Use of psoralen as extinguisher of contaminated DNA in PCR

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Contamination of DNA is a serious problem of PCR. Enzymes themselves are often contaminated with DNA. This becomes a persistent obstacle, especially when amplifying a small amount of DNA, e.g., microdissected chromosomal DNA (1). To overcome this problem, several improved methods have been developed that adopt an ultraviolet (UV) treatment (2), and a DNaseI or restriction enzyme treatment (3) before PCR.

Here we show another treatment to solve this problem, that is, the use of a psoralen to extinguish the template activity of contaminated DNAs. Psoralens are known to intercalate into double-stranded nucleic acids and form a covalent interstrand crosslink after photoactivation with incident light of wavelength of 320-400 nm. Therefore, it is expected that the effect of psoralens is double-stranded DNA/RNA-specific. The procedure consists of two steps: incubation of DNAs with a psoralen, 8-methoxypsoralen (8-MOP) in the dark for 30 min to overnight and subsequent irradiation of long wave (365 nm) UV for 1 hr. The DNA incubated with 460 μM 8-MOP was irradiated with a conventional UV-handmonitor in 10 μl to 200 μl in a transparent plastic tube from the bottom at room temperature or 4°C, or in a freezer. *Sau*3AI-restricted pUC19 was used as target sequences in PCR. This amplification method is described elsewhere (1). Briefly, *Sau*3AI fragments are ligated with an oligonucleotide and amplified with PCR using the same oligonucleotide as a primer.

The psoralen/UV-treatment clearly eliminated the template activity of the DNA (lane 1), while psoralen or UV alone had no effect on the template activity of the DNA (lanes 2 and 3). This procedure was effective in 50% glycerol solution at −20°C to the DNA at various concentrations ranging 20 pg/μl to 0.2 pg/μl (lanes 6-8). When the DNA was treated in an enzyme preparation (*Eco*RI), the enzyme did not apparently lose its activity by this treatment (data not shown). Finally, the treatment was performed in a PCR reaction mixture effectively (lane 11). On the other hand, the treatment did not seem to injure Taq polymerase and dNTPs (lane 12) and a primer oligonucleotide (lane 16). It was estimated that more than 99.9% of the DNAs are inactivated with the psoralen/UV-treatment (data not shown).

This procedure has obvious advantages, compared with those previously reported (2, 3). Restriction enzymes to be used in order to eliminate contaminated DNA may bring DNA by themselves. In addition, enzymes are useless to treat the DNA contaminated in proteinase K. So far as the results of our experiments are concerned, the method of UV-irradiation with a combination of 254 nm and 365 nm did not work well to suppress the template activity of contaminated DNA.

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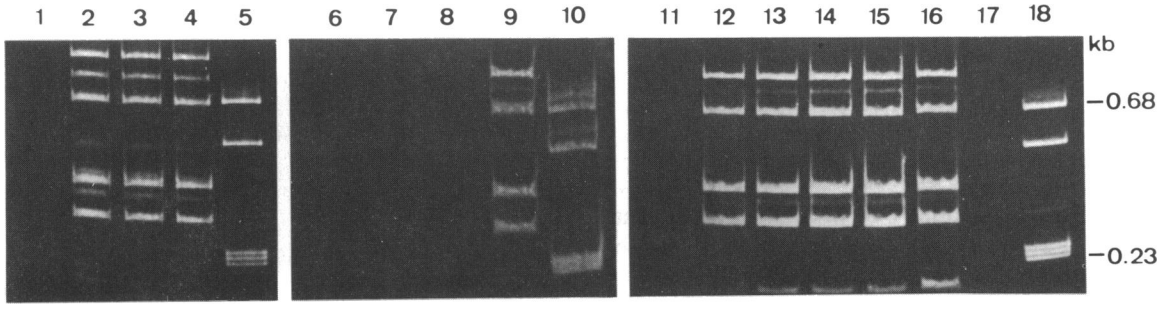

DNA (*Sau*3AI digest of pUC19) was treated with 8-MOP and UV under various conditions: psoralen-treatment in TE at room temperature for 30 min (lanes 1 and 2), in an enzyme preparation (*Eco*RI) at −20°C overnight (lanes 6-8), or in the PCR mixture at 4°C for 30 min after the DNA was ligated with a primer (lanes 11-13 and 16). UV-irradiation was performed at 365 nm for 1 hr (lanes 1, 3, 6-8, 11, 14 and 16). The treated DNAs were ligated with a primer and amplified with PCR by 21 cycles (for 1 pg of the template, lanes 11-15) or 26 cycles (for 0.2 pg and 0.1 pg of the template, lanes 1-9, and 16 and 17, respectively). Lanes 1, 6-8 and 11 are psoralen/UV-treated target DNAs; lanes 2 and 13, psoralen treatment alone; lanes 3 and 14, UV-irradiation alone; lanes 4, 9 and 15, positive controls; lane 12, psoralen/UV-treatment of the PCR reaction mixture before adding the ligation mixture containing target DNAs and the primer; lane 16, the addition of 10-fold reduced amount of the target DNAs and primer to the same pre-treated PCR reaction mixture as in lane 11; lane 17, the addition of the same amount of the target DNAs and primer as in lane 16 to a fresh PCR mixture containing Taq polymerase, dNTP and buffer; lanes 5, 10 and 18, the pUC19-*Alu*I size marker.