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Multiplex Polymerase Chain Reaction

INTRODUCTION

The Polymerase Chain Reaction (PCR) has revolutionized modern biology as it enables the amplification of a single DNA fragment into a large number of copies. A variant of this traditional PCR method was developed, the Multiplex PCR (MPCR), in which one or more fragments can be amplified using a varying number of primers, within a single reaction. This extension results in a significant reduction in time, cost and overall effort and therefore, in certain situations, it represents an improvement to the technique [1].

TYPES OF MULTIPLEX PCR

1. Multiple primers pairs on a single template;
2. Multiple primers pairs on multiple templates;
3. Multiplex Ligation-Dependent Probe Amplification (MLPA).

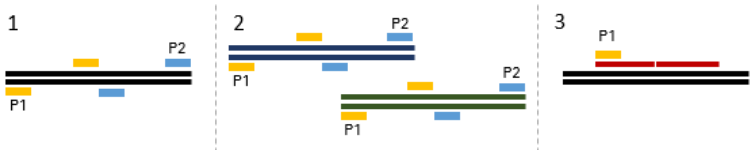

Figure 1 - Types of MPCR

ADVANTAGES

The use of multiple amplicons provides **internal controls** for each amplified fragment, allowing for a better recognition of artifacts and false negatives, which can arise due to reaction failure. The amplification of some of the fragments indicates that the reaction didn't fail. Moreover, Multiplex PCR gives a better indication of **template quality and quantity** than single locus PCR. A degradation of the template (i.e., loss of amplification efficiency) can be indicated by a reduced amplification an abundant control sequence. The templates can be quantified, usually by comparing the signal intensity of a reference sequence to the signal from another sequence in the same reaction. Finally, multiplexing is **more efficient** due to the conservation of PCR reagents, less preparation time and overall effort. This is especially relevant in situations where the template is in short supply.

DISADVANTAGES

Since multiplexing frequently requires the use of many different primers, this increases the risk of primer dimerization resulting in non-specific amplification. Consequently, when compared to standard ones, multiplex PCR primers must be **more specific** [2]**.** Furthermore, each amplicon must be a **different size** (for electrophoresis analysis) or labelled with a **fluorescent dye** that is spectrally distinct from the others used in the reaction. In addition, different templates **compete for resources** leading to the detection of highly abundant templates in detriment of less abundant ones, which may fade into the background [3], [4].

MULTIPLEX LIGATION DEPENDENT PROBE AMPLIFICATION

The MLPA is a type of one primer on multiple templates MPCR. The key difference is the use of probes, which hybridise with DNA and are amplified instead of the sample DNA. These probes are constituted by two probe oligonucleotides, which must hybridize adjacently in order to be ligated. The probes can then be conventionally MPCR amplified using primers that target said probes. The probe's signal is indicative of not only the presence or absence of a sequence, but it will also report small deletions, insertions, and mismatches. This variant is used mainly for the detection of copy number variations. Lastly, this technique, being a one primer multiple template MPCR, does not have the disadvantages of primer dimerization or competition for resources [5].

APPLICATIONS

MPCR can be utilized for many different applications such as: pathogen identification; high throughput SNP genotyping; mutation analysis; gene deletion analysis; template quantitation; linkage analysis; RNA detection; forensic studies [4]. A specific example is the use of MPCR for the detection of tetracycline resistant genes. In Ng *et al*., 2001 [5] specific primer pairs were selected for the PCR amplification of 14 common tetracycline resistant genes. This approach allowed for the identification of the type of resistance genes and in which strains they were present. For instance, in figure 2, lane 2 it is evident that the bacterial group used for the MPCR has three separate resistance genes (in this case tet(B), (C) and (D)).

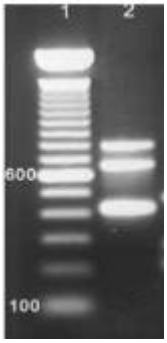

Figure 2 - MPCR amplicons of different classes of tetR. Lane 1, 100-bp DNA ladder; lane 2, Bacterial group with tet(B), (C) and (D). Adapted from Ng et al., 2001.

REFERENCES

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