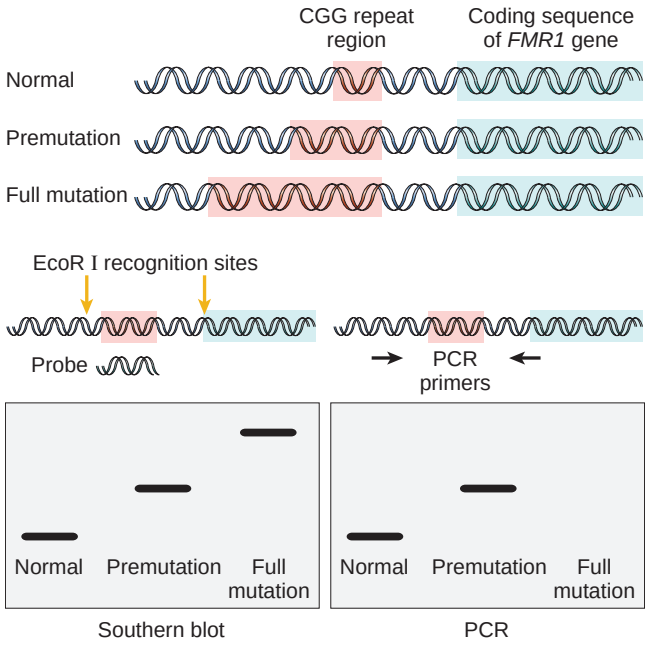

Figure 5-30 Diagnostic application of PCR and Southern blot analysis in fragile X syndrome. With PCR the differences in the size of CGG repeats between normal and premutation give rise to products of different sizes and mobility. With a full mutation, the region between the primers is too large to be amplified by conventional PCR. In Southern blot analysis the DNA is cut by enzymes that flank the CGG repeat region, and is then probed with a complementary DNA that binds to the affected part of the gene. A single small band is seen in normal males, a band of higher molecular weight in males with premutation, and a very large (usually diffuse) band in those with the full mutation.

Fluorescence in Situ Hybridization (FISH)

FISH uses DNA probes that recognize sequences specific to particular chromosomal regions. As part of the Human Genome Project, large libraries of bacterial artificial chromosomes that span the entire human genome were created. The human DNA inserts in these clones are on the order of 100,000-200,000 base pairs. These DNA clones are labeled with fluorescent dyes and applied to metaphase chromosome spreads or interphase nuclei that are pretreated so as to "melt" the genomic DNA. The probe hybridizes to its homologous genomic sequence and thus labels a specific chromosomal region that can be visualized under a fluorescent microscope. The ability of FISH to circumvent the need for dividing cells is invaluable when a rapid diagnosis is warranted (e.g., when deciding to treat a patient with acute myeloid leukemia with retinoic acid, which is only effective in a particular subtype with a chromosomal translocation involving the retinoic acid receptor gene [Chapter 13]). FISH can be performed on prenatal samples, peripheral blood cells, touch preparations from cancer biopsies, and even fixed archival tissue sections. FISH is used to detect numeric abnormalities of chromosomes (aneuploidy) (Fig. 5-19); subtle microdeletions (Fig. 5-20) or complex translocations that are not demonstrable by routine karyotyping; and gene amplification (e.g., *HER2* in breast cancer or *NMYC* amplification in neuroblastomas). Chromosome painting is an extension of FISH, whereby probes are prepared that span entire chromosomes. The number of chromosomes that can be detected simultaneously by chromosome painting is limited by the availability of fluorescent dyes that emit different wavelengths of visible light. This limitation has been overcome by the introduction of spectral karyotyping (also called multicolor FISH). Use of different mixtures of five fluorochromes in probes that are specific for each chromosome permits visualization of entire human genome. So powerful is spectral karyotyping that it might well be called "spectacular karyotyping."

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA blends DNA hybridization, DNA ligation, and PCR amplification to detect deletions and duplications of any size, including anomalies that are too large to be detected by PCR and too small to be identified by FISH. Briefly, each MLPA reaction uses a pair of probes that can hybridize side-by-side to one strand of the target DNA. Once bound, the probes are covalently joined via a ligase reaction. In addition to the target sequence, the probes also contain additional sequences at their ends that can be used as primer sequences in a PCR. The ligated probes thus create a template that can then be amplified by PCR. Quantification of the amplified DNA yields highly accurate information regarding the original amount of genetic starting material at the particular probe location (e.g., a probe at the site of a heterozygous deletion will produce only a 50% signal). Because it involves PCR amplification, MLPA can be performed on very small amounts of genomic DNA, and because each probe-set can be designed with identical primer sequences, many probe-sets can be applied and amplified in one reaction tube. Looking back to the cystic fibrosis example presented earlier, an MLPA probe-set covering the genomic coordinates corresponding to each of the 27 exons of the *CFTR* gene can readily detect deletions affecting one or more exons that would escape identification by PCR and conventional DNA sequencing.

Southern Blotting

Changes in the structure of specific loci can be detected by Southern blotting, which involves hybridization of radiolabeled sequence-specific probes to genomic DNA that has been first digested with a restriction enzyme and separated by gel electrophoresis. The probe usually detects one germ line band in normal individuals, and a different size band depending on the genetic anomaly. With the advent of FISH, MLPA, and microarray technology, Southern blotting is rarely used but remains useful in the detection of certain large-trinucleotide-expansion diseases, including the fragile X syndrome (Fig. 5-30).

Cytogenomic Array Technology

FISH requires prior knowledge of the one or few specific chromosomal regions suspected of being altered in the test sample. However, **genomic abnormalities can also be detected without prior knowledge by using microarray technology to perform a global genomic survey.** First generation platforms were designed for comparative genomic hybridization (CGH), while newer platforms incorporate SNP genotyping approaches, offering multiple benefits.

Array-Based Comparative Genomic Hybridization (Array CGH). In array CGH, the test DNA and a reference