US/EG Bulletin 1745

Inactivation of Contaminating Templates in PCR* Using High Intensity Ultraviolet Light

Introduction

Amplification of DNA through the polymerase chain reaction (PCR) is a highly sensitive method capable of copying millions of DNA molecules from just a single molecule.[1] Because of the extreme sensitivity of this method, any carry-over product from previous reactions can result in false positives or extra bands. Carry-over contamination can be avoided by using positive displacement pipettes, sterile and autoclaved reagents, and dedicated areas for template preparation away from template amplification.

In addition, previous reports have noted that UV light is also an effective means of preventing carry-over contamination.[2,3,4] High intensity UV light induces the formation of thymine dimers which block the progress of the *Taq* polymerase, and thus contaminating carry-over products are not amplified. In this bulletin, we describe a modified sterilization method using the GS Gene Linker® UV chamber to destroy contaminating amplicon or carry-over molecules in microcentrifuge tubes prior to amplification.

Materials and Methods

Amplification of DNA was performed using genomic DNA from lambda phage and purified amplified human β-actin DNA. All of the tubes used in the amplification of a 500 bp fragment of lambda phage contained 50 ng of total lambda genomic DNA suspended in 5 µl of distilled water. All of the tubes for the amplification of the amplicon DNA contained 20 ng of amplified β-actin product suspended in 2 µl of distilled water. For the experimental UV tests, tubes were placed in the middle of the GS Gene Linker oven and were either capped and placed on their side or kept open and held in place by a plastic microfuge rack. UV light was delivered by the GS Gene Linker chamber for 30 minutes. After UV irradiation was initiated, both a capped and an open tube were removed every 10 minutes from each of the lambda and β-actin samples. Control tubes for the lambda and β-actin templates consisted of untreated tubes that were not irradiated (0 seconds).

After UV irradiation, an aliquot from a master solution mix, containing AmpliTaq® enzyme, appropriate primers, reaction buffer, and dNTPs was added to each tube. The tubes were then subjected to 30 cycles of PCR and analyzed on a 3% NuSieve® 3:1 gel.

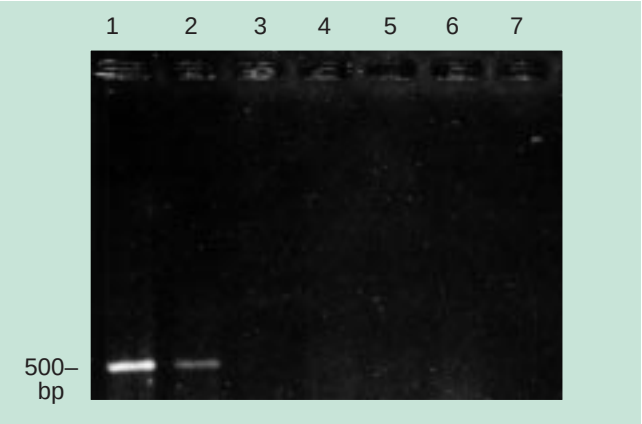

Fig. 1. Effect of UV irradiation on lambda genomic DNA. Lane 1: Control, 0 minutes; **Lane 2:** 10 min, tube open; **Lane 3:** 10 min, tube closed; **Lane 4:** 20 min, tube open; **Lane 5:** 20 min, tube closed; **Lane 6:** 30 min, tube open; **Lane 7:** 30 min, tube closed.

Results

Amplification of the lambda genomic DNA results in a 500 bp fragment, which can be seen in the control lane (Figure 1, lane 1), and amplification of the β-actin product results in a 268 bp fragment which can also be seen in the control lane (Figure 2, lane 1). With increased levels of UV irradiation, the amount of detectable PCR product decreases, as visualized by ethidium bromide staining. After 30 minutes of UV irradiation, both of the templates have been inactivated. As demonstrated in Figure 1 and 2, UV irradiation is an effective means of inactivating templates, whether the tubes are capped or closed. However, our data suggest that capping the tube and placing it on its side in the middle of the chamber is more effective than leaving the tube open in an upright position.

For best results, we suggest the following procedure:

- Cap the desired number of microfuge tubes
- Place the tubes in the middle of the GS Gene Linker oven.
- Select 900 seconds (15 minutes) and press start.
- At the end of 900 seconds, press start again for another 900 seconds.

This will complete the sterilization procedure. The tubes are now ready to be used for the PCR process.

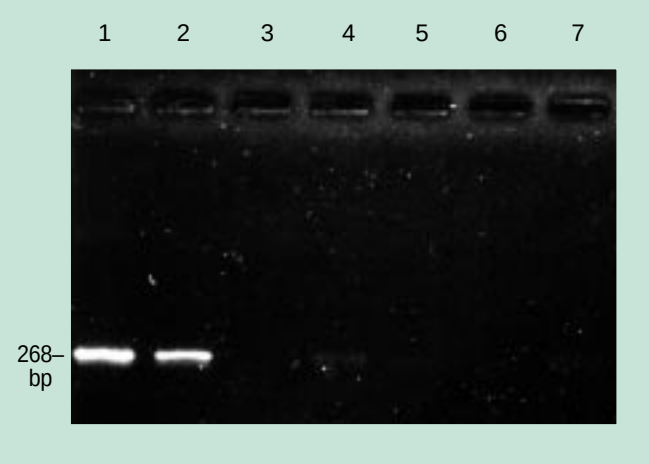

Fig. 2. Effect of UV irradiation on amplified β-Actin DNA. Lane 1: Control, 0 minutes; **Lane 2:** 10 min, tube open; **Lane 3:** 10 min, tube closed; **Lane 4:** 20 min, tube open; **Lane 5:** 20 min., tube closed; **Lane 6:** 30 min, tube open; **Lane 7:** 30 min., tube closed.

References

1. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R. Horn, G. T., Mullis, K. B. and Erlich, H. A., *Science,* **239**, 487-491 (1988).

2. Sakar, G. and Sommer, S. S., *Nature*, **343**, 27 (1990).

3. Sakar, G. and Sommer, S. S., *BioTechniques*, **10**(5), 591-593, (1991).

4. Ou, C., Moore, J. L. and Schochetman, G., *BioTechniques*, **10**(5) 442-445 (1991).

* PCR is covered by U.S. Patents issued to Cetus Corporation.

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Ordering Information

Catalog Number	Product Description
165-5031	**GS Gene Linker UV Chamber**, 20 VAC/60 Hz, includes five 254 nm bulbs and instructions
165-5032	**GS Gene Linker UV Chamber,** 220 VAC/50 Hz, includes five 254 nm bulbs and instructions
165-5033	**GS Gene Linker UV Chamber,** 240 VAC/50 Hz, includes five 254 nm bulbs and instructions
165-5034	**GS Gene Linker UV Chamber,** 100 VAC/50 Hz, includes five 254 nm bulbs and instructions
165-5035	**GS Gene Linker UV Chamber Replacement Bulbs,** 254 nm, 5 bulbs

For more information on the GS Gene Linker chamber, request bulletin 1667.

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