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Application Note

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Using Vivacon® 500 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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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 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 require some form of PCR cleanup. However, typical processes for this – such as precipitation, chromatography and electrophoresis – are generally time consuming and risk incomplete contaminant removal or loss of yield of the target, amplified DNA.

Here, we demonstrate the effective removal of primers using Vivacon® 500 ultrafiltration devices. With a 30 kDa MWCO Hydrosart® membrane, Vivacon® 500 is effective at retaining 300 bp DNA fragments, while removing the 25 bp primers.

Materials and Methods

To evaluate the effectiveness of primer removal after PCR, a mock reaction mixture was prepared with 100 µg/mL of a 300 bp DNA fragment – representing the amplified DNA – and 2 µM of 25 bp primers in TE buffer, pH 8.0.

50 µL PCR sample and 450 µL TE buffer were applied onto each of four Vivacon® 500 devices, then centrifuged for 15 minutes at 5,000 *g*. To mimic removal of reaction salts, the devices were refilled with 450 µL TE buffer, pH 8 and centrifuged for another 15 minutes at 5,000 *g*.

The effectiveness of primer removal was determined with analysis of samples collected before, during and after ultrafiltraiton by 12% TBE-Polyacrylamide SDS gel electrophoresis. 5 µL samples of the initial PCR mixture, first concentrate, and concentrate after wash step were applied to the SDS gel. Duplicates were prepared for each step.

Results

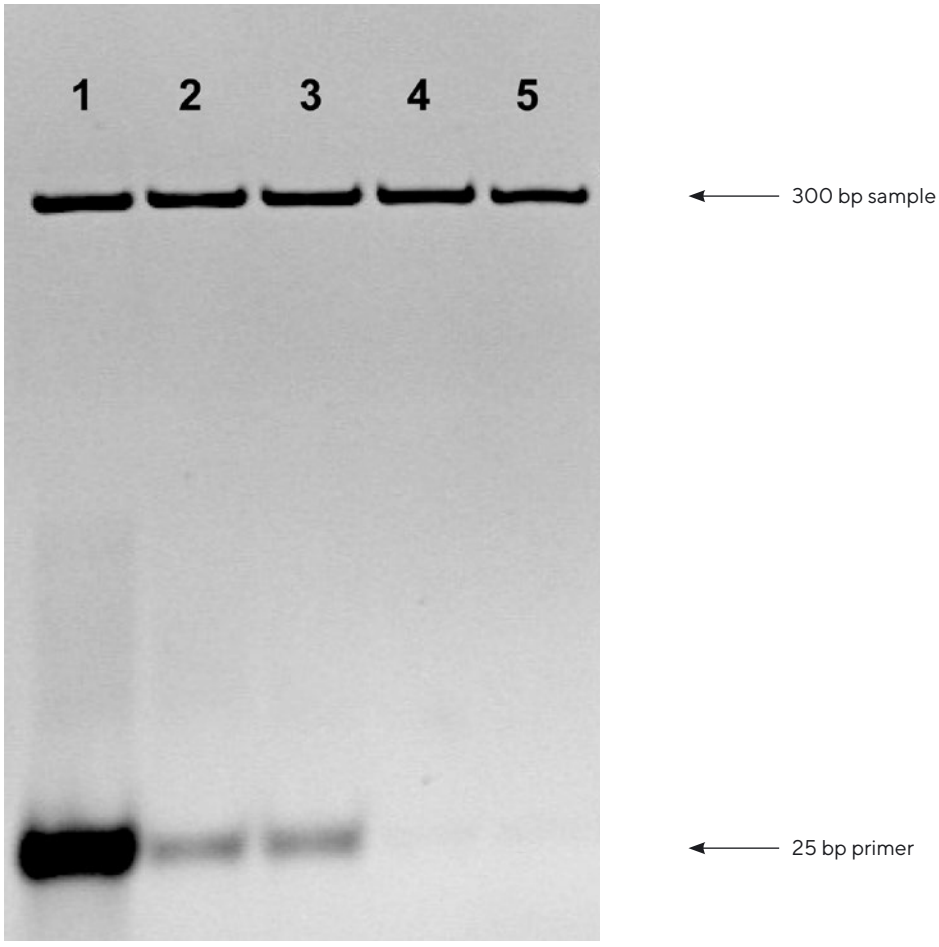
The SDS gel analysis showed effective primer removal with a 30 kDa MWCO Vivacon® 500, with quantitative recoveries of the 300 bp PCR fragment in a 30 minute procedure. In a single spin, 80% of the primers are removed. After a second spin, > 95% of the primers from the PCR mixture are removed.

Conclusions

Using a 30 kDa Vivacon® 500, primers and PCR reaction components can effectively be removed from a PCR sample containing 300 bp DNA fragments and larger, prior to subsequent downstream applications.

Figure 1:

12% TBE polyacrylamide SDS gel analysis of samples prior to, during and after ultrafiltration with Vivacon® 500 (30 kDa MWCO Hydrosart®) for the removal of PCR primers.



Lane 1	300 bp DNA fragment + 25 bp Primer – original sample
Lane 2	300 bp DNA fragment + 25 bp Primer – concentrate (1)
Lane 3	300 bp DNA fragment + 25 bp Primer – concentrate (2)
Lane 4	300 bp DNA fragment + 25 bp Primer – concentrate after wash (1)
Lane 5	300 bp DNA fragment + 25 bp Primer – concentrate after wash (2)

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