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Application Note

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Isolation of DNA Fragments From Agarose Gels Using Sartobind® D Anion Exchanger

Ion Exchange Chromatography With Sartobind® Membrane Adsorbers

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Introduction

The isolation of DNA fragments from agarose gels is a standard method frequently used in molecular biology. A variety of isolation methods have been developed, all with advantages and disadvantages. This procedure describes the use of Sartobind® D membranes for electrophoretic isolation of DNA fragments by insertion of small pieces of the weak anion exchange membrane into the agarose gel. It includes more "hands-on" time than spin columns but it has the advantage of being inexpensive, efficient (70–90% for DNA fragments between 200 and 5000 bp) and has no contamination of agarose in the purified fragment. It is also easy to perform. The membrane can be easily applied, as it is stiff enough to insert into slits.DNA purified by this method can be used for a variety of applications such as restriction digestions, ligations, probe-labeling, PCR, etc.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/membrane-chromatography

Materials

Weak anion exchanger:
Sartobind® D Sheet A4 (210×297 mm, order No. 94IEXD42-001) with diethylamine ligand

Elution buffer :
6.7 mM Tris, pH 8.0
0.07 mM EDTA
1.7 M NaCl

Method

1. Separate the DNA fragments on an agarose gel.

2. View EtBr-stained gel with a hand UV lamp (long wavelength UV) and cut a slit in the gel immediately above or below the band. Try to keep the UV exposure of the DNA to a minimum. To isolate the lower of two bands running close to one another, make the slit between the bands and reverse the run direction.

3. Cut a piece of Sartobind® D membrane to match the length and depth of the slit. Pre-wet the membrane in gel running buffer and avoid trapping air bubbles in the membrane. (see also the remarks on p. 3)

4. Using flat tweezers, insert the membrane into the slit. Make sure that the membrane is inserted completely to the bottom of the gel and that there are no air bubbles trapped between the gel and the membrane.

5. Perform electrophoresis to bind the DNA onto the membrane. This takes about 3–5 min at 90 V. Keep the time to the minimum necessary to get the DNA onto the membrane (can be checked with the hand lamp).

6. Remove the membrane from the slit and wash it briefly in a low salt buffer like TE or in H_2O to remove the gel running buffer and clinging pieces of agarose. You can also trim away excess parts of the membrane where no DNA is bound.

7. Cut the membrane into pieces so that they fit loosely at the bottom of a 1.5 mL Eppendorf tube. Elute the DNA by adding elution buffer (1.7 M NaCl in TE) to the moist membrane and incubating at 65 °C for 20–30 min. The total elution volume should be kept to a minimum and should not exceed 300 µL for one 1.5 mL tube.
   The membrane pieces should swim freely but be just covered by the buffer. The elution time can be extended and may help for fragments larger than 3 kb.

8. Transfer the liquid to a new tube and extract, first with 1 volume phenol: $CHCl_3$ 1 : 1 and then with 1 volume $CHCl_3$. Depending on what is planned with the purified fragment, it is possible to skip this extraction step.

9. Precipitate the DNA by adding 3 volume ethanol.
   The pellet is usually larger than expected for the amount of DNA isolated.

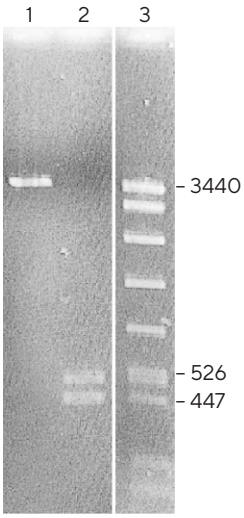

Fig.1 Purification of DNA from agarose gels with Sartobind® D membrane

1 = purified 3440 bp band
2 = purified 526 + 447 bands
3 = unpurified mixture of bands

Results and Remarks

Fig.1 shows that DNA fragments from an agarose gel were purified effectively with Sartobind® D membrane.

If you have problems with the yield, you can try pre-treating of the membrane. Pre-wet the membrane in isopropanol for a minute or two before soaking in gel running buffer appears to improve the yield slightly.
Alternatively, pre-treating the membrane by washing for 10 min in 10 mM EDTA, then 5 min in 0.5 M NaOH and then several times in H_2O may help. Pre-treated membranes can be stored for several weeks at 4 °C.

Reference

This Application Note is courtesy of Dr. Peter Nielsen, Max Plank Institute for Immunobiology, Freiburg, Germany: Isolation of DNA fragments from agarose gels using Sartobind® D membranes. Protocol

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