AIM

Isolation of plant (Onion) genomic DNA by a crude method.

INTRODUCTION

A general difficulty in isolating DNA from plant cells is the presence of a cell wall, which requires degradation either physically or enzymatically. Specific plant species and tissues may have additional challenges due to starches or phenolic compounds.

OBJECTIVE

✔Extract genomic DNA from onion.

PRINCIPLE

All DNA isolation methods consist of three steps:

✔Lysis of cells.

✔Denaturation of histones associated with DNA.

✔Precipitation of DNA using an organic solvent like ethanol.

In the described method:

1. A kitchen blender (mixer Grinder) breaks open the cell walls.
2. Detergent degrades the phospholipid membranes around the nuclei, releasing the DNA.
3. Detergent, combined with heating, denatures the histones associated with the DNA.
4. Protease hydrolyses the denatured histones into peptides and amino acids.
5. DNA is then precipitated in ice-cold ethanol, which disrupts DNA-water interactions, causing DNA to precipitate out of solution.

REQUIREMENTS

MATERIALS

- ☐ Onion
- ☐ Washing-up liquid (Detergent)
- ☐ Table salt (3 g)
- ☐ Water (tap water is suitable)
- ☐ Very cold ethanol or Isopropanol (10 ml) (keep in the freezer)
- ☐ a protease enzyme, (2–3 drops) (Optional)
- ☐ Ice
- ☐ Cheesecloth

REQUIREMENTS (cont)

EQUIPMENT

- ☐ Plastic funnel
- ☐ Beakers, 2 (250 ml)
- ☐ Test tubes, 2
- ☐ Plastic spoon for stirring the mixture
- ☐ Chopping board
- ☐ Knife for chopping onion
- ☐ Water bath, maintained at 60°C
- ☐ Mixer Grinder or liquidiser

PROTOCOL

Dissolve 3g salt in 90ml (about 3.04 oz) water with washing-up liquid.

Add chopped onion (5mm (about 0.2 in) x 5mm) to the solution in a beaker.

Place the beaker in a 60°C water bath for 15 minutes.

Cool the mixture in an ice water bath for 5 minutes, stirring frequently.

Blend the mixture for 5 seconds on high speed in a mixer grinder.

Filter the mixture into another beaker to obtain the DNA-containing filtrate.

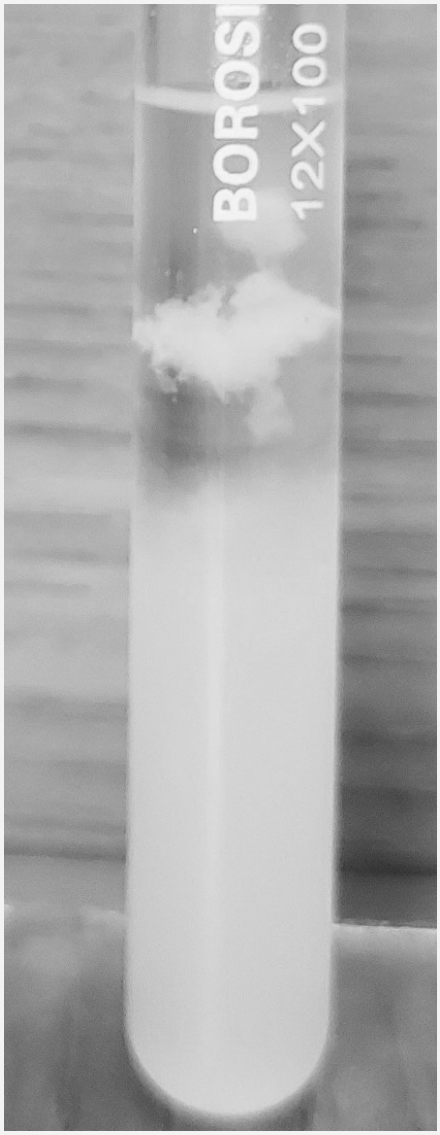
Optionally, add 2–3 drops of protease to 10ml (about 0.34 oz) of onion extract in a boiling tube and mix well.

Carefully pour ice-cold ethanol down the side of the boiling tube.

Let the tube sit undisturbed for a few minutes.

DNA will precipitate into the upper ethanol layer, appearing as white material.

RESULT



OBSERVATIONS

The photo shows the result of mixing a part of an onion with detergent and salt, and then layering ice-cold ethanol on top of the mixture. DNA appears as the white precipitate in the alcohol layer.

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