Comparative genomic hybridization allows imbalances anywhere in a genome to be detected

Fluorescence *in situ* hybridization is a powerful technique, but it requires one to know in advance which chromosomal location is to be tested, so that an appropriate specific probe can be used. Comparative genomic hybridization (CGH) removes that limitation. CGH uses three sets of DNA sequences: the test DNA, a normal control DNA, and a collection of known and characterized DNA fragments immobilized on a microarray (**Figure 15.6**). Used in this way, the technique is termed array-CGH (aCGH).

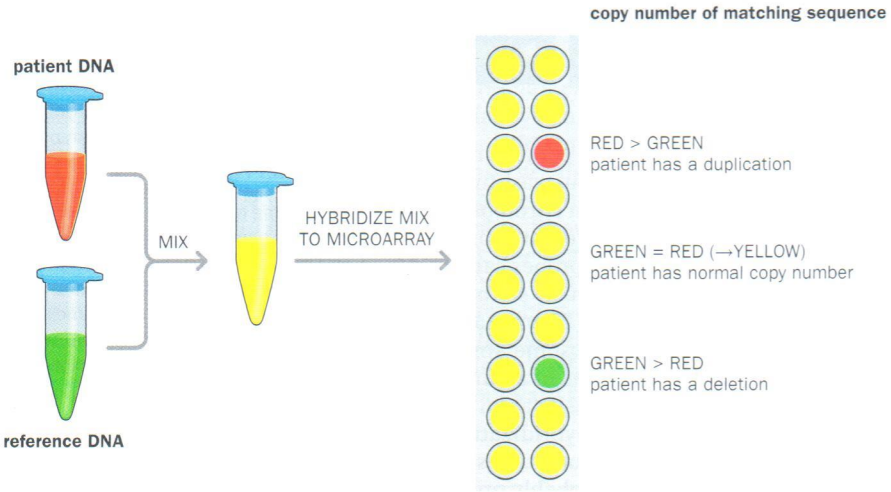

Figure 15.6 Principle of array-comparative genomic hybridization (array-CGH). Test (patient) and reference DNA samples are labeled with different colored fluorophores, fragmented, and made single-stranded. They are mixed in equal genomic amounts and allowed to hybridize to the microarray. Each cell of the array contains a large number of identical single-stranded DNA molecules from a known genomic location. Corresponding fragments of the differently colored test and reference DNA compete to hybridize to the molecules on the array. The average color of a cell of the array after hybridization is a measure of the relative amounts of the corresponding fragments in the test and reference samples. (From Read A & Donnai D [2015] *New Clinical Genetics*, 3rd edn. With permission from Scion Publishing.)

The beauty of array-CGH is that the resolution, and the choice of whether to check the whole genome or some particular part of it, depends purely on the choice of molecules that were used to construct the microarray. To look for imbalances anywhere in the genome, 50,000 long probes fairly regularly spaced across the genome could be used. The average probe spacing of 60 kb would determine the resolution. For a high-resolution examination of, say, just chromosome 9, the same number of probes might be used, but they would be oligonucleotides closely spaced across just that chromosome. Various companies sell standard arrays or will make custom arrays for special purposes.

The array scanner software will present the results in a way that allows one to immediately spot deletions or duplications and see which genes might be involved (**Figure 15.7**). Mosaicism might sometimes be apparent if the color ratios do not fit a simple deletion or duplication, although deep sequencing would be a more sensitive tool for that purpose. Note, however, that array-CGH cannot detect balanced abnormalities such as inversions or balanced translocations. Array-CGH is now the default technique for routine cytogenetics in most diagnostic laboratories.

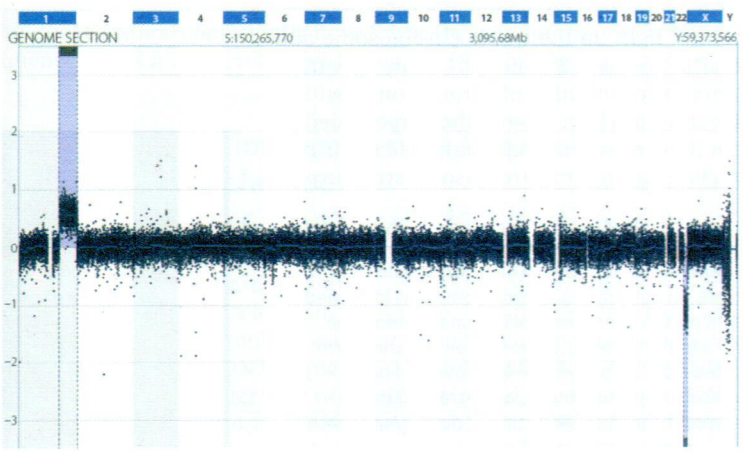

Figure 15.7 Typical result of an array-CGH analysis. Relative intensity of the two fluorescent dyes is plotted on the vertical axis. The reading from each cell of the array is shown as a black dot. Results have been sorted along the horizontal axis by chromosomal location (shown at the top). The result shows that this person has three copies of part of chromosome 1, and only a single copy of part of chromosome 22. (From Read A & Donnai D [2015] *New Clinical Genetics*, 3rd edn. With permission from Scion Publishing. Data produced using an Oxford Gene Technology 8*60 array; courtesy of Lorraine Gaunt and Ronnie Wilson, St Mary's Hospital, Manchester.)

SNP chips can provide similar information to array-CGH

SNP chips (**Figure 15.8**; see **Box 20.1**) use noncompetitive hybridization of just the test DNA. Deletions and duplications are identified by the differing intensity of hybridization, compared to probes from normal diploid sequences. SNP chips have the advantage that they can detect copy-neutral uniparental disomy (UPD). As described in Chapter 10, occasionally, although a sequence is present in the correct two copies per genome, both copies are inherited from just one of the parents. For most chromosomal regions this is only important to the extent that it can create homozygosity for recessive conditions, but for some regions it directly causes developmental abnormalities because of the presence of imprinted genes. When the mechanism producing UPD is trisomy rescue (see Section 15.2), there would be homozygosity for an entire chromosome. Alternatively, mitotic recombination (an abnormal event: normally recombination occurs only in prophase I of meiosis) followed by segregation at cell division can produce segmental UPD, affecting a terminal portion of a chromosome. On a SNP-chip analysis as in **Figure 15.8**, UPD would be seen as a segment having only two genotypes (1-1 or 2-2, depending on the individual SNP) as in Track 3 of the figure, but without any deletion in Tracks 1 or 2. Runs of homozygosity are also seen without UPD when a person inherits both copies of a chromosomal segment from a common ancestor through different lines of descent (**autozygosity**), but except in a closely inbred person the runs are short. If necessary, UPD could be proved unambiguously by genotyping the parents.

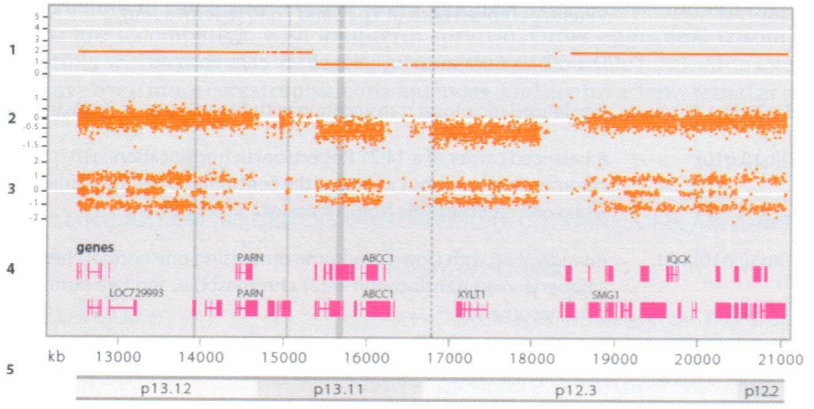

Figure 15.8 SNP-chip data showing a microdeletion at 16p13.11. Across the bottom is an ideogram of chromosome 16, showing the bands and physical distance from 16pter. As with the CGH data in **Figure 15.7**, dots in Track 2 represent the data from each cell, with hybridization intensity, summed across both alleles of each SNP, plotted vertically and chromosomal location horizontally. Track 1 shows the interpretation: there is only a single copy of the central part of the sequence, between positions 15,400 and 18,200. Track 3 shows the genotype at each SNP. In the nondeleted regions there are three possible genotypes, 1-1, 2-1, or 2-2 (arbitrary numbering of alleles), while in the deleted region there are only two, 1 or 2. In summary, there is a 2.8 Mb deletion encompassing the genes shown in Track 4. (From Read A & Donnai D [2015] *New Clinical Genetics*, 3rd edn. With permission from Scion Publishing. Data generated using an Affymetrix SNP 6® microarray, courtesy of Lorraine Gaunt, St Mary's Hospital, Manchester.)

15.2 GROSS CHROMOSOME ABNORMALITIES

Nowadays the widespread use of molecular cytogenetic techniques and whole-genome sequencing has removed any clear dividing line between changes traditionally described as chromosomal and changes thought of as molecular or DNA defects. Nevertheless, in this section we will describe the large-scale abnormalities that are visible under the microscope and traditionally described as chromosomal. We will deal with microdeletions and microduplications in Section 15.3. One might consider an alternative definition of a chromosomal abnormality as an abnormality produced by a specifically chromosomal mechanism, such as incorrect segregation of chromosomes during mitosis or meiosis, improper recombination events, or misrepair of broken chromosomes.

Like all other genetic abnormalities, chromosomal abnormalities can be constitutional or mosaic. Constitutional abnormalities are present in all cells of the body and most likely were present in the original fertilized egg, the result of an abnormal sperm, an abnormal ovum, or a mishap during fertilization. Mosaic abnormalities result when something goes wrong with a single cell in a post-zygotic embryo—most likely, nondisjunction during mitosis. Chromosomal abnormalities, whether constitutional or mosaic, can be classified into numerical and structural abnormalities (**Table 15.2**).

Numerical chromosomal abnormalities include polyploidy and aneuploidy

Polyploidy

Normal somatic cells are diploid, having two genomes. Gametes (sperm or egg) are haploid, with a single genome (23 chromosomes). Polyploid cells have more than two complete genomes. Out of all recognized human pregnancies, 1–3% involve a triploid