

Plasmid Isolation

Protocol

Plasmid Isolation

1. Obtain a 1.5ml microcentrifuge tube containing 600µl of bacterial culture grown in LB media.
2. **Complete the next two steps within 2 minutes of each other:**
 - a. Add 100µl Cell Lysis Buffer (CLC) to your tube. Invert 6 times to mix or until the solution completely changes from opaque to clear blue.
 - b. Add 350µl of cold Neutralization Solution (NSC) to your tube. Invert 4 times or until the solution completely changes from clear blue to yellow.
3. Centrifuge in a large, high-speed centrifuge at top speed for 3 minutes at room temperature.
4. During the spin, insert a Spin Column into a Collection Tube and label BOTH with your initials.
5. Using a P1000 micropipette (set to 500µl) and/or a P200 micropipette (set to 200µl), transfer your supernatant into the spin column. Be careful not to transfer any of the pellet into the Spin Column.
6. Centrifuge at room temperature for 1 minute in a small benchtop centrifuge.
7. Discard your 1.5ml tube containing the cell pellet.
8. Discard the liquid that flows through the Spin Column into the Collection Tube (flow-through) by dumping the liquid waste into the biohazard bag. Reinsert the Spin Column into the Collection Tube.
9. Add 200µl of Endotoxin Removal Wash (ERB) to the Spin Column. Centrifuge at room temperature for 1 minute in a small benchtop centrifuge.
10. Discard the flow-through by dumping the liquid waste into the biohazard bag. Reinsert the Spin Column into the Collection Tube.
11. Add 400µl of Column Wash Solution (CWC) to the Spin Column. Centrifuge in a large, high-speed centrifuge at top speed for 30 seconds at room temperature.
12. During the spin, collect a clean 1.5ml tube and label the cap with your initials. This is your Elution Tube.
13. Discard flow-through by dumping the liquid waste into the biohazard bag. Discard the Collection Tube.
Reinsert the Spin Column into the Elution Tube.
14. Add 50µl of Nuclease-Free Water to the Spin Column. Let stand for 1 minute at room temperature.
15. Centrifuge in a large, high-speed centrifuge at top speed for 30 seconds at room temperature.
16. Discard Spin Column.
17. Cap Elution Tube and keep for downstream analysis and applications.

Appendix

1. Protocol adapted from Promega PureYield™ Plasmid Miniprep System (A1220, A1221, A1222 or A1223).