Use of Miniprep Kits in High School Teaching Labs

Background information:

DNA plasmid preps are performed routinely in molecular biology research laboratories as a quick and economical way to maintain pure quantities of DNA. Minipreps involve a process of using alkaline compounds to extract the plasmid DNA from bacterial sources in large enough quantities to be useful to the laboratories.

Transformation and restriction activities are now commonly used in many high schools. However, at this point plasmid minipreps have not become as popular in the high school lab as this writer thinks they should be. Extracting DNA (miniprep activities) in the high schools can add an additional dimension to one's biotechnology program. The miniprep system can be used in the classroom as a separate skill (see lab write-up that follows), or made part of a larger skill set. It can serve as an important connection between transformation and restriction activities. Qualitative analysis studies can be developed by the teacher for students who know the techniques involved in these three activities. For example, students may be expected to transform an unknown plasmid (known only by the teacher) into a bacterial sample, transfer a transformed colony to a sterile liquid medium, and incubate the culture overnight. After overnight growth, the student could extract the plasmid via a miniprep activity, restrict the DNA sample, electrophorese the DNA, and then identify the "unknown" sample. These activities reinforce the skills learned in the lab, as well as give students the opportunity to do the work of research scientists.

Quantum Prep™ Plasmid Miniprep Kit (Catalog number 732-6100EDU)

Introduction:

Plasmids are circular pieces of DNA located in various types of bacteria. Plasmids are relatively small in size, ranging from 2,000 to 10,000 base pairs. Because they are easily purified, conveniently manipulated in microfuge tubes, and can be made to carry, express, and replicate specific genes from any source in living bacteria, they are essential tools in molecular biology.

A plasmid miniprep is a technique involving the use of alkaline materials to extract plasmid DNA from bacteria. It is a quick way to obtain a relatively pure quantity of DNA, usually less than 2 µg. Plasmid miniprep activities are carried out routinely in research laboratories as a method of extracting and purifying DNA.

The Quantum Prep plasmid miniprep kit eliminates many of the problems associated with standard miniprep procedures. For example, since chloroform and phenol are not used in this kit, it is much less hazardous to use than other standard miniprep systems. Also, the entire process can be completed easily in a standard 50-minute high school classroom period.

Materials:

Per Group

- 4 sterile microfuge tubes
- 1 miniprep filter
- 0–20 µl (P-20) pipettor
- 0–1,000 µl (P-1000) pipettor
- yellow (0–20 µl) tips
- blue (0–1,000 µl) tips
- miniprep kit solutions
- marking pen
- 3 ml of bacterial culture containing the plasmids of interest
- 1x TE buffer (10 mm Tris, 1 mm EDTA, pH 8), 100 µl

Per Class

- Microfuge
- 95% or 100% ethanol, 63 ml
- Quantum Prep plasmid miniprep system containing the following:

1. Cell resuspension solution

2. Cell lysis solution
3. Neutralization solution
4. Binding matrix solution
5. Column wash solution
6. Miniprep filters
7. Loading buffer

Procedure:

A. Extraction method (see Quantum Prep plasmid miniprep kit instruction manual for more details)

1. Label the lid of a clean microfuge tube as #1. Fill the microfuge tube with a bacterial culture containing the plasmid DNA.
2. Centrifuge the bacterial sample at 10,000 rpm or full speed for 1 minute or until a large pellet forms at the bottom. (Be sure the microfuge is balanced properly before it is turned on.)
3. Discard all the supernatant (fluid above the pellet) by pouring and pipetting. (The pellet is composed of millions of bacterial cells concentrated into a small area.) Save the pellet.
4. Add 200 µl of the resuspension solution. Resuspend the pellet by slowly and carefully drawing the solution up the pipet tip and releasing it back to the microfuge tube. Repeat until the pellet is completely dissolved. (The resuspension solution contains Tris-HCl and EDTA, which buffer and protect the DNA, and RNase, which will destroy RNA in the sample.)
5. Add 250 µl of cell lysis solution. Close the cap, and mix by inverting the tube slowly and carefully about 10 times. Do not vortex. (Mixing too vigorously will break up and release genomic DNA.) The solution should become viscous and slightly clear if cell lysis has occurred. (The lysis solution contains NaOH and sodium dodecyl sulfate (SDS) detergent. These reagents break up the bacterial cells and release the inner contents.)
6. Add 250 µl of the neutralization solution and mix by gently inverting the capped tube about 10 times (do not vortex). A visible precipitate should form. (Potassium acetate in the neutralization solution alters the pH from a basic to a slightly acidic condition. At this stage the plasmid DNA is preferentially renatured.)
7. Pellet the cell debris for 5 minutes by centrifugation at 10,000 rpm or full speed. A compact white pellet will form on the side or bottom of the tube. The supernatant (cleared lysate) at this step contains the desired plasmid DNA.
8. While the centrifugation at step 7 is running, insert a spin filter into a clean 2 ml microfuge tube and label the tube with tape as #2.
9. Transfer the cleared lysate from step 7 to the spin filter. Vigorously shake the bottle containing the Quantum Prep matrix and then add 200 µl of the matrix to the spin filter. Pipet the mixture in the filter up and down several times to mix it well, and make sure the whole filter becomes wet. (The matrix is composed of diatomaceous earth, which binds the plasmid DNA.)
10. Let the mixture saturate the filter. Then centrifuge the sample for 30 seconds at 10,000 rpm or full speed.
11. Remove the spin filter from tube #2, discard the filtrate at the bottom of the tube, and replace the filter in the same tube. Add 500 µl of column wash solution and wash the matrix by centrifugation for 30 seconds at 10,000 rpm or full speed. (Make sure you have added 63 ml of 95% or 100% ethanol to the entire bottle of column wash solution before using it.)
12. Remove the spin filter from tube #2 once again. Discard the filtrate at the bottom of the tube and replace the filter in the same tube. Add another 500 µl of column wash solution and centrifuge the matrix for a full 3 minutes to remove residual traces of ethanol.
13. Remove the spin filter and discard the microfuge tube. Label a clean microfuge tube #3. Place the filter in tube #3. Add 50 µl of 1x TE buffer or sterile H_2O to the filter. Elute the DNA by centrifugation for 2 minutes.
14. Discard the spin filter. Label the side of the tube with the type of DNA plasmid you have extracted and store the sample at -20°C (standard freezer temperature) or prepare the DNA for electrophoresis.

B. Electrophoresis of the DNA sample

1. Transfer 4 µl of the extracted plasmid sample to a clean microfuge tube. Add 5 µl of distilled water, and l µl of loading buffer.

2. Load your 10 µl sample in the well assigned to you by your teacher. Record the well number you use.

3. After all groups have loaded their samples, the DNA will be electrophoresed. The DNA will be stained with

ethidium bromide and photographed.

Cleanup:
Clean up your lab station. Remove the tape labels from the microfuge tubes and place the tubes and tips in waste containers according to your teacher's instructions.

Follow-up:
Observe the gel when it is finished. Then answer the following questions:

1. What is the purpose of the mini filter in the spin column?

2. What role does SDS play in the plasmid miniprep procedure?

3. What extension of this lab activity would you design to determine the exact size of the plasmid removed from the bacteria? Please describe.

4. Explain why it was necessary to have ampicillin in the bacterial culture containing the plasmid you extracted.

5. How could a plasmid miniprep procedure be useful following bacterial transformation?

For the Teacher:

1. The Quantum Prep miniprep kit is made by Bio-Rad. The kit may be purchased as a part of Bio-Rad's high school Biotechnology Explorer program. The kit, which contains 100 preps, includes 20 ml cell resuspension solution, 25 ml cell lysis solution, 25 ml neutralization solution, 20 ml Quantum Prep matrix, 63 ml column wash solution, 100 spin filters, wash tubes, collection tubes, and instructions.

2. After the students complete this activity you may want to give each group of 2–3 students a different "mystery plasmid" if you have others available. Each group could transform bacteria with the plasmid, clone the plasmid and bacteria, plate out, select a bacterial colony, grow up the bacteria overnight and finalize with a mini prep, restriction and electrophoresis to determine the "mystery plasmid" they have (a lab practical exam).

Questions and Comments:

Contact Bio-Rad Laboratories, Biotechnology Explorer Program, at http://explorer.bio-rad.com/ or call 1-800-424-6723