## APPENDIX—3.I

# COLONY HYBRIDIZATION

This technique is used to identify those bacterial colonies in a plate, which contain a specific DNA sequence. These bacterial colonies are obtained from bacterial cells into which this sequence was introduced through genetic engineering, and the given sequence is represented by the probe used in the hybridization experiment. The procedure for colony hybridization is briefly described below.

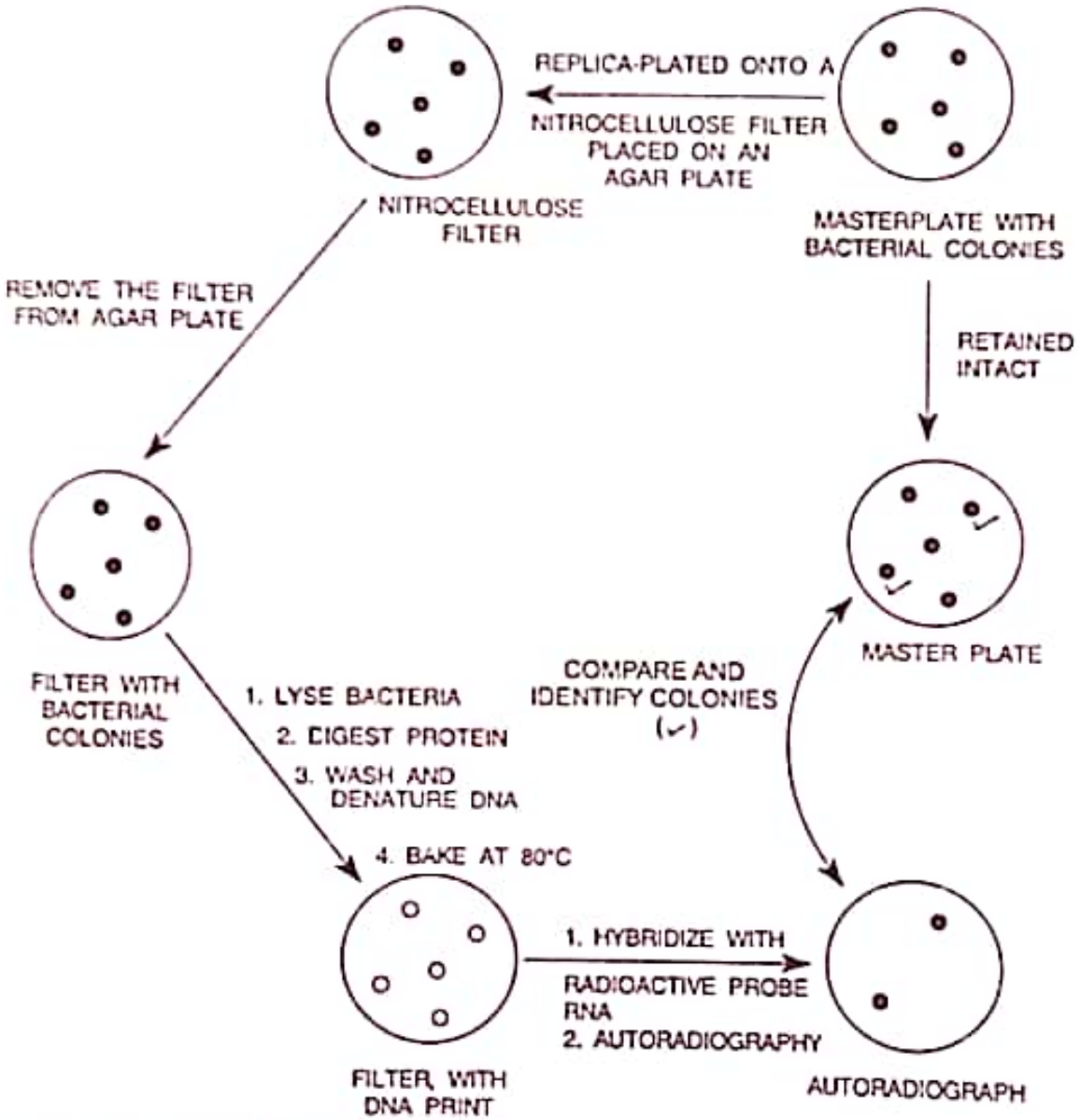


**Fig. 3.I.1.** Colony hybridization scheme is used to identify bacterial colonies containing a specific sequence of DNA, which is represented by the radioactive probe used in the test.

1. The bacterial cells subjected to transformation are plated onto a suitable agar plate; this is the ***master plate.***
2. The colonies of master plate are replica-plated onto a nitrocellulose filter membrane placed on agar medium. For ***replica plating,*** a block of wood or cork, of suitable

diameter for the master plate, is covered with velvet cloth. This block is sterilized and then lowered into the master plate till the velvet touches all the colonies; the block is withdrawn and gently lowered onto the nitrocellulose filter so that the bacterial cells sticking on to the velvet are transferred onto the filter. The master plate is retained intact for later use. A reference point is marked both on the master plate and on the replica plate to facilitate later comparisons.

3. After the colonies appear, the filter is removed from the agar plate and treated with alkali to lyse the bacterial cells. This also denatures the DNA released from these cells.
4. The filter is treated with proteinase K to digest and remove the proteins; the denatured DNA remains bound to the filter.
5. The filter is now baked at 80° C to fix the DNA; this yields the DNA-print of bacterial colonies in the same relative positions as those of the colonies themselves in the master plate.
6. The filter is now hybridized with the radioactive probe; the probe represents the sequence of DNA segment used for transformation. The unhybridized probe is removed by repeated washing.
7. The colonies whose DNA hybridizes with the probe are detected by autoradiography; only these colonies show up in the autoradiograph.

The positions of colonies showing up in the autoradiograph are compared with the masterplate to identify the colonies that contain the DNA segment in question. The colonies are then picked up for further studies. A variation of this procedure can be applied to phage plaques as well.

## APPENDIX–3.II

# SOUTHERN HYBRIDIZATION

The name of this technique is derived from the following: (1) the name of its inventor, E.M. Southern, and (2) the DNA-DNA hybridization that forms its basis. It is also called ***Southern blotting*** since the procedure for transfer of DNA from the gel to the nitrocellulose filter resembles blotting. This technique has since been extended to the analysis of RNA (***northern blotting***) and proteins (***western blotting***); these names are only jargon terms, *i.e.*, reverse of Southern being northern and so on, and do not reflect any functional or historical significance.

In Southern hybridization, a sample of DNA containing fragments of different sizes is subjected to electrophoresis using either polyacrylamide or agarose gel. The DNA sample may either be subjected to mechanical shearing or to restriction endonuclease digestion in order to generate the fragments. Agarose gel is useful in separating DNA fragments of few hundred to 20 kb in size, while polyacrylamide is preferred for smaller fragments. Very large DNA fragments of upto 1000-2000 kb are separated in agarose gel with pulsed electrical fields, or field inversion.

The gel provides a complex network of polymeric molecules through which DNA fragments migrate, depending on their sizes, under an electric field since DNA molecules are negatively charged. Smaller molecules of DNA migrate relatively faster than the larger ones. Marker DNA fragments of known sizes are run in a separate lane; this permits an accurate determination of the size of an unknown DNA molecule by interpolation. The gels are stained with the intercalcating dye ethidium bromide which, gives visible fluorescence on illumination of the gel with UV light; as little as 0.05 μg of DNA in one band can be detected by using this dye. This approach is useful when few DNA fragments with considerable length differences are to be separated and studied. This approach also separates the closed circular (supercoiled), nicked (relaxed) and linear configurations of a single DNA molecule.

In many situations, it is critical to detect and identify DNA fragments in a sample that are complementary to a given DNA sequence, *e.g.*, to demonstrate the presence of the gene in question in transgenics, to detect and stuty RFLP (restriction fragment length polymorphism), etc. This is achieved by Southern hybridization in which the following steps are performed.

1. The restriction fragments of DNA present in agarose gel (after electrophoresis) are denatured into single-stranded form by alkali treatment.
2. They are then transferred onto a nitrocellulose filter membrane; this is done by placing the gel on top of a buffer saturated filter paper, then laying the nitrocellulose filter membrane on the top of gel, and finally placing some dry filter papers on top of this membrane. The buffer moves, due to capillary action, from the bottom filter paper through the gel carrying with it the denatured DNA present in the gel; the DNA becomes trapped in the nitrocellulose membrane as the buffer phases through it. This process is known as ***blotting*** and takes several hours to complete. The relative positions of the bands on the membrane remain the same as those in the gel and there is a minimal loss in their resolution (sharpness).
3. The nitrocellulose membrane is now removed from the blotting stack, and the DNA is permanently immobilized on the membrane by baking it at 80°C *in vacuo*.
4. Single-stranded DNA has a high affinity for nitrocellulose filter membrane. (Note that RNA lacks this affinity). Therefore, the baked membrane is treated with a solution containing 0.2% each of Ficoll (an artificial polymer of sucrose), polyvinylpyrrolidone and bovine serum albumin; this mixture is often supplemented with an irrelevant nucleic acid, *e.g.*, tRNA ***(pretreatment)***. This treatment prevents nonspecific binding of the radioactive probe (to be used in the next step) probably by attaching macromolecules to all the free binding sites on the membrane. Often the above mixture is included in the hybridization reaction itself.
5. The pretreated membrane is placed in a solution of radioactive, single-stranded DNA or an oligodeoxynucleotide (a DNA segment having few to several nucleotides) called ***probe***. The name probe signifies the fact that this DNA molecule is used to detect and identify the DNA fragment in the gel/membrane that is complementary to the probe. The conditions during this step are chosen so that the probe hybridizes with the complementary DNA on the membrane to the greatest extent with a low nonspecific binding on the membrane; this step is known as ***hybridization reaction***.

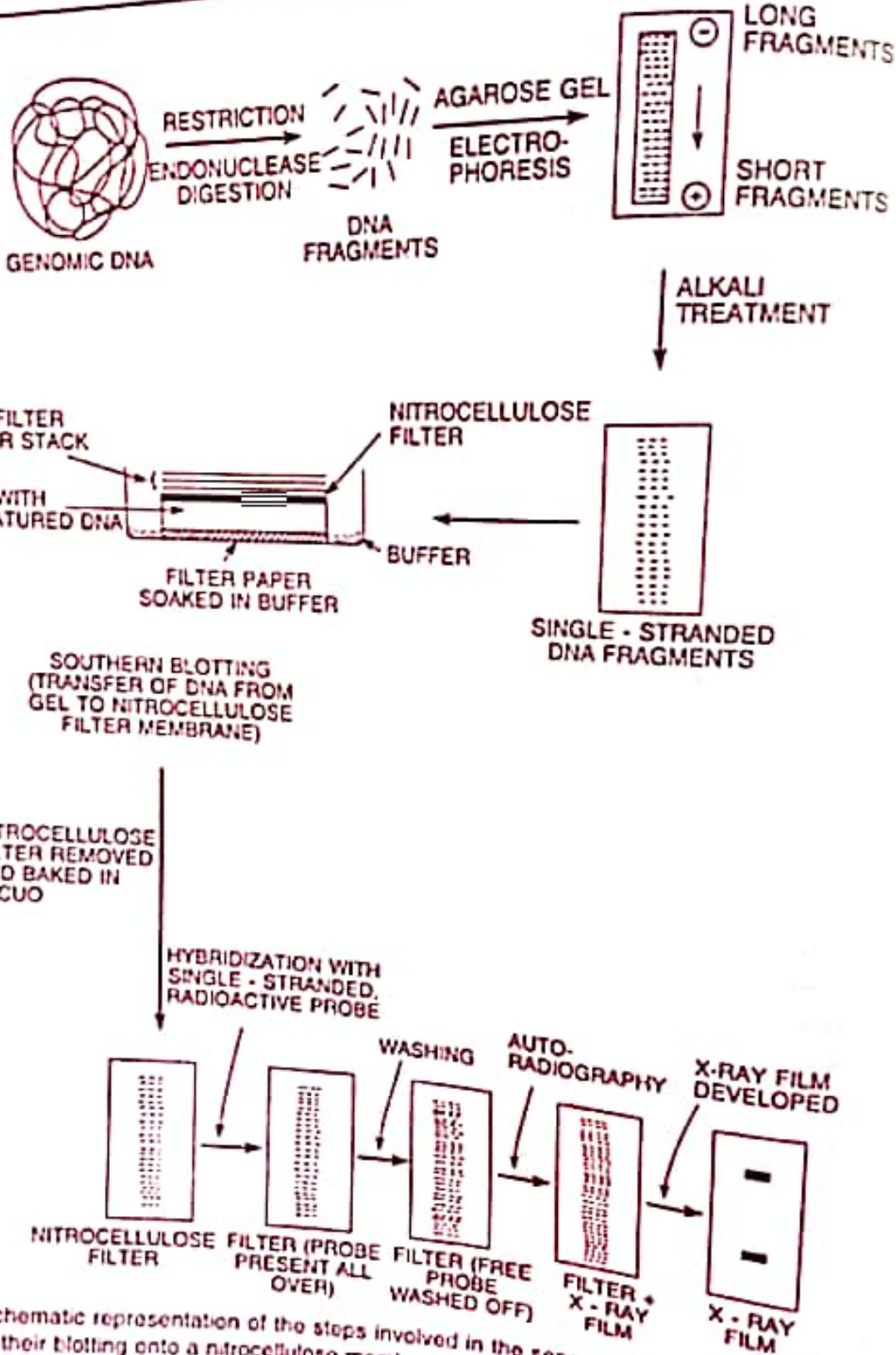


**Fig. 3.II.1.** A schematic representation of the steps involved in the separation of DNA fragments on a gel, their blotting onto a nitrocellulose membrane filter and detection of a specific sequence through hybridization with a radioactive single-stranded probe.

Usually, the initial hybridization reaction is carried out under conditions of relatively low stringency of hybridization to permit a high rate of hybridization; this is followed by a series of post-hybridization washes of increasing stringency, *i.e.*, higher temperature or, more commonly, lower ionic strength, with a view to eliminate the

pairing of radioactive probe to related sequences and to allow only perfectly complementary pairing.

6. After the hybridization reaction, the membrane is washed to remove the unbound probes.
7. The membrane is now placed in close contact with an X-ray film and incubated for a desired period to allow images due to the radioactive probes to be formed on the film. The film is then developed to reveal distinct band(s) indicating positions in the gel of the DNA fragments that are complementary to the radioactive probe used in the study.

It should be kept in mind that electrophoresis of sheared or restricted DNA produces a smear in which the fragments are distributed in a continuum according to their size, and there are no distinct bands. The distinct bands are produced by the hybridization reaction of the selected probe with one or few fragment sequences present in the gel. The Southern blotting technique is extremely sensitive. It can be used to map the restriction sites around a single copy gene sequence in any genome (even of man). It is used for DNA fingerprinting, preparation of RFLP maps, detection and identification of the transferred genes in transgenic individuals, etc.

Recently some new membrane materials, *e.g.*, nylon membranes, have been developed which have the following advantageous features: (1) They are physically robust in comparison to nitrocellulose filter membranes, (2) both DNA and RNA become cross-linked to them by a brief exposure to UV light, which (3) saves the time needed for baking *in vacuo* in the case of nitrocellulose membranes, and (4) the same membrane blot, *e.g.*, a membrane onto which DNA/RNA has been transferred from a gel and cross-linked by UV exposure, can be used for search with more than one probe after removing the earlier probe by high temperature washing or some other denaturing procedure; in other words, the nylon membranes are reusable.

## APPENDIX–3.III

# DOT BLOT TECHNIQUE

This technique is used to detect the presence of a given sequence of DNA/RNA in the nonfractionated (not subjected to gel electrophoresis) DNA. Sample DNA's from several tissues/individuals can be tested in a single test-run. Dot blots are useful in detecting the presence of the sequence being transferred in a number of suspected transgenic individuals, and the presence of a specific mRNA in several such individuals or in different tissues of a single individual. The generalized procedure of dot blot is briefly described below.

1. The sample DNAs (or RNAs) from different individuals or tissues are transferred onto a nitrocellulose filter in form of dots; several samples are dot-blotted onto a single filter (Fig. 3.III.1). A sample DNA or RNA is the total DNA or RNA extracted from an individual or a tissue.
2. The DNA is first denatured and then the filter is backed at 80°C to fix the DNA

firmly onto the filter. The filter is now pretreated appropriately to prevent nonspecific binding of the probe to the filter (see, Southern hybridization).

3. The filter is then treated with the appropriate radioactive single-stranded DNA probe under conditions favouring hybridization. The filter is now washed repeatedly to remove the free probe.

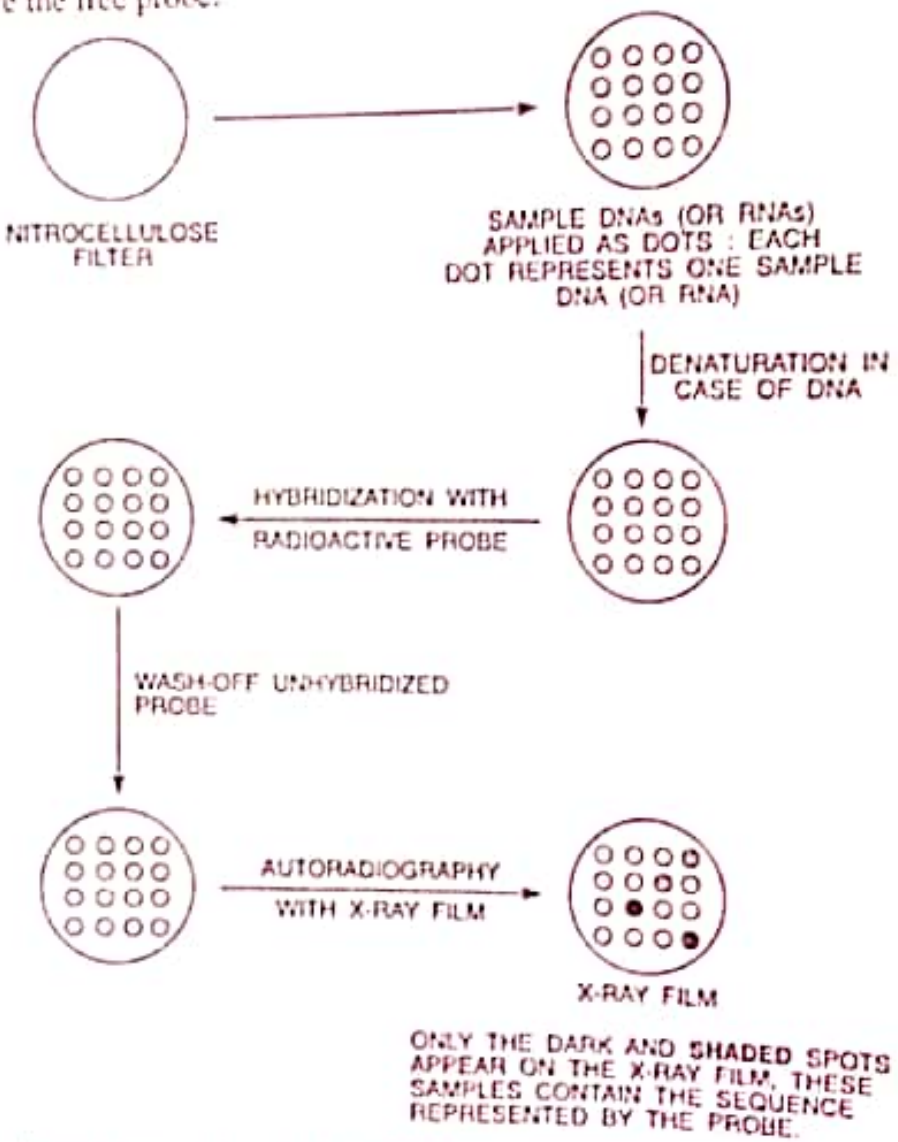


Fig. 3.III.1. A schematic representation of the dot blot technique for detecting the presence of a specific sequence in DNA (or RNA) from several individuals or tissues.

4. Dots having the appropriate DNA or RNA sequence will hybridize with the radioactive probes. These dots are detected by autoradio-graphy; the intensity of dot in the autoradiograph corresponds fairly well with the extent to which DNA or RNA is represented in the sample.

The dots that show up in the autoradiograph denote the individuals or tissues in which the DNA or RNA sequence corresponding to the probe is represented. *In case of transgenic individuals, Southern hybridization may then be used for the dot blot positive individuals to derive more precise information on whether and where the sequence in question is integrated into the genome.*

## APPENDIX—3.V

# NORTHERN HYBRIDIZATION

In this technique, RNAs are separated by gel electrophoresis, the RNA bands are transferred onto a suitable membrane, e.g., diazobenzyloxymethyl (DBM) paper or nylon membranes, and immobilized; the bands are hybridized with radioactive single-stranded DNA probes, and the bands showing hybridization are detected by autoradiography.

Clearly, northern blotting/hybridization is simply an extension of the Southern blotting technique (Fig. 3.II.1). The two techniques, however, show the following differences: (1) in Southern hybridization, DNA is separated by gel electrophoresis, while in northern blotting RNAs are separated; (2) as a result, in Southern hybridization DNA has to be denatured before blotting, while this step is not needed in northern hybridization; (3) nitrocellulose membrane is generally not used for northern, while it is often used for Southern hybridization; and finally (4) hybridization with the probe produces DNA : DNA hybrid molecules in Southern hybridization but RNA : DNA molecules in northern hybridization.

Initially, specially prepared paper (diazobenzyloxymethyl, DBM, paper prepared by diazotization of aminobenzyloxymethyl paper) was used for northern blotting since RNA did not bind to nitrocellulose membrane. RNA becomes covalently bound to DBM paper due to which these blot-transfers are reusable. DBM is also equally effective in binding to denatured DNA, and is more efficient than nitrocellulose in binding to small DNA fragments. Recently developed nylon membranes have superceded the use of DBM paper as they are robust, reusable and bind (by cross-linking) to RNA on a brief exposure to UV light.

Northern hybridization is useful in the identification and separation of the RNA that is complementary to a specific DNA probe; this is a sensitive test for the detection of transcription of a DNA sequence that is used as probe.

## APPENDIX—3.VI

# WESTERN BLOTTING

In western blotting, proteins are electrophoresed in polyacrylamide gel, transferred onto a nitrocellulose or nylon membrane (to which they bind strongly), and the protein bands are detected by their specific interaction with antibodies, lectins or some other compounds. The various steps of this technique are briefly described below.

1. Protein bands are separated by polyacrylamide gel electrophoresis.
2. The protein bands are transferred onto a nitrocellulose or nylon membrane; initially this was achieved by a capillary movement of buffer similar to Southern blotting (***capillary blotting***), but nowadays it is usually done by electrophoresis (***electrophoretic blotting***). Electrophoresis has been applied for the blotting step in Southern and northern hybridizations as well; in such cases buffer of low ionic strength (to avoid overheating during electrophoresis) and nylon membranes (since nucleic acids bind to nitrocellulose membrane only under conditions of high ionic

strength) are used. The electrophoretic blotting, both of proteins and nucleic acids is much faster and more efficient than capillary blotting.

3. The specific protein bands are identified in a variety of ways. (*i*) Antibodies are the most commonly used as probes for detecting specific antigens. (*ii*) Lectins are used as probes for the identification of glycoproteins. These probes may themselves be radioactive or a radioactive molecule may be tagged to them. Often the identification process is based on a ***'sandwich' reaction.*** In such an approach, a species-specific second antibody or protein A of *Staphylococcus aureus* (protein A binds to certain subclasses of IgG antibodies) or streptavidin (it binds to biotinylated antibodies) is used to bind to the antibodies bound to the protein bands. These second molecules may be labelled with radioactive, enzyme or fluorescent tags; a single preparation of these labelled molecules can be employed as a general detector for various probes.

## APPENDIX–3.VII

# PROBES

***Probes*** are small (15—30 bases long) nucleotide sequences used to detect the presence of complementary sequences in nucleic acid samples. This is achieved by permitting the probes to base pair with the sample nucleic acids and then identifying the samples that show base pairing with the probes, *i.e.*, ***hybridization***. The detection of hybridization is highly precise and extremely sensitive provided the probes are suitably labelled for an easy detection. Clearly hybridization can occur only when the base sequence of a probe is present within the gene or DNA segment, which it is aimed to detect.

Both DNA and RNA are used as probes. Single-stranded DNA probes are more convenient and preferable, but denatured double-stranded DNA molecules can also be used. RNA probes are ordinarily single-stranded.

***Preparation of Probes***. Probes can be obtained in several ways; some of the important ones are briefly described below.

1. Highly purified mRNA can be used as probe; mRNA's are naturally single-stranded.
2. Single-strand RNA probes can be readily prepared by cloning the corresponding DNA sequence inserted into a special vector like *p*GEM or phage M13 vectors. *p*GEM has a different and specific prokaryotic promoter on the two ends of the DNA insert. The recombinant DNA is linearized and transcribed *in vitro* with the appropriate prokaryotic RNA polymerase to obtain RNA molecules complementary to one or the other strand of the DNA insert.
3. DNA segments isolated from the genome of an organism or cDNA molecules prepared by using mRNAs can be cloned in *E. coli* and used as probes. These probes, of necessity, will be double-stranded.
4. Single-stranded cDNA probes can be prepared by limiting the copying of mRNA by reverse transcriptase to only one strand.

development necessary for detection; these tails have a nonspecific base sequence so that the same tail can be added to any probe. These probes are hybridized with the test nucleic acid fixed to a solid support and the nonhybridized probes are washed off. The hybridized samples are detected by the development of blue colour following a series of cytochemical reactions, which basically utilize the affinity of egg white glycoprotein avidin for biotin. This approach has the advantage of being much faster than that with radioactive probes, which requires autoradiography. But its chief disadvantage relates to the inability to reuse the filter (solid support) and the nucleic acids fixed to it for hybridization with other probes since the reactions leading to the colour development produce insoluble precipitates. In contrast, the radioactive probes are easily removed by washing under conditions favouring denaturation, e.g., high pH, and the same filter can be reused for hybridization with a series of probes, used one at a time.

Nucleotides conjugated with digoxigenin, a plant derived chemical, may be used in nick-translation to produce digoxigenin labelled probes. The probes are used in hybridization; after washing away the free probes, the filter is incubated in a detection buffer containing a digoxigenin specific antibody (anti-digoxigenin) coupled with an enzyme (usually alkaline phosphatase). After appropriate washing, the alkaline phosphatase activity is detected by using a suitable substrate that yields colour due to the enzyme action.

Nucleotides have been conjugated with other ligands that produce some detectable signal, e.g., fluorescence, enzyme activity, etc. The signals from probes can be amplified by any one of several approaches, e.g., (*i*) attaching multiple enzyme molecules to each probe molecule, (*ii*) adding nonspecific labelled tails to the probes. (*iii*) using multiple secondary probes that hybridize with multiple target specific primary probes (Christmas tree or forest approach), etc.

### *Applications of Probes*

1. Identification of the recombinant clones carrying the desired DNA inserts; this is the most critical step in DNA cloning (techniques used: Southern, northern, colony, dot blot hybridizations).
2. Confirmation of the integration and detection of copy number (by Southern hybridization) of a DNA insert in the host genome, and its expression in transformed cells (northern hybridization).
3. Development of RFLP (restriction fragment length polymorphism) maps (Chapter 7).
4. DNA finger-printing for the unequivocal identification of plant varieties, criminals, parental relationships, etc.
5. *In situ* hybridization (Appendix-3.X) for determining the locations of specific sequences in specific chromosomes.
6. Accurate diagnosis of diseases caused by parasites, pathogens or defective viruses (Chapter 9).
7. Preparation of genome maps of eukaryotes, including man.

wax, sectioned, stained with a suitable stain, *e.g.*, haematoxylin and eosin, and the slides are viewed under light microscope. The sections are then exposed to specific nonradioactive probes and viewed again under the light microscope. This permits the correlation of conventional microscope data with that obtained with the probes, and determination of the location of concerned pathogens in the tissues and cells.

Alternatively, *in situ* hybridization can be carried out using fluorescent labelling in the place of radioactive labelling; this strategy is called ***fluorescence in situ hybridization (FISH)***. If different fluorochromes are used, two or more genes can be used for simultaneous *in situ* hybridization; this is referred to as ***multicolour fluorescene in situ hybridization (McFISH)***. McFISH allows determination of the relative locations of two or more genes (or any other DNA sequences) in the chromosomes. It also permits a more precise analysis of chromosomal rearrangements; in such a case, such probes are used whose chromosomal locations are already known; this approach yields much quicker results than do the conventional staining techniques.

## APPENDIX—3.XI

# GEL ELECTROPHORESIS

***Electrophoresis*** is the technique of separation of charged molecules under the influence of an electrical field so that they migrate in the direction of electrode bearing the opposite charge, *viz.*, cationic (positively charged) molecules move toward cathode (–ve electrode) and anionic (negatively charged) molecules travel towards anode (+ve electrode). The molecules to be separated are maintained in aqueous phase. The speed of migration (***electrophoretic mobility***) of a molecule depends on its charge and molecular mass. Charge of a molecule is influenced by the following: (1) the type, concentration and pH of buffer, (2) the temperature, (3) strength of the electrical field, and (4) the nature of the support material (***matrix***) used for electrophoresis.

## 3.XI.1. Types of Electrophoresis

There are basically three different electrophoretic methods as follows: (1) electrophoresis (sometimes called zone electrophoresis), (2) isotachophoresis and (3) isoelectric focussing. A huge variety of electrophoretic methods have been devised to achieve specific objectives; for greater details and procedural directives/variations, the reader is advised to consult one of the following books.

1. Andrews, A.T. 1986. *Electrophoresis, Theory, Techniques, and Biochemical and Clinical Applications*. Clarendon Press, Oxford.
2. Chrambach, A. 1985. *The Practice of Quantitative Gel Electrophoresis*. VCH, Weinheim.
3. Mosher, R.A., Saville, D.A. and Thorman, W. 1992. *The Dynamics of Electrophoresis*. VCH, Weinheim.
4. Westermeier, R. 1993. *Electrophoresis in Practice*. VCH, Weinheim.

Electrophoresis can be carried out in free solutions, *e.g.*, capillary electrophoresis, or in a stabilizing support material like thin-layer plates, films and gels. A brief description of gel electrophoresis is given below.

## 3.XI.2. Gel electrophoresis

Gel matrices should have adjustable and regular pore size, should be chemically inert and should not exhibit electroosmosis. ***Electroosmosis*** is the phenomenon of migration of water toward an electrode as a result of the supporting medium and/or the surface of the separation equipment, *e.g.*, of capillaries, also carrying charge. The gel can be as vertical rods, as plates or horizontal slabs. The following types of gels are commonly used.

1. ***Agarose Gels.*** These gels have large pores, and are used for analysis of molecules of over 10 nm diameter. Agarose is a polysaccharide obtained from red seaweed. When agaropectin is removed, agarose gells with melting points from 35°C to 95°C and varying degrees of electroosmosis are obtained. Agarose dissolves in hot water. When this solution is cooled, double helices form and become arranged laterally the produce thick filaments; these filaments become cross-linked to form the gel (Fig. 3.XI.1). Pore size depends on agarose concentration (w/v): in general, a 1% (w/v) gel will have a pore size of 150 nm, while a 0.16% gel has pore size of 500 nm.

Gels having 0.7 to 1% agarose have large pore size. Such horizontal agarose gels are used for the separation of high molecular weight proteins, *e.g.*, serum proteins, and enzymes, *e.g.*, isoenzymes of diagnostic importance, and of large (few to several kb) fragments of DNA. Proteins separated in agarose gels can be subjected to immunofixation, immunoprinting and immunoblotting. Agrarose gels are also used for immunoelectrophoresis and affinity electrophoresis.

Agarose gels above 1% concentration are cloudy and exhibit high electro-osmotic flow. These gels are, therefore used for the separation of very high molecular weight proteins or protein aggregates. However, they are the standard medium for separation, identification RFL-analysis, and purification of DNA and RNA fragments; for these applications, horizontal submarine agarose gels are used to prevent the gel from drying out.

2. ***Polyacrylamide Gels.*** These gels are obtained by copolymerization of acrylamide ($CH_2$=CH—$CONH_2$) monomers with a cross-linking reagent (usually, N, N'-methylenebisacrylamide, *bisacrylamide* in short) (Fig. 3.XI.2). Polyacrylamide gels are transparent, chemically inert and, particularly, mechanically stable, and they exhibit very little electroosmosis. The temperature during polymerization should be maintained above 20°C in order to prevent incomplete polymerization. In addition, polymerization should take place under an inert atmosphere since oxygen can act as a free radical trap. Oxygen absorption is minimized by casting the gels in vertical chambers, *e.g.*, molds formed by two glass plates sealed together around the edges in the case of flat gels. *The monomers are toxic; therefore they should be handled with the utmost care.*

Horizontal polyacrylamide gels polymerized on ultra-thin films are used to separate low molecular weight compounds, *e.g.*, dyes with molecular weights of 500 Daltons. Polyacrylamide gels are also used for analysis of nucleic acids, *e.g.*, DNA sequencing, for

3. The polypeptides are unfolded and stretched (they form ellipsoids); their separation gives high resolution and sharp bands.
4. The bands are easy to fix.
5. ***Separation of protein molecules is based on their molecular mass.***
6. The proteins treated with SDS fix dyes better than the native proteins.
7. Charge differences between isoenzymes are cancelled by SDS; therefore, each enzyme forms one band only, which includes all the isozymes.
8. SDS solubilizes all proteins, including very hydrophobic and even denatured proteins.

## APPENDIX–3.XII

# MICROARRAYS

An ***array*** is a systematic arrangement of a group of data. In case of ***microarrays***, *a series of probes are immobilized on a glass slide as microdots, which are then hybridized with a mixture of test DNA sequences that are labelled with a fluorochrome*. An extremely large number of probes is spotted on to the slide, each probe is a pure preparation, the test DNA is a mixture of sequences and the results are visualized by confocal microscopy.

Microarrays were first used in case of yeast, which has 6,000 genes. Every yeast gene was obtained as an individual clone and a sample of each gene was spotted onto glass sides in arrays of 80 spots × 80 spots. In order to determine the identity of genes active in yeast cells under a set of given conditions, mRNA is extracted from these cells. The mRNA is converted into cDNA and the cDNA is fluorescently labelled. The labelled cDNA is used for hybridization with the microarray, and the identity of spots showing fluorescene, *i.e.*, hybridization is determined by confocal microscopy. The spots showing fluorescence represent the genes that were expressed in the cells (Fig. 3.XII.1). It may be pointed out that single-stranded DNA preparations are spotted using a laboratory robot.

## 3.XII.1. Types of Microarrays

At present, microarrays are basically of two types: (1) DNA microarrays (being widely used), and (2) antibody microarrays. The DNA microarrays are of two basic types: (1) spotted microarrays and (2) oligonucleotide microarray or DNA chips. In case of ***spotted DNA microarrays***, *DNA sequences representing different genes of an organism are spotted onto the slides; these microarrays are used to determine genes being transcribed in the cells concerned, i.e., their transcriptome.* ***Transcriptome*** is the total mRNA content (= composition) of a cell, and reflects the overall pattern of gene expression in that cell. The DNA microarrays can be prepared by using DNA sequences derived in one the following ways.

1. Sequences obtained from individual cloned genes.
2. A systematized collection of cDNAs, often derived from collections of expressed

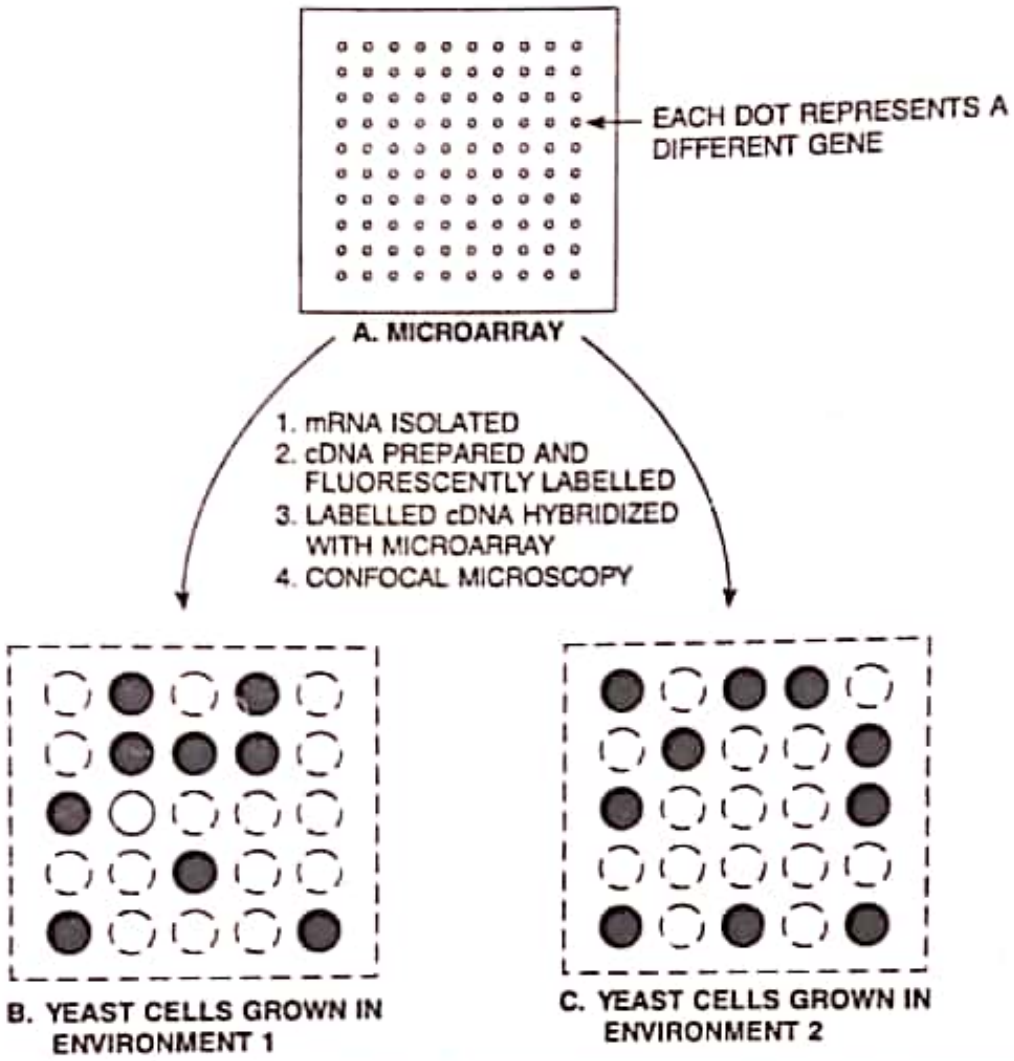


**Fig. 3.XII.1.** Use of DNA microarrays in yeast. **A.** A DNA microarray (only 10 × 10 spots are shown). **B** and **C.** Microarrays as seen by confocal microscopy; the dark spots represent fluorescence (= hybridization with labelled cDNA) and hence the transcribed genes. A comparison of B and C reveals the identity of genes that are differentially expressed under these two environments.

sequence tags (ESTs). ***ESTs*** are short sequences obtained from the ends of cDNAs, *i.e.*, partial gene sequences. Thus each EST represents a different gene.

3. ESTs or cDNAs from a related species.

The ***antibodies microarrays***, on the other hand, consit of spots of different antibodies and are used to measure the abundance of thousands of different proteins in the samples; these are in developmental stages.

## 3.XII.2. DNA Chips (Oligonucleotide Microarrays)

***DNA chips*** or ***oligonucleotide microarrays*** are thin wafers of silicon glass carrying many different oligonucleotides synthesized at a very high density (300,000 to over 1 million oligonucleotides/cm$^2$) directly onto the wafer. The oligonucleotides are synthesized at a high spatial resolution and in precise locations; each oligonucleotide has the sequence of a different gene present in the genome. Therefore, sequence information for the genes to be represented in the DNA chip must be available. The oligonucleotide synthesis is based on two

techniques called photolithography and solid-phase DNA synthesis; it uses a series of building blocks that contain photochemically removable protective groups. The DNA chips are inverted and mounted in a temperature-controlled hybridization chamber into which fluorescently labelled cDNA preparation is injected and allowed to hybridize with the oligonucleotides. Laser excitation enters through the back of the glass support focussed at the interface of the array surface and target solution. Fluorescent emission is collected by a lens and passed on to a sensitive detector and a quantitative assay of hybridization intensity is obtained. DNA chips present an alternative to the DNA microarrays.

### 3.XII.3. Applications of Microarrays

1. Studies of the gene expression pattern of an organism as affected by the stage of development and/or environment.
2. Identification of common regulatory elements shared by coregulated genes.
3. DNA chips can be prepared for the detection of SNPs (single nucleotide polymorphisms; Chapter 15); these are called ***SNP chips.***
4. DNA chips can be used for the detection of genetic diseases, *e.g.*, for detection of mutant alleles causing cystic fibrosis, mutant alleles of gene *BRCA1* (gene involved in breast cancer), etc.

### 3.XII.4. Advantages of Microarrays/DNA Chips

1. The analyses based on microarrays/DNA chips are very rapid and highly sensitive.
2. All the genes present in the genome are analyzed in one test.
3. The assay also provides quantitative data, *viz.*, the level of expression of different genes.
4. Multiple colour test (= target) samples using a single DNA chip/ microarray.

## APPENDIX—3.XIII

# ENZYMES USED IN RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology employs a variety of enzymes to achieve specific goals. The features and uses of restriction enzymes have been adequately described in Chapter 2 (Section 2.3). A brief description of the other important enzymes is given below.

### 3.XIII.1. DNA Ligase

*DNA ligase* enzyme seal nicks between adjacent nucleotides in a double-stranded DNA molecule. It is sometimes termed as ***molecular glue***. Usually, DNA ligase is prepared from *E. coli* cells infected with phage T4. In fact, all living cells produce ligase that joins together the neighbouring nucleotides flanking a discontinuity in a DNA strand by forming phosphodiester bond. A ***discontinuity*** in a DNA strand is the site at which the phosph[illegible] between two adjacent nucleotides is broken. *In vitro* DNA