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TECHNICAL NOTE

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The Application of Ultraviolet Irradiation to Exogenous Sources of DNA in Plasticware and Water for the Amplification of Low Copy Number DNA

ABSTRACT: Using high sensitivity forensic STR polymerase chain reaction (PCR) typing procedures, we have found low concentrations of DNA contamination in plasticware and water assumed to be sterile, which is not detected by standard DNA procedures. One technique commonly used to eliminate the presence of DNA is ultraviolet (UV) irradiation; we optimized such a protocol used in the treatment of water, tubes, plates, and tips for low copy number DNA (LCN) amplification. UV light from a Stratalinker® 2400 was administered to 0.2, 1.5 mL tubes, and PCR plates contaminated with up to 500 pg of DNA. They were subsequently quantified with an ALU-based real-time PCR method using the Rotorgene 3000. Overall, there was a decrease in concentration of DNA recovered as the duration of treatment increased. Nonetheless, following 45 min of irradiating a PCR plate with 500 pg of DNA, nearly 6 pg were still detected. However, when the plate was raised within an inch of the UV source, less than 0.2 pg of DNA was detected. Additionally, lining the area around the samples with aluminum foil further reduced the amount of time necessary for irradiation, as only 30 min eliminated the presence DNA in the raised PCR plate. Similar experiments were conducted using tubes filled with a solution of DNA and water in equivalent concentrations for 50, 15, and 1.5 mL tubes with comparative results. It is plausible that the aluminum foil increased the amount of reflection in the area thereby enhancing penetration of UV rays through the walls of the plasticware. This protocol was tested for the possibility of inhibitors produced from irradiation of plastic tubes. As our protocols require less irradiation time than previous studies, PCR sensitivity was not affected. Moreover, the lifespan of the UV lamps was extended. Our findings demonstrate that this method is useful as an additional precautionary measure to prevent amplification of extraneous DNA from plasticware and water without compromising the sensitivity of LCN DNA amplifications.

KEYWORDS: forensic science, DNA, contamination, ultraviolet irradiation, polymerase chain reaction, low copy number DNA

Polymerase chain reaction (PCR) is a widely used *in vitro* method for the replication of specific DNA segments. Methods for avoiding contamination are essential particularly where amounts less than 100 pg of DNA can be detected as in low copy number (LCN) DNA amplifications which use specialized methodologies or in mitochondrial DNA typing. Because of the increase in sensitivity of these types of amplification, we have found that contamination of presumably sterile tubes and water can be present in very low concentrations not previously detected by standard forensic PCR STR techniques.

One method commonly used as an additional precaution to sterilize pre-PCR laboratory equipment, reagents, and surfaces is ultraviolet (UV) irradiation (1–3). This type of practice can be observed in forensic mitochondrial DNA laboratories. UV rays degrade DNA by creating cyclobutane rings that form intrastrand pyrimidine dimers, which prevent polymerase mediated chain elongation (4). However, UV treatment of PCR tubes (1), pipette tips (5), and reagents (6) for extended periods of time, has been shown to negatively affect subsequent amplification of targeted DNA. For example, free radical formation has been demonstrated in UV-treated mineral oil, which reduced the sensitivity of amplification (3,7). This inhibition was reversed with the addition of antioxidant 8-hydroxyquinoline, a free radical scavenger (3). While this represents a cumbersome solution to targeted DNA inhibition, and considering that the extent of UV damage is dose dependent (1), further investigation into UV treatment as a method of decontamination is warranted.

The objective of this study was to develop a UV irradiation protocol for treatment of water, tubes, and PCR plates used in the amplification of LCN DNA. In theory, the detection of extraneous DNA is eliminated by the irradiation of tubes and water with UV light before PCR. This reduces the presence of spurious alleles in the amplification product. Although forensic mitochondrial DNA laboratories routinely implement UV irradiation methods for decontamination (8), the recommended irradiation times may be excessive. Therefore, an efficient means for irradiating before amplifications with an increased sensitivity should be defined. In order to construe an isolated contamination, small volumes of DNA were added to 0.2, 1.5 mL tubes, and PCR plates. To emulate the irradiation of contaminated water, parallel experiments were conducted with water containing comparable amounts of DNA in 50, 15, and 1.5 mL tubes. In addition, a variety of pipette tips were either washed with DNA to determine the amount of

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DNA retained in the tip or washed with water alone testing the cleanliness of the tips. Finally, the likelihood of inhibitors produced as a result of this type of irradiation was assessed by taking into account whether the sensitivity of LCN DNA amplification was compromised. All samples were treated for various times to establish the optimum conditions for irradiation.

Materials and Methods

Irradiation of Labware

The following amounts of DNA (Human Genomic DNA, G772A or G147A; Promega, Madison, WI, or AmpF*l*STR® Positive Control DNA 9947A; Applied Biosystems, Foster City, CA, and Ultra Pure™ DNAse/RNAse-Free Distilled Water (Gibco, New York, NY) were irradiated within the described labware: 500 pg of DNA (G772A) in 2.5 μL of water inside clear thin-walled 0.2 mL ABI MicroAmp® autoclaved reaction tubes (Applied Biosystems), 500 pg of DNA (G772A) in 2.5 μL of water or 50 ng of DNA (G772A) in 500 μL of water inside 1.5 mL homopolymer tubes (Axygen Scientific Inc., Union City, CA), 500 ng of DNA (G147A) in 5 mL of water inside Blue Max™ Jr. 15 mL polypropylene conical tubes (Becton Dickinson Labware, Franklin Lakes, NJ), 1500 ng of DNA (G147A) in 15 mL of water inside Blue Max™ 50 mL polypropylene conical tubes (Becton Dickinson Labware), and 500 pg of DNA (G772A) in 2.5 μL of water inside each well of a clear, blue, yellow, or red Thermo-Fast® 96 well semi-skirted PCR plate (ABgene, Epsom, Surrey, UK).

The samples containing only 2.5 μL were used to simulate a contamination of an empty tube. During UV exposure, 2.5 μL would almost entirely evaporate. By this means, the effectiveness of irradiating a dry tube was investigated. After irradiation, water was added to the tube to reconstitute the DNA for quantitation. All other samples contained a solution of 100 pg/μL DNA and were used to compare UV penetration through different volumes of water.

Samples were irradiated using the Stratalinker® UV Crosslinker 2400 (Stratagene, La Jolla, CA) delivering a total of 4000 μW/cm^2. Tubes were irradiated upright and open inside a rack or capped lying on their sides as indicated. Plates were irradiated on the floor of the Stratalinker® or raised within 1 in. of the UV bulbs. The floor and all sides of the Stratalinker® were lined with aluminum foil. Furthermore, in addition, foil was placed directly underneath some of the PCR plates tested.

DNA Detection

Following UV treatment, the remaining DNA was measured with real-time PCR on the Rotorgene-3000 (Corbett Research, Sydney, Australia) according to the method developed by Buel and Nicklas (9,10) with the exception of a 25 μL reaction volume and the use of 0.28 μL of 100 × SYBR® Green I. In order to maximize DNA detection, 5 μL of the sample were used for quantitation. For those samples in dry tubes that only contained 2.5 μL, the resulting volume after evaporation was reconstituted by adding water. Therefore, for the dry tubes containing 500 pg of DNA, the 5 μL volume generated a concentration of 100 pg/μL, which was equivalent to that of the tubes with water.

Testing of Concentration Devices and Pipette Tips

A concentration of 500, 25, or 6.25 pg DNA (9947A) was added to each Microcon® 100 (Millipore, Bedford, MA) membrane pretreated with 1 ng of PolyA RNA (Sigma, St. Louis, MO). Following centrifugation at relative centrifugal force (RCF) of 470 × g for 15 min, the DNA was eluted with 20 μL of water, and analyzed in triplicate. Before any treatment the Microcon® tubes were irradiated upright with aluminum foil lining for 15 and 30 min; a control group of Microcons was not irradiated.

A 5 μL volume of 100 ng of DNA (G772A) was pipetted up and down 10 times inside ART p10 tips (Molecular BioProducts, San Diego, CA). These tips were subsequently irradiated for 15, 30, 45, and 60 min. In the same way, but with less DNA as 100 ng of DNA proved to be excessive, a 5 μL volume of 50 ng of DNA (G772A) was pipetted in p1000 tips (Molecular BioProducts). The p1000 tips were irradiated for 10, 15, and 30 min. Further, 50 ng of DNA (G772A) in 5 μL were pipetted in 50 μL Tecan Compatible Conductive Filter tips (Corbett Research). The p50 tips were treated in the same fashion as the p10 tips. All tips were irradiated in their containers while turned upside down, and raised on racks close to the bulbs of the Stratalinker®, which was lined with aluminum foil. After irradiation, the tips were washed with 5 μL of water aspirated and dispensed 10 times to recover any remaining DNA. Controls consisted of tips washed with DNA and tips washed with 5 μL of water only, both of which were not irradiated. The latter revealed any DNA inherent to the tips themselves. All samples were analyzed in triplicates except the p10 tips, which consisted of four replicates.

Effect of UV Treatment of Labware or Water on Subsequent Amplification

The 0.2 mL tubes were left untreated or were exposed to UV light, while closed and lying on their side in an aluminum foil lined Stratalinker® for 10 min. A concentration of 25, 12.5, or 6.25 pg DNA (9947A) was added to each tube for amplification with AmpF*l*STR® Identifiler™ (Applied Biosystems) using LCN DNA analysis parameters in a 13 μL reaction volume as follows: 31 cycles of 94°C for 1 min, 59°C for 2 min, and 72°C for 1 min. Amplification was followed by incubation at 60°C for 60 min. Electrophoresis was conducted using 5 μL of the PCR products on an ABI Prism® 3100 Genetic Analyzer with an injection of 3 kV for 20 sec, and subsequently analyzed using GeneScan® and Genotyper® (Applied Biosystems) software. The minimum relative fluorescence unit (RFU) threshold was set to 75. Through the use of a 10% global filter, the highest peak of each locus was determined and peaks that were less than 10% of this height were not assigned allelic values.

Dilutions of 25, 12.5, or 6.25 pg DNA (9947A) made with either untreated or irradiated water (Gibco) were amplified, separated, and analyzed as described above with the exception of a 2 min extension time, and an injection of 4 μL of the PCR product. Data was evaluated based on the quality of the DNA profiles. In addition, the raw data from quantitative real-time PCR for negative controls comprised of either non-treated or irradiated water was assessed for evidence of inhibition as defined by the shape of their curves.

Results and Discussion

Exposure to sufficient UV light before amplification inhibits the replication of any DNA present. Presumably, the treatment of pre-PCR products such as tubes and water with UV irradiation inhibits exogenous DNA from consequent amplification thus reducing the presence of spurious alleles in our target DNA.

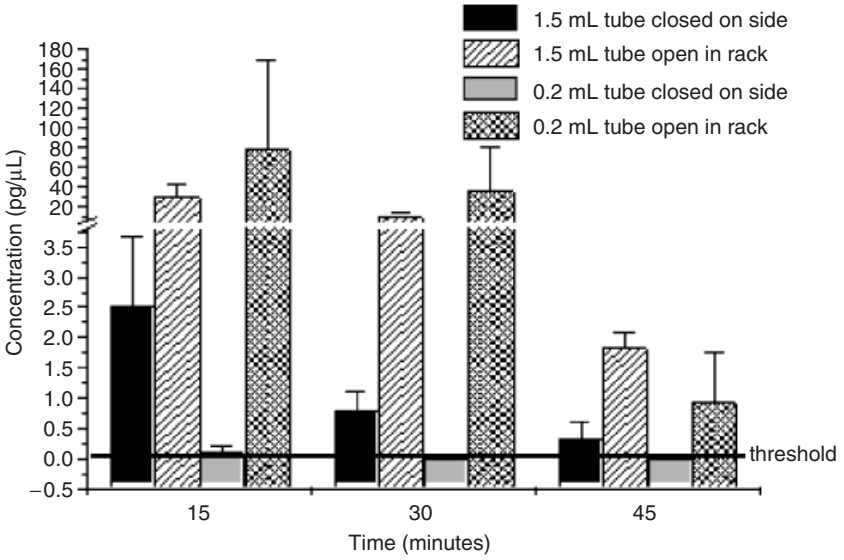

FIG. 1—*Effects of tube orientation on DNA degradation by ultraviolet irradiation; 0.2 mL and 1.5 mL dry tubes which contained 500 pg of DNA from an initial volume of 2.5 μL before evaporation, were irradiated for the indicated times. Tubes either were laid on the floor of the Stratalinker® on their sides with closed caps or upright inside a tube rack with their caps open. Data is expressed as the mean concentration of DNA recovered from each sample ± SD, where n = 3.*

Tube Orientation

Contamination was defined as recovery of DNA greater than 0.04 pg/μL of DNA recovered from the sample. The threshold of our amplification system, 0.24 pg or 5 μL of 0.048 pg/μL of DNA, represents the template amount where one allele can be detected in at least two of three replicate amplifications optimized for LCN DNA. In general, as the time of irradiation increased, the amount of DNA recovered decreased. The amount of DNA recovered from the dry 1.5 and 0.2 mL tubes capped, lying on their side in a Stratalinker® was significantly lower for both tube types at every time interval than those that were positioned upright and open inside the rack (Fig. 1). It is plausible that the position in the rack reduces the overall exposure to UV, as the rays have to penetrate through the walls of the rack to reach the sides of the sample tube. Regardless, only the 0.2 mL samples closed laying on their side at 30 and 45 min were below the threshold value (Fig. 1). Similarly, none of the other sample types fell below the desired concentration (Fig. 2). One approach to improve these results is to increase the time of treatment. Alternatively, the distance to the UV bulbs and the level of reflection within the Stratalinker® were explored.

Enhancement of Irradiation

The results for the clear PCR plates were especially of concern. After 15 min, more than 48 pg/μL of DNA was recovered, and samples irradiated up to 45 min were still above our threshold concentration. Presuming that the distance to the UV source is a limiting factor, the plate was raised towards the bulbs; the values for all time intervals were reduced (Fig. 3). To further enhance the effects of irradiation, the Stratalinker® was lined with aluminum foil with the premise that the foil would reflect the UV rays potentially increasing UV penetration of the sample. As illustrated in Fig. 3, the altered conditions within the Stratalinker® did improve the results. After 30 min, the recovered DNA concentration was well below the threshold number. Moreover, when a sheet of aluminum foil was placed directly under the raised PCR plate, no significant difference among the 30, 45 and 60 min time points resulted (Fig. 3).

Tests were conducted for other sample types employing the aluminum foil and, as expected, the amount of recovered DNA decreased for them as well (Figs. 4 and 5); the desired concentration was obtained for all samples. In addition, for those samples that were previously below threshold without the use of foil, the time needed to reach this point was reduced. For instance, the time required to eliminate the detection of DNA in the 0.2 mL tubes was reduced from 30 to 10 min. Although the desired effect was achieved for a number of our samples without altering the conditions inside the Stratalinker®, efficiency in time and bulb life must be taken into consideration. Lining the Stratalinker® with aluminum foil decreased the necessary time of exposure overall, therefore it would be best to implement the aluminum foil lining with all of the UV decontamination protocols. Also, when irradiating a PCR plate, it is necessary to bring the plate within an inch of the UV light source. One possible reason for this is that UV rays cannot easily penetrate the samples through the sides of the plate

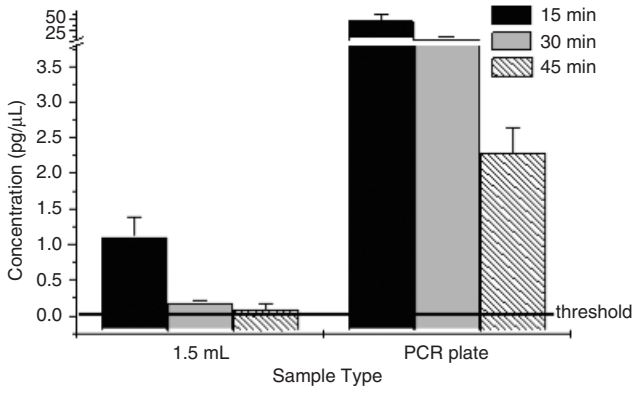

FIG. 2—*Efficacy of ultraviolet irradiation for DNA degradation in a solution of water or in polymerase chain reaction (PCR) plates; 50 ng of DNA in 500 μL of water in a 1.5 mL tube or 500 pg of DNA in 2.5 μL of water in a clear PCR plate were irradiated as indicated. All tubes were positioned on their sides with their caps closed, and the PCR plate was on the floor of the Stratalinker®. Shown is the mean concentration of DNA recovered from each sample ± SD, where n = 3.*

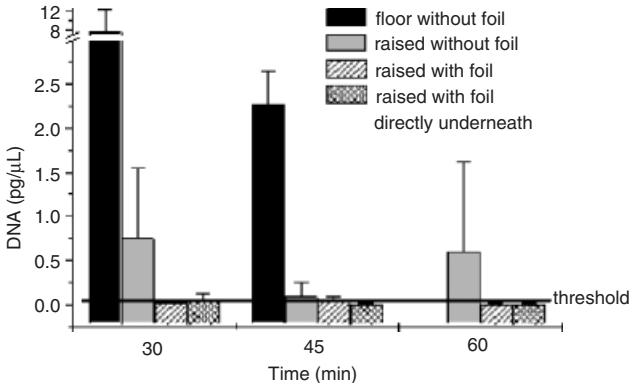

FIG. 3—*Irradiation enhancers: Proximity to the radiation source and the use of aluminum foil; clear polymerase chain reaction plates containing 500 pg of DNA were irradiated either on the floor of the Stratalinker® or within 1 in. of the ultraviolet bulbs. In addition, the Stratalinker® was lined with aluminum foil for a study with raised plates, some of which were lined with foil directly underneath. Results are expressed as the mean concentration of DNA in 2.5 μL of water ± SD for three replicates. (Plates on the floor were not tested for the 60 min interval.)*

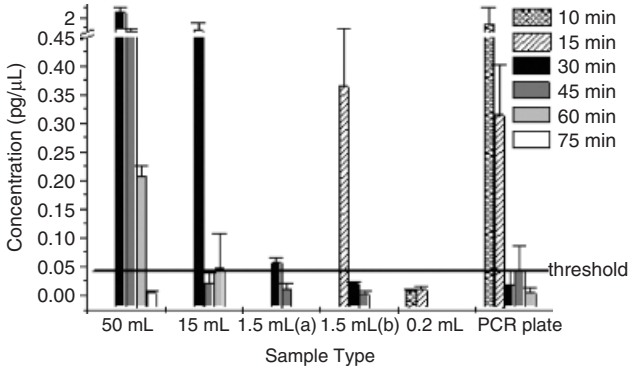

FIG. 5—*DNA degradation by ultraviolet irradiation under optimized conditions. The following concentrations of DNA and labware were irradiated in a Stratalinker® lined with foil; 100 pg/μL of DNA either in 0.5 mL (1.5 mL (a) tube), 5 mL (15 mL tube), or 15 mL (50 mL tube), or 200 pg/μL of DNA in 2.5 μL in a 1.5 mL (b) tube or 0.2 mL tube or a polymerase chain reaction plate. All tubes were lying capped on their sides, and the polymerase chain reaction plate was raised within 1 in. of the bulbs. Data represents the mean concentration of DNA recovered from each sample ± SD, where n = 3. (Where no bars are shown, time point was not tested with particular labware.)*

much like tubes inside a rack. Additionally, the color of the PCR plate posed no considerable effect on the success of the irradiation treatment (data not shown).

Testing of Concentration Devices and Pipette Tips

The irradiation process also did not seem to influence the performance of the Microcons. When 500, 25, or 6.25 pg of DNA in triplicate was purified with a non-treated Microcon® 100 membrane, or a Microcon® 100 membrane irradiated for 15 or 30 min, no difference in DNA recovery was detected (data not shown). Yet, additional experimentation may be warranted; according to the manufacturer, irradiation could dry the membrane compromising recovery.

In order to ascertain whether irradiation of pipette tips was necessary, p1000 and p10 ART tips and p50 Tecan tips were washed with 0, 50, or 100 ng of DNA in 5 μL of water. The tips were left untreated or were irradiated for 0, 10, 15, 30, 45, or 60 min. Subsequently, 5 μL of water was used to recover any DNA from the tips and was quantified. No significant amount of DNA was detected from control tips; for example, p10 tips with no added template generated 0.066 pg/μL of DNA. Considering that the quantitation assay used has an inherent error margin of 30% to 50% for DNA template less than 0.24 pg, a value of 0.066 pg/μL recovered from the p10 tips could qualify below our threshold (data not shown).

Conversely, untreated P10 ART tips retained 1% or 1 ng of the applied 100 ng of DNA. Out of all the samples irradiated, only the 50 μL Tecan compatible tips treated for 45 or 60 min generated less than the threshold value of 0.04 pg/μL of DNA. These results may appear high due to the fact that the tips were washed with *c.* 100–200 times more DNA than our previous studies performed with tubes and a solution of water containing DNA. Furthermore, the tips were irradiated while still inside their box and much like tubes inside a rack, the box may have inhibited the UV rays from effectively penetrating the tips. Irradiating the tips outside of the box would likely increase UV exposure. On the other hand, contamination could be introduced when handling the tips outside of the box after irradiation. Additional studies should be conducted employing a UV treatment method for pipette tips that minimizes the handling after irradiation. Ultimately, as DNA was not detected in the control pipette tips tested, untreated ART barrier pipette tips and tips designed for the CAS 1200 robot (Tecan compatible tips from Corbett Research) proved adequate for our LCN DNA typing.

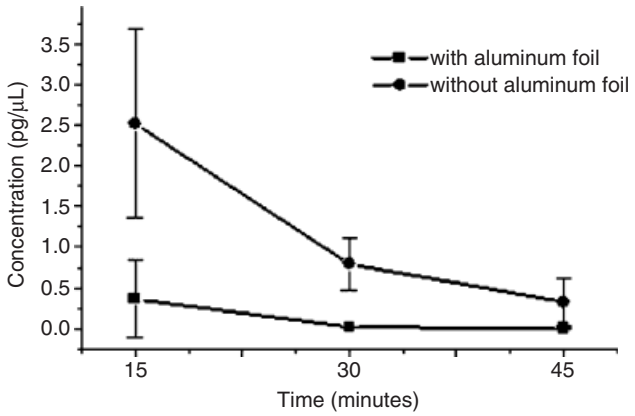

FIG. 4—*The impact of lining the Stratalinker® with aluminum foil on the efficiency of ultraviolet irradiation of tubes; 1.5 mL tubes, containing 500 pg of DNA in 2.5 μL, were irradiated lying closed on their sides in a bare Stratalinker® or one lined with aluminum foil. Shown is the concentration of DNA recovered from each sample as a mean of three replicates ± SD.*

Effect of UV Treatment of Labware and Water on Subsequent Amplification

To test for possible inhibition, PCR tubes were either untreated or irradiated and subsequently used for amplification. The number of allelic dropouts and the average peak heights were analyzed and compared. Out of 288 alleles amplified in total for triplicate amplifications of template amounts of 25, 12.5, and 6.25 pg for each condition, 2, 7, and 37 alleles were not present from the control, untreated samples whereas 1, 11, and 29 alleles were absent from the irradiated samples, respectively. The irradiated samples comprised 47.1% of the total drop out rate. 44.8% of the total dropouts were exhibited in the CSF, TH01, D16S539, D18S51, and FGA loci, all of which are known to have high dropout incidences (data not shown). To asses the amount of DNA that was

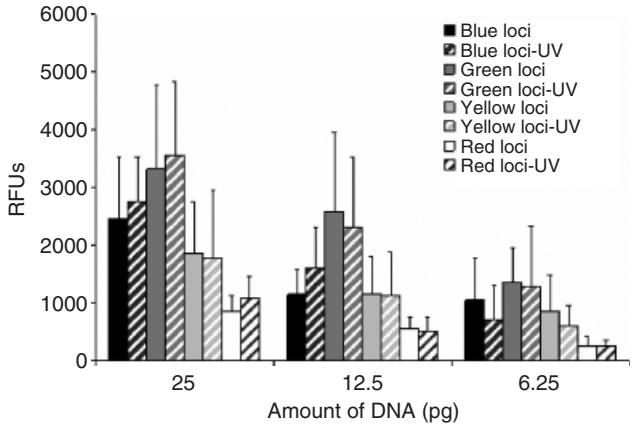

FIG. 6—*The effect of ultraviolet irradiation of polymerase chain reaction (PCR) tubes on amplification; Using untreated or irradiated clear PCR tubes (10 min), the indicated amounts of AmpFlSTR® control DNA 9947A were amplified using AmpFlSTR® Identifiler™ reagents for 31 cycles, and profiles were generated using the ABI Prism® 3100 Genetic Analyzer. Data is expressed as the mean relative fluorescence unit (RFU) for each color included in the Identifiler™ kit ± SD, where n = 3. (Peak heights for the homozygous peaks were halved.)*

amplified under the two conditions, the peak heights were compared (Fig. 6). There was no significant difference between the number of allelic dropouts and the peak height values of samples from treated or untreated tubes. Thus, our PCR sensitivity was not affected. This may be in view of the fact that our protocols require less irradiation time than previous studies (1).

Regarding the effects of irradiated water, both treated and non-irradiated water was used to dilute DNA to 25, 12.5, and 6.25 pg, and amplified with Identifiler™. No difference in the sensitivity of amplification with either solution of DNA was observed (data not shown). Overall, less spurious alleles were detected for amplifications employing irradiated water. Furthermore, negative controls comprised of either non-treated or irradiated water were measured with real-time PCR. No difference in yield was observed, and no inhibition was apparent from the raw data.

Conclusions

According to our optimized conditions, the UV sterilization parameters for pertinent labware in a Stratalinker® 2400 lined with foil are as follows: 10 min for dry 0.2 mL tubes, 30 min for dry 1.5 mL tubes and PCR plates, 45 min for 1.5 and 15 mL tubes filled with water, and 75 min for 50 mL tubes with water. Moreover, tubes must be positioned prone on their sides and PCR plates should be within 1 in. of the UV light source.

Through PCR, contamination can be introduced from a wide array of sources that are impossible to completely avoid. Spurious contamination can result from the manufacturer during packaging of tubes, tips, and even from water that is used for DNA amplification. Although this problem may not have been detected previously with standard PCR methods, exogenous DNA is especially apparent when the sensitivity of amplification is increased with LCN DNA typing protocols. Reducing the occurrence of sporadic contamination or drop-ins with LCN DNA typing requires improvements to sterilization methodologies. Utilizing our described UV irradiation protocols, we have noted a decrease in the incidence of drop-ins observed found within our target DNA. These UV irradiation methods can be regarded as a safe means for decontamination without compromising the sensitivity of our LCN PCR DNA amplifications.

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