Over the last 12 years we have seen data from restriction fragment length polymorphism (RFLP) testing, DQA1 and PM testing, and short tandem repeat (STR) testing using silver staining, challenged on the issue of general acceptance in many courts. As criminal cases make their way into court systems in the various states using data from the thirteen STR CODIS loci, questions of admissibility will once again become important. Two issues which will usually require testimony are whether the STR method in question enjoys general scientific acceptance and whether the tests done in the specific case were done correctly. The laboratory documentation will generally serve to answer the question of “Was the test done correctly?” Answering the question of “Are the multiplex PCR reaction kits and the instrumentation used for STR analysis generally accepted in the relevant scientific community” requires that the witness be able to review for the court the information, which the witness used to reach this conclusion.

How is general acceptance defined from a scientific perspective? For the area of DNA identification there are several important resources. These are: 1) Publications in the scientific literature of underlying theory upon which procedures are based 2) Publication of non-forensic applications 3) Publication of forensic applications 4) General use in the fields of genetics and molecular biology 5) General use in forensic applications including paternity testing and 6) Concordance of results demonstrated by proficiency testing. This paper addresses how these resources apply to multiplex amplification protocols for the thirteen CODIS STR loci and analysis using fluorescent detection methods with slab gel or capillary electrophoresis. The accompanying list of selected references is organized by topic with references in chronological order by publication date.

GENERAL ACCEPTANCE: THE LOCI

STR loci are part of a larger class of polymorphic loci, which are based on length polymorphisms arising from the presence of alleles having varying numbers of tandem repeats. These include both variable number of tandem repeat (VNTR) loci used for RFLP testing and STR loci used with PCR amplification. The references listed in section A include information about the organization of repeated sequences in the genome (Section A1), selected historical references for RFLP typing (Section A3), genome database web sites for accessing up-to-date information regarding map position of loci and the National Institute of Standards and Technology (NIST) web site (STRbase). The NIST site contains a comprehensive reference list for the thirteen CODIS STR loci and associated typing methods (Section A4). The 800-plus peer-reviewed publications listed on this site, by themselves, demonstrate general scientific acceptance of STR typing.
GENERAL ACCEPTANCE: FEATURES OF THE PCR AND FORENSIC APPLICATIONS

There are enormous advantages to the application of multiplex amplification procedures to forensic samples the most important of which is the conservation of sample for future testing by other interested parties. Out of necessity, most multiplex PCR reactions are done under conditions that are not optimal for all loci being amplified, yet are sufficiently optimized to yield results from small DNA samples (50 to 200 cells). This is true of all the commercially available kits for forensic STR testing. While it is correct that different primer pairs may have different conditions under which they will work optimally, conditions can be determined which will allow a primer pair to work well in the presence of other primer pairs even though the optimum reaction is not individually achieved for each pair. When multiple primer pairs are required to work together in a multiplex reaction, the primers must work sufficiently well together so that amplification is achieved with all of the primer pairs that are present. The references in section B address the development of multiplex reactions (Section B1), known complications which occur as a result of the PCR reaction chemistry (Section B2), and selected references demonstrating that successful and reproducible results can be achieved using multiplex systems and forensic samples (Section B3).

GENERAL ACCEPTANCE: ELECTROPHORESIS AND FLUORESCENT DETECTION

Electrophoresis is used to separate PCR products from different loci and alleles for each locus which differ in length due to the variation in the number of tandem repeats. There are two commonly used electrophoresis methods for the analysis of STRs. One method uses an acrylamide slab gel, the second uses an acrylamide derivative in a narrow capillary. Both use acrylamide as a separation medium and include chemicals which maintain the DNA in a denatured (strand-separated) condition. The concentration of acrylamide, presence or absence of cross-linking agents, and buffer used in the electrophoresis may vary between methods. There are numerous buffer and acrylamide formulations that could be used in these types of separation systems. Regardless of which specific separation system is used, the general principles of the separation remain the same.

Detection of DNA fragments after or during electrophoresis follows one of several possible variations:

a) Amplify DNA, separate alleles on an acrylamide slab gel and detect allele position with silver stain.
b) Amplify DNA using fluorescently labeled primers, analyze DNA on an acrylamide slab gel and detect allele position by fluorescent signal.
c) Amplify DNA using fluorescently labeled primers, analyze DNA on an acrylamide slab gel or acrylamide-filled capillary and detect allele position by fluorescent signal.

The same basic method for determining allele type following electrophoresis is used in all of the above formats. This method utilizes an allelic ladder that is a collection in one sample of all
common alleles known for a locus. The allele type is determined by comparing the position of the alleles in the unknown sample to the position of the alleles in the allelic ladder. This method can use direct visual comparison or comparison of sizes in base pairs of the sample alleles and the ladder alleles. The separation and detection methods mentioned above, as well as the methods for calculating size in base pairs and allele identification using an allelic ladder, are widely used in paternity and forensic testing and are generally accepted. The accompanying references in Section C cover the general principles of electrophoresis, calculation of DNA fragment size, and use of capillary electrophoresis and fluorescent detection. It is interesting to note that the original publication on fluorescent detection (Smith et al.) is as early as 1986 and the original STR publication in 1991 (Edwards et al.) uses a precursor instrument to the ABI 377.

While validation does not equal general scientific acceptance, validation followed by general use does contribute to general acceptance. When the scientific principles underlying a kit and analysis method are generally scientifically accepted, and numerous laboratories have validated the use of the kit and analysis method, then general acceptance can be inferred from general use. If general acceptance cannot be inferred from general use, one would have to suppose that the many forensic laboratories which are either already using these kits and fluorescent analysis methods or in the process of validating them are proceeding forward with a technology which they do not believe is generally accepted. The extensive scientific literature, general use in other applications of molecular biology as well as forensic applications and concordance of results in proficiency testing (not addressed here) give substantial scientific weight to the conclusion that analysis of STR loci using multiplex amplifications followed by electrophoresis and fluorescent detection are accepted by the scientific community.
REFERENCES:

A. GENERAL ACCEPTANCE: THE LOCI

1. Reviews of Organization and Structure of Repetitive DNA Sequences:


2. Original Reference for a DNA Polymorphism:


3. Original DNA Typing Methods, Length Polymorphisms:


4. Short Tandem Repeat Polymorphisms:

Literature references for STR loci in general and specifically the thirteen CODIS loci can be found and organized by locus on the NIST web site.

   Short Tandem Repeat DNA Internet Database (www.cstl.nist.gov/div831/strbase/)
   Sequence and Mapping data can be accessed on the following two sites:
   The Genome Database (http://gdbwww.gdb.org/)
   National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/)


B. GENERAL ACCEPTANCE: FEATURES OF THE PCR AND FORENSIC APPLICATIONS

1. Use of Multiplex Reactions With the PCR:


2. Complications to the PCR:

Preferential Amplification:


Mutations:


Stutter:


Non-templated nucleotide addition (minus A):


3. PCR and Forensic Samples, Degraded DNA, Matrix Studies:


C. GENERAL ACCEPTANCE: ELECTROPHORESIS AND FLUORESCENT DETECTION

1. Electrophoresis General:


2. Fluorescent Detection, Capillary Electrophoresis:


