Pcr and southern blotting: applications in medicine

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Introduction

The 1970's introduced an inspirational technique in which a specific sequence could be determined from a sample of DNA via the process of Southern blotting. This method, named after Edwin Southern, provided the basis for a range of common laboratory techniques such as western blotting, eastern blotting and northern blotting [1].

Shortly after, in 1983, a revolutionary method was invented by Kary Mullis, called the polymerase chain reaction (PCR) [2]. This procedure was originally used to amplify and identify DNA sequences in the human genome. Its use in genetic analysis was immediately recognised, as one of the first publications of its use was of prenatal diagnosis of sickle-cell anaemia [3]. Since then it has been manipulated and a whole array of techniques have been derived from this invention.

Both PCR and Southern blotting have been used widely in understanding and identifying microbes which in turn assists the diagnosis and management of patients suffering from infectious diseases.

PCR Procedure

The sample of DNA is heated up to 90°C to separate the two strands of DNA thereby exposing the nucleotide bases on each strand. A primer is then annealed to each strand from the 5′ region at around 60°C and the temperature is increased once more. The thermostable DNA polymerase enzyme, Taq, prevents contamination by binding free complementary base pairs to the original strand of DNA at temperatures up to 70°C. The strands

are then cooled and double the amount of DNA is synthesised, and the cycle restarts until a sufficient amount of DNA is produced.

Reverse-transcriptase PCR (RT-PCR) is used when the original sample of RNA is transcribed so that DNA is the product of amplification. The sensitivity of PCR is great, as detection is from a single nucleotide base whilst its quantitative ability is derived from the proportional expansion of amplified DNA from its original size [5].

Southern Blot Procedure

Southern blotting begins with a sample of DNA which is first broken up by a restriction endonuclease into smaller, varying fragments. The DNA is then placed into wells to undergo agarose gel electrophoresis where the fragments diffuse across a polarised field according to their size. The DNA is denatured by sodium hydroxide and transferred to a sheet of nitrocellulose or nylon and incubated with a hybridisation probe of single-stranded DNA. This radiolabelled probe binds to the exposed complementary base pairs and can be detected by autoradiography [6].

Southern Blotting Applications in Medical Microbiology

Southern blotting is primarily used for DNA fingerprinting, gene sequencing and genetic engineering.

It has been used in the identification of strains in microbes such as concluding the type of human papillomavirus extracted from a condyloma. However in this case it provided to be unreliable as it produced false-negatives, as PCR and in-situ hybridisation were deemed to be more efficient [7]. Another use of Southern blotting was in the detection of a strain of

Listeria monocytogenes. In this study it was deemed an important technique in verifying species identification and in the characterisation of epidemic strains [8].

This method can be used to DNA fingerprint most microbes and determine a diagnosis and treatment for a patient suffering from their pathogenicity. However it is found to be too laborious, time consuming and requires large amounts of high quality DNA for most routine laboratories yet variations of this technique are still widely performed. The western blot, which uses antibodies as the probe to detect proteins instead of DNA, is a confirmatory test in the diagnosis of a human immune-deficiency virus(HIV) infection [9]. Since the established use of PCR there has become a decreased need for these laborious techniques in DNA sequencing [10].

PCR Applications in Medical Microbiology

PCR can be used in detecting the genetic sequence of all microbes. It is useful in detecting organisms in early cultures where organisms are otherwise difficult to isolate, for example in enteroviruses RT-PCR is more sensitive than culture and the ‘ gold standard’ is detection of the this genome in cerebral spinal fluid (CSF) by PCR [11].

PCR is also used in detecting genes encoding antibiotic resistance such as in Helicobacter pylori and Methicillin-resistant Staphylococcus aureus. However its use is currently unsuitable for the diagnosis of H. pylori as clinical samples may contain inhibitors which can generate false-negatives10.

PCR is used in quantifying the viral load of HIV within an infected person therefore determining the effectiveness of their treatment. The problem

occurs when the genome sequence of the HIV changes therefore the PCR method needs to be altered and the current test would prove useless9. Currently immunoassays are used in the diagnosis of a HIV infection, however early in infection there is a variable period of time until anti-HIV antibodies can be detected and this provides a potential area of identification of the HIV by PCR [12].

Pitfalls of PCR include that the sample must be kept cold during storage and transport to the laboratory, the expertise required for analysing and interpreting results as well as standardising between different laboratories and finally the expensive cost of the procedure compared to available techniques9.

Conclusion

The applications for PCR and Southern blotting are enormous however they have limitations which prevent it from being routinely used in the diagnostic laboratory. With the advance of technology both are being developed, especially PCR, and have endless applications in medical microbiology.