Genetics 101: cytogenetics and FISH

Alison Sinclair

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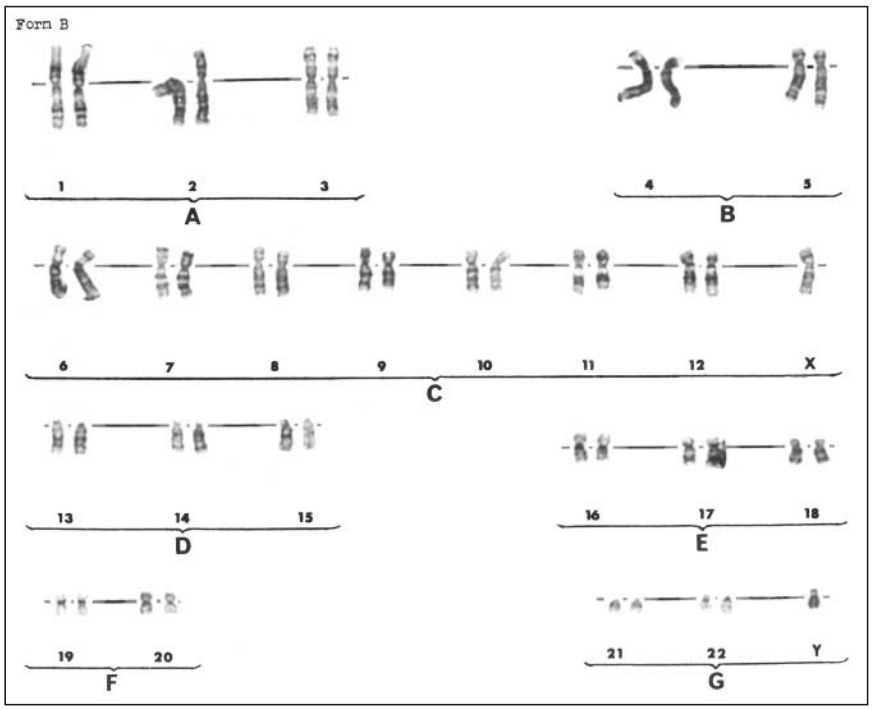
Karyotyping is the isolation, staining and visual examination of chromosomes to find chromosomal rearrangements. Karyotyping is used in prenatal diagnosis (particularly amniocentesis for trisomy 21), investigation of repeat pregnancy loss, investigation of children and adults with features suggestive of a syndrome (dysmorphic features or other associated anomalies, mental retardation, learning disabilities or behavioural anomalies), and subtyping of lymphomas, leukemias and solid tumours[1,2] for the purpose of exact diagnosis, treatment stratification and prognosis.



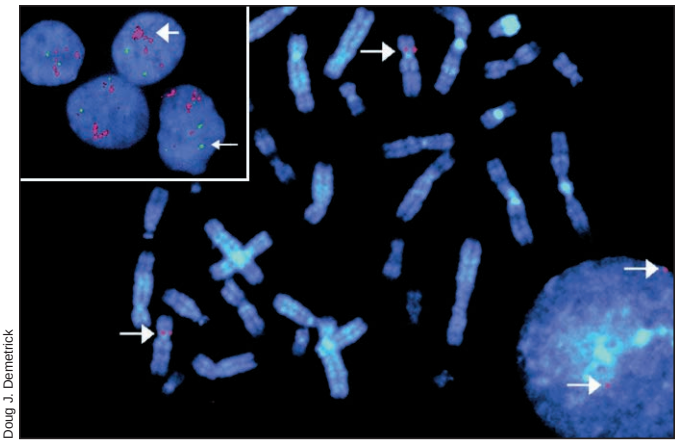
Eric Woolfrton

Fig. 1: Standard G-banded karyotype: each chromosome has a characteristic banding pattern, allowing the identification of gross duplications, deletions, additions and translocations.

Cells are cultured from samples of blood, bone marrow, amniotic fluid, tissue or tumour under sterile conditions for 4–7 days, depending upon the cell type. For karyotyping the chromosomes must be isolated from cells in the metaphase, which is the stage of the cell cycle in which the chromosomes assume their characteristic condensed, discrete shape. Various chemical agents may be added to the cultured cells to synchronize their cell cycles and make it possible to bring the maximum number to a predictable metaphase. The cells are then swollen by the addition of hypotonic solution to disperse the chromosomes, which are fixed chemically, examined microscopically and then stained. Standard G-banding (Giemsa, Leishman's or variant) produces a banding pattern that is characteristic of the individual chromosomes and allows identification of abnormalities in the number and morphology (deletion, addition, translocation of large segments of DNA) of chromosomes (Fig. 1).

Cytogenetic analysis by G-banding cannot resolve structural abnormalities that are small (best resolution is about 2000–3000 kilobases),[3] within G-negative bands or involve translocations between regions that have similar staining patterns. Resolution is also dependent upon the cell of origin. Resolution from blood lymphocytes is better than that from fibroblasts grown from amniotic fluid, and both are generally better than cells from tumours.

Resolution of very small deletions (microdeletions) and small translocations is possible with fluorescence in situ hybridization (FISH).[4] This technique uses short sequences of single-stranded DNA (probes) that carry fluorescent tags to detect chromosomal DNA with a complementary sequence. Gene-specific probes, also known as "locus specific," bind to single areas of a chromosome, whether a gene, or a repetitive sequence such as a centromere or telomere. Such locus-specific probes can detect abnormal duplication of a gene (Fig. 2) or chromosome. The use of 2 different gene-specific probes can detect translocations too small to detect by normal karyotyping, such as when genetic material from the c-*abl* oncogene on chromosome 9 is inserted into the *BCR* gene on chromosome 22 as occurs in chronic myelogenous leukemia. Whole chromosome probes are mixtures of smaller, chromosome-specific probes, which allow each chromosome to be "painted" a different colour.[5,6] Chromosome painting does not detect translocations within chromosomes or allow precise identification of breakpoints. Various extensions of the technique are under development such as spectral banding, which creates a multicoloured banding pattern.[7]



Doug J. Demetrick

Fig. 2: Visualization of genes using locus-specific FISH probes: red signals (see arrows) hybridized to the chromosomes of a metaphase cell and an adjacent interphase nucleus demonstrate normal numbers of the cyclin D1 gene. Inset: excess copies of the *RB1* gene (green) and cyclin D1 gene (red) are demonstrated in human breast cancer cells.

The potential of FISH to detect much smaller chromosomal abnormalities than can be detected with karyotyping has already been mentioned. FISH does not require cells to be in the metaphase before analysis, because it relies upon the presence or absence of a fluorescent signal to identify chromosomes or parts of chromosomes, rather than a specific banding pattern. It allows the surveillance of more cells and requires a much smaller sample than karyotyping — even down to a single cell. Increasingly, this technique has moved from the research laboratory into routine use, particularly when specimens are in limited quantity (such as those for prenatal diagnosis) or give poor-quality results (tumours) or negative results (microdeletion syndromes) on karyotyping.

Dr. Sinclair is a medical writer in Victoria, BC. She was formerly an Editorial Fellow with *CMAJ*.

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References

1. Pedersen-Pjergaard J. Molecular cytogenetics in cancer. *Lancet* 2001;357:492-3.
2. Olopade O, Sobulo O, Rowley JD. Recurring chromosomal arrangements in human cancer. In: Bast RC Jr, Gansler TS, Holland JF, Frei E, editors. *Cancer medicine e.5.* Hamilton (ON): BC Decker; 2000.
3. McNeil N, Ried T. Novel molecular cytogenetic techniques for identifying complex chromosomal rearrangements: technology and applications in molecular medicine. *Exp Rev Mol Med* 2000;Sept 14. Available: www.ermm.cbcu.cam.ac.uk/00001940.htm (accessed 2002 July 19).
4. Demetrick DJ. Gone FISHin' for genes. *CMAJ* 1999;161(9):1138.
5. Uhrig S, Schuffenhauer S, Fauth C, Wirtz A, Daumer-Haas C, Apacik C, et al. Multiplex-FISH for pre- and postnatal diagnostic applications. *Am J Hum Genet* 1999;65(2):448-62.
6. Bayani J, Squire JA. Advances in the detection of chromosomal aberrations using spectral karyotyping. *Clin Genet* 2001;59:65-73.
7. Kakazu N, Ashihara E, Hada S, Ueda T, Sasaki H, Terada M, et al. Development of spectral colour banding in cytogenetic analysis. *Lancet* 2001; 357(9255):529-30.

Correspondence to: *Dr. Alison Sinclair, 406-337 St. James St., Victoria BC V8V 1J7; fax 250 380-6717; alisonsinclair@shaw.ca*