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Drop Dialysis

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ABSTRACT

This protocol is modified from the Drop Dialysis protocol available at http://www.neb.com.

Introduction

Many enzymes are adversely affected by a variety of residues in typical DNA preparations (e.g. minipreps, genomic and $CsCl_2$ preparations). The following method has been successfully used to remove inhibitory substances (e.g. SDS or excess salt) from substrates intended for subsequent DNA manipulations. It is particularly effective for:

- Preparation of templates for DNA sequencing
- Assuring complete cleavage of DNA by restriction endonucleases
- Increasing the efficiency of ligation

Reference

1. Silhavy, T., Berman, M. and Enquist, L. *Experiments with Gene Fusions*, Cold Spring Harbor, N.Y. Press (1984).

EXTERNAL LINK

https://www.neb.com/protocols/2013/09/16/drop-dialysis

MATERIALS

NAME	CATALOG #	VENDOR
Nuclease-free water (e.g. MilliQ or HPLC grade water)		
Type-VS Millipore membrane (MF type pore size = 0.025 µm)	VSWP02500 or VSWP01300	Millipore Sigma

1 Pour 30–100 ml of dialysis buffer, usually double-distilled water or 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), into a Petri dish or beaker.

2 Float a 13mm or 25 mm diameter, Type-VS Millipore membrane (MF type, VS filter, mean pore size = 0.025 µm, Millipore, Inc. #VSWP02500 or #VSWP01300) shiny side up on the dialysis buffer. Allow the floating filter to wet completely (~5 minutes) before proceeding. Make sure there are no air bubbles trapped under the filter.

3 Pipette a few µl of the DNA droplet carefully onto the filter. Depending on the size of the filter, you can dialyze several samples at the same time.

Notes

- If the sample has too much phenol or chloroform, the drop will not remain in the center of the membrane and the dialysis should be discontinued until the organics are further removed. In most cases, this is performed by alcohol precipitation of the sample. If the test sample remains in the center of the membrane, pipette the remainder on to the membrane.
- Step 3 may be tricky for those with shaky hands or poor hand-eye coordination. The filter has a tendency to move briskly around the surface as you touch it with the pipette tip. Practice with buffer droplets to master the technique before using a valuable sample.
- Dialysis against double-distilled water is also recommended, especially if proceeding to another manipulation where EDTA might be a problem.
- Steps 2 to 4 can be repeated with fresh buffer or for longer times if additional dialysis is required.

4 Cover the Petri dish or beaker and dialyze for 15 minutes to 4 hours. Do not allow the sample to flip or become covered with dialysis buffer.

5 Carefully retrieve the DNA droplet with a micro-pipette and place it in a microcentrifuge tube. Rinse the spot on the membrane with 50 µl of water or 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and add to the microcentrifuge tube.

6 Estimate the concentration of the DNA product using agarose gel electrophoresis or a spectrophotometer.