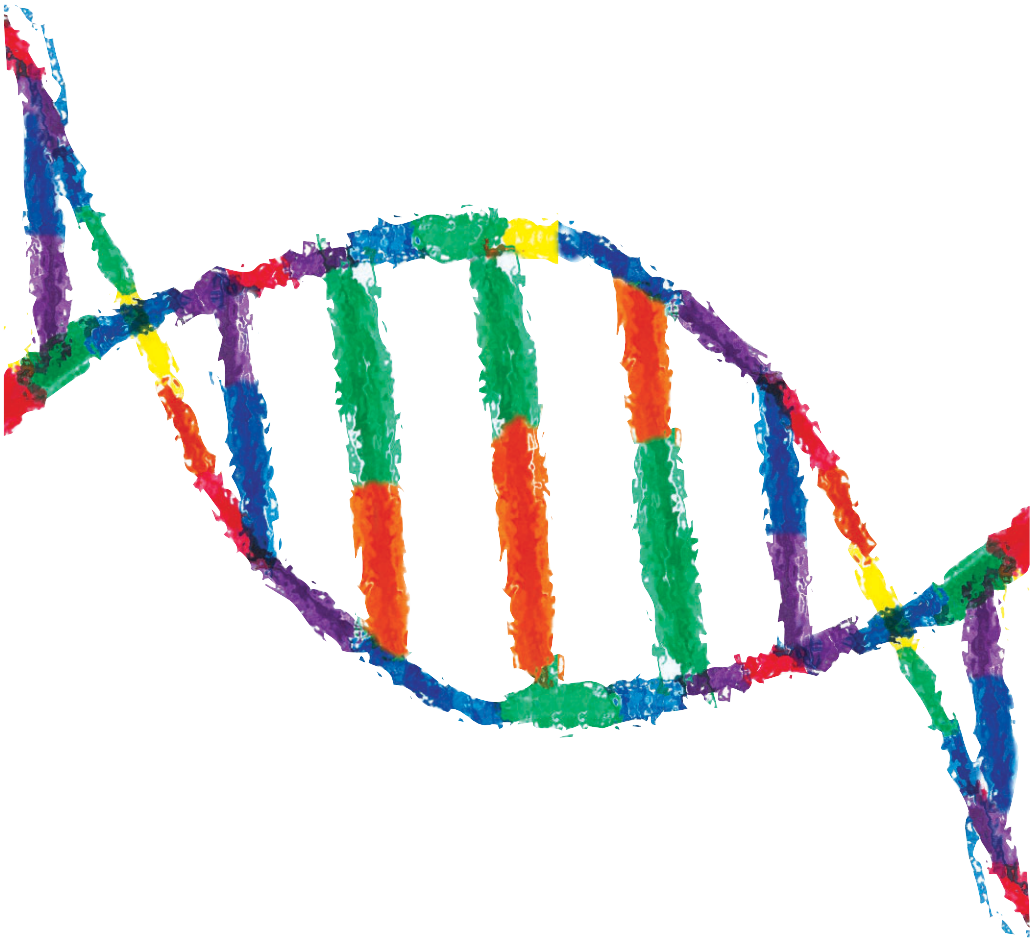
15-4773

# Extremely Easy DNA Extraction Kit

**TEACHER'S MANUAL WITH STUDENT GUIDE**



by Priya and Shiladitya DasSarma

CAROLINA
*World-Class Support for Science & Math*

# Extremely Easy DNA Extraction Kit

## Teacher's Manual

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About the authors

Priya and Shil DasSarma have studied *Halobacterium* sp. NRC-1 for over 20 years. They are enthusiastic about making these safe, convenient microbes accessible to educators and students at all levels. Visit their website at http://www.carolina.com/life_science/halobacteria for more information, ideas, and contact information.

**On the cover:** DNA double helix by Anjali DasSarma, age 6, using colored pens.

# Extremely Easy DNA Extraction Kit

## Summary

In this activity, students extract deoxyribonucleic acid (DNA) from *Halobacterium* sp. NRC-1, an organism of the domain Archaea. Extracting and observing DNA gives students hands-on experience that can demystify DNA and enable them to better understand some of the properties of this amazing molecule. This activity is ideal for introducing *AP® Biology Lab 6: Molecular Biology*, for teaching the structure and function of DNA, and for demonstrating the process of DNA extraction.

Using techniques similar to those used in top research laboratories, students lyse *Halobacterium* cells, transfer the lysate to a test tube, and then add alcohol to the lysate to bring the DNA out of solution. As the DNA strands precipitate out at the interface between the aqueous cell lysate and the alcohol, students wind the DNA strands onto a spooling stick, forming a mass of DNA that can be observed without magnification.

This Teacher's Manual contains preparation and classroom management information, an overview of *Halobacterium* sp. NRC-1, lists of sources of additional information, a glossary, and a reproducible blackline master Student Guide. The Student Guide includes background information for students, step-by-step DNA extraction instructions, assessment questions, and a bioinformatics link. Materials in this kit are sufficient for 32 students working individually.

**Note:** This kit requires the purchase of Carolina item RN-15-4772, the *Basic Microbiology Skills Part 2* kit, as a source of *Halobacterium* sp. NRC-1.

## Content Standards

This kit is appropriate for intermediate students from middle school to college and meets the following National Science Content Standards:

**Grades 5–8**
**Life Science**
- Structure and function in living systems
- Reproduction and heredity

**Grades 9–12**
**Life Science**
- The cell
- Molecular basis for heredity

## Background

**Deoxyribonucleic acid (DNA)**, the genetic material in all living cells, is a **double helix** formed from two **antiparallel polynucleotide** chains or strands made up of **nucleotide** bases, **Adenine** (A), **Cytosine** (C), **Guanine** (G), and **Thymine** (T). The bases obey the strict Watson and Crick rules of base pairing: A always pairs with T, and G always pairs with C. DNA resembles a ladder that is twisted so that there is one turn for every ten and one-half rungs, or bases.

DNA forms very long molecules, much longer than the cell in which it is found. The length of DNA can be calculated by multiplying the distance between bases, 3.4 **Ångstroms** (Å) (100,000,000 Å = 1 cm), by the number of bases in the chromosome, which typically is 1–5 million for **prokaryotes** and about 3 billion for humans. For prokaryotic microorganisms, which are only one or a few micrometers ($\mu$m) long, the chromosomal DNA may be 1 millimeter (mm), or almost one thousand times longer than the cell! In order to fit inside the cell, chromosomal DNA is packaged into coils and supercoils.

While DNA can form very long molecules, the thickness of a DNA ladder is only 20 Å, or about 1/50,000$^{th}$ the width of a human hair (50–150 $\mu$m). Because of its minute size, individual DNA molecules must be visualized using a special microscope called an electron microscope. In order to be visualized by an electron microscope, the DNA must be coated with proteins and a heavy metal, such as platinum. This coating thickens the DNA strand and creates enough contrast between the DNA and the surrounding area for it to be seen with the electron microscope (Figure 1).

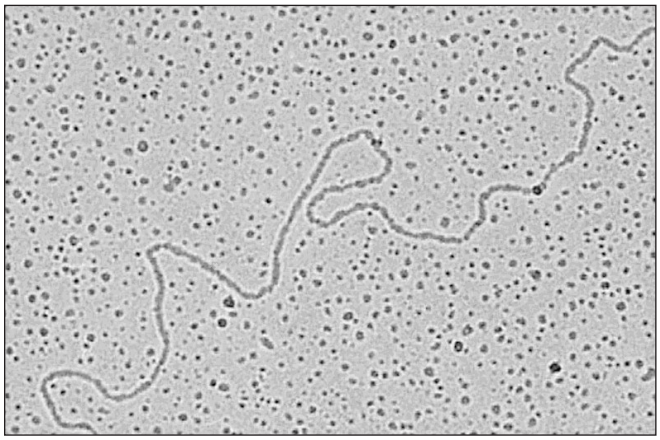

**Figure 1**. Electron micrograph of DNA by Shiladitya DasSarma.

DNA possesses the special property of replication, forming two identical progeny molecules from a single parental molecule (this is the reason that it has evolved to serve as the genetic material in cells). For this process, the two antiparallel DNA strands are first partly **denatured**, or separated, by the action of proteins called DNA **helicases**. Then, each DNA strand serves as the template for synthesis of the **complementary** strand using Watson and Crick base pairing of nucleotide building blocks, utilizing an **enzyme** known as **DNA polymerase**. A complication in this process is that DNA synthesis does not occur *de novo* (from scratch). The DNA polymerase must have something to which it attaches the first base pair in the DNA chain. So, first, a short piece of RNA, a **primer**, is laid down by another enzyme, called **primase**. In creating this primer, analogous Watson and Crick rules are followed, except that the RNA base **Uracil** (U) is used in place of the DNA base T. The DNA polymerase extends the chain of DNA from this primer. Later, the RNA primer is removed and replaced by DNA. Another twist in the DNA replication story is that DNA chains are always synthesized in one direction, called 5′ to 3′, and never in the opposite direction, 3′ to 5′ (Figure 2). This

leads to there being a **leading strand** in replication, which is formed using only one RNA primer, and a **lagging strand**, whose synthesis requires many RNA primers. During replication, the accuracy of the DNA replication is checked by the editing activity of DNA polymerase itself, as well as by a host of other repair proteins. In this way, genetic information is faithfully transmitted from one generation to the next.

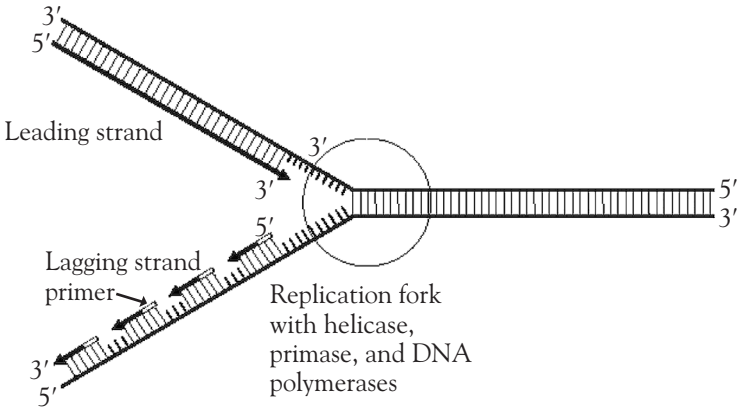


**Figure 2**. DNA synthesis.

In this lab, students wind DNA strands onto spooling sticks, forming a mass of DNA that can be observed without magnification. What is the relationship between the mass of DNA seen on the sticks and the DNA visualized and manipulated by scientists in labs? If prepared for observation by electron microscopy, individual DNA strands could be visualized. If you conducted electrophoresis of the DNA on an agarose gel and then stained it with a DNA stain, it could be visualized, but only as a collection of molecules (a smear on the gel). During a crude DNA prep, the DNA is broken into a heterogeneous mixture of strands of different sizes. This is because most cellular DNA is broken into pieces by the forces used to break open the cells. In addition, the DNA is degraded by nucleases, enzymes that are found naturally in all cells. In order to see the three components of the *Halobacterium* genome (see below), the cells would have to be lysed while embedded in a gel to avoid mechanical breakage of the DNA.

For restriction enzyme digestion, the DNA would require further purification. Otherwise, the proteins (including nucleases) and other impurities associated with the DNA would inhibit the restriction enzyme digestion and/or degrade the DNA.

## Extracting DNA from *Halobacterium* sp. NRC-1

Many cells have tough cell walls to protect them from their environment. DNA studies require breaking open the cell in order to extract the DNA. Breaking down cell walls often requires harsh chemical or physical methods that are technically challenging and frequently damage the DNA. However, with extreme **halophiles** like ***Halobacterium* sp. NRC-1**, the cells can be broken open easily, as the cell membrane is weakened with **hypotonic** solutions—solutions possessing lower solute concentration than the cell's interior (e.g., pure water).

Students will use water to lyse the *Halobacterium* cells. The cell envelope of *Halobacterium* cells has a unique composition that is easily broken down in the presence of pure water. In addition, osmotic pressure will cause water to flood into the high-salt interior of the cell. (**Note:** *Halobacterium* cells maintain an internal salt concentration equal to the high-salt concentration of their environment.) The cell **lysate** that results from cell lysis contains a solution of cell components including RNA, proteins, and, most importantly for the purpose of this lab, DNA.

As soon as the DNA is released, it becomes susceptible to breakage. Therefore, your students should handle the cell lysate gently. The more the solution is stirred and shaken, the smaller are the resulting DNA fragments. If the fragments become too small, the students will not be able to spool them.

To precipitate the DNA so it can be wound onto a spooling stick, students must add alcohol to the cell lysate. DNA is highly soluble in water, but is insoluble in alcohol (such as ethanol and isopropanol) and in mixtures of alcohol and water. Thus, as alcohol is added to the cell lysate, the insoluble components, including the DNA, **precipitate** out of the solution. The DNA that precipitates is in the form of long, fibrous molecules, which can be spooled by winding them around a stick. Remember, the DNA that students extract will be crude and will include a lot of proteins and RNA. If one wished to analyze the DNA, it would have to be further purified.

## *Halobacterium* sp. NRC-1

*Halobacterium* sp. NRC-1 was among the first organisms to have its **genome** sequenced. As a result of this sequencing, scientists have discovered many interesting features, among them the microbe's three **chromosomes**: one large chromosome and two minichromosomes. In total, the genome of *Halobacterium* sp. NRC-1 contains 2,570,010 nucleotide base pairs and 2,682 predicted genes.

*Halobacterium* sp. NRC-1 is a member of the domain **Archaea**. Like other members of this domain, *Halobacterium* sp. NRC-1 has similarities to both bacteria (its small cell size and lack of a membrane-bound nucleus) and to higher eukaryotic organisms (such as its DNA replication and transcription systems). This microbe is an extreme halophile (salt lover), requiring a nearly saturated concentration of salt (~4 M NaCl) to maintain optimal membrane stability. *Halobacterium* sp. NRC-1 can survive the fluctuating conditions of its natural habitat, places like the Great Salt Lake and the Dead Sea. These conditions include extremes of desiccation and exposure to UV radiation, as well as low oxygen levels and low levels of organic material. The microbe also uses **bacteriorhodopsin**, found in regions of its cell membrane (called purple membrane) to produce ATP in a process similar to photosynthesis. This process is called phototrophy. Other pigments called **carotenoids**, which help protect the microbe from UV light, are what give the microbe its distinctive color. All of the characteristics that allow the microbe to live in the conditions discussed above are created and controlled by genes contained within the organism's genome (DNA).

### *Halobacterium* in the Classroom

Because it cannot survive outside of its extreme environment, *Halobacterium* sp. NRC-1 is ideal for use in the science classroom. It is destroyed by exposure to the low salt concentrations found in the human body, tap water, etc., and the media it does grow in is not tolerated by most other organisms. The American Type Culture Collection (ATCC) classifies the microbe at Biosafety Level 1 (BSL-1). Materials classified at BSL-1 "are not known to cause disease in healthy adult humans."

## Materials

The materials in this kit are sufficient for 32 students working individually to perform the activity. The materials are supplied for use with the educational exercises in this kit only. Carolina Biological Supply Company disclaims all responsibility for other uses of these materials.

*Included in the kit*

32 15-mL test tubes
64 3-mL bulb pipets (for adding alcohol and water)
32 spooling sticks
16 funnels
10 spreaders
Teacher's Manual with photocopy masters

*Needed, but not supplied*

*Halobacterium* sp. NRC-1 lawns (cultured using *Basic Microbiology Skills Part 2* kit, Carolina Biological Supply item RN-15-4772)
70% isopropyl alcohol or 95% ethyl alcohol, chilled
tap water
timer, clock, or watch
containers with ice
forceps (optional)

## Time Requirements

This activity can be completed in one class period. Some pre-lab preparation, including *Halobacterium* culturing (7–14 days or more, depending on growth conditions), is required.

## Teacher Preparation and Presentation

You will need to

- read the entire booklet to familiarize yourself with the information and instructions given to the students.
- photocopy sets of student guides for individuals or teams.
- decide how and when to share additional background information with students.
- set up workstations for students.

This kit is designed to allow instructors to tailor the lab to meet their own unique classroom conditions. Students may perform the DNA extraction in pairs, but will benefit most from individual, hands-on experience with the process. Use the *Basic Microbiology Skills Part 2* kit (Carolina item RN-15-4772) to culture one *Halobacterium* sp. NRC-1 lawn for each student or pair of students. Materials sufficient for at least 32 students are supplied in the *Basic Microbiology Skills Part 2* kit. If you use the *Basic Microbiology Skills Part 2* kit solely as a source of *Halobacterium*, you do not need to perform the serial dilution portion of that kit.

In addition to one *Halobacterium* lawn, each student or group should have one test tube, two 3-mL bulb pipets, and one spooling stick. The 16 funnels and 10 spreaders in this kit are to be shared among students. Devise a system to make tap water available to students, as they will pour approximately 5 mL onto each petri plate. You may wish to distribute water in paper cups, or have students use their 3-mL bulb pipets to measure and deliver the desired volume of water. Likewise, implement a method of storing, chilling, and distributing the alcohol that students will add to their test tubes. It is crucial that the alcohol is cold when it is added to the lysate. You may wish to set up individual cups or extra test tubes of alcohol in a container of ice, or have students draw alcohol into their pipets from a chilled central container. Alternatively, we suggest that you supply students individually or have several supply locations. This will avoid bottlenecks in the classroom. Finally, a timekeeping device is needed to monitor the cell lysing time; wall clocks, stopwatches, or wristwatches will suffice.

## Answers to Student Worksheet Questions

1. Answers will vary, but may include any of the following:
   - DNA may be isolated from any organism to study the organism or to use its DNA to bioengineer another organism.
   - DNA may be isolated from crime sites to find evidence to help solve the crimes.
   - DNA may be isolated from ancient material, such as insects trapped in amber, in order to study and solve archaeological problems. (Read more about this at http://www.archaeology.su.se/arklab/dnaextr.htm, or read *Jurassic Park* for a fictionalized account.)
   - DNA may be isolated from organisms, including humans, to understand, diagnose, and treat genetic diseases.
2. Drawings may vary, but should resemble a ladder with rungs. Make sure that the bases are paired and labeled correctly.
3. Answers will vary, but should be an accurate representation and interpretation of what students saw. Students should draw and label the two solutions, and indicate where the interface layer and DNA are found.
4. Most other organisms require harsher conditions for cell lysis, such as the addition of detergents and other denaturing chemicals, and/or strong mechanical forces. This is because of the difference in the cell membrane and cell wall and the intracellular ionic conditions of *Halobacterium* as compared to nearly all other organisms.

## Online Resources

The DasSarma Team would be happy to discuss projects with students and educators and can be reached by email: dassarma@comcast.net.

**Note:** *At the time of this printing, the following Web sites are active. You may wish to have students conduct additional Internet research.*

For more information on halophiles, consult the HaloEd Project's Web site: http://halo.umbi.umd.edu/~haloed

For more information on the genetic sequencing of *Halobacterium* sp. NRC-1, consult the *Halobacterium* genome database: http://halo.umbi.umd.edu

## Further Reading

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Ng, W.V., S.P. Kennedy, G.G. Mahairas, B. Berquist, M. Pan, H.D. Shukla, S.R. Lasky, N.S. Baliga, V. Thorsson, J. Sbrogna, S. Swartzell, D. Weir, J. Hall, T.A. Dahl, R. Welti, Y.A. Goo, B. Leithauser, K. Keller, R. Cruz, M.J. Danson, D.W. Hough, D.G. Maddocks, P.E. Jablonski, M.P. Krebs, C.M. Angevine, H. Dale, T.A. Isenbarger, R.F. Peck, M. Pohlschroder, J.L. Spudich, K.H. Jung, M. Alam, T. Freitas, S. Hou, C.J. Daniels, P.P. Dennis, A.D. Omer, H. Ebhardt, T.M. Lowe, P. Liang, M. Riley, L. Hood, S. DasSarma. 2000. Genome sequence of *Halobacterium* species NRC-1. *Proc. Natl. Acad. Sci*. USA 97:12176–81.

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## Glossary

**Adenine** one of the four nucleotide bases in DNA and RNA; adenine pairs with either thymine (in DNA) or uracil (in RNA).

**Ångstrom** a unit of length equal to $10^{-10}$ meter or 0.00000001 centimeters.

**Antiparallel** a term describing DNA strands that run in opposite directions in a double-stranded molecule.

**Archaea** a domain of prokaryotic life distinct from the Bacteria. This domain includes the extreme halophiles, organisms that grow in high-salt environments; methanogens, anaerobes that produce methane; and thermophiles, which thrive in high-temperature environments.

**Bacteriorhodopsin** a red pigment that enables some microbes to convert solar energy into chemical energy and grow phototrophically.

**Carotenoid** a common type of red-orange pigment found in *Halobacterium* sp. NRC-1 and many other organisms.

**Chromosome** (from Greek *chroma*, "color," and *soma*, "body") DNA and associated proteins carrying the genetic information of a cell.

**Complementary** in DNA, nucleotide sequences which pair through Watson and Crick base pairing, resulting in a double helix.

**Cytosine** one of the four nucleotide bases in DNA and RNA; cytosine pairs with guanine.

**Denature** to separate the two strands of DNA into two single strands, usually by heating or exposure to alkaline conditions.

**Deoxyribonucleic acid (DNA)** a chemical substance found in all cells, consisting of two polynucleotide chains forming a double-helix with Watson and Crick pairing of the nucleotide bases. DNA functions as the hereditary or genetic material of the cell.

**DNA polymerase** an enzyme, found in all cells, which functions in the replication and repair of DNA.

**Double helix** the structure of DNA, in which two chains are wrapped around each other like the sides of a twisted ladder.

**Enzyme** a subcellular component, usually a protein, which accelerates (catalyzes) chemical reactions important for the cell's metabolic activities.

**Genome** the complete genetic hereditary material (DNA) of cells. It is composed of chromosomes and, in some organisms, extrachromosomal genetic elements.

**Guanine** one of the four nucleotide bases in DNA and RNA; guanine pairs with cytosine.

***Halobacterium* sp. NRC-1** an extremely halophilic microorganism (an Archaeon) found in extremely salty environments all over the world.

**Halophile** an organism that requires salt concentrations above that found in seawater for optimal growth. Those that require nearly saturating salinity are called "extreme halophiles."

**Helicase** an enzyme that promotes the unwinding of DNA (or RNA) chains.

**Hypotonic** a term describing a solution in which the concentration of dissolved salts (or solutes) is less than that found in another solution or medium to which it is being compared.

**Lagging strand** in DNA replication, the chain that is synthesized in the 3′ to 5′ direction and in many small chains.

**Leading strand** in DNA replication, the chain that is synthesized in the 5′ to 3′ direction and in one long chain.

**Lysate** the solution/mixture of cell components that results after cells are broken open (lysed).

**Nucleotide** any one of the building blocks of DNA (a deoxyribonuclotide) or RNA (a ribonucleotide).

**Polynucleotide** a chain of nucleotides forming a strand of DNA or RNA.

**Precipitate** to separate a dissolved material out of a solution or suspension so that it can be physically collected.

**Primase** an enzyme that initiates the synthesis of a short RNA chain (primer) that allows DNA polymerase to begin making polynucleotide chains in DNA replication.

**Primer** in DNA replication, a short RNA chain which serves to prime DNA synthesis. DNA polymerase attaches the first nucleotide in DNA synthesis to the primer.

**Prokaryote** a microorganism that lacks a nucleus.

**Thymine** one of the four nucleotide bases in DNA; thymine pairs with adenine.

**Uracil** one of the four nucleotide bases in RNA; uracil pairs with adenine.

Student Guide

15-4773

Name _____

Date _____

# Extremely Easy DNA Extraction

## Summary

In this activity, you will extract **deoxyribonucleic acid (DNA)** from ***Halobacterium* sp. NRC-1**, a member of the domain **Archaea** and one of the first organisms to have its **genome** sequenced. Using techniques similar to those used in top research laboratories, you will lyse *Halobacterium* cells, transfer the **lysate** to a test tube, and then add alcohol to the lysate to bring the DNA out of solution. As the DNA strands **precipitate** out at the interface between the aqueous cell lysate and the alcohol, you will wind the DNA strands onto a spooling stick, forming a mass of DNA that can be observed without magnification.

## Background

Deoxyribonucleic acid (DNA), the genetic material in all living cells, is a **double helix** formed from two **antiparallel polynucleotide** chains or strands made up of **nucleotide** bases, **Adenine** (A), **Cytosine** (C), **Guanine** (G), and **Thymine** (T). The bases obey the strict Watson and Crick rule of base pairing: A always pairs with T, and G always pairs with C. DNA resembles a ladder that is twisted so that there is one turn for every ten and one-half rungs, or bases.

DNA forms very long molecules, much longer than the cell in which it is found. The length of DNA can be calculated by multiplying the distance between bases, 3.4 **Ångstroms** (Å) (100,000,000 Å = 1 cm), by the number of bases in the chromosome, which typically is 1–5 million for **prokaryotes** and about 3 billion for humans. For prokaryotic microorganisms, which are only one or a few micrometers (μm) long, the chromosomal DNA may be 1 millimeter (mm), or almost one thousand times longer than the cell! In order to fit inside the cell, chromosomal DNA is packaged into coils and supercoils.

While DNA can form very long molecules, the thickness of a DNA ladder is only 20 Å , or about 1/50,000$^{th}$ the width of a human hair (50–150 μm). Because of its minute size, individual DNA molecules must be visualized using a special microscope called an electron microscope. In order to be visualized by an electron microscope, the DNA must be coated with proteins and a heavy metal such as platinum. This coating thickens the DNA strand and creates enough contrast between the DNA and the surrounding area for it to be seen with the electron microscope (Figure 1).

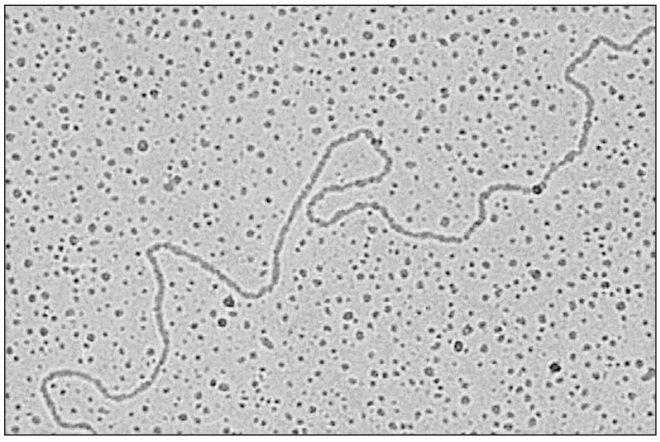

**Figure 1**. Electron micrograph of DNA by Shiladitya DasSarma.

DNA possesses the special property of replication, forming two identical progeny molecules from a single parental molecule (this is the reason that it has evolved to serve as the genetic material in cells). For this process, the two antiparallel DNA strands are first partly **denatured**, or separated, by the action of proteins called DNA **helicases**. Then, each DNA strand serves as the template for synthesis of the **complementary** strand using Watson and Crick base pairing of nucleotide building blocks, utilizing an **enzyme** known as **DNA polymerase**. A complication in this process is that DNA synthesis does not occur *de novo* (from scratch). The DNA polymerase must have something to which it attaches the first base pair in the DNA chain. So, first, a short piece of RNA, a **primer**, is laid down by another enzyme, called **primase**. In creating this primer, analogous Watson and Crick rules are followed, except that the RNA base **Uracil** (U) is used in place of the DNA base T. The DNA polymerase extends the chain of DNA from this primer. Later, the RNA primer is removed and replaced by DNA. Another twist in the DNA replication story is that the DNA chains are always synthesized in one direction, called 5′ to 3′, and never in the opposite direction, 3′ to 5′ (Figure 2). This leads to there being a **leading strand** in replication, which is formed using only one RNA primer, and a **lagging strand**, whose synthesis requires many RNA primers. During replication, the accuracy of the DNA replication is checked by the editing activity of DNA polymerase itself, as well as by a host of other repair proteins. In this way, genetic information is faithfully transmitted from one generation to the next.

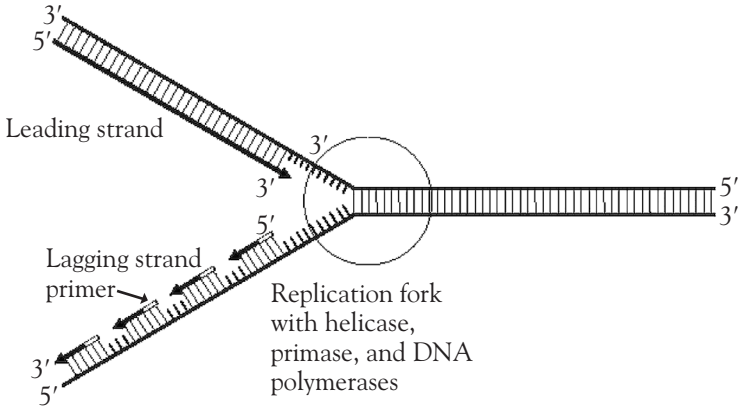


**Figure 2.** DNA synthesis.

Why would scientists want to extract DNA from cells? In research, DNA is used to study fundamental aspects of organisms and their genes and to understand, diagnose and treat genetic diseases. Isolated DNA may also be used to bioengineer cells for applications in biotechnology. For example, bacteria can be engineered to produce proteins such as insulin for diabetics. In addition, DNA is also used in forensic labs for genetic fingerprinting, and is being used to exonerate innocent people and to convict criminals.

The DNA that is extracted in this activity is from a microbe that tolerates and even requires extremely high salinity for survival. The DNA of *Halobacterium* sp. NRC-1 is contained in three c**hromosomes**, one large chromosome and two minichromosomes. However, the type of DNA extraction you are going to do will not allow you to isolate unbroken chromosomes in their entirety. The *Halobacterium* cells would have to be lysed while embedded in a gel in order for the fragile structure of the chromosomes to be maintained; the DNA could then be separated by electrophoresis on gels for further study.

## Extracting DNA from *Halobacterium* sp. NRC-1

Many cells possess a tough cell wall to protect them from their environment. DNA studies require breaking open the cell in order to extract the DNA. Breaking down cell walls often requires harsh chemical or physical methods that are technically challenging and frequently damage the DNA. However, with extreme **halophiles** like *Halobacterium* sp. NRC-1, the cells can be broken open easily as

the cell envelope can be weakened with **hypotonic** solutions—solutions possessing lower solute concentration than the cell's interior (e.g., pure water).

You will use water to lyse the *Halobacterium* cells. The cell envelope of *Halobacterium* cells has a unique composition that is easily broken down in the presence of pure water. In addition, osmotic pressure will cause water to flood into the high-salt interior of the cell. (**Note:** *Halobacterium* cells maintain an internal high salt concentration equal to the high-salt concentration of their environment.) The cell lysate that results from cell lysis contains a solution of cell components including RNA, proteins, and, most importantly for the purpose of this lab, DNA.

As soon as the DNA is released, it becomes susceptible to breakage. Therefore, you should handle the cell lysate gently. The more the solution is stirred and shaken, the smaller are the resulting DNA fragments. If the fragments become too small, you will not be able to spool them onto the stick.

To precipitate the DNA so it can be wound onto a spooling stick, you will add alcohol to the cell lysate. DNA is highly soluble in water, but is insoluble in alcohol (such as ethanol and isopropanol) and in mixtures of alcohol and water. Thus, as alcohol is added to the cell lysate, the insoluble components, including the DNA, precipitate out of the solution. The DNA that precipitates is in the form of long, fibrous molecules, which can be spooled by winding them around a stick. Remember, the DNA you extract will be crude and will include a lot of proteins and RNA. If you wished to analyze the DNA, it would have to be further purified.

## Procedure

1. Use a 3-mL graduated bulb pipet or other device to add 5 mL (about 100 drops) of tap water onto the *Halobacterium* lawn on the petri plate. Tilt the petri plate so that water covers the entire surface of the *Halobacterium* lawn. Distribute the water as an even layer over the cells.
2. Let the petri plate sit on the bench top for three minutes to give the cells time to lyse.
3. With a back-and-forth motion, use a spreader to gently loosen the remaining cell material from the petri plate.
4. Place a funnel into the mouth of the test tube. Pour the lysate gently and carefully into the test tube; try to minimize breakage of the fragile DNA strands. Remove the funnel from the test tube.
5. Using a bulb pipet, add 3–4 mL of chilled alcohol to the test tube. To prevent the aqueous and alcohol layers from mixing, tip the tube at a 45° angle, hold the pipet against the side of the tube, and allow the alcohol to flow slowly down the side of the tube onto the aqueous layer. Discard the pipet.
6. Let the test tube sit for about 20 minutes to allow the DNA to precipitate out of solution. It should appear as a stringy, gelatinous mass at the interface between the water and alcohol.
7. Hold the test tube at a 45° angle and carefully insert the spooling stick. Position the stick at the interface between the alcohol and the lysate. **In one direction only**, rotate the stick in a slow, even, circular motion, **gently** mixing the material in the interface layer. (**Note:** Be very careful. If you are too rough, the DNA strands will break and you will be unable to spool the strands.)
8. You should see an accumulation of a viscous material on the stick. This is the DNA! Use a pair of forceps or the spooling stick to pull out the fibers of DNA.

Name _____

Date _____

# Extremely Easy DNA Extraction

## DNA Extraction

1. From where, and why, do scientists extract DNA? Give several examples.

2. In the space below, draw a simple model of DNA. Make sure to label the bases correctly.

3. In the space below, draw what you saw in the test tube. Make sure to label the two solutions, the interface layer, and the DNA.

4. Why would extracting DNA from an organism that is not an extreme halophile require more effort than extracting DNA from *Halobacterium*?

Extension Activity

15-4773

Name _____

Date _____

# *Halobacterium* sp. NRC-1 Bioinformatics Link

You have just extracted DNA from the extreme halophile, *Halobacterium* sp. NRC-1. The DNA of this microbe has been sequenced in its entirety. Access the *Halobacterium* genome database at the Web site http://halo.umbi.umd.edu. Click on the "Database" link. Select the "Gene table" option, or alternatively, select the "Search" option and perform a keyword search by typing the name of a particular gene into the keyword box (for example, "bop" for the bacteriorhodopsin gene, or "gvp" for a list of the gas vesicle protein genes). You can find out more about these genes by clicking on each gene's name. Select one gene and conduct an Internet search to see what you can find out about the gene. If you find references to journal articles, you may wish to visit a science library to find the articles. In the space below, summarize what you have discovered about the gene that you chose to research and discuss how the gene might be important to *Halobacterium* sp. NRC-1.

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