Introduction

The use of DNA methods for establishing paternity in the United States has become commonplace. GeneScreen has been performing DNA testing for parentage determination since August of 1988. Since that time, the technology available for performing paternity testing has undergone significant changes and improvements. Many paternity testing laboratories across the United States have abandoned Restriction Fragment Length Polymorphism (RFLP) technology and have implemented one or more multiplex Short Tandem Repeat (STR) systems for human parentage. GeneScreen is a high volume laboratory that has experienced a variety of different technologies, including RFLP, PCR coupled with various silverstain STR systems, PowerPlex™ 1.1 and 2.1, AmpF/STR™ Profile™ and Cofiler™, and SNP detection by GBA®. This manuscript will examine the advantages and disadvantages of DNA typing using multiplexed, fluorescently-labeled STR systems. Parameters such as ease of implementation, gains in testing efficiencies, comparative cost, ease of interpretation and data analysis, and reliability will be presented.

Advantages of FL-STRs

For many years RFLP technology was the method of choice for most paternity testing laboratories. The availability of well-characterized RFLP markers and the relative power of these genetic systems to exclude falsely accused alleged fathers fueled the widespread use of this technology among many laboratories. With the implementation of buccal swab collection as the accepted method for collecting human biological specimens, the need for an alternative technology became necessary. While it was possible to perform RFLP on a large majority of buccal swab specimens, it was not possible to perform RFLP testing on all samples, due either to lack of sufficient DNA or the isolation of partially or extensively degraded DNA from the swab. This change in collection device caused our laboratory to consider a number of testing platforms to replace RFLP. For a variety of reasons, some of which will be discussed, GeneScreen decided to use the PowerPlex™ 1.1 system from Promega.

The advantages of multiplexed, fluorescent STR systems are shown in Figure 1. The use of PCR has allowed DNA profiling to be successful on most of the buccal swab specimens collected for paternity testing. In addition, the kits provided for parentage testing by both Promega and Perkin Elmer are extremely robust, providing sufficient amplification and subsequent typing from a variety of specimen sources yielding DNA of various degrees of quality.

One of the most notable benefits recognized by our laboratory has been the increased speed and efficiency gained in the testing process. Decreasing case turn-around-time (TAT) has significantly increased our capacity without the need for increasing laboratory or support staff. For the sake of comparison, during the last several months prior to switching from RFLP to PCR, the average TAT for a case in our laboratory was 18 calendar days from collection of specimen to reporting. Since switching to PCR and fluorescent STRs over nine months ago, our average case is reported in less than 10 calendar days. As one might expect, these improvements in laboratory efficiency have also led to a significant decrease in laboratory testing costs. Since converting to the PowerPlex™1.1 system our laboratory costs have decreased significantly in spite of added equipment purchases (e.g., a Hitachi FMBIO®) and PCR license fees. Furthermore, the use of more powerful multiplex STR systems, like the PowerPlex™2.1 system from Promega, should further reduce laboratory costs by virtually eliminating the need for
additional genetic systems to meet either internal or contract-mandated paternity indices or minimum power of exclusions.

**Disadvantages of FL-STRs**

While there are several advantages to the multiplexed, fluorescent STR systems, there are a few disadvantages. These disadvantages are shown in Figure 2. Due to the fact that buccal swab specimens collected from different individuals often yield different amounts of DNA, the quality of a gel result may be affected by imbalance of the DNA samples being electrophoresed on the same gel. One solution to this problem would be to quantify the DNA isolated from all specimens prior to amplification and then adjust the volume of each sample to be amplified based upon the amount of DNA present. While this solution would yield the most consistent results, it does involve the addition of another step in the process adding both additional time and resources to the case. In our laboratory we have chosen to live with the occasional imbalance seen from lane to lane and have instead chosen to optimize the isolation procedure so that more consistent quantities of DNA are isolated from each swab on a regular basis. While not quantifying the DNA does lead to occasional reamplifications of some DNA samples, the number is sufficiently low that it does not present a major problem in case flow or laboratory cost.

Another disadvantage of multiplexed STRs is the difficulty in automating a gel-based detection platform. While some strides have been made in this arena, such as multi-channel gel loaders, the need for more automation is real. Compared to other DNA testing platforms, like plate-based systems or microarrays, automation in STR systems is lagging behind and is limited almost exclusively to the front-end portion of the test, i.e., DNA isolation. Even that aspect of automation presents significant challenges using buccal swabs as the collection method of choice.

As mentioned previously, the available multiplexed STR systems are very sensitive and quite robust. This robustness, however, is applicable over a fairly narrow range of conditions required for amplification. This means that the products are manufactured to work well within the range and conditions for which they were designed. Slight modifications in these required conditions (shorter PCR cycle times or decreased amount of Taq, for example) could lead to failure of the testing procedure. Simply stated, this means that slight changes or alterations in the laboratory’s standard testing protocol could lead to sample amplification failure. While failures of this type do not lead to erroneous results, they can create a situation of mass reruns that increase laboratory costs and decrease productivity and efficiency. The simple solution to this potential problem is to adequately train laboratory staff to pay close attention to all details of the procedure and to put in place a quality plan that monitors all factors impacting the testing process.

One final disadvantage worthy of mention is not one that is relegated solely to PCR or more specifically to STR systems. It is well understood that most genetic systems display some level of genetic recombination or mutation. Because some mutations may occur under PCR amplification primers, it is incumbent upon the reviewer of STR results to take this fact into account when examining unusual DNA results. One such result would be single, or even double exclusions, where two of the tested parties demonstrate one or two non-matches and both individuals are homozygous for different alleles and yet all other tested genetic systems are matches. These non-matches could be referred to as “null” alleles and might possibly be explained by the presence of mutations under the amplification primers leading to non-amplification of the “null” allele. Figures 3 and 4 demonstrate the frequency of “null” alleles present in the PowerPlex™ 1.1 system in mothers and possible biological fathers, respectively. Along these same lines, it is important for a laboratory performing PCR/STR testing to use well-characterized genetic markers and to have a working knowledge of the mutation rates for the genetic systems in use.

**Conclusions**

In summary, multiplexed fluorescent STR systems represent a fast, efficient, and relatively inexpensive means of performing parentage testing. While there are a few minor disadvantages, none of these represent serious problems that could potentially lead to the release of erroneous results. With care taken in design of the standard operating protocol and the laboratory’s quality plan, the few potential
disadvantages discussed can be readily overcome. Knowledge of the system’s intricacies such as optimal operating conditions, possibility of null alleles, and mutation rates will greatly assist the laboratory in data interpretation and in maximizing the potential of this DNA methodology.

Figure 1
Advantages of FL-STRs

- Compatible with buccal swabs
- Multiplex amplification systems are extremely robust
- Increased speed and efficiency
- Decreased lab costs

Figure 2
Disadvantages of FL-STRs

- Quantification of DNA is necessary to balance lanes
- Difficult to automate process
- Systems are robust only within a narrow range
- Mutations may occur under amplification primers
### Figure 3
Maternal Single Exclusions
Due to “Null” Alleles

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<th>Genetic Locus</th>
<th>Mutation Rate</th>
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<tr>
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### Figure 4
Paternal Single Exclusions
Due to “Null” Alleles

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<tr>
<th>Genetic Locus</th>
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