

## **Protocol: Minipreparation of Plasmid DNA, Restriction Digest of Potential Recombinants, and Gel Electrophoresis**

**NOTE:** in this protocol, it is important to *pay attention* to the incubation times and whether the incubation is on ice or on the bench top.

For centrifugation in the microfuge, the hinge on the eppy tube should point out from the rotor center. The material will collect below the hinge.

### A. Minipreparation of Plasmid DNA

1. The day before lab, 1 mL of rich medium (LB Broth) containing the appropriate antibiotic was inoculated with a single colony of transformed bacteria. The cultures were incubated overnight at 37°C with vigorous shaking.
2. Each student will take two glass test tubes with overnight cultures. Keep the contents separate, DO NOT mix them. Label two eppy tubes "M1" and "M2" (miniprep 1 & 2) plus your initials. Keep your two samples separate throughout the procedure, and note on the line below what plasmid you are isolating. Aliquot 1 mL of the culture into an eppy tube. Centrifuge at maximum speed (10K rpm) for 30 seconds in a microcentrifuge to pellet the cells.  
M1 \_\_\_\_\_  
M2 \_\_\_\_\_

Clean up: take glass test tubes to the sink and put them on the rack. Remove tops and put them into the plastic bucket. Remove any tape and throw it into the trash. Give each culture tube a good squirt of bleach solution and let it sit.

3. Pour off or draw off the culture medium with a pipettor, leaving the bacterial pellet as dry as possible.
4. Resuspend the bacterial pellet in 100 uL of ice-cold GTE (solution I) by vigorous vortexing or pipetting. Resuspend the pellet completely.
5. Add 200 uL of freshly prepared NaOH/SDS (solution II) to each bacterial suspension. (This stock solution should NOT be put on ice.) Close the tube tightly, and mix the contents by inverting the tube rapidly five times. *Do not vortex!* The solution should become slimy and translucent. Store the tube on ice while you obtain the next reagent.

6. Add 150  $\mu\text{L}$  of ice-cold acetic acid/K acetate (solution III). Close the tube and invert the tube 5 times to disperse the solution through the viscous bacterial lysate. Store the tube on ice for 3-5 minutes. The solution forms a white precipitate.
7. Centrifuge the bacterial lysate at 10k rpm for 5 minutes in a microcentrifuge. CAREFULLY Transfer the supernatant to a fresh tube. DO NOT bring along any of the white precipitate.

Use a P200 to draw up the 400  $\mu\text{L}$  of supernatant because it has a narrower tip and will be easier to avoid the "goop". Wipe off the tip with a small piece of Kim wipe each time before transferring to a clean eppy tube.

If you see any particles floating in the tube, spin and transfer a *second time*.

8. Precipitate nucleic acids from the supernatant by adding 400  $\mu\text{L}$  of isopropanol at room temperature. Mix the solution by vortexing and then allow the mixture to stand for 2 minutes at room temperature. The next step is a centrifugation, so be sure before you add the alcohol that a microfuge is available for use in 2 minutes (coordinate with your lab mates).
10. Collect the precipitated nucleic acids by centrifugation at 10K rpm for 5 minutes in a microcentrifuge. The hinge on the eppy tube should point out from the rotor center.
11. Carefully remove the supernatant with a pipettor. Do not suck up your small pellet! Stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away. Use a small piece of Kim wipe or disposable pipette tip to remove any drops of fluid adhering to the walls of the tube.
12. Add 200  $\mu\text{L}$  of 95% ethanol to the pellet and invert the closed tube several times. Recover the DNA by centrifugation at maximum speed for 2 minutes in a microcentrifuge. (If we could not finish the protocol, the DNA can be stored safely in alcohol at  $-20^{\circ}\text{C}$  for later).
13. Carefully remove all of the supernatant with a pipettor. *Take care with this step*, as the pellet sometimes does not adhere tightly to the tube.
14. Remove any beads of ethanol that form on the sides of the tube. Blow warm air over the top of the tubes with a hair dryer until the ethanol has evaporated, no fluid is visible in the tube, and the alcohol smell is gone (sniff test). The small pellet should be located *under the hinge*, and will change from translucent (wet) to opaque (dry). This takes about 5-10 minutes.
15. Dissolve the nucleic acids in 30  $\mu\text{L}$  of TE (pH 8.0) containing 20  $\mu\text{g/ml}$  DNase-free RNase A (pancreatic RNase). Resuspend the pellets by scraping them with the pipet tip and pipetting up and down, especially below

the hinge region. Do not be too vigorous as you can shear (break) the DNA with pipetting. Rinse down the sides of the tube. Check that all of the DNA has dissolved. Store the plasmid DNA solution on ice.

## B. Restriction Analysis of Purified Plasmid Recombinants

1. Set up restriction digests:

Use a permanent marker to label four eppy tubes. Use the table below as a checklist when adding reagents. The total will be 20 uL.

| Tube:         | M1       | M2       | 10x Buffer | H <sub>2</sub> O | RE #1 =  | RE #2 =  |
|---------------|----------|----------|------------|------------------|----------|----------|
| M1(-)         | 10 uL    | 0        | 2 uL       | uL               | 0        | 0        |
| M2(-)         | 0        | 10 uL    | 2 uL       | uL               | 0        | 0        |
| M1(+)         | 10 uL    | 0        | 2 uL       | uL               | 1 uL     | 1 uL     |
| M2(+)         | 0        | 10 uL    | 2 uL       | uL               | 1 uL     | 1 uL     |
| <b>Order:</b> | <b>3</b> | <b>3</b> | <b>2</b>   | <b>1</b>         | <b>4</b> | <b>4</b> |

**M1** and **M2** are miniprep **one** and **two**; the **(-)** or **(+)** are **without** or **with** restriction enzyme respectively.

2. Add H<sub>2</sub>O, 10x buffer, and then DNA. Always add enzymes *last* (keep them cold). Make sure digest reagents are mixed; pulse in the microfuge if needed and then incubate at 37°C for at least 40 minutes.

3. When it is time to load the gel, add 4 uL of 6x tracking dye to each tube. Load 20 uL of each sample.

Your instructor will load the controls: undigested and digested control plasmid(s) and a ladder/marker lane.