RAPID MEASUREMENT OF HUMAN-SPECIFIC DNA IN MIXED DNA SAMPLES THROUGH THE USE OF A COUPLED ENZYME SYSTEM

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The accurate analysis of STR fragments that have been amplified by PCR is dependent on using a narrow range of DNA concentration in the amplification reaction. For casework samples, determining DNA concentrations can be further complicated by contamination with microbial, fungal, and occasionally animal DNA. The current available human-specific DNA quantitation kits use hybridization of a repetitive sequence probe to sample DNA attached to membranes. While these systems provide the necessary sensitivity, they have a limited dynamic range frequently compared by eye with known standards. In addition, these techniques require the manipulation of membranes and suffer the inherent problems of filter hybridization.

As an alternative approach, Promega has modified its READIT™ SNP detection system to quantitate human-specific DNA. This system hybridizes probes to repetitive DNA sequences in solution. An enzyme mix is then added to analyze the hybridized fragments. A polymerase pyrophosphorylates (depolymerizes) hybrids containing perfect base pairing near the 3' terminus of the probe. The second enzyme then transfers the terminal phosphates of the resulting dNTPs to ADP to produce ATP in proportion to the amount of hybrid. A subsequent Luciferase/luciferin reaction is used to generate a light signal proportional to the amount of ATP generated in the first reaction. This signal is measured by an inexpensive luminometer.

This approach has several advantages over other systems. First, solution hybridization is used instead of filter hybridization. This eliminates the need to work with membranes and provides a more consistent reaction. Second, no washing is required. The specificity resides in both the hybridization and the necessity to have perfect base pairing at the 3' end of the probe. Third, the results are obtained as quantitative numbers with a large dynamic range. This large dynamic range reduces the problem of saturation observed in the other systems. Fourth, the entire analysis takes approximately one hour. Finally, the system is completely solution based. Our system involves only simple pipetting steps while the other systems require several manipulations with membranes. The use of solution-based chemistry also allows for automation.

Our initial experiments with this system using DNA from both blood as well as forensic samples suggest that this system will work in the nanogram to subnanogram range. Bacterial and mouse DNA present in mixed samples do not appear to interfere with the human quantitation.