



Isolation of DNA from *Nyctanthus arbor tris tris*

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Abstract

Reliable measurement of DNA concentration and purity is important for almost all molecular genetics studies. Different plant species have varying levels of polysaccharides, polyphenols, and other secondary metabolites which combine with nucleic acids during DNA isolation and further affect the quality of the extracted DNA. The current extraction protocol is based upon extraction of DNA using tris HCl. The cell walls must be broken (or digested away) in order to release the cellular constituents. This is usually done by grinding the tissue in dry ice or liquid nitrogen with a mortar and pestle or a food grinder.

Keywords: DNA concentration, HCl, Dry ice, Liquid nitrogen, mortar and pestle

Introduction

Good quality DNA is a prerequisite for all experiments of DNA manipulation. All plant DNA extraction protocols comprise of the basic steps of disruption of the cell wall, cell membrane and nuclear membrane to release the DNA into solution followed by precipitation of DNA while ensuring removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites. This is brought about by disruption of the tissue in a mortar and pestle. The various components of the homogenization or extraction buffer were precipitated and purified with the methods employed. Since DNA can be extracted from various types of tissues such as seedlings, leaves, cotyledons, seeds, endosperm, tissue culture callus, roots etc., the tissue type along with the concentration of DNA finally required determine the methodology of DNA extraction to be followed by the experimenter. The most commonly used basic plant DNA extraction protocols are those of Dellaporta *et al.* 1983^[3] and Saghai Maroof *et al.*, 1984 along with many others that are modifications of the components of these protocols to suit a particular tissue type or downscaling them for mini preparation. The genomic DNA isolation needs to separate total DNA from RNA, protein, lipid, etc. Initially the cell membranes must be disrupted in order to release the DNA in the extraction buffer. SDS (sodium dodecyl sulphate) is used to disrupt the cell membrane.

Once cell is disrupted, the endogenous nucleases tend to cause extensive hydrolysis. Nucleases apparently present on human fingertips are notorious for causing spurious degradation of nucleic acids during purification. DNA can be protected from endogenous nucleases by chelating Mg²⁺⁺ ions using EDTA. Mg²⁺⁺ ion is considered as a necessary cofactor for action of most of the nucleases. Nucleoprotein interactions are disrupted with SDS, phenol or proteinase K. Phenol and chloroform are used to denature and separate proteins from DNA. Chloroform is also a protein denaturant, which stabilizes the rather unstable boundary between an aqueous phase and pure phenol layer. The denatured proteins form a layer at the interface between the aqueous and the organic phases which are removed by centrifugation. DNA released from disrupted cells is precipitated by cold absolute ethanol or isopropanol. This

process can be done in several ways, depending on the type of the sample and the downstream application, the most common methods are: mechanical, chemical and enzymatic lysis, precipitation, purification, and concentration. The specific method used to extract the DNA, such as phenol-chloroform extraction, alcohol precipitation, or silica-based purification. For the chemical method, many different kits are used for extraction, and selecting the correct one will save time on kit optimization and extraction procedures. PCR sensitivity detection is considered to show the variation between the commercial kits.

There are many different methods for extracting DNA, but some common steps include:

- 1. Lysis:** This step involves breaking open the cells to release the DNA. For example, in the case of bacterial cells, a solution of detergent and salt can be used to disrupt the cell membrane and release the DNA. For plant and animal cells, mechanical or enzymatic methods are often used.
- 2. Precipitation:** Once the DNA is released, proteins and other contaminants must be removed. This is typically done by adding a precipitating agent, such as alcohol (such as ethanol or isopropanol), or a salt. The DNA will form a pellet at the bottom of the solution, while the contaminants will remain in the liquid.
- 3. Purification:** After the DNA is precipitated, it is usually further purified by using column-based methods. For example, silica-based spin columns can be used to bind the DNA, while contaminants are washed away. Alternatively, a centrifugation step can be used to purify the DNA by spinning it down to the bottom of a tube.
- 4. Concentration:** Finally, the amount of DNA present is usually increased by removing any remaining liquid. This is typically done by using a vacuum centrifugation or a lyophilization (freeze-drying) step.

Material and method

Flowers of *Nyctanthus arbortris tris* were collected from the campus of college.

They were crushed and the following procedure was followed.

1. 1M Tris HCL 1 liter (MW 157.64)
 - It maintains pH.
 - Adjust pH with HCL 8.0.
2. 0.25 M EDTA 1 liter (MW 372.24)
 - It helps in chelation of divalent cation and inhibition of metal dependant enzymes.
 - Adjust pH to 7.0 with 6 M NaOH.
3. 3 M Sodium Acetate 250ml (MW 136.08) Adjust pH to 7.0 with glacial acetic acid.
4. 5 M Potassium Acetate 200ml (MW 94.14)

Autoclave (121 psi, 20 min).

All above reagents are autoclaved at 121 pounds per square inch(psi), for 20 minutes.

5. 10% w/v SDS Sodium lauryl sulfate. It is an anionic detergent helps in denaturation of proteins.
6. 5M Sodium Chloride (MW 58.4) 1liter.
7. Chloroform: Iso-amyl alcohol (24:1): it helps in protein precipitation.
8. TE buffer pH 8.0 1L

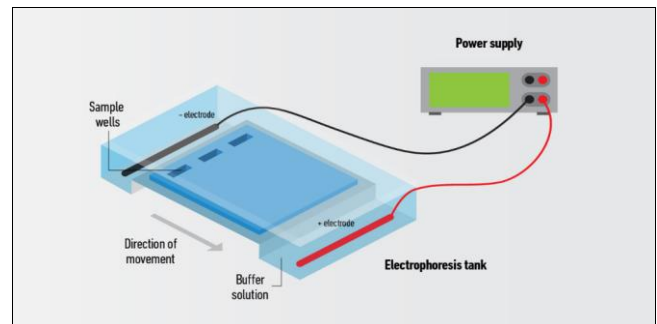
Tris HCl (1 M, pH 8.0) 10ml Make up the volume to 1000ml with double distilled water.

9. Extraction buffer: 1l
10. Isopropanol: for precipitation of DNA complex
11. Absolute ethanol: for precipitation of DNA
12. 12.70% ethanol: for washing DNA

1. Grind plant tissues (about 8-10 leaf punches) using mortar and pestle. Do not allow the sample to thaw.
2. Add 600 of pre- heated (65°C) extraction buffer with sodium bisulfite.
3. Incubate at 65°C in water bath for 30 minutes with vigorous shaking once in 10 minutes.
4. Allow to cool at room temperature, adding 240 (40% volume of extraction buffer) of 5M potassium acetate. Mix well and let it be at room temperature for 1-2 minutes.
5. Incubate at -20°C for 20 minutes.
6. Centrifuge at 8000 rpm for 10 minutes at 5°C.
7. Transfer 500 of supernatant from step 6 into new micro centrifuge tube without disturbing the cell debris pellet.
8. Add at least 400 -600 of isopropanol to the supernatant.
9. Mix gently. 10. Incubate tubes at -20°C least 15 min or 4°C for overnight.
10. Incubate tubes at -20 C least 15 min or 4 C for overnight.
11. Centrifuge tubes at 12000rpm for 10 minutes to pellet the DNA.
12. Remove and discard supernatant, being very careful not to dislodge the pellet.
13. Add 500 70% ethanol. Gently invert tube several times to wash pellet.
14. Centrifuge at 12000 rpm for 5 minutes.
15. Remove and discard the supernatant. The pellet will be loose so take care not to lose the pellet.
16. Repeat steps 13-15 once.

17. Let the pellet air dry (at least 15- 20minutes) by leaving the cap of the tube open.
18. Resuspend DNA in 50 to 100 of TE buffer pH 8.0(depending on amount of cells used).
19. Incubate at 55°C for 2 hours to help dissolve the DNA and then store at 40°C.
20. Set 0.8% agarose gel with preferably wide comb and load 6 of DNA sample and in each well with 2 of loading dye.
21. Electrophoresis at constant voltage. Compare the intensity of band on UV transilluminator to assess relative concentration of DNA in each sample.

Demonstration of agarose gel electrophoresis



Preparation of a 0.8% agarose gel

Rinse and dry the gel casting tray (with 95% ethanol).

Adjust the level of the comb so it rests evenly with a few mm of space between teeth and the tray; this will allow wells to form in the agarose. The 0.8% agarose has already been prepared (240µg agarose powder per 30 mL 1x TAE), and melted in microwave oven. Gently swirl, make sure there are no particles. Using a 50 ml beaker, measure out about 30 ml. Return agarose stock to water bath. Pour the gel when the agarose has cooled to about 55° C and add drop (2 µl -3µl) of ethidium bromide. Pour the agarose into the casting tray. Allow the gel to solidify; then remove the comb. Cover the gel with 1X TAE running buffer.

Loading and running the gel

Loading dye was added to each sample. Samples were loaded, taking care not to puncture the well bottoms. Do not attempt to load a sample if there is an air bubble in your pipet tip. Attach lid, make sure cords are correctly plugged into the power supply (red to red, black to black) Plug in the power supply. Turn the power on and adjust the voltage control knob to 50 or 100 volts. Electrophorese (run) until the bromophenol blue has migrated to within ¾ of the positive electrode end of the gel. Shut off the power supply, unplug the leads, and unplug the power supply.





Fig 1: Observe the DNA bands in UV transilluminator

Result

The bands were separated but were little merged. Identification of the plant was not able to done properly. This paper of the botanical traits, methods for extracting essential oil, yield, chemical makeup, and pharmacological and biological effects of *N. arbor-tris tris* extracts and essential oil is given in this work. While the analysis of barks has to be addressed or given more thought, the production of essential oils and the chemical compositions of the plant are mostly focused on the leaves and flowers. Of the various extraction techniques available, hydro distillation is the main method used to extract essential oils. The specific method used to extract the DNA, such as phenol-chloroform extraction, alcohol precipitation, or silica-based purification. For the chemical method, many different kits are used for extraction, and selecting the correct one will save time on kit optimization and extraction procedures. PCR sensitivity detection is considered to show the variation between the commercial kits.

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