Primer removal after a PCR reaction with Vivacon® 500

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.

Here, we show in an experiment the effective removal of primers using Vivacon® 500 ultrafiltration devices and show that the 30 kDa Hydrosart® membrane is effective at retaining 300 bp DNA fragments, while removing the 25 bp primers.

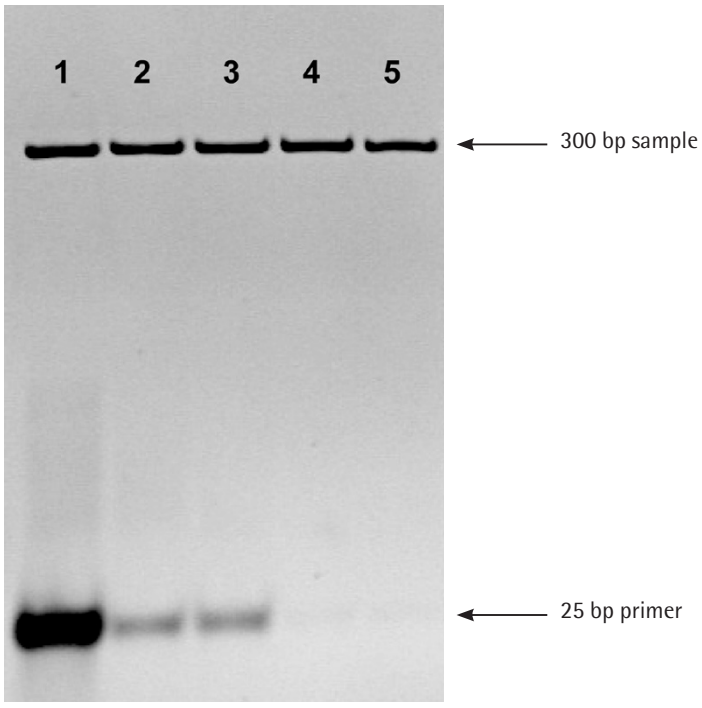
Matrials and Methods
PCR sample was prepared with 100 µg/ml 300 bp PCR fragment and 2 µM 25 bp primers in TE buffer, pH 8.0.

50 µl PCR sample and 450 µl TE buffer were loaded onto each of 4 Vivacon® 500 devices, then centrifuged for 15 minutes at 5,000 × g. The devices were then filled up with 450 µl TE buffer, pH 8 and centrifuged for another 15 minutes at 5,000 × g. The respin was performed at 2,500 × g for 20 s.
The effectiveness of the primer removal was analysed using a 12% TBE-Polyacrylamid SDS gel.

5 µl samples of the initial sample, first concentrate and concentrate after 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® 500, with quantitaive 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 reaction are removed.

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 for subsequent applications.



12 % TBE Polyacryamid SDS gel
30 kDa Vivacon® 500

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)

Sartorius Lab Instruments
GmbH & Co. KG
Weender Landstrasse 94-108
37075 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius.com

Publication No.: SL-4059-e160202
Order No.: 85032-539-58
Ver. 02 | 2016