ANALYSIS OF POLYACRYLAMIDE GEL MATRIXES FOR THE DETECTION OF FLUORESCENT MEGAPLEX STR ALLELES

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The remarkable resolution of polyacrylamide gel electrophoresis (PAGE) for the separation of proteins and nucleic acids over the past three decades has led to its universal use. Recent technological advances in the manufacture and purification of polyacrylamide gels have provided enhanced separation of nucleic acids for both sequencing and allele detection. The separation of fluorescently-tagged Short Tandem Repeat (STR) alleles from biological samples associated with forensic evidence can be accomplished with flat bed PAGE and the alleles detected using a laser-based instrument. Currently, STRs may be purchased as a megaplex system in which eight or more loci are amplified simultaneously. In a static PAGE, it is important that single base pair resolution of the high molecular weight alleles be attained in order to assure accurate interpretation of the DNA profile.

The Promega PowerPlex™ 2.1 (PP2.1) STR fluorescent Megaplex system includes the following loci (Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, and vWA) in which the lowest molecular weight fragment is vWA allele 10 at 123 base pairs and the highest molecular weight fragment is Penta E at 474 base pairs. Consideration must be given therefore to the amount of sample loaded on a gel, the thickness, the length, width, wattage, time-of-run and most importantly, the type of gel matrix used for the separation of such a wide range of DNA fragments.

The goal of these studies was to systematically evaluate each parameter of PAGE of the PowerPlex™2.1 Megaplex system in order to optimize the separation of all alleles and allow the automated allele detection software, STARCALL, to aid in the identification of all alleles in the allelic ladder, plus detect and accurately call allele microvariants. Results from these studies include the discussion of: different matrix concentrations (4,5%, 5% and 6%) in FMC Long Ranger gels, Hitachi R3 Disposable gels, Amresco PagePlus, Amresco GenePage, and Amresco ThermoPage; the effect of gel length (32cm vs. 43cm vs. 44.3cm), different polymerization times (30 min, 45 min, 1:00h and overnight), different pre-electrophoresis times (15 min, 20 min, 30 min, and 1:00h) variations in the wattage (55W, 60W and 65W), time-of-run (1:25h, 1:30h, 1:35h, 1:40h, 1:45h, 1:50h, 2:00h, and 2:05h); the utilization of different comb/spacer widths (.4mm, .25mm, and .2mm) and also the evaluation of the efficacy of STARCALL to detect all alleles in the PowerPlex™2.1 allelic ladders and samples.

In summary, although there are several parameters that may offer sufficient DNA fragment separation, the most dramatic and reproducible results occurred when using a 0.25mm comb/spacer set with a 6% Amresco PagePlus PAG matrix using 60 watts for 100 minutes. This system allowed the STARCALL software to consistently call all alleles without the need for manual banding or moving of autobands to accommodate placing manual bands on the gel file. Use of these studies to optimize the separation of the PowerPlex™16 fluorescent STR Megaplex will also be discussed.