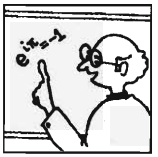

In this section of **Resonance**, *we invite readers to pose questions likely to be raised in a classroom situation. We may suggest strategies for dealing with them, or invite responses, or both. "Classroom" is equally a forum for raising broader issues and sharing personal experiences and viewpoints on matters related to teaching and learning science.*

A Simple and Rapid Method for Isolation of Cellular DNA

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A simple method has been standardised to isolate cellular DNA rapidly within 1 to 2 hours with common chemicals and simple instruments. This experiment can be carried out at laboratories in undergraduate colleges and high schools.

Introduction

The elucidation of the structure of DNA in 1953 by James Watson and Francis Crick was one of the most exciting discoveries in the history of genetics and molecular biology. After understanding the functional properties of DNA viz, replication, transcription, mutation, recombination and repair, it became possible to manipulate DNA.

In India, we have now introduced a number of new courses at undergraduate and postgraduate levels in life sciences. We teach DNA technology in almost all disciplines of biology. However, the infrastructure to provide practical knowledge of DNA technology to the students is inadequate in most of the colleges due to various reasons. In view of this, I have standardised and demonstrated a few simple and rapid methods to teach recombinant DNA technology at undergraduate and postgraduate colleges by using commonly available chemicals and

Box 1. Merits and Demerits of the Protocol

Compared to standard methods this is a simple and low cost procedure, where a few common chemicals are sufficient for the extraction. The method does not require expensive enzymes like proteinase K and RNAse or expensive equipment. DNA extraction can be completed faster (within 1-2 hours) than by standard methods. This method does not require handling of hazardous chemicals like phenol and chlorotoform. This method can be introduced at the pre university level and even at the high school levels. Readers are warned that the yield of DNA by this method is low and the DNA extracted by this method cannot be used for research work without further purification.

equipment. Here, I describe the isolation of eukaryotic DNA.

Principle

Most methods of DNA isolation involve the breakage or lysis of the cells to release nuclei and further breakage of nuclei to release the chromatin. DNA in cells exists as nucleoprotein complexes and therefore isolation of DNA involves removal of proteins and carbohydrates (if any) associated with it. Finally, the polymeric nature of DNA is utilised to precipitate it and make it free of small molecular contamination.

Reagents, Supplies and Equipment Required

(1) Tissue: spleen/heart/testis/kidney of any vertebrate or coconut endosperm; (2) Mortar and pestles or glass homogeniser; (3) Glass distilled water; (4) Centrifuge (range 3000 to 10,000 rpm); (5) pH meter (optional); (6) 10 ml centrifuge tubes; (7) 30 ml test tubes; (8) Test tube rack ; (9) bent glass rod; (10) Sodium saline citrate solution (SSC–85ml of 0.9% sodium chloride solution + 15ml of 0.5% sodium citrate solution usually gives pH 7.4, if not adjust pH.); (11) 12% Sodium chloride solution (Dissolve 12 gms of sodium chloride in 100ml of distilled water); (11) Absolute alcohol (double distilled alcohol).

Laboratory Protocol

(1) Grind about 200mg of the tissues in about 5ml of SSC in a homogeniser or with a mortar and pestle. (2) Transfer the homogenate into a centrifuge tube and make up the volume to 10ml with SSC. (3) Centrifuge at 3000rpm for 8 minutes and discard the supernatant.(4) Rehomogenise the sediment with 5ml of SSC.(5) Adjust the volume to 10ml, centrifuge at 3000rpm for 8 minutes and discard the supernatant.(6) Then, suspend the sediment in 10ml of 12% sodium chloride solution and centrifuge at 10,000rpm (at least 7000 rpm) for 15 minutes. (7) Transfer the supernatant into a 30ml test tube and add 2-3 volumes of absolute alcohol.(8) Gently mix it by inverting the tube. The white fibrous DNA precipitates. (9) Spool the **fibrous**

white DNA by winding around a clean sterile bent glass rod.

The presence of DNA in solution can be checked by the following methods:

(a) Transfer the spooled fibrous DNA into a 1.5ml eppendorf tube, add 1ml of 70% alcohol, centrifuge for 5 minutes at 10,000 rpm and discard the supernatant. Then the pellet containing the DNA is dried, dissolved in distilled water and optical density is read in a spectrophotometer at 260nm wavelength.

(b) The DNA in solution can be colorimetrically estimated by using diphenylamine colouring reagent [1]. In brief, the deoxyribose purine in DNA in presence of acid forms hydroxylevulinic aldehyde which reacts with diphenylamine to give a blue colour. The formation of blue colour indicates the presence of DNA and intensity of the colour gives the concentration of the DNA in solution.

(c) The DNA is sheared by violent agitation by passing through the small gauged needles as well as by boiling the DNA solution for 10 minutes and chilling it immediately on ice. This is needed to break the high molecular weight DNA, otherwise it cannot get into the gel. For agarose gel electrophoresis, the agarose gel can be prepared by dissolving 0.8% agarose with tris-acetate buffer {4 mM Tris, 2 mM acetic acid, 0.2. mM EDTA, pH 8.1 (TAE)} on boiling and pouring on to the casting tray after cooling the solution to 45°C and placing a slot creating comb before the polymerisation of the gel. Then place the polymerised gel into submarine electrophoretic chamber containing TAE buffer and load the DNA sample into the wells of the gel after mixing with tracking dye. After this connect the power supply and run the gel at 80 volts for 20-30 minutes. Remove the gel and stain with ethidium bromide (et Br), a DNA intercalating dye. The DNA-et Br complex can be seen as an orange coloured fluorescent streaking band under ultraviolet light on a device called transilluminator.

Suggested Reading

[1] R L Rodriguez and R C Tart. *Recombinant DNA Techniques – An Introduction.* Benjamin/Cummings Publishing Company, Inc., 1983

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[3] B R Glick and J J Pasternak. *Molecular Biotechnology–Principles and Applications of Recombinant DNA.* ASM Press, Washington DC, 1994.

[4] J Jayaraman. *Laboratory Manual in Biochemistry.* Fifth reprint. Wiley Eastern Limited. New Delhi, 1996.