TECHNICAL NOTE

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AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® COfiler™ Analysis of Tissues Stored in GenoFix™, a New Tissue Preservation Solution for Mass Disaster DNA Identification

REFERENCE: Frégeau CJ, Vanstone H, Borys S, McLean D, Maroun JA, Birnboim HC, Fourney RM. AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® COfiler™ analysis of tissues stored in GenoFix™, a new tissue preservation solution for mass disaster DNA identification. J Forensic Sci 2001;46(5):1180–1190.

ABSTRACT: A preliminary study was conducted to assess the capability of a new alcohol-based tissue fixative, GenoFix™, to preserve DNA from biopsy tissues stored at room temperature and/or −20°C in a freezer, for subsequent short tandem repeat (STR) DNA typing analysis. Fresh human smooth muscle samples were stored at room temperature in GenoFix™ for one month and up to one year and seven months before being processed using the megaplex STR systems, AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® COfiler™. Alternatively, muscle tissues in GenoFix™ were placed at −20°C in a freezer for up to 3½ years following two to three months in the fixative at room temperature. DNA analysis was also carried out on tissues stored in GenoFix™ for one month at room temperature and subsequently paraffin-embedded and stored at room temperature for four years. The AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® COfiler™ STR profiles produced, using DNA extracted from all fixed tissue samples, were of very good quality. The fluorescent signals were well balanced across the nine STR loci or six loci comprised in the megaplexes surveyed and profiles showed no differences with those observed for the control blood of the respective donor patients. Continuous exposure to GenoFix™ at room temperature (up to one year and seven months) did not compromise the STR typing analysis of the fixed tissues. No adverse effects were noted on the STR typeability of tissues fixed with GenoFix™ and stored at −20°C in a freezer for up to 3½ years. STR profiles generated from the paraffin-embedded tissues fixed in GenoFix™ were of excellent quality. This preliminary study suggests that GenoFix™ can be used to store tissue samples at room temperature for up to one year and seven months or at −20°C in a freezer for longer storage (up to 3½ years). This new and odorless tissue fixative promotes tissue and DNA preservation in a very effective manner and as such may prove useful in criminal investigations or mass disaster identifications carried out in remote locations and in which a small or large number of tissue samples are collected for further analyses.

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KEYWORDS: forensic science, short tandem repeat (STR), multiplex, fluorescence, polymerase chain reaction (PCR), sequencer, DNA analysis, AmpFℓSTR® Profiler Plus™, AmpFℓSTR® COfiler™, tissue preservation, GenoFix™, paraffin-embedded tissue, fixative, room temperature, disaster identification, major crime scene

Tissue preservation is very important in clinical settings for subsequent histological studies or genetic diagnoses (1–6). It is also critical in forensic investigations where human remains are collected for positive identification following plane crashes (7–11), terrorist bombings (12,13), homicides (14,15), and mass murders (16–19). The type of fatal injuries inflicted to or suffered by the victims as well as the environmental conditions in which the remains are kept until recovery present major challenges for subsequent forensic analysis. Indeed, human remains may have been exposed to very high temperatures and prolonged incineration, explosion, and chemical insult. Alternatively, human remains may have been exposed to cycles of warm and cold temperatures or have been visited by scavenging animals and insects and consequently may be found in an advanced state of putrefaction or totally skeletonized. These remains, even badly decomposed or putrid, may constitute a suitable source for DNA extraction and subsequent DNA typing analysis if properly collected and analyzed. Special care should be taken for an efficient conservation of the material. Low ambient temperatures and rapid recovery of human remains represent ideal conditions to promote tissue preservation and ensure successful subsequent DNA typing analysis. However, such conditions are rarely met in disaster areas and the reality of forensics. As part of a routine procedure to optimize the chance of successful analysis, human remains are normally recovered and kept at 4°C in a morgue. Alternatively, refrigerated trucks are used at the scene of a disaster when the number of victims is large and when the location of a forensic institute or hospital is remote. Various tissue samples are collected by the forensic pathologist and sent to a forensic laboratory for further toxicological and/or DNA analyses. Tissue samples are also collected for histological purposes and routinely placed in vessels containing liquid nitrogen (−150°C) or formalin. The remains are sometimes kept at −20°C

in freezers following examination until a positive identification has been issued.

Alternative approaches to preserving tissue structure and DNA at room temperature for clinical or forensic genetic analysis are not yet available. However, a new alcohol-based tissue fixative, GenoFix™, has recently been developed which allows storage of biopsy tissues at room temperature for subsequent histopathological or DNA diagnostic studies (20). The ease of use of this tissue fixative as well as the convenience afforded by working in an ambient temperature environment without the requirement of a refrigerator or a freezer, while maintaining tissue and DNA integrity, represent potential benefits for the forensic community. A preliminary study was therefore conducted to determine the effect of GenoFix™ on DNA analysis using short tandem repeat (STR) methodology following long term storage of tissues in the fixative. Smooth muscle samples were stored at room temperature in GenoFix™ for one month up to one year and seven months before being processed using the megaplex STR systems, AmpFℓSTR® Profiler Plus™ (7,21–24) and AmpFℓSTR® COfiler™ (25). Tissues were also placed at −20°C in a freezer for up to 3½ years following two to three months in the fixative at room temperature. This study was also extended to DNA recovered from paraffin-embedded tissues maintained at room temperature for four years after fixation in GenoFix™ at room temperature for one month.

Materials and Methods

Samples—Samples of fixed normal colon tissue were obtained from the Ottawa Colorectal Tumor Bank. Immediately following surgical resection of segments of colon, the tissue was placed into an appropriate vessel (i.e., a 5 L container) containing 3 L of GenoFix™ (DNA Genotek, Inc., 1350 Sherbrooke Street West, Suite 1020, Montréal, Québec, H3G 1J1; further details about the fixative may be obtained from the author, Dr. Birnboim). Once samples were taken for diagnostic purposes, the container was tightly sealed and the remainder of the tissue was stored for a period of two to three months at room temperature at which time samples of tumor-free mucosa and muscularis mucosa (smooth muscle) were taken and transferred into a 1 mL vial containing "spent" GenoFix™ (i.e., GenoFix™ from the original collection vessel) for prolonged storage at room temperature (up to one year and seven months) or at −20°C in a freezer (up to 3½ years).

To prepare paraffin-embedded tissue samples, portions of tumor-free mucosa and muscularis mucosa (smooth muscle, approximately 3 by 3 by 3 mm) were taken after one month of resection and embedded in paraffin using a Shandon Tissue Processor, omitting the formalin cycle. Thereafter, paraffin blocks were stored at room temperature for four years before DNA was extracted as described below. Examples of histological use of GenoFix™ are provided in Ref 26.

Blood samples used as controls were obtained from patients after surgery and were collected in 7 mL Vacutainers™ (containing the anticoagulant EDTA).

DNA Extraction—A small portion of each biopsy sample (approximately 2 by 4 by 5 mm) was subjected to a one-step DNA extraction protocol using organic solvents and subsequent concentration with Microcon-100 size-exclusion columns [Amicon, Inc., MI] (27). DNA was extracted from blood following standard procedures involving hypotonic lysis (28). DNA was recovered from paraffin-embedded tissue essentially as described previously (29). Briefly, ten 5 μm sections were extracted three to five times with 0.5 mL toluene, then twice with 1 mL 70% ethanol. Excess ethanol was removed under vacuum for 15 min, then 0.5 mL of DNA extraction solution (0.5 M LiCl, 1 *M* urea, 0.25% SDS, 1.5 mM $CaCl_2$, 50 mM BES [N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid] buffer, pH 6.8) was added along with 0.5 mg/mL of proteinase *K*. Samples were incubated at 55°C for 18 h. If necessary, additional proteinase *K* and further incubation was carried out. The digest was extracted with phenol/chloroform and DNA was precipitated with ethanol.

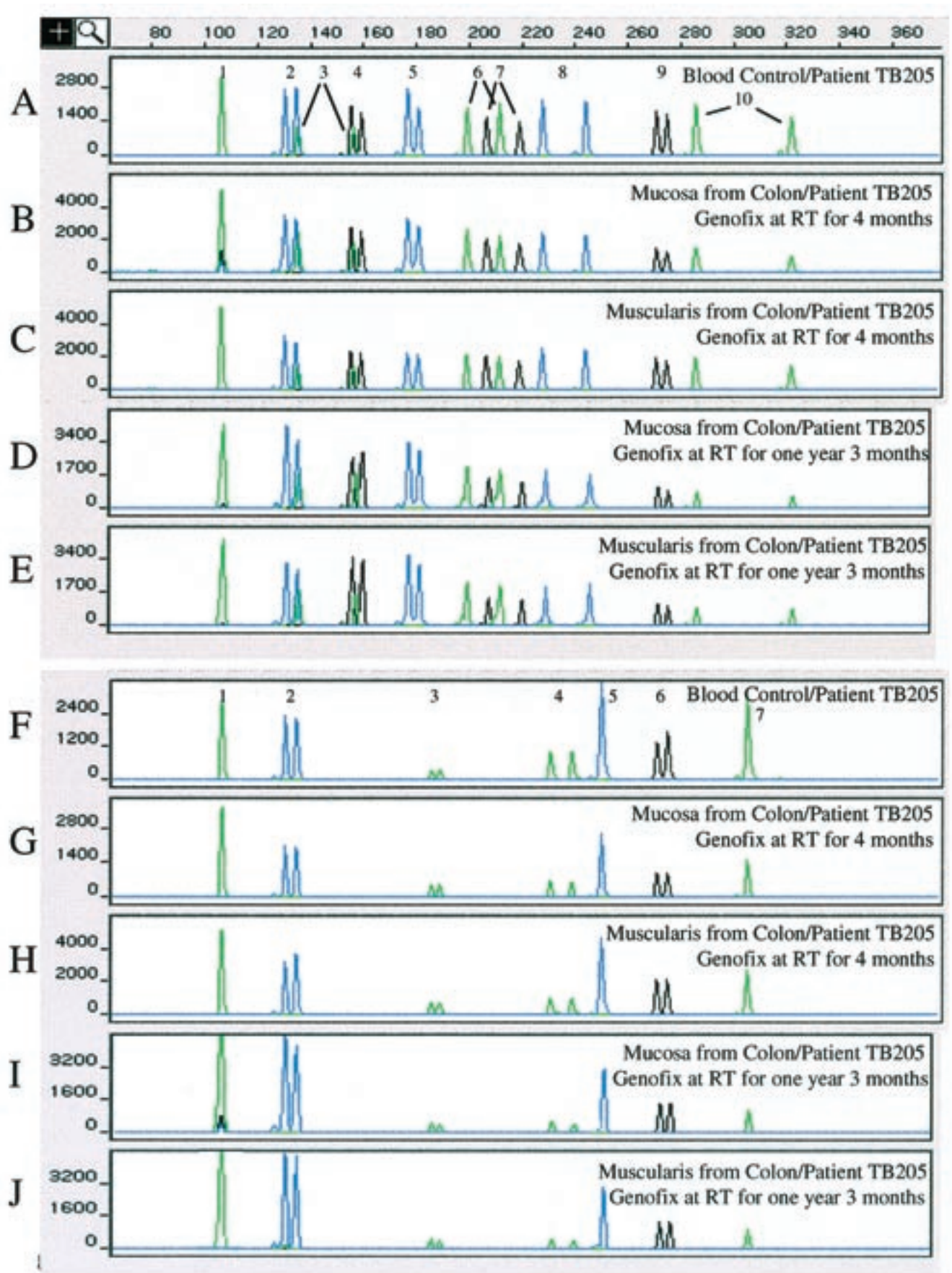
DNA Quantitation—Quantitation of human genomic DNA extracted from tissue, blood, and paraffin-embedded tissue blocks was determined using a slot blot hybridization procedure with chemiluminescence-based detection (30). A biotinylated human-specific D17Z1 α-satellite probe was used to hybridize to unknown and reference samples (i.e., two-fold serial dilutions of control cell line DNA) immobilized on a membrane.

Amplification Conditions—Simultaneous amplification of the nine STR systems comprised in the AmpFℓSTR Profiler Plus™ PCR amplification kit or the six STR systems included in the AmpFℓSTR COfiler™ PCR amplification kit was conducted in a 10 μL final reaction volume containing 1 ng of genomic DNA (in a total sample volume of 4 μL with FAD [filtered, autoclaved, deionized] water completing the volume), 3.8 μL of the AmpFℓSTR PCR Reaction Mix, 2 μL of the AmpFℓSTR Profiler Plus™ Primer Set Solution or AmpFℓSTR COfiler™ Primer Set Solution and 0.2 μL of AmpliTaq Gold™ DNA Polymerase (5 U/μL stock). The reaction mixtures were subjected to a hot start at 95°C for 11 min in order to activate the AmpliTaq Gold™ DNA Polymerase. Amplifications were carried out for 28 cycles using the following parameters: denaturation for 60 s at 94°C, annealing of primers for 90 s at 59°C and extension for 90 s at 72°C. A final extension at 60°C for 45 min, followed by an overnight incubation at room temperature, were also included as these conditions were required to promote the 3′ terminal transferase activity of the AmpliTaq Gold™ DNA Polymerase. All amplifications were conducted using a Perkin Elmer GeneAmp™ PCR System 9600 thermal cycler and thin-walled 0.2 mL MicroAmp™ Reaction Tubes.

The control cell line GM9947A (31; NIST Standard Reference Material #2391 PCR-based DNA Profiling Kit) served as the positive amplification control and FAD water as the negative amplification control.

The amplification conditions and the cycling parameters described herein differ from those recommended by the manufacturer of the AmpFℓSTR Profiler Plus™ and AmpFℓSTR COfiler™ Amplification kits. Following an extensive PCR volume reduction study, reliable STR typing results were obtained using a 10 μL amplification reaction volume instead of the recommended 50 μL (32). An increase in the annealing step and the extension step by 30 s (i.e., using 90 s instead of the recommended 60 s for each cycle) was found to enhance the yield of amplicons by a factor of 2 to 3 in such reduced amplification volumes. The final extension step at 60°C for 45 min followed by an overnight incubation at room temperature were found to be necessary to promote the complete addition of the nucleotide at the 3′ end of the blunt-ended amplicons. Shorter extension times did not support the complete conversion of the n products into $n+1$ products.

Analysis of Fluorescent Amplicons—An aliquot of 1 μL of each PCR reaction was mixed with 0.5 μL of ABI GeneScan-500 Internal Lane Size Standard (labeled with 6-carboxy-X-rhodamine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Genofix at RT for 4 months
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Muscularis from Colon/Patient TB205
Genofix at RT for 4 months
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Mucosa from Colon/Patient TB205
Genofix at RT for one year 3 months
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[ROX, a fluorescent dye from ABI]) and 4 μL of denaturing loading buffer (20 mg/mL blue dextran, 7.3 M urea, 2X TBE, 20 mM EDTA). Following denaturation at 95°C for 2 to 3 min, samples were snap-cooled in ice-cold water and 1.5 μL aliquots were loaded on a 4% (19:1) acrylamide:bisacrylamide gel containing 6 M urea (36 cm well-to-read glass plate format; prerun at constant voltage (1000 V) for 30 min and equilibrated to 51°C). Electrophoresis was conducted for 2 h at constant voltage (3000 V) in 1X TBE using an ABI PRISM® 377 DNA Sequencer with the laser power set at 40 mW. Allele sizes were determined using the GeneScan® Analysis v.2.1 software and the Local Southern size calling method. Automatic allele designation was achieved using the Genotyper® v.2.0 software (Applied Biosystems Division of Perkin Elmer).

Results

Long Term Storage in GenoFix™ at Room Temperature

Examples of AmpFℓSTR Profiler Plus™ and AmpFℓSTR COfiler™ profiles generated from DNA extracted from smooth muscle samples stored at room temperature in GenoFix™ for four months or one year and three months are shown in Fig. 1. Tissues processed after four months in GenoFix™ generated fluorescent signals that were very balanced across the nine STR loci included in the AmpFℓSTR Profiler Plus™ (see panels *B* and *C*) and relatively balanced across the six STR loci surveyed in AmpFℓSTR COfiler™ with HumTHO1 showing reduced amplification efficiency (see panels *G* and *H*). Similar observations were made with tissues examined after one year and three months in GenoFix™ at room temperature (see panels *D* and *E* for AmpFℓSTR Profiler Plus™ and panels *I* and *J* for AmpFℓSTR COfiler™). For all STR loci that presented a heterozygous profile, the fluorescence intensity of both alleles within each profile was very balanced (see panels *D, E, I,* and *J*). However, a small gradient in fluorescence intensity from amelogenin to D18S51 was noted for the AmpFℓSTR Profiler Plus™ megaplex. This gradient was not observed in profiles generated from tissues stored in GenoFix™ at room temperature for four months (compare panels *B* and *C* with panels *D* and *E*). A small reduction in fluorescence intensity was also apparent with the AmpFℓSTR COfiler™ megaplex (compare panels *G* and *H* with panels *I* and *J*). This signal reduction never compromised the STR analysis of the fixed tissues as full profiles were observed in all instances. No allele dropout or extraneous bands were detected in profiles generated from the DNA of the fixed tissues. No inhibitory effect on the PCR process or interference with the fluorescence-based detection procedure was noted. Profiles were essentially identical to those of the control blood DNA. An additional ten sets of smooth muscle tissues examined in this study showed the same trend (see Table 1 and data not shown).

Long Term Storage in GenoFix™ at −20°C

Ten sets of mucosa and muscularis samples in GenoFix™ were placed at −20°C in a freezer for up to 3½ years following two to three

TABLE 1—*Effects of long term storage in GenoFix™ at room temperature on STR DNA typing analysis.*

Sample #	Length of time in GenoFix™ at room temperature before processing	Full AmpFℓSTR Profiler Plus™ profiles	Full AmpFℓSTR COfiler™ profiles
1	5 months 8 months 1 year and 7 months	Yes for all time periods	Yes for all time periods
2	4 months 7½ months 1 year and 6½ months	Yes for all	Yes for all
3	4 months 7 months 1 year and 6 months	Yes for all	Yes for all
4	3½ months 7 months 1 year and 6 months	Yes for all	Yes for all
5	3 months 6 months 1 year and 5 months	Yes for all	Yes for all
6	2½ months 6 months 1 year and 5 months	Yes for all	Yes for all
7	2 months 6 months 1 year and 5 months	Yes for all	Yes for all
8	1½ month 5 months 1 year and 4 months	Yes for all	Yes for all
9	1 month 4 months 1 year and 3 months	Yes for all	Yes for all
10	1 month 4 months 1 year and 3 months	Yes for all	Yes for all
11	1 month 4 months 1 year and 3 months	Yes for all	Yes for all

FIG. 1—*STR profiles of smooth muscle specimens from one colon cancer patient which were stored at room temperature in GenoFix™ for four months (panels* B, C, G, *and* H*) or one year and three months (panels* D, E, I, *and* J*). PCR amplifications were performed using 1 ng of template DNA in a 10 μL PCR reaction volume as detailed in Materials and Methods. Panels* A *to* E: *AmpFℓSTR Profiler Plus™ profiles. Panels* F *to* J: *AmpFℓSTR COfiler™ profiles. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-500 [ROX] using the ABI GeneScan® Analysis version 2.1 software. For the AmpFℓSTR Profiler Plus™ profiles, the genetic markers observed from left to right, in order of size, are: Amelogenin (1), D3S1358 (2), D8S1179 (3), D5S818 (4), HumvWA (5), D21S11 (6), D13S317 (7), HumFGA (8), D7S820 (9), and D18S51 (10). For the AmpFℓSTR COfiler™ profiles, the genetic markers observed from left to right, in order of size, are: Amelogenin (1), D3S1358 (2), HumTHO1 (3), HumTPOX (4), D16S539 (5), D7S820 (6), and HumCSF1PO (7).*

months in the fixative at room temperature. Representative AmpFℓSTR Profiler Plus™ and AmpFℓSTR COfiler™ profiles obtained for this series of fixed samples are shown in Figs. 2 and 3. For both patient TB62 (Fig. 2) and patient TB67 (Fig. 3), the genetic profiles from normal mucosa and muscularis tissues were complete and showed well balanced peak heights across all nine STR loci included in AmpFℓSTR Profiler Plus™ (see Fig. 2, panels *A* to *C* and Fig. 3, panels *A* and *B*). The same was true for the AmpFℓSTR COfiler™ profiles generated using these samples (see Fig. 2, panels *A* to *C* and Fig. 3, panels *A* and *B*). The efficiency and specificity of the PCR amplification were essentially the same between the frozen fixed tissues and the control blood DNAs. Minor differences noted in the fluorescence intensity of some samples most likely originated from differences in pipetting during the quantitation step and/or preparation of the DNA samples for PCR amplification and/or preparation of the amplified DNA aliquots for gel electrophoresis and analysis.

Tumor material dissected from the resected colon of patient TB62 and patient TB67 showed significant imbalances in peak heights of heterozygous STR alleles when compared to their respective control DNA (see Fig. 2, compare panels *D* with *A* and panels *H* with *E* and Fig. 3, panels *C* with *A* and panels *F* with *D*). Many STR loci were affected as shown by the large number of asterisks identifying the most significant differentials in allele peak heights observed. For patient TB62, the STR loci D3S1358, HumvWA, D21S11, D13S317, and HumTHO1 showed unequal amplification of alleles whereas the STR loci D5S818, D7S820, D13S317, HumFGA, D18S51, HumTPOX, and HumCSF1PO appeared to be affected in patient TB67. Loss of heterozygosity was noted at HumTHO1, D5S818, D18S51, and HumCSF1PO. These characteristics, i.e., significant imbalances in peak heights of heterozygous STR alleles as well as loss of heterozygosity have been noted before for other types of cancers (33–36).

Paraffin-Embedded Tissue Blocks Fixed in GenoFix™

Nine paraffin-embedded tissue blocks, prepared from tissues (eight mucosa, one muscularis) stored in GenoFix™ for one month at room temperature, were processed for STR DNA typing analysis. High quality AmpFℓSTR Profiler Plus™ and AmpFℓSTR COfiler™ profiles were obtained from all tissue blocks examined. Representative samples are shown in Figs. 4 and 5. The genetic profiles of the fixed tissues and those of the respective control blood presented the same characteristics. The fluorescent signals were very well balanced across all STR loci and no extraneous bands were detected. The efficiency and specificity of the PCR amplification were essentially the same for both AmpFℓSTR Profiler Plus™ and AmpFℓSTR COfiler™.

Discussion

The preservation of valuable human remains in difficult forensic recovery situations such as disaster areas has always been a challenging task. In recent years, the additional need for definitive human identification using DNA analysis has added yet another qualifier to the list of characteristics of an ideal tissue preservative. Indeed, previous reports have indicated that a good tissue preservative may not be conducive to efficient and high quality DNA analysis (5,6). Likewise, a safe and inexpensive tissue storage solution derived for DNA analysis may not provide the necessary tissue morphology preservation characteristics required for histological analysis (37–40). These observations prompted the development of suitable methods and/or tissue preservatives to maintain both the DNA and tissue in good condition for histological and molecular genetic analyses.

In this preliminary report, a new alcohol-based tissue fixative capable of preserving tissue morphology was evaluated for its ability to preserve DNA for subsequent forensic STR analysis. Clearly, from the results presented in Fig. 1 and Table 1, a continuous exposure (up to one year and seven months) to GenoFix™ at room temperature did not compromise the STR typing analysis of the mucosa and muscularis tissue samples tested (eleven different sets). The fixation regimen did not result in alterations in the allele profiles of the individuals that donated tissues and blood for this study. Although a small reduction in fluorescence intensity was apparent for the larger STR amplicons in profiles generated from tissues stored in GenoFix™ at room temperature for over one year, full profiles were obtained in all instances. Results presented in Figs. 2 and 3 indicated that DNA, suitable for STR DNA typing analysis, could be recovered from tissues fixed and stored in GenoFix™ for at least 3½ years at −20°C in a freezer. GenoFix™ had no deleterious effect on the PCR amplification of the 13 STR systems included in both AmpFℓSTR Profiler Plus™ and AmpFℓSTR COfiler™. Accurate and reliable STR profiles were also obtained from nine different paraffin-embedded tissue blocks, fixed in GenoFix™.

Currently, the most commonly used method of preserving tissues for subsequent analyses remains the storage of pathological specimens in a liquid nitrogen vapor-cooled freezer at −150°C or electric freezer maintained at −80°C. Very few alternative approaches have been developed to preserve tissue structure and DNA at room temperature for clinical or forensic genetic analysis. Takahashi et al. (37) used freeze-drying methods to preserve rat liver specimens at room temperature for 24 weeks prior to DNA and protein analysis. Although more convenient and economical, their approach required a tight control of the moisture and temperature during long-term storage to optimize stability of the cellular molecules in tissues. Schultz et al. (39) used a simple, low-cost lysis storage and transportation (LST) buffer to maintain human clinical lymphoid tissue samples at room temperature for up to four weeks before DNA-based diagnostic testing. As no histological studies were attempted on the tonsils stored in LST buffer, the structural integrity of tissues may potentially be compromised using this lysis-based storage buffer. Structural integrity of tissues is optimal with formalin but the stability of DNA exposed to formalin is significantly influenced by pH, salt concentration, temperature, and storage time (5,6). Many published studies have indicated the difficulties of extracting DNA from materials in standard histological fixatives such as formalin (5,6,41–44). Birnboim and colleagues (20) developed GenoFix™ to optimize the results of both histological and molecular diagnostic tests performed on resected colon tissues from cancerous patients. These authors found that GenoFix™ could provide all the advantages of formalin without formalin-based health issues and complicated neutralization and disposal procedures. Indeed, GenoFix™ facilitated transport and storage of the resected tissues at room temperature and preserved tissue and DNA integrity allowing histological and molecular genetic analyses to be performed 13 months after fixation. In addition, the odor was significantly reduced compared with formalin. A histological study of colon tissues fixed in GenoFix™ is presented in Ref 26. Corach and colleagues (40) recently reported the use of inorganic salt mixtures to preserve putrid cadaver tissues for more than two months at room temperature. The authors mentioned that the reduction of offending odors was remarkable and that they successfully typed tissues using the classical RFLP markers as well as some STR systems.

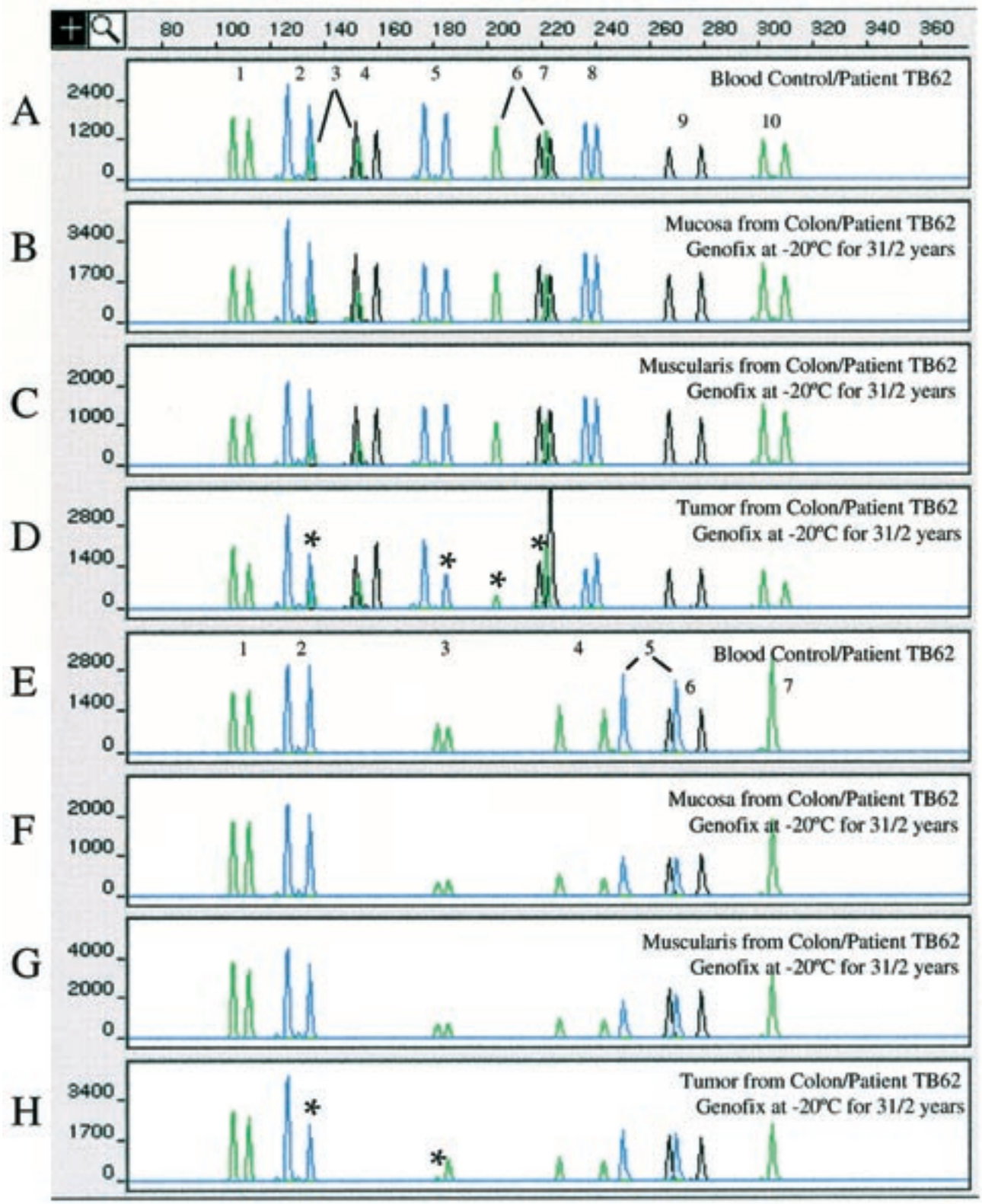

FIG. 2—*STR profiles of smooth muscle specimens from one colon cancer patient which were stored at −20 °C in GenoFix™ for 3½ years. PCR amplifications were performed using 1 ng of template DNA in a 10 μL PCR reaction volume as detailed in Materials and Methods. Panels* A *to* D: *AmpFℓSTR Profiler Plus™ profiles. Panels* E *to* H: *AmpFℓSTR COfiler™ profiles. In panels* D *and* H, *the alleles from heterozygous profiles that showed the most significant differentials in peak heights when compared to the control blood DNA profiles have been marked with an asterisk. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-500 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed are as indicated in the legend of Fig. 1.*

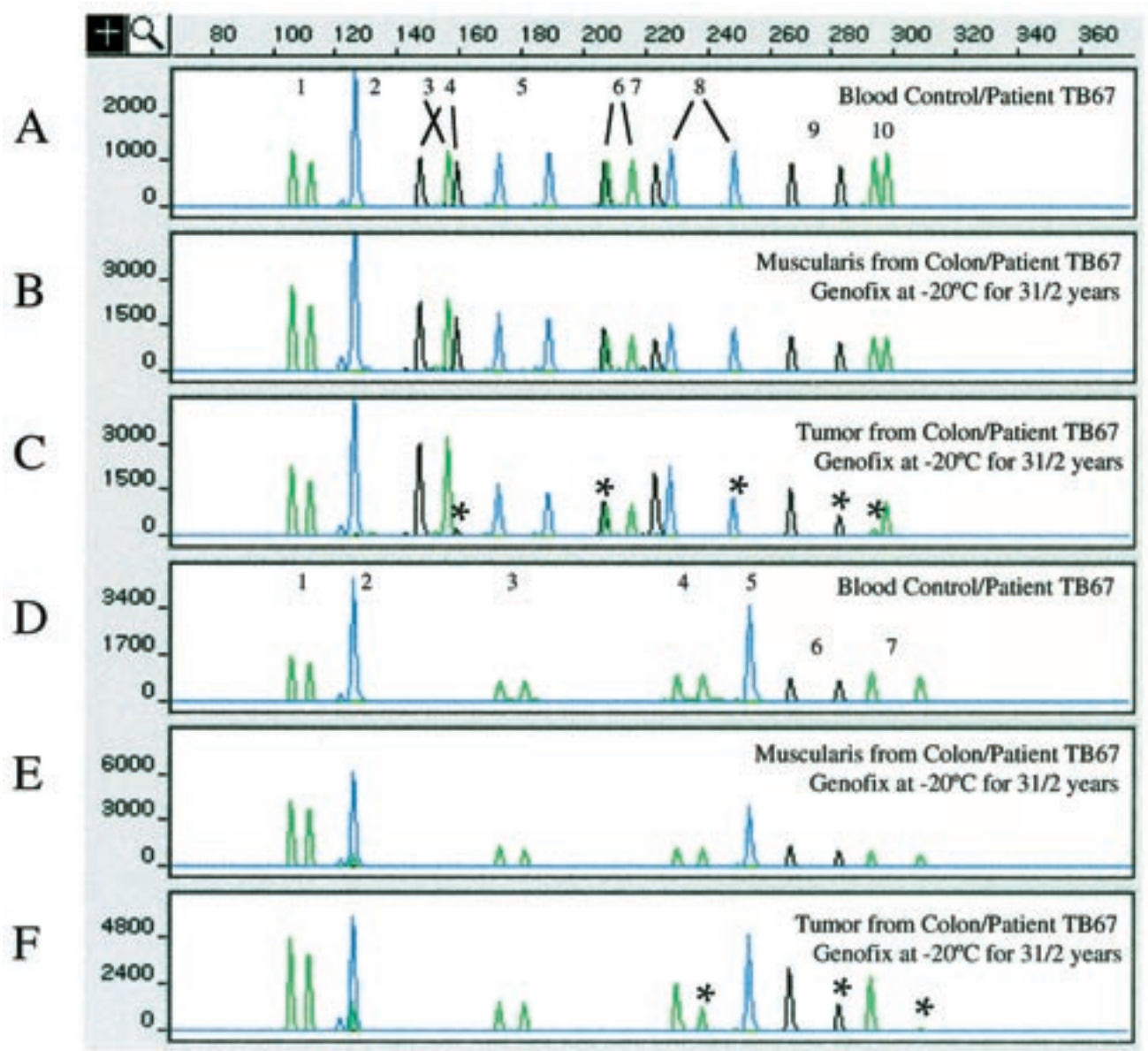

FIG. 3—*STR profiles of smooth muscle specimens from one colon cancer patient which were stored at −20 °C in GenoFix™ for 3½ years. PCR amplifications were performed using 1 ng of template DNA in a 10 μL PCR reaction volume as detailed in Materials and Methods. Panels* A *to* C: *AmpFℓSTR Profiler Plus™ profiles. Panels* D *to* F: *AmpFℓSTR COfiler™ profiles. In panels* C *and* F, *the alleles from heterozygous profiles that showed the most significant differentials in peak heights when compared to the control blood DNA profiles have been marked with an asterisk. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-500 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed are as indicated in the legend of Fig. 1.*

The results presented in this preliminary report complement those obtained by Genik et al. (20) and indicate that GenoFix™ can preserve tissues and DNA for at least one year and seven months at room temperature and up to 3½ years in a freezer and supports the production of STR profiles of very high quality. Our results also suggest that tissues fixed in GenoFix™, embedded in paraffin without exposure to formalin and stored at room temperature for up to four years could serve as archival material. Such blocks proved to be a reliable source of control DNA and could assist in criminal investigations for the identification of human remains or biological stains from a missing person. Previous reports indicated that archived formalin-fixed tissues have been used to prove paternity or identity in cases where they represented the only available source of control DNA (45,46).

The STR profiling results generated from tumor material fixed in GenoFix™ corroborated observations made by other investigators studying somatic instability of STRs in various types of cancers (33–36). Although limited in number, these results indicate that tumor specimens stored in GenoFix™ for 3½ years can easily be examined for allele gains and losses using the STR methodology described in this paper.

In light of the results shown in this report, GenoFix™ represents an effective alternative to snap-freezing human tissue samples and storing specimens in ultracold conditions before extraction of ge-

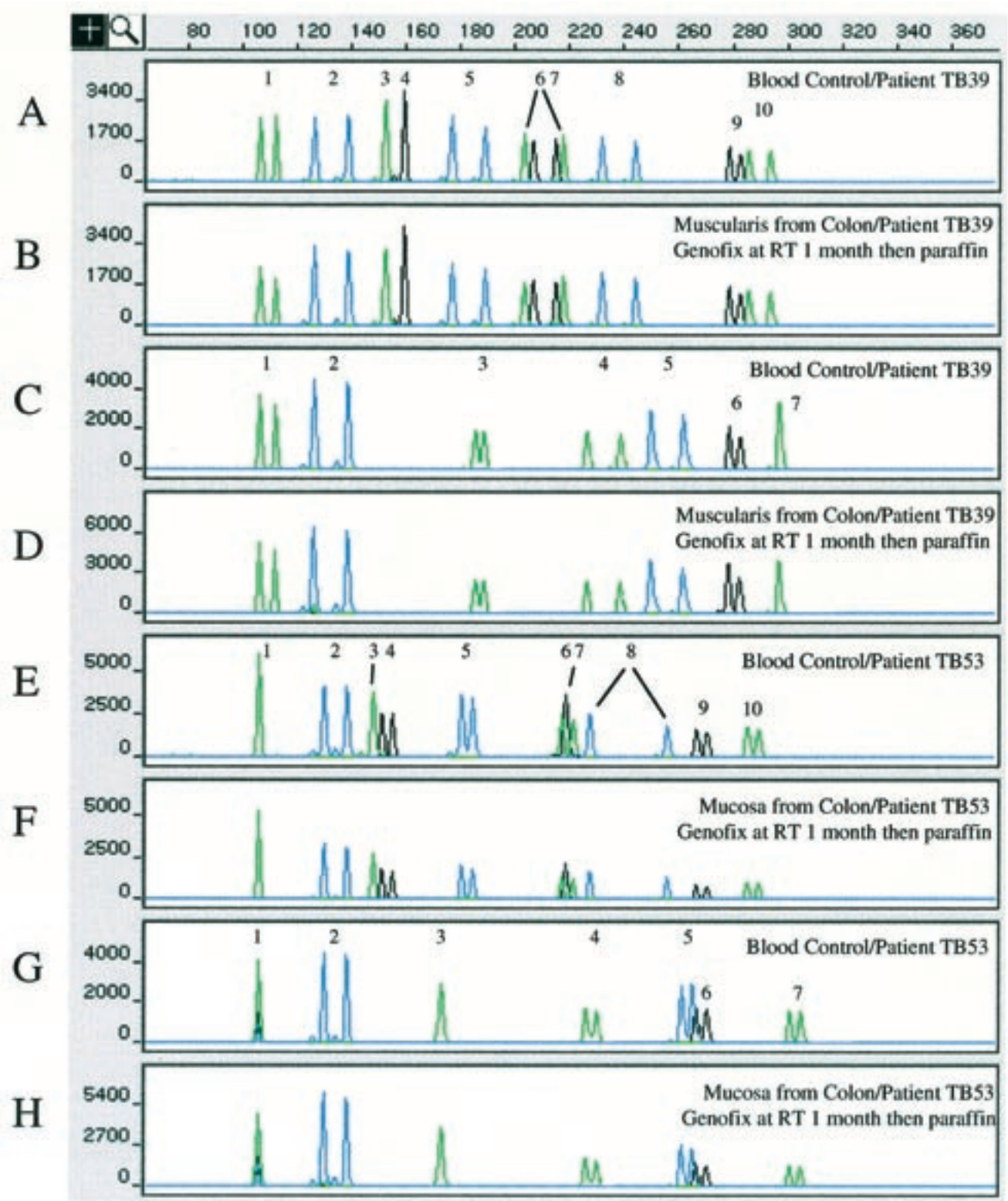

FIG. 4—*STR profiles of paraffin-embedded tissues from two colon cancer patients which were prepared from tissues stored in GenoFix™ for one month at room temperature and four years at room temperature in the paraffin blocks. PCR amplifications were performed using 1 ng of template DNA in a 10 μL PCR reaction volume as detailed in Materials and Methods. Panels* A, B *and* E, F: *AmpFℓSTR Profiler Plus™ profiles. Panels* C, D *and* G, H: *AmpFℓSTR COfiler™ profiles. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-500 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed are as indicated in the legend of Fig. 1.*

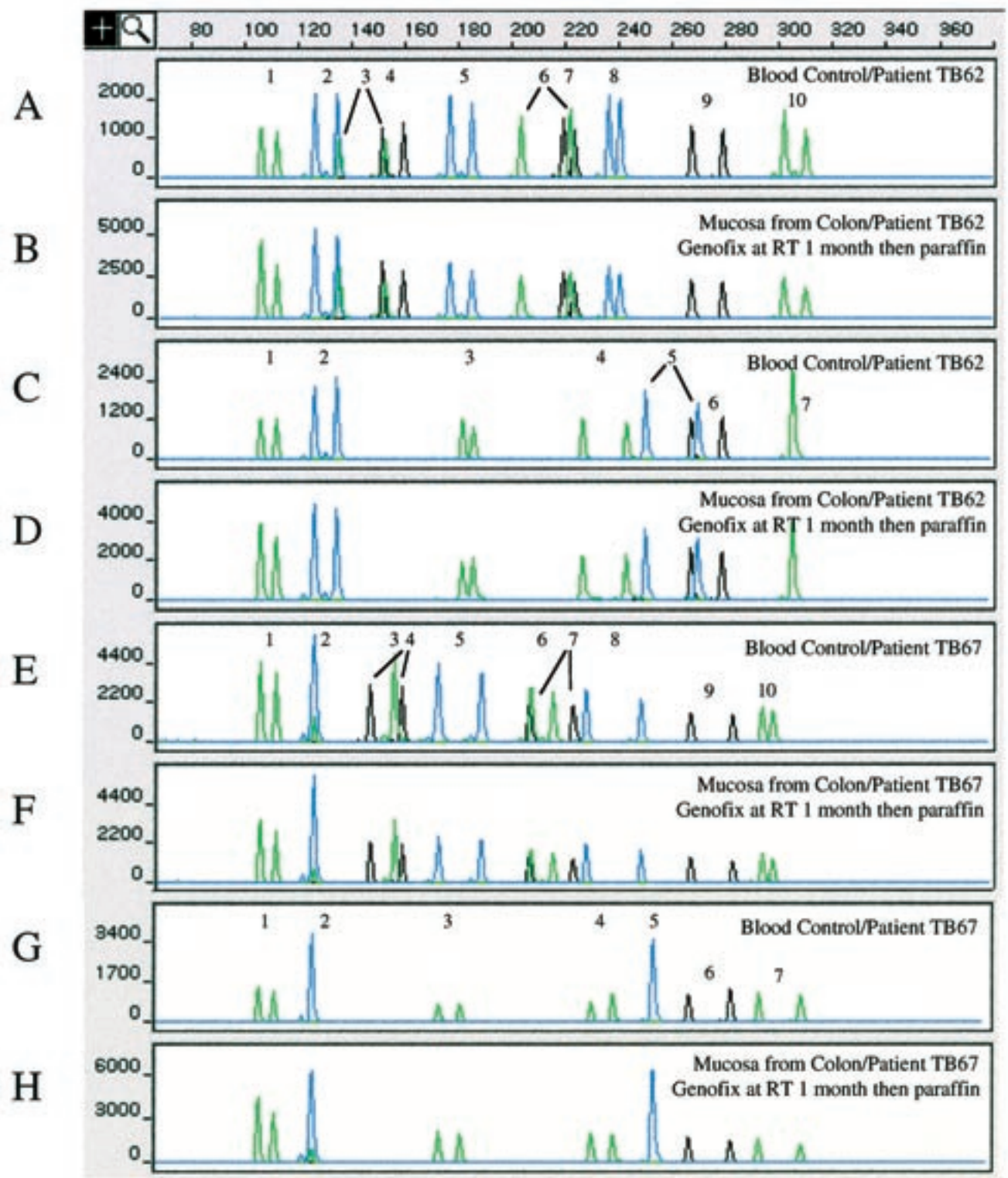

FIG. 5—*STR profiles of paraffin-embedded tissues from two colon cancer patients which were prepared from tissues stored in GenoFix™ for one month at room temperature and four years at room temperature in the paraffin blocks. PCR amplifications were performed using 1 ng of template DNA in a 10 μL PCR reaction volume as detailed in Materials and Methods. Panels* A, B *and* E, F: *AmpFℓSTR Profiler Plus™ profiles. Panels* C, D *and* G, H: *AmpFℓSTR COfiler™ profiles. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-500 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed are as indicated in the legend of Fig. 1.*

nomic DNA. Tissue samples can be collected in GenoFix™ using a simple screw-cap container at room temperature. Furthermore, the odorless and nontoxic properties of GenoFix™ makes it a very attractive agent for the preservation of biological materials for molecular analysis. Shelf-stable preservation and uncomplicated storage methodology are major advantages of immersion preservation of tissues using GenoFix™. They allow convenient and inexpensive shipment of samples at ambient temperature. In mass disaster situations, often encountered in geographically remote regions of the world, GenoFix™ could facilitate the transportation of the samples to a laboratory equipped to perform molecular studies without the need for refrigeration or specialized equipment at the time of collection. As such, GenoFix™ could help eliminate substantial barriers for tissue collection, storage and transportation that otherwise would preclude testing for some human remains recovered in very isolated locations. Experiments using GenoFix™ on real human remains or cadaveric tissues are being pursued to determine the benefits of such a fixative on stabilizing the cellular environment and preventing further tissue and DNA degradation.

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