Biotechnology Science for the New Millennium by Ellyn Daugherty

Genomic DNA Isolation

(Lab 4h)

(Cat. # BE-317)

Developed in partnership with

Genomic DNA Isolation (Lab 4H)

Teacher's Guide

The following laboratory activity is adapted from "Laboratory 4h: DNA Extraction from Bacteria" from *Biotechnology: Laboratory Manual* by Ellyn Daugherty. For more information about the program, please visit www.emcp.com/biotechnology. This kit is produced under license from Paradigm Publishing, Inc., a division of New Mountain Learning.

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About Ellyn Daugherty: Ellyn Daugherty is a veteran biotechnology educator and recipient of the Biotechnology Institute's National Biotechnology Teacher-Leader Award. She is the founder of the San Mateo Biotechnology Career Pathway (SMBCP). Started in 1993, SMBCP has instructed more than 10,000 high school and adult students. Annually, 30-40 SMBCP students complete internships with mentors at local biotechnology facilities.

About G-Biosciences: In addition to the Biotechnology by Ellyn Daugherty laboratory kit line and recognizing the significance and challenges of life sciences education, G-Biosciences has initiated the BioScience Excellence™ program. The program features hands-on teaching kits based on inquiry and curiosity that explore the fundamentals of life sciences and relate the techniques to the real world around us. The BioScience Excellence™ teaching tools will capture the imagination of young minds and deepen their understanding of various principles and techniques in biotechnology and improve their understanding of various social and ethical issues.

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Upon receipt, store the materials as directed in the package literature.

MATERIALS INCLUDED

This kit has enough materials and reagents for 8 lab groups (8 student pairs or 4 groups of 32 students).

- 9 vials of *E. coli*, cell pellet
- 1 vial LB Broth, 2ml
- 1 culture tube with LB Agar Slant
- 1 sterile loop
- 8 tubes of deionized water, sterile, 2 mL
- Transfer pipets (80), small
- 8 tubes of Protease, dry
- 8 tubes DNA Release Buffer, 800 µL
- 8 tubes of NaCl, 5*M*, 500 µL
- 8 tubes of Alcohol, 75%, 2 mL, store in freezer until ready to use
  CAUTION: *Alcohol is flammable. Keep away from flame or ignition sources.*
- 8 tubes DNA Precipitation Solution, 4 mL
- Centrifuge tubes (16), 2 mL
- 8 Weigh boats (3.5" x 3.5")
- 8 tubes of Nucleic DotMETRIC™ Dye, 0.6 ml
- 16 1µl Capillary Tubes
- 16 Nucleic dotMETRIC™ Assay strips
- 8 dotMETRIC™ Standard

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

- Permanent marker
- Paper towel
- Incubator or water bath, 37°C
- Beaker with tap water
- Water bath or beaker with water, floating micro test tube rack and thermometer, 60°C
- Vortexer
- Microcentrifuge (12,000xg)
- Disinfectant towels

OPTIONAL MATERIALS:

- P1000, P200, P10 micropipet and tips
- DNA Gel Electrophoresis Equipment and Reagents

SPECIAL HANDLING INSTRUCTIONS

- Store the tubes of ethanol (EtOH) in the freezer until ready to use.
- Store *E. coli* cell pellets and protease frozen until ready to use
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

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GENERAL SAFETY PRECAUTIONS

- The reagents and components supplied in the *Biotechnology by Ellyn Daugherty*™ kits are considered non-toxic and are safe to handle (unless otherwise noted), however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located and be versed in general lab safety.
- Remind students to read all instructions including Safety Data Sheets (SDSs) before starting the lab activities. A link for SDS for the chemicals in this kit is posted at www.gbiosciences.com.
- At the end of the lab, all laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory.

TEACHER'S PRE EXPERIMENT SET UP

1. Set up a class demonstration to show that the cell pellet is made up of bacteria cells.
   1) Preheat a heat block or water bath and/or shaking incubator to 37°C prior to starting the preparation of the bacterial broth culture.
   2) Wipe down the lab tabletop with disinfectant towels before starting broth culture. In the presence of the class, pour the sterile LB broth into the vial containing the cell pellet. Invert the vial several times until the cell pellet has dissolved and is suspended.
   3) Place the vial in a 37°C shaking incubator or water bath for 4-6 hours. Alternatively, leave at room temperature overnight.
      NOTE: **If possible, every few hours, swirl the vial vigorously to swirl in the air above the culture in the broth culture. Do not open the vial while swirling. Aeration improves cell division and increases the concentration of bacteria in the broth culture.*
   4) The next day, use a sterile loop to take a loopful of the cell broth culture and streak it back and forth on the surface of the LB agar in the slant tube.
   5) Place at 37°C and allow the slant culture to grow overnight. The next day, observe the colonies and bacteria in the streaks on the agar.
2. Briefly centrifuge all small tubes and vials before opening to prevent waste of reagents.
3. Place the 8 tubes of EtOH on ice to chill at least 30 minutes prior to students starting the lab protocol.

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4. Distribute the materials to each group:
    - *E. coli*, cell pellet, vial
    - Deionized water, sterile, 2 mL
    - Transfer pipets (10), small
    - DNA Release Buffer, 800 µL
    - Protease, dry
    - NaCl, 5*M*, 500 µL
    - DNA Precipitation Solution, 4 mL
    - Alcohol 70%, 2 mL, store in freezer until ready to use
    - ***CAUTION:*** *Alcohol is flammable. Keep away from flame or ignition sources.*
    - Centrifuge tubes (2), 2 mL
    - Weigh boat
    - Nucleic DotMETRIC™ Dye (1), 0.6 ml
    - 1µl Capillary Tubes (2)
    - Nucleic dotMETRIC™ Assay strips (2)
    - dotMETRIC™ Standard (1)
    - 1 piece of paper towel
5. Place all bacteria-contaminated pipets and tips into a 10% bleach solution. Allow contaminated items to soak for 30 minutes before disposing.

TIME REQUIRED

- 1 hour for teacher preparation of cultures and distribution of reagents
- 2-hour lab period for genomic bacterial DNA isolation
- 1-hour lab period for DNA analysis (G-Biosciences Nucleic dotMETRIC™ Assay)
- 1 hour for post lab analysis

NEXT GENERATION SCIENCE STANDARDS ADDRESSED

- HS-LS1: From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

For more information about Next Generation Science Standards, visit: http://www.nextgenscience.org/

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EXPECTED RESULTS

Observations of Sample during Genomic DNA Isolation

Step in Protocol	Appearance	Color	Viscosity	Comments/Explanations
Water mixed with bacteria pellet	homogenous	light brown or tan	thick	cloudy, no lumps cells/sample suspended
Cell Suspension after adding Release Buffer, protease, NaCl and heat	no longer homogenous	clear with white floating	goopy white in clear liquid	like egg whites in water
Sample after centrifugation at Step 7	pellet with liquid (supernatant) on top	white pellet with clear liquid	dense pellet/smear on bottom (precipitant)	cellular waste (protein and other cell structures) precipitate. DNA in supernatant
Supernatant after adding DNA Precipitation Solution supernatant solution	liquid with some bubbles trapped	clear to opaque white liquid	Maybe some goopy strands visible	pure DNA is clear so it may be hard to see in solution
Sample after centrifugation at Step 10	small, clear pellet with liquid (supernatant) on top	clear pellet	loose, goopy DNA pellet	pure DNA is clear so it may be hard to see the pellet

NUCLEIC dotMETRIC™ indicator = close to 0.9µg/30µl (= 0.03 µg/µl) of sample depending on the cell pellet concentration

ANSWERS TO DATA ANALYSIS QUESTIONS

- Describe the appearance and quantity of DNA (pellet) extracted from the bacterial cell sample compared with the starting cell pellet sample.
    - Answer: A clear, smeared precipitant of pure DNA is the desired outcome at Step 10. The original cell pellet is slightly bigger and a light brown color due to the other macromolecules in cells. These are removed during the isolation protocol.
- Approximately 1 mg of DNA is needed to see a DNA band on an agarose gel. Based on the indicator testing, does your sample yield suggest that you should be able to visualize your isolated DNA samples during a gel electrophoresis?
    - Answer: Answers will vary but if there is a concentration of 0.1 µg/µl of genomic DNA in a sample, then loading a 10-20 microliters of sample on a gel should result in a visible band or smear after staining.
- Protein makes up about 75% of the dry weight of a cell. What steps in the protocol are used to try to decrease the amount of protein contamination in the final sample?
    - Answer: The steps in the protocol that are used to try to decrease the amount of protein contamination in the final sample include using a protease, DNA release buffer, NaCl treatment, heating the sample, and pelleting the protein waste by centrifugation.

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OBJECTIVES

What concentration of genomic DNA can be isolated from a bacteria cell sample?

BACKGROUND

DNA molecules carry the instructional code for how all molecules are constructed in an organism.

Sections of DNA, called genes, are responsible for protein production. Since proteins do the work in cells, the characteristics of an organism are due to which proteins are made at a given time. When DNA molecules are passed on to other cells, the DNA genetic code for protein production is passed.

DNA molecules are called nucleic acids since they are acidic in nature and were first found localized in the nucleus of most cells. Nucleic acids are very long strands composed of smaller molecules called nucleotides. The nucleotides are chained together like beads on a necklace. Each nucleotide is made up of 3 smaller molecules (a nitrogenous base, a sugar, and a phosphate group). In solution, phosphate groups have a negative charge and since phosphate groups stick out along the nucleic acid chain, it makes the long strands of DNA negatively charged. DNA's characteristic negative charge makes it rather easy to separate DNA from other molecules.

Negatively charged DNA molecules are hydrophilic and dissolve well in watery solutions. On the other hand, charged DNA molecules are repelled by nonpolar solutions such as alcohol. Scientists exploit this characteristic, and use alcohol to isolate DNA by precipitating (separating) it out of solution. This kit uses alcohol precipitation and centrifugation to isolate DNA strands from other molecules found in cells.

Maybe you have experienced spooling DNA strands from prepared solutions. Alcohol precipitation and DNA spooling can be used to pull long strands of DNA out of a solution and wrap them around a glass rod. The tighter the strands are spooled around the rod, the more water is pushed out of the solution and the DNA around the rod goes from looking like goopy mucous to white strands. Pure, dry crystalline DNA is white.

In research labs, DNA spooling is not done. Instead, DNA isolation is done in micro test tubes using centrifugation and alcohol precipitation. In this activity, bacteria cells are broken open using a buffer containing a detergent and then a protease treatment. When the bacteria cells burst, they release DNA, and the detergent and protease initiate the breakdown of contaminant proteins. Heat and high salt washes denature more protein contaminants. Using a centrifuge, cellular waste is pelleted and discarded. The genomic DNA, floats in the supernatant above the waste and is pipetted off and precipitated into a clean pellet using alcohol and high-speed centrifugation. The pelleted DNA is dissolved in TE Buffer and stored at -20°C for later use. Samples of the purified genomic DNA may be studied using indicators or gel electrophoresis.

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MATERIALS FOR EACH GROUP

- *E. coli*, cell pellet, vial
- Deionized water, sterile, 2 mL
- Transfer pipets (10), small
- DNA Release Buffer, 800 µL
- Protease, dry
- NaCl, 5*M*, 500 µL
- DNA Precipitation Solution, 4 mL
- Alcohol 70%, 2 mL, store in freezer until ready to use
  CAUTION: *Alcohol is flammable. Keep away from flame or ignition sources.*
- Centrifuge tubes (2), 2 mL
- Weigh boat
- Nucleic DotMETRIC™ Dye (1), 0.6 ml
- 1µl Capillary Tubes (2)
- Nucleic dotMETRIC™ Assay strips (2)
- dotMETRIC™ Standard (1)

ADDITIONAL MATERIALS FOR EACH GROUP

The following standard lab equipment should be available for each group.

- Permanent marker
- Paper towel
- Beaker with tap water
- Water bath or beaker with water, floating micro test tube rack and thermometer, 60°C
- Microcentrifuge (12,000xg)
- Disinfectant towels

PROCEDURE

* ***NOTE***: *If micropipets and tips are available, use them instead of the transfer pipets.*

1. Using a small transfer pipet, transfer 1 mL of sterile water to the bacteria pellet vial. Vortex or shake the vial vigorously to suspend the bacteria sample in a homogenous (no lumps) mixture.

2. Using a different small transfer pipet, add 500 µL of sterile water to the dry protease tube. Mix by inverting five times.

3. Label two 2 mL centrifuge tubes with a group ID and using a small transfer pipet, transfer 0.2mL of the *E. coli*, cell suspension to one of the 2 labeled tubes. Save the other tube for Step 8.

4. Using a different small transfer pipet, add 200 µL of DNA Release Buffer to the *E. coli* cell suspension. Mix by gently inverting five times. The DNA Release Buffer has detergent in it. Avoid foaming. The release buffer breaks the cells open and releases DNA into the suspension.

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5. Using a different small transfer pipet, add 200 µL of protease to the tube. Make sure the cap is completely closed. Invert gently 6 times to mix and place tightly capped tube in the 60°C water bath for 15 minutes.

6. After 15 minutes, using a different small transfer pipet, add 100 µL of the NaCl solution to the tube. Invert 3 times to mix.

7. Move the tube to a microcentrifuge. Find another group's tube to balance your tube on the centrifuge. Centrifuge the tubes for 5 minutes at 5000xg. The protease, salt solution and centrifugation precipitate protein waste at the bottom of the tube as a white, cream, or gray pellet. DNA should be suspended in the supernatant liquid above the protein pellet.
   NOTE: *Orient the tube so that the hinge of the cap faces out. This way the precipitated protein waste pellet will be pushed out and be on the bottom of the tube, under the hinge.*

8. Using a different small transfer pipet, carefully pipet the clear supernatant to the other labeled tube (from Step 3).

9. Using a different small transfer pipet, add 800 µL of DNA Precipitation Solution to the clear DNA supernatant solution. Invert gently, while watching the tube contents. White DNA strands may appear.

10. To prepare the bacteria genomic DNA for additional analysis, centrifuge the DNA sample tube (with another team's sample as a balance tube) at 12,000Xg for 10 min. A clear or whitish pellet of DNA will form on the bottom/side of the tube.
    NOTE: *Orient the tube so that the hinge of the cap faces out. This way the DNA pellet will be pushed out and be on the bottom of the tube, under the hinge.*

11. Using a transfer pipet, remove all of the DNA Precipitation solution and using another transfer pipet wash the pellet with 500 µL of 70% alcohol. Centrifuge the DNA sample tube (with another team's sample as a balance tube) again at 12,000Xg for 5 min. Remove all of the alcohol (gently invert the open tube touching a paper towel to draw off excess alcohol). When no odor of alcohol is evident, use a fresh pipet to resuspend the pellet with 30 µL sterile deionized water. Let the DNA go back into solution overnight at 4°.

12. After 2 days, using the G-Biosciences' NUCLEIC dotMETRIC™ indicator and test strips, estimate the concentration of DNA in the sample:

    a) Place an indicator test strip in the center of a weigh boat.

    b) Place the tip of a 1µl Capillary tube into the sample DNA and watch the solution move up the tube.

    c) Hold the capillary tube vertical and touch the tip on the G-Biosciences' NUCLEIC dotMETRIC™ indicator test strips and allow all the solution to flow into the strip.

    d) Using the same capillary tube, repeat 2 more times so that there are 3 sample dots.

    e) Use a new capillary tube to make 3 dots with tap water (negative control) as above.

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f) Use a small transfer pipet to cover the strip with G-Biosciences' NUCLEIC dotMETRIC™ indicator solution. Wait 30 seconds to one minute and rinse off the indicator by dunking in a beaker of tap water.

g) Use the G-Biosciences' NUCLEIC dotMETRIC™ indicator key to estimate the concentration of DNA in the sample by matching the sample dots to the key's standards. Record the estimated concentration of the sample in the Data section.

Optional:

13. After several days, the DNA samples can be visualized on a 0.8% agarose gel. Use 20 µL samples mixed with DNA loading dye. The loading dye to be used is dependent on the electrophoresis buffer used. See www.gbiosciences.com for electrophoresis materials.

DATA ANALYSIS AND CONCLUSION:

Observations of Sample during Genomic DNA Isolation

Step in Protocol	Appearance	Color	Viscosity	Comments/Explanations
Water mixed with bacteria pellet				
Cell Suspension after adding Release Buffer, protease, NaCl and heat				
Sample after centrifugation at Step 7				
Supernatant after adding DNA Precipitation Solution supernatant solution				
Sample after centrifugation at Step 10				

Estimation of the concentration of the bacteria genomic DNA sample using the G-Biosciences' NUCLEIC dotMETRIC™ indicator _____

Data Analysis

1. Describe the appearance and quantity of DNA (pellet) extracted from the bacterial cell sample compared with the starting cell pellet sample.
2. Approximately 1 mg of DNA is needed to see a DNA band on an agarose gel. Based on the indicator testing, does your sample yield suggest that you should be able to visualize your isolated DNA samples during a gel electrophoresis?
3. Protein makes up about 75% of the dry weight of a cell. What steps in the protocol are used to try to decrease the amount of protein contamination in the final sample?

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