Miniprep overnight cultures

Aim:

- Isolating plasmids from liquid bacterial cultures.

Timeframe:

- Preparation: 15 minutes
- Wait-time: 15 min
- Overall: 30 min

Materials:

- Overnight liquid cultures (see overnight liquid culture protocol)
- NEB Monarch Miniprep kit
- Resuspension Solution
- Lysis Solution
- Neutralisation Solution
- Wash Solution
- Elution Buffer
- GeneJET Spin Columns
- 1.5ml eppendorf tubes

Procedure:

*all centrifuge steps for Miniprep (in eppendorf tubes or spin columns) should be carried out on table-top microcentrifuges at the highest speed (12500 rpm)

1. Aliquot 1.5ml of each overnight liquid culture into individual labelled 1.5 ml eppendorf tubes.
2. Spin down aliquots in a table-top microcentrifuge at highest speed for 3 mins.
3. Decant growth medium into waste bottles.
4. Resuspend (pipette up/down + vortex) pelleted cells in **250 µl of Resuspension Solution** (*make sure no clumps remains).
5. Add **250 µl of Lysis Solution** and mix gently by inverting tube 4-6 times until solution. becomes viscous and slightly clear. Avoid leaving Lysis Solution in any sample for more than 5 mins.
6. Add **350 µl of Neutralisation Solution** and mix immediately by inverting the tube 4-6 times. Neutralised bacterial lysate should become cloudy.
7. Centrifuge for 5 mins to pellet cell debris and chromosomal DNA.
8. Transfer all supernatant (~850 µl) to a labelled GeneJET spin column. Avoid contact with the pellet.
9. Centrifuge for 1 min. Discard flow-through and place column back into same collection tube.

10. Add **500 µl of Wash Solution**. Make sure Wash Solution has been diluted with ethanol; there should be a checkmark on the lid of the bottle.
11. Centrifuge for 1 min. Discard flow-through and place column back into same collection tube.
12. Repeat Wash step (steps 10-11).
13. Centrifuge for an additional 1 min to remove residual Wash Solution.
14. Transfer GeneJET spin column into fresh 1.5 ml eppendorf tube (make sure either the column or new tube is labelled).
15. Add **25 µl of Elution Buffer** to center of GeneJE spin column membrane (take care not to contact the membrane with the pipette tip).
16. Incubate for 2 mins at room temperature.
17. Centrifuge for 2 mins. Keep the flow-through.
18. Repeat steps 15-17 to give a total of **50 µl** in flow-through.
19. Discard column and store purified plasmid DNA at -20°C.

Steps for storing remaining overnight liquid cultures:

20. Spin down samples in the falcon tubes at 5000 rpm for 5 mins.
21. Decant supernatant in waste bottles.
22. Store falcon tubes containing bacterial pellet at -20°C.