High Pure PCR Product Purification Kit: Changed Protocol for Purification of Large DNA Fragments (4.5 kb to >30 kb)

Rolf Stucka
Neurological Clinic and Polyclinic, Friedrich-Baur-Institute, Laboratory of Molecular Myology
Ludwig-Maximilians University, Munich, Germany
rolf.stucka@med.uni-muenchen.de

Introduction

The High Pure Technology allows rapid purification of DNA fragments from complex mixtures (including PCRs and restriction nuclease digests) and from agarose gel slices using the High Pure PCR Product Purification Kit [1].

Selective adsorption of the DNA fragments to the fiberglass fleece is achieved in the presence of chaotropes such as guanidine thiocyanate (provided in the binding buffer 1). Soluble samples such as PCR product mixes are thoroughly mixed with five volumes of binding buffer 1, whereas agarose gel slices are solubilized with three volumes of binding buffer 1 at 55°C (1 volume [µl] equals the weight of the agarose slice in milligram). According to the standard kit protocol, 1.5 volumes of isopropanol are then added to the samples. The isopropanol-containing solutions are pipetted directly into the upper buffer reservoir of the High Pure Filter Tubes and the tubes are centrifuged for 30 seconds at 13.000 x *g*, followed by washing steps (500 µl wash buffer 2) and a final elution step (50 µl or more elution buffer 3).

In this article, I propose a changed protocol omitting the isopropanol-addition step for the purification of large DNA fragments. Furthermore, I provide evidence that without the addition of isopropanol, a higher recovery – in particular of very large DNA fragments (>30 kb) – is obtained using two standard DNA purification procedures (the purification of DNA from restriction nuclease digest solutions and from agarose gel slices).

Materials and Methods

The plasmid DNA CMV-pAdEasy-1 (37.2 kb) was isolated from *E.coli* BJ5183-AD-1 cells (AdEasy XL Adenoviral Vector System, Stratagene) grown in Luria Bertani (LB) medium with kanamycin. This low-copy number plasmid was isolated using the Genopure Plasmid Maxi Kit. The restriction-nuclease digest with *Pac* I yields a large 32.7-kb fragment containing a full-length adenoviral genome together with an expression cassette and a 4.5-kb fragment with a pBR322 origin of replication and kanamycin resistance cassette.

Whole restriction nuclease digests or agarose gel extracted DNA fragments were separated electrophoretically on 0.8% agarose gels in 1x Tris/acetate/EDTA (TAE) buffer.

Results and Discussion

To compare the recovery of DNA material from High Pure Filter Tubes with or without adding isopropanol prior to filter loading, the plasmid CMV-pAdEasy-1 was digested by *Pac* I and analyzed. This restriction analysis yields two fragments of 32.7 kb and 4.5 kb, and was chosen for the following reasons:

- The 32.7-kb fragment is within the upper size limit of DNA fragments usually to be handled in PCRs or in other applications such as restriction analysis.
- The large 32.7-kb and the smaller 4.5-kb fragments can be isolated in one tube thus enabling a direct comparison of recovery efficiency.

To test whether DNA recovery depended on the amount of material loaded on the High Pure Filter, different amounts of DNA were loaded (3 µg or 1 µg DNA per filter) and

Rolf Stucka

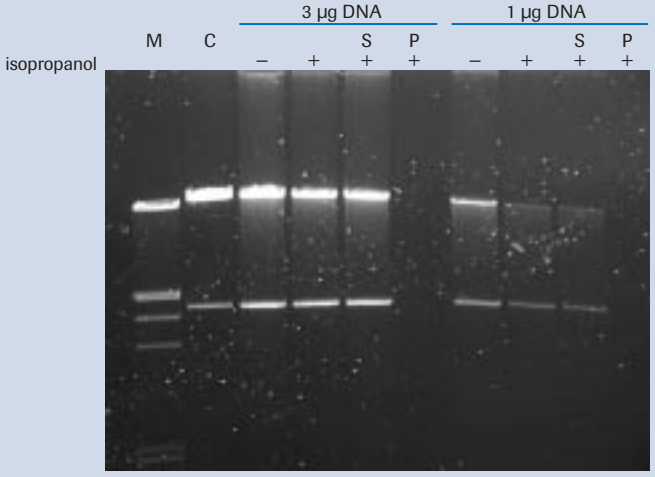

Figure 1: Recovery of restriction nuclease digested plasmid DNA with High Pure PCR Product Purification Kit. Samples were processed with isopropanol or without isopropanol. Selected samples were centrifuged at full speed after addition of isopropanol (*S,* supernatant) and the potential precipitates were redissolved in water (*P*, precipitate). As a control (*C*), *Pac* I digested CMV-pAdEasy-1 plasmid DNA was used, yielding 32.7-kb* and 4.5-kb fragments (*M,* marker: I *Hind* III-*Eco* RI DNA).

*Due to limitations in the resolution of large fragments on 0.8% agarose gels, the 32.7-kb band migrates next to the 21-kb marker of the λ *Hind* III-*Eco RI* DNA ladder.

analyzed. Furthermore, the addition of isopropanol might precipitate large DNA, thereby reducing the binding/adsorption to the fiberglass. Therefore, following the addition of isopropanol, samples were either loaded directly onto the filter or centrifuged at full speed for 10 minutes. The supernatant was loaded onto the filter and processed; any precipitates were directly checked by gel electrophoresis.

Purification from restriction nuclease digest mix

In a total volume of 200 µl, 13.3 µg CMV-pAdEasy-1 DNA was completely digested with *Pac* I. Three 45-µl aliquots (3 µg DNA each) and three 15-µl aliquots (1 µg DNA each) were adjusted with water to 100 µl each. The remaining 20 µl of the digest was used in gel electrophoresis as control sample.

Next, 500 µl binding buffer 1 was added to each sample and the samples were mixed thoroughly. Isopropanol (150 µl) was added to the isopropanol samples. Samples were centrifuged at 20,000 x *g* for 10 minutes, and the supernatants were carefully removed and transferred to new cups. Pelleted material was dissolved with 50 µl water and analyzed by agarose gel electrophoresis.

After the remaining samples had been applied to High Pure Filter Tubes, which were inserted into collection tubes, the entire High Pure Tube assemblies were centrifuged at 13,000 x *g* for 30 seconds. The flow-through was discarded, 500 µl wash buffer 2 was added and the filter tubes were centrifuged again. Then the filter tubes were removed and inserted into clean 1.5 ml microcentrifuge tubes. To each upper reservoir, 50 µl elution buffer 3 was added and the tube assemblies were centrifuged at 13.000 x *g* for 30 seconds. Eluted DNA as well as unpurified control and the isopropanol "pellet" samples were loaded onto an 0.8% agarose gel (containing ethidium bromide) and separated electrophoretically (100 V, 12 hours run).

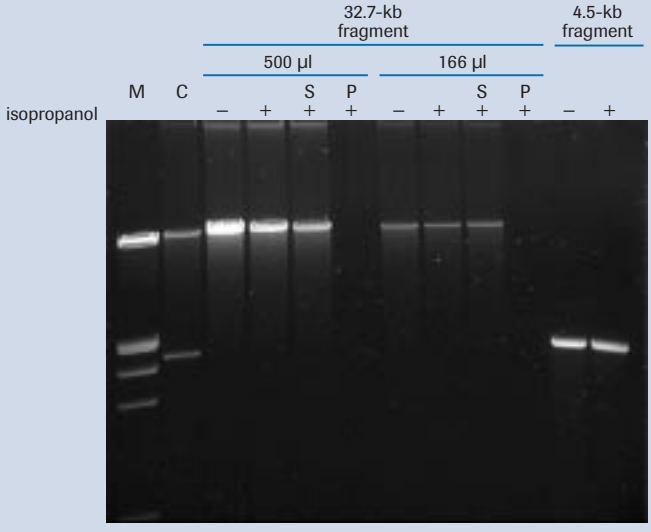

Figure 2: Recovery of DNA fragments extracted from agarose gel slices using the High Pure PCR Product Purification Kit. Samples were processed with isopropanol or without isopropanol. Selected samples were centrifuged at full speed after addition of isopropanol (*S*, supernatant) and the potential precipitates were redissolved in water (*P*, precipitate). As a control (*C*), *Pac* I digested CMV-pAdEasy-1 plasmid DNA was used (*M*, marker: λ *Hind* III-*Eco* RI DNA).

Total DNA recovery can be judged by visual inspection of the intensity of the stained DNA bands in Figure 1. Using the purification protocol without isopropanol, more of the large DNA fragment can be recovered. This is independent of the amount of DNA loaded on the filter. Interestingly, the addition of isopropanol does not seem to precipitate any DNA material.

Extraction from agarose gel slices

DNA fragments (32.7 kb and 4.5 kb) of 13.3 µg CMV-pAdEasy-1 digested with *Pac* I were separated by agarose gel electrophoresis and slices were cut under UV light illumination.

The 32.7-kb fragment. Three volumes of binding buffer 1 (1,500 µl) were added to the large 32.7-kb DNA fragment slice (500 µg) and the agarose slice was completely dissolved at 55°C. Then three 500-µl aliquots ("high load" samples) and three 166-µl aliquots ("low load" samples) were processed: Two samples (500 µl and 166 µl) without isopropanol were loaded directly onto the High Pure Filter and the other samples were mixed with 1.5 x volumes isopropanol (187 µl and 62.5 µl, respectively). Samples were then either loaded onto the High Pure Filter or centrifuged at full speed prior to loading the supernatant. Potential precipitates from this centrifugation were dissolved in water and analyzed directly.

The remaining steps (washing and elution) were performed according to the standard protocol. Fifty microliters of elution buffer was used.

The biological activity of the isolated large DNA fragments containing the adenoviral construct was tested and confirmed by transfecting 293 cells and obtaining infectious viral particles (data not shown).

The 4.5-kb fragment. The smaller 4.5-kb fragment was obtained as a 312-µg slice and 936 µl binding buffer 1 was added. Then, the agarose slice was solubilized at 55°C. Two 625-µl aliquots were removed and 234 µl isopropanol was added to one of the samples. Both samples were loaded directly to High Pure Filters and processed in parallel to the large 32.7-kb fragment.

DNA was recovered from High Pure Filters with a higher efficiency – at least when a larger amount of DNA was loaded – from material that was not treated with isopropanol (Figure 2). The difference is not immediately obvious when only small amounts of DNA are loaded. Nevertheless, repeated experiments with low amounts of DNA showed reduced recovery only for the isopropanol treated samples (data not shown). There is no visible difference in the recovery of the 4.5-kb fragment with or without isopropanol.

How can the specific loss of large DNA material (>30 kb) after the addition of isopropanol be explained? It seems very likely that the addition of isopropanol alters the hydrophobicity of the binding solution. A more hydrophobic environment, however, does not result directly in precipitation of DNA. This was checked by the centrifugation of isopropanol-treated samples (Figures 1, 2). One cannot rule out that the adsorption of DNA to the fiberglass in the isopropanol containing binding buffer is reduced, and therefore some material may simply be lost in the flow-through. This possibility needs to be checked, for example by performing binding studies with radioactively labeled DNA fragments.

Conclusion

A simple modification of the standard protocols for purification of DNA fragments with the High Pure PCR Product Purification Kit makes this commonly used application faster. By omitting the isopropanol addition step, a reduction of the total volumes to be passed through the High Pure Filters can be achieved. More importantly, the efficiency of recovery for various DNA fragment sizes equals or – in the case of very large fragments – even exceeds the efficiency reached with the standard isopropanol protocol. ■

References

1. Roche Applied Science; Nucleic Acid Isolation and Purification (2003) 2nd edition

Order INFO

Product	Pack Size	Cat. No.
High Pure PCR Product Purification Kit	50 purifications	11 732 668 001
	250 purifications	11 732 676 001
Genopure Plasmid Maxi Prep	10 purifications	03 143 422 001

NEW: MagNA Pure Compact RNA Isolation Kit (Tissue): Automated Preparation of Total RNA from Mammalian Tissue

*Agnes Aschenbrenner, Irene Huber, Elke Holzner, Werner Malmberg, Klaus Geißler, Vera Nieswandt, Walter Eberle, and Thomas Kirschbaum**
Roche Applied Science, Penzberg, Germany
**Corresponding author: thomas.kirschbaum@roche.com*

Introduction

The newly developed MagNA Pure Compact RNA Isolation Kit (Tissue) for the MagNA Pure Compact System allows the isolation of highly purified total RNA from up to 10 mg of mammalian tissue. The total RNA is suitable for highly sensitive and quantitative RT-PCR analysis using the LightCycler® Instrument or other PCR platforms, and for array applications in gene-expression studies. The ready-to-use kit contains prefilled reagent cartridges, reagents such as DNase and Tissue Lysis Buffer, and all the necessary disposables. All nucleic acid isolation steps are performed automatically using the MagNA Pure Compact Instrument.

The reagents and the protocol of the new kit are optimized to yield high amounts of RNA from mammalian tissue. The complete isolation procedure takes about 38 minutes of instrument time for up to eight samples. The elution volume may be set to either 50 µl or 100 µl.

Table 1: RNA yields from different sample materials. RNA was isolated from 10 mg of tissue and analyzed as described in Materials and Methods (CV, coefficient of variation).

Sample material	RNA yield [µg]	Purity [OD_{260nm}/OD_{280nm}]	CV yield [%]	CV RT-PCR [%]	No. of replicates
Mouse liver	42.2	1.99	9.1	1.1	24
Mouse spleen	57.5	1.97	8.7	1.4	8
Mouse lung	11.6	2.03	14.9	2.3	8
Mouse heart	9.2	1.97	13.7	1.2	8
Human placenta	12.7	2.10	3.8	1.1	8
Human colon	14.9	2.05	10.3	0.9	8