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Drop Dialysis

Many enzymes are adversely affected by a variety of residues in typical DNA preparations (e.g. minipreps, genomic and CsCl₂ 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

PROTOCOLS:

Purification of genomic, miniprep DNA or standard templates for DNA sequencing

Phenol extract, phenol/chloroform extract and then alcohol precipitate the DNA. Pellet the DNA by microcentrifugation; decant the supernatant and rinse the pellet with 70% ethanol. Recentrifuge the DNA, decant the supernatant and dry the pellet. Resuspend the pellet in 50 μ l H₂O. (Proceed to Step 1)

Purification of PCR products as templates for DNA sequencing

Phenol extract and then phenol/chloroform extract the aqueous layer of the PCR reaction. Follow this with an alcohol precipitation. Pellet the DNA by microcentrifugation, pour off the supernatant and rinse the pellet with 70% ethanol. Dry the pellet and resuspend it in 50 μ l of 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Alternatively, purify the PCR product through an appropriate spin column, precipitate and recover the DNA as described above. PCR products which are not a single band on an agarose gel should be gel purified in low-melt agarose and then treated with β -Agarase I (NEB #M0392) or an appropriate purification column technology, followed by extraction, precipitation and recovery as described above.

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 plate or beaker.
2. Float a 25 mm diameter, Type-VS Millipore membrane (MF type, VS filter, mean pore size = 0.025 μ m, Millipore, Inc. #VSWP 02500) 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 μ l of the DNA droplet carefully onto the center of the filter. 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.
4. Cover the petri plate or beaker and dialyze for 1 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 in a microcentrifuge tube. Rinse the spot on the membrane with 50 μ l of 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.

Notes

Step 4 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.

Reference

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