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Intricacies of fluorescence labelled primer in the molecular biology research

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Fluorescence-labeled primers are short DNA sequences (primers) with a fluorescent dye attached, used for real-time monitoring and detection of nucleic acid amplification like in PCR. The fluorescence signal changes as the primer is incorporated into new DNA strands, with the signal increasing with more double-stranded DNA produced, which can be detected by a fluorescence-based amplification system. During PCR, the fluorescently labeled primer is incorporated into the newly synthesized DNA strand. Fluorescent primers are used in PCR to detect and quantify DNA amplification in real-time by emitting a fluorescent signal. This signal increases as more double-stranded DNA is produced, allowing for the detection of the target sequence and quantification of its amount without needing a separate post-PCR analysis. They offer an advantage over standard PCR and DNA-binding dyes by providing more specific detection, which minimizes interference from artifacts like primer dimers and enables multiplexing (detecting multiple sequences in a single reaction).

Reddy *et al.,* (2017) reported that Commiphora wightii is a valuable endangered tree that produces a gum called guggul-resin, used in the alternative systems of medicine for its anti-inflammatory and cholesterol-reducing activities. RAPD analysis of the Commiphora wightii, taken up to record and characterize the genetic diversity of the dwindling population revealed highly variable identified markers. The study was taken up with three fluorescent-labelled RAPD primers by a high speed automated DNA fragment analysis device based upon the automated fluorescent DNA sequencer with the molecular taxonomy software Free Tree and Tree View. The program computes the distance matrix, constructs the phylogenetic or phonetic tree (dendrogram) using unweighted pair group method with arithmetic averages (UPGMA) or neighbour-joining and computes bootstrapping values for internal branches of tree. Pairwise comparison between accessions based on the proportion of shared bands produced by the primers used, were calculated using Jaccards similarity.

Walker *et al.,* (2005) have designed and developed *Xylella fastidiosa* (Xf) Simple Sequence Repeat primers. Thirty-four of them have been validated and are available to public. These primers are Xf-specific and powerful for detecting polymorphism among and within crop-associated Xf strains and can be used for Xf 14 genotyping, population structure and genetic diversity studies. Recently, they used fluorescent-labelled primers for PCR and an ABI 3100 genetic analyser in combination with our rapid sample preparation protocol to create a high throughput Xf pathogen diagnostic and genetic analysis platform.

They used this marker system to study the geographic population structures of grape Xf strains in California. We also used this marker system as a tool to study interactions between Vitis and Xf in Pierces disease resistant and susceptible grapes. Metin *et al.*, (2014) studied phylogenetic relationships between 14 Colchicum taxa spread throughout Turkey was performed using a fluorescent based amplified fragment length polymorphism technique. Five primer pair combinations were used in AFLP reactions. The data set was analysed statistically using the NTSYS 2.1 software and the neighbour-joining and maximum parsimony methods were implemented to generate phylogenetic trees. These analyses clustered the samples into 3 main clades. Both the neighbour-joining and maximum parsimony analyses resulted in similar topologies. Furthermore, supporting the phylogenetic trees, a similar grouping of 14 taxa was generated by principal component analysis.

The AFLP analysis with 5 primer combinations was carried out to assess 14 taxa. Fragment sizes ranged from 54 to 462 bp in length for each primer combination. The average was 166 fragments per primer pair. Smith, (1993) reported that random primed labelling of DNA has now almost superseded the method of nick translation of DNA. Random primed labelling, based on the method of Feinberg and Vogelstein, is a method of incorporating radioactive nucleotides along the length of a fragment of DNA. Random primed labelling can give specific activities of between 2 × 109 and 5 × 109 dpm/μg. The following method is essentially that described by Feinberg and Vogelstein, in which a DNA fragment is denatured by heating in a boiling water bath. Then, random sequence oligonucleotides are annealed to both strands. Klenow fragment polymerase is then used to extend the oligonucleotides, using three cold nucleotides and one radioactively labelled nucleotide provided in the reaction mixture, to produce a uniformly labelled double-stranded probe. Each batch of random oligonucleotides contains all possible sequences (for hexamers, 15 which are most commonly employed, this would be 4096 different oligonucleotides) and so any DNA template can be used with this method. Theresa *et al.,* (2013) studied that the technique was successfully used to developed microsatellite markers in several plant species. Microsatellites amplified with this multiplexing process were identical to those generated from PCR using individual primer pairs and with traditional methods using a prior labelled fluorescence primers. This technique is an efficient and economical way to fluorescently labelled multiple microsatellite primers in the same reaction. It is also applicable to other markers used in PCR amplification of genetic material.

Blacket *et al.,* (2012) explain that more cost-effective end-labelling of PCR products can be achieved through a three primer PCR approach, involving a fluorescently labelled universal primer in combination with modified locus specific primers with 5 universal primer sequence tails. In this study, they reported a suite of four high-performance universal primers that can be employed in a three primer PCR approach for efficient and cost-effective fluorescent end labelling of PCR fragments. They demonstrated that these universal primers can be combined with multiple fluorophores to co-amplify multiple loci efficiently via multiplex PCR. This method provides a level of multiplexing and PCR efficiency similar to microsatellite fluorescent detection assays using directly labelled primers while dramatically reducing project costs. Primer performance is tested using several alternative PCR strategies that involve both single and multiple fluorophores in single and multiplex PCR across a wide range of taxa. Fraser and Shah, (2010) studied that improvements in methods of DNA separation by capillary electrophoresis and advancements in cost effective

automated platforms for genome wide studies are a driving force behind the development of newer and more sophisticated software packages for handling the data analysis needs of a dedicated genotyping laboratory. Gene Mapper v4.1 software is designed to provide high performance fragment analysis for low to high throughput labs by combining the functions of the older Genescan and
Genotyper software packages into one. Using any one of the available sizing standards, a researcher can more complicated calculations and reporting, Gene Mapper v4.1 was designed to analyze genotype applications data from techniques 16 including amplified fragment length polymorphism (AFLP), microsatellite and loss of heterozygosity (LOH), deletion and duplication analysis (ex: aneuploidy), conformation analysis (ex: SSCP) and analysis of single nucleotide polymorphisms (SNPs) assays. The purpose of this poster is to compare and demonstrate the advances in genotype analysis available through the Gene Mapper® software v4.1 with sample files originally analyzed with Genescan® and Genotyper® software. Imle, (2005) study successful application of capillary electrophoresis technology to the genotyping of various types of polymorphism has been well documented.

The flexibility and automation of the Applied Biosystems 3100 genetic analyzer make it an excellent capillary electrophoresis platform for the generation of high quality genotype data. These data are readily applied to pharmacogenomic investigations of various types. This chapter included a protocol for the generation of genotype data using minimal template DNA and maximizing the automation of both data analysis and genotype assignment through the use of the Applied Biosystems Gene Mapper 3.0 software.

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