Mini-prep Isolation of Plasmid DNA

Experiment Objective:

The objective of this experiment is to introduce the principles of extracting plasmid DNA from bacterial cells. Students will develop an understanding of the structure and function of plasmid DNAs.

See page 3 for storage instructions.
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Safety Data Sheets can be found on our website: [www.edvotek.com/safety-data-sheets](http://www.edvotek.com/safety-data-sheets)
## Experiment Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage</th>
<th>Check (✓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Tris Buffer concentrate</td>
<td>Room temp.</td>
<td></td>
</tr>
<tr>
<td>B Sodium Hydroxide solution</td>
<td>Room temp.</td>
<td></td>
</tr>
<tr>
<td>C SDS solution (Sodium dodecyl sulfate, 10%)</td>
<td>Room temp.</td>
<td></td>
</tr>
<tr>
<td>D Resuspension buffer</td>
<td>Room temp.</td>
<td></td>
</tr>
<tr>
<td>E Potassium Acetate solution</td>
<td>Room temp.</td>
<td></td>
</tr>
<tr>
<td>F RNase solution</td>
<td>Room temp.</td>
<td></td>
</tr>
<tr>
<td>Plasmid Extraction LyphoCells</td>
<td>-20° C Freezer, with desiccant</td>
<td></td>
</tr>
</tbody>
</table>

**Store the following components at room temperature.**

- 10x Gel Loading Solution
- Practice Gel Loading Solution
- UltraSpec-Agarose™ powder
- 50x concentrated electrophoresis buffer
- FlashBlue™ DNA Stain
- InstaStain™ Blue cards
- 1 ml pipets
- Microcentrifuge tubes
- Microtipped Transfer pipets

Experiment #202 contains reagents for 20 plasmid isolations (Mini-preps) and enough electrophoresis reagents to prepare and run seven agarose gels based upon the use of Horizontal gel electrophoresis apparatus, Model #M12.

All experiment 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.

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Requirements *(not included with this kit)*

**PLASMID ISOLATION**
- Microcentrifuge
- Automatic micropipets with tips
- Pipet pumps
- 95-100% Isopropanol
- 70% Ethanol
- Ice

**AGAROSE GEL ELECTROPHORESIS**
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Microwave, hot plate or burner
- Small plastic trays or large weigh boats for gel staining
- DNA visualization system (Cat. #552 White Light Box, recommended)
- 5 or 10 ml pipets
- Pipet pumps
- 250 ml beakers or flasks
- Hot gloves
- Disposable gloves and safety goggles
- Marking pens
- Distilled or deionized water
Background Information

Many types of bacteria contain plasmid DNA. Plasmids are extrachromosomal, double-stranded circular DNA molecules generally containing 1,000 to 100,000 base pairs. Even the largest plasmids are considerably smaller than the chromosomal DNA of the bacterium, which can contain several million base pairs. Certain plasmids replicate independently of the chromosomal DNA and can be present in hundreds of copies per cell. A wide variety of genes have been discovered in plasmids. Some of them code for antibiotic resistance and restriction enzymes. Plasmids are extremely important tools in molecular cloning because they are useful in propagating foreign genes. When plasmids are used for these purposes, they are referred to as vectors. Through the use of recombinant DNA technology, hundreds of artificial vectors have been constructed from elements of naturally occurring plasmids. These vectors have specifically designed properties that make them useful in solving particular experimental problems. For example, synthetic oligodeoxynucleotide linkers have been incorporated into many plasmid vectors. These linkers contain many different restriction enzyme recognition sites to facilitate the insertion of foreign DNA. The linkers are often placed near characteristic marker genes or high efficiency transcriptional promoters, both of which aid in the isolation and expression of the cloned DNA.

Plasmid DNA naturally exists as a supercoiled molecule. Supercoiling arises from alterations in the winding of the two DNA strands around each other. In certain areas of the molecule, the DNA strands are wound around each other less frequently than in non-supercoiled DNA. The strain caused by these alterations create deformations in the DNA. These deformations partially relieve the strain and ultimately lead to supercoiling. Supercoiled DNA is folded onto itself and has a more condensed and entangled structure than the same DNA which is relaxed. As an analogy to supercoiling, consider a rubber band. When the rubber band is twisted, it eventually becomes knotted and collapses onto itself as an entangled ball.

Purified DNA must be a covalently closed circle to exist as a supercoiled molecule. Supercoiled plasmid DNA is often called Form I DNA. Supercoiling in the cell is caused by the action of enzymes called DNA gyrases. These enzymes use the chemical energy in ATP to introduce supercoiling into a relaxed molecule. In addition, there are enzymes that relax supercoiled DNA and are called unwinding or relaxing enzymes. Supercoiling has important biological consequences. Very large DNA molecules would simply not fit in the cell if they were not supercoiled. Gene expression can also be influenced by supercoiling.

If one or more phosphate bonds anywhere in the backbone of supercoiled DNA are broken, the molecule unravels to a relaxed form called open circular DNA or Form II DNA. These breaks in the phosphate backbone are called nicks. Nicked double-stranded DNA is not covalently closed.
The two strands of nicked DNA are still held together by hydrogen bonds between the bases. With time, purified supercoiled DNA slowly develops nicks and converts to Form II. This is because supercoiled DNA is not as stable as its relaxed or open circular forms. Endonucleases, such as DNAse I, will randomly nick supercoiled DNA when used in low amounts. Nicking can also be introduced by mechanical manipulations during plasmid purification.

During replication, several of the same plasmid molecules can form interlocked rings. These multimers of plasmid are called catenanes. A catenane containing two of the same plasmid molecules is called a dimer. Similarly, those containing three or four molecules are called trimers and tetramers, respectively. Each plasmid molecule in a catenane can be supercoiled, however, for clarity, they are represented as relaxed circles.

Agarose gel electrophoresis is a powerful separation method frequently used to analyze plasmid DNA. The agarose gel consists of microscopic pores that act as a molecular sieve. Samples of DNA can be loaded into wells made in the gel during molding. When an electric field is applied, the DNA molecules are separated by the pores in the gel according to their size and shape. Generally, smaller molecules pass through the pores more easily than larger ones. Since DNA has a strong negative charge at neutral pH, it will migrate towards the positive electrode in the electrophoresis apparatus. The rate at which a given DNA molecule migrates through the gel depends not only on its size and shape, but also on the type of electrophoresis buffer, the gel concentration and the applied voltage. Under the conditions that will be used for this experiment, the different forms of the same plasmid DNA molecule have the following rates of migration (in decreasing order):

Supercoiled > linear > Nicked Circles > dimer > trimer > etc.

Supercoiled DNA has the fastest migration rate of the different forms of plasmid. In the plasmid extraction experiment you will be doing, there will be some residual, degraded RNA which consists of transfer RNA and digested ribosomal and messenger RNA. Degraded RNA has a faster migration rate than supercoiled plasmid DNA because it is much smaller in size.

In the beginning of this experiment, RNase and cell lysis solution are added to the suspended bacterial cells. RNase will survive under these conditions because it is a robust protein. The cell lysis solution contains the detergent sodium dodecylsulfate (SDS) which dissolves the cell membrane and denatures proteins. The solution is very alkaline (pH > 12) due to the presence of sodium hydroxide. The high pH aids in denaturing proteins and causes the cleavage of the phosphate bonds in RNA. This eliminates interference from high molecular weight RNA during the plasmid purification. Under highly alkaline conditions, the two strands in non-supercoiled DNA (linear fragments of chromosomal DNA, relaxed and nicked circular DNA) separate and are partially removed from solution. However, this does not occur with supercoiled forms of plasmid DNA because the two strands are intertwined and entangled in a way that prevents them from coming apart.

The potassium acetate neutralization buffer contains acetic acid and potassium salts. The acidic buffer neutralizes the alkaline conditions created by the sodium hydroxide. The potassium causes the SDS, with its associated membrane fragments and proteins, to precipitate. The chromosomal DNA of E.coli is attached at several points to the cell membrane. Centrifugation of the potassium-SDS-membrane complexes also removes large amounts of entrapped chromosomal DNA.

The addition of isopropanol precipitates the plasmid and remaining RNA. Tris buffer (diluted buffer concentrate for RNase) is used to resuspend the DNA precipitate in a higher concentration. The buffer contains the enzyme RNase, which further degrades RNA. The concentrated gel loading solution prepares the sample for electrophoresis by making it denser than the electrophoresis buffer. This enables the sample to sink into the wells of the submerged gel. A negatively charged, blue tracking dye is also present to monitor the electrophoresis and to make sample loading easier.

In this experiment, a 3000 base pair plasmid will be extracted from E. coli cells. The restriction map for this plasmid has a single site for Eco RI. Digestion with the enzyme will yield a single band measuring 3,000 ± 300 nucleotides. The multiple forms of the plasmid will be converted to the linear form.
**Experiment Objective:**

The objective of this experiment is to introduce the principles of extracting plasmid DNA from bacterial cells. Students will develop an understanding of the structure and function of plasmid DNAs. **NOTE:** The extracted plasmid does not contain restriction enzyme sites that are suitable for mapping. We recommend Cat. #206 for mapping analysis.

**Laboratory Safety:**

1. Wear gloves and goggles while working in the laboratory.
2. Exercise extreme caution when working in the laboratory - you will be heating and melting agar, which could be dangerous if performed incorrectly.
3. **DO NOT MOUTH PIPEt REAGENTS - USE PIPEt PUMPS OR BULBS.**
4. The *E. coli* bacteria used in this experiment is not considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.
   - **A.** Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
   - **B.** All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
     - **Autoclave at 121°C for 20 minutes.**
       Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
     - **Soak in 10% bleach solution.**
       Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
5. Always wash hands thoroughly with soap and water after working in the laboratory.
6. If you are unsure of something, ASK YOUR INSTRUCTOR!
Module I: Isolation of Plasmid DNA

1. **LABEL** a microcentrifuge tube of *E. coli* cells with your initials or group number on it. **PLACE** tube on ice.

2. **CENTRIFUGE** the sample at full speed (10,000-14,000 rpm) for 2 minutes at room temperature.

3. **REMOVE** the supernatant. **ADD** 200 μl of Resuspension Buffer to the bacterial pellet. **RESUSPEND** the pellet by pipetting up and down.

4. **ADD** 5 μl of RNAse solution to the cell suspension and **MIX** by tapping the tube.

5. **INCUBATE** the cell suspension for 5 minutes at room temperature.

6. **ADD** 350 μl of freshly prepared Lysis Buffer to the cell pellet. **CAP** the tube and **MIX** well by inverting gently 4 to 6 times. To avoid damaging the plasmid DNA, DO NOT vortex the sample.

7. **ADD** 200 μl of Potassium Acetate Solution to the suspension. **CAP** the tube and **MIX** thoroughly by inverting the tube 4 to 6 times. A white precipitate should form.

8. **INCUBATE** the sample in ice for 5 minutes. DO NOT shake the tube during this incubation.

9. **CENTRIFUGE** the sample at full speed for 5 minutes at room temperature.

10. Carefully **TRANSFER** the supernatant into a clean 1.5 ml microcentrifuge tube. Avoid transferring the white cellular debris with the supernatant. **DISCARD** the tube containing the pellet.

11. **ADD** 0.6 volume of 100% isopropanol to the supernatant (i.e. 0.6 ml isopropanol for 1 ml of supernatant). **MIX** gently by inverting the tube 4 to 6 times.

12. **INCUBATE** the sample for 5 minutes at room temperature.
Module I: Isolation of Plasmid DNA, continued

13. **INSERT** the tubes in the rotor so that the hinges are facing towards the outside edge. **CENTRIFUGE** the sample for 5 minutes at full speed. The precipitated plasmid DNA will form a small, white pellet at the bottom of the hinge side of tube after centrifugation.

14. **REMOVE** and **DISCARD** the supernatant. To prevent pellet loss, we recommend gently pouring the supernatant onto a paper towel.

15. **WASH** the DNA pellet by adding 350 μl of ice-cold 70% ethanol to the tube. **CENTRIFUGE** at full speed for 3 minutes.

16. **REMOVE** the supernatant and air dry the pellet for 5-10 minutes to remove the residual ethanol.

17. **RESUSPEND** the pellet in 60 μl of 1x Tris-EDTA buffer. **CAP** the tube and **MIX** by shaking and vortexing. Briefly centrifuge to get all the contents to the bottom of the tube.

18. **PREPARE** purified plasmid DNA for electrophoresis.
   a. Sample 1: Concentrated plasmid – **TRANSFER** 40 μl of the purified plasmid DNA to a fresh microcentrifuge tube. **ADD** 5 μl of the 10x gel loading solution to the tube. **LABEL** this tube “S1”. **PLACE** sample on ice.
   b. Sample 2: Diluted plasmid – **ADD** 20 μl 1X TE and 5 μl 10x gel loading solution to the tube containing the remaining 20 μl of the purified plasmid. **LABEL** this tube “S2”. **PLACE** sample on ice.

19. **PROCEED** to Module II – Agarose Gel Electrophoresis

**OPTIONAL STOPPING POINT:**
The sample can be stored in the freezer for analysis at a later time. If digesting the plasmid with restriction enzymes after extraction, **STOP** at step 17. Assume the plasmid DNA concentration to be approximately 0.02 μg/μl. (Restriction enzymes are NOT included with this kit).
After electrophoresis, transfer gel for staining InstaStain® Blue or FlashBlue™ DNA stain.

Attach safety cover, connect leads to power source and conduct electrophoresis.

Load each sample in consecutive wells.

Remove end blocks & comb, then submerge gel under buffer in electrophoresis chamber.

Prepare agarose gel in casting tray.

After electrophoresis, transfer gel for staining.

Analysis on white light source.

Gel pattern will vary depending upon experiment.
Module II: Agarose Gel Electrophoresis

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. **WAIT** for 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

<table>
<thead>
<tr>
<th>Size of Gel Casting Tray</th>
<th>Concentrated Buffer (50X) + Distilled Water + Amt of Agarose (g) = TOTAL Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 ml 29.4 ml 0.23 g 30 ml</td>
</tr>
<tr>
<td>7 x 10 cm</td>
<td>1.0 ml 49.0 ml 0.39 g 50 ml</td>
</tr>
<tr>
<td>7 x 14 cm</td>
<td>1.2 ml 58.8 ml 0.46 g 60 ml</td>
</tr>
</tbody>
</table>

**CASTING THE AGAROSE GEL**

**IMPORTANT:**

Each gel is shared by 2-3 groups.

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at [www.edvotek.com](http://www.edvotek.com)

**Wear gloves and safety goggles**
Module II: Agarose Gel Electrophoresis, continued

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** 40 µl of the sample into the well in the order indicated by Table 2, at right.

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.

**RUNNING THE GEL**

- **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.
- **Load** 40 µl of the sample into the well in the order indicated by Table 2, at right.
- **Place** safety cover on the unit. **Check** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
- **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.
- After electrophoresis is complete, **Remove** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **Staining** the agarose gel.

**Table 2: Gel Loading**

<table>
<thead>
<tr>
<th>Lane</th>
<th>Samples 1</th>
<th>Samples 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group 1</td>
<td>Concentrated Plasmid</td>
</tr>
<tr>
<td>2</td>
<td>Group 1</td>
<td>Diluted Plasmid</td>
</tr>
<tr>
<td>3</td>
<td>Group 2</td>
<td>Concentrated Plasmid</td>
</tr>
<tr>
<td>4</td>
<td>Group 2</td>
<td>Diluted Plasmid</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>M6+</th>
<th>M12 (new)</th>
<th>M12 (classic) &amp; M36</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>15/20 min.</td>
<td>20/30 min.</td>
<td>25 / 35 min.</td>
</tr>
<tr>
<td>125</td>
<td>20/30 min.</td>
<td>30/35 min.</td>
<td>35 / 45 min.</td>
</tr>
<tr>
<td>75</td>
<td>35 / 45 min.</td>
<td>55/70 min.</td>
<td>60 / 90 min.</td>
</tr>
</tbody>
</table>

**REMINDER:** Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.
Module III-A: Staining Agarose Gels Using FlashBlue™

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. Carefully **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.

**ALTERNATIVE PROTOCOL:**

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
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.
Module III-B: Staining Agarose Gels Using InstaStain® Blue

1. Carefully REMOVE the agarose gel and casting tray from the electrophoresis chamber. SLIDE the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
2. MOISTEN the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, PLACE the blue side of the InstaStain® Blue card on the gel.
4. With a gloved hand, REMOVE air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. PLACE the casting tray on top of the gel/card stack. PLACE a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. STAIN the gel for 10 minutes.
6. REMOVE the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.
7. TRANSFER the gel to a small, clean gel-staining tray. COVER the gel with about 75 mL of distilled water and DESTAIN for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.
8. Carefully 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.

ALTERNATIVE PROTOCOL:

1. Carefully SLIDE the agarose gel from its casting tray into a small, clean tray containing about 75 ml of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.
2. Gently FLOAT the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm² of gel (7 x 7 cm).
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. VISUALIZE results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.
Study Questions

1. What are some reasons for isolating plasmid DNA?

2. What are the functions of sodium hydroxide and SDS in the cell lysis solution? What is the function of the potassium acetate solution?

3. What structural property of plasmid DNA allows it to be separated from chromosomal DNA during alkaline cell lysis?

4. Was more than one band observed in your plasmid sample after electrophoresis and staining?
## ADVANCE PREPARATION:

<table>
<thead>
<tr>
<th>Preparation For:</th>
<th>What to do:</th>
<th>When:</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Module I</td>
<td>Prepare and aliquot reagents</td>
<td>One day to 30 minutes before performing the experiment.</td>
<td>20 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare and aliquot cells</td>
<td>No more than 2 hours before experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td>Module II</td>
<td>Prepare diluted electrophoresis buffer</td>
<td>Any time before the class period.</td>
<td>10 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gels</td>
<td>One day to 30 minutes before performing the experiment.</td>
<td>45 min.</td>
</tr>
<tr>
<td>Module III</td>
<td>Prepare staining components</td>
<td>The class period or overnight before the class period.</td>
<td>10 min.</td>
</tr>
</tbody>
</table>
Module I: Pre-Lab Preparations

DNA ISOLATION

General Preparation of Biologicals and Reagents

- The SDS in solution (C) and components of the potassium acetate neutralization buffer (E) may have come out of solution during shipping. Warm the solutions until the precipitate redissolves.

Resuspension of Plasmid Extraction LyphoCells

Prepare within two hours of the lab:

1. Add 4.7 ml distilled water to the vial of Plasmid Extraction LyphoCells.
2. Mix until all the material of the vial is resuspended. The suspension of cells will look turbid.
3. Add 0.5 ml of Tris Buffer concentrate (A) to the cells. Cap and mix by inversion.
4. Aliquot 0.2 ml of resuspended cells to each of 20 microcentrifuge tubes.
5. Place cells on ice until needed.

Preparation of Plasmid Extraction Reagents

The following experiment reagents can be aliquoted for groups of four students. Alternatively, the reagents can be measured by students directly from the stock preparations. Set up a pipetting station with a designated 1 ml pipet for each reagent.

1. Dispense 1 ml Resuspension Buffer (D) for each group of students. Label these 5 tubes "Resuspension Buffer".
2. Preparation of Cell Lysis Solution
   - In a beaker, add all of the Sodium Hydroxide Solution (B) to 8 ml of distilled water.
   - Add all of the SDS solution (C). Mix.
   - Label this "Cell Lysis Solution" and keep at room temperature.
   - Dispense 1.6 ml for each group of students. Label these 5 tubes "Lysis Solution".
3. Dispense 1 ml of Potassium Acetate Solution (E) for each group of students. Label these 5 tubes "Potassium Acetate".
4. Preparation of 1XTE Solution
   - Add 1 ml of Tris Buffer Concentrate (A) to 9 ml distilled water. Mix well. Dispense 400 μl for each group of students. Label these 5 tubes "1XTE".
5. Dispense 20 μl of RNase Solution (F) for each group of students. Label these 5 tubes "RNase".
6. Dispense 2 mL isopropanol into 5 tubes. Label these tubes "Isopropanol"
7. Dispense 1.5 mL ethanol into 5 tubes. Label these tubes "Ethanol"
8. Dispense 50 μl 10x Gel Loading Solution into 5 tubes. Label these tubes "10x Gel"

FOR MODULE I
Each Group Requires:
- 1 tube containing 0.2 ml of resuspended cells
- 1 clean 1.5 ml snap top tube

Reagents to be Shared by group of 4 students:
- Resuspension Buffer 1 ml
- Prepared Cell Lysis Solution 1.6 ml
- Potassium Acetate Solution 1 ml
- 1XTE Solution 400 μl
- 95-100% Isopropanol 2 ml
- 70% Ethanol 1.5 ml
- RNase solution 20 μl
- 10x Gel Loading Solution 50 μl

NOTE:
The resuspension of Plasmid Extraction LyphoCells should be performed within 2 hours of the lab.
All other biologicals and reagents can be prepared any time on the day of the lab.
Module II: Pre-Lab Preparations

AGAROSE GEL ELECTROPHORESIS

This experiment requires 0.8% agarose gels for analysis by electrophoresis. Each gel can be shared by up to three groups. 7 x 7 cm gels are recommended. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I in the Student’s Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20°C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be “anchored” back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.
Module III: Pre-Lab Preparations

**MODULE III-A: STAINING AGAROSE GELS WITH FLASHBLUE™**

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

**MODULE III-B: STAINING AGAROSE GELS WITH INSTASTAIN® BLUE**

The easiest and most convenient DNA stain available is InstaStain® Blue. InstaStain® Blue does not require the formulation, storage and disposal of large volumes of liquid stain. Each InstaStain® Blue card contains a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution simultaneously stains and destains the gel, providing uniform gel staining with minimal liquid waste and mess.

You can use a White Light Visualization System (Cat. #552) to visualize gels stained with InstaStain® Blue.

**PHOTODOCUMENTATION OF DNA (OPTIONAL)**

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.
Experiment Results and Analysis

The figure to the left is an idealized schematic. The example in Lane 1 shows the relative positions of some possible extraction products. Extractions performed by the various student groups may yield different band compositions.

The example of undigested plasmid at left shows the following:

<table>
<thead>
<tr>
<th>Lane 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Degraded RNA</td>
</tr>
<tr>
<td>B</td>
<td>Supercoiled plasmid</td>
</tr>
<tr>
<td>C</td>
<td>Nicked plasmid (may not be visible)</td>
</tr>
<tr>
<td>D</td>
<td>Plasmid dimer</td>
</tr>
<tr>
<td>E</td>
<td>Higher catenanes (may not be visible)</td>
</tr>
<tr>
<td>F</td>
<td>Residual chromosomal DNA (may not be visible)</td>
</tr>
</tbody>
</table>

If the plasmid preparation is digested with the restriction enzyme, Eco RI, it will yield a single band measuring 3,000 ± 300 nucleotides. The multiple forms of the plasmid will be converted to the linear form. The following would be detected.

A No change.
B-E Will form the single linear form of the plasmid since they will all be cut with Eco RI.
F Will be less or will not be present since chromosomal DNA has many Eco RI sites and therefore the fragments that are generated will diffuse in the gel or give a "smearing" effect.

Actual results will yield broader bands of varying intensities. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.
Please refer to the kit insert for the Answers to Study Questions
Appendices

A  EDVOTEK® Troubleshooting Guide
B  Bulk Preparation of Agarose Gels

Safety Data Sheets:
Now available for your convenient download on www.edvotek.com/safety-data-sheets
## PLASMID PREPARATION

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>There is a precipitate in my Lysis Buffer.</td>
<td>The Lysis Buffer was placed on ice, and the SDS precipitated out.</td>
<td>Gently warm the Lysis Buffer to dissolve the SDS. Keep the solution at room temperature before use.</td>
</tr>
<tr>
<td>No/small DNA pellet after extraction.</td>
<td>Not enough cells used for lysis.</td>
<td>Be sure the bacterial cell pellet is the size of a match head before moving forward. Make sure cell pellet is completely resuspended in the resuspension buffer before adding the Lysis Buffer.</td>
</tr>
<tr>
<td></td>
<td>Cells did not lyse.</td>
<td>Lysis solutions did not mix well. Make sure to thoroughly but gently mix the sample after each buffer addition.</td>
</tr>
<tr>
<td></td>
<td>Plasmid DNA was sheared.</td>
<td>Lysis solutions were mixed too vigorously. Be sure to mix the solutions gently by inverting the tube 4-6 times. The cells should not be incubated in the lysis solution for more than five minutes.</td>
</tr>
<tr>
<td></td>
<td>Pellet was aspirated during wash.</td>
<td>Try gently pouring the supernatant out of the tube instead of using a pipet.</td>
</tr>
<tr>
<td>Pellet is hard to resuspend</td>
<td>Pellet was dried for too long.</td>
<td>Dry the pellet for 5-10 minutes</td>
</tr>
<tr>
<td></td>
<td>Some of the white precipitate was left behind in the supernatant.</td>
<td>Make sure to avoid the white pellet when transferring the supernatant to a fresh tube.</td>
</tr>
</tbody>
</table>
# Appendix A

**EDVOTEK® Troubleshooting Guides**

## ELECTROPHORESIS

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands are not visible on the gel.</td>
<td>The gel was not prepared properly.</td>
<td>Ensure that the electrophoresis buffer was correctly diluted.</td>
</tr>
<tr>
<td></td>
<td>The gel was not stained properly.</td>
<td>Repeat staining.</td>
</tr>
<tr>
<td></td>
<td>Malfunctioning electrophoresis unit or power source.</td>
<td>Contact the manufacturer of the electrophoresis unit or power source.</td>
</tr>
<tr>
<td>After staining the gel, the DNA bands are faint.</td>
<td>The gel was not stained for a sufficient period of time.</td>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td></td>
<td>The background of gel is too dark.</td>
<td>Destain the gel for 5-10 minutes in distilled water.</td>
</tr>
<tr>
<td>DNA bands were not resolved.</td>
<td>Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.</td>
<td>Be sure to run the gel at least 3.5 cm before staining and visualizing the DNA (approximately one hour at 125 V).</td>
</tr>
<tr>
<td>DNA bands fade when gels are kept at 4°C.</td>
<td>DNA stained with FlashBlue™ may fade with time</td>
<td>Re-stain the gel with FlashBlue™</td>
</tr>
<tr>
<td>There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.</td>
<td>The wrong percent gel was used for electrophoretic separation.</td>
<td>Be sure to prepare the correct percent agarose gel. For reference, the DNA samples should be analyzed using a 0.8% agarose gel.</td>
</tr>
</tbody>
</table>
Appendix B
Bulk Preparation of Agarose Gels

To save time, the 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 solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

**Table D**

<table>
<thead>
<tr>
<th>50x Conc. Buffer</th>
<th>Distilled Water</th>
<th>Total Volume Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 ml</td>
<td>2,940 ml</td>
<td>3000 ml (3 L)</td>
</tr>
</tbody>
</table>

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer.

2. Pour 3.0 grams 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. Measure 30 ml for a 7 x 7 cm tray, 50 ml for a 7 x 10 cm tray, and 60 ml for a 7 x 14 cm tray. *For this experiment, 7 x 7 cm gels are recommended.*

7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

**Table E**

<table>
<thead>
<tr>
<th>Amt of Agarose (g)</th>
<th>Concentrated Buffer (50x) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>7.5</td>
<td>382.5</td>
<td>390</td>
</tr>
</tbody>
</table>

*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.