Real Time Paternity Testing Utilizing a Novel 11-System STR Multiplex Analyzed on an ABI Prism 377 DNA Sequencer

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The validity and utility of PCR-STR technology in paternity determination is well established. STR results can be rapidly obtained and are easily interpreted. However, limitations in the use of STRs in paternity testing relate to lower powers of exclusion of individual STR loci as compared to those obtained through the analysis of VNTR loci (RFLP). As a result if has been necessary to analyze many more STR loci than RFLP loci to report paternity inclusions. This is both costly and labor intensive. Consequently, researchers have endeavored to develop multiplex STR systems with higher powers of exclusion obtained from a single amplification. However, since most STR products are produced within a fairly narrow size range (from ~100bp to ~350bp), the number of systems that can be visualized on standard silver-stained polyacrylamide gels is constrained to three or four systems. Current technology now permits the labeling of the oligonucleotides used to prime PCR reactions with a number of different fluorescent molecules (dyes). These dyes emit light when excited by laser radiation and are readily detectable by Charge-Coupled Devices (CCDs). The development of multiple colored dyes allows several loci, even those with overlapping alleles, to be analyzed from a single PCR amplification.

Two laser platforms have been developed to take advantage of fluorescence technology. One is engineered to allow laser energy to scan an entire gel, in total, for tagged PCR products after the completion of a gel run. Fragment detection is accomplished independently of electrophoresis. The second platform uses a laser to scan across a fixed, limited area of a gel, while it is running, detecting labeled products as they pass before it during electrophoresis. The former system trades resolution of fragments at high molecular weights for speed of throughput, while the latter sacrifices turnaround speed for increased read accuracy, which is especially significant at higher molecular weights.

In a bid to capitalize on these developments we are in the final stages of developing an undecaplex fluorescent STR test (IdentiPlex) for use in the paternity market. The 11 loci examined are D9S302, D22S683, D18S535, D7S1804, D3S2387, D4S2366, D5S1719, HUMCSF1PO, HUMFESFPS, HUMTH01 and HUMLIPOL. PCR primers have been labeled with the ABI fluorescent dyes FAM, TET, and HEX. Amplification conditions have been worked out to allow the robust co-amplification of all 11 loci in a single reaction tube. Results are read from an ABI Prism® 377 DNA Sequencer equipped with a 64-well XL upgrade and running GeneScan® and GenoTyper® automated software. Paternity data derived from our initial validation studies for the undecaplex indicate that it provides a cumulative power of paternity exclusion greater than 0.9999. The numerous advantages of adopting this strategy include:

- Increased efficiency (fewer gels are required to obtain a paternity result.)
- Higher throughput (50% more cases can be reported in a single day.)
- Faster turn-around times (high powers of exclusion obtained from the products of a single amplification.)
- Automation (reduced likelihood of technician error.)
- High order of discrimination (single base pair resolution of PCR fragments at all molecular weights scanned by IdentiPlex.)

We have found the last advantage especially beneficial for the analysis of D9S302, a locus based on GATA repeats. This

We find our STR IdentiPlex to be a very powerful testing battery for resolving paternity disputes, and the use of an ABI Prism® 377 DNA Sequencer for fragment analysis enables us to quickly and effectively process cases through our laboratory.