FINAL - 4X Miniprep

Introduction

Using the standard miniprep column, we can achieve a yield of >20 ug. The timing of this protocol is 2.5 to 3 hrs for 24 minipreps.

Materials

- Reagents
- NEB Miniprep (NEB T1010L)
- If you get to the scale that you want to make your own reagents for miniprep -- we make our own resuspension buffer and wash buffers using this protocol https://people.mbi.ucla.edu/sumchan/qiagenbuffer.html#:~:text=Buffer%20P1%20%2D%20Resuspension%20Buffer&text=Prep%20%2D%20Dissolve%206.06g%20Tris,A%20per%20liter%20of%20P1.

Procedure

4x MiniPrep

Preparing Preps:

1. In a 50 ml conical, grow 15 ml of bacteria
2. After 20 to 24 hours of growth, spin down the conical at 6000g for 15 minutes
3. Remove supernatant
4. Using a p1000, remove any additional supernatant that doesn't come out when poured out of the tube

PAUSE You can either go into miniprep from this step or the samples can be frozen for future use.

Lysis

5. Resuspend pellet in 400 µl Plasmid Resuspension Buffer within the 50 mL conical.
6. Vortex conicals for 20-30 seconds to ensure cells are completely resuspended. There should be no visible clumps.
7. Transfer 425 ul of resuspended sample into a clean 2 ml tube.
8. Lyse cells by adding 425 µl Plasmid Lysis Buffer. Invert tubes immediately (15-20 times) until the solution is clear and viscous.

CRITICAL Do not vortex!

9. Incubate for one minute at room temperature.

Neutralization

10. Neutralize the lysate by adding 850 µl of Plasmid Neutralization Buffer. Invert the tube until the color is uniformly yellow and precipitate forms (likely 15-20 times).

    For many samples, you should place all tubes into a tube rack and invert them all at one time...mix them aggressively
    CRITICAL Do not vortex!

11. Incubate for 2 minutes at room temperature.

12. Spin down tubes for 5 minutes at 16,000 rcf

13. Transfer 400 ul to the spin column and centrifuge for 1 minutes at 16,000 rcf. Discard flow-through while keeping columns inside centrifuge for ease.

4X Wash & Elution

14. **[ROUND 1]** Transfer 400 ul to the spin column and centrifuge for 1 minutes at 16,000 rcf.

15. Discard flow-through.

16. Re-insert columns in the collection tubes and add 180 µl of Plasmid Wash Buffer 1 to the column.

17. Centrifuge for 45 seconds at 16,000 rcf

18. Add 380 µl of Plasmid Wash Buffer 2

19. Centrifuge for 1.5 minutes at 16,000 rcf

20. Transfer column to a clean and labeled 1.5 mL tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If there is ANY doubt, re-spin the column for 30 seconds before inserting it into the clean microfuge tube.

21. Add 60 µl UltraPure distilled water to the center of the matrix.

22. Let matrix sit for one minute

23. Spin for 1.5 minutes at 10,600 rcf without centrifuge lid to elute DNA.

24. **[ROUND 2]** Transfer another 400 ul of the neutralized mixture to the spin column and centrifuge for 1 minutes at 16,000 rcf.

25. Discard flow-through.

26. Re-insert columns in the collection tubes and add 180 µl of Plasmid Wash Buffer 1 to the column.

27. Centrifuge for 45 seconds at 16,000 rcf

28. Add 380 µl of Plasmid Wash Buffer 2

29. Centrifuge for 1.5 minutes at 16,000 rcf

30. Transfer column to the same 1.5 ml tube in step 20

31. Using the same elution material in Step 23, transfer to the matrix of the column.

32. Let matrix sit for one minute

33. Spin for 1.5 minutes at 10,600 rcf without centrifuge lid to elute DNA.

34. **[ROUND 3]** Transfer another 400 ul of the neutralized mixture to the spin column and centrifuge for 1 minutes at 16,000 rcf.

35. Discard flow-through.

36. Re-insert columns in the collection tubes and add 180 µl of Plasmid Wash Buffer 1 to the column.

37. Centrifuge for 45 seconds at 16,000 rcf

38. Add 380 µl of Plasmid Wash Buffer 2

39. Centrifuge for 1.5 minutes at 16,000 rcf

40. Transfer column to the same 1.5 ml tube in step 20

41. Using the same elution material in Step 23, transfer to the matrix of the column.

42. Let matrix sit for one minute

43. Spin for 1.5 minutes at 10,600 rcf without centrifuge lid to elute DNA.

44. **[ROUND 4]** Transfer the last 400 ul of the neutralized mixture to the spin column and centrifuge for 1 minutes at 16,000 rcf.

45. Discard flow-through.

46. Re-insert columns in the collection tubes and add 180 µl of Plasmid Wash Buffer 1 to the column.

47. Centrifuge for 45 seconds at 16,000 rcf

48. Add 380 µl of Plasmid Wash Buffer 2

49. Centrifuge for 1.5 minutes at 16,000 rcf

50. Transfer column to the same 1.5 ml tube in step 20

51. Using the same elution material in Step 23, transfer to the matrix of the column.

52. Let matrix sit for one minute

53. Spin for 1.5 minutes at 10,600 rcf without centrifuge lid to elute DNA.