Protocol for Ultraviolet Irradiation of Surfaces to Reduce PCR Contamination

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False-positive PCRs arise from contamination with exogenous genomes, plasmids, or PCR products.⁽¹⁾ Contaminated laboratory surfaces represent one of the many potential sources of exogenous DNA.

UV irradiation of dry DNA provides just one tool in the arsenal necessary to prevent PCR contamination. Although this type of decontamination was recommended previously as a way to “quickly damage any DNA left on exposed surfaces,”⁽¹⁾ further work has revealed a slow time course and sequence dependence.^(2–4) UV irradiation has also been proposed for decontaminating DNA in reagent solutions,^(5–7) a procedure that has met with mixed reviews.^(4,8,9)

Most UV-induced DNA damage occurs via the formation of cyclobutane rings between neighboring pyrimidine bases, thymidine or cytidine. The cyclobutane rings form intrastrand pyrimidine dimers that inhibit polymerase-mediated chain elongation. Dimer formation is reversible, establishing a steady-state equilibrium that favors monomers over dimers. As such, <10% of the possible pyrimidine dimers actually exist in irradiated DNA at one time.⁽¹⁰⁾

UV irradiation of laboratory surfaces has some important limitations. First, the surface must be perpendicular to the light source to achieve optimal light intensity. Skewed surfaces dilute the intensity, and three-dimensional objects, such as pipettors, cannot be effectively decontaminated by UV light because only a fraction of the surface actually faces the light source. This drawback is compounded by the fact that almost all laboratory surfaces, such as pipettors, centrifuges, door handles, test tube racks, and so forth, present potential sources of contamination.⁽¹¹⁾ Second, other materials dried with the target DNA, such as irrelevant DNA and nucleotides, can shield the target, making inactivation less efficient.⁽⁹⁾ Third, very short PCR products may not contain adequate numbers of neighboring pyrimidines to make them susceptible targets. The UV sensitivity of an amplified region can be estimated by counting the number of dimerizable sites (neighboring pyrimidines: CT, TT, TC, CC) in each single strand of the sequence. Based on theoretical considerations⁽¹⁰⁾ and limited experimental data,^(2,7) sequences with <10 dimerizable sites will be relatively UV resistant.

Meticulous technique remains the most important way to prevent contamination. However, UV irradiation can provide an additional margin of safety for keeping the PCR setup laboratory contamination free.

This procedure describes a method for reducing DNA contamination on laboratory surfaces by using UV light to inactivate dried DNA.⁽²⁾ Different procedures have been proposed for UV inactivation of DNA in solutions.^(5–7)

PROTOCOL

Safety precaution: UV irradiation is mutagenic and can cause visual loss or blindness. Wear UV-protective glasses and cover exposed skin when working with UV light.

Supplies

UV light ballast UF-36-2, American Ultraviolet Co., Santa Anna, CA
Two UV lamps, model G36T6L, American Ultraviolet Co.
Markline timer switch, M.H. Rhodes, Inc., Avon, CT
UV meter J-225, Black-Ray Co., San Gabriel, CA
Purified template DNA
35×10-mm tissue culture dishes, Corning Glass Works, Corning, NY

Installation

1. Mount the ballast and two lamps ~1 meter over the work surface. The

UV light source can be located at any distance from the surface; but as the distance increases, stronger lights will be necessary to achieve the same light intensity at the work surface. Installation of an in-line timer switch for automatic lamp shutoff can help to conserve the limited UV lamp life.

2. Document the UV light (254 nm) intensity at the work surface by measuring it with a UV meter. This measure of UV intensity will establish the baseline performance of your UV lamp installation. Lamp performance can then be checked by comparing future light intensity measurements with this one. We achieved effective decontamination with an intensity of 400 $\mu\text{W}/\text{cm}^2$ at the work surface using the above equipment.

Measuring DNA Inactivation

Standards

1. Obtain a concentrated solution of purified template DNA, such as genomic DNA, plasmid DNA, or PCR products.
2. Establish the minimum amplifiable concentration by making duplicate 10-fold dilutions of the DNA in 10 mM Tris (pH 8) and then amplifying an aliquot of each dilution.
3. Determine the most dilute specimen that was PCR positive in duplicate and call the DNA concentration in that dilution the minimum amplifiable concentration.
4. Prepare a concentrated DNA standard from the original DNA solution that is 10^6 – 10^8 times more concentrated than the minimum amplifiable concentration.
5. Prepare 12 test targets, each composed of 100 μl of the concentrated DNA standard spread in the center of a plastic petri dish and dried at room temperature.

Experimental Protocol

1. Place all 12 uncovered petri dishes with dry DNA in the area to be decontaminated. When ready to begin this 8-hr experiment, remove three dishes from the area and cover them. Turn on the UV lights.
2. After each UV irradiation time point (2, 4, and 8 hr), remove and cover three more dishes. Resuspend the DNA by adding 100 μl of 10 mM Tris (pH 8) to each dish. Agitate thoroughly by pipetting repeatedly and swirling the dish for several minutes, and remove the liquid to a labeled tube.

Determining UV Sensitivity

1. Quantitate the amount of amplifiable DNA in each sample by amplifying serial 10-fold dilutions as described above.
2. Plot the results with time on the x-axis (0, 2, 4, and 8 hr) and the number of 10-fold dilutions to achieve the minimum amplifiable concentration on the y-axis.⁽²⁾

The data should reveal a time-dependent decrease in DNA concentration. For instance, if the minimum amplifiable concentration of the 0-hr time point were reached at a 10^{-7} dilution and the minimum amplifiable concentration of the 4-hr time point were reached at a 10^{-4} dilution, then 4 hr of irradiation would have resulted in a 1000-fold reduction. Although inactivation of even 10-fold could be considered useful, susceptible targets can routinely be inactivated by 10,000-fold or more.

Decontamination Procedure

Decontaminate the work space after use by turning on the UV lights. Turn off the UV lights before resuming work. The minimum duration of UV illumination required for effective DNA inactivation can be determined from the measurement of DNA inactivation procedure described above. Alternatively, the UV lights can remain on at all times when the work area is not in use.

TROUBLESHOOTING

If contamination persists, look for shadowed work space areas and nonperpendicular surfaces that will escape effective irradiation, and seek other contamination sources, such as reagents, equipment, or surfaces outside of the immediate work area that could contact the operator during setup.

If contamination recurs after it was eliminated by UV irradiation, remeasure the UV intensity at the work surface as described above and compare it with the original intensity. Replace the UV lamps as necessary. UV lamps will still look blue even though their UV output has decreased.

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