EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #

203

Isolation of *E. coli* Chromosomal DNA

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to isolate high molecular weight DNA suitable for spooling.

Table of Contents

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

Experiment Components

This experiment contains reagents for ten groups to perform the isolation of chromosomal DNA. The same reagents can be used for twenty isolations if supplemented with additional tubes, pipets and spooling rods.

There are enough electrophoresis reagents to prepare and run six agarose gels based upon the use of Horizontal gel electrophoresis apparatus, Model #M12. Several groups can share each gel for electrophoresis of their isolated DNA samples.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

Store all components below at room temperature.

	Component	Check (✓)
A	Buffer for cell resuspension	❑
B	EDTA Buffer, 0.25 M, pH 8.0	❑
C	RNase A (DNAse-free)	❑
D	Sarkosyl, 15%	❑
E	Protease	❑
F	NaCl solution, 5 M	❑
G	Buffer concentrate for RNase, Protease and DNA resuspension (Tris-HCl, EDTA, pH 7.5)	❑
•	Vial of DNA extraction LyphoCells™ (freeze-dried)	❑
•	UltraSpec-Agarose™	❑
•	Electrophoresis Buffer (50x)	❑
•	10x Gel Loading Solution	❑
•	InstaStain® Blue	❑
•	FlashBlue™ Liquid Stain	❑
•	Practice Gel Loading Solution	❑
•	1 ml pipet	❑
•	Microtipped Transfer Pipets	❑
•	Large plastic tubes	❑
•	Spooling rods	❑

Experiment Requirements **(NOT included in this experiment)**

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Water bath (45°C)
- Balance
- Hot plate, Bunsen burner or microwave oven
- DNA visualization system (white light)
- Small plastic trays or large weigh boats (for gel destaining)
- Safety goggles and disposable laboratory gloves
- 1 and 10 ml pipets
- Pipet pumps
- 20 ml and 250 ml beakers or flasks
- Test tubes
- Hot gloves
- Marking pens
- Distilled or deionized water
- 95-100% isopropanol (70% isopropyl alcohol can be substituted)
- Ice

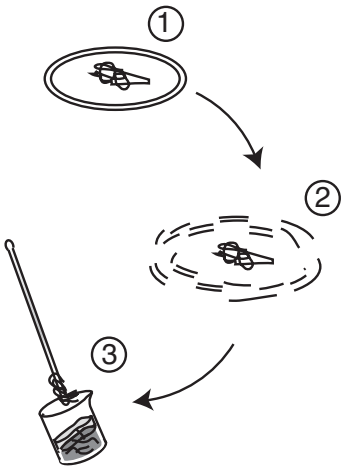
Isolation of *E. coli* Chromosomal DNA

Background Information

When scientists study DNA, cells are chemically lysed (broken open) and DNA from chromosomes is released. This procedure is known as cell lysis. The DNA is then isolated from a solution and redissolved. The isolation of chromosomal DNA is frequently the first step in DNA and molecular cloning experiments.

E. coli chromosomal DNA is a large circular molecule containing approximately 3,000,000 base pairs attached to the bacterial cell wall at several points. Large DNA is very sensitive to mechanical shear which causes random breaks in the phosphodiester backbone of the molecule. However, if the extraction procedure is performed carefully, large fragments of chromosomal DNA can be obtained with an average fragment length of 100,000 to 200,000 base pairs. Since the average length of a gene is about 2,000 base pairs, there is a high probability that genes of interest will remain intact in one of the fragments of DNA. In subsequent steps, specific genes can be amplified and cloned.

The objective of this experiment is to isolate bacterial chromosomal DNA. The resuspended cells are first mixed with a solution of ethylenediamine tetraacetic acid (EDTA). EDTA forms complexes (chelates) with several kinds of metal ions. Divalent metal cations, such as Mg^{+2} are required cofactors for the majority of DNases. DNA being extracted is protected from DNase degradation since the complexed Mg^{+2} cannot be utilized by the enzyme.



1. The DNA is inside the cell.
2. The cell wall is disrupted.
3. The DNA is spooled onto a rod.

Addition of the ionic detergent sarkosyl dissolves the cell membrane and denatures many proteins. RNase is also present to degrade RNA. A proteolytic enzyme (protease) is added to the cell lysate in order to digest proteins that are free in solution or bound to the DNA. RNases and proteases are exceptionally stable enzymes. They remain partially active in the presence of denaturing detergents such as sarkosyl and at high temperatures. Eventually, RNase will be degraded by the protease. However, it is in high enough concentration so that most of the RNA is degraded before significant proteolytic inactivation occurs.

In this experiment, the aqueous cell lysis solution that contains DNA is overlaid with very cold isopropanol (one volume of 91% or two volumes of 70% isopropyl alcohol available in drugstores can be used). In the presence of salts, high molecular weight DNA precipitates onto a rod when it is mixed at the interface of the two liquids. Due to its size and abundance, chromosomal DNA forms a viscous mass that is easily collected. This process is known as DNA spooling. Smaller molecules such as sugars and amino acids remain in solution. The spooled DNA can be redissolved in buffer and analyzed by electrophoresis.

Isolation of *E. coli* Chromosomal DNA

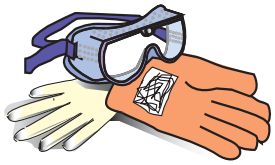
Background Information

Agarose gel electrophoresis is a convenient procedure for analyzing DNA in this size range. The gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting. The gel is placed in an electrophoresis chamber containing a buffer. Direct current is applied from a power supply. Since DNA has a strong negative charge at neutral pH, it will migrate through the gel towards the positive electrode. Pores in the gel separate linear fragments of DNA according to their size. The smaller the fragment, the faster it will migrate. If many fragments are present in a wide range of sizes (such as occurs with badly sheared chromosomal DNA) the DNA appears as a smear instead of a distinct band after electrophoresis.

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to isolate high molecular weight DNA suitable for spooling.



Wear gloves and safety goggles

IMPORTANT

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.

The Experiment

Isolation of DNA from *E. coli* Cells - Option A

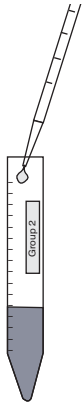
of Groups **10**

If you are sharing stock reagents, use designated pipets and do not cross-contaminate reagents.

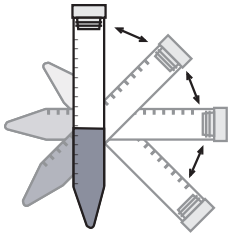
If the reagents have been aliquoted, be sure to rinse the pipet thoroughly before adding a new reagent.

ISOLATE THE DNA

1. Put your initials or group number on a tube containing **5 ml** of resuspended cells.



2. Add **2 ml** of EDTA Buffer to the cell suspension.



3. Add **0.5 ml** of RNase Solution to digest the RNA. Tightly cap and gently mix by inverting the tube several times.

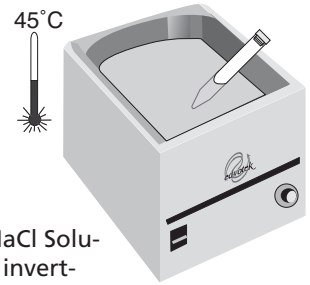
4. Incubate the cells for 5 minutes at room temperature.

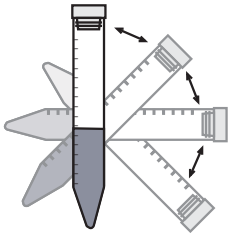
The combination of the sarkosyl and protease breaks up the cell wall. The protease further cuts up intracellular proteins, especially the unfolded ones.

5. Add **0.5 ml** of Sarkosyl Solution.

6. Add **1 ml** of Protease Solution. Tightly cap and gently mix by slowly inverting the tube 3 times.

7. Incubate the tube for 20 minutes in a 45°C water bath.

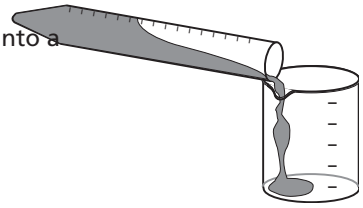




8. Add **0.6 ml** of 5 M NaCl Solution. Gently mix by inverting.

   This prepares DNA almost to the point of coming out of solution.

9. Slowly pour the viscous DNA solution into a clean 50 ml beaker.



Proceed to Spool the DNA, page 10

Isolation of DNA from *E. coli* Cells - Option B

of Groups **20**

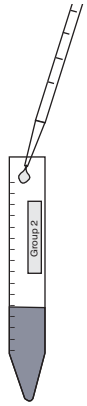
The Experiment

ISOLATE THE DNA

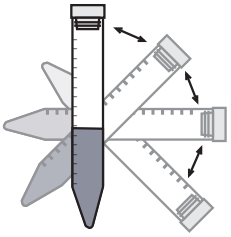
If you are sharing stock reagents, use designated pipets and do not cross-contaminate reagents.

If the reagents have been aliquoted, be sure to rinse the pipet thoroughly before adding a new reagent.

1. Put your initials or group number on a tube containing **2.5 ml** of resuspended cells.



2. Add **1 ml** of EDTA Buffer to the cell suspension.



3. Add **0.25 ml** of RNase Solution to digest the RNA. Tightly cap and gently mix by inverting the tube several times.

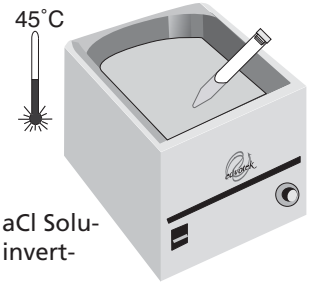
4. Incubate the cells for 5 minutes at room temperature.

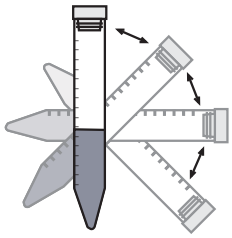
The combination of the sarkosyl and protease breaks up the cell wall. The protease further cuts up intracellular proteins, especially the unfolded ones.

5. Add **0.25 ml** of Sarkosyl Solution.

6. Add **0.5 ml** of Protease Solution. Tightly cap and gently mix by slowly inverting the tube 3 times.

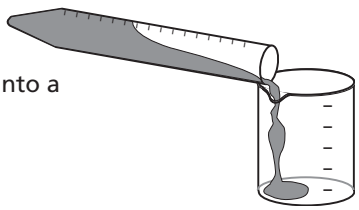
7. Incubate the tube for 20 minutes in a 45°C water bath.





8. Add **0.3 ml** of 5 M NaCl Solution. Gently mix by inverting.

   This prepares DNA almost to the point of coming out of solution.



9. Slowly pour the viscous DNA solution into a clean 50 ml beaker.

Proceed to Spool the DNA, page 10

The Experiment

Isolation of DNA from *E. coli* Cells

(Both Options A & B)

SPOOL THE DNA

Check with your instructor regarding whether you are using 95-100% isopropanol or 70% isopropyl rubbing alcohol for spooling DNA. Use the appropriate volume as follows:

Option A (10 groups)

10 ml of Isopropanol
20 ml of 70% isopropyl rubbing alcohol

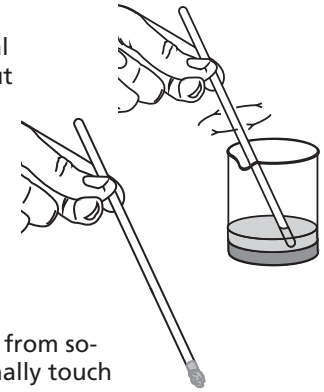
Option B (20 groups)

5 ml of Isopropanol
10 ml of 70% isopropyl rubbing alcohol

1. Carefully overlay the viscous DNA solution with the specified volume of ice cold isopropanol. (See reminder box at left.)

   Let the isopropanol slowly stream down the inside wall of the beaker or tube. Isopropanol is less dense than water and will remain in the upper layer.

2. Submerge the end of a glass rod just below the interface of the isopropanol and the aqueous DNA solution.



3. Quickly swirl and twirl the rod several times in a circular motion to spool out the DNA.

4. Remove the rod to see if the precipitate is being collected. The precipitate will appear translucent and gelatinous in texture.

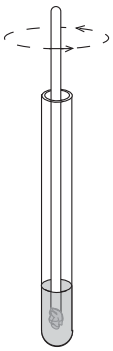
5. Continue swirling rod to collect DNA from solution. Allow end of rod to occasionally touch the bottom of the beaker or tube.

6. Note appearance of spooled DNA. As DNA adheres to rod, its initial gelatinous texture (as it appeared in the previous step) will become more compact and fibrous in appearance.

Preparation of DNA for Electrophoresis

(Both Options A & B)

RE-DISSOLVE THE SPOOLED DNA



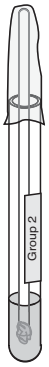
1. Remove the rod from the beaker or tube, making sure to let the excess isopropanol drip off.

Optional Procedure

Rinsing the spooled DNA on the rod with 95% ETOH will draw out excess proteins. This will enable the DNA to re-dissolve faster.

2. Add 1 or 2 ml of Tris Buffer (diluted buffer G) to a clean test tube.

3. Submerge the coated end of the rod into the buffer.



4. Twirl the rod several times to dislodge some of the DNA.

5. Cover the test tube, with the rod still inside, with plastic wrap, parafilm or foil to prevent evaporation.

6. Put your initials or group number on the tube.

7. Allow the DNA to rehydrate at room temperature. High molecular weight DNA can take several days to completely rehydrate and dissolve. Check the tube during your next lab period to see if some of the DNA precipitate has dissolved.

Optional Stopping Point:

The prepared DNA sample can be stored in the freezer for electrophoresis at a later time.

The Experiment

The Experiment

Preparation of DNA for Electrophoresis

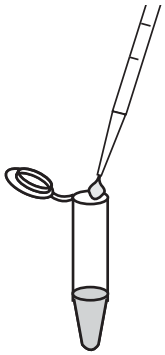
(Both Options A & B)

PREPARE THE DNA FOR ANALYSIS BY ELECTROPHORESIS

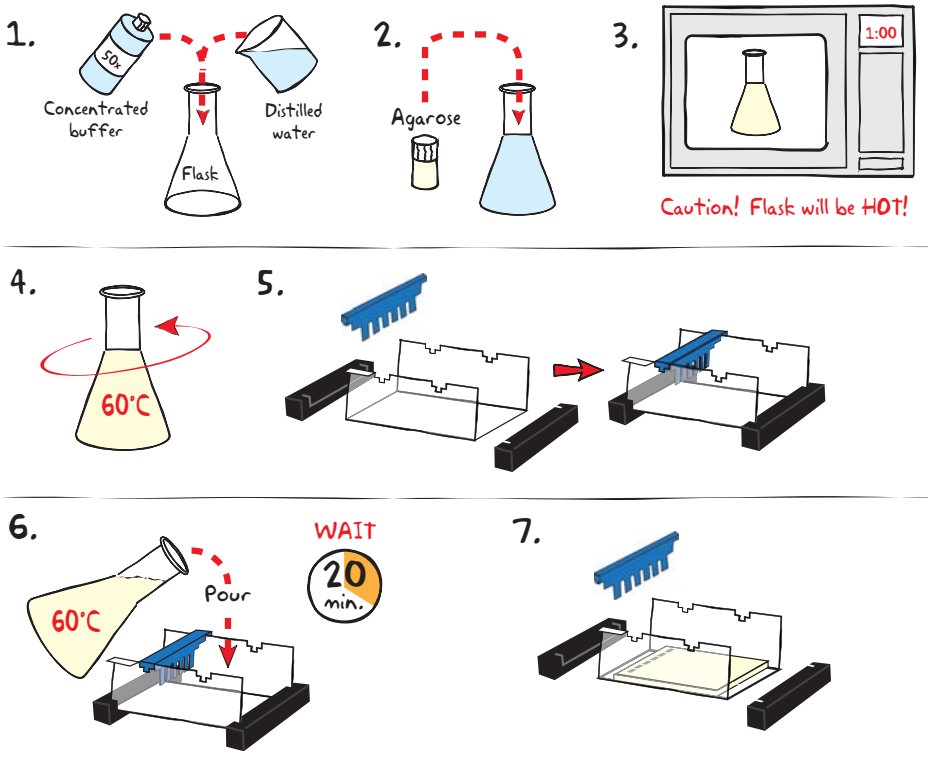
The DNA can be analyzed by electrophoresis at a time determined by your instructor.

To prepare the DNA for electrophoresis:

1. Transfer 0.2 ml of the dissolved DNA to a fresh test tube. Label the tube #1.
2. Transfer 0.2 ml of the dissolved DNA to a second fresh test tube. Label the tube #2.
3. To tube #1, add one-tenth the volume (20 µl) of 10x Gel Loading Solution. Mix by tapping. (If you are using a transfer pipet, add 1-2 drops.)
4. Dilute the DNA in tube #2 by adding 0.4 ml of diluted Buffer G.
5. To tube #2, add one-tenth the volume (60 µl) of 10x Gel Loading Solution. Mix by tapping.
6. Two samples are ready for electrophoresis. Deliver 40 µl of each prepared sample to an agarose gel for electrophoresis.



Electrophoresis - Agarose Gel Preparation



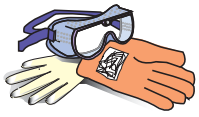
IMPORTANT:

This experiment requires 0.8% agarose gels.

Place well-former templates (combs) in the first set of notches.

Groups can share gels. 2 wells per group.

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**



Wear gloves and safety goggles

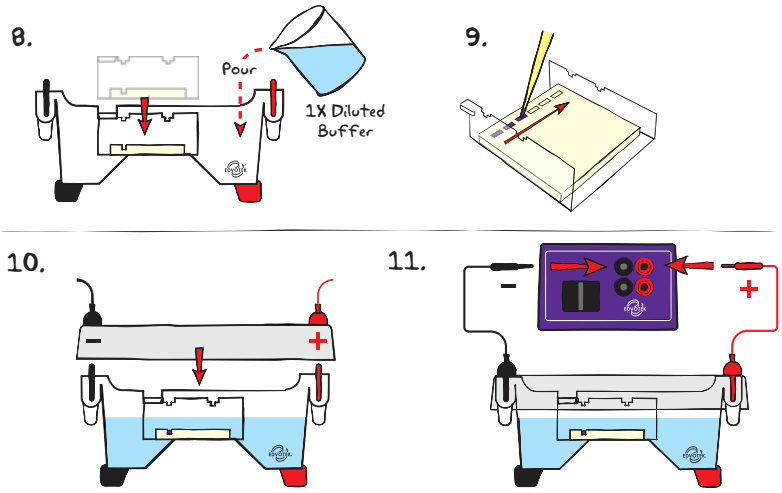
1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

The Experiment

Electrophoresis - Conducting Electrophoresis



Reminder:

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Wear gloves and safety goggles

8. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** the entire sample (30 µL) into the well. **RECORD** the position of the samples in Table 1, below.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3.5 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **STAINING** the agarose gel.

Table 1: Samples Loaded

Lane	Recommended Sample Name	
1	Student Group 1	Chromosomal DNA (Dilution 1)
2	Student Group 1	Chromosomal DNA (Dilution 2)
3	Student Group 2	Chromosomal DNA (Dilution 1)
4	Student Group 2	Chromosomal DNA (Dilution 2)
5	Student Group 3	Chromosomal DNA (Dilution 1)
6	Student Group 3	Chromosomal DNA (Dilution 2)

Table B 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+ & M12 (new)	300 ml	6 ml	294 ml
M12 (classic)	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C Time & Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model		
	M6+	M12 (new)	M12 (classic) & M36
Volts	Min. / Max.	Min. / Max.	Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

Method 1: Staining Agarose Gels using FlashBlue™

Wear gloves and safety goggles

DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.

1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 mL of water in a flask and **MIX** well.

2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.

3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**

4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.

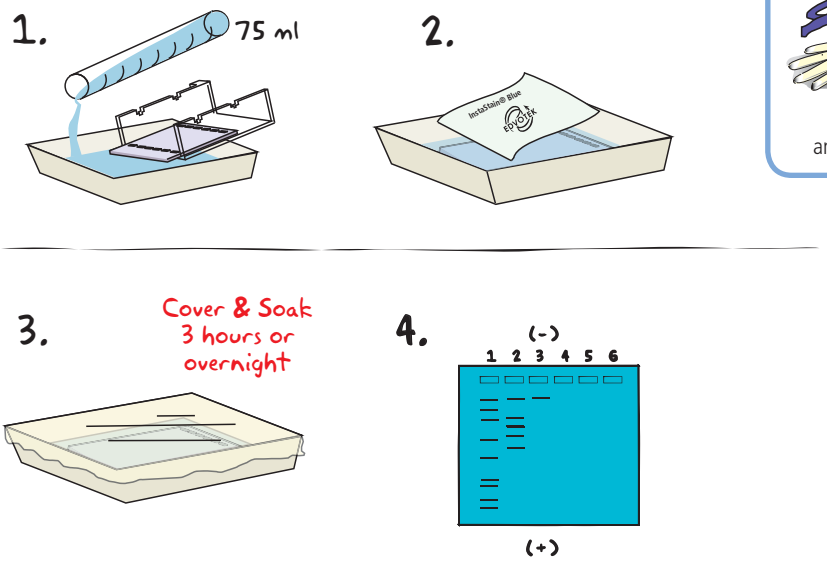
5. **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Alternate Protocol:

1. **DILUTE** one mL of concentrated FlashBlue™ stain with 149 mL dH_2O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.

The Experiment

Method 2: Staining Agarose Gels using InstaStain® Blue



Wear gloves and safety goggles

DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.

1. Carefully **SLIDE** the agarose gel from its casting tray into a small, clean tray containing at least 75 ml of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.

   Note: Appropriate staining trays include large weigh boats and small, plastic food containers.

2. Gently **FLOAT** the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing the gel. Each InstaStain® Blue card will stain 49 cm^2 of gel (7 x 7 cm). **REMOVE** the InstaStain® card(s) after 30 seconds.

3. **COVER** the tray with plastic wrap to prevent evaporation. **SOAK** the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.

4. Carefully **REMOVE** the gel from the staining tray and **DOCUMENT** results.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Did your chromosomal DNA preparation appear as a single band after agarose gel electrophoresis? What does it mean if the DNA appears as a smear after electrophoresis?
2. What is the purpose of sarkosyl, protease and EDTA in this extraction procedure?
3. Is DNA more or less soluble in alcohol (isopropanol) than in water? How can this property be used to your advantage during the purification of DNA?
4. Why is badly degraded genomic DNA a poor source of genes for cloning experiments?

Instructor's Guide

Notes to the Instructor:

This experiment contains reagents for ten groups to perform the isolation of chromosomal DNA. The same reagents can be used for twenty isolations if supplemented with additional tubes, pipets and spooling rods.

There are enough electrophoresis reagents to prepare and run six agarose gels using Horizontal gel electrophoresis apparatus, Model #M12. Several groups can share each gel for electrophoresis of their isolated DNA samples.

EDVOTEK Experiment # S-44, Micropipetting Basics, focuses exclusively on the use of micropipets. Students learn and practice pipeting techniques by preparing and delivering various dye mixtures to a special Pipet Card™.

APPROXIMATE TIME REQUIREMENTS

1. Prelab preparation and dispensing of biologicals and reagents will take approximately 1 hour.

2. Students should be able to complete the isolation of DNA and prepare the samples for electrophoresis in 50 minutes.

3. **Gel preparation:** Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

4. **Practice Gel Loading:** If your students are unfamiliar with using micropipets and sample loading techniques, a practice activity is suggested prior to conducting the experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. This activity can require anywhere from 10 minutes to an entire laboratory session, depending upon the skill level of your students.

5. **Conducting Electrophoresis:**
The approximate time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Different models of electrophoresis units will separate DNA at different rates depending upon its configuration and the distance between the two electrodes. Generally, the higher the voltage applied the faster the samples migrate. However, the maximum amount of voltage significantly depends upon the design of the electrophoresis apparatus and should not exceed manufacturer's recommendations. Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.

Table C — Time & Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model		
	M6+	M12 (new)	M12 (classic) & M36
Volts	Min. / Max.	Min. / Max.	Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

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Pre-Lab Preparations for DNA Isolation

Pre-lab preparations for reagents and materials are outlined below for ten groups to perform the isolation of chromosomal DNA. If increasing the number of isolations to twenty groups, additional tubes, pipets and spooling rods will be required (not included in this kit). For 20 groups, prepare reagents as specified below (the same as for 10 groups) and aliquot half the volume of reagents per group.

IMPORTANT NOTE:

Preparation of DNA Extraction Lyphocells, RNase Solution and Protease Solutions should be done **NO MORE than ONE hour** before performing the experiment.

Other reagents can be prepared and aliquotted up to one day before performing the experiment.

Quick Reference
Components for DNA Isolation

- DNA Extraction LyphoCells

A Buffer for cell resuspension
B EDTA Buffer, 0.25 M, pH 8.0
C RNase A (DNAse-free)
D Sarkosyl, 15%
E Protease
F NaCl solution, 5 M
G Buffer concentrate (Tris-HCl, EDTA, pH 7.5) for RNase, Protease and DNA resuspension.

RESUSPENSION OF DNA EXTRACTION LYPHOCELLS
(Prepare NO more than 1 hour prior to starting the experiment)

1. Add 5 ml of buffer for cell resuspension (A) to the vial of DNA Extraction LyphoCells™.
2. Allow the lyophilized cells to re-hydrate for several minutes, until all the material is resuspended. The suspension of cells will look turbid. (Do not be concerned if a few undissolved clumps remain.)
3. Cap and very gently invert the vial several times to mix the cells.
4. Transfer the entire contents of the vial to the remainder of Buffer A (approximately 47 ml) in a beaker. Gently mix.
5. Aliquot 5 ml of resuspended cells to each of 10 large plastic tubes.

TRIS BUFFER

Buffer Concentrate (G) is used to prepare Tris buffer for the preparation of RNase and protease solutions. The Tris Buffer is also used by the students for DNA resuspension after spooling.

7. In a small beaker or flask, add all of the concentrated Tris buffer in bottle G to 54 ml of distilled water. Mix.
8. Use prepared Tris buffer to prepare the RNase and Protease solutions.
9. Aliquot 3 ml in 10 small test tubes for each student group. Store on ice or in the refrigerator until needed.

Instructor's Guide

Pre-Lab Preparations for DNA Isolation

RNase SOLUTION
(Prepare NO more than 1 hour prior to starting the experiment)

10. Add 6 ml of diluted Tris buffer to a large plastic tube.
11. With a transfer pipet, add all the concentrated RNase (C) to the Tris buffer.
12. Rinse the tube and the pipet in the buffer to remove any residual RNase solution which might remain.
13. Label 10 tubes "RNase Solution" .
14. Aliquot 0.6 ml for each group. Store on ice or refrigerate.

PROTEASE SOLUTION
(Prepare NO more than 1 hour prior to starting the experiment)

15. Add 12 ml of diluted Tris buffer to a large plastic tube.
16. Add all the powdered protease (E) to the Tris buffer (rinse the tube with buffer). Cap and mix by inverting.
17. Label 10 tubes "Protease Solution".
18. Aliquot 1.0 ml for each group. Store on ice or refrigerate.

Reminders:

Chill the isopropanol well before starting the experiment. Spooling results are more successful when the isopropanol is very cold.

Allow ample time to equilibrate a water bath at 45°C on the day of the experiment.

PREPARATION OF ISOPROPANOL:

19. Place the isopropanol in a flask in the freezer so it is thoroughly chilled before students spool the DNA.
    - If using 95-100% isopropanol or 91% Isopropyl Rubbing Alcohol, place 110 ml in the freezer.
    - If using 70% Isopropyl Rubbing Alcohol, place 220 ml in the freezer.
20. When students are ready to spool:
    - Place the chilled isopropanol on ice in a centrally located pipetting station.
    - Label a designated 10 ml pipet for students to use.

Pre-Lab Preparations for DNA Isolation

10 DNA Isolations Each Group Requires: For **10** Groups

Resuspended cells	5.0 ml
EDTA Buffer (B)	2.2 ml
Prepared RNase solution	0.6 ml
Prepared Protease solution	1.0 ml
Sarkosyl solution (D)	0.7 ml
NaCl solution (F)	0.8 ml
Tris Buffer	2.0 ml
1 ml pipet and pump	
25 or 50 ml beaker	
Spooling Rod	

20 DNA Isolations Each Group Requires: For **20** Groups

Resuspended cells	2.5 ml
EDTA Buffer (B)	1.1 ml
Prepared RNase solution	0.3 ml
Prepared Protease solution	0.5 ml
Sarkosyl solution (D)	0.4 ml
NaCl solution (F)	0.4 ml
Tris Buffer	1.0 ml
1 ml pipet and pump	
25 or 50 ml beaker	
Spooling Rod	

Recommended aliquoted amounts may include a small amount of "excess" to enable students to accurately measure volumes needed for the procedure.

OTHER REAGENTS:

21. Label appropriately sized glass test tubes or plastic microtest tubes and aliquot the following kit components which are ready to use as provided.

EDTA Buffer (B)	2.2 ml
Sarkosyl Solution (D)	0.7 ml
5 M NaCl solution (F)	0.8 ml

SUMMARY OF REAGENTS AND MATERIALS FOR THE ISOLATION OF DNA

Recommended amounts for dispensing reagents for 10 or 20 lab groups are summarized in the tables below. Alternatively, students can share stock tubes placed in a central location. However, note that sharing stock tubes increases the risk of spills and/or contamination of reagents, as well as the possibility of reagent shortages if students do not pipet accurately.

Quantity Preparations for Agarose Gel Electrophoresis

To save time, electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel can be remelted.

BULK ELECTROPHORESIS BUFFER

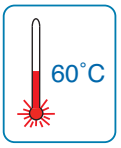
Bulk preparation of 1X electrophoresis buffer is outlined in Table D.

BATCH AGAROSE GELS (1.5%)

Bulk preparation of 1.5% agarose gel is outlined in Table E.

Note:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour the appropriate amount of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Proceed with electrophoresis (Module II) or store the gels at 4° C under buffer.

Table D — Bulk Preparation of Electrophoresis Buffer

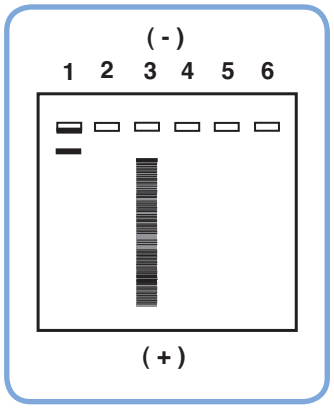
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

Table E — Batch Preparation of 0.8% UltraSpec-Agarose™

Amt of Agarose	+	50x Conc. Buffer	+	Distilled Water	=	Diluted Buffer (1x)
3.2 g		8.0 ml		392 ml		400 ml

Experiment Results and Analysis

GEL ELECTROPHORESIS RESULTS



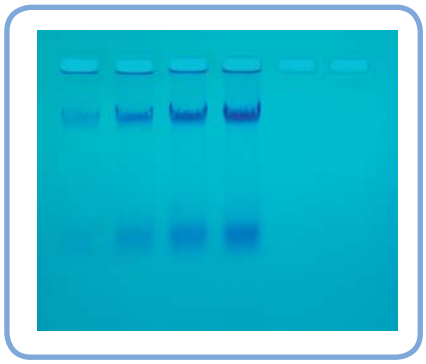
The idealized schematic shows the relative mobility and positions of the chromosomal DNA sample bands, but are not depicted to scale.

Results obtained by students will vary.

Lane 1: Intact Chromosomal DNA

In this example, a band near the edge of the well may sometimes be observed. This extra band can be due to residual bound proteins that retard migration of the DNA into the gel.

Lane 3: Example of badly sheared chromosomal DNA



Photographic example of gel results.

Instructor's Guide

Please refer to the kit insert for the Answers to Study Questions