POWERPLEX® Y

The PowerPlex® Y System

By Benjamin E. Krenke, Patricia M. Fulmer, Katharine Driftmier Miller and Cynthia J. Sprecher, Promega Corporation

The new Promega PowerPlex® Y System allows for the co-amplification and three-color detection of 12 Y-STR loci, which include both the European minimal haplotype and the Scientific Working Group on DNA Analysis Methods recommended panel of Y-STR loci.

INTRODUCTION

Short tandem repeat (STR) analysis has become the leading technology for genetic human identification. Multiplex STR typing is used in forensic, paternity and anthropological studies and is a cornerstone of criminal databasing. Although autosomal STR analyses have been a primary focus for human identification, some cases can benefit from the analysis of sex-specific Y-STR markers.

Utility of Y-specific human identification tools is based on the difference between the Y and X chromosomes and the autosomes (1–7). Although possibly evolving from a pair of homologous autosomes, the X and Y chromosomes exhibit genetic specialization and related sequence differentiation that allows development of Y-specific PCR[b] assays. However, homology exists between the X and Y chromosomes, and these regions must be avoided for effective Y-specific analysis. Differences between the X and Y chromosomes are maintained due to a lack of recombination between much of the two chromosomes. Roughly 95% of the Y chromosome does not undergo recombination (nonrecombining region, NRY). The Y markers of greatest forensic interest are within the NRY. These loci are genetically linked and inherited unaltered from father to son. Barring a mutation event, all male relatives will share the same Y profile.

Y analysis has both advantages and drawbacks for human identification. The most important advantage is that female DNA does not amplify with a properly designed Y-specific assay. This allows easy profiling of the male contributor in a male/female mixture. Additionally, a single-peak haploid (all loci reported together) profile observed with most Y-STRs is easy to interpret. The greatest drawback of Y-specific analysis is the reduced power of discrimination obtained from genetically linked markers. This linkage rules out the use of the product rule to obtain the frequency of a given combination of alleles at multiple loci. Instead, more conservative methods must be employed if estimating the prevalence of a haplotype in a population of unrelated males, such as the “counting method”, is desired. Finally, diminishing returns can be expected from continued analysis of a sample with an ever-increasing number of linked loci.

The primary application of Y-STRs in forensics is the analysis of sexual assault samples (8,9). The preferred autosomal-STR analysis often requires deconvolution of results from male/female mixtures. Use of differential extraction can adequately isolate sperm cells from female epithelial cells in many cases, allowing for a clear indication of the primary donor(s). However, in a number of cases, differential extraction is neither possible nor effective. Samples collected after an extended postcoital interval can experience degradation and dilution to a point where sperm are not microscopically detectable, yet the male DNA persists (often detected in the ”epithelial fraction” of a differential extraction). Other samples, such as fingernail scrapings, azoospermic ejaculate and saliva on skin, require detection of male DNA from nonsperm cell types. By removing an overriding female profile seen in the autosomal analysis, Y-STR analysis can add clarification to "multiple-male"

POWERPLEX® Y

mixtures. The number of male contributors can be easier to resolve with Y-STRs. Furthermore, because the amount of DNA added to an amplification can be normalized to the quantity of male DNA in a sample (rather than total human DNA), increased signal from these additional males can be expected. Many laboratories use the ratio of X- and Y-specific Amelogenin signals as a simple means to help normalize the amount of male DNA to add to an amplification reaction.

Immigration, paternity and anthropological studies also benefit from Y-STR analysis, particularly when individuals related through male bloodlines are separated by one or more generations. Y-STR analysis can aid in anthropological kinship studies, add to current Y-SNP phylogenetic studies or complement mitochondrial DNA maternal lineage studies. A major database of Y-STRs can be found at: **www.ystr.org** (4).

THE POWERPLEX® Y SYSTEM

The PowerPlex® Y System[a] (Cat.# DC6761 and DC6760) allows co-amplification and three-color detection of 12 Y-STR loci: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439 (10,11). One of the two primers for DYS389I/II, DYS391 and DYS439 is labeled with fluorescein; one primer for DYS19, DYS392, DYS437 and DYS438 is labeled with 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (JOE); and one primer for DYS385a/b, DYS390 and DYS393 is labeled with carboxy-tetramethylrhodamine (TMR). Amplicon size is determined by use of the Internal Lane Standard 600, which is labeled with carboxy-X-rhodamine (CXR).

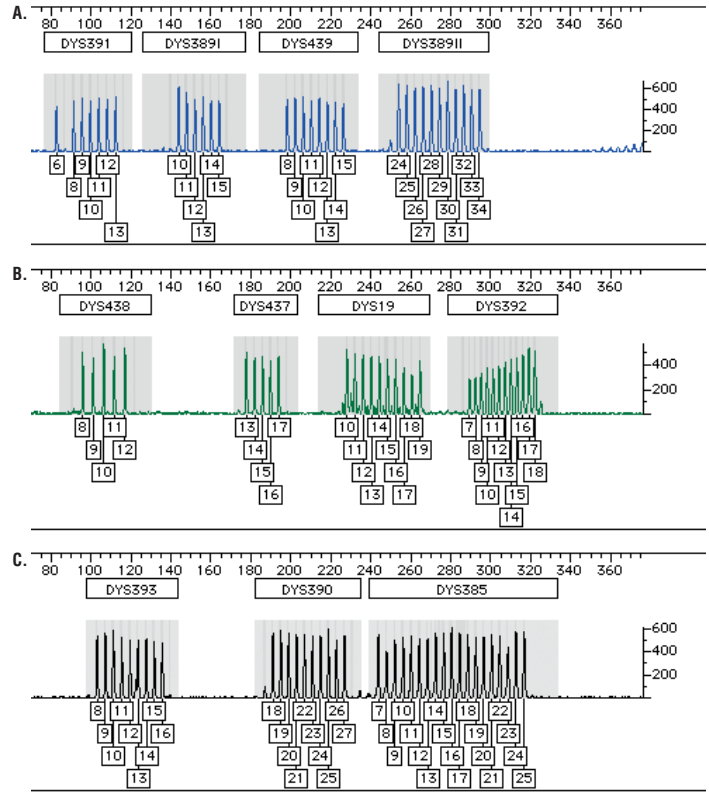

Figure 1. The PowerPlex® Y Allelic Ladder Mix. The PowerPlex® Y Allelic Ladder Mix was separated with the ABI PRISM® 310 Genetic Analyzer using a 3-second injection time. The GeneScan® sample file was analyzed with the Genotyper® software and the PowerTyper™ Y Macro. **Panel A.** The fluorescein-labeled allelic ladder components (DYS389I/II, DYS391 and DYS439) and their allele designations. **Panel B.** The JOE-labeled allelic ladder components (DYS19, DYS392, DYS437 and DYS438) and their allele designations. **Panel C.** The TMR-labeled allelic ladder components (DYS385a/b, DYS390 and DYS393) and their allele designations.

The PowerPlex® Y System's four-color chemistry allows analysis on the ABI PRISM® 377 DNA Sequencer and the ABI PRISM® 310 and 3100 Genetic Analyzers. Color deconvolution can be performed with available color matrices (Cat.# DG2680 and DG3380). Laboratories currently using FL-JOE-TMR-CXR PowerPlex® chemistries (11,12) can use the same matrix or spectral files for the PowerPlex® Y System.

We have created allelic ladders following International Society for Forensic Genetics (ISFG) recommendations (3). To increase confidence in allele designation, a total of 102 alleles have been included (Figure 1). The PowerTyper™ Y Macro automatically labels fragments from GeneScan® data using the supplied allelic ladder and size standard. This file has been designed to operate within the Genotyper® software (version 2.5 or higher). The PowerTyper™ Y Macro is available on the PowerTyper™ Macros CD-ROM (Release 2.0, Cat.# DG3470) or can be downloaded at: **www.promega.com/geneticidtools/powertyper/**

POWERPLEX® Y

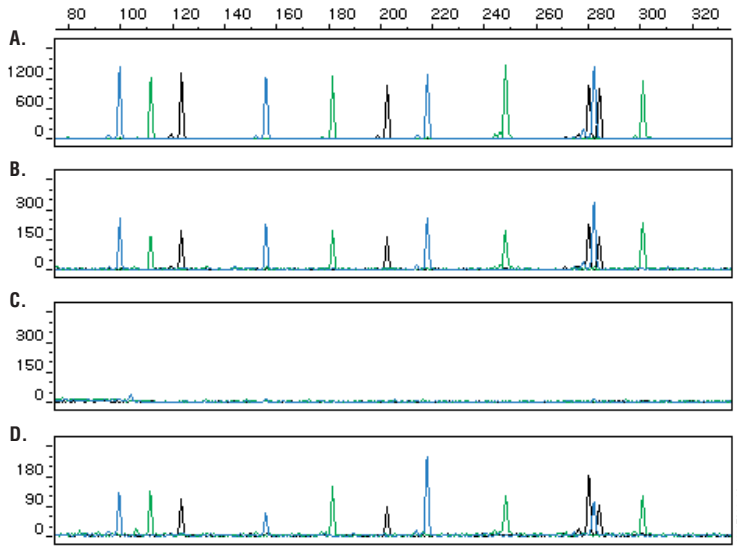

Figure 2. PowerPlex® Y System amplification. Amplifications were performed with a variety of templates and run on the ABI PRISM® 310 Genetic Analyzer using a 2-second injection time. GeneScan® software was used to view and analyze samples. **Panel A.** Amplification results with 1ng of male template. **Panel B.** Amplification results with 0.125ng of male template. **Panel C.** Amplification results with 100ng of female DNA. **Panel D.** Amplification results with a 1,000-fold excess of female DNA (100ng) over male DNA (0.1ng).

The PowerPlex® Y System is available in two sizes: 50 and 200 reactions of 25µl and includes the Gold ST★R 10X Buffer and two control DNAs: 9948 Male DNA and 9947A DNA (female). Also included is the Blue Dextran Loading Solution for use on the ABI PRISM® 377 DNA Sequencer.

DEVELOPMENT OF THE POWERPLEX® Y SYSTEM

Development of the PowerPlex® Y System began with identification of the required loci. A survey was distributed to laboratories worldwide that had been working with Y-STRs. A single-reaction 13-locus 4-color product concept was proposed, and the 16 respondents clarified the desired features and characteristics. The initial concept included the Y-STR loci generally accepted for forensic use along with several other loci. These loci included the nine-locus "Y-STR European minimal haplotype" of DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393 plus DYS388, DYS437, DYS438 and DYS439 and a number of optional loci. Respondents agreed that the core set, minus the uninformative DYS388 locus, would be necessary for forensic casework. No consensus was reached for the inclusion of other loci. Product specifications including sensitivity with as little as 250pg of template, maximum amplicon size of 350bp and no reactivity with at least 100ng female DNA were established by the responses.

In December 2002/January 2003, a prototype was sent to eight alpha test sites. The sites included four European and four U.S. laboratories that were familiar with Y-STR analysis. Laboratories included ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 and 3100 Genetic Analyzer users. Alpha test sites gave high marks for system sensitivity, male specificity and allelic ladders. Changes made to the sytem were based on alpha-test feedback and included additional citations and information in the technical manual, correction of a macro error in the DYS19 ladder recognition, more alleles in the allelic ladder and a modification to the 3′-end of the unlabeled oligonucleotide in the DYS438 primer pair. The removal of one base from the DYS438 primer avoids a single-base deletion described by Gusmão as producing a rare null allele (13). Revised materials were reviewed by some of these laboratories. Seventeen additional laboratories participated in further testing.

The marker Amelogenin was intentionally omitted to ensure maximum yield from the male-specific Y-STRs with “heavy” male/female mixtures (those where the amount of the female component is many times greater than that of the male). A major concern was that, in the heaviest mixtures (100- to 1000-fold more female than male DNA), amplification of Amelogenin would outcompete and reduce the signal of Y-STRs in the PCR. Additionally, in reactions with high levels of female DNA, the X-specific Amelogenin signal may be strong enough that bleedthrough renders that region of the assay range unusable in other channels. Finally, because Amelogenin is more robust than some STR loci, it is a poor control for amplification failure versus no male DNA. However, Amelogenin is available as a monoplex for analysis if desired.

In January 2003, the Scientific Working Group on DNA Analysis Methods (SWGDAM) announced a recommended panel of 11 Y-STRs, consisting of the European minimal haplotype plus DYS438 and DYS439. The PowerPlex®

POWERPLEX® Y

Y System is compatible with these SWGDAM recommendations and with the European minimal haplotype.

POWERPLEX® Y SYSTEM PERFORMANCE

The PowerPlex® Y System is sensitive, specific and appropriate for use in forensic casework. The allelic ladder contains 102 alleles with an average of 35bp separating each locus from the next to reduce the potential for locus overlap (Figure 1). Primers yield amplification products that are less than 335bp in length. In general, shorter amplicons are preferred for analysis of degraded DNA or samples containing PCR inhibitors.

The PowerPlex® Y System is optimized for amplification of 0.5–1.0ng of male DNA (Figure 2, Panel A). However, product specifications ensure that it will amplify as little as 250pg. Internal results show that full profiles can be observed with <250pg (Figure 2, Panel B). The PowerPlex® Y System minimizes the impact of female DNA in the reaction. Product specifications ensure no amplification products are observed with up to 100ng female DNA (Figure 2, Panel C). We have observed no reactivity with >100ng of female DNA. This high level of specificity allows easy amplification and interpretation of male DNA in the presence of >100-fold excess of female DNA (Figure 2, Panel D).

CURRENT STUDIES FOR THE POWERPLEX® Y SYSTEM

Seven collaborating laboratories are performing an initial validation study to determine 1) North American haplotype frequencies of the 12 PowerPlex® Y loci, 2) concordance with other Y-STR products, 3) mutation rates and 4) linkage between different Y-STRs and nonlinkage between Y-STRs and autosomal STRs. The population study involves several thousand samples from different populations, and in the future, a new haplotype reference database with the haplotype frequencies will be available at: **www.promega.com/geneticidentity/**

A second validation study is underway at collaborating laboratories to focus on sensitivity, female reactivity, mixtures (male/female, male/male, etc.), variation in reaction and cycling conditions and other commonly performed "developmental validation" experiments. Results of both validation studies will be published in peer-reviewed journals.

CONCLUSION

The PowerPlex® Y System is available for the analysis of Y-STRs in forensic, paternity and anthropology studies. The 12 loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439) include both the European minimal haplotype and the Scientific Working Group on DNA Analysis Methods recommended panel of Y-STR loci. This system has a high level of male specificity and sensitivity appropriate for use with forensic casework. Allelic ladders and size standard are included, and a system-specific PowerTyper™ Macro is available. Current validation studies will help support use of PowerPlex® Y use worldwide. With a single-tube amplification, the PowerPlex® Y System represents a new and useful tool for human identification laboratories.

REFERENCES

1. Kayser, M. *et al.* (1997) Evaluation of Y-chromosomal STRs: A multicenter study. *Int. J. Legal Med.* **110**, 125–33.
2. Jobling, M.A., Pandya, A. and Tyler-Smith, C. (1997) The Y chromosome in forensic analysis and paternity testing. *Int. J. Legal Med.* **110**, 118–24.
3. Gill, P. *et al.* (2001) DNA commission of the International Society of Forensic Genetics: Recommendations on forensic analysis using Y-chromosome STRs. *Int. J. Legal Med.* **114**, 305–9.
4. Roewer, L. *et al.* (2001) Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes. *Forensic Sci. Int.* **118**, 106–13.
5. Ruitberg, C.M. *et al.* (2001) STRBase: a short tandem repeat DNA database for the human identity testing community. *Nucl. Acids Res.* **29**, 320–2.
6. Butler, J.M. *et al.* (2002) A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. *Forensic Sci. Int.* **129**, 10–24.
7. Gusmão, L. and Carracedo, A. (2003) Y chromosome-specific STRs. *Profiles in DNA* **6**(1), 3–6.
8. Prinz, M., Boll, K., Baum, H. and Shaler, B. (1997) Multiplexing of Y chromosome specific STRs and performance for mixed samples. *Forensic Sci. Int.* **85**, 209–18.
9. Prinz, M. *et al.* (2001) Validation and casework application of a Y chromosome specific STR multiplex. *Forensic Sci. Int.* **120**, 177–88.
10. Ayub, Q. *et al.* (2000) Identification and characterization of novel human Y-chromosomal microsatellites from sequence database information. *Nucl. Acids Res.* **28**, e8.
11. *PowerPlex® ES System Technical Manual* #TMD017.
12. *PowerPlex® 16 System Technical Manual* #TMD012.
13. Gusmão, L. *et al.* (2002) Point mutations in the flanking regions of the Y-chromosome specific STRs DYS391, DYS437 and DYS438. *Int. J. Legal Med.* **116**, 322–6.