G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Onion Genomic DNA Isolation

Teacher's Handbook

(Cat. # BE-316)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students.)

- 6 Tubes & Pestles
- 6 Razor Blades
- 1 vial Protease: Dry Proteases
- 1 bottle DNA Release Buffer
- 1 bottle Precipitation Solution
- 1 bottle DNA Salt Solution
- 60 2ml Centrifuge Tubes

SPECIAL HANDLING INSTRUCTIONS

- All reagents can be stored at room temperature

The majority of reagents and components supplied in the *BioScience Excellence*™ kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
344P	Precipitation Solution	Flammable

ADDITIONAL EQUIPMENT

- Onion
- Waterbath or beaker and thermometer
- Mini Centrifuge
- Agarose Gel Equipment (optional)
- DNA Loading Buffer (optional)
- 70% Ethanol (Optional)

TIME REQUIRED

- **Day 1:** 2 hours

OBJECTIVES

- Isolate onion genomic DNA.

BACKGROUND

DNA, deoxyribonucleic acid, is the molecule of life. Every living organism has DNA in each cell of the organism and each molecule of DNA carries the blueprint for that organism. The DNA molecule is also responsible for heredity, passing on genetic information from parents to child.

DNA molecules are large strands or chains of small molecules known as nucleic acids, which are localized in the nucleus of a cell. This kit allows students to break open plant cells and their nuclei to release the genomic DNA using a mechanical disruption and a protease to digest away the cell and nuclear walls. Once released, the genomic DNA is visualized by the addition of a precipitating solution (alcohol) and high salt, which causes the DNA to precipitate and become visible.

TEACHER'S PRE EXPERIMENT SET UP

1. Heat a waterbath or heating block to 50-55°C is required for efficient release of the genomic DNA. A beaker with warm water and a thermometer can also be used.

2. Using a fresh onion, supply each group with six 1cmx1cm pieces of the onion layer. Each group will need a hard surface, such as a plate, to dice their onion pieces.

3. If extra vials are available, aliquot the reagents for each group as indicated in the following section.

4. Prior to the commencement of the experiment, add 0.5ml Sterile Water to the vial of dry protease to rehydrate. Mix by inverting the vial several times until a white suspension is visible. This solution can be stored frozen for up to 1 week.

MATERIALS FOR EACH GROUP

- 1 Tube and Pestle
- 6 Onion Samples
- 1 Razor Blade
- 0.8ml DNA Release Buffer
- 80µl Protease
- 0.5ml DNA Salt Solution
- 4ml Precipitation Solution
- 8 2ml Centrifuge Tubes

PROCEDURE

1. Label two 2ml Centrifuge Tubes with your name.
2. Taking it in turns finely dice your onion piece with the razor blade and transfer to the tube with the pestle. Crush and grind the onion pieces until a homogenous, smooth paste is achieved.
3. Add 0.2ml DNA Release Buffer to the tube containing the onion paste. Invert the tube several times to slowly mix and transfer the sample from the grinding tube to one of your labeled tubes. The DNA Release Buffer breaks open the onion cells releasing the DNA.
4. Add 0.02ml Protease to the tube to digest and remove the cellular material and protein and release the genomic DNA.
5. Close the cap. Briefly mix by inverting the tube 5-6 times and then place in a 50-55°C waterbath or heating block for 1 hour.
6. After 1 hour, add 0.1ml DNA Salt Solution to the tube and mix by inverting the tube several times. The salt solution aids in the precipitation of the DNA.
7. Centrifuge the tube for 5 minutes at 5,000xg to pellet the cell debris. Transfer the supernatant to your other labeled tube.
8. Add 0.8ml Precipitation Solution, close the tube and, whilst watching, slowly invert the tube several times to mix. White DNA strands may appear.
   OPIONAL: The genomic DNA can be visualized on an agarose gel. Follow the steps below to prepare genomic DNA for agarose electrophoresis.
   OPTIONAL: To pellet the DNA centrifuge the tube at 14,000rpm for 10 minutes. A tight white pellet should be visualized.
   OPTIONAL: Remove the Precipitation Solution and wash the pellet with 0.5ml 70% ethanol and centrifuge as before. Remove the 70% ethanol and leave the open tube at room temperature for 10-15 minutes to dry. Resuspend in 30μl water and load 10-20μl on a 1% agarose gel to visualize the genomic DNA.

RESULTS, ANALYSIS & ASSESSMENT

What is the role of the DNA release buffer?

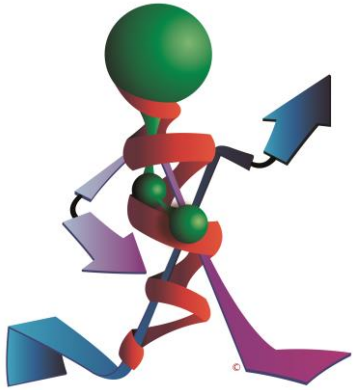
The DNA Release Buffer is responsible for breaking open the onion cells to release the genomic DNA into the solution.

Describe the genomic DNA:

The genomic DNA forms thin white strands on addition of the Precipitation Solution, which condense into a tight white pellet on centrifugation.

This page is intentionally left blank

This page is intentionally left blank



www.GBiosciences.com

G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Onion Genomic DNA Isolation

Student's Handbook

(Cat. # BE-316)

OBJECTIVES

- Isolate onion genomic DNA.

BACKGROUND

DNA, deoxyribonucleic acid, is the molecule of life. Every living organism has DNA in each cell of the organism and each molecule of DNA carries the blueprint for that organism. The DNA molecule is also responsible for heredity, passing on genetic information from parents to child.

DNA molecules are large strands or chains of small molecules known as nucleic acids, which are localized in the nucleus of a cell. This kit allows students to break open plant cells and their nuclei to release the genomic DNA using a mechanical disruption and a protease to digest away the cell and nuclear walls. Once released, the genomic DNA is visualized by the addition of a precipitating solution (alcohol) and high salt, which causes the DNA to precipitate and become visible.

MATERIALS FOR EACH GROUP

- 1 Tube and Pestle
- 6 Onion Samples
- 1 Razor Blade
- 0.8ml DNA Release Buffer
- 80µl Protease
- 0.5ml DNA Salt Solution
- 4ml Precipitation Solution
- 8 2ml Centrifuge Tubes

PROCEDURE

1. Label two 2ml Centrifuge Tubes with your name.

2. Taking it in turns finely dice your onion piece with the razor blade and transfer to the tube with the pestle. Crush and grind the onion pieces until a homogenous, smooth paste is achieved.

3. Add 0.2ml DNA Release Buffer to the tube containing the onion paste. Invert the tube several times to slowly mix and transfer the sample from the grinding tube to one of your labeled tubes. The DNA Release Buffer breaks open the onion cells releasing the DNA.

4. Add 0.02ml Protease to the tube to digest and remove the cellular material and protein and release the genomic DNA.

5. Close the cap. Briefly mix by inverting the tube 5-6 times and then place in a 50-55°C waterbath or heating block for 1 hour.

6. After 1 hour, add 0.1ml DNA Salt Solution to the tube and mix by inverting the tube several times. The salt solution aids in the precipitation of the DNA.

7. Centrifuge the tube for 5 minutes at 5,000xg to pellet the cell debris. Transfer the supernatant to your other labeled tube.

8. Add 0.8ml Precipitation Solution, close the tube and, whilst watching, slowly invert the tube several times to mix. White DNA strands may appear.

OPTIONAL: Remove the Precipitation Solution and wash the pellet with 0.5ml 70% ethanol and centrifuge as before. Remove the 70% ethanol and leave the open tube at room temperature for 10-15 minutes to dry. Resuspend in 30µl water and load 10-20µl on a 1% agarose gel to visualize the genomic DNA.

RESULTS, ANALYSIS & ASSESSMENT

What is the role of the DNA release buffer?

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

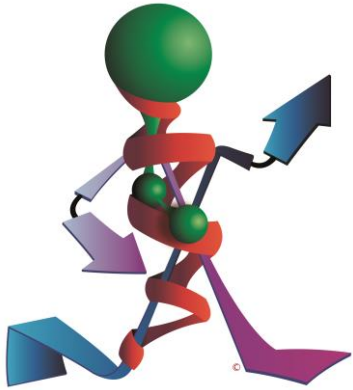
Describe the genomic DNA:

_____
_____
_____
_____
_____
_____
_____
_____
_____

Last saved: 8/11/2014 CMH

This page is intentionally left blank

This page is intentionally left blank



www.GBiosciences.com