Gel Extraction

Introduction

For the extraction of our DNA samples from agarose gels, we used the "Monarch DNA Gel Extracton kit" from NEB.

For more information check the following link: [https://international.neb.com/-/media/nebus/files/manuals/manualt1020.pdf?rev=200eb4b2504c4a29af767f5502afcab5&hash=11B846CE931C0D55644AE323610A515B]

Materials

- Materials
  - Gel Dissolving Buffer
  - DNA Wash Buffer
  - Elution Buffer
  - Agarose gel slice containing the DNA fragment
- Equipment
  - Pipette & tips
  - 1.5 ml microcentrifuge tubes (2 per reaction)
  - Collection tube and column (1 per reaction)
  - Incubator

Procedure

Excision of the DNA fragment

1. Weigh as many eppedorfs as the samples, in order to calculate the weight of the gel sample. Excise the DNA fragment to be purified from the agarose gel using a razor blade, scalpel, or other clean cutting tool. Use care to trim excess agarose. Transfer it to a 1.5 ml microcentrifuge tube and weigh the gel slice.

   Note: Using UV light to visualize the slice is common, but exposure time should be kept as short as possible to minimize damage to the DNA. Use long-wave UV when possible, as shorter wavelengths induce greater damage. Also, trim off excess agarose from the perimeter of the band to minimize the amount of dissolving buffer needed, and to reduce the time necessary to extract the DNA.

Dissolution of the gel

2. Add 4 volumes of Monarch Gel Dissolving Buffer to the tube with the slice (e.g., add 400 µl Gel Dissolving Buffer per 100 mg agarose). If the gel slice is > 150 mg, consider reducing the amount of Gel Dissolving Buffer to 3 or 3.5 volumes to minimize the guanidine salt present in the workflow.

   Note: If the volume of the dissolved sample exceeds 800 µl, the loading of the sample onto the column should be performed in multiple rounds to not exceed the volume constraints of the spin column

3. Incubate the sample between 37–55°C (typically 50°C), inverting periodically until the gel slice is completely dissolved (generally 5– 10 minutes).

   Note: For DNA fragments > 8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA (e.g., 100 mg gel slice: 400 µl Gel Dissolving Buffer: 150 µl water). Failure to dissolve all the agarose will decrease the recovery yield due to incomplete extraction of the DNA and potential clogging of the column by particles of agarose.

Wash of DNA

4. Insert the column into collection tube and load sample onto the column. Spin for 1 minute, then discard flow-through. To save time, spin for 30 seconds, instead of 1 minute. If using a vacuum manifold* instead of centrifugation, insert the column into the manifold and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

   ** Make sure to follow the manifold manufacturer's instructions to set-up the manifold and connect it properly to a vacuum source.*

5. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional. If using a vacuum manifold, add 200 µl of DNA Wash Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off

6. Repeat wash (Step 5)

7. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If in doubt, re-spin for 1 minute before placing into clean microfuge tube. If using a vacuum manifold: Since vacuum set-ups can vary, a 1-minute centrifugation is recommended prior to elution to ensure that no traces of salt and ethanol are carried over to the next step.

8. Add ≥ 6 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

   Note: Typical elution volumes are 6–20 µl. Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated. For larger size DNA (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency. To save time, spin for 30 seconds, instead of 1 minute.