

Usage of DNA Fingerprinting Technology for Quality Control in Molecular Lab Bench Work

Linda Y. McIntosh, MS, MLS (ASCP), Janella E. Lal, BS, MLS (ASCP),
and Dahui Qin, MD, PhD

Abstract: One of the major quality assurance (QA) goals in many molecular laboratories is to avoid sample pipetting errors on the lab bench; especially when pipetting into multiwell plates. A pipetting error can cause a switch in patient samples, which can lead to recording the wrong results for the patient samples involved. Such pipetting errors are difficult to identify when it happens in lab bench work. DNA fingerprinting is a powerful tool in determining sample identities. Our laboratory has explored the usage of this technology in our QA process and successfully established that DNA fingerprinting can be used to monitor possible sample switch in gene rearrangement lab bench work. We use florescent light to quench the florescence in the gene rearrangement polymerase chain reaction products. After that, DNA fingerprinting technology is used to identify the sample DNA in the gene rearrangement polymerase chain reaction plate. The result is compared with the corresponding patient's blood sample DNA to determine whether there is a sample switch during the lab bench work.

Key Words: DNA fingerprint, laboratory QC, quality control, florescence quench, gene rearrangement

(*Appl Immunohistochem Mol Morphol* 2018;26:79–81)

One of the major quality assurance (QA) goals in many molecular laboratories is to avoid sample pipetting errors on the lab bench; especially when pipetting into multiwell plates. A pipetting error can cause a switch in patient samples, which can lead to recording the wrong results for the patient samples involved. Such pipetting errors are difficult to identify when it happens in lab bench work. An effective QA procedure to monitor such pipetting errors can help improve laboratory quality control. DNA fingerprinting is a powerful tool in

determining sample identification and it has been used in many labs for post-transplant chimerism test.^{1,2} It can be potentially used in monitoring lab bench pipetting errors and accidental sample switches. Our laboratory has explored the use of this technology in our QA process and has successfully established that DNA fingerprinting can be a fast and reliable method in determining possible pipetting errors in our gene rearrangement bench work.

MATERIALS AND METHODS

Quench of the Florescence in Gene Rearrangement Polymerase Chain Reaction (PCR) Products

Previously amplified gene rearrangement PCR products were deidentified and randomly selected for this study. The PCR products of the gene rearrangement assay were exposed to 25 W fluorescent lights for approximately 20 hours to quench the 6-FAM fluorescence in the reaction (InVivoScribe, San Diego, CA) before using DNA fingerprinting to verify sample identification. The distance between the fluorescent light tube and the PCR product plate is 8.26 cm.

DNA Fingerprinting Test on the Sample in Gene Rearrangement PCR Products

DNA fingerprinting was accomplished by addition of 1 μ L of the quenched sample from gene rearrangement PCR plate directly into 11.5 μ L of the Promega PowerPlex 16 HS Amplification PCR admixture. These samples then went through a second round of PCR amplification (Promega, Madison, WI, per the manufacturer's protocol) on the C1000 Touch thermal cycler (Bio-rad, Hercules, CA). After the amplification, 1 μ L of the product was used for fragment analysis. The fragment analysis was performed on AB 3130 \times L. Data are analyzed using GeneMapper version 4.1 software (Life Technologies, Grand Island, NY). The DNA fingerprint was then compared with the original DNA fingerprint to check for possible sample mix-up during the workbench process.

RESULTS

Quench of the Florescence in Gene Rearrangement PCR Products

Analysis of the electropherograms showed that the florescence from the gene rearrangement assay was

Received for publication February 25, 2016; accepted April 5, 2016.
From the Molecular Diagnostic Laboratory, H. Lee Moffitt Cancer Center, Tampa, FL.

The authors declare no conflict of interest.

Reprints: Dahui Qin, MD, PhD, M2Gen 312E, Molecular Diagnostic Laboratory, Department of Pathology, H. Lee Moffitt Cancer Center, 10902 North McKinley Drive, Tampa, FL 33612 (e-mail: dahui.qin@moffitt.org).

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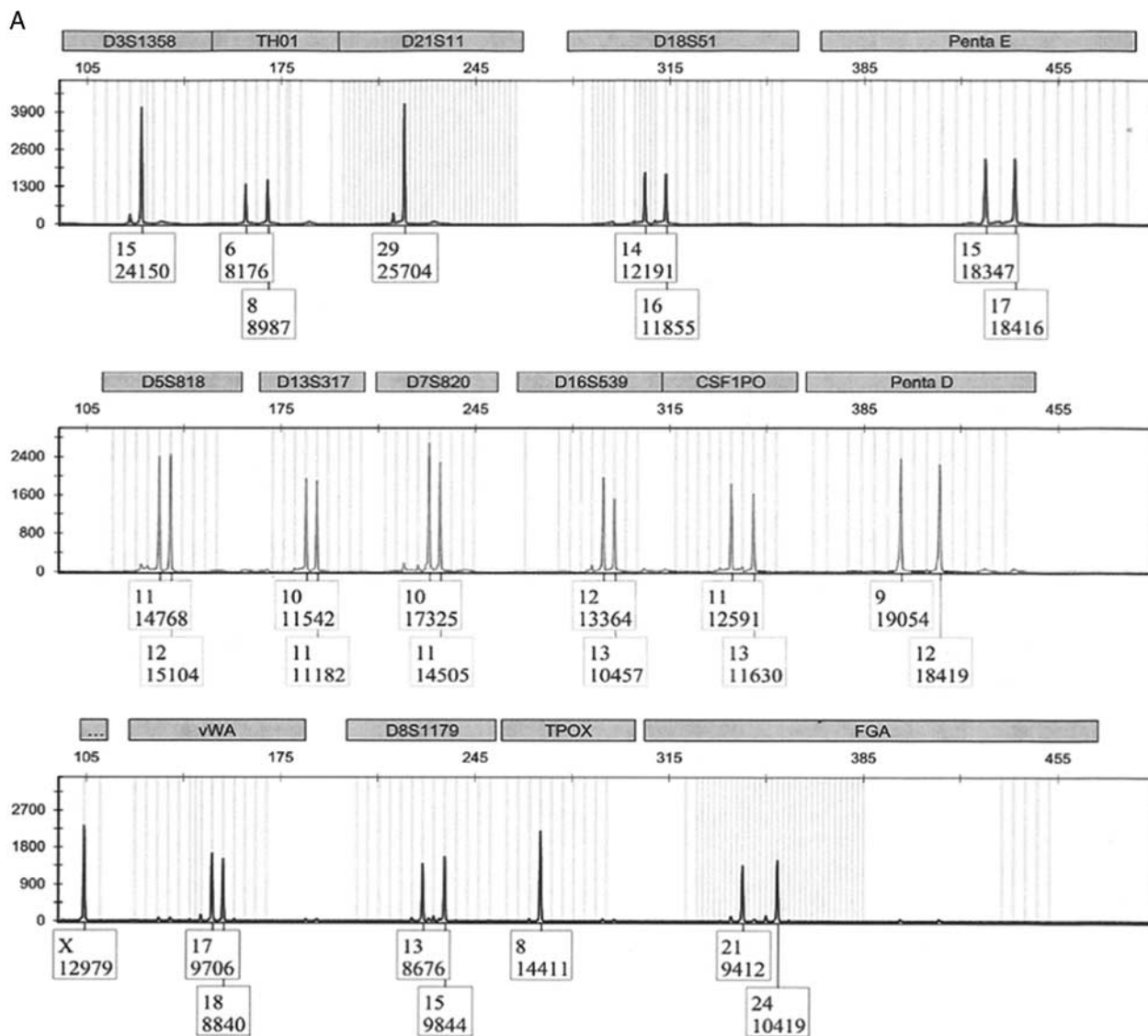


FIGURE 1. (Continued)

largely quenched with minimal residual fluorescence (Fig. 1B).

DNA Fingerprinting Test on the Sample in Gene Rearrangement PCR Products

The genomic DNA in the gene rearrangement (previously amplified) PCR products could be directly amplified a second time in a DNA fingerprinting assay and generate fingerprinting patterns for analysis (Fig. 1B).

Comparative analysis of the PCR product fingerprint to the original DNA fingerprint (Fig. 1A), which was ran in parallel, showed matching allelic markers between the 2 electropherograms—confirming proper sample identification. Background interference was minimal in the quenched PCR product graphs; however, some residual background peaks were displayed but did not

interfere with the overall sample identification. Five cases were tested using this method and the results are consistent.

DISCUSSION

In a clinical molecular laboratory, a pipetting error can cause a switch in patient samples, which can lead to recording the wrong results for the patient samples involved. Usually, such pipetting errors are difficult to identify when it happens. There is an urgent need to find a way to monitor the pipetting process on the lab bench. DNA fingerprinting is a powerful tool in determining sample identification^{1,2} and it can be potentially used in lab bench pipetting error and accidentally sample switch. The gene rearrangement assay is a large volume test in our laboratory. We set out to explore the use of this technology in our QA process.

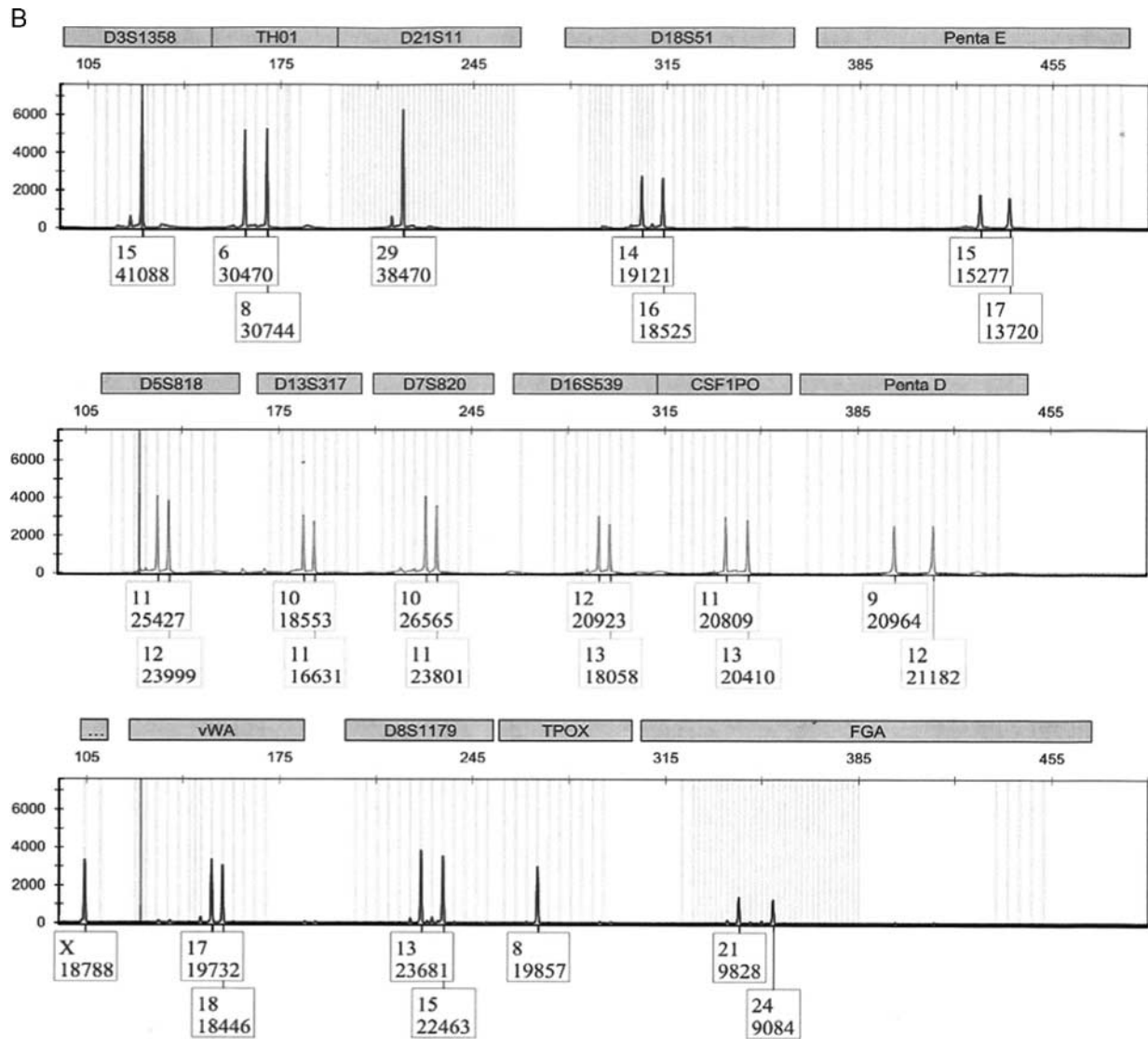


FIGURE 1. A, The DNA fingerprint from original DNA sample. B, The DNA fingerprint from the gene rearrangement polymerase chain reaction product of the sample. The florescence from the gene rearrangement assay was largely quenched.

In gene rearrangement assay, genomic DNA should exist in the PCR plate. The challenges are: (1) if we can amplify the genomic DNA from the PCR plate using DNA fingerprinting kit, regardless the possible interference from the prior PCR process and the PCR products: (2) how can we minimize the florescence interference from prior PCR. To minimize the florescence interference from prior PCR, we expose the PCR plate to fluorescent light to quench the florescence from prior PCR. The quench effect is proportional to the distance between the fluorescent light and the PCR plate and the time of exposure. When the PCR plate was placed 8.26 cm from the fluorescent light tube and exposed for 20 hours, the florescence from the prior PCR was largely quenched (Fig. 1B). The residual florescence is minimal and will not affect the DNA fingerprinting data analysis. Our results show that the genomic DNA from the gene rearrangement PCR plate can be amplified using

DNA fingerprinting kit (Fig. 1B), regardless the possible interference from the prior PCR process and the PCR products. The DNA fingerprint can be reliably analyzed.

The results showed that DNA fingerprinting from previously amplified gene rearrangement products can be utilized as a fast and reliable technique for monitoring lab bench pipetting error and sample switch in gene rearrangement assay. This method may potentially be used to monitor pipetting error-associated sample switch in other molecular assays.

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