ARIA

Miniprep an OD Measuremnt Protocol

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Miniprep and OD measurement

Introduction

Miniprep kits are designed for the rapid, small-scale preparation of highly pure plasmid DNA from recombinant Escherichia coli strains. Miniprep procedure is based on the **alkaline lysis** of bacterial cells followed by **adsorption** of DNA onto silica in the presence of high salt. The plasmid DNA is selectively adsorbed in the silica gel-based plasmid spin column and other impurities such as proteins, salts, nucleotides, and oligos (<40-mer) are washed away.

Materials

- Recombinant *E.Coli* culture
- **NZYMiniprep:**
- Buffer A1
- Buffer A2
- Buffer A3
- Buffer AY
- Buffer A4
- Buffer AE (does not contain EDTA)
- RNase A
- NZYTech Spin Columns
- Collection Tubes (2 mL)

Procedure

Before Starting

1. To isolate DNA from **low copy number plasmids,** BACs, or cosmids, or to obtain higher DNA concentrations, use 10 mL of E. coli cultures and double the volumes of Buffers A1, A2, and A3 [1].

2. **Add 1** mL of Buffer A1 to the **RNase A vial** and vortex. Transfer the resulting solution into the Buffer A1 bottle and mix thoroughly. Buffer A1 with RNase should be stored at 4 °C for frequent use and at -20 °C for infrequent use.

   - Add 32 mL (MB01001) of 100% molecular biology grade ethanol to each bottle of **buffer A4.**
   - Buffer A2 may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37 °C.
   - Buffers A3 and **AY** contain guanidine hydrochloride. Wear gloves and goggles when using this kit.

3. All **centrifugations** should be carried out at **room temperature** in a table-top microcentrifuge at >12000 xg (10000-15000 rpm depending on the rotor type).

Miniprep for plasmid purification

4. **Pellet** 1-5 mL of an E. coli LB culture for 30 s (centrifugue). Discard supernatant. Remove as much media as possible.

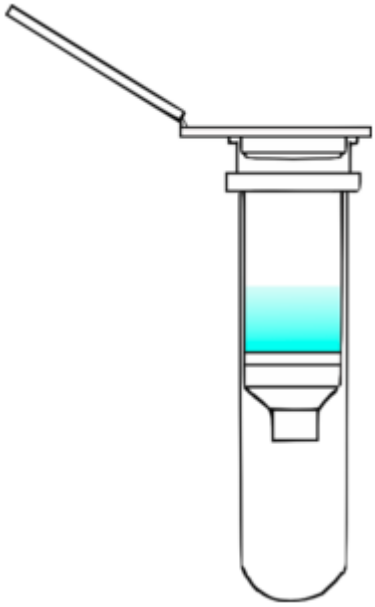
5. **Cell lysis:** Re-suspend cell pellet in **250 µL Buffer A1** by vigorous vortexing.

6. Add **250 µL of Buffer A2** and mix gently by inverting the tube for 6-8 times. Incubate at room temperature for a maximum of 4 min. Do not vortex.

7. Add **300 µL Buffer A3**. Mix gently by inverting the tube for 6-8 times. Do not vortex.

8. **Clarification of lysate:** Centrifuge for 5-10 min at room temperature, depending on initial culture volume.

9. **Bind DNA:** Place NZYTech spin column in a 2 mL collecting tube and load the supernatant from step 3 onto the column. Centrifuge for 1 min at 11,000 xg. Discard flow-through.



10. **Wash silica membrane:** Add 500 µL of Buffer AY onto the column. Centrifuge for 1 min. Discard flow-through. This step is crucial to increase the reading length of DNA sequencing reactions and to improve the performance of critical enzymatic reactions. When using endA+ strains, such as JM series, HB101 and its derivatives, or any wild-type strain, use pre-warmed Buffer AY (50 °C). Add 600 µL of Buffer A4 (make sure ethanol was previously added). Centrifuge for 1 min. Discard flow-through.

11. **Dry** silica membrane Re-insert the NZYTech spin column into the empty 2 mL collecting tube and centrifuge for 2 min.

12. **Elute** highly pure DNA Place the dried NZYTech spin column into a clean 1.5 mL microcentrifuge tube and add 50 µL of Buffer AE. Incubate 1 min at room temperature. Centrifuge for 1 min. By repeating this step the overall yield will increase by 15-20%. To obtain a highly concentrated miniprep (1.3 times higher) reduce the volume of elution buffer to 30 µL.

OD Measurement

13. **Mesure OD** in the NanoDrop.

    - **Blank**: for blanking the spectrophotometer we used the solution where the DNA has been resuspended in (1-2uL).
    - **NanoDrop:** to measure your samples, place 1-2μL of mini-prepped DNA onto the pedestal

- **Close** the lid, click measure and record the concentration and purity.
-- A good purity ranges from 1.80-2.00) --
- **Repeat** for each sample.
- **Clean** the pedestal

14. **Store** the purified DNA at -20 °C.

Note: It is extremely important to add the **Elution Buffer into the center part** of the column. Incubating the column with the Elution Buffer at higher temperatures (37 to 50 °C) may slightly increase the yield especially of large (>10,000 bp) DNA Plasmids. Pre-warming the Elution Buffer at 55 to 80 °C may also slightly increase elution efficiency. If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield [2].

Bibliography

1. NZYMiniprep - miniprep - NZYTech. (2018, May 19). Retrieved October 17, 2021, from Nzytech.com website: https://www.nzytech.com/products-services/molecular-biology/dna-rna-purification/plasmid-dna-purification/miniprep/mb010/
2. DNA Quantification. (n.d.). Retrieved October 17, 2021, from Addgene.org website: https://www.addgene.org/protocols/dna-quantification/