

GenePrint™ Custom Protocols

By Isobel Maciver, James Lloyd,
Julie Pederson, Ann Lins and Kimberly A. Huston
Promega Corporation
e-mail genetics@promega.com

INTRODUCTION

The "Technical Tips" article in this issue of *Profiles in DNA* focuses on using the Internet to obtain information on various topics pertinent to genetic identity testing. One of the sites featured is the Promega website (www.promega.com or www.euro.promega.com), where up-to-date information on GenePrint™ products, meetings, training and symposium proceedings are available. Recent additions to this site include Custom Protocols for the use of GenePrint™ STR Systems.

These protocols are provided in a format that allows the user to key in information such as the STR system, detection instrument, enzyme and thermal cycler used. The program then generates a specific protocol for each system based on the chosen parameters.

The GenePrint™ Systems have been developed in multiple formats, allowing the user to choose a system that will perform well with their preferred detection method and instrumentation. Currently available GenePrint™ STR Systems can be classified into three groups based on the complexity of the system and the method used for detection of amplified fragments. Selected examples from each of these groups are shown in Figure 1.

The first group comprises the GenePrint™ PowerPlex™ System (Figure 1, Panel A). This system contains eight loci that can be amplified in a single reaction. Four of the loci (D16S539, D7S820, D13S317 and D5S818) are labeled with fluorescein and the other four (CSF1PO, TPOX, TH01 and vWA) are labeled with carboxy-tetramethylrhodamine (TMR). The PowerPlex™ 1.1 System may be detected using the Hitachi FMBIO® Fluorescent Scanner.

The second group comprises the GenePrint™ Fluorescent STR Systems. These quadriplex systems each contain four loci that are labeled with fluorescein. The three currently available quadriplex systems are the CTTv Multiplex (CSF1PO, TPOX, TH01 and vWA), the GammaSTR™ Multiplex (D16S539, D7S820, D13S317 and D5S818) (Figure 1, Panel B) and the FFFL Multiplex (F13A01, FESFPS, F13B and LPL). The amplification products of these systems can be detected using the Hitachi FMBIO® Fluorescent Scanner, the ABI PRISM™ 310 Genetic Analyzer, the ABI PRISM™ 377 and ABI 373 DNA Sequencers and the Molecular Dynamics FluorImager™ Fluorescent Scanner.

The third group, the GenePrint™ STR Systems, are designed for silver stain detection. These are the CTT Multiplex (CSF1PO, TPOX and TH01) (Figure 1, Panel C), the FFv Multiplex (F13A01, FESFPS and vWA) and the SilverSTR™ III Multiplex (D16S539, D7S820 and D13S317). The nine loci available in the silver multiplex detection format offer significant discriminating power to laboratories with lower cost, lower throughput needs.

The development of both silver stain and fluorescent detection methods for the same set of loci allows each user the flexibility to choose the detection method best suited to his/her throughput needs and budget. Each GenePrint™ System contains the appropriate 10X Primer Pairs, STR 10X Buffer, loading solution, the appropriate allelic ladders, K562 DNA (positive control) and a detailed Technical Manual.

TECHNICAL MANUALS

Extensive instructions for use of the GenePrint™ Systems are given in the Technical Manuals provided with each product. These are the GenePrint™ STR Systems (*Silver Stain Detection*) Technical Manual #TMD004, the GenePrint™ Fluorescent STR Systems Technical Manual #TMD006, and the GenePrint™ PowerPlex™ 1.1 System Technical Manual #TMD008. These

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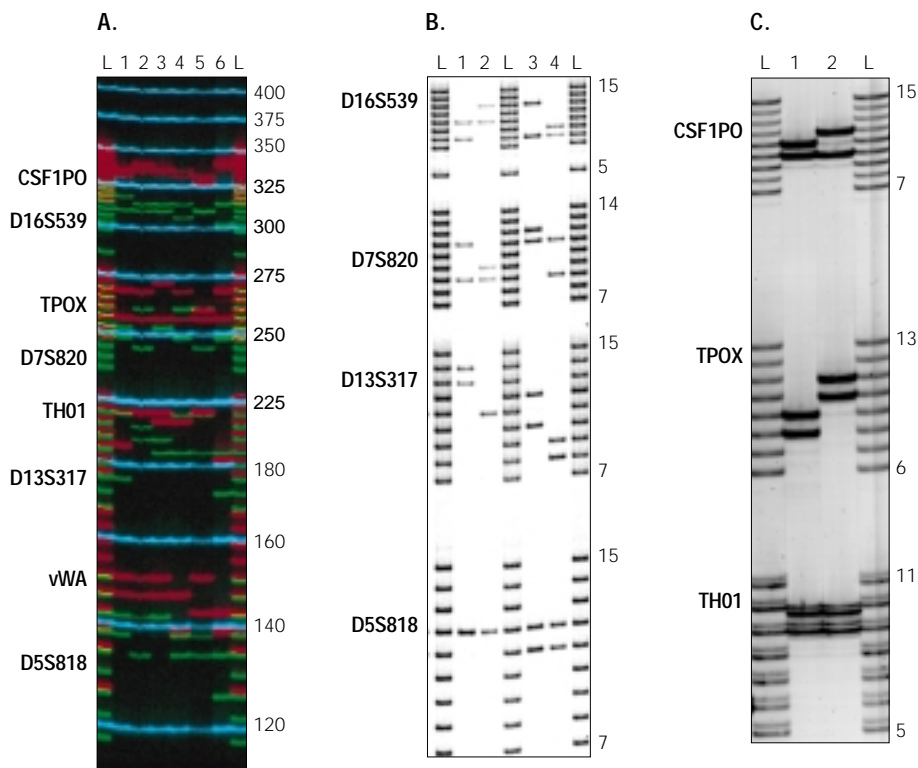


Figure 1. Representative *GenePrint*™ STR Systems. **Panel A:** STR analyses performed using the *GenePrint*™ PowerPlex™ 1.1 System. The amplified products of the four fluorescein-labeled loci (D16S539, D7S820, D13S317 and D5S818) are shown in green, while the four TMR-labeled loci (CSF1PO, TPOX, TH01 and vWA) are shown in red. The products of six amplification reactions (lanes 1-6) were separated on a 4% polyacrylamide denaturing gel and detected using the Hitachi FMBIO® II Fluorescent Scanner. An additional size marker, the Fluorescent Ladder (CXR) is shown in blue. Allelic ladders for the corresponding loci are shown in the lanes labeled L. **Panel B:** STR analyses performed using the GammaSTR™ Fluorescent Multiplex System (D16S539, D7S820, D13S317 and D5S818). Four DNA samples (lanes 1-4) were amplified, separated on a 4% polyacrylamide denaturing gel and detected using the Hitachi FMBIO® II Fluorescent Scanner. Allelic ladders for the corresponding loci are shown in the lanes labeled L. **Panel C:** STR analyses performed using the *GenePrint*™ STR System-CTT (CSF1PO, TPOX, TH01). Two DNA samples (lanes 1-2) were amplified, separated on a 4% polyacrylamide denaturing gel and detected by silver staining. Allelic ladders for the corresponding loci are shown in the lanes labeled L. In Panels B and C, the number of repeats for the largest and smallest alleles are noted to the right of the gels.

manuals are also available on the Internet at www.promega.com/tbs/. Each technical manual contains extensive information on the STRs used in each system, detailed DNA extraction methods, protocols for DNA amplification and detection, representative data, extensive reference listings, and population data for all of the alleles in each system.

CUSTOM PROTOCOLS

The custom protocols available on the Internet are designed as a supplement to the Technical Manuals, and are not intended as a substitute for them. For background information, information on DNA extraction, references, population data and detailed explanation of terms, please refer to the manual specific for the system used. Each manual provides information on the use of several different *GenePrint*™ Systems and provides a number of options for DNA amplification and detection for each system. These protocol options vary depending on the user's choice of system, enzyme, thermal cycler and detection instrumentation. On the Internet a dynamic protocol that is designed to be specific to a single user's need is created. These custom protocols ask a series of questions of the user, then gather the corresponding usage

information relevant to these requirements from the larger Technical Manual and distill it into a protocol that is tailored specifically to the needs of a single user.

Custom protocols are provided for the PowerPlex™ 1.1 System, for all of the *GenePrint*™ Fluorescent STR Systems and for the *GenePrint*™ STR Systems for silver stain detection. The user selects their choice of enzyme (AmpliQa® or AmpliQa Gold™ DNA polymerase), thermal cycler (Perkin-Elmer 480 or the GeneAmp® PCR System 9600), *GenePrint*™ System and detection method. Protocols also offer the option of including or excluding BSA from the amplification reaction and including the Fluorescent Ladder (CXR) as a size marker with the fluorescent systems.

After the user selects choices from a series of pull-down menus, the program generates a unique set of usage instructions based on these parameters. These instructions include a list of the loci in the system of choice, a complete protocol for setting up amplification reactions and a thermal cycler program. They also include the appropriate protocols for electrophoresis and detection on the instrument of choice.

We hope that users will find the speed and convenience of obtaining custom-designed protocols through this website to be of value. Historically, *GenePrint*™ products have always been provided as systems customized for multiple instrument platforms and detection formats. We are now expanding this approach and are beginning to use the Internet as a means to provide protocol information in a way that is tailored to meet the needs of each individual user. The custom protocols are a pilot project in this area. Please visit the web site at www.promega.com/geneticidentity/ and let us know what you think.

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