I. Southern blotting

Method to detect specific DNA fragment among a complex mixture.
Based on ability of complementary single strands to anneal (hybridize).

1) Separate DNA fragments on an agarose gel.
2) Make DNA single-stranded by soaking in alkaline solution.
3) Transfer ssDNA from gel to solid support (nitrocellulose or nylon **membrane**, or filter), usually by capillary action (**blotting**).
4) Make a radioactive copy of the sequence we want to detect = **probe**.
5) Incubate single-stranded probe with blot in conditions that allow hybridization between probe and target sequence. Wash excess (unbound) probe away.
6) Expose blot to X ray film (**autoradiography**); develop film.

Low-stringency hybridization can be used to find DNA with similar, but not exact sequence. For example, a probe from the human alpha hemoglobin gene may hybridize to the chicken alpha hemoglobin gene, if appropriate conditions are used. The hybrids will be mismatched in some positions.

Library screening:

1) Grow colonies representing different clones in the library.
2) "Lift" imprints of the colonies onto a membrane (some cells will remain on the plate).
3) Bacteria on the membrane are lysed to release DNA; DNA is denatured.
4) Probe the membrane like a Southern blot to detect which colonies have the desired clone.
5) Align the X ray film with the original plate to isolate the appropriate colony.

Chromosome walking: Start with a desired DNA probe.

1) Screen library of genomic DNA for clones that overlap the probe.
2) Map these clones to determine overlap (*e.g.* map restriction enzyme sites).
3) Make a new probe from the end of one of these clones. Go to step 1.

II. Northern blotting

Similar to Southern blotting, but RNA is on the membrane.
Information obtained from northern blotting includes:
size(s) of mRNAs
expression patterns (by using mRNA samples from different tissues, times, conditions, etc.)
abundance (highly expressed genes will have many mRNAs, and give a stronger signal)

III. Western blotting

Proteins on membrane.
Proteins are usually separated on a polyacrylamide gel electrophoresis (PAGE).
Proteins are detected using antibodies that recognize specific proteins.
Provides information about protein expression (time, place, abundance), size, modification, etc.
Can use western blotting to screen an "expression library", in which protein from each clone is expressed in the bacteria.

IV. Expression profiling using microarrays

Microarray = DNA spotted in a grid pattern at very high density (10,000 on a standard microscope slide). Each position has DNA from a known source.

Arrays are used to explore differential expression – increases or decreases in mRNA levels at different times, in different tissues, or in different conditions.

1) mRNA is isolated from each of the two samples to be compared.
2) 1st strand cDNAs are made and labeled with fluorescent tags;
   one sample is labeled with a red tag, the other green.
3) The labeled cDNAs are hybridized to DNA on the array.
4) A laser is used to read the relative levels of red and green at every location:
   yellow = no difference between the two samples
   red = gene expressed more highly in sample one
   green = gene expressed more highly in sample two

Examples:

Alizadeh *et al*. (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403: 503-511.

17,856 cDNA clones (12,069 from a B-cell cDNA library) were analyzed in 96 normal and malignant cell types: 1.8 million measurements made. The results allow precise diagnosis of very specific classes of B-cell lymphoma.

Perou *et al.* (2000) Molecular Portraits of Human Breast Tumors. *Nature* 406: 747-52.

8,102 genes analyzed for 65 breast cancer tissue specimens (some before and after treatment). Allows classification of tumor type by comparison of genes expressed.

White *et al*. (1999) Microarray analysis of Drosophila development during metamorphosis. *Science* 286: 2179-2184.

6,240 cDNA clones (4,500 unique – about 30-40% of genes represented) were analyzed at six time points after pupation, to determine what genes are turned on and off during metamorphosis.

Cirelli and Tononi (1999) Differences in brain gene expression between sleep and waking as revealed by mRNA differential display and cDNA microarray technology. *J Sleep Res*. 8:44-52.