A150 Development and Validation of a PCR Amplification Kit: An STR Multiplex System for Teaching and Research Purposes

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After attending this presentation, attendees will have a basic understanding of the methodologies and techniques needed to create a 4-loci Short Tandem Repeat (STR) multiplex kit for teaching and research purposes.

This presentation will impact the forensic science community by providing the groundwork that teaching and research facilities need to create a cost-effective STR kit. By lowering the cost of each STR reaction, teaching facilities can perform more reactions and therefore, provide their students with more opportunities to improve their techniques in a DNA laboratory with the ability to determine the success of research projects at a fraction of the cost.

Although numerous commercial kits have been developed to amplify STR loci, commercial kits are often expensive. Since these kits are used for discrimination purposes, 16-loci provide the statistical confidence needed for testimony. Since these loci are spread throughout the genome, every allele is inherited independently and the markers are not linked. In this way, a complete profile has powerful discriminatory capability as the probability of individuals with the identical alleles decreases as more loci are analyzed.

Not all facilities that use these kits, require such power of discrimination, resulting in wasted resources. Teachers can demonstrate multiplex STR amplification and analysis using reactions that amplify fewer loci. The purpose of most forensic biology laboratory exercises is to teach the basics of PCR amplification and provide students with hands-on experience setting up and performing the reactions. Although these exercises may also include examining and comparing allelic profiles, making an identification with a high statistical level of confidence is not necessarily an objective. Therefore, using the commercial kits to perform classroom laboratory experiments could be considered wasteful and unnecessarily expensive. A kit that amplifies at least three loci using two dyes would be useful for classes conducting multiplex reactions.

From a research standpoint, a simple kit could be used to screen samples and predict the success of a commercial kit. For forensic DNA research projects, often the objective is to determine whether amplifiable DNA is present in a given sample. The results from less expensive monoplex and multiplex reactions would provide data about whether amplifiable DNA exists at chosen STR loci. A less expensive kit would allow investigators with a limited budget to process more sets of samples and include more samples in a given set. Often, evidence samples, or mock evidence samples, will contain small amounts of DNA (fingerprint residues) or be exposed to harsh environments (fired cartridges). These conditions can result in variation of results within a data set, where some samples may amplify all STR loci included in the reaction, while other samples will not contain any amplifiable DNA. Including more samples in each data set will help investigators better evaluate the variability of results within a given set of samples.

The goal of this project was to develop and validate a more cost-effective STR amplification kit specifically designed for research, screening, and teaching purposes. For this study, 4 STR loci were chosen for amplification, D3S1358, Th01, D8S1179, and vWA. Dye-labeled primers were designed for each loci based on previously published sequence data. A ladder was developed for analysis. Monoplex reactions were performed in order to evaluate the best amplification conditions for each locus. Once the multiplex reaction was balanced to produce even amplification among the 4-loci, a validation study was performed, evaluating thermal cycling parameters, precision, and sensitivity.

STR Multiplex, Teaching Kit, DNA

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