

Short Note on Usage of Genetic Probes for DNA Analysis through the Polymerase Chain Reaction

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DESCRIPTION

Genetic or DNA probes are oligonucleotides (short DNA sequences) that are used to detect the presence of complementary sequences. They are typically 100-1000bp length. A fluorescent dye or radioactive isomer is usually used to label the probe. When a DNA sample is combined with the probe, any regions/fragments with a complementary sequence will hybridize (stick to). The target sequence can be observed under UV light or on an X-ray film when using a probe. The use of DNA probes to identify particular DNA fragments separated by Gel Electrophoresis is common. The identification of unique nucleotide sequences in a microorganism's DNA or RNA is the basis for genetic probes. When a unique nucleotide sequence is discovered, it is isolated and inserted into a cloning vector (plasmid), which is then inserted into *Escherichia coli* to produce multiple copies of the probe. After that, the sequence is isolated from plasmids and labeled with an isotope or substrate for diagnostic purposes. Following the cleavage of the microorganism's double-stranded DNA in the specimen, the sequence is hybridized with a complementary sequence of DNA or RNA.

The Duplexes or triplexes are generated with targets in solution or on solid substrates as hybridization products. Hybridization acceleration and branch migration responses are among the other topics covered. The second portion covers the manufacture or biosynthesis of labeled probes, as well as their detection and sensitivity and specificity limitations. Radioactive probes are used to demonstrate direct labeling. The only feasible technique to identify a complementary target sequence in a complex nucleic acid mixture is to use nucleic acid hybridization with a tagged probe. The thermodynamics and kinetics of nucleic acid hybridization are covered quantitatively. Oligonucleotides or polynucleotides, DNA or RNA, single-stranded or double-stranded, natural or modified, either in the nucleotide bases or in the backbone is among the probes studied.

The introduction of gene amplification techniques, such as the Polymerase Chain Reaction (PCR), has improved the use of

molecular technology in the diagnosis of infectious diseases. DNA polymerase can duplicate a strand of DNA by elongating complementary strands of DNA begun by a pair of closely spaced oligonucleotide primers. This method has been used to identify infections caused by microbes that are difficult to culture (e.g., the human immunodeficiency virus) or have not yet been cultivated effectively (e.g., the Whipple's disease bacillus). The use of genotyping pathogenic isolates in the study of suspected outbreaks, the discovery of undetected transmission, the tracing of infectious agents within a community, and the identification of likely sources of infection for newly diagnosed cases has long been recognized. Fingerprinting allows strains from various geographic locations to be compared and the movement of particular strains to be traced at a national or worldwide level. The fingerprinting approach necessitates high-quality genomic DNA, which is not only difficult to obtain but also necessitates organism culture, resulting in a lengthy turnaround time. Furthermore, fingerprint matching and interpretation can be difficult, requiring sophisticated computer algorithms for large-scale study[1-4].

Nucleic acid amplification-based assays, on the other hand, do not require the organisms to be cultured, allowing for real-time analysis of samples. The target DNA of interest is amplified and labeled by PCR in many PCR-based typing tests, and the labeled products are hybridized to an array of immobilized diagnostic probes. This approach has proven successful in detecting mutations in Mycobacterium TB drug resistance genes as well as identifying Mycobacterium species. Spoligotyping, a reverse dot blot assay that identifies the presence of a set of distinct spacers in the Direct Repeat (DR) locus, fills a need in the market for a quick and easy way to differentiate *M. tuberculosis* complex strains. Spoligotyping, on the other hand, has a far lower discriminating power than fingerprinting.

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