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Primer removal after a PCR reaction with Vivacon® 2

Application Note

PCR (Polymerase Chain Reaction) is one of the most versatile methods used today in molecular biology for a multitude of applications like preparing fragments for cloning or amplifying DNA sequences, for e.g. forensic analysis.

These applications may be more or less sensitive to the remaining components of the PCR reaction mixture. PCR reaction mixtures contain a variety of salts, free nucleotides, glycerol, proteins, and primers. Certain restriction enzymes as well as DNA ligase 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 show in an experiment the effective removal of primers using Vivacon® 2 ultrafiltration devices and show that the 30 kDa Hydrosart membrane Vivacon® 2 is effective at retaining 300 bp DNA fragments, while removing the 24 bp primers.

Matrials and Methods
In order to evaluate the effectiveness of primer removal after a PCR reaction, two PCR sample mock ups with excess of 24 bp primers (for optimal visability 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.

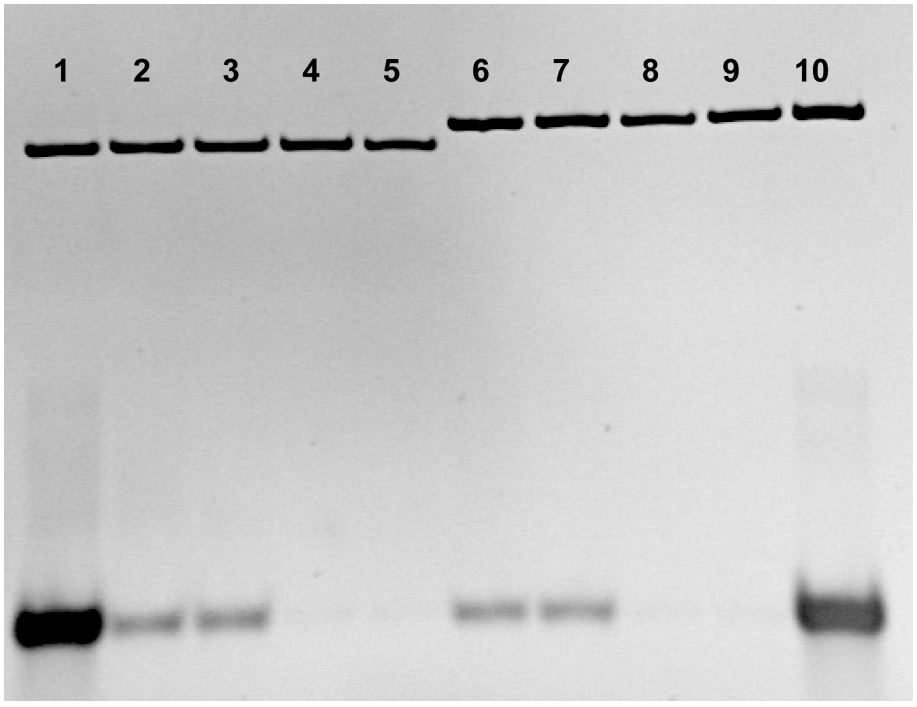
200 µl of a 75 µg/µl 24 bp primer solution in deionized water was added to 90 µl of a 51 µg/µl 400 bp DNA fragment in deionized water and diluted with 320 µl TE buffer, pH 8 to a final volume of 430 µl.

For primer removal, 200 µl of each mixture was diluted with 1800 µl TE buffer, pH 8 in a Vivacon® 2 with a 30 kDa membrane, then centrifuged for 20 minutes at 2500 × g. The sample was filled up again to 2 ml with TE buffer, pH 8 and centrifuged for additional 20 minutes at 2500 × g. The effectiveness of primer removal was analysed using a 12% TBE-Polyacrylamid SDS gel.

5 µl samples of the initial sample, concentrate and concentrate after wash step were applied to the SDS gel. Duplicates were prepared of each step.

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

For smaller sample volumes, Vivacon® 500 with a maximal volume of 500 µl can be used accordingly.



12 % TBE Polyacryamid SDS gel
30 kDa Vivacon® 2

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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Publication No.: SL-4047-e10112
Order No.: 85032-535-16
Ver. 11 | 2010