Miniprep: Plasmid purification

Guide for curing plasmids from bacteria. Three possible kits.

MN was phased out during the iGEM period. Use QIA when doing the vacuum protocol, E.Z.N.A when using the centrifuge.

QIA kit (homemade buffers)

P1 buffer must be kept on ice at all times outside the fridge.

<u>N.B.:</u> 1 min centrifugation at 13,000 rpm can be used instead of vacuum at each step – only use vacuum if you have received proper instructions.

- Pellet 4 mL culture at > 8,000 rpm for 3 min.
- Resuspend in 250 µL P1 buffer and transfer to microcentrifuge tube.
- Add 250 µL P2 buffer and mix by inverting 4-6 times.
- Add 300 µL N3 buffer and mix by inverting 4-6 times.
- Centrifuge for 5 min at 13,000 rpm. **N.B.: Start cleaning spin column meanwhile.**
- Transfer supernatant to new centrifuge tube and spin for 5 min at 13,000 rpm. Discard pellet.
- **Prepare vacuum suction by cleaning 1x used spin columns:**
    - Insert a 1x used spin column into the vacuum for each sample.
    - Make sure all unused valves are closed.
    - Apply 1 mL 0.1 M HCl to each column and suck them dry. **N.B.:** When using the vacuum open a valve just before turning off the vacuum, do this as soon as the spin column is sucked dry.
    - Apply 1 mL MQ H_2O 3 times. The column should now be clean and ready for use.
- Apply the supernatant to the spin column and suck dry using vacuum.
- Wash by applying 0.5 mL PB buffer and sucking dry using vacuum.
- Wash by applying 075 mL PE buffer and sucking dry using vacuum.
- Transfer column to collection tube and centrifuge for 1 min at 13,000 rpm. to remove residual washing buffer.
- Place spin column in 1.5 mL microcentrifuge tube.
- To elute DNA add 50 µL EB buffer to the center of the column (one drop at a time) and let it stand on the table for 1 min.
- Centrifuge for 1 min at 13,000 rpm.

- Remove spin column and place in 2x used box. DNA sample should now be in tube.
- Store sample at -20ºC. Can be thawed and re-frozen multiple times.

Buffers

Generally made and stored in 50 mL falcon tubes.

P1 (15 mL falcon)

Must be stored at 5ºC and kept on ice at all times when outside.

- 50 mM Tris-HCL pH 8.0 (7.88 g/L)
- 10 mM EDTA
- 100 µg/mL RNase A
- Fill with MQ H_2O

P2

- 200 mM NaOH (8 g/L)
- 1% SDS (1 g/L)
- Fill with MQ H_2O

N3

- 4.2 M Guanidine HCl (401.226 g/L)
- 0.9 M Potassium Acetate (88.335 g/L)
- Fill with MQ H_2O

PB

For 50 mL:

- 35 mL Guanidine HCl
- 15 mL isopropanol

PE (pH 7.5)

For 50 mL:

- 0.5 mL 1M Tris pH 7.5
- 42 mL 96% EtOH
- 7.5 mL MQ H_2O

EB

- 10 mM Tris-HCl pH 8.5
- Fill with MQ H_2O

E.Z.N.A kit

Guide for isolation of plasmid DNA from E. coli grown in an overnight 1-5 mL LB culture, following manufacturers instructions.

Get the kit, containing all buffers, from the cupboard in the gel room. Solution I containing RNAse is found in the fridge in the gel room.

Protocol

1. Using the protocol for inoculations, prepare a liquid inoculation of a colony in 5 ml LB. Grow over night before starting this protocol.

2. Centrifuge at 10,000 x g for 1 minute at room temperature.

3. Decant or aspirate and discard the culture media.

4. Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution I before use.

5. Transfer suspension into a new 1.5 mL microcentrifuge tube.

6. Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

7. Add 350 µL Solution III. Immediately invert several times until a focculent white precipitate forms.

8. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

10. Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the fltrate and reuse the collection tube.

13. Add 500 µL HB Bufer.

14. Centrifuge at maximum speed for 1 minute.

15. Discard the fltrate and reuse collection tube.

16. Add 700 µL DNA Wash Bufer diluted with 100% ethanol prior to use.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the fltrate and reuse the collection tube.

Repeat Steps 16-18 for a second DNA Wash Bufer wash step.

19. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix, as residual ethanol may interfere with downstream applications.

20. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

21. Add 25 µL Elution Buffer directly to the center of the column membrane.

22. Let sit at room temperature for 1 minute.

23. Centrifuge at maximum speed for 1 minute.

Repeat step 21-23 for a second elution, yielding a higher amount of purified plasmid.

24. Store DNA at -20°C.

NucleoSpin Plasmid QuickPure

Guide for curing plasmids from bacteria.

Find the kit in the gel room. (The kit was phased out from September. Use E.Z.N.A kit instead)

Protocol

1 Cultivate and harvest the cells

Use 1–3 mL of a saturated E.coli LB culture, pellet cells in a standard benchtop microcentrifuge for 30 s at 11,000 x g. Discard the supernatant and remove as much of the liquid as possible. 11,000 x g, 30 s

2 Cell lysis

Add 250 µL Buffer A1. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30–40 °C until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature (18–25 °C).

Add 250 µL Buffer A2. Mix gently by inverting the tube 6–8 times. Do not vortex to avoid shearing of genomic DNA. Incubate at room temperature for up to 5 min or until lysate appears clear. Add 300 µL Buffer A3. Mix thoroughly by inverting the tube 6–8 times until blue samples turn colorless

completely! Do not vortex to avoid shearing of genomic DNA!

Make sure to neutralize completely to precipitate all the protein and chromosomal DNA. LyseControl should turn completely colorless without any traces of blue.

3 Clarification of lysate

Centrifuge for 5 min at 11,000 x g at room temperature.

4 Bind DNA

Place a NucleoSpin® Plasmid QuickPure Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum of 750 µL of the supernatant onto the column. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the NucleoSpin® Plasmid QuickPure Column back into the collection tube.

Repeat this step to load the remaining lysate.

5 Wash silica membrane

Add 450 µL Buffer AQ (supplemented with ethanol). Centrifuge for 3 min at 11,000 x g.

Very carefully discard the collection tube and the flow-through and make sure the spin cup outlet does not touch the wash buffer surface. Otherwise repeat the centrifugation step.

6 Dry silica membrane

The drying of the NucleoSpin® Plasmid QuickPure Column is performed by the 3 min centrifugation in step 5.

7 Elute DNA

Place the NucleoSpin® Plasmid QuickPure Column in a 1.5 mL microcentrifuge tube (not provided) and add 50 µL Buffer AE. Incubate for 1 min at room temperature. Centrifuge for 1 min at 11,000 x g.