

Isolation of *Plasmid DNA*

INTRODUCTION

This lab activity is unlike all the others in this manual. Each of the other activities posed some problem for you to solve and provided techniques for you to use to solve it. This activity is limited to the techniques only. However, the main technique presented, the plasmid miniprep, is a very useful and common technique in modern genetics. It is presented here because your instructor may choose to have you use this technique to isolate plasmid DNA that will be used in Lab Activity 11, entitled “Genetic Transformation of Yeast” (interestingly, yeast, which is a eukaryote, can be transformed using plasmid DNA from a bacterium!). In this activity, you will isolate plasmid DNA from a liquid culture of bacteria and characterize it via electrophoresis.

PROCEDURE

Isolating the DNA

Step 1: Your instructor will provide you with a sample of an “overnight” bacteria culture containing a dense population of bacteria. Add 1.5 ml of this solution to a sterile microcentrifuge tube.

Step 2: Place your tube in a microcentrifuge so that it is balanced with another tube, and run the microcentrifuge for 1 minute.

Step 3: Remove the supernatant using a micropipette with a sterile pipette tip. A creamy pellet of bacteria should be visible at the bottom of the tube.

Step 4: Add 100 μl of a lysis solution to the tube with the pellet, and resuspend the pellet with a vortex mixer or by repeatedly flicking the tube with your finger. The lysis solution has osmotic properties that help to break the bacterial cells open.

Step 5: Add 200 μl of a solution containing the detergent agent sodium dodecyl sulfate (SDS). This substance is a detergent-like material that will help to solubilize proteins that may be associated with the DNA. Mix the contents by inverting the tube back and forth. During this stage, the cloudy solution should become somewhat clear.

Step 6: Let the solution stand for about 3 minutes, and then add 150 μl of a sodium acetate solution. Sodium acetate will cause the proteins and other substances to precipitate out of the solution. At this point, you will begin to see a white, clumpy material start to form in the solution.

Step 7: Incubate the tube in an ice bath for about 20 minutes.

Step 8: Centrifuge the tube for 5 minutes in a microfuge.

Step 9: Remove the supernatant (approximately 400 μl should be present) using a 200- μl micropipette. Transfer this supernatant into a fresh microcentrifuge tube. This solution contains the plasmid DNA that you are attempting to isolate.

Step 10: Add 1 ml of 95% ethanol to the solution in the clean tube and mix well. The ethanol will cause the DNA to precipitate out of the solution.

Step 11: Centrifuge this solution in microfuge for about 15 minutes. The goal of this step is to obtain a tiny (in some cases, almost invisible) DNA pellet at the bottom of the tube. To ensure that you will be able to locate the DNA pellet after centrifuging, place the tube into the centrifuge in the following way. Place the tube into the rotor with the closure of the tube (i.e., the “hinge”) facing downward (i.e., toward the center of the rotor). This way, when the outward force of the centrifuge causes the pellet to form on the lower wall of the tube, you will know where to look for it. The pellet (whether you are able to see it or not) should be located at the bottom of the tube on the opposite side from the closure.

Step 12: Using a micropipette, carefully remove the supernatant and discard it. Place the tip of the micropipette at the bottom of the tube on the same side as the closure when removing the supernatant. This way, you will be less likely to dislodge the DNA pellet.

Step 13: After removing the supernatant, add 500 μl of an 80% ethanol solution. This solution is added as a “wash” solution to help remove materials other than the DNA.

Step 14: Centrifuge the tube as before for about 5 minutes, and carefully remove the wash solution without disturbing the pellet.

Step 15: Now do a final wash with 95% ethanol. Add 500 μl of 95% ethanol. Centrifuge for about 5 minutes and remove the supernatant as before.

Step 16: Remove any remaining ethanol solution by floating the open microcentrifuge tube in a water bath at a temperature between 50° and 65°C for about 10 minutes.

Step 17: Add 100 μl of TE buffer to the pellet to dissolve the DNA. To ensure that the DNA becomes completely dissolved in the TE buffer, repeatedly aspirate the solution by slowly sucking it up and down through a micropipette tip. Do this gently to avoid shearing the DNA.

Step 18: If an appropriately sensitive spectrophotometer is available, your instructor will show you how to use it to determine the concentration and relative purity of your DNA.

First, you will dilute some of your sample into distilled water and use that to obtain your measurement. If possible, you should measure the absorbance of your dilute sample at both 260 and 280 nm (nanometers). You can use the absorbance at 260 nm to calculate the concentration of DNA in your sample, since it is known that 1 absorbance unit equals 50 $\mu\text{g}/\text{ml}$ of DNA (remember to factor in the dilution factor when determining the concentration of your original sample). You can also determine the relative purity of your sample by calculating a value for it based on the absorbance value at 260 nm divided by the value at 280 nm. For completely pure DNA, this value will equal 1.8.

Visualizing the Plasmid DNA on an Agarose Gel

Determining the concentration of DNA using a spectrophotometer is one way to see how well your plasmid miniprep worked.

Another way is to run your DNA sample on an agarose gel. If you successfully isolated only the plasmid, and it is mostly intact, you will obtain a single band on your agarose gel (corresponding in size to the approximate size of the plasmid). If your sample is contaminated with other DNA from the bacteria, or if the plasmid was fragmented during isolation, you will see more smearing on your gel. You should run your samples on a gel, as described later. You will then be instructed how to estimate the size of the plasmid, based on the location of the band in the gel.

Step 1: Set aside 20 μl of your plasmid DNA in a microfuge tube to use as a 1X concentrated sample for electrophoresis. Also prepare 20 μl or more of a 0.1X concentration for electrophoresis. Prepare both dilutions for electrophoresis as described here. If your plasmid miniprep DNA is highly concentrated, the bands you see on your gel may be more distinct and reliable for the 0.1X diluted sample.

Your instructor will tell you what volume of DNA solution you need to load the sample wells on your agarose gel. In a separate microfuge tube, mix a small volume of your sample together with a 6X loading dye in the appropriate ratio (i.e., five parts sample to one part 6X loading dye). The dye will give your sample a blue color so that you can see it when you load it into the gel, and it will also make your sample more dense, so that it will sink through the aqueous buffer in the gel tank.

Step 2: Set your micropipette for the volume indicated by your instructor, and remove that amount of your DNA/dye solution.

Step 3: Add your sample to one well in the gel (note which well[s] you load), by submerging the tip below the buffer solution and directly into the opening of a well. Do not expel the solution until you are sure that your tip is inserted into a well, and do not insert your tip too far into the well (be careful to avoid puncturing the bottom of the gel with your pipette tip). When you expel the sample into the well, you should be able to see the colored solution settling into the well and filling it.

One student should also load one lane (on each gel used) with a molecular marker for size determination.

Step 4: After the gel is loaded, your instructor will set the voltage. The gel should be allowed to run until the dye front is close to the opposite end of the gel (unless a somewhat long gel is used).

Step 5: View the gel(s) on an ultraviolet light box or use a gel documentation system to photograph the gel (wear gloves when handling the gel — it contains a potent mutagen, ethidium bromide). If you view the gel directly (rather than viewing an image of the gel), *wear appropriate plastic eye protection*. Since prolonged exposure to UV light may also harm the skin, students may wish to wear a plastic face shield and appropriate clothing to cover exposed skin.

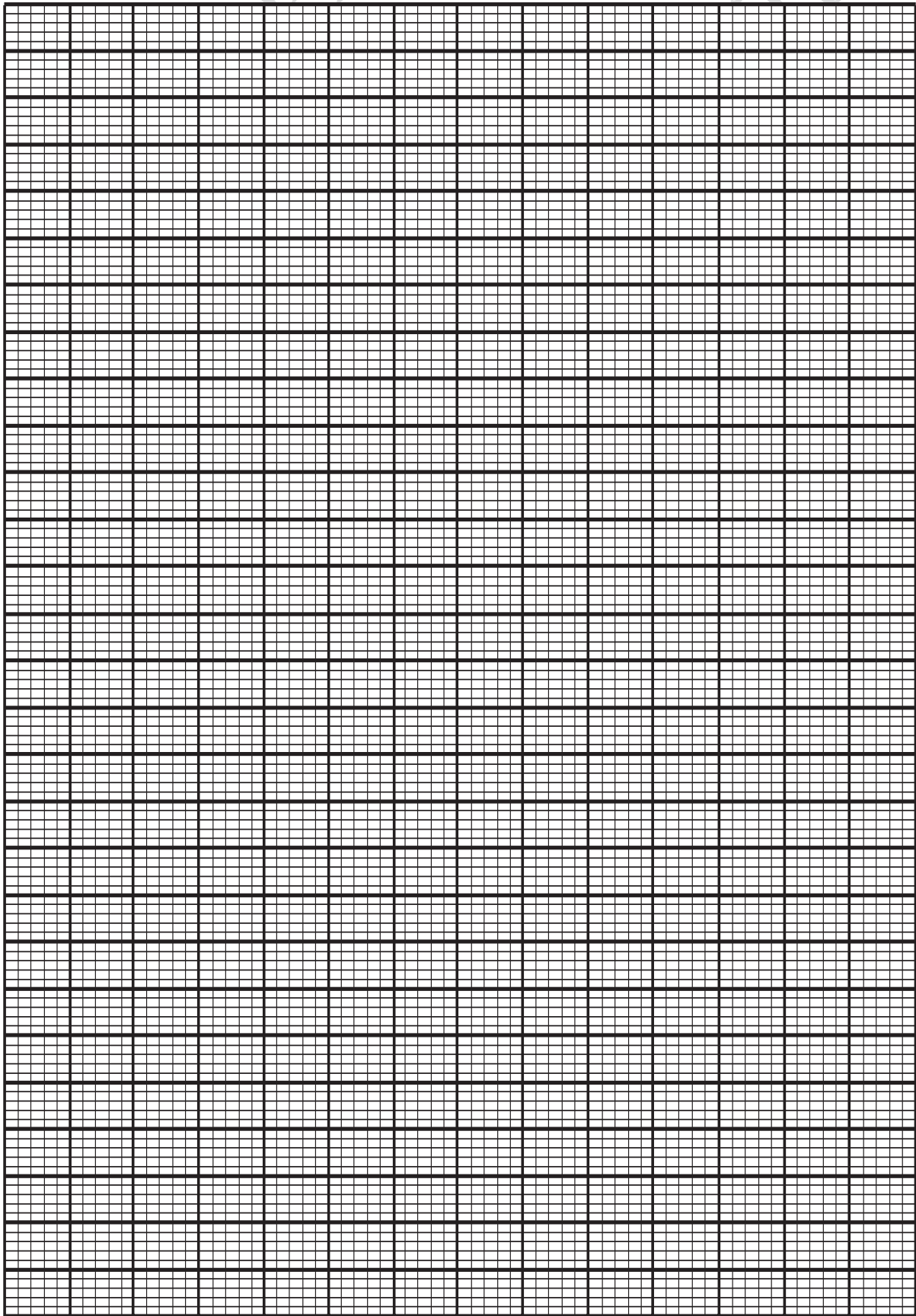
Measure how far each band migrated from the well. You will use this information to determine the size of the plasmid (see next step).

Measure the distance from the well to the location on the gel where your plasmid seems to be located (if several bands or a smear are present, measure the largest and the most distinct band[s] present; measure the distance to the center of the bands). Also measure the distances between the well and each of the bands in the lane containing the reference DNA. Your instructor will tell you the sizes of the DNA fragments represented by each band in the reference DNA lane.

Step 6: To estimate the size of the DNA you isolated, compare the distance traveled by the band representing the plasmid to that of each of the reference bands. Do this by plotting a standard curve for the reference DNA fragments on semilog graph paper and comparing the measurement for your plasmid DNA to these. Plot the sizes of the reference fragments (in base pairs) on the log scale axis and the distances migrated on the standard scale axis. You should be able to draw a straight line passing through (or nearby) each of the points. Now use the standard curve to estimate the size of the plasmid you isolated.*

Q1 How large was the plasmid you isolated according to the estimate from the standard curve? Hand in a copy of your standard curve showing distance migrated by your plasmid and its estimated size on the graph paper on the next page.

* Note: If you isolated the plasmid without breaking the circular DNA molecule, it will be closer to the bottom of the gel than you would predict, based on its known size. This occurs because uncut plasmids are supercoiled, and supercoiled DNA travels through an electrophoresis gel faster than linear DNA of the same size.



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