

Recent Developments in Cytogenetics

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The major advance that facilitated rapid growth of the field of cytogenetics was the development of techniques for examining chromosomes in peripheral blood lymphocytes. The "banding era in human cytogenetics began in 1970 with the publication of Q-banding of human chromosomes. Q-banding represented a tremendous breakthrough in chromosome identification and clinical diagnosis. In the 1970s, newer banding techniques were introduced that significantly improved the resolution of chromosome morphology. C-banding, which was an extension of Pardue and Gall's *in situ* hybridization technique allowed the study of chromosome polymorphisms. The introduction of G-banding in 1971 by Sumner et.al was another major breakthrough in the field of cytogenetics. Today Giemsa banding is the most widely utilized staining technique for chromosome analysis. The standard banding techniques developed in the 1970s and our knowledge of the significance of chromosome abnormalities in cancer and genetic diseases, derived from their use, still remain the cornerstone of medical cytogenetics.

Fluorescence *in situ* hybridization (FISH) provides one of the most effective and rapid approaches for assigning and ordering DNA fragments within single eukaryotic chromosome bands. These techniques have wide applications not only for the mapping of the human genome and the genomes of other organisms, but also in clinical cytogenetics, somatic and germ cell genetics, cancer diagnosis and gene expression studies. The development of FISH methods and a diverse array of molecular probes has brought a new emphasis and refinement to cytogenetics and the types of questions that can be addressed. FISH techniques are now widely used in clinical diagnostic laboratories and provide an important adjunct to classical cytogenetic procedures. Recent applications of fluorescence *in situ* hybridization (FISH) using chromosome and centromere specific DNA libraries have given more insight into the mechanisms of induction of chromosomal aberrations. The detection of telomere repeats at the chromosomal breakage sites by FISH indicates a possible role of telomerase dependent or independent processes in chromosome healing and telomere capture in mammalian cells. FISH technique using chromosome specific DNA libraries has also been combined with PCC and applied to study radiation induced translocations in human quiescent fibroblasts and lymphocytes.

Comparative genomic hybridization (CGH), reported in 1992 was the first molecular cytogenetic tool that allowed comprehensive analysis of the entire genome. The technique had attracted wide spread interest especially among cancer researchers. Today the method of Spectral karyotyping (SKY) or multiplex-FISH (m FISH) approaches based on chromosome painting probes combinatorially labeled to distinguish all 24 human chromosomes in the human karyotype by the unique spectral signatures provide a powerful tool to detect rearrangements between different chromosome classes. Further, microdissected chromosome region specific DNA libraries and different fluochrome labeling enable one to characterize chromosome rearrangements in finer detail.

The estimation of aneuploidy rate in human gametes is a subject of interest and research because non-disjunctions make a major contribution to the chromosomal abnormalities found in man. Investigations of the chromosomal constitution of spermatozoa address the important question of the paternal contribution to aneuploidy. FISH with chromosome specific DNA probes provides a rapid technique for detecting aneuploidy in human sperm. Finally, multicolour FISH analysis is much more accurate than single-colour FISH because it provides internal controls to detect a lack of hybridization and to distinguish disomy from diploidy. The Primed *In Situ* labeling (PRINS) technique provides a fast and efficient alternative to conventional FISH for nucleic acid detection. Based on the use of chromosome-

specific primers, the PRINS method combines the high sensitivity of the PCR reaction with the cytological localization of DNA sequences.

The *in vitro* tests for chromosomal aberrations (CA), Sister Chromatid Exchanges (SCE) and Micronuclei (MN) have undergone considerable evolutionary changes over the last 20 years. Since its description in 1985, the cytokinesis-block micronucleus (CBMN) technique in human lymphocytes has been adopted by many laboratories world-wide as a means for rapidly assessing baseline chromosome damage in human populations and for *in vitro* genotoxicity testing. In its current basic form the CBMN assay can provide, using simple morphological criteria, the following measures of genotoxicity and cytotoxicity: chromosome breakage, chromosome loss, chromosome rearrangements (nucleoplasmic bridges), cell division inhibition, necrosis and apoptosis. The use of molecular probes enables chromosome loss to be distinguished from chromosome breakage and importantly non-disjunction in non-micronucleated binucleate cells can be efficiently measured. However the appropriate implementation of this assay requires a thorough understanding of both experimental variables and biological factors that can have impact on micronucleus (MN) frequency. It is also important to establish standardized protocols that would enable more reliable data comparison between laboratories world-wide. The International human micronucleus (HUMN) project launched in 1997 has created a network of laboratories interested in increasing the understanding of human MN assay and in its improvement.