Comparison of PCR Kleen™ Spin Columns to Traditional Methods for Purification of PCR Products Prior to Sequencing

Jolanta Kolodziejek, MSc, and Professor Norbert Nowotny, PhD, Clinical Virology Group, Institute of Virology, University of Veterinary Medicine, Vienna, Veterinaerplatz 1, A – 1210 Vienna, Austria

Introduction

The purity of the DNA template employed in sequencing PCR (cycle sequencing) is the most critical factor in nucleotide sequencing. The presence of contaminants such as enzymes (nucleases, polymerases), salts, primers, primer-dimers, nucleotides (dNTPs or ddNTPs), ethanol, and others decreases the quality of the sequencing scans significantly. Primer- or RNA-contaminated samples exhibit a high background and lead to misinterpretation of sequencing data. Contamination with nucleases, salts, nucleotides, and ethanol decreases read length and signal intensity and sometimes results in unreadable sequence data.

We tested Bio-Rad's PCR Kleen spin columns for purification of PCR products prior to sequencing PCR in two different experiments, and compared the resulting nucleotide sequences obtained from these samples with the sequences obtained after purification by traditional methods.

Methods

Experiment 1. Comparison of Effectiveness of Purification Method Prior to Sequencing PCR

A 510 bp RT-PCR product (100 µl) was prepared using Borna disease virus as template, and three of four equal-volume aliquots of the PCR product were purified by different methods prior to sequencing. The fourth aliquot was left as an unpurified control.

The first aliquot of the PCR product was purified using PCR Kleen spin columns according to the instructions. The DNA from the other two aliquots was recovered from agarose slices using commercial gel extraction kits from two other suppliers (suppliers A and B).

Following purification, PCR aliquots were analyzed on a 1.2% TAE agarose gel and compared with the unpurified PCR product (aliquot 4). The DNA concentration of all three purified PCR products was evaluated spectrophotometrically and compared with the concentration of the unpurified PCR product. The DNA concentrations were: unpurified product, 0.978 µg/µl; product purified by PCR Kleen spin column, 0.064 µg/µl; by supplier A gel extraction kit, 0.016 µg/µl; by supplier B gel extraction kit, 0.014 µg/µl.

Following sequencing PCR, the resulting nucleotide sequences were analyzed on an ABI PRISM 310 automated sequencer and compared.

Experiment 2. Sequencing Quality of Longer PCR Product After Sequencing PCR and Dye Terminator Removal

In order to evaluate the effectiveness of PCR Kleen spin columns on the quality of longer nucleotide sequences, three aliquots of a 750 bp PCR product derived from Borna disease virus were purified using PCR Kleen spin columns. After sequencing PCR, the recovered products were purified for sequencing by three different methods: We tested one classical purification method (precipitation in the presence of C_2H_5OH and 0.5 mM $MgCl_2$) in parallel with two modern commercially available dye removal kits from two other suppliers.

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Results

Experiment 1

The best yield of DNA was achieved after purification of the PCR product with the PCR Kleen spin column (see figure).

In contrast, both PCR products obtained by gel extraction, using either the protocol of supplier A or B, were only weakly detected by agarose gel analyses. These results correlated with the amount of DNA in each sample: the PCR product purified with the PCR Kleen column yielded 4 times more of the specific DNA compared to the other samples. Also, the use of PCR Kleen spin columns for purification gave rise to excellent sequencing results (the entire sequence of 510 bp was nicely readable), whereas only 200–300 bases were readable in the sequences derived from the other two samples.

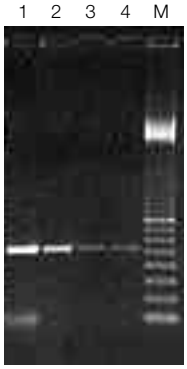

Figure. Agarose gel showing PCR products prior to sequencing PCR. Lane 1, unpurified 510 bp PCR product; lane 2, PCR product purified on PCR Kleen spin column; lane 3, PCR product purified by gel extraction kit from supplier A; lane 4, PCR product purified by gel extraction kit from supplier B; lane M, molecular weight marker.

Experiment 2

Independently of the purification method used after sequencing PCR (classical or one of the dye removal kits), we achieved the best sequencing results when the PCR products had been purified by PCR Kleen spin columns prior to sequencing PCR: 700 bases were readable, which is the maximum achievable with the ABI PRISM 310 automated sequencer.

Discussion

The results of direct nucleotide sequencing depend on the quality and purity of the PCR products; therefore, the DNA purification step prior to sequencing plays the most important role. The purification method used for the sequencing PCR product is of secondary importance.

The use of PCR Kleen spin columns led to excellent sequencing results. Other advantages of this method are:

- A simple and rapid procedure (4 min for PCR Kleen column vs. 3 hr for gel purification)
- The purified PCR product is immediately available for sequencing PCR
- No exposure to ethidium bromide or UV light
- It is possible to obtain sequencing results for a PCR product within 1 day

Only one specific PCR product should be present after amplification; in cases where agarose gel electrophoresis exhibits multiple bands, purification methods based on excision of the desired specific band from the gel may be advantageous.

To summarize, we have used PCR Kleen spin columns for purification of many different PCR products, and have found this method very reliable and superior to other techniques.

Information in this tech note was current as of the date of writing (2002) and not necessarily the date this version (rev A, 2003) was published.

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