

Advances in Molecular Cytogenetic Technology: Comparative Genomic Hybridization (CGH)



Mark Adams

COMPARATIVE GENOME HYBRIDIZATION (CGH) IS A TECHNIQUE USED TO VISUALIZE DIFFERENCES BETWEEN TWO GENOMES. IT HOLDS PROMISE FOR IDENTIFYING GENES THAT ARE AMPLIFIED OR DELETED — USEFUL FOR FINDING SPECIFIC SYNDROMES OR EXAMINING DIFFERENCES THROUGHOUT THE ENTIRE GENOME. THIS ARTICLE DISCUSSES THE DEVELOPMENT, MECHANISM, AND APPLICATION OF CGH.

DEVELOPMENTS LEADING UP TO COMPARATIVE GENOME HYBRIDIZATION

Chromosomal abnormalities are one cause of phenotypic differences between normal and affected individuals. The technique of chromosomal banding was developed in the early 1970s to identify these abnormalities (Shaffer & Bejjani, 2004). Banding uses chemicals that alter the colorimetry of DNA pairs to produce differently stained regions on chromosomes. These regions appear as bands of varying shades of grey under light or fluorescence microscopy. This allows the detection of structural rearrangements such as translocations, deletions, duplications, polymorphic variations, or ploidy differences (Shaffer & Bejjani, 2004). However, with time it has become evident that routine banding methods cannot uncover all abnormalities, as only rearrangements greater than 10 megabases can be reliably detected (Spiecher & Carter, 2005). As a result, numerous technologies have been developed to provide a higher resolution in the delineation of subtle rearrangements.

Molecular cytogenetics was developed in the 1980s and is based on the hybridization of DNA probes to target DNA (Levsky & Singer, 2003). These probes are labeled using fluorochromes for a variety of different regions of the chromosome, including the centromere, telomere, specific genes, or even the entire chromosome (Shaffer & Bejjani, 2004). Application of fluorescence in-situ hybridization (FISH) in conjunction with routine banding vastly improved the detection of changes in DNA (Albertson & Pinkel, 2003). FISH is a popular molecular cytogenetic technique that hybridizes the labeled probe to the target DNA, through a series of denaturing and re-annealing steps. Currently there are commercially available probe kits for common aneuploidies, specific deletions, duplications, fusion gene rearrangements in cancers, and telomeric regions (Shaffer & Bejjani, 2004). Though this technology provides a higher resolution of 5 to 10 megabases, the largest limitation is that one must decide beforehand on specific regions in which to look (Shaffer & Bejjani, 2004).

COMPARATIVE GENOME HYBRIDIZATION

A new technology called Comparative Genome Hybridization (CGH) has a resolution power equivalent to FISH. Although CGH is limited to using metaphase chromosomes, it has many other advantages (Shaffer & Bejjani, 2004). CGH compares differences in two different genomes; DNA is extracted from both the test subject and a normal reference subject. The two samples are then labeled, usually green (cyanine 3, or Cy3) and red (cyanine 5, or Cy5). The ratio of the two fluorochromes on the metaphase chromosomes are then compared (Spiecher & Carter, 2005). To illustrate its application, consider a red test sample and a green reference sample. If a region is amplified in the test sample, the corresponding region of hybridized chromosome appears red. However, if a region is deleted in the test sample, the corresponding region of the hybridized chromosome would appear green. The ratio of the test to reference fluorochromes is quantified by a computer mapped to each chromosome pair and visualized with a digital image (Spiecher & Carter, 2005).

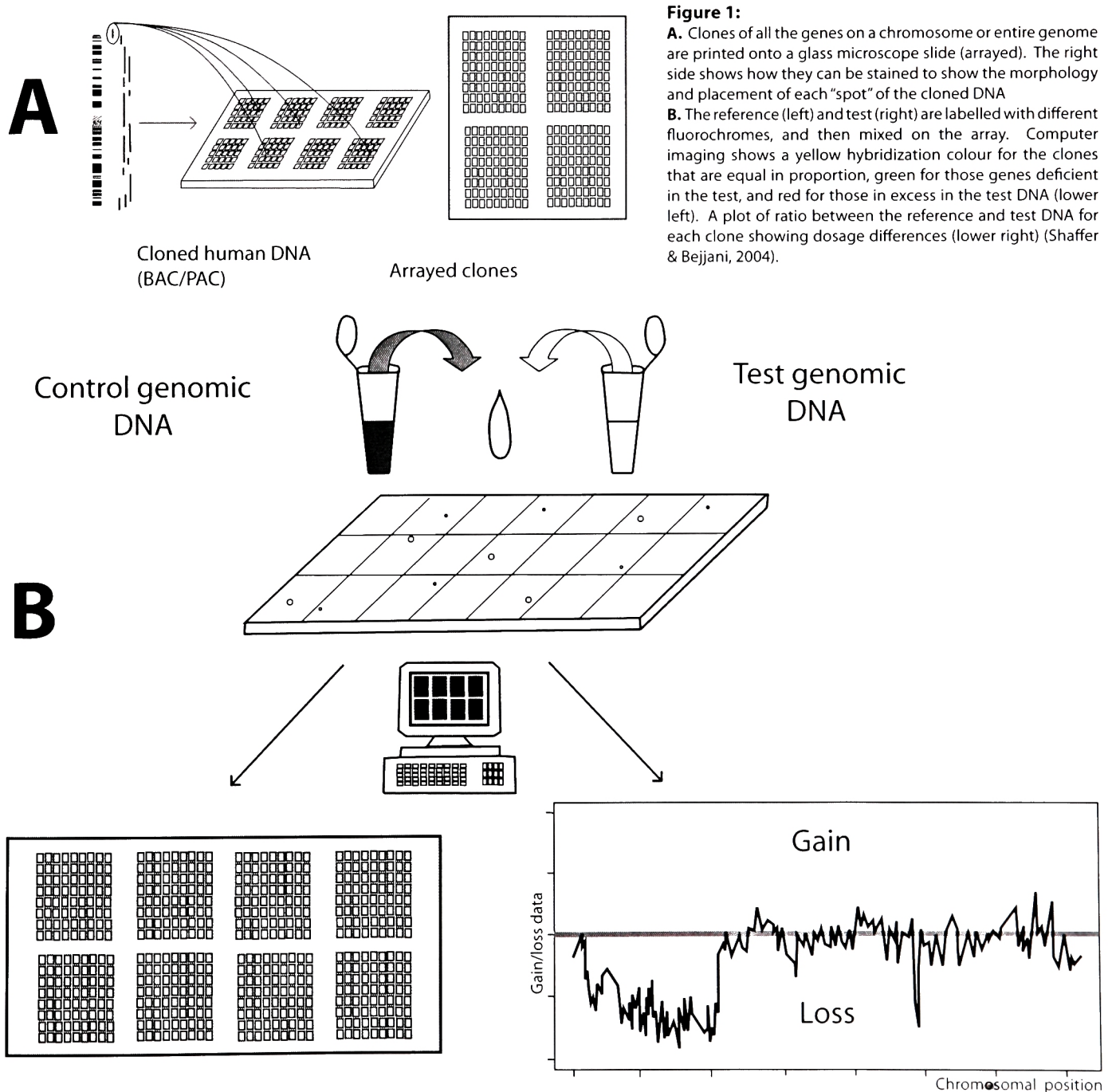
The major advantage of this technology is that it does not require a prior hypothesis of a genetic defect, and can thus identify regions of genetic imbalance. CGH is especially useful in detecting deletions, duplications, non-reciprocal translocations, and gene amplifications throughout the entire genome (Spiecher & Carter, 2005). However, CGH does come with limitations such as failure to detect balanced translocations and inversions due to incomplete genomic information. Also, CGH analysis does not provide possible sites of imbalance, thus locating the site of the imbalance can be a concern without the availability of routine cytogenetics methods (Spiecher & Carter, 2005).

CGH has been improved through use with microarrays. For Array CGH (aCGH), large numbers of mapped clones (cDNA, Bacterial Artificial Chromosomes, PCR-generated sequences, or oligonucleotides) spotted onto a glass slide are used instead of metaphase chromosomes. This increases the resolution of screening for gains or losses of genomic copy numbers so that

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the resolution is only limited by the size and density of the target sequence. Typically, one clone is used per megabase (Carter & Vetrie, 2004). The test and reference genomes are labeled and co-hybridized onto a microarray (Shaffer & Bejjani, 2004). The array is imaged and the fluorescence intensities are calculated for each mapped clone, with the resulting ratio reflecting the differences in DNA copies (Shaffer & Bejjani, 2004). Computer imaging reveals a yellow hybridization colour for all clones that are in equal proportion between the test and reference. Clones that are deleted in the test DNA will appear green, and those duplicated will appear red. A computerized plot of the ratio between test and reference is made to reveal dosage differences. It is visualized as a deviation of the ratio from zero (Shaffer & Bejjani, 2004).

Array CGH is advantageous because of its higher resolution and dynamic range, and there is direct mapping of aberrations to the genome sequence because the alterations are immediately linked to genomic markers via the clones used (Albertson & Pinkel, 2003). aCGH is also advantageous because it can be automated for high-throughput application, and it has a low false-positive count (Shaffer & Bejjani, 2004). There is also wide flexibility with the type of array available, ranging from whole-genome arrays to specialized arrays for specific diseases (Spiecher & Carter, 2005). The highest resolution aCGH is provided by oligonucleotide arrays, produced by spotting oligonucleotides onto a slide, or synthesizing them directly onto the glass. This allows for a resolution as specific as 15 to 20 kilobases (Albertson & Pinkel, 2003). It is even possible to




find single nucleotide polymorphisms (SNP) using SNP arrays, which are high-density oligonucleotide-based arrays. These are useful in identifying Loss of Heterozygosity which is the loss of a single allele at a given locus as a result of genomic mutations (Spiecher & Carter, 2005). The primary drawback of aCGH is the cost; routine testing of the equipment has been quoted at \$90 000, and each array costs more than \$1 200 in some cases (Kolomietz, 2006).

Currently CGH has been used for the analysis of gains or losses in tumours, but remains largely a tool for research purposes. CGH has also been important in identifying the presence and levels of normal genomic variation, which are assumed to be responsible for individual differences in gene expression, phenotypic variation, and susceptibility to disease (Spiecher & Carter, 2005).

APPLICATION TO THE REAL WORLD

There are no genetic techniques that can be used to find every variation. For example, routine G-Banding has been used as the standard for decades in screening for polyploidy, aneuploidy, or rearrangements. However, its low resolution makes it challenging to detect microdeletions, microduplications, and subtle rearrangements; it is also reliant on the adequate preparation and morphology of the chromosomes. FISH can be quite effective for clinical laboratories in identifying specific syndromes, provided that

FISH probes are commercially available (Spiecher & Carter, 2005). CGH is very useful for searching the entire genome and for indicating the presence of genetic abnormalities, but remains very expensive, allowing only well-funded labs to utilize CGH technology.

Each technique described seems to be useful under different circumstances, while lacking in terms of resolution or economics. At this time, it is best to combine techniques in order to provide a comprehensive analysis, if the tests are available. An example given by Speicher & Carter (2005) illustrates this point best using the example of a child exhibiting mental disability and dysmorphic features due to an unknown chromosomal rearrangement. First, G-banding was able to show a complex chromosomal rearrangement, but could not identify the structural chromosomal rearrangements. FISH could identify the rearrangement involving the chromosomes 2, 5, 6, 8, and 14, but could not tell anything about the imbalance. Lastly, array CGH was able to identify four genomic deletions, and allowed direct mapping of the deletion breakpoints onto the reference. The number of genes involved could be determined using internet based genome browsers (Spiecher & Carter, 2005). With strategies similar to this, it is plausible that the origin of some genetic disorders can be confirmed. Also, the possibility of developing a more cost efficient technology will allow genetic disorders to be diagnosed in the most economic manner under the tight restraint of health care dollars. 

REFERENCES

- Albertson, D. & Pinkel, D. (2003). Genomic micro arrays in human genetic disease and cancer. *Human Molecular Genetics*. 12(2), 145-152.
- Carter, N. & Vetrie, D. (2004). Applications of genomic micro arrays to explore human chromosome structure and function. *Human Molecular Genetics*. 13(2), 297-302.
- Kolomietz, E. (2006, May 19). Array CGH: Expanding resolution of cytogenetics analysis. Paper presented at the 2006 Great Lakes Chromosome Conference, Toronto, Ont.
- Levsky J. & Singer, R. (2003). Fluorescence in situ hybridization: past, present and future. *Journal of Cell Science*. 116, 2833-2838.
- Shaffer, L. & Bejjani, B. (2004). A cytogenetist's perspective on genomic microarrays. *European Society of Human Reproduction and Embryology*, 10(3), 221-226.
- Spiecher, M. & Carter, N. (2005). The new cytogenetics: Blurring the boundaries with molecular biology. *Nature* 6, 782-792.

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Project Supervisor
Viola Freeman
Assistant Professor
Dept. of Pathology and Molecular Medicine,
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