Miniprep

Introduction

The miniprep uses silica gel to isolate plasmid DNA from an E. coli culture

Materials

- Buffer P1 (resuspension buffer)
  - Retrieve from refrigerator. If you are opening a new miniprep kit, **add the RNAse and LyseBlue reagent and check the box on the cap.**
- Buffer P2 (lysis buffer)
  - Open the cap and look at the lysis buffer. Swirl it around. If it appears cloudy, the SDS has fallen out of solution; warm it for a few minutes in the 55°C water bath.
- Buffer N3 (neutralization buffer)
- Buffer PB (binding buffer)
- Buffer PE (rinse buffer)
  - Make sure the "Ethanol added?" box has been checked. If you are opening a new miniprep kit, **add absolute ethanol as per the kit instructions and check the box on the cap.**
- Buffer EB (elution buffer)
- Miniprep waste container
  - Miniprep buffers contain salts that can't go down the sink.
- Per miniprep: two microcentrifuge tubes and one **blue** spin column, with collection tube.

Procedure

Harvest and resuspension

1. For each culture, label two microcentrifuge tubes **on the cap** and one **blue** spin column **on the side.**

   The spin columns should be in their (cap-less) collection vials.

2. Pipette 1.6 mL of each culture into the corresponding microcentrifuge tubes.

   (This is for a 1.7 ml microcentrifuge tube; set your pipettor to 800 ul and do two transfers. If we ever go back to 2-ml tubes, do 1.9 ml (2x950 ul))

3. Centrifuge at maximum speed (10,000 or 13,000xg) for three minutes.

4. Aspirate the supernatant, or pour it off into the bucket.

5. Pipette ANOTHER 1.8 ml of each culture into the corresponding microcentrifuge tubes.

6. Centrifuge at maximum speed for three minutes.

7. While the centrifuge is running , move the remaining cultures to 4degC.

8. Aspirate the supernatant off with the bench aspirator. Be careful not to disturb the pelleted E. coli.

   We use an aspirator here because the less extra salt and protein we put in the miniprep, the better the yield is.

9. Add 250 μl **Buffer P1** to each tube.

10. Resuspend the E. coli pellet. The preferred way is with the roto-mixer at the other end of the lab.

    Alternately, if you have just a few tubes, you can resuspend on a vortex.
    Make sure to resuspend fully and thoroughly. The resulting suspension should be smooth and cloudy; if there is particulate matter floating around, vortex some more.

Lysis

11. Add 250 μl **Buffer P2** to each tube.

    Work quickly; the lysis step should take less than 5 minutes.

12. Snap the tubes closed and invert them 4-6 times, until the tube is thoroughly mixed and the entire solution turns blue.

    If you have many many tubes, you can stack a second tube rack on top of them and invert the entire thing.

13. Add 350 ul **Buffer N3** to each tube.

14. Snap the tubes closed and invert 4-6 times, until the solution is thouroughly mixed and no longer blue.

    The solution will become cloudy or flocculent.

15. Centrifuge on high speed for 10 minutes.

Separation

16. Remove the tubes from the microcentrifuge, being careful not to disturb the white pellet.

17. Using P-1000 micropipettor set to 850 ul, carefully transfer the supernatant from each centrifuge tube to the corresponding **blue** spin column.

18. Centrifuge the spin columns for 30 seconds at maximum speed.

    Don't forget to put the lid on the rotor! Some of the salts get aerosolized because the spin columns don't have caps.

19. Pour the flow-through from each column into the miniprep waste container.

20. Pipette 500 ul of **Buffer PB** onto each spin column.

21. Centrifuge the spin columns for **30 seconds** at maximum speed.

22. Pour the flow-through from each column into the miniprep waste container.
23. Pipette 750 ul of **Buffer PE** onto each spin column
24. Wait 1-3 minutes.

    This allows some of the salt that's still bound to the silica matrix to resuspend in the buffer.
25. Centrifuge the spin columns for **30 seconds** at maximum speed.
26. Pour the flow-through from each column into the miniprep waste container.
27. Return each spin column to its collection tube and centrifuge **an additional 1 minute** at high speed.

    This removes every last trace of buffer PE; the ethanol can screw up downstream steps.
28. Transfer each spin column to a clean labelled microcentrifuge tube.
29. Pipette 50 ul of **Buffer EB** onto the **center of each column.**

    The volume of EB is comparable to the volume of silica gel matrix; if you pipette down the side, you might not get the entire transfer to the matrix.
30. Wait 1-3 minutes.

    This gives the DNA a chance to dissociate from the silica matrix.
31. Centrifuge the spin columns, in their collection tubes, for **one minute** at maximum speed.
32. Vortex, then pulse spin down the samples.
33. Proceed directly to analyze the samples on the Nanodrop.