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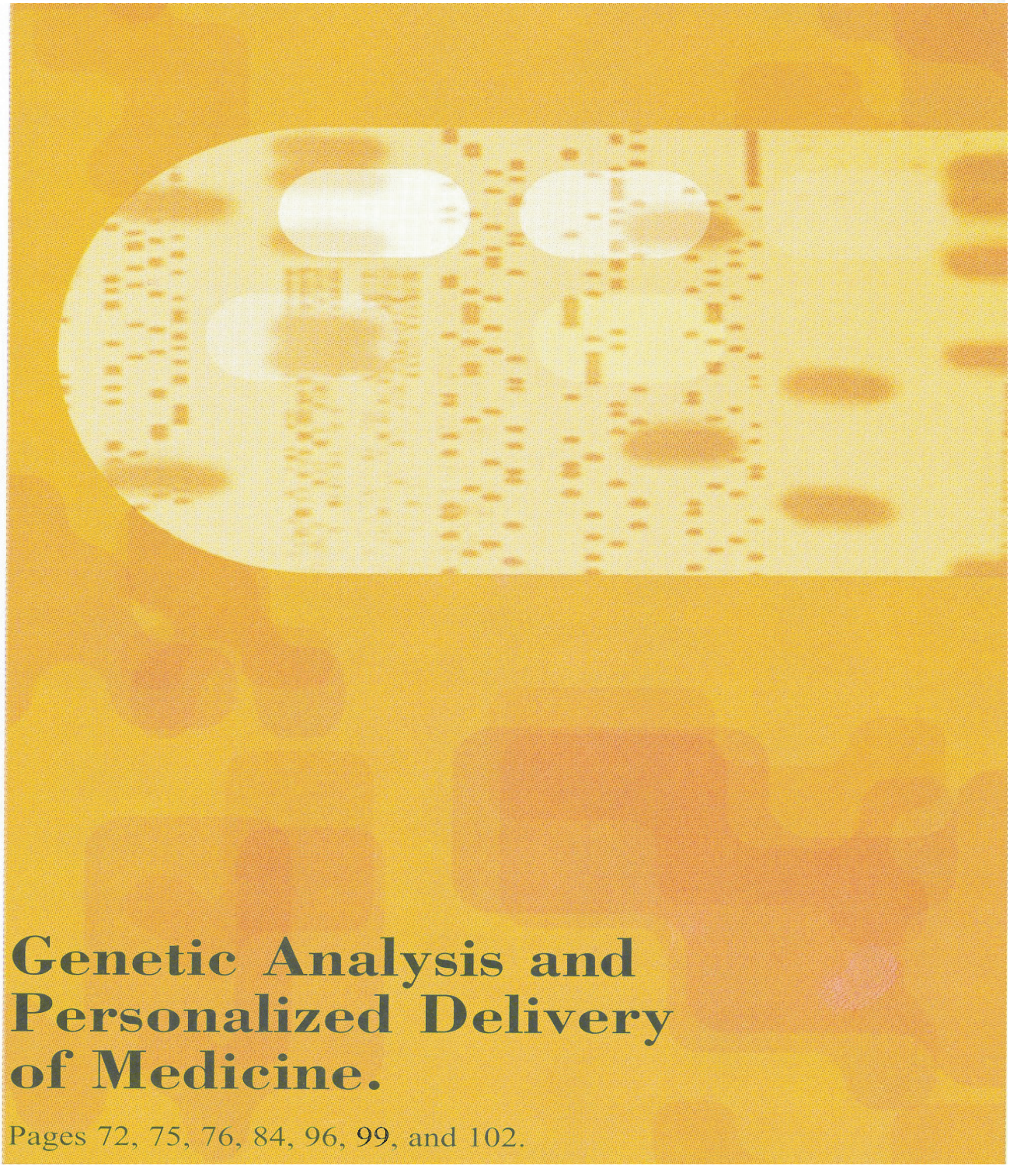

Genetic Analysis and Personalized Delivery of Medicine.

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Karen Peng

High resolution definition of chromosome abnormalities with probes designed from genome sequences

Joan H.M. Knoll and Peter K. Rogan*

Laboratories of Human Molecular Genetics and Genomic Disorders, Children's Mercy Hospitals and Clinics, University of Missouri, Kansas City, 64108, USA
* Correspondence: progan@cmh.edu

Numerical and structural chromosome abnormalities are a common cause of inherited and acquired diseases in humans. Cytogenetic detection of genomic imbalances and rearrangements is standard diagnostic practice, and is used both prognostically and for treatment stratification, especially for neoplastic disorders. Abnormalities are recognized by chromosome banding and by fluorescence in situ hybridization (FISH). FISH permits examination of specific DNA sequences within single or multiple chromosome bands on a metaphase cell or within an interphase cell.

Locus-specific FISH probes have traditionally been composed of recombinant DNA segments that span large chromosomal targets of hundreds of thousands of base pairs, about an order of magnitude smaller than the length of a typical chromosomal band. These probes, which contain either repetitive sequences, single copy sequences or combinations of both, have been developed to hybridize to a wide spectrum of chromosomal targets -- encompassing a single gene to an entire chromosome. Commercially available clones are generally useful for detecting more common abnormalities, whereas, detection or characterization of rare chromosomal abnormalities by FISH has relied on clones obtained from research laboratories.

Combinations of probes can be scored simultaneously within a cell if the sequences are detected with different fluorescent moieties or are distinguishable from one another based upon their chromosomal locations. Changes in copy number can be scored at multiple individual loci with array comparative genomic hybridization (CGH) (Pinkel et al., 1998). CGH is a modification of FISH where the patient's DNA is hybridized to an array of probes that are attached to a solid support such as a microscope slide. Both FISH on interphase cells and CGH detect changes in locus copy number, but neither technique detects abnormalities that solely alter the context or position of the sequence in the genome. Metaphase FISH detects changes in the position of the hybridized sequence as well as differences in copy number, but the lengths of commonly-used probes prohibit the precise, high-resolution definition of structural abnormalities.

DNA probes designed directly from genome reference sequences overcome these limitations. We have produced short repeat-free probes to detect chromosome rearrangements and determined the distribution of probe intervals across the sequenced, euchromatic human genome (Rogan et al., 2001). The technology involves computationally identifying the boundaries of single copy intervals within a specified disease locus, selecting primers for long PCR amplification of one or more of these intervals, then purification of the amplicon either as a probe for FISH or for other genomic applications, e.g., as microarray targets (Figure 1). In contrast to conventional recombinant DNA probes, these probes can be designed and produced quickly, at a high rate of throughput.

Single copy fluorescent in situ hybridization (or scFISH) delineates chromosome abnormalities at a resolution equivalent to genomic Southern analysis, which is approximately 100-fold greater than conventional FISH probes. This is because scFISH probes are substantially shorter (typically 1,500-3,500 bp) than conventional recombinant DNA-derived probes (100-300 kb), potential probe sequences are more densely arranged on chromosomes (average of 1 per 20 kb for chromosomes 21 and 22), and suppression of repetitive sequences is not required. The reproducibility of data obtained using related hybridization techniques such as array CGH, which also utilize locus-specific probes, has been compromised by batch-to-batch variability of highly reiterated DNA, i.e., Cot1 DNA, used for repetitive sequence suppression (Carter et al., 2002). Recent array CGH studies have confirmed our initial findings that single copy amplicons significantly improve signal-to-noise ratios and increase the genomic resolution for abnormality detection (Mantripragada et al., 2004).

We have developed single copy sequence probes (>1,500 bp) for both inherited and acquired disorders for more than 30 chromosomal regions, and have used these probes

to detect abnormalities and localize sites of structural chromosome rearrangements (Knoll and Rogan, 2003). For example, we have utilized both individual and combinations of probes to detect congenital microdeletion syndromes such as Angelman syndrome and DiGeorge syndrome on chromosomes 15 and 22; to characterize imprinting center deletions in Prader-Willi syndrome on chromosome 15; to detect cryptic subtelomeric rearrangements for many chromosomes; to identify atypical chromosome breakpoints in Smith-Magenis syndrome on chromosome 17; and to delineate translocation breakpoints in chronic myelogenous leukemia in chromosomes 9 and 22. Additionally, we have designed probes that hybridize to multiple cis paralogs, i.e., a family of adjacent related sequences, on chromosome 16, and that detect the chromosome inversion breakpoint in acute myelogenous leukemia-M4 by splitting the family of related sequences onto both chromosome arms.

Rapid and precise characterization of cytogenetic abnormalities is feasible because of the sequence-defined properties and dense euchromatic organization of scFISH probes. These properties will facilitate numerous other clinical molecular cytogenetic applications, including: (a) improving on existing commercial probes by selecting scFISH probes that result in lower rates of false negative detection of abnormalities; (b) detecting or characterizing abnormalities for which commercial probes are not available; (c) delineating precise breakpoints of common chromosome rearrangements (as this information has become more important in diagnosis and treatment, especially in various forms of leukemia where deletions have been found adjacent to translocation breakpoints); (d) customizing development of probes for detection of private or familial rearrangements, for example, in pre-implantation diagnosis; and (e) therapeutic or pharmacogenetic screening, for example, probes that detect inherited duplications or deletion alleles of drug metabolizing genes, treatment-related amplification of drug transporter loci, or that identify tumor-associated rearrangements in drug metabolism genes.

scFISH permits the high resolution definition of chromosome abnormalities, obviating the requirement to screen recombinant DNA libraries for clones containing breakpoints from derivative chromosomes. This capability to differentiate among multiple intragenic breakpoints will allow for more precise cytogenetic and ultimately clinical characterization of acquired and inherited disorders.

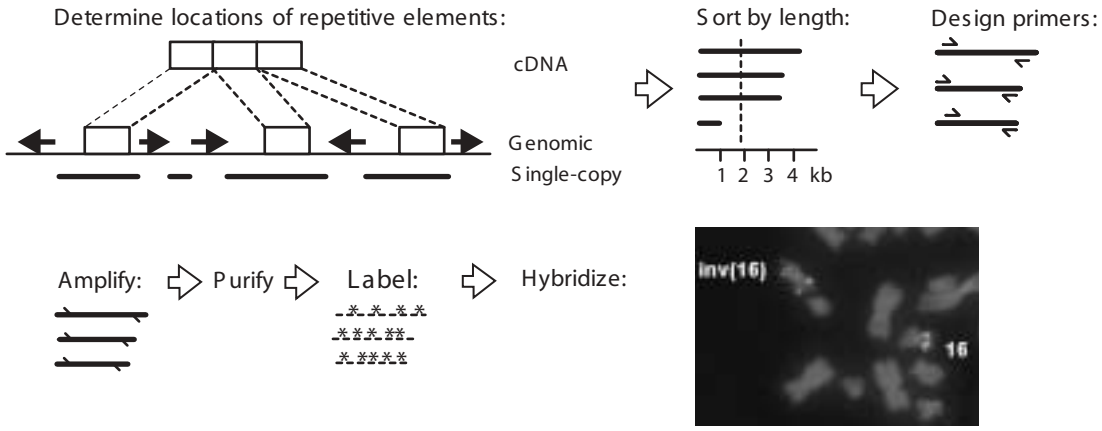

Figure 1: Design and production of single copy FISH probes.

References

Carter NP, Fiegler H, Piper J.
Comparative analysis of comparative genomic hybridization microarray technologies: report of a workshop sponsored by the Wellcome Trust.
Wellcome Trust Sanger Institute, Hinxton, UK.
Cytometry 49:43-48, 2002.
Summary: The study presents results from a multi-center study of the reproducibility of array-CGH in laboratories developing this technology. PCR-based methods for amplification of large insert clones for arraying and hybridizations performed in simple wells with gentle rocking were the most effective, however commercial $C_{o}t1$ preparations exhibited variable suppression of hybridization to repeat sequences.

Knoll JH and Rogan PK.
Sequence-based, in situ detection of chromosomal abnormalities at high resolution.
Children's Mercy Hospital and Clinics, University of Missouri-Kansas City, MO, USA.
Am J Med Genet 121A:245-257, 2003.
Summary: This study documents the design, production, validation and application of single and low-copy FISH probes for detection of numerous distinct chromosomal disorders. Applications highlight the detection of cryptic and subtle aneuploidies, refinement of chromosome breakpoints, and development of probes with low copy paralogs whose distributions are optimized for detection of chromosome rearrangements.

Mantripragada KK, Tapia-Paez I, Blennow E, Nilsson P, Wedell A, Dumanski JP.
DNA copy-number analysis of the 22q11 deletion-syndrome region using array-CGH with genomic and PCR-based targets.
University of Upsalla, Sweden.
Int J Mol Med 13:273-279, 2004.
Summary: The authors compare hybridization of fixed arrays of genomic clones and single copy probes derived from chromosome 22q11 to labeled chromosomal DNA from patients and controls. Repeat-free genomic amplicons of 11.5 kb and cDNAs of 3.5 kb were found to reliably detect deletions in patients with DiGeorge syndrome.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG.
High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays.
University of California-San Francisco, CA, USA.
Nat Genet 20:207-211, 1998.
Summary: This study introduced analysis of chromosome aneuploidy by reverse hybridization of labeled patient genomic DNA to microarrays comprised of mapped, locus-specific recombinant clones of human genomic sequences. The genomic resolution of the analysis (~3 Mb) was based on the size and distribution of the DNA clones.

Rogan PK, Cazcarro PM, Knoll JH.
Sequence-based design of single-copy genomic DNA probes for fluorescence in situ hybridization.
Children's Mercy Hospital and Clinics, University of Missouri-Kansas City, MO, USA.
Genome Res 11:1086-1094, 2001.
Summary: The authors describe the computational and laboratory methods used to develop and hybridize single copy probes (> 2,000 bp in length) to chromosomes of normal individuals and those with congenital abnormalities. The probes are designed directly from the human genome reference sequence. The study also demonstrates that suppression of repetitive sequence hybridization is not required for use of these probes. The distribution of probe intervals in finished human chromosome sequences establishes the feasibility of a genome-wide application for the detection of chromosome abnormalities.