Novel molecular cytogenetic techniques for identifying complex chromosomal rearrangements: technology and applications in molecular medicine

Nicole McNeil and Thomas Ried

Molecular cytogenetic techniques that are based on fluorescence in situ hybridisation (FISH) have become invaluable tools for the diagnosis and identification of the numerous chromosomal aberrations that are associated with neoplastic disease, including both haematological malignancies and solid tumours. FISH can be used to identify chromosomal rearrangements, by detecting specific DNA sequences with fluorescently labelled DNA probes. The technique of comparative genomic hybridisation (CGH) involves two-colour FISH. It can be used to establish ratios of fluorescence intensity values between tumour DNA and control DNA along normal reference metaphase chromosomes, and thereby to detect DNA copy-number changes such as gains and losses of specific chromosomal regions and gene amplifications. Spectral karyotyping (SKY) is a novel molecular cytogenetic method for characterising numerical and structural chromosomal aberrations. SKY involves the simultaneous hybridisation of 24 differentially labelled chromosome-painting probes, followed by spectral imaging and chromosome classification, and produces a colour karyotype of the entire genome. The use of SKY has contributed significantly to the identification of chromosomal anomalies that are associated with constitutional and cancer cytogenetics, and has revealed many aberrations that go undetected by traditional banding techniques. In this article, we have reviewed these new molecular cytogenetic techniques and described their various applications in molecular medicine.

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The study of human chromosomes has been going on for well over a 100 years. However, progress has been impeded by difficulties associated with the preparation of high-quality chromosomes from mammalian cells. It was not until 1956 that Tjio and Levan reported the correct number of human chromosomes (Ref. 1). In the 1960s, the addition of phytohaemagglutinin to blood lymphocyte cultures was found to stimulate cell division (i.e. mitosis), significantly increasing the number of metaphase spreads (Refs 2, 3). These discoveries improved the analysis of human chromosomes and led to the realisation that the analysis of chromosomes was indispensable to the study of human disease. After the observation of an extra copy of chromosome 21 (i.e. trisomy) in patients suffering from Down syndrome, other trisomies were found in the D and G group chromosomes and were linked to certain characteristic phenotypes that are associated with Patau syndrome and Edwards syndrome, respectively. Another milestone was the discovery of the Philadelphia chromosome and its association with chronic myelogenous leukaemia (Ref. 4), which invigorated the field of cancer cytogenetics.

The 1970s saw the introduction and successful application of a variety of staining techniques that gave chromosomes a banding pattern. Banding, by greatly improving the accuracy of chromosome analysis (Refs 5, 6), permitted the analysis of many different tissues and diseases. Such banding patterns facilitated the analysis of subchromosomal regions. High-resolution banding methodology made possible the detection of chromosomal deletions and the identification of small translocations (Ref. 7).

Until the advent of molecular cytogenetic techniques in the 1980s, chromosomal analysis had relied on the study of chromosomal bands. However, it was often difficult to discern the origins of the chromosomes that were involved in chromosomal rearrangements (e.g. translocations), particularly in the case of solid tumours and leukaemia. Modern cytogenetic techniques have improved the diagnosis of chromosomal aberrations. Fluorescence in situ hybridisation (FISH) probes have been designed to detect specific regions of DNA, and thus to elucidate abnormalities even at the level of the gene, which cannot be detected by conventional banding techniques. Some of the benefits of FISH include the confirmation of chromosome breakpoints, an assessment of specific nucleic acid sequences and the ability to detect such sequences in non-dividing cells (i.e. interphase cytogenetics). Although FISH is an extremely useful technique, until recently only a few target sequences could be visualised simultaneously.

By contrast, comparative genomic hybridisation (CGH) is a molecular cytogenetic technique that screens for whole-genomic imbalances in tumour samples. It identifies chromosomal gains and losses (e.g. deletions, duplications or amplifications) by using differentially labelled tumour DNA and normal DNA (Ref. 8). On visualising the two different fluorochromes (i.e. molecules that are fluorescent when appropriately excited), differences in the intensity of fluorescence along the chromosome correspond to the loss or gain of genetic material in the tumour sample.

Spectral karyotyping (SKY) is a technique that combines the power of conventional chromosome analysis with the specificity of FISH. SKY can be used to identify marker chromosomes (i.e. chromosomes that are important in the diagnosis of a disease) and detect telomeric translocations, which are sometimes difficult to identify using traditional banding analysis. This technique has also proven to be beneficial in elucidating the complex rearrangements that are observed in cancer genomes (for examples, see Refs 9, 10, 11, 12). SKY entails a single multicolour FISH analysis, which can be used to yield 24 different-coloured chromosomes in a human metaphase spread. SKY involves a combination of epifluorescence microscopy, charge-coupled device (CCD) imaging and Fourier spectroscopy to measure the complete emission spectra at all image points (Refs 13, 14).

These three molecular cytogenetic techniques and their applications in the analysis of chromosomal aberrations that are associated with human disease are discussed in more detail below.

Fluorescence in situ hybridisation (FISH)

The FISH technique is based on the discovery that labelled ribosomal RNA hybridises to acrocentric chromosomes (i.e. chromosomes that have a non-centrally located centromere, and therefore unequal chromosome arms; Refs 15, 16). Initially, radioactive isotopes were used for this technique; however, the use of fluorochromes is safer, requires a shorter reaction time and can give rise

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to different colours. FISH involves a fluorescently labelled DNA probe being hybridised to genomic DNA sequences, and can be used to study a specific site on a chromosome.

Methodology of FISH

The first consideration in FISH is the availability of a DNA probe. Once pure DNA has been isolated, a fluorochrome must be incorporated into the DNA probe in a reaction known as labelling. DNA probes can be labelled by enzymatic procedures such as nick translation, random priming or the polymerase chain reaction (PCR). During nick translation, the exonuclease activity of DNA polymerase causes a single-strand break (or nick) in the DNA, by removing a nucleotide (i.e. adenine, guanine, thymidine or cytosine). Subsequently, a specific fluorescently labelled nucleotide is incorporated into the nicked strand by DNA polymerase, which uses the DNA sequence of the non-nicked strand as a template. When a probe is produced from genomic DNA, Cot-1 DNA is added to the mixture. Human Cot-1 DNA consists of the repetitive sequences of the genome, and it is used to suppress the hybridisation of repetitive sequences. Failure to use Cot-1 DNA often results in non-specific hybridisation, making it difficult to distinguish the 'signal' from the 'background noise'.

Metaphase chromosome spreads are prepared by using a spindle inhibitor such as Colcemid to arrest cultured cells during mitosis. Hypotonic solution (0.075 M potassium chloride) and fixative (methanol and acetic acid in a 3:1 ratio) are then applied sequentially. Finally, the chromosome suspension is dropped onto glass slides (Ref. 17). Before hybridisation, metaphase chromosomes and interphase nuclei are pretreated enzymatically to enhance their accessibility to the probe and to reduce the amount of cytoplasm (Ref. 18). The pretreated slide is heated to denature the DNA.

The previously prepared probe and Cot-1 DNA are mixed and denatured together and then applied to the slide for hybridisation. Denaturation of the probe and Cot-1 DNA allows the repetitive sequences to anneal to the Cot-1 DNA before hybridisation. This can then be followed by the site-specific binding of the single-copy probe sequences to the denatured target DNA. Hybridisation between the probe and target DNA (on the slide) takes place during an incubation period of ~16–48 hours at 37°C. This incubation time can vary depending on the probes used: shorter hybridisation times can be used for repetitive DNA probes or chromosome-painting probes, whereas longer incubation times are needed for the hybridisation of complementary DNA (cDNA) sequences or complete genomes.

Detecting the probe permits the visualisation of target DNA sequences. Detection starts with post-hybridisation washes of the slide in formamide and SSC (sodium chloride, sodium citrate) salt solutions to remove any excess probe that is non-specifically bound. However, a detection step is not required for all DNA probes. In a direct-label reaction, a fluorochrome-conjugated nucleotide [e.g. 2'-deoxyuridine 5' triphosphate (dUTP) conjugated to fluorescein isothiocyanate (FITC)] is incorporated into the strands. In an indirect-label reaction, reporter molecules such as biotin or digoxigenin are incorporated into the DNA. Such indirect labels require a detection step in which the reporter molecule or hapten is labelled with an agent such as avidin or anti-digoxin that is conjugated to a fluorochrome. The detection methods for biotinylated probes employ avidin–fluorochrome conjugates, whereas those for digoxigenin-labelled probes employ anti-digoxin–fluorochrome conjugates.

FISH microscopy

FISH signals are visualised by fluorescence microscopy using a light source that illuminates the fluorescently labelled specimens. Mercury vapour lamps and xenon lamps both emit light that excites fluorochromes. To visualise the fluorescence signal of the probe, a variety of specific filter sets can be used to separate the different fluorochromes (Refs 19, 20, 21). Digital imaging systems, such as a CCD camera, capture the image and quantify fluorescent signals (Ref. 22). Resultant images are analysed on commercially available systems (e.g. from Vysis, Leica Microsystems or Applied Spectral Imaging; see box entitled 'Further reading, resources and contacts').

Clinical applications of FISH

Conventional high-resolution chromosome banding techniques as used in cytogenetic laboratories can result in up to 1000 bands per genome; however, even at such high resolution, disease-associated deletions, duplications or translocations can be difficult to discern. By

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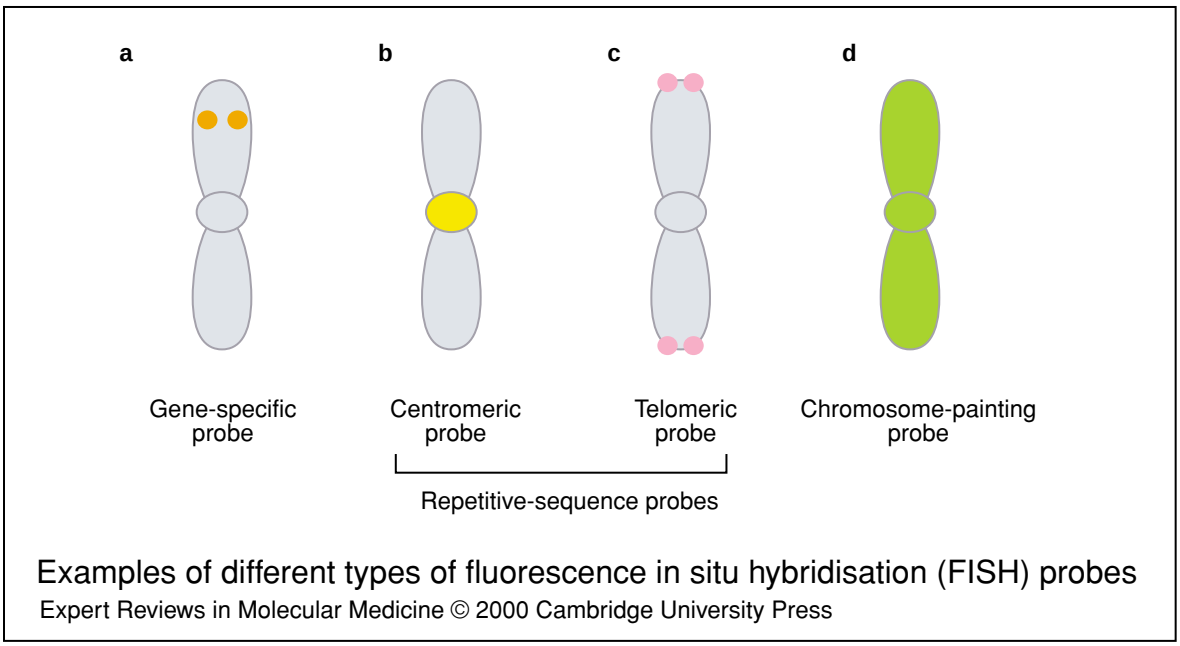

Figure 1. Examples of different types of fluorescence in situ hybridisation (FISH) probes. (a) Gene-specific probes target specific nucleic acid sequences on a chromosome. (b) Centromeric probes bind to repetitive sequences that are specific to the centromeric regions. (c) Telomeric probes recognise the repetitive sequence TTAGGG, and can be used to visualise all telomeres simultaneously. Chromosome-specific telomeric probes hybridise to subtelomeric, chromosome-specific repeats. (d) Chromosome-painting probes consist of pools of chromosome-specific probes **(fig001trn)**.

contrast, FISH can be used to determine the origin of marker chromosomes and to confirm numerical and structural aberrations. Using banding techniques on highly extended chromosomes, the smallest chromosome abnormality detectable is ~2000–3000 kilobases (kb), whereas FISH probes can detect regions as small as 0.5 kb on metaphase chromosomes. The increased resolution provided by FISH has particular relevance in the study of microdeletion syndromes (e.g. Prader-Willi) because the size of the DNA region that has been deleted is often too small to be detected by conventional banding techniques.

FISH probes are highly specific for their target or cDNA sequence, and can be divided into four main types: gene-specific probes, repetitive-sequence probes, whole-genomic DNA probes and chromosome-painting probes (see Fig. 1).

Gene-specific probes

Gene-specific probes target specific nucleic acid sequences within chromosomes. Examples of such probes include bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) probes and cosmids. These probes have proven particularly useful in the study of microdeletion syndromes, where the absence of a gene often goes undetected by conventional banding methods. For instance, Duchenne muscular dystrophy is a progressive muscular degenerative disease that results from a deletion at Xp21 (i.e. band 21 on the short arm of the X chromosome) in affected males. Although the deletion can vary in size, exon 45 of the dystrophin gene is missing in 60% of patients. FISH probes that can detect deleted regions of this gene are elegant tools for determining the carrier status of clinically unaffected women (Refs 23, 24, 25). Gene-specific probes are also useful for mapping genes on chromosomes.

Repetitive-sequence probes

Repetitive-sequence probes bind to regions that are rich in repetitive base-pair sequences. Examples of such probes include centromeric and telomeric probes. Centromeres frequently contain A–T-rich tandem repeats, whereas telomeres are recognised by the short repetitive sequence TTAGGG. Centromeric probes have applications in the identification of marker chromosomes

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and numerical chromosome abnormalities in interphase nuclei and when specimens are sex mismatched. Telomeric probes and subtelomere-specific probes (Ref. 26) are commonly used to identify cryptic chromosomal translocations such as those occurring in cases of unknown mental retardation.

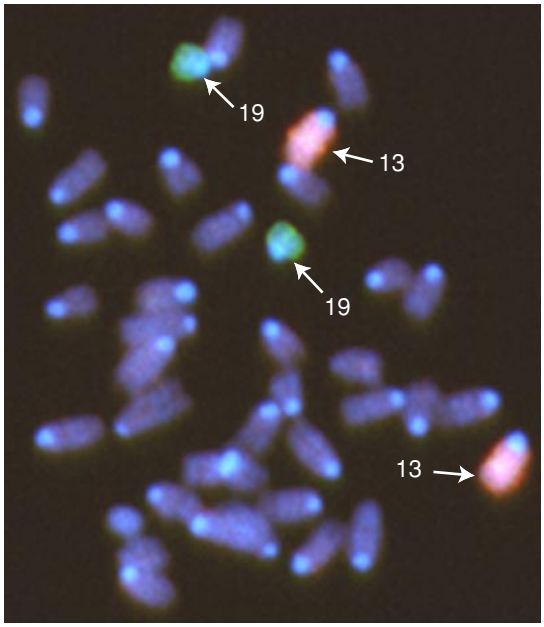
Whole-genomic DNA probes

Whole-genomic DNA probes are used for the FISH-based technique CGH (see below). They can be used to detect genomic imbalances in tumour genomes by combining tumour and normal DNA to analyse gains and losses.

Chromosome-painting probes

Chromosome-painting probes contain sequences that are specific to either a single chromosome (i.e. whole-chromosome-painting probes) or an arm of a chromosome (i.e. chromosome-arm-painting probes). After hybridisation, one or more chromosomes of interest are 'lit up' in different colours, which are dependent on which particular fluorochromes have been used (see Fig. 2). This technique is particularly useful for identifying chromosome arms that are involved in translocations, as well as for marker chromosomes and ring chromosomes. Ring chromosomes are formed when breaks occur in the short and long arms of a chromosome, and the broken ends re-join to form a ring.

Whole-chromosome-painting probes are also used in SKY (as discussed later). By assigning an individual colour to each of the 23 pairs of human chromosomes, an entire karyotype (i.e. all 46 chromosomes) can be differentially labelled. As well as being useful for visualising all aberrations in a simple experiment, SKY can be used to elucidate cryptic translocations that often go undetected by conventional cytogenetic analysis.



Fluorescence in situ hybridisation (FISH) using whole-chromosome-painting probes

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Figure 2. Fluorescence in situ hybridisation (FISH) using whole-chromosome-painting probes. Chromosome-painting probes were used to rule out the presence of an extra copy of chromosome 19 (i.e. trisomy 19) in this metaphase spread, which was derived from a glial cell taken from the brain of a mouse. Chromosome 19 appears green [it was labelled with avidin conjugated to fluorescein isothiocyanate (FITC)] against the blue background stain of 4,6-diamidino-2-phenylindole (DAPI). Chromosome 13, which was used as a normal control chromosome, has also been identified and appears red [it was labelled with avidin conjugated to tetramethylrhodamine isothiocyanate (TRITC)] **(fig002trn)**.

Comparative genomic hybridisation (CGH)

CGH involves the differential labelling of test and reference DNA to measure genetic imbalances in entire genomes. CGH is based on quantitative two-colour FISH (Ref. 27). It has become an invaluable technique for studying chromosomal aberrations that occur in solid tumours and other malignancies. A major advantage of the CGH technique is that only DNA from the tumour samples is needed for analysis; this avoids the often-difficult preparation of tumour metaphase chromosomes, which can have a poor morphology and resolution. Instead, karyotypically normal metaphase chromosomes are used to detect tumour-associated chromosomal gains and losses. Another advantage of CGH is that formalin-fixed tissue sections can be used; thus, comparisons can be made between a phenotype and genotype, and genetic changes can be correlated with the clinical course of a disease.

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Methodology of CGH

For CGH, whole-genomic DNA is isolated from a tumour by standard extraction protocols. Control or reference DNA is isolated from an individual who has either a normal 46, XX karyotype or a normal 46, XY karyotype. The DNA that has been extracted from the two genomes is differentially labelled (for example using biotin conjugated to dUTP for the tumour genome and digoxigenin conjugated to dUTP for the normal genome). The tumour and normal DNA samples are combined, and an excess of unlabelled human Cot-1 DNA is added into the hybridisation mixture, to suppress the repetitive sequences that are present in both genomes. The Cot-1 DNA is essential because hybridisation of the repetitive DNA would impair the evaluation of the unique sequences that are either overrepresented or underrepresented in the tumour genome. This probe mixture is hybridised to normal human reference metaphase chromosomes. Avidin coupled with FITC (which fluoresces green) is used for the detection of biotin-labelled tumour DNA, whereas anti-digoxin coupled to rhodamine (which fluoresces red) is used for the digoxigenin-labelled control DNA. The relative colour intensities of the two flurochromes reflect DNA copy-number alterations in the tumour genome (Ref. 8; see Fig. 3).

CGH analysis

To determine copy-number imbalances within a tumour, CCD images must be acquired from several metaphase spreads. Variations in the fluorochrome intensity along the chromosomes in a metaphase spread reflect copy-number changes (i.e. gains and losses of DNA along a chromosome) in the tumour sample. For example, using the fluorochromes mentioned above, a loss of DNA within the tumour genome shifts the colour of that region to red. A gain of a chromosomal region would be shown by an increased intensity of the green fluorescence in the reference metaphase preparation. If chromosomes or subchromosomal regions are balanced with respect to DNA content in both tumour and control samples, the intensity of the red and green fluorescence is similar. Digital image analysis must be used to quantify fluorochrome intensity, especially in cases where changes involve low copy numbers or where many gains and losses have occurred. A CCD camera and fluorochrome-specific optical filters are used for image acquisition with rhodamine and FITC labels. A variety of filter sets can be purchased through commercial vendors (e.g. Chroma Technology Corp.). Specialised software selects metaphases that have an adequate signal intensity, segments them along the chromosomal axis, and correctly identifies and orientates each chromosome. For each chromosome, a ratio value of fluorochrome intensity is generated from a minimum of five metaphase spreads. Finally, an average ratio profile for each chromosome is produced, indicating the calculated gains and losses (Refs 28, 29; see Fig. 4).

Experimental investigations with CGH

CGH is useful for the analysis of chromosomal gains and losses in solid tumours. Recurrent patterns of gains and losses have been observed in the malignant tissue from virtually all human cancers (Refs 30, 31, 32, 33). For example, using CGH to study pancreatic carcinomas, Ghadimi and colleagues found recurrent gains on the chromosome arms 3q, 5p, 7p, 8q, 12p and 20q. Losses were observed on the chromosome arms 8p, 9p, 17p, 18q and 19p and on chromosome 21 (Ref. 10). Heselmeyer and colleagues studied tissue from the cervical epithelium at various stages of dysplasia (Ref. 34). Their results showed that the gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma.

Spectral karyotyping (SKY)

SKY is a novel molecular cytogenetic FISH-based technique that allows colour karyotyping of human and mouse chromosomes (Refs 14, 35). Whereas FISH limits analysis to specific chromosomes or regions of chromosomes, and CGH visualises only those changes that result in variations in copy number, SKY permits the visualisation of all chromosomes at one time, 'painting' each pair of chromosomes a different fluorescent colour. Before the development of this technique, molecular cytogeneticists analysed chromosomes with staining techniques that produced a black-and-white banding pattern. However, the identification of all chromosomal aberrations in a complex karyotype was often not possible from such patterns. SKY has been applied to a variety of human malignancies and mouse model systems (Refs 36, 37), and it has been highly effective in deciphering many complex karyotypic rearrangements.

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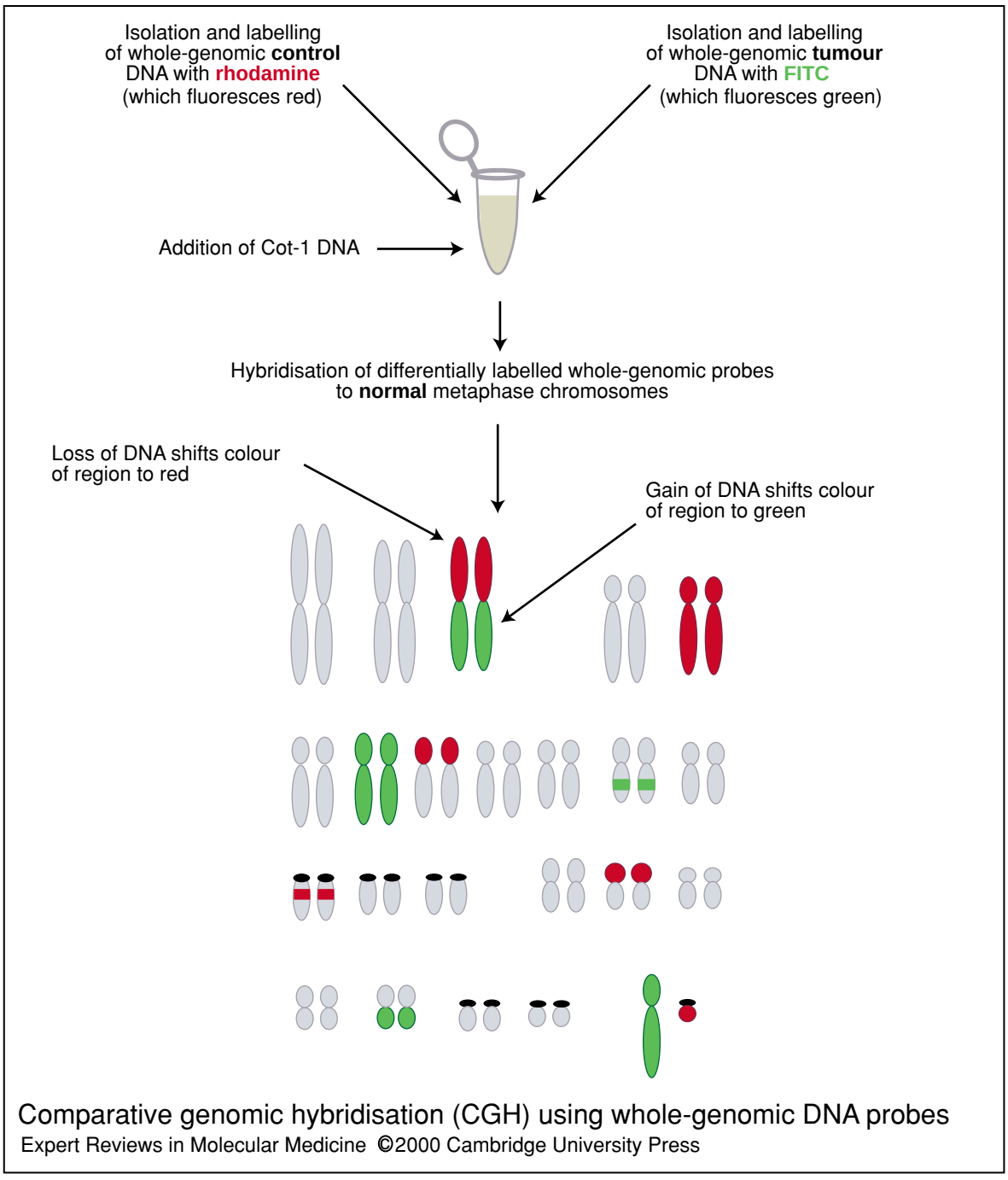

Figure 3. Comparative genomic hybridisation (CGH) using whole-genomic DNA probes. CGH begins with the isolation of both genomic DNA from a tumour sample and genomic DNA from an individual who has a normal karyotype (the control DNA). Next, the two genomes are differentially labelled; for example, the control DNA with the red fluorochrome rhodamine, and the tumour DNA with the green fluorochrome fluorescein isothiocyanate (FITC). Excess unlabelled human Cot-1 DNA is added to the probe mixtures to suppress the repetitive DNA sequences that are present in both genomes. The differentially labelled genomes are then combined and hybridised to normal metaphase chromosomes. The relative intensities of the green and red fluorochromes reflect the actual copy-number changes that have occurred in the tumour genome. DNA losses and gains are indicated by a shift to red (dark grey) and green fluorescence (light grey), respectively **(fig003trn)**.

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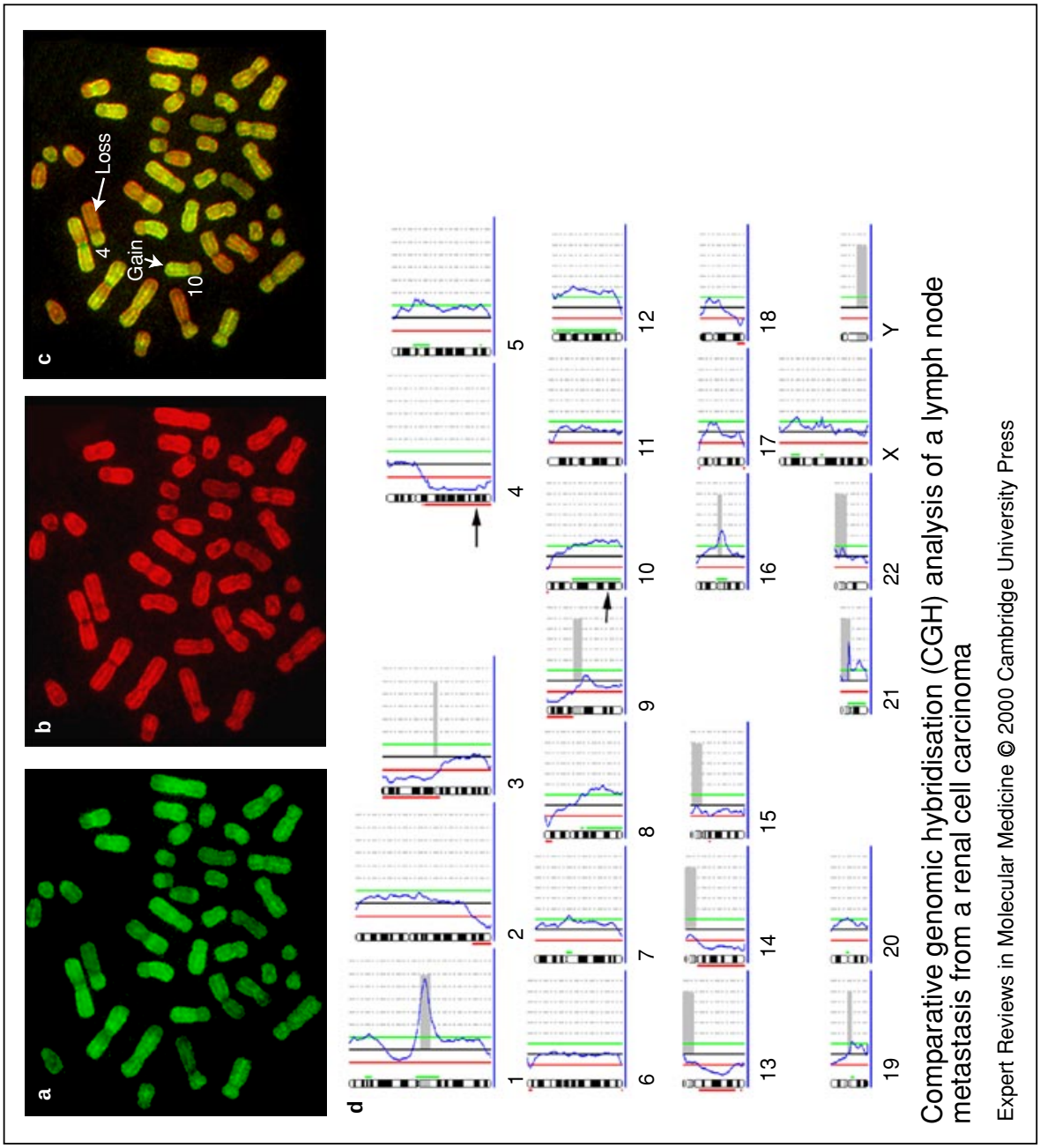

Figure 4. Comparative genomic hybridisation (CGH) analysis of a lymph node metastasis from a renal cell carcinoma. The metaphase spreads shown in this figure were derived from a patient suffering from von Hippel-Lindau syndrome, a hereditary form of renal cell carcinoma. (a) This metaphase spread has been visualised using a green fluorochrome that, in this particular sample, is specific for tumour DNA. (b) The same metaphase spread has been visualised using a red fluorochrome that, in this particular sample, is specific for the control DNA. (c) This image shows an electronically blended composite of the metaphases shown in parts '(a)' and '(b)', which are labelled with green and red fluorochromes, respectively. A chromosomal gain in the tumour is reflected by a stronger intensity of the green fluorescence, whereas a loss is reflected by a stronger intensity of the red fluorescence. (d) This profile shows the ratio of the metaphases that are shown in parts '(a)' and '(b)'. The most prominent loss can be seen at chromosome 4q, whereas the most prominent gain is at chromosome 10q. The grey boxes in the profile represent chromosomal regions that are rich in heterochromatin, which cannot be interpreted owing to the abundance of highly repetitive DNA **(fig004trn)**.

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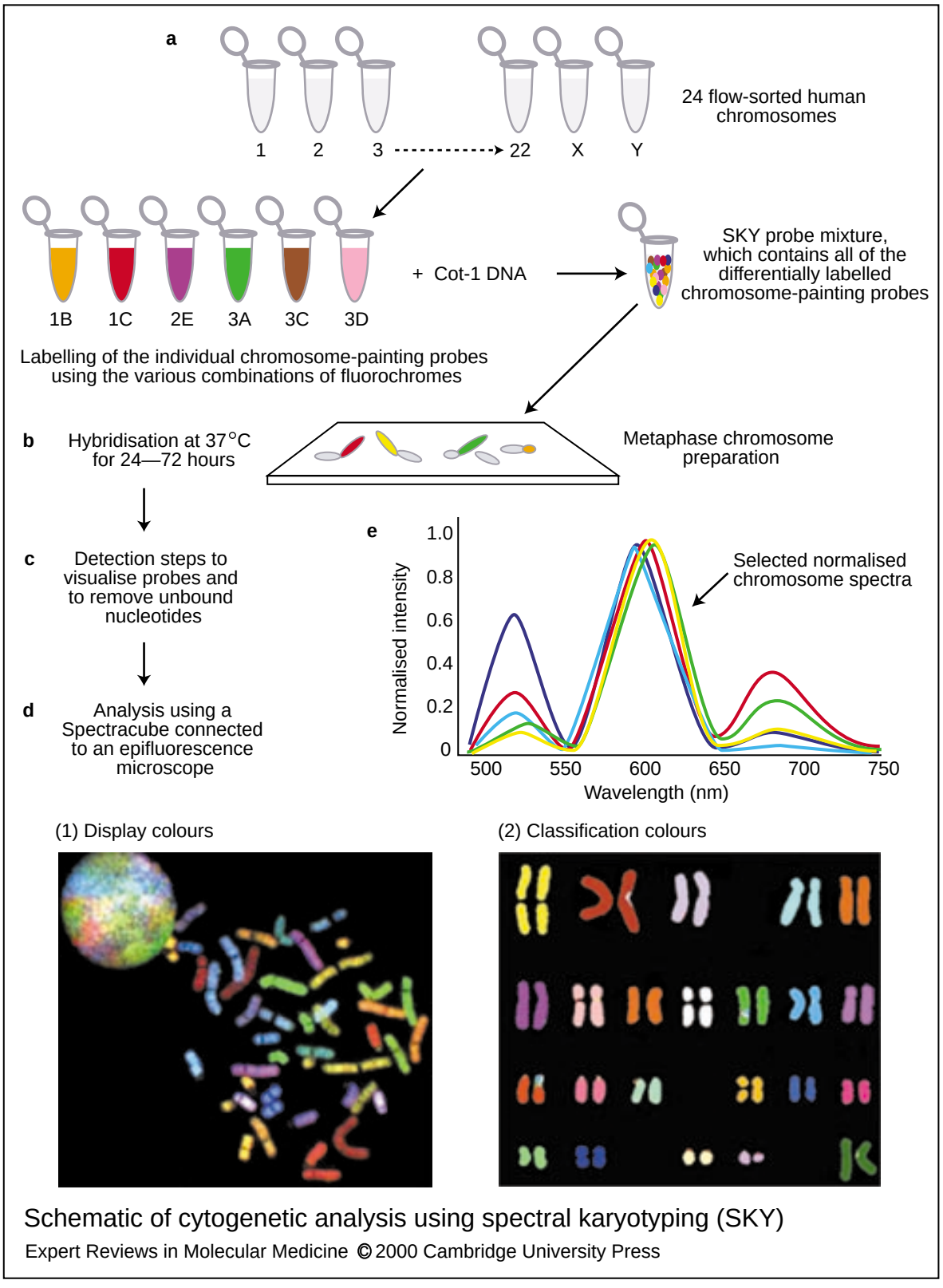

Schematic of cytogenetic analysis using spectral karyotyping (SKY)

Figure 5. Schematic of cytogenetic analysis using spectral karyotyping (SKY) (*see next page for legend*) **(fig005trn).**

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Figure 5. Schematic of cytogenetic analysis using spectral karyotyping (SKY). (a) First, 24 flow-sorted human chromosomes (chromosomes 1–22, X and Y) are combinatorially labelled with at least one, and as many as five, fluorochrome combinations to create a unique spectral colour for each chromosome pair. Aliquotes of the 'painted' chromosomes are pooled together with an excess of Cot-1 DNA. Cot-1 DNA is used to suppress repetitive sequences in the genome. (b) The SKY probe mixture is then hybridised to a metaphase chromosome preparation at 37°C for 24–72 hours. (c) Next, detection of the hybridised sample takes place by washing the slide in solutions containing formamide and SSC (sodium chloride, sodium citrate) to remove unbound nucleotides. Incubation with fluorochrome-conjugated antibodies is used to visualise the probes. (d) Visualisation of the SKY hybridisation requires the use of a Spectracube connected to an epifluorescence microscope. All of the fluorochrome dyes within the sample are simultaneously excited within a single exposure and are imaged with a custom-designed triple band-pass optical filter. The light is sent through a Sagnac interferometer that generates an optical path difference for each pixel that is specific for a certain fluorochrome or fluorochrome combination in the image. (e) Fourier transformation is used to recover the spectrum from this interferogram. A spectral classification is then used to assign a discrete colour to all pixels with identical spectra. The visualisation software applies an RGB (red, green, blue) look-up table to the raw spectral image, resulting in the infra-red emission being displayed in red, the red emission in green and the green emission in blue. The spectral measurement can then be converted to a display image that is based on the fluorescence intensities of all of the chromosome-painting probes (1); classification colours are also assigned to the chromosomes, based on the spectra from every pixel (2). This measurement forms the basis for automated chromosome identification. (See also Fig. 6) **(fig005trn).**

Methodology of SKY

SKY is based on the simultaneous hybridisation of 24 chromosome-specific composite probes. Such chromosome-painting probes are generated from flow-sorted human (or mouse) chromosomes (see Fig. 5). Chromosome-specific spectra are produced by labelling each chromosome library either with a single fluorochrome or with specific combinations of multiple fluorochromes. The use of different combinations of fluorochromes for DNA labelling increases the number of targets that can be discerned. A DOP–PCR (degenerate oligonucleotide primer–polymerase chain reaction) labelling reaction is used to incorporate the fluorochromes into the DNA. Using a combinatorial labelling scheme and five different fluorochromes, 31 different targets can be distinguished. The chromosome-painting probes are pooled with an excess of Cot-1 DNA, to suppress the repetitive sequences, and hybridised onto metaphase chromosome preparations. After incubation for 24–72 hours at 37°C, detection steps are used to visualise the fluorochromes, and residual probe is removed by post-hybridisation washes. Image acquisition is based on a spectral imaging system using an interferometer and a CCD camera. The hybridised sample is illuminated, using a custom-designed triple band-pass optical filter, and the light that is emitted from each point of the sample is collected with the microscope objective. The collected light is transferred to a Sagnac interferometer in which an optical path difference is produced. This is performed at all pixels using a CCD camera. Using Fourier transformation, the emission spectrum is retrieved and processed by a computer. This process allows the spectral signature to be measured at all image points (pixels). The measurement forms the basis for chromosome classification by assigning all pixels with identical spectra-unique colours (Refs 38, 39). The technique results in a specific colour being assigned to each chromosome in the image (see Fig. 6). One advantage of the system is that the image is acquired with a single filter set.

SKY as a diagnostic tool

The applications of SKY for identifying chromosomal aberrations that are involved in human disease are manifold. Karyotype analysis based on chromosomal banding is routinely performed in prenatal and postnatal cytogenetic laboratories. SKY is suitable for the identification of subtle chromosomal aberrations, such as the translocation of telomeric chromatin, which is difficult to discern using banding alone, and the identification of small markers, which remain elusive after conventional banding analysis (Refs 40, 41). In a recent study by Schröck and colleagues, 16 samples that had constitutional chromosome abnormalities were analysed by GTG banding (also known as Giemsa–trypsin–Giemsa banding or G banding). GTG banding is a technique that uses an enzyme to degrade some of the proteins that bind to the chromosomes.

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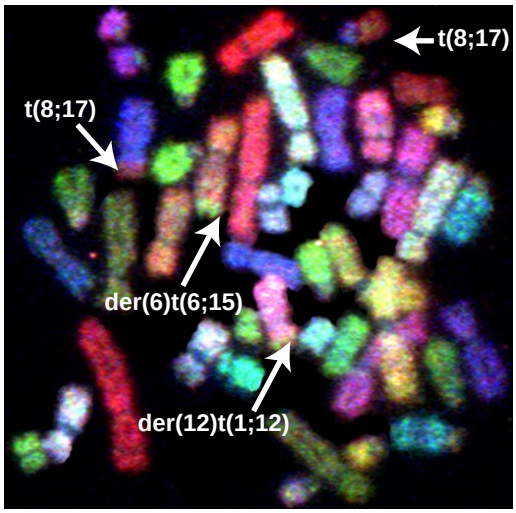

Spectral image of the chromosomes of the primary bladder tumour cell line RT4

Figure 6. Spectral image of the chromosomes of the primary bladder tumour cell line RT4. This image shows balanced translocations between chromosome 8 and chromosome 17. Spectral karyotyping (SKY) also reveals that chromosome 6 is involved in an unbalanced translocation with chromosome 15. A derivative chromosome 12 that is involved in an unbalanced translocation with chromosome 1 is also observed. A derivative chromosome refers to a structurally rearranged chromosome generated from the fusion of two or more chromosomes or by multiple aberrations within a single chromosome. It always refers to the chromosome with the intact centromere. Without SKY, these complex rearrangements would be difficult to detect because the chromosomes that are involved have similar banding patterns. Abbreviations used: 'der' = derivative; 't' = translocation **(fig006trn).**

After enzyme treatment with the protease trypsin, Giemsa dye selectively binds to the A–T nucleotides, creating a black-and-white banding pattern. Using SKY, G-banding diagnosis was redefined in nine of these cases, revealing translocations that were previously unobserved. In five cases, SKY supported the G-banding results. As for the remaining two cases, G-banding results suggested subtle rearrangements that went undetected by SKY. FISH experiments were conducted with locus-specific probes that were optimal for detecting submicroscopic intrachromosomal translocations and deletions (Ref. 42). In another study involving SKY, Padilla-Nash and colleagues (Ref. 43) studied the bladder cancer cell line BK-10 to identify multiple structural aberrations: SKY redefined 11 aberrations that had previously been detected by G banding, detected four hidden chromosomal rearrangements and confirmed the presence of 12 identified markers.

SKY has also been developed for mouse models of human cancers (Ref. 35). Weaver and colleagues used SKY to analyse chromosomal aberrations in mammary gland tumours in mice that were carrying the mammary tumour virus (MMTV)–*cmyc* transgene (Ref. 44). A recurring pattern of chromosome alterations was observed. The loss of chromosome 4 was detected in five of the eight tumours. Mouse chromosome 4 has regions that are orthologous with region 1p31–p36 in humans, which is deleted in some human breast carcinomas. Also detected in four of the tumours were translocations involving the distal region of chromosome 11, which is homologous to human chromosome 17q, which is where the *BRCA1* gene is located. These findings were significant because they showed that similar chromosomal loci are involved in mouse and human breast tumours, therefore validating this mouse model for human carcinomas.

Conclusions

Cytogenetic research and diagnostics have greatly benefited from employing molecular techniques. In particular, the development of FISH techniques has widened the plethora of applications considerably. The considerable gap in resolution between traditional cytogenetic techniques (megabase pairs) and molecular biology techniques (base pairs) has been closed to a large extent by molecular cytogenetics, which allows the assessment of genetic changes on chromosome preparations. The issue of sensitivity has been successfully tackled by devising new protocols for the detection of fluorescently tagged probes as small as 200 base pairs. The third defining technical parameter, multiplicity, has been solved by recent developments of colour karyotyping that allow the visualisation of all human or mouse chromosomes simultaneously in different colours. One very important development in molecular cytogenetic techniques was the introduction of CGH, which has proved

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to be an exceedingly valuable tool for the characterisation of chromosomal imbalances in solid tumours. Together, these developments have contributed to the refinement of the field of molecular cytogenetics and have, in an unpredictable way, increased the flexibility with which experiments can be designed. Thus, the applications of these techniques have been extended beyond the mere diagnosis of chromosomal abberrations to functional and comparative basic research.

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Further reading, resources and contacts

The following **publications** provide a useful source of background information on cytogenetic methodology and molecular cytogenetics and their applications.

Heim, S. and Mitelman, F. (1995) Cancer Cytogenetics, Wiley Liss

Knutsen, T. and Ried, T. (2000) SKY: A Comprehensive Diagnostic and Research Tool: A Review of the First 300 Published Cases. J Assoc Genet Technol 26 (1), 3-15

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Verma, R.S. and Babu, A. (1995) Human Chromosomes: Principles and Techniques, McGraw-Hill

The following **companies** may be contacted for hardware and software as well as for information on cytogenetics applications.

Applied Spectral Imaging Inc., USA (2120 Las Palmas Drive, Suite D, Carlsbad, CA 92009, USA; Tel: +1 760 929 2840; Fax: +1 760 929 2842; E-mail: asi-inc@spectral-imaging.com)

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Chroma Technology Corp. (72 Cotton Mill Hill, Unit A9, Brattleboro, VT 05301, USA; Tel: +1 800 824 7662 or +1 802 257 1800; Fax: +1 802 257 9400; E-mail: sales@chroma.com)
http://www.chroma.com

Leica Microsystems (Imaging Solutions Ltd, Clifton Road, Cambridge, CB1 3QH, UK; Tel: +44 1223 411411; Fax: +44 1223 412526; E-mail: ia-support@leica.co.uk)
http://www.leica.co.uk/ia/homepage.htm

Vysis, Inc. (3100 Woodcreek Drive, Downers Grove, IL 60515-5400, USA; Tel: +1 800 553 7042 or +1 630 271 7000; Fax: +1 630 271 7138; E-mail: vysis_help@vysis .com)
http://www.vysis.com

Features associated with this article

Figures

Figure 1. Examples of different types of fluorescence in situ hybridisation (FISH) probes (fig001trn).
Figure 2. Fluorescence in situ hybridisation (FISH) using whole-chromosome-painting probes (fig002trn).
Figure 3. Comparative genomic hybridisation (CGH) using whole-genomic DNA probes (fig003trn).
Figure 4. Comparative genomic hybridisation (CGH) analysis of a lymph node metastasis from a renal cell carcinoma (fig004trn).
Figure 5. Schematic of cytogenetic analysis using spectral karyotyping (SKY) (fig005trn).
Figure 6. Spectral image of the chromosomes of the primary bladder tumour cell line RT4 (fig006trn).

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