

## A HIGHER SENSITIVITY STR TYPING MULTIPLEX USING FLUORESCENCE ENERGY TRANSFER DYE LABELS

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Current commercial STR multiplex systems employ single fluorescent dye labeled primers for multi-color detection, resulting in sub-optimal sensitivity due to the disparity in excitation efficiencies among fluorescent dyes. This same problem was faced 15 years ago in the Human Genome Project and solved by the introduction of energy-transfer (ET) labels which reduced color cross talk and boosted signal strength up to 14 fold relative to single-dye labels<sup>1,2</sup>. ET labeling uses a pair of dye molecules, one of which absorbs light and then donates the absorbed energy to an acceptor that releases it as fluorescence. By pairing one donor dye with 4 or 5 different acceptor dyes, a single laser can be used to easily get high fluorescence intensities at four or more distinctive wavelengths<sup>3</sup>. These superior spectral properties are even more valuable for forensic STR typing where multi-color detection of minute amounts of DNA is routinely performed.

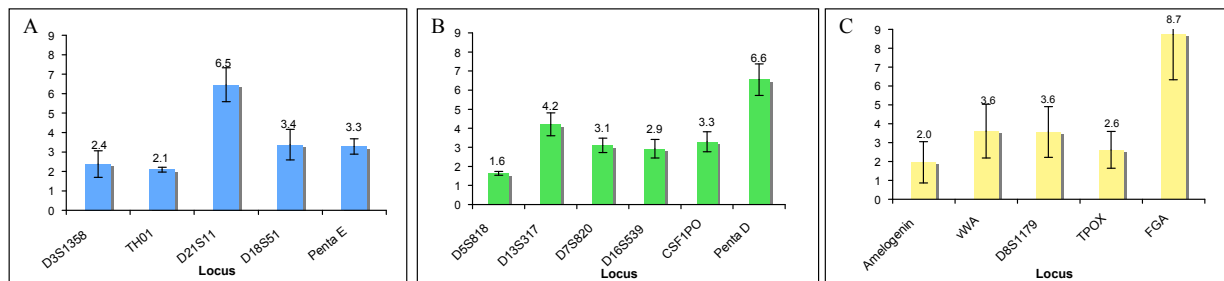
To evaluate ET dye labels for forensic applications, we have constructed and balanced a 16-plex STR typing system using a universal ET cassette labeling method<sup>4</sup>. To maintain the standard fluorescence color scheme, FAM labeled primers in the PowerPlex 16 kit were labeled with a FAM-FAM cassette, JOE with FAM-R6G and TMR with FAM-TMR. The final primer concentration for each locus was the same or lower than PowerPlex 16 primer concentrations. Using a 96-lane microfabricated capillary array electrophoresis (mCAE) system previously evaluated for forensic STR typing<sup>5</sup>, STR profiles were obtained from 0.25–0.5 ng of human DNA amplified with the ET 16-plex and PowerPlex 16 in a parallel fashion. The ET 16-plex PCR products display 2–9 fold increase in fluorescence intensity over PowerPlex 16 samples (Figure 1).

A sensitivity study was performed by typing ET 16-plex and PowerPlex<sup>®</sup> 16 samples amplified using serially diluted DNA templates (0.25, 0.125, 0.0625, 0.0313, 0.0156, 0.0078 and 0.0039 ng). All ET labeled STR alleles were successfully typed down to 0.0625 ng of input DNA while only 91% of the same profile was detected using PowerPlex 16. We were even able to obtain 97% of the ET labeled profile from only 0.0313 ng of DNA. This increase in sensitivity over the single fluorescent dye labeled multiplex system clearly demonstrates the capability of ET primers to achieve higher-sensitivity STR analyses.

With this increased sensitivity it should be possible to reduce the number of PCR cycles to speed analyses to reduce false amplification products and background. We therefore performed experiments to type 0.5 ng of DNA templates at 30, 29, 28 and 27 cycles in 12.5-mL PCR reactions. All STR alleles were successfully detected and called for samples amplified at 28 cycles. The use of fewer PCR cycles is particularly beneficial for low copy number (LCN) DNA typing in which allele drop in is often observed when increased PCR cycles are used<sup>5,6</sup>.

To demonstrate the capabilities of the ET 16-plex system to amplify real-world forensic DNA samples, we have typed 6 samples from sexual assault case evidence that had previously proved difficult to analyze at the Palm Beach County Sheriff's Office. The DNA samples for two cases were extracted from a variety of commonly encountered sources, including swabs from a towel, underwear, and both the victims and suspects. DNA (0.5 ng) was amplified using the ET 16-plex and PowerPlex 16 systems. Successfully typed STR alleles using the ET 16-plex system outnumbered those obtained using PowerPlex 16 for all samples. Although samples from one casework yielded comparable allele calls between the two typing systems, partial profiles (4–14 alleles) were obtained for samples from the second casework using the ET 16-plex while only 1-2 alleles were typed using PowerPlex 16. The results of this study illustrate the potential of using ET primers to enhance successful calls for challenging samples.

Our demonstration of STR analyses using a ET 16-plex typing system establishes the feasibility of using ET labels for higher-sensitivity forensic STR typing from limited amount of DNA. This accomplishment provides a convenient approach for enhancing LCN typing methods without developing new typing systems or increasing PCR cycles.



**Figure 1.** Fluorescence intensity ratios of ET labels versus single-dye labels at each STR locus. Specifically, (A) 2–6.5X for FAM-FAM labeled loci, (B) 1.6–6.6X for FAM-R6G loci and (C) 2–8.7X for FAM-TMR loci.

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## References

- Ju, J. Y., Ruan, C. C., Fuller, C. W., Glazer, A. N. & Mathies, R. A. Fluorescence Energy-Transfer Dye-Labeled Primers For DNA-Sequencing and Analysis. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4347-4351 (1995).
- Ju, J. Y., Glazer, A. N. & Mathies, R. A. Energy transfer primers: A new fluorescence labeling paradigm for DNA sequencing and analysis. *Nature Medicine* **2**, 246-249 (1996).
- Hung, S. C., Mathies, R. A. & Glazer, A. N. Optimization of spectroscopic and electrophoretic properties of energy transfer primers. *Analytical Biochemistry* **252**, 78-88 (1997).
- Yeung, S. H. I., Greenspoon, S.A., McGuckian, A.B. Crouse, C.A., Emrich, C.A., Ban, J. and Mathies, R.A. Rapid and high-throughput forensic short tandem repeat typing using a 96-lane microfabricated capillary array electrophoresis microdevice. *Journal of Forensic Sciences* **51**, 740-747 (2006).
- Gill, P., et al. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Science Int* **112**, 17-40 (2000).

6. Whitaker, J. P., E.A. Cotton, and P. Gill. A comparison of the characteristics of profiles produced with the AMPF\_STR® SGM™ Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. *Forensic Science Int* **123**, 215-223 (2001).