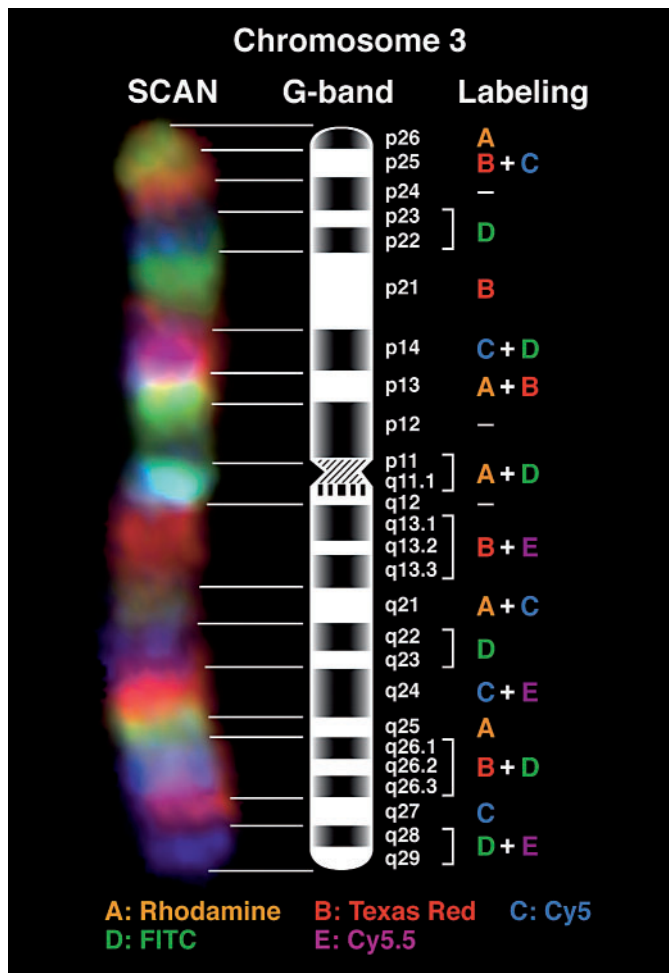


in situ hybridization (M-FISH) (Speicher et al., 1996). SKY analysis is a FISH-based molecular cytogenetic technique that allows simultaneous discrimination of all 24 human chromosomes in different colors in a single hybridization, making use of the SKY probe mixture containing differentially labeled painting probes for each chromosome. This approach has been particularly useful for the comprehensive identification of chromosomal aberrations, which are difficult to analyze by G-banding (Veldman et al., 1997; Kakazu et al., 1999, 2000). Multicolor karyotyping techniques have been spreading rapidly at clinical laboratories as an effective diagnostic tool. However, multicolor karyotyping also involves three limitations associated with the use of whole chromosome painting probes: (1) identification of the origin of chromosomal segments is possible only in units of chromosomes, which is at much lower resolution than G-banding; (2) intrachromosomal aberrations such as inversion, duplication, or terminal or interstitial deletion cannot be detected as color changes in aberrant chromosomes; and (3) chromosomal breakpoints are difficult to determine by multicolor karyotyping analysis alone. To overcome these limitations of multicolor karyotyping analysis and to elevate the accuracy of cytogenetic analysis, several multicolor banding techniques have been developed recently. To date, many reports have been published on methods such as multicolor chromosome bar code (Müller et al., 1997), cross-species color banding (Müller et al., 1998; Teixeira et al., 1999) and multicolor-banding (MCB) (Chudoba et al., 1999; Liehr et al., 2002). However, banding patterns in these methods are not identical to those in G-banding. The ideal method is the one which is capable of detecting aberrations in units of bands identical to G-bands corresponding to the ISCN (2005) and of distinguishing between different bands in terms of their unique colors. The authors have been work-

ing to develop a new chromosome color banding technique with these ideal capabilities. As the result, we have developed a multicolor FISH technique capable of detecting chromosomal segments in units of bands corresponding to G-bands. This new method is named 'spectral color banding (SCAN)' (Kakazu et al., 2001). This review article presents this method, citing examples of its applications to clinical cytogenetic analysis.

### Methodology of SCAN

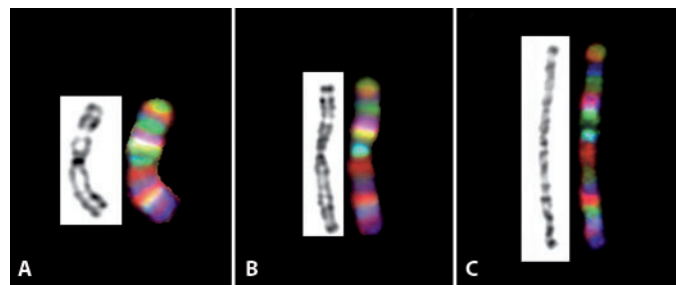
The microdissection technique can cut out and collect a region identical to a specific band from G-banded chromosomes. This is followed by extraction and purification of genomic DNA to serve as a template for degenerate oligonucleotide-primed PCR (DOP-PCR) (Telenius et al., 1992), accompanied by direct labeling with fluorochrome. The amplification product can be used as a chromosome band-specific painting probe for FISH (Fig. 1). In this way, band-specific genomic DNAs covering each band of a given chromosome are labeled with one or two of five different fluorochromes (Rhodamine, FITC, Texas Red, Cy5.5 and Cy5). When doing so, each band-specific painting probe is differentially labeled. All probes prepared in this way are combined to yield a probe cocktail. The cocktail is then hybridized with human metaphase chromosomes and analyzed using a detection system identical to that used for SKY analysis. We expect that in this way we can distinguish between different band units by means of different colors. Initially, we obtained six band-specific genomic DNAs for chromosome 10 and prepared a banding probe, followed by analysis of normal metaphase chromosomes by SCAN. As a result, chromosome 10 was successfully detected as a multicolor



**Fig. 2.** SCAN analysis for normal chromosome 3. From left to right: SCAN image of chromosome 3, the G-banded chromosome 3 ideogram (defined by ISCN 2005) corresponding to the approximately 400-band level per haploid set, fluorescence labeling scheme for a total of 15 band-specific painting probes of chromosome 3.

image in which individual bands were depicted in different colors corresponding to the G-band pattern (Kakazu et al., 2001). In addition to chromosome 10, we were able to generate a multicolor banding pattern for chromosome 3 using 15 differentially labeled band-specific painting probes (Fig. 2) (Kakazu et al., 2003).

For SKY analysis, the fluorescence signals from each chromosome show spectral patterns unique to the combination of labeling fluorochromes used for each chromosome painting. When these unique patterns of signals are recognized by spectral analysis, it is possible to automatically determine the chromosome from which a given signal originates. For SCAN analysis, under the same principle, the signals from each band must have spectral patterns unique to individual combinations of fluorochromes. Therefore, if the algorithm for spectral pattern recognition by SKY analysis is used for SCAN analysis, it will be possible to automatically determine the chromosomal band from which a given signal originates.



**Fig. 3.** SCAN images for various band levels of chromosome 3. Chromosomes 3 at the 250- (A), 400- (B), and 550- (C) band levels per haploid set. These normal chromosome samples, previously analyzed by G-banding, were derived from one bone marrow sample with a 250-band level per haploid set and two lymphocyte samples from peripheral blood with different levels of banding resolution (400- and 550-band levels). Multicolor banding patterns are detected in a reproducible manner, irrespective of the degree of chromosome condensation or quality of G-banding. For each chromosome, the G-band-like image (inverted DAPI counterstained image) (left) and spectral color image (right) are shown.

### Reproducibility in condensed chromosomes

So that cytogenetic analysis in clinical diagnosis can be performed appropriately using G-banding, the resolution needs to be at least at the approximately 400-band level per haploid set. Cytogenetic analysis in cases of hematological malignancies often uses bone marrow or lymph node samples, but the metaphase chromosomes in these samples tend to be more condensed than those in peripheral blood samples, often resulting in unsatisfactory resolution less than the 400-band level. This trend is more marked in solid tumors. Furthermore, in tumor-derived chromosome samples, the quality of G-banding itself is poor. Because of these unfavorable factors, the accuracy of G-band analysis of tumors tends to be lower than that of G-band analysis of congenital diseases using peripheral blood samples. Bearing this in mind, we applied SCAN to chromosome samples of varying degrees of condensation and examined whether or not color band patterns of a certain resolution could be obtained in a reproducible manner. In this evaluation, the use of SCAN allowed cytogenetic analysis at a resolution close to the 400-band level even for condensed chromosomes for which the resolution with previous G-banding was below the 250-band level per haploid set (Fig. 3). Furthermore, detection of evident multicolor banding patterns was possible with SCAN even from chromosome samples with unclear G-bands. On the basis of these results, SCAN is expected to allow cytogenetic analysis with a constant adequate resolution level irrespective of the degree of chromosome condensation or quality of G-banding. In MCB for chromosome 5, a banding pattern comparable to the 500-band level per haploid set has been observed independently of the length of the chromosomes (Chudoba et al., 1999).

## Applications of SCAN to cytogenetic analysis

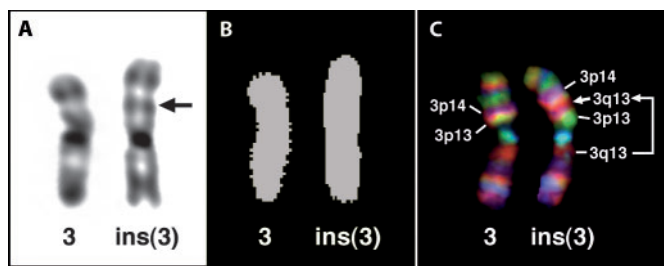
SCAN was applied to the analysis of chromosomal aberrations in tumors and shown to be useful in the following three respects.

### *Identification of the origin of the chromosome band of abnormal segments*

If the origin of an abnormal chromosomal segment cannot be identified by G-banding, it is relatively easy to identify the chromosomal origin by using SKY analysis. However, it is difficult with SKY analysis alone to determine the chromosomal band from which the segment originates. If the segment is short, it is also difficult even by reevaluation of the G-band pattern of the segment and the original chromosome. As stated above, SCAN can overcome this limitation and allows identification of the origin of abnormal chromosomal segments in units of bands. A model case study is presented below. We have analyzed the origin of double minute chromosomes (DMs) of bone marrow metastases of gastric cancer using the SCAN technique (Kakazu et al., 2001). DMs are minute chromosomal segments that lack a centromere region and are barely detectable with G-banding. If dozens (occasionally hundreds) of DMs are detected in tumor metaphase cells, the DMs are usually considered to contain some particular oncogene. Therefore, DMs are also known as a chromosomal indication of intense amplification of oncogenes. However, DMs are too small to allow identification of their origin with G-band analysis alone. When DMs from a case with gastric cancer were first subjected to SKY analysis, chromosome 10 was identified as the origin, but the band of chromosome 10 from which the DMs originated remained unidentified. We then analyzed the DMs using SCAN for chromosome 10. The signals on the DMs were identical to band q26 of normal chromosome 10 in terms of spectral pattern, suggesting that the DMs originate from this band. Band 10q26 was found to bear the fibroblast growth factor receptor 2 (*FGFR2*, previously termed *K-sam*) gene known to be particularly amplified in poorly differentiated gastric cancer. Following this finding, we conducted FISH using a probe containing this gene and found numerous signals on the DMs, confirming the SCAN results.

### *Detection of intrachromosomal aberrations*

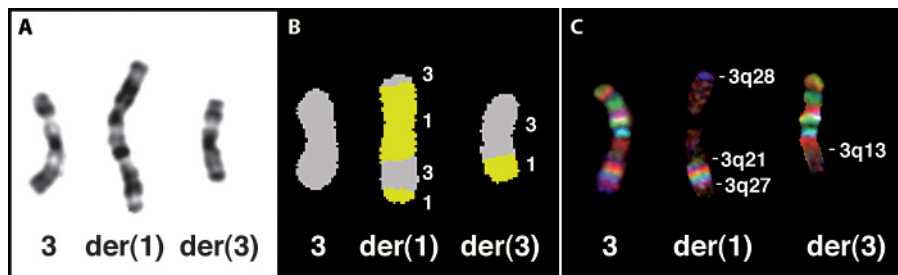
Some structural aberrations involve different chromosomes (e.g., translocation) and others take place within the same chromosome (e.g., duplication). SKY is useful for analysis of the former type of aberrations, but it is difficult to detect the latter type of aberrations using SKY analysis alone. This is because SKY analysis using whole chromosome painting probes cannot detect intrachromosomal aberrations as changes in the color of fluorescence signals. However, if SCAN is used, these aberrations can be easily detected on the basis of color banding patterns. In practice, SCAN analysis not only detected the single band-sized insertion within the short arm of chromosome 3 in a case of malignant lymphoma, but also precisely identified the origin of the chromosomal band as 3q13 (Fig. 4) (Kakazu et al., 2003).



**Fig. 4.** Analysis of an intrachromosomal aberration. (A) G-band image. Insertion of a small segment of unknown origin (arrow) in the short arm of chromosome 3 [ins(3)] is revealed. The left is a normal homolog of chromosome 3. (B) SKY image. The ins(3) chromosome cannot be detected as a change in color because the inserted segment originates from chromosome 3. (C) SCAN image. The ins(3) chromosome possesses an inserted segment with excessive red signals (not seen in the normal chromosome 3, shown on the left) between bands 3p13 and 3p14. The spectral color of signals of the inserted segment is identical to that of signals of band q13 of the long arm of the same chromosome 3. Thus, SCAN has revealed that the inserted segment originates from band 3q13. (Reprinted with permission from Molecular Medicine, Vol. 39 (suppl): Kakazu et al., Copyright 2002 by Nakayama-Shoten Co., Ltd.).

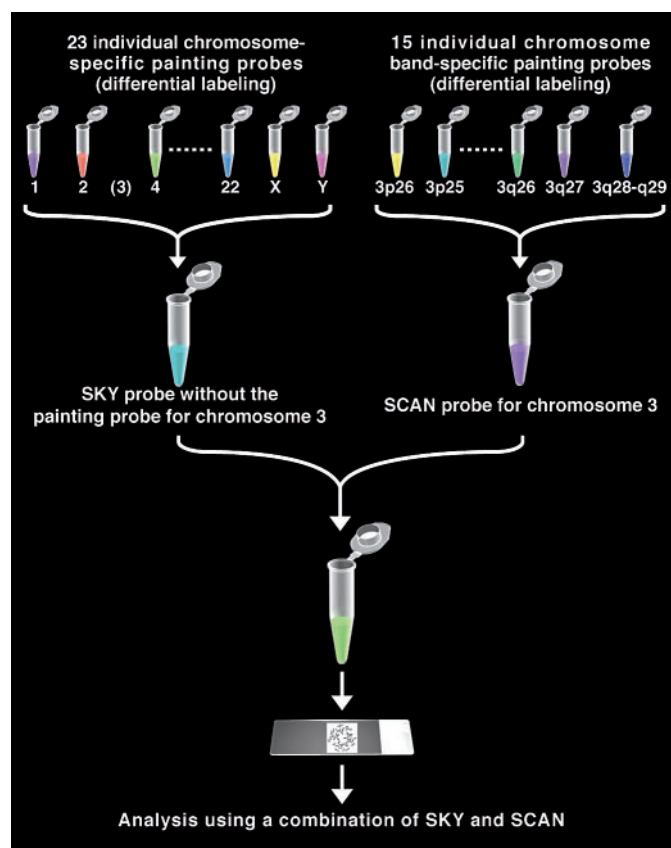
### *Precise determination of breakpoints of chromosomal rearrangements*

Numerous recurrent chromosomal translocations specific to the subtypes of hematological malignancies have been found. The molecular approach of identifying their breakpoints has led to the cloning of the causative genes that were closely associated with tumorigenesis. Therefore, precise determination of breakpoints is important for diagnosis of subtype, prediction of prognosis and determination of treatment strategy in patients with hematological malignancies. With SKY analysis alone, however, definite determination of breakpoints is difficult even when the chromosome from which a given translocated segment originates can be identified. If SCAN is used in such cases, the translocation breakpoints can be easily determined on the basis of identification of the band from which a given translocated segment originates. In many cases of hematological malignancies, especially in malignant lymphoma cases, translocations were too complex to allow easy identification of breakpoints even with G-banding analysis. We analyzed the complex translocations in a case of B-cell lymphoma using SCAN for chromosome 3 and unveiled all of four breakpoints of chromosome 3, which had not been detected by G-banding, in a single analysis (Fig. 5). In this analysis, we identified the translocation involving band 3q27, serving as one of the four breakpoints. Such 3q27 translocation is seen relatively frequently in cases of B-cell lymphoma and is reported to be specific for a subtype of diffuse large-cell lymphoma. It is significant in clinical diagnosis of hematological malignancies that SCAN detected the subtype-specific chromosomal translocation that had been overlooked by G-banding. MCB has also been shown to be useful for precise determination of chromosomal breakpoints (Lemke et al., 2001; Weise et al., 2002).



**Fig. 5.** Precise determination of breakpoints for complex rearrangements. (A) G-band image: Two aberrations derived from chromosomes 1 and 3 [der(1): ?del(1)(p36); der(3): add(3)(q?21)] were revealed in a malignant lymphoma case. At this stage, these aberrations appear to have no relation to each other. (B) SKY image: These two aberrations were shown to be a complex translocation between chromosomes 1 and

3. However, it was not possible to determine breakpoints of this translocation. (C) SCAN image: In the der(1) chromosome, region 3q28~q29 was translocated to the short arm and region 3q21~q27 was inserted into the long arm. In the der(3) chromosome, the segment from chromosome 1 was translocated to region q13. All of four breakpoints of chromosome 3 can be determined in a single SCAN analysis.



**Fig. 6.** Scheme of a probe cocktail composed of the banding probes for SCAN and the painting probes for SKY.

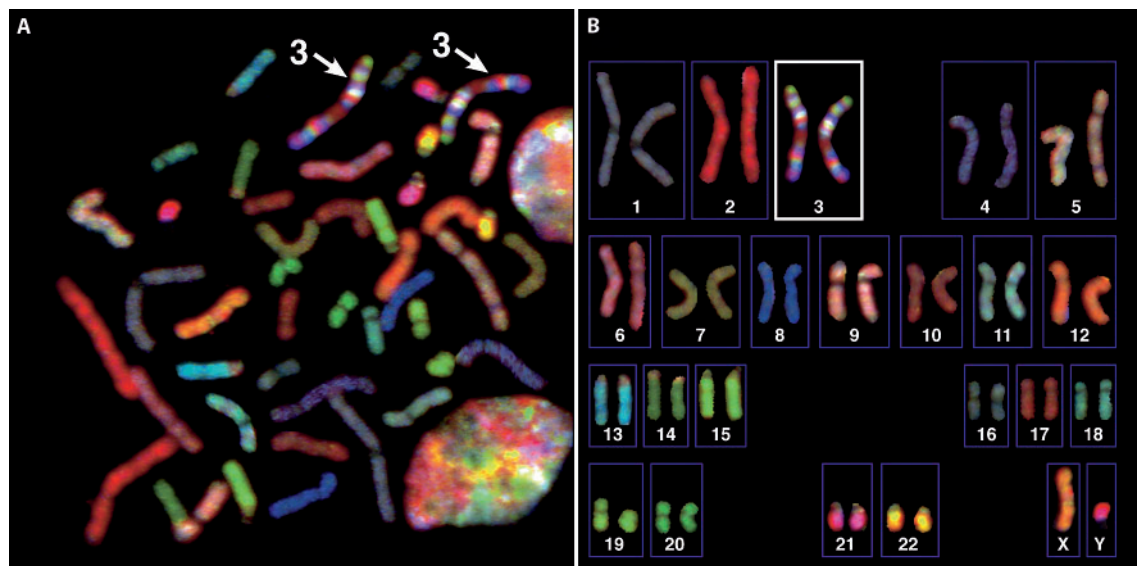
### Conclusions and perspectives

We have developed a new method called SCAN on the basis of the conventional SKY analysis. We applied this method to analysis of chromosomal aberrations related to tumors and demonstrated its usefulness in cytogenetic diagnosis. SCAN, which allows precise identification of the

origin of chromosomal segments in units of bands corresponding to G-bands, is expected to remarkably improve the accuracy of cytogenetic analysis as compared to conventional banding techniques. However, this method does not completely replace G-banding or SKY analysis, but it serves as an auxiliary method making up for the shortcomings of existing methods. We summarized the features and usefulness of microdissection-based multicolor banding techniques compared with conventional chromosome banding and multicolor karyotyping techniques (Table 1).

SCAN is a technique for analysis of a particular chromosome and is not capable of detecting aberrations of the other 23 chromosomes. To overcome this shortcoming of SCAN analysis, we prepared a probe cocktail composed of the banding probes for the particular chromosome used for SCAN and the painting probes for SKY analysis after elimination of the painting probe for the particular chromosome for SCAN (Fig. 6). Using this cocktail, we succeeded in simultaneously displaying multicolor banding images for chromosome 3 and unique spectral color images identical to those of SKY analysis alone for the other 23 chromosomes in the same metaphase cells (Fig. 7). This method, involving a combination of SKY and SCAN, makes use of the advantages of both methods. It is particularly suitable for simultaneous characterization of complex rearrangements and their breakpoints in cancer cytogenetics (Kakazu et al., in preparation). We have presented SCAN for only chromosomes 3 and 10 so far. In addition, we have prepared SCAN probes for another eleven human chromosomes (chromosomes 1, 5, 7–9, and 11–16) (data not shown). This technique will be available for all human chromosomes in the near future.

On the other hand, in MCB simultaneous use of two sets of chromosome-specific probes could detect a balanced translocation between chromosomes 5 and 13, with the respective breakpoints at q13 and q14 (Liehr and Claussen, 2002). Furthermore, multitude MCB (mMCB) has been developed (Weise et al., 2003). The mMCB probe set contains a total of 138 chromosome region-specific microdissection-



**Fig. 7.** Representative images of a normal male metaphase analyzed using a combined method of SCAN and SKY. (A) Chromosomes 3 (arrows) are displayed in a multicolor banding image of SCAN. The other 23 chromosomes are detected in their unique colors of SKY. (B) Karyogram of the metaphase depicted in A. SCAN image of chromosomes 3 is framed in white.

**Table 1.** Comparison among conventional banding, multicolor karyotyping and microdissection based multicolor banding techniques

| Description  | Technique  |   |  |
|--|--|---|--|
|  | Conventional banding   | Multicolor karyotyping                                | Multicolor banding   |
| Typical method(s)  | G-banding  | spectral karyotyping (SKY)<br>multiplex-FISH (M-FISH) | spectral color banding (SCAN)<br>multicolor-banding (MCB)  |
| Labeled probes   | unnecessary  | chromosome-specific painting probes (flow-sorted)     | chromosome band (region)-specific painting probes (microdissected)   |
| Identification   | morphological identification   | automated identification                              | automated identification   |
| Objects in a single analysis                               | all 24 human chromosomes   | all 24 human chromosomes                              | some particular chromosomes (SCAN, MCB), all 24 human chromosomes (mMCB)   |
| Intrachromosomal aberrations                               | detectable   | difficult to detect                                   | detectable   |
| Interchromosomal aberrations                               | detectable, but sometimes overlooked if the translocation involves similarly banded segments or segments are too small | very useful   | difficult with the probe for a particular chromosome alone<br>detectable with probe sets for more than two different chromosomes (MCB) or for all human chromosomes (mMCB) |
| Identification of the origin of chromosomal segments       | possible in units of bands   | possible in units of chromosomes                      | possible in units of bands identical to G-bands (SCAN)   |
| Determination of breakpoints of chromosomal rearrangements | possible, but sometimes difficult in the case of complex rearrangements  | difficult with multicolor karyotyping analysis alone  | possible even in the case of complex rearrangements  |

derived libraries covering all human chromosomes. Thus, mMCB allows simultaneous visualization of multicolor banding patterns unique for each of the 24 human chromosomes in a single analysis at a banding resolution corresponding to about 370 bands per haploid set. This method was applied to two myelodysplastic syndrome cases and two

solid tumor cell lines and has been shown to be useful for comprehensive characterization of complex structural abnormalities involving at least three chromosomes (Weise et al., 2003). Moreover, mMCB revealed cryptic chromosomal abnormalities even in acute lymphoblastic leukemia cases with normal karyotype (Karst et al., 2006). This approach

is also a powerful tool for high-resolution analysis of karyotype evolution in chimpanzee (Gross et al., 2006).

As a remarkable outcome of the human genome project, draft sequencing of the entire genome was completed and published in 2001 (International Human Genome Sequencing Consortium, 2001). Through the genomic era, genome analysis technologies such as array comparative genomic hybridization (array CGH) have been advancing remarkably (Ishkanian et al., 2004). Now, huge amounts of information about the human genome are available. However, for more effective utilization of abundant genomic information in molecular cytogenetics, it is necessary to establish

more highly sophisticated analysis systems in combination with multicolor karyotyping and multicolor banding techniques so that they can efficiently and comprehensively detect multiple specific genetic changes involved in tumors and congenital diseases.

## Acknowledgements

We wish to thank Dr. Irit Bar-Am (Applied Spectral Imaging Ltd.) for preparing the SCAN probe and Mr. Humitsugu Yamane for technical assistance.

## References

- Chudoba I, Plesch A, Lorch T, Lemke J, Claussen U, Senger G: High resolution multicolor-banding: a new technique for refined FISH analysis of human chromosomes. *Cytogenet Cell Genet* 84:156–160 (1999).
- Gross M, Starke H, Trifonov V, Claussen U, Liehr T, Weise A: A molecular cytogenetic study of chromosome evolution in chimpanzee. *Cytogenet Genome Res* 112:67–75 (2006).
- International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 409:860–921 (2001).
- ISCN (2005): An International System for Human Cytogenetic Nomenclature. Shaffer LG, Tommerup N (eds) (S. Karger, Basel 2005).
- Ishkanian AS, Malloff CA, Watson SK, DeLeeuw RJ, Chi B, et al: A tiling resolution DNA microarray with complete coverage of the human genome. *Nat Genet* 36:299–303 (2004).
- Kakazu N, Taniwaki M, Horiike S, Nishida K, Tatekawa T, et al: Combined spectral karyotyping and DAPI banding analysis of chromosome abnormalities in myelodysplastic syndrome. *Genes Chromosomes Cancer* 26:336–345 (1999).
- Kakazu N, Kito K, Hitomi T, Oita J, Nishida K, et al: Characterization of complex chromosomal abnormalities in B-cell lymphoma by a combined spectral karyotyping (SKY) analysis and fluorescence in situ hybridization (FISH) using a 14q telomere probe. *Am J Hematol* 65:291–297 (2000).
- Kakazu N, Ashihara E, Hada S, Ueda T, Sasaki H, et al: Development of spectral colour banding in cytogenetic analysis. *Lancet* 357:529–530 (2001).
- Kakazu N, Bar-Am I, Hada S, Ago H, Abe T: A new chromosome banding technique, spectral color banding (SCAN), for full characterization of chromosomal abnormalities. *Genes Chromosomes Cancer* 37:412–416 (2003).
- Karst C, Gross M, Haase D, Wedding U, Hoffken K, et al: Novel cryptic chromosomal rearrangements detected in acute lymphoblastic leukemia detected by application of new multicolor fluorescent in situ hybridization approaches. *Int J Oncol* 28:891–897 (2006).
- Lemke J, Chudoba I, Senger G, Stumm M, Loncarevic IF, et al: Improved definition of chromosomal breakpoints using high-resolution multicolour banding. *Hum Genet* 108:478–483 (2001).
- Liehr T, Claussen U: Current developments in human molecular cytogenetic techniques. *Curr Mol Med* 2:283–297 (2002).
- Liehr T, Heller A, Starke H, Rubtsov N, Trifonov V, et al: Microdissection based high resolution multicolor banding for all 24 human chromosomes. *Int J Mol Med* 9:335–339 (2002).
- Müller S, Rocchi M, Ferguson-Smith MA, Wienberg J: Toward a multicolor chromosome barcode for the entire human karyotype by fluorescence in situ hybridization. *Hum Genet* 100:271–278 (1997).
- Müller S, O'Brien PCM, Ferguson-Smith MA, Wienberg J: Cross-species colour segmenting: a novel tool in human karyotype analysis. *Cytometry* 33:445–452 (1998).
- Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, et al: Multicolor spectral karyotyping of human chromosomes. *Science* 273:494–497 (1996).
- Speicher MR, Gwyn Ballard S, Ward DC: Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 12:368–375 (1996).
- Teixeira MR, Micci F, Dietrich CU, Heim S: Cross-species color banding characterization of chromosomal rearrangements in leukemias with incomplete G-band karyotypes. *Genes Chromosomes Cancer* 26:13–19 (1999).
- Telenius H, Palmear AH, Tunnacliffe A, Carter NP, Behmel A, et al: Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* 4:257–263 (1992).
- Veldman T, Vignon C, Schröck E, Rowley JD, Ried T: Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. *Nat Genet* 15:406–410 (1997).
- Weise A, Starke H, Heller A, Tönnies H, Volleth M, et al: Chromosome 2 aberrations in clinical cases characterised by high resolution multicolour banding and region specific FISH probes. *J Med Genet* 39:434–439 (2002).
- Weise A, Heller A, Starke H, Mrasek K, Kuechler A, et al: Multitude multicolor chromosome banding (mMCB) – a comprehensive one-step multicolor FISH banding method. *Cytogenet Genome Res* 103:34–39 (2003).