Midiprep

Introduction

Once a plasmid has been built and verified, we midiprep it so we have a lot of DNA for future use.
This is also a procedure done to prepare for transfecting cells.

Materials

- Buffer P1 (resuspension buffer)
  - Retrieve from refrigerator. If you are opening a new midiprep 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 S3 (neutralization buffer)
- Buffer BB (binding buffer)
- Buffer ETR
- 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)
- Per midiprep: Two 50 mL blue-capped tubes, one microcentrifuge tube, and one midiprep spin column, with collection tube.

Procedure

Materials Setup (Fresh Overnight Cultures)

1. Warm up the LB to at least room temperature (if it came from the fridge), but not warmer than 37°C.
2. Label one round-bottom culture tube for each miniprep. Use "NAME-1, NAME-2, ..."etc for the naming convention, where NAME is a shortened name of the plasmid (eg, "hEF1a:mKate").

   Your impulse is to just use number, or initials and number, but trust me -- you will want to be able to identify this tube in three weeks when you've forgotten what you were doing.
3. Using a sterile pipette, transfer 5 ml of LB to the mixing container for each culture PLUS 5 ML.
4. Add antibiotic stock to a final concentration of 1X (1 µl stock for each 1 ml in the mixing container.)
5. Cap tightly and mix well.

Culture Setup

6. Using a sterile pipette, transfer 5 ml of LB+antibiotic to each round-bottom culture tube.

   If you are making cultures with different antibiotics, **take care that the right media goes in each tube.**

7. Pipette 1 uL of the old culture into its corresponding round-bottom culture tube.

8. Transfer to an incubating shaker at 37°C and incubate 14-16 hours.

   Don't over-grow too badly, or your yield will suffer.
   If you need to grow longer, you can grow at 30°C instead for 20 hours.

50 mL Culture Setup

9. In the morning, check that the 5 mL overnight cultures have grown and are cloudy.

   If any of the overnight cultures did not grow, put them aside. They will have to be regrown and midiprepped another day.

10. Grab a sterilized 250 mL flask for each round-bottom culture tube.

11. Label each 250 mL flask with tape for each round-bottom culture tube.

12. Using a pipette gun, transfer 45 ml of LB to each 250 mL flask.

13. Add the appropriate antibiotic stock to a final concentration of 1X (1 µl stock for each 1 ml in the mixing container.)

14. Pour out the 5 mL of culture into the corresponding 250 mL flask.

    Each flask should have a volume of 50 mL in it.

15. Transfer to an incubating shaker at 37°C and incubate 3 - 4 hours.

    During the day, e very 60 - 90 minutes, use the Nanodrop to analyze the OD.
    You want the OD to be between 2 and 3.

Harvest and Resuspension

16. For each culture, label one 50 mL blue-capped tube, one 15 mL blue-capped tube, one midiprep spin column, and one microcentrifuge tube.

    There's a lot of labelling, but if you do it now, the rest of the protocol will go by quicker.

17. Once the flask cultures are ready, pour them into labeled 50 mL blue-capped tubes.

    The tube will be filled to the very top. Just don't let the tube overflow.
    You don't really need to get all the foam into the tube.

18. Centrifuge the 50 mL blue-capped tubes in the big centrifuge at 6000 x g for **15 min** at 4°C.

    Make sure the centrifuge is balanced.

19. Slow pour out the supernatant into the bleach bucket.

20. Add 4 mL **Buffer P1** to each 50 mL tube and resuspend the pelleted bacteria using a pipette gun.

    Vortex the tube until it is uniform and cloudy.
    The bacteria are completely resuspended when you don't see any white clumps of bacteria floating around.

Lysis

21. Add 4 mL **Buffer P2** to each 50 mL tube.

22. Cap the tubes and gently mix by inverting each tube 4 - 6 times until the solution appears blue and is thoroughly mixed.

23. Incubate at room temperature (15 - 25°C) for **3 minutes**.

24. After incubation, add 4 mL **Buffer S3** to the first set of 50 mL tubes.

25. Cap the tubes closed and gently mix by inverting each tube 4 - 6 times until the solution is thoroughly mixed and no longer blue

    The solution will become cloudy or flocculent.

26. Place a QIAfilter Cartridge into the set of labeled 50 mL tubes on a rack.

    The filter should sit comfortably in the tubes.

27. Pour the lysate into the QIAfilter Cartridge.

28. Incubate at room temperature for 10 minutes.

29. Place a midiprep spin columns into the QIAvac 24 Plus. Unplug a hole for each spin column that you are using.

    The "QIAvac 24 Plus" is in the hood right across from the PCR machines.
    It was made by one of the past iGEM teams and it is made out of white PVC pipes.

30. Insert a Tube Extender into each midiprep column.

Separation

31. After incubation, gently insert a plunger into each QIAfilter Cartridge.

32. Push down on the plunger until there is only white clumps trapped in the plunger.

    This step filters the cell lysate into the tube. A little resistance is expected but keep going.

33. Discard the QIAfilter Cartridge and plungers.

34. Add 2 mL **Buffer BB** to each 50 mL tube.

35. Cap the tubes closed and gently mix by inverting each tube 4 - 6 times.

    After this step, take your rack of 50 mL tubes over to the QIAvac 24 Plus.

Be sure to bring Buffer ETR, Buffer PE, a P1000 pipette, and a biological waste bin.

36. Pour the lysate into the Tube Extenders on the QIAvac 24 Plus.

    Make sure the vacuum is not on when you do this step.
    Make sure that there are no empty holes on the QIAvac 24 Plus.

37. Apply vacuum until the liquid has been drawn through all the columns.

    On your left, turn the yellow vacuum knob to the left until it's slightly loose.

38. Turn off the vacuum and add 700 µL **Buffer ETR** to each tube.

    On your left, turn the yellow vacuum knob to the right until it's tight.

39. Apply vacuum until the liquid has been drawn through all the columns.

40. Turn off the vacuum and add 700 µL **Buffer PE** to each tube.

41. Wait **3 minutes**.

42. Apply vacuum until the liquid has been drawn through all the columns.

43. Discard the Tube Extenders.

44. Place the midiprep columns back into their corresponding midiprep tubes.

45. Centrifuge at maximum speed (10,000 or 13,000xg) for one minute in a tabletop centrifuge.

46. Place each spin column into a clean labelled microcentrifuge tube.

47. Add 200 µL **Buffer EB** to the center of each spin column.

48. Wait 1 - 3 minutes.

49. Centrifuge at maximum speed (10,000 or 13,000xg) for one minute in a tabletop centrifuge.

50. Vortex, then pulse spin down the samples.

51. Proceed directly to analyze the samples on the Nanodrop.