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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**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 ultrafiltration 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)