NEB Monarch Miniprep (Purifications)

Materials

Reagents

- NEB Monarch Miniprep Kit
- Make sure that you have enough spin columns and that ethanol has been added to your wash buffer.
- Store Plasmid Neutralization Buffer (B3) at 4°C after opening.

Equipment

- Autoclaved Microcentrifuge tubes
- p1000, p200, and p2 for subsequent nanodrop afterwards
- 50mL Falcon tubes for convenient waste disposal in between steps

Procedure

1. Pellet 1–5 mL bacterial culture by centrifugation for 30 seconds. Discard supernatant.
    - Note: For a standard miniprep to prepare DNA for restriction digestion of PCR, we recommend 1.5mL of culture.
2. Resuspend pellet in 200µL Plasmid Resuspension Buffer (B1) (pink). Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.
3. Lyse cells by adding 200µL Plasmid Lysis Buffer (B2) (blue/green). Invert tube immediately and gently 5–6 times until color changes to dark pink and the solution is clear and viscous. **Do not vortex!** Incubate for one minute.
    - Note: Care should be taken not to handle the sample roughly and risk shearing chromosomal DNA, which will co-purify as a contaminant. Avoid incubating longer than one minute to prevent irreversible plasmid denaturation.
4. Neutralize the lysate by adding 400µL of Plasmid Neutralization Buffer (B3) (yellow). Gently invert tube until color is uniformly yellow and a precipitate forms. **Do not vortex!** Incubate for 2 minutes.
    - Note: Be careful not to shear chromosomal DNA by vortexing or vigorous shaking. Firmly inverting the tube promotes good mixing, important for full neutralization.
5. Clarify the lysate by spinning for 2–5 minutes at 16,000 g.
    - Note: Spin time should not be less than 2 minutes. Careful handling of the tube will ensure no debris is transferred and the 2 minute recommended spin can be successfully employed to save valuable time.
    - For culture volumes > 1 mL, we recommend a 5 minute spin to ensure efficient RNA removal by RNase A. Also, longer spin times will result in a more compact pellet that lowers the risk of clogging the column.
6. Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow-through.

7. Re-insert column in the collection tube and add 200µL of Plasmid Wash Buffer 1. *If the DNA will be used in transfection, incubate 5 minutes*. Centrifuge for 1 minute. Discarding the flow-through is optional.
    - Plasmid Wash Buffer 1 removes RNA, protein, and endotoxin.
    - Note: The collection tube is designed to hold 800µL of flow-through fluid and still allow the tip of the column to be safely above the top of the liquid. Empty the tube whenever necessary to ensure the column tip and flow-though do not make contact.
8. Add 400µL of Plasmid Wash Buffer 2 and centrifuge for 1 minute.
9. Transfer column to a clean 1.5mL microfuge 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 1 minute before inserting it into the clean microfuge tube.
10. Add ≥ 30 µL DNA Elution Buffer to the **center** of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.
    - Note: Nuclease-free water (pH 7–8.5) can also be used to elute the DNA (37℃).
    - Delivery of the Monarch DNA Elution Buffer should be made directly to the center of the column to ensure the matrix is completely covered for maximal efficiency of elution.
    - Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated as a result of dilution.
    - For larger plasmids (≥ 10 kb), heating the DNA Elution Buffer to 50°C prior to eluting and extending the incubation time after buffer addition to 5 minutes can improve yield.
11. Proceed to Nanodrop for DNA quantification. Be sure to blank with the appropriate elution substance (ie. Elution Buffer OR Nuclease-free water).

Acknowledgements

This protocol was sourced from NEB: *Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010),* https://international.neb.com/protocols/2015/11/20/monarch-plasmid-dna-miniprep-kit-protocol-t1010