

Lab 8 – Isolation of DNA from an Agarose Gel

Quantum Prep™ Freeze ‘N Squeeze DNA Gel Extraction Spin Columns

Background

Purification of double-stranded DNA fragments from TAE or TBE agarose gels with the Quantum Prep™ Freeze ‘N Squeeze DNA Gel Extraction Spin Column is quick and efficient. Unlike other DNA purification methods which are often time-consuming or involve toxic chaotropic materials, the Quantum Prep Freeze ‘N Squeeze DNA Gel Extraction Spin Column method purifies via filtration in a spin column format. The spin column consists of a filter cup (with 0.45 µm cellulose acetate filter) contained within a special 2.0 ml “dolphin” tube for collection of purified sample. The distinctive bottle-nosed shape of the dolphin tube means recovered samples remain well below the bottom of the filter cup to insure purity. The extra long strap allows the cap to fit over either the filter cup during purification or to cap the collection tube itself for storage of the purified sample. In this method, a DNA band of interest is excised from an agarose gel, and the gel slice cut into small pieces and placed into the filter cup. The cup plus gel pieces is put in a -20 °C freezer for 5 minutes (the freeze), then removed and immediately centrifuged at 13,000 x g for 3 minutes at room temperature (the squeeze). Agarose debris is retained within the filter cup; the liquid at the bottom of the tube contains the recovered DNA. This method allows for recovery of DNA over a wide range of fragment sizes (50 bp to 23 kbp). The recovered DNA can be used for PCR, ligations, labeling or other enzymatic reactions without further purification or sample preparation.

Purpose:

To practice purifying DNA strands from an agarose gel and understand the implications of this technique.

Protocol

1. Obtain your sample of isolated pGLO (13µL).

In an eppendorf tube, put:	Volume (µL)
Your sample of pGLO	10
DNA Orange Loading Buffer (5X conc)	4.5
Sybr Stain (20X conc)	2.5
Final Volume	17
In a separate tube put:	
pGLO standard	5
Sterile water	5
DNA Orange Loading Buffer (5X conc)	2.5
Sybr Stain (20X conc)	2.5
Final Volume	15

2. Pour a 1% agarose gel using 50mL TAE buffer using a 6-well comb. When solidified, electrophorese your pGLO sample and the standard pGLO sample at 100 V for ~30 minutes.
3. Place gel on the UV plate and compare your band distance to the p-glo standard band distance. If your band looks like pGLO based on distance traveled, then using a clean spatula, carefully cut the band of interest. If not, then use the standard band as your sample and make a note in your data. Trim excess agarose from all six sides of the DNA band to maximize recovery and purity.
4. Chop the trimmed gel slice and place the pieces into the filter cup of the Quantum Prep Freeze 'N Squeeze DNA Gel Extraction Spin Column. Place the filter cup into the dolphin tube.
5. Place the Quantum Prep Freeze 'N Squeeze DNA Gel Extraction Spin Column (filter cup nested within dolphin tube) in a -20° C freezer for 5 minutes.
6. Spin the sample at 13,000 x g for 3 minutes at room temperature. What is this value in RPMs if the rotor radius is 6 cm? _____
7. Collect the purified DNA from the collection tube; the agarose debris will be retained within the filter cup of the Quantum Prep Freeze 'N Squeeze DNA Gel Extraction Spin Column. Discard the filter cup.
8. Measure and record the volume of each of the purified samples in μL .
9. Transfer 50 μL of a sample to a cuvette. Measure the absorbance at 260 nm of your sample three times. (Don't forget to blank the spectrophotometer with water before measuring your sample.) If your sample is less than 50 μL , then add water to your sample to bring the total volume up.
10. Measure the absorbance at 280 nm, making sure you blank the spectrophotometer first.
11. Repeat the measurement for your other sample.

Data/Results:

The absorbance readings.

Conclusion:

1. Calculate the DNA concentration for each of the 260 nm readings and obtain an average concentration.
2. Calculate the purity of the sample (Based on the average the three 260 nm readings) and indicate what it is contaminated with if not pure.
3. Using the concentration of the standard sample provided by the instructor, calculate the percent yield of the purified standard sample. To do this your divide the grams of purified pGLO by the grams of pGLO that your started with and multiply by 100.
4. Why would this be an important technique to know?