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Application Note

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Using Vivacon® 2 for Primer Removal after PCR

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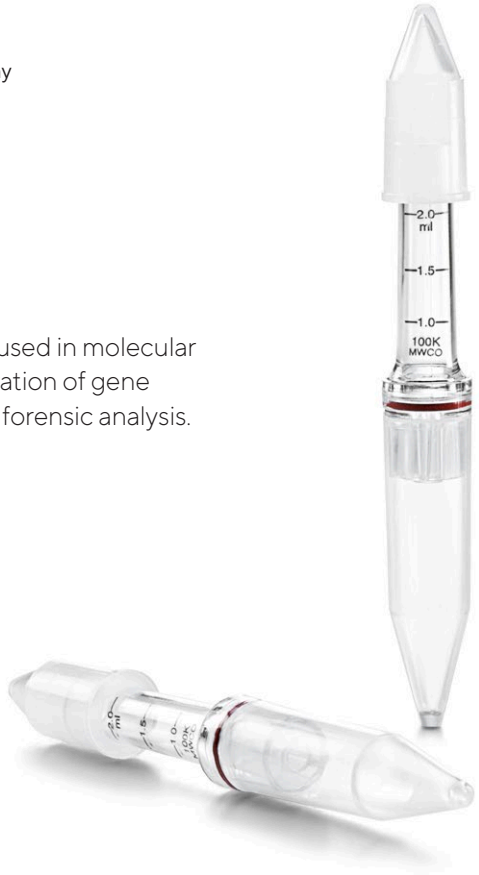

Abstract

PCR (Polymerase Chain Reaction) is one of the most versatile methods used in molecular biology today. It is relevant to a multitude of applications , such as preparation of gene fragments for cloning or amplification of DNA sequences, for example in forensic analysis.

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www.sartorius.com/products/lab-filtration-purification

Introduction

Out of necessity, polymerase chain reaction (PCR) mixtures contain a variety of salts, free nucleotides, glycerol, proteins, and primers. PCR is typically followed by further downstream processing of the amplified DNA and these applications may be more of less sensitive to the remaining components of the PCR mixture. For example, in certain processes such as restriction endonuclease digestion and DNA ligation, the enzymes used are particularly sensitive to the presence of contaminants in DNA samples. Due to this, most downstream applications will require some sort of PCR cleanup.

The PCR cleanup can be performed in a variety of ways:

1. Precipitation, using chemical solubility properties to selectively separate DNA from other sample components is a frequently used method. Its main disadvantage is the rather lengthy procedure along with the incomplete removal of co-precipitating buffer components and contaminants.

2. Chromatography, using size exclusion particles or affinity to glass to purify DNA from the PCR mixture components is a very sensitive and effective method. This technique is however costly, generally requires significant handling, and dilutes samples, so that they must be concentrated after elution from the matrix.

3. Ultrafiltration involves the isolation and concentration of PCR products using membrane devices. It is rapid, requires very little handling, achieves high recoveries, leaves DNA undamaged, and the concentrated DNA is free of contaminants that may inhibit downstream reactions.

Here we demonstrate the effective removal of primers using Vivacon® 2 ultrafiltration devices. With a 30 kDa MWCO Hydrosart membrane, Vivacon® 2 is effective at retaining 300-400 bp DNA fragments, while removing the 24 bp primers.

Materials and Methods

To evaluate the effectiveness of primer removal after a PCR reaction, two PCR sample mock ups with an excess of 24 bp primers (to aid visibility on a SDS gel) were prepared in the following way: 20 µL of a 75 µg/µL 24 bp primer solution in deionized water was added to 90 µL of a 40 µg/µL 300 bp DNA fragment in deionized water and diluted with 320 µL TE buffer, pH 8 to a final volume of 430 µL.

200 µL of a 75 µg/µL 24 bp primer solution in deionized water was added to 90 µL of a 51 µg/µL 400 bp DNA fragment in deionized water and diluted with 320 µL TE buffer, pH 8 to a final volume of 430 µL.

For primer removal, 200 µL of each mixture was diluted with 1,800 µL TE buffer, pH 8, added to a Vivacon® 2 device with 30 kDa MWCO membrane, then centrifuged for 20 minutes at 2,500 *g*. To mimic the removal of reaction salts, the sample volume was adjusted to 2 mL with TE buffer, pH 8 and centrifuged again for 20 minutes at 2,500 *g*. The effectiveness of primer removal was analysed using a 12% TBE polyacrylamide SDS gel.

5 µL samples of the initial sample, retentate and retentate after wash step were analyzed by polyacrylamide gel electrophoresis, in duplicate.

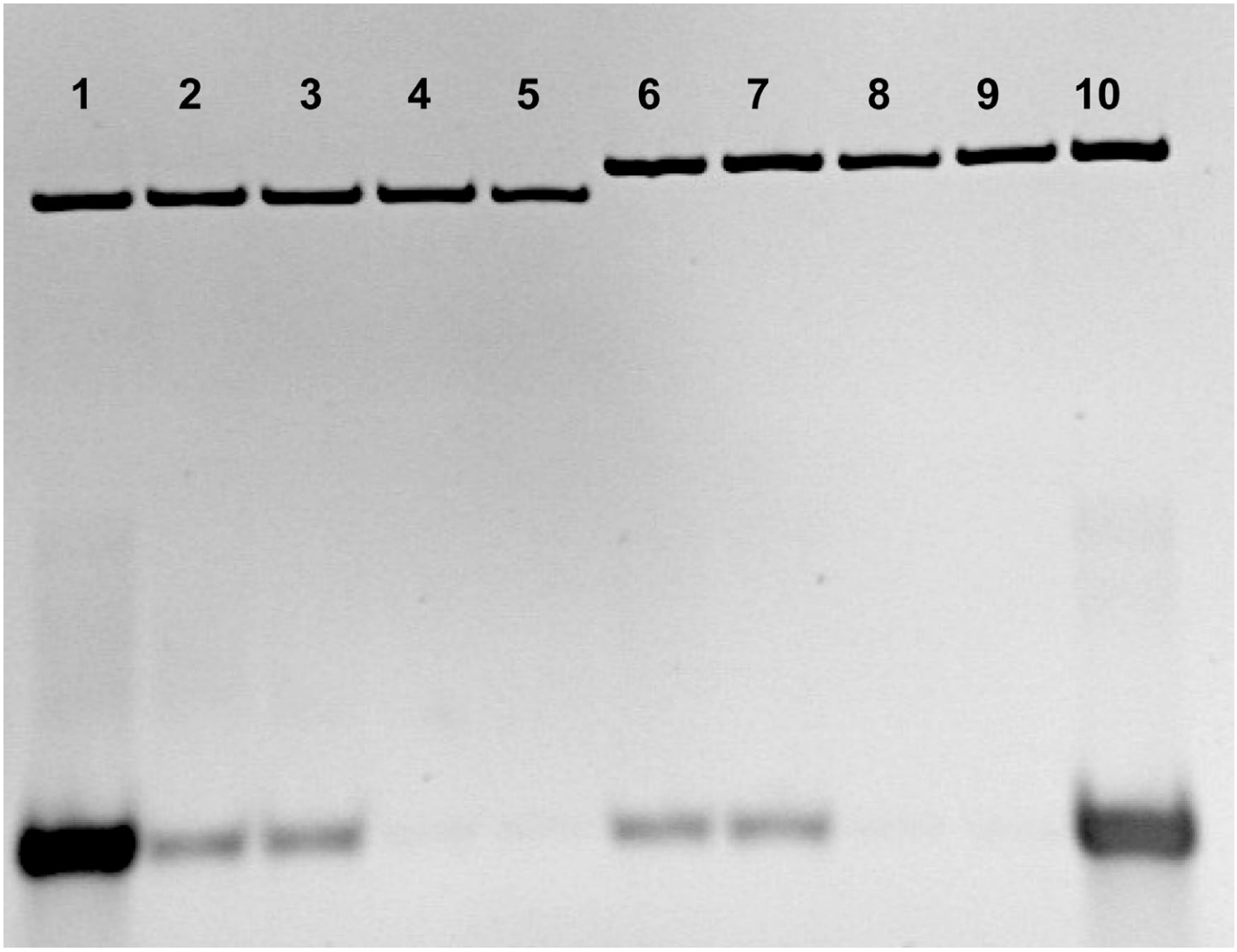
Results

The SDS gel shows the effectiveness of primer removal using Vivacon® 2, with quantitaive recoveries of both the 300 and 400 bp PCR fragments in a 40 minute procedure (Figure 1). Using a 30 kDa Vivacon® 2 device, primers and PCR reaction components can effectively be removed from a PCR sample containing 300 bp and larger DNA fragments for subsequent applications.

For smaller sample volumes, Vivacon® 500 accommodates samples of up to 500 µL.

Figure 1

12% TBE polyacrylamide SDS gel analysis of samples prior to, during and after ultrafiltration with Vivacon® 2 (30 kDa MWCO Hydrosart®) for the removal of PCR primers.



Lane 1	300 bp DNA fragment + 24 bp Primer – original sample
Lane 2	300 bp DNA fragment + 24 bp Primer – concentrate (1)
Lane 3	300 bp DNA fragment + 24 bp Primer – concentrate (2)
Lane 4	300 bp DNA fragment + 24 bp Primer – concentrate after wash (1)
Lane 5	300 bp DNA fragment + 24 bp Primer – concentrate after wash (2)
Lane 6	400 bp DNA fragment + 24 bp Primer – concentrate (1)
Lane 7	400 bp DNA fragment + 24 bp Primer – concentrate (2)
Lane 8	400 bp DNA fragment + 24 bp Primer – concentrate after wash (1)
Lane 9	400 bp DNA fragment + 24 bp Primer – concentrate after wash (2)
Lane 10	400 bp DNA fragment + 24 bp Primer – original sample

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