• Then the membrane is exposed to hybridization probe. But the DNA probe is labeled so that it can easily detect, when the molecule is tagged with a chromogenic dye.

• After hybridization process, excess probe is washed away by using SSC buffer and it can be visualized on X-ray film with the help of autoradiography.

Applications

i) It is used in the technique called RFLP (Restriction fragment length polymorphism) mapping.

ii) Also used in phylogenetic analyze.

iii) To identify the gene rearrangements.

2) Western blotting

Western blotting is named after W. Neal Burnette. This method is used for detection and analysis of protein in a given sample. It involves the following steps:

• Firstly, isolating the protein from particular sample.

After that beta- mercaptoethanol (BME) and Sodium dodecyl Sulfate (SDS) is added into the protein suspension.

• Then, protein- SDS complex is placed on top of the gel in the well. A molecular weight marker is also loaded in one of the well in order to determine the molecular weight of other proteins. After that the samples are added in the remaining wells.

• Once the samples and markers are loaded then current is passed across the gel. Protein is pulled down to the positive pole of the well because it is tightly bound to SDS which is negatively charged. Movement of protein is inversely proportional to its size.

• After this step, gel is placed against a membrane and current is passed across the gel so that all the proteins are transferred onto the membrane.

• Then Immunoblotting has to be done. In this method, firstly block the membrane with non-specific protein in order to prevent antibody from binding to the membrane where the protein is not present.

• After that primary antibody is added to the solution. These antibodies are responsible for recognizing a specific amino-acid sequence. Then wash it to remove unbound primary antibody and add secondary antibody.

• Now these antibodies are conjugated with an enzyme and recognize the primary antibody. Lastly, another wash is done to remove unbound secondary antibody.

• Here, chemiluminescent substrate is used for detection. The light is being emitted once the substrate has been added and can be detected with film imager.

Microbial Diagnostic Microarray – specific oligonucleotides, 35-70 nucleotides, in length are chemically synthesized – the oligonucleotides are spotted, at defined positions, onto a glass slide to construct the array – binding of fluorescently labelled probes to the oligonucleotides is detected spectrophotometrically

Genes USED ON MDM? – Housekeeping genes – 16S rRNA, – 16S-23S rRNA intergenic transcribed spacer region (ITS), – rpoB; RNA polymerase Beta – Hsp60/groEL; Heat shock protein – recA; Recombinase A – gyrB; Gyrase Beta

Virulence genes – bacterial toxins – adhesins – important virulence factors – facilitate colonisation – cause tissue damage

Bacteria detection

The complex interaction between a pathogen and its host is the molecular basis of infectious diseases. Microarray technology is a powerful tool to investigate the crosstalk between a pathogen and the host as it assesses whole genome expression profiles in response to disease

1. Tuberculosis – Gryadunov et al : developed a biochip for detection of rifampicin-resistant and isoniazid-resistant strains of M. tuberculosis . – The biochip identifies over 95% of rifampicin-resistant and more than 80% of isoniazid-resistant M. tuberculosis strains in sputum samples. – The biochip has 77 gel elements and detects the 27 most-common mutations in the rpoB gene responsible for rifampicin resistance as well as 11 mutations in the katG gene, five mutations in the promoter region of the inhA gene, and five mutations in the intergenic regulatory region of the ahpC-oxyR genes all of which can cause resistance to isoniazid.

sensitivity of 80% and a specificity of 100% compare to traditional testing for rifampicin resistance. – Disadvantage: rare mutations or unknown mutations not detectable by the microarray probes The newest generation of TB-biochips identifies mutations responsible for the emerging resistance of M. tuberculosis so the highly effective second-line fluoroquinolone antibiotics can be administered

2. Meningitis – Using the sequencing array, the scientists were able to correctly classify 45 samples that were previously identified by conventional methods. But more importantly, was able to classify 12 previously unclassifiable samples into existing meningitis serotypes. – resequencing microarrays provide results in just 48 hours, much faster than traditional methods. – The meningitis resequencing array can now be used to quickly identify new meningitis strains, as well as for epidemiological studies and vaccine research.

Virus detection:

Based on publications, microarray approaches can be summarized into four viral infection groups to focus on: 1) respiratory diseases 2) hemorrhagic fever (HF) 3) neurotropic infection 4) HIV

1. Respiratory Diseases Detection of etiological agents : – Some influenza microarrays are designed to detect DNA. For instance, a universal microchip was developed for genotyping influenza A viruses with