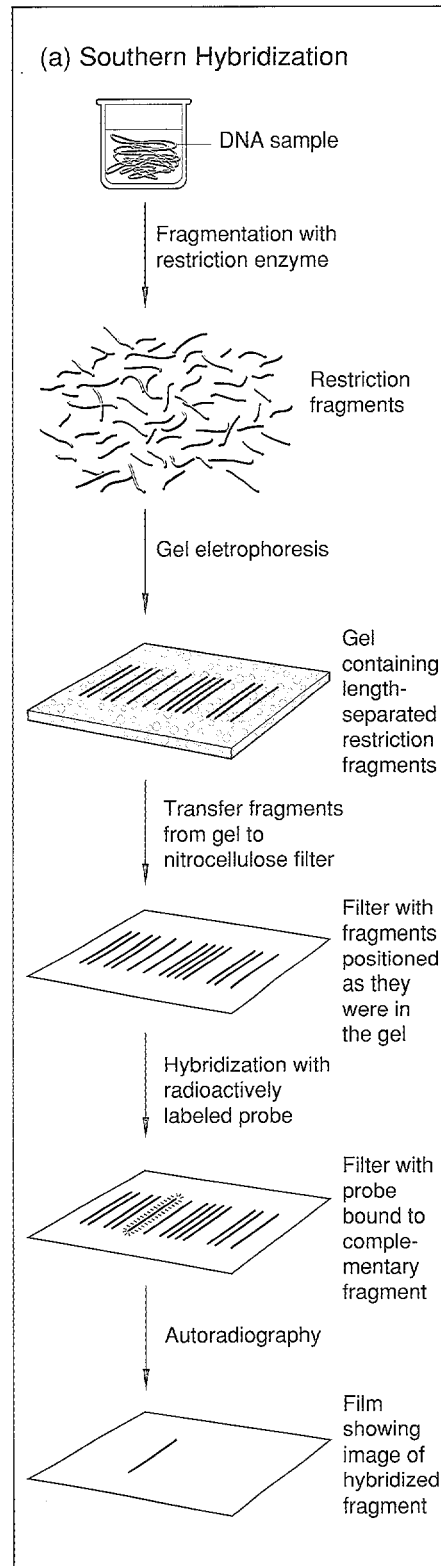


## HYBRIDIZATION TECHNIQUES

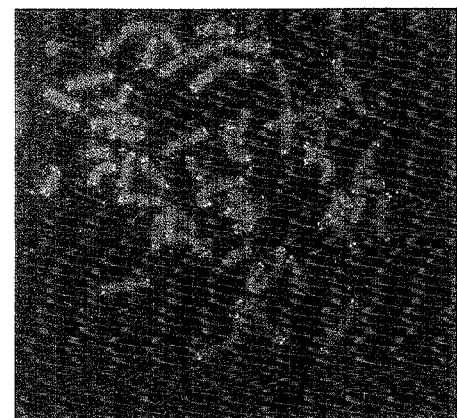
**S**outhern hybridization is a technique for identifying, among a sample of many different DNA fragments, the fragment(s) containing a particular nucleotide sequence. As depicted in (a), the sample has typically been fragmented with a restriction enzyme. The restriction fragments are subjected to gel electrophoresis to separate them by length and immobilize them. The length-separated fragments are then transferred to a filter paper made of nitrocellulose, a procedure called blotting. (Note that blotting preserves the locations of the fragments.) The filter is washed first with a solution that denatures the fragments and then with a solution containing many copies of a radioactively labeled, single-stranded "probe" whose sequence is identical to or complementary to some unique portion of the sequence of interest. The probe hybridizes (hydrogen bonds) to only the denatured fragments containing the complement of its sequence and hence the sequence of interest. The unbound probe is washed away, and the filter is dried and placed in contact with x-ray film. The radioactivity of the bound probe exposes the film and creates an image, an autoradiogram, of the fragment(s) to which the probe has bound. Southern hybridization is particularly useful for detecting variations among different members of a species in the lengths of the restriction fragments originating from a particular region of the organism's genome (see "Modern Linkage Mapping with Polymorphic DNA Markers" in "Mapping the Genome").

The number of fragments "picked out" by a probe depends on the number of times the sequence of interest occurs in the sample DNA. If the sequence occurs only once (if a probe for, say, a single-copy gene is being used), the probe picks out one or at most two fragments (provided the probe is shorter than any of the fragments in the sample). On the other hand, if the sequence of interest occurs more than once (if a probe for a multiple-copy gene or a repeated sequence is being used), the probe picks out a larger



number of fragments. Furthermore, the hybridization conditions (temperature and salinity of the probe solution) can be adjusted so that either exact complementarity or a lesser degree of complementarity is required for binding of the probe.

In-situ hybridization is a variation of hybridization in which the sample consists of the complement of chromosomes within a cell arrested at metaphase. The metaphase chromosomes are spread out and partially denatured on a microscope slide, the probe is labeled with a fluorescent dye, and the bound probe is imaged with a fluorescence microscope. Shown in (b) is the fluorescence signal resulting from in-situ hybridization of a probe for the human telomere to human metaphase chromosomes. (A telomere is a special sequence at each end of a eukaryotic DNA molecule that protects the molecule from enzymatic degradation and prevents shortening of the molecule as it is replicated. The sequence of the human telomere was discovered by Robert K. Moyzis and his colleagues, who also provided evidence that all vertebrates share the same telomeric sequence. Note that, as expected, the probe has bound only to the terminal regions of each chromosome. (Micrograph courtesy of Julie Meyne.)



(b) Results of In-Situ Hybridization of Human-Telomere Probe to Human Chromosomes