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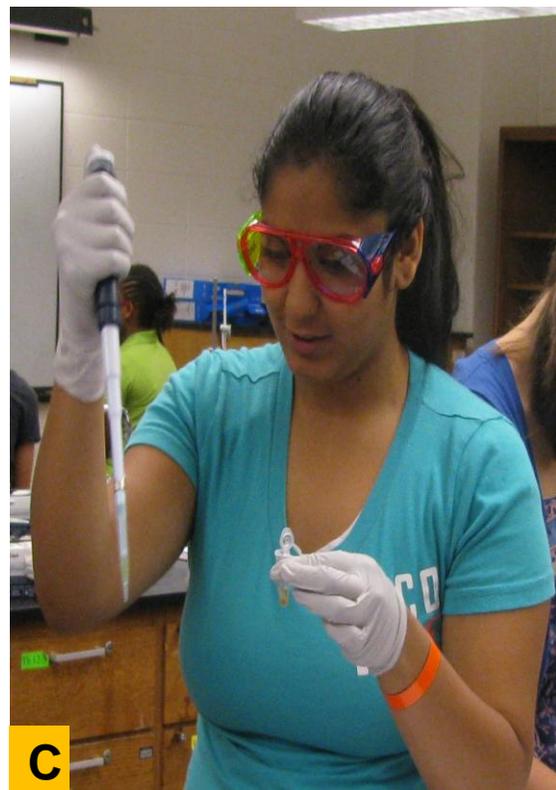
A

## DNA Extraction from Extremophiles

Life on the Edge



B



C

# Acknowledgements

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## Photography Credits:

- A. Picture of a tank with a *Halomonas halodenitrificans* bacteria.
- B. Picture of a student loading samples into the microcentrifuge machine.
- C. Picture of a student pipetting.

# National Standards Correlation

## Life Science Content Standard C

### The Cell

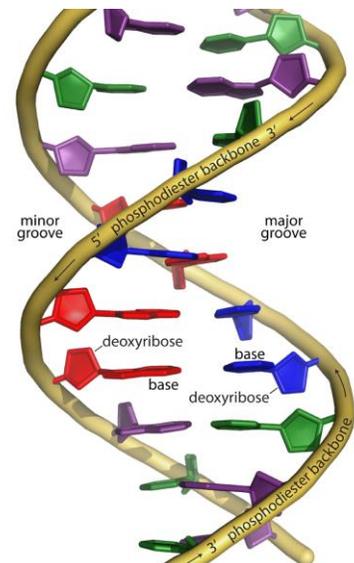
Cells store and use information to guide their functions. The genetic information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.



SK-N-SH cells grown in the NASA RCCS.

### The Molecular Basis of Heredity

In all organisms, the instructions for specifying the characteristics of the organism are carried in the DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular “letters”) and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome. Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms. Only mutations in germ cells can create the variation that changes an organism’s offspring.



DNA's double helix.

## The Teacher's Manual

### Purpose

The purpose is to develop an understanding of DNA by extraction and physical manipulation, and to classify organisms based on their genetic makeup by testing for the presence of DNA.

### Key Concepts

- Students will be introduced to prokaryotic extremophiles and learn their importance.
- Students will learn where prokaryotic extremophiles fit on the phylogenetic tree and what distinguishes them from other organisms.
- Students will learn and understand how prokaryotic extremophiles are able to survive extreme conditions.

### Common Misconceptions

- DNA is difficult to extract.

### Overview

In today's lab, you will break apart the cells of bacterial extremophiles and release their DNA into solution. You will remove other cellular components and precipitate the DNA to form large aggregates that are visible to the naked eye.

An extremophile is an organism that lives in an extreme environment that is detrimental to most organisms on Earth. Extremophiles can thrive in environments of extreme heat, cold, salt, sugar, pH, desiccation, etc.

Today, you will extract DNA from a psychrophile (cold-loving) or a thermophile (heat-loving) bacteria. The species you will be using are *Psychrobacter urativorans* and *Thermus thermophilus*. *P. urativorans* lives in glaciers and permafrost environments, while *T. thermophilus* lives in hot springs or thermal vents. The existence of these extreme organisms is exciting because they demonstrate that life can exist at similar temperatures on other planets.

In this lab, you will be given the task of extracting DNA from either the psychrophile or the thermophile. The procedures for the extractions are similar because both bacteria are gram negative, meaning that they have a relatively thin and fragile cell wall.

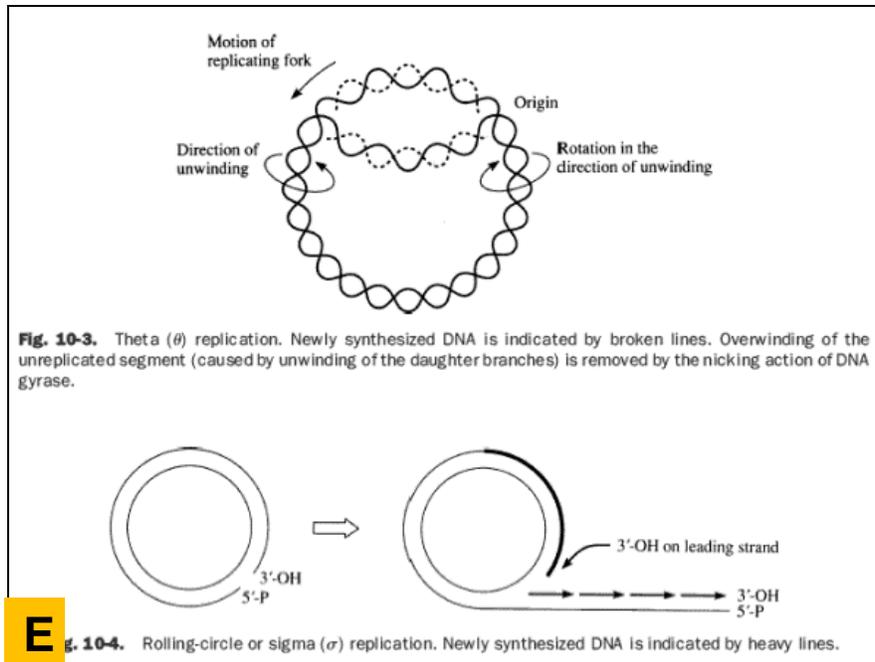
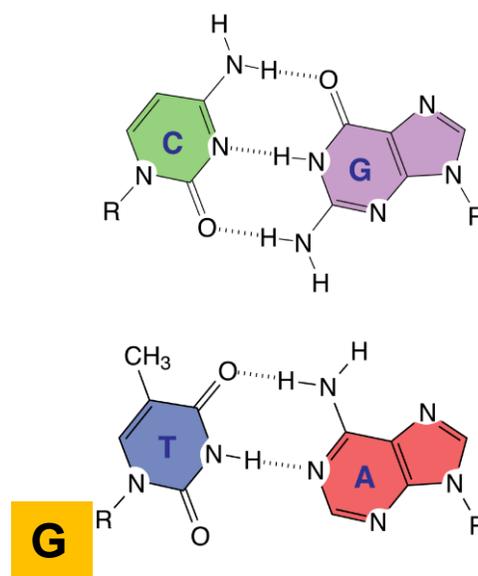
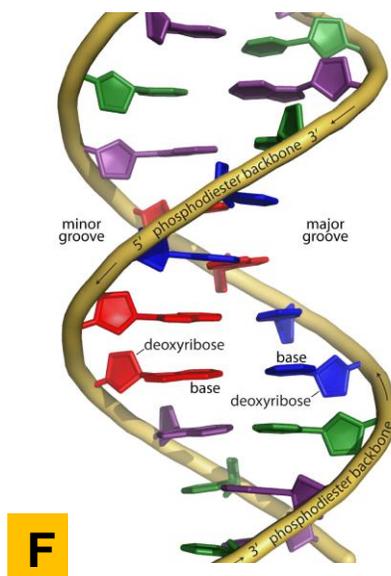


Image of bacterial DNA replication from: <http://www.education.com/study-help/article/bacterial-dna-replication-cell-division/>

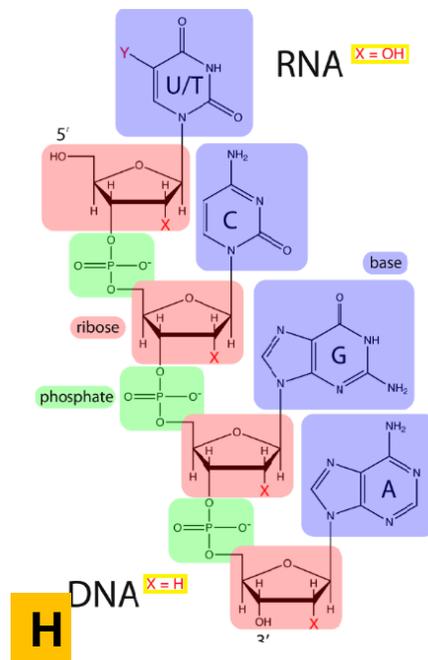
The DNA found in all living systems is a double-stranded helix. The bases are paired and stacked, like pennies in a penny roll. However, unlike the DNA found in complex organisms (eukaryotes), which is linear and is encased in membrane bound nucleus, the DNA found in bacteria is circular and is not bound in a nuclear membrane (see image above). Human DNA denatures and becomes single stranded above 70°C. Thermophiles, however, are found at very high temperatures because they have DNA with more G-C base pairs, which are more stable than A-T base pairs.



## Steps for DNA Extraction

Several steps are required to extract the bacterial DNA so that it will precipitate out in a visible form. First, the cell wall must be broken open by adding the lysis solution. Unlike DNA, which is formed from nucleotide monomers made of deoxyribose, phosphate and a nitrogenous base, cell and nuclear membranes contain primarily fats and proteins. Because the chemical nature of the membranes and DNA is different, the extraction buffer disrupts them while leaving the DNA intact. The purpose of this step is to separate the DNA from the nucleoid region and from other proteins.

The second step is to add the RNase to the cell lysate to break down RNA into smaller components. It is important to obtain the most pure DNA sample possible because when performing a PCR and running a gel, the bands will show neat DNA bands with no RNA interference. The image below shows the structures of DNA and RNA. RNA differs from DNA by the structure of the sugar molecule; RNA contains an OH and DNA has a H. The phosphate groups are in green, sugars are in pink and the nitrogenous bases are in purple.



The third step precipitates the proteins but leaves the DNA in the solution. The last step is to recover the DNA, which is achieved by precipitating the DNA with the addition of isopropanol. DNA is soluble in water, but is insoluble in mixtures of isopropanol and water. Therefore, the careful addition of isopropanol in step 13 to the supernatant, followed by gentle inversion, will cause the DNA to form visible, insoluble threads, representing aggregated DNA.

## I. Extracting DNA

### Skills

1. Predicting the outcome of an experiment
2. Controlling variables
3. Conducting an experiment
4. Collecting, recording, and graphing data
5. Drawing conclusions and communicating them to others

**Prep Time for teachers:** 30 minutes (aliquot solutions)

**Class Time:** 45 minutes

### Objectives

- Students will be able to isolate DNA from extremophiles.
- Students will be able to describe the role of the DNA and genes.

### Themes

- Heredity
- Diversity

### Materials Per Group

1. 8ml of bacterial culture
2. 3 microcentrifuge tubes
3. 80 C water bath (500mL beaker set on a hot plate with a thermometer in it)
4. 37 C water bath
5. Room temperature isopropanol (approximately 30 ml for a class of 30 students)
6. Room temperature 70% ethanol
7. A small beaker or container full of ice
8. Vortex
9. Microcentrifuge

### Procedure

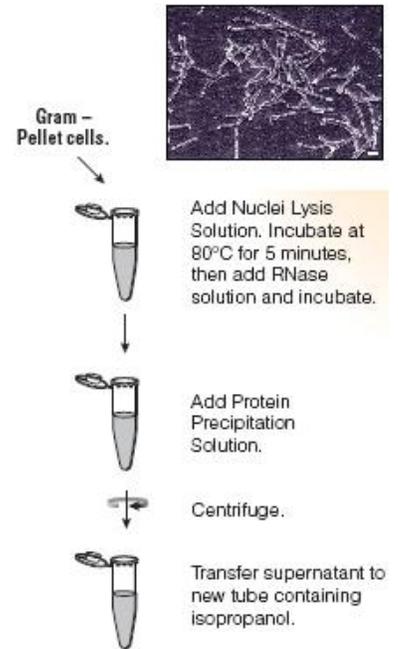
**READ ALL INSTRUCTIONS BEFORE BEGINNING EACH STEP IN THE LAB (ADD STEPS)**

Step 1- Add 8ml of a healthy culture to a 15ml centrifuge tube.

Step 2-Centrifuge at 4500 g for 20 minutes and at 4°C.

Step 3- Add 600 µl of Nuclei Lysis Solution. Gently pipet up and down until all the cells are re-suspended.

Step 4- Transfer the mixture to a clean 1.5 ml microcentrifuge tube.



Step 5- **For Psychrobacter, incubate at 80 degrees C for 5 minutes to lyse the cells, then cool them to room temperature.**

**For Thermus and Halo, incubate at 90 degrees C for 5 minutes to lyse the cells, then cool them to room temperature.**

Step 6- Add 6 µl of RNase Solution to the cell lysate. Invert 2-5 times to mix.

Step 7- Incubate at 37°C for 15 minutes. Cool to room temperature.

Step 8- Add 200 µl of Protein Precipitation Solution to the RNase-treated cell lysate.

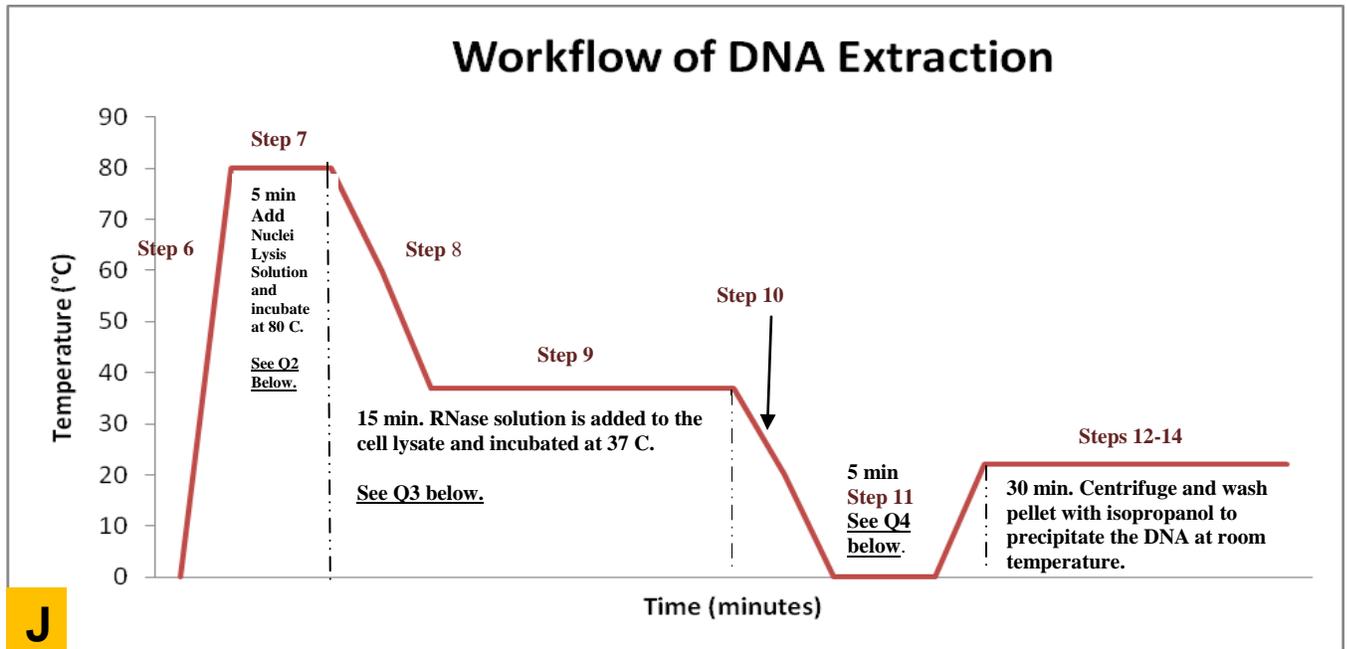
Step 9- Vortex rigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate.

Step 10- Incubate the sample on ice for 5 minutes.

Step 11- Centrifuge at 13,000-16,000 g for 3 minutes.

Step 12- Transfer the supernatant containing the DNA to a clean 1.5 ml microcentrifuge tube containing 600ul of room temperature isopropanol. Place the remaining content to the side for the DNA indicator test in part 2 of this lab.

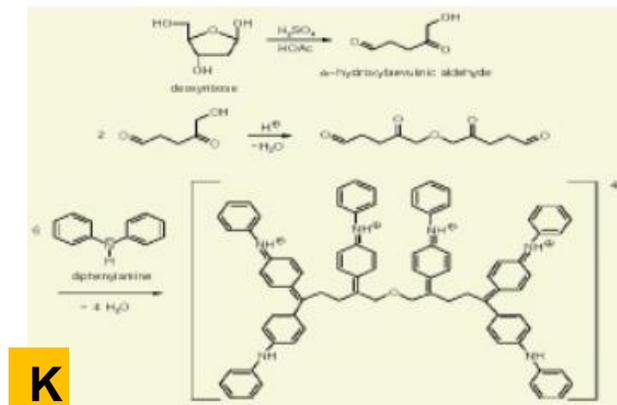
Step 13- Gently mix by inversion until the thread-like strands of DNA form a visible mass.



## II. Testing your Extracted DNA

Cells contain a wide variety of molecules. Some of these are relative small (ATP, NAD, etc) but others are large polymers (DNA, RNA, protein and complex carbohydrate).

**How can a scientist be certain that he or she has isolated pure DNA during the extraction process?** To confirm that DNA has been purified, scientists use various indicator tests such as ethidium bromide (EtBr) Dot test and DPA as well as using spectroscopy to test for the presence of DNA and contaminants. To test the sample extracted in the above protocol, the indicator test Dische diphenylamine (DPA) will be used to confirm the presence of DNA. DPA turns blue when it binds to DNA. The blue color change is the result of diphenylamine reacting with the sugar deoxyribose in DNA. The blue color change does not occur with RNA due to the difference between the sugars of DNA and RNA: DNA contains deoxyribose, whereas RNA contains ribose. Concentration of DNA present can be determined based upon the intensity of the blue color change.



## Procedure: (STEPS)

Step 1 - Using your extracted DNA sample from Part I, add 1ml (approximately 20 drops) of DPA reagent to your microcentrifuge tube containing the DNA and mix.

Step 2 - In microcentrifuge tube II add 500  $\mu$ l (approximately 10 drops) of your positive control DNA.

Step 3 - In microcentrifuge tube III add 500  $\mu$ l of supernatant.

Step 4 - In microcentrifuge tube IV add 500  $\mu$ l (approximately 10 drops) of distilled water (this will serve as your negative control).

Step 5 - Add 1 ml (approximately 20 drops) of DPA reagent (\*See Appendix for instructions on making the DPA solution) to microcentrifuge tubes II, III, and IV and mix.

Step 6 - Using a boiling hot water bath, heat the test tube contents for approximately 25 minutes in a chemical fume hood.

Step 7 - Observe the color of test tube contents and record observations in your data table.

Step 8 - Assess the amount of DNA in your sample by the degree of color compared with your positive standard.

### Table with Answers:

Tube #	Tube Contents	Color Observed	Conclusion
1	Extracted DNA	Blue	DNA present
2	DNA (Positive Control)	Dark Blue	DNA present
3	Supernatant	No color change	No DNA
4	Distilled Water	No color change	No DNA

## Lab Questions Part I:

1. Why do you think DNA Extraction is important to learn?

DNA extraction is often the first step for scientists who study genes. The extracted DNA is used as a pattern to make copies or clones of particular genes. These copies can be selectively separated away from the total extracted DNA, and used to study the function of those particular genes. In humans, once the genes have been studied, genomic DNA extracted from the person can be used to solve genetic disorders.

2. What do you think is the purpose of adding the nuclei lysis solution?

The nuclei lysis solution lysis or break open the cells so that the DNA can be released from the nucleus.

3. Why do you think adding RNase to the cell lysate is important?

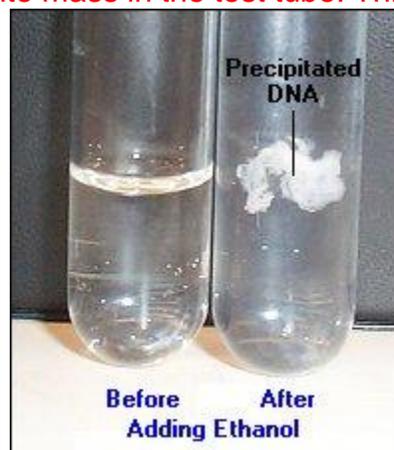
If you don't add RNase, you will not successfully denature RNA in your sample. when you run your sample in an electrophoretic gel, instead of only seeing bands for DNA, you'll get giant RNA bands. This will ruin your results from your gels.

4. What is the purpose of adding the protein precipitation solution in step 10?

The purpose of adding the protein precipitation solution is to remove any contaminants.

5. Sketch your DNA precipitate and describe what you see (qualitative):

Students should see a white mass in the test tube. This is the DNA precipitate.



Picture from: <http://www.vivo.colostate.edu/hbooks/genetics/biotech/manip/conc.html>

## Lab Questions Part II:

1. The DNA that was isolated should be a pure sample. If the extracted DNA test results are not as expected, what could be contaminating your sample?

RNA

2. If the results if testing a known DNA solution are negative, what might be the reason?

Didn't follow the instructions to extracting DNA. Ex. didn't add the Nuclei Lysis solution.

3. Were the colors in the extracted DNA and the given DNA identical? Why? Or Why not?

No, the extracted DNA had a darker hue of blue than the DNA sample because it was more concentrated.

4. What was the purpose of testing the distilled water with DPA?

The distilled water served as the negative control.

5. Could the DPA test be used to distinguish between ribose and deoxyribose? Explain your answer.

Yes, blue indicates the presence of DNA (deoxyribose) and green indicates the presence of RNA (ribose).

6. When running the DPA test, what is the amount of blue color dependent upon?

The intensity of the blue color depends on the concentration of DNA present in the sample. The darker the hue of the blue color, the more DNA is present in the sample.

7. Why are the test tubes placed in a boiling water bath when running the DPA test?

The tubes are placed in a boiling water bath during the DPA test so that the 2-deoxyribose can be converted to *w*-hydroxylevulinyl aldehyde, which reacts with the compound, diphenylamine, to produce a blue-colored compound.

## Appendix

How to make Diphenylamine (DPA) Solution out of powdered DPA:

**Method:**

1. Measure 1g of DPA and place it in a 100ml bottle with a screw cap.
2. Add 2.5 ml of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to the bottle.
3. Add 100 ml of glacial acetic acid ( $\text{CH}_3\text{COOH}$ ) to the bottle.
4. Shake until dissolved.

**Notes:**