Biotechnology
Science for the New Millennium
by Ellyn Daugherty

Genomic DNA Spooling and DNA Estimation

(Lab 4B)

(Cat. # BTNM-4B)

Developed in partnership with

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Teacher's Guide

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

This kit is produced under license from Paradigm Publishing, Inc., a division of New Mountain Learning.

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.

Permission granted to make unlimited copies for use in any one school building. For educational use only. Not for commercial use or resale.

Copyright 2017 Geno Technology, Inc. All rights reserved.

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Teacher's Guide

MATERIALS INCLUDED 4

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED 4

OPTIONAL MATERIALS: 4

SPECIAL HANDLING INSTRUCTIONS 4

GENERAL SAFETY PRECAUTIONS 5

TEACHER'S PRE EXPERIMENT SET UP 5

TIME REQUIRED 6

NEXT GENERATION SCIENCE STANDARDS ADDRESSED 6

EXPECTED RESULTS 6

ANSWERS TO DATA ANALYSIS QUESTIONS 7

OBJECTIVES 8

BACKGROUND 8

MATERIALS FOR EACH GROUP 9

ADDITIONAL MATERIALS FOR EACH GROUP 9

PROCEDURE 9

DATA ANALYSIS AND CONCLUSION: 12

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Teacher's Guide

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).

- 8 Mini Beakers, plastic, capped
- 8 tubes of DNA, salmon testes, 2 mg/ml in TE buffer, 2 ml
- 24 Transfer pipets, large
- 8 tubes of NaCl, 5*M*, 250 µl
- 16 Transfer pipets, small
- 8 tubes of Ethanol (EtOH), 95%, 2 mL, store in freezer until ready to use
  CAUTION: *Alcohol is flammable. Keep away from flame or ignition sources.*
- 32 Wooden Spooling Sticks
- 8 6ml Non-sterile tubes
- 8 tubes of TE Buffer, 2 ml
- 8 Weigh boats (3.5" x 3.5")
- 8 tubes of Nucleic DotMETRIC™ Dye, 0.6 ml
- 16 1µl Capillary Tubes
- 8 Forceps
- 16 Nucleic dotMETRIC™ Assay strips
- 8 dotMETRIC™ Standard

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

- Permanent markers
- Paper towels
- Beakers of tap water
- Beakers with ice

OPTIONAL MATERIALS:

- DNA Gel Electrophoresis Equipment and Reagents

SPECIAL HANDLING INSTRUCTIONS

- Store the tubes of ethanol (EtOH) in the freezer until ready to use.
- Store tubes of salmon testes DNA, NaCl, and TE Buffer at 4°C (refrigerator) until ready to use. These reagents need to be kept chilled throughout the lab. Place vials on ice.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Teacher's Guide

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 EtOH and other 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. Briefly centrifuge all small tubes and vials before opening to prevent waste of reagents.

2. Distribute the reagents and other materials to each group:
   - Mini Beaker, plastic, capped
   - DNA, salmon testes, 2 mg/ml in TE buffer, 2 ml (***Keep on ice***)
   - Transfer pipets, large (3)
   - NaCl, 5*M*, 250 µl (***Keep on ice***)
   - Transfer pipet, small (2)
   - Ethanol (EtOH), 95%, 2 mL, chilled (***Keep on ice***)
     CAUTION*: Alcohol is flammable. Keep away from flame or ignition sources.*
   - Wooden spooling stick
   - 6ml Non-sterile tube
   - TE buffer, 2 ml
   - Weigh boat
   - Nucleic DotMETRIC™ Dye, 0.6 ml
   - 2 Nucleic dotMETRIC™ Assay strips
     NOTE: *The strips have a protective paper folded over them. Make sure to supply test strip in its protective paper.*
   - 1 forcep
   - 8 dotMETRIC™ Standard
   - 1µl Capillary Tube (2)
   - Permanent marker
   - Paper towel
   - Beaker with tap water
3. Demonstrate for students how to trickle the ethanol down the side of the mini beaker to make two distinct layers: DNA on the bottom and ethanol on the top.

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Teacher's Guide

4. Demonstrate good spooling technique, including the following: tilting the mini beaker to at least 45°, holding the spooling stick parallel to the floor, scooping up DNA from the bottom layer through the alcohol layer, and twirling it on the spooling stick.

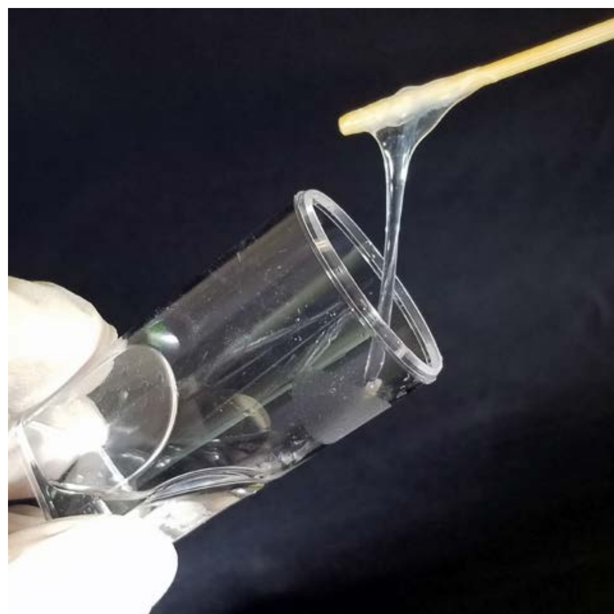
TIME REQUIRED

- 30 minutes for distribution of reagents and demonstration of spooling technique.
- 1-hour lab period for salmon testes DNA precipitation/spooling
- 2-day waiting period for spooled DNA sample to go back into solution
- 1-hour lab period for DNA analysis (G-Biosciences Nucleic dotMETRIC™ Assay)
- 30 minutes 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/.



EXPECTED RESULTS

Mucous-like globs of salmon testes DNA should be visible, trapped in bubbles at the interface between alcohol and the DNA solution. Long strands of clear salmon testes DNA should easily pull up out of solution to be spooled around the rod. Make sure students spool up and not let the strands slide off. Upon rehydration, samples should be approximately 1-2 mg/ml and can be run on an agarose gel where they will appear as a fat smear near the wells.

Observations of Genomic DNA during Alcohol Precipitation through Spooling

Step in DNA Spooling	Appearance	Color	Viscosity	Other Observations
Salmon DNA in Tube	not visible	clear	watery	
DNA with EtOH Layer	2 layers with bubbles at the interface	clear to whitish	goopy around bubbles	some strands from bottom to top layer
Spooled DNA	mucous-like	clear to whitish	thick goopy, stringy	more white as the sample is spooled tighter
Spooled DNA in TE Buffer (day of spooling)	thick goopy, stringy, bubbly	clear to whitish	Blobs floating in clear liquid	looks like it is getting less bubbly and goopy
Spooled DNA in TE Buffer (2 days after spooling)	not visible	clear	watery	looks like the DNA has disappeared into solution

Estimation of the concentration of the spooled DNA sample in TE Buffer after 2 days using the G-Biosciences' NUCLEIC dotMETRIC™ indicator ______1-2 µg/µl (= 1-2 mg/ml)______

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Teacher's Guide

ANSWERS TO DATA ANALYSIS QUESTIONS

1) Discuss how easily the DNA could be pulled out of solution in long, spoolable strands. If 100% of the salmon testes DNA was recovered during spooling and transferred to the 2 mL of TE buffer in step 9, what would the expected concentration of DNA be in that final sample.

Answer: The salmon testes DNA is easily pulled out solution if there are long, spoolable strands present, that are repelled by the cold ethanol, and a wooden spooling stick is present to scoop and spool the strands. If 100% of the salmon testes DNA were recovered during spooling and transferred to the 2 mL of TE buffer, the approximate concentration of DNA in the final sample would be 2 mg/ml (same as the starting sample). 2 mg/ml is equal to 2 µg/µl.

2) In this activity, you precipitated purified DNA, ordered from a biological supply house, out of a relatively pure solution. Which molecules did the supply company have to remove from the original cell source to purify this DNA?

Answer: Salmon testes cells are the original source of the spooled salmon testes DNA. Male salmon are given a low level electrical shock and they release salmon sperm cells into a vessel. The cells are exploded and the DNA is purified from the other cell macromolecules (mainly protein).

3) Discuss the value of learning how to isolate pure DNA molecules from a liquid solution. How would this technique be valuable to biotechnologists? For what purposes could the isolated DNA be used?

Answer: Isolated, pure DNA molecules are important for gene studies, PCR, DNA sequencing and fingerprinting, genetic engineering and many other molecular biology applications. Contaminant molecules such as RNA or proteins often interfere with these methods.

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Student's Guide

OBJECTIVES

Can strands of salmon testes DNA be recovered using alcohol precipitation and DNA spooling?

BACKGROUND

Each cell of every organism contains one or more copies of DNA. DNA molecules carry the instructional code for how all molecules will be constructed in that organism. The code is called the genetic code since 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 genetic code is passed.

DNA molecules are called nucleic acids since they are acidic in nature and are localized in the nucleus of most cells. Nucleic acids are very long strands composed of smaller molecules called nucleotides. Each nucleotide is made up of 3 groups; a nitrogenous base, a sugar, and a phosphate. Phosphate groups have a negative charge in solution and since phosphate groups stick out along the nucleic acid chain, it makes the long strands of DNA negatively charged.

Negatively charged DNA molecules are hydrophilic and go into watery solutions rather easily. However, charged DNA molecules are repelled by nonpolar solutions such as alcohol. Scientists exploit this characteristic, and use alcohol to isolate DNA by precipitating it out of solution. This kit uses alcohol precipitation to isolate long genomic DNA strands from a solution so that they may be spooled around a glass rod or wooden spooling stick and stored in a buffered solution for further study.

A known amount of salmon testes DNA solution is dissolved in TE buffer, mixed with NaCl, and placed in a vessel (beaker or tube). A layer of 95% ethanol is added to the DNA mixture. Using a glass rod or wooden spooling stick, the ethanol can be gently pushed down into the DNA solution. The charged DNA molecules move away from the ethanol and can be scooped up and swirled around a glass rod or wooden spooling stick. This is call **DNA spooling**. DNA spooling can isolate long strands of DNA from a solution. The tighter the strands are spooled, the more water is pushed out and, the DNA goes from looking like goopy mucous to white strands. Pure, dry crystalline DNA is white. Spooled DNA is scraped off the rod and mixed into a small amount of TE buffer for storage. After a day or two in the TE buffer, the DNA strands unwind and dissolve back into solution. Samples of the salmon DNA may be studied using indicators or gel electrophoresis.

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Student's Guide

MATERIALS FOR EACH GROUP

- Mini Beakers, plastic, capped
- DNA, salmon testes, 2 mg/ml in TE buffer, 2 ml
- Transfer pipets, large (3)
- NaCl, 5*M*, 250 µl
- Transfer pipet, small (2)
- Ethanol (EtOH), 95%, 2 mL, chilled
  CAUTION: *Alcohol is flammable. Keep away from flame or ignition sources.*
- Wooden Spooling Sticks (4)
- 6ml Non-sterile tube
- TE buffer, 2 ml
- Weigh boat
- 1µl Capillary Tubes
- Nucleic DotMETRIC™ Dye, 0.6 ml
- 1 Forceps
- 2 Nucleic dotMETRIC™ Assay strips
  NOTE: *The strips have a protective paper folded over them. Make sure to use the test strip not the protective cover.*
- 1 dotMETRIC™ Standard

ADDITIONAL MATERIALS FOR EACH GROUP

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

- Permanent marker
- Paper towel
- Beaker with tap water

PROCEDURE

1. Using a permanent marker, just below the cap of a capped mini beaker, label the mini beaker "S DNA" and add a group ID and the date. Label the 6ml Non-sterile tube in the same manner.

2. Using a large transfer pipet, transfer the entire contents of salmon testes DNA (2 ml) into the labeled mini beaker. Discard the pipet.

3. In the data table, describe and record the appearance, color, and viscosity of the 2-mg/ml salmon testes DNA sample.

4. Using a small transfer pipet, add the entire contents of the 5*M* NaCl solution (250 µl) to the salmon sperm DNA. Mix by swirling. Discard the pipet.

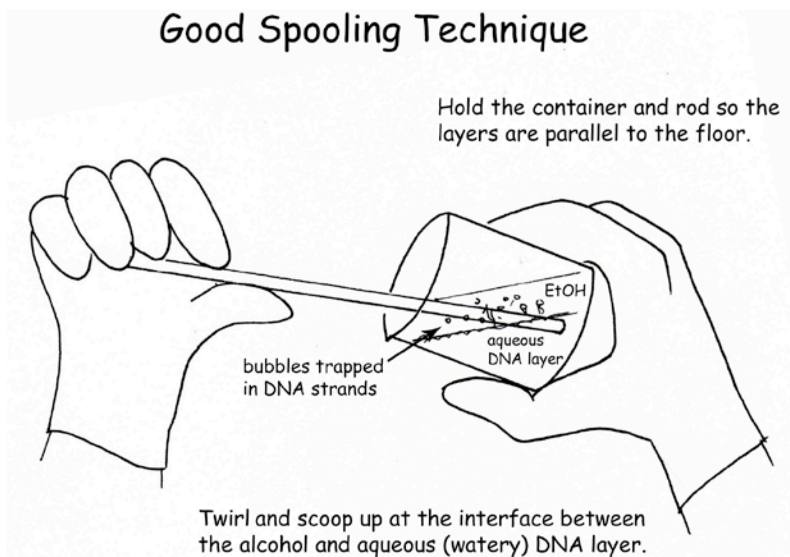
Genomic DNA Spooling and DNA Estimation (Lab 4B)

Student's Guide

5. Keep everything as cold as possible. Hold the plastic mini beaker near the rim to keep hands from warming the solutions." Using a large transfer pipet, slowly trickle the entire contents of EtOH (2 ml) down the side of the beaker containing the DNA and NaCl mixture. **Do not mix the alcohol and DNA layers.** Discard the pipet.

6. Observe the interface between the two solutions. You should see a layer of alcohol on the top of the layer containing the DNA and NaCl. Do not mix the two layers. Describe and record what each layer looks like.

7. Tilt the mini beaker without spilling the solutions or mixing the layers. Place the wooden spooling stick at the interface of the two-layered solution. Holding the mini beaker tilted to the right 45°, push the wooden spooling stick into the DNA layer and wind (spool) and pull up the DNA that comes out of solution onto the wooden spooling stick. Watch the interface as you rotate the stick. These are not single DNA molecules, but thousands of molecules. Spool the strands tighter and tighter to wind them around the stick and push the EtOH out. If there is still DNA in the mini beaker, have a lab partner use another wooden spooling stick to spool even more DNA. To increase the amount of DNA collected, gently stir the interface with the spooling stick to allow more ethanol to interact with the DNA.



8. Remove excess ethanol from the spooled DNA by placing a piece of paper towel just below the spooled sample and letting the EtOH absorb into the towel. Get rid of as much ethanol as possible without losing the DNA sample.

9. Examine and touch the DNA on the wooden spooling stick. Record the appearance of the DNA, including color, texture, and other characteristics. Touching the samples exposes it to the enzyme, DNase, which chops DNA. Normally, random chopping is not good for a DNA sample, but in this case it will shorten strands, which will facilitate further testing.

10. Scrape the spooled DNA into the labeled 6ml Non-sterile tube. Spool again to get all the remaining DNA out of the beaker. Make sure to remove excess ethanol.

11. Once all the DNA is spooled from the mini beaker, use a large transfer pipet, transfer the entire contents of TE buffer (2 ml) into the 6ml Non-sterile tube containing the spooled DNA. Cap the 6ml Non-sterile tube tightly so that no

leakage occurs. Keep the mini beaker upright. Observe the spooled DNA in the TE buffer. Can you see evidence of the DNA in the buffer?

12. Store the labeled tube, upright at 4°C (refrigerate) for several days. During that time, the DNA will unwind and go back into solution. At this time, the DNA is ready to use for indicator testing or gel electrophoresis.

13. After 2 days, using the G-Biosciences' Nucleic DotMETRIC™ Dye and assay strips, estimate the concentration of DNA in the spooled DNA sample in TE Buffer:

    a) Place an indicator test strip in the center of a weigh boat.
    NOTE: *The strips have a protective paper folded over them. Make sure to use the test strip not the protective cover.*

    b) Drop a a 1µl Capillary tube into the sample DNA and watch the solution move up the tube. Using the forceps, gently grip the 1µl Capillary tube and pull from the tube.

    c) Hold the capillary tube vertical with your fingers and touch the tip on the G-Biosciences' NUCLEIC dotMETRIC™ assay strips and allow all the solution to flow into the strip. You can use the forceps to hold the strip in place.

    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.

    f) Use a small transfer pipet to cover the strip with G-Biosciences' NUCLEIC dotMETRIC™ dye. 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™ standard 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:

14. After several days, the stored DNA samples in TE buffer 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.

Genomic DNA Spooling and DNA Estimation (Lab 4B)

Student's Guide

DATA ANALYSIS AND CONCLUSION:

Observations of Genomic DNA during Alcohol Precipitation through Spooling

Step in DNA Spooling	Appearance	Color	Viscosity	Other Observations
Salmon DNA in Tube				
DNA with EtOH Layer				
Spooled DNA				
Spooled DNA in TE Buffer (day of spooling)				
Spooled DNA in TE Buffer (2 days after spooling)				

Estimation of the concentration of the spooled DNA sample in TE Buffer after 2 days using the G-Biosciences' NUCLEIC dotMETRIC™ indicator: ____________

1) Discuss how easily the DNA could be pulled out of solution in long, spoolable strands. If 100% of the salmon testes DNA was recovered during spooling and transferred to the 2 mL of TE buffer in step 9, what would the expected concentration of DNA be in that final sample.

2) In this activity, you precipitated purified DNA, ordered from a biological supply house, out of a relatively pure solution. Which molecules did the supply company have to remove from the original cell source to purify this DNA?

3) Discuss the value of learning how to isolate pure DNA molecules from a liquid solution. How would this technique be valuable to biotechnologists? For what purposes could the isolated DNA be used?

Last saved: 6/6/2018 CMH

www.GBiosciences.com