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Automation Protocol for Plasmid DNA Extraction from *E. coli*

Forked from Automation Protocol for Plasmid DNA Extraction from E. coli

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We use this protocol and it's working

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Abstract

Here, we describe an adapted protocol for automated extraction of plasmid DNA from 24 E. coli cultures. It is adapted from a NIST DNA Extraction method based on the QIAprep Spin Miniprep Kit (Qiagen, 27106). It results in a sample volume of 130µL of DNA.

This protocol requires an automated liquid handler (Hamilton Robotics, STAR) with both 8-channel and 96-channel pipetting heads and positive pressure filter press (Hamilton, MPE2). It also requires a centrifuge with a 96-well plate rotor capable of at least 3878 *g*.

Guidelines

Supernatant removal, cell resuspension, and cell sample transfer (steps 2-4) is performed using the automated liquid handler’s 8-channel head with which each channel is capable of independent movement and liquid-level sensing to allow for variations in cell culture volume and density recovered from each sample. Most of the subsequent pipetting (steps 5-11) is performed using the automated liquid handler’s 96-channel head with an offset pickup of 24 pipette tips so that the timing for each sample will be identical. Pipetting the water for elution (step 13) is performed using the automated liquid handler’s 8-channel head to allow for individual channel movement.

Materials

Starting cultures:

- *E. coli* cultures in 96-well deep-well plate (Eppendorf, cat. no. 951033405)
  24 cultures, approximately 1.7 mL each

Reagents:

- Resuspension Buffer: 50 mmol/L Tris-Cl, pH 8.0 (Invitrogen, cat. no. 15-568-025), 10 mmol/L EDTA (Fisher BioReagents, cat. no. 1311-200), 100 µg/mL RNase A (Qiagen, cat. no. 19101)
- Lysate Buffer: 200 mmol/L NaOH (Millipore Sigma, cat. no. 106462), 10 g/L SDS (Millipore Sigma, cat. no. 24802350)
- Neutralization Buffer (Qiagen, cat. no. 19064)
- Binding Buffer (Qiagen, cat. no. 19066)
- Wash Buffer: 8 mmol/L Tris-Cl, pH 7.5 (Fisher Bioreagents, cat. no. BP1757-100), 80 %Absolute ethanol (Fisher Bioreagents, cat. no. BP2818500)
- Nuclease-free water (Thermo Scientific, cat. no. AM9938)

Labware:

- 96-well reagent plate, 1.2 mL per well (Abgene, cat. no. AB-1127)
- 96-well filter plate (Agilent, cat. no. 201702-100)
- 96-well deep well plate (Eppendorf, cat. no. 951033588)
- 96-well glass fiber binding plate (Nunc, cat. no. 278010)
- Low-binding 96-well elution plate (Eppendorf, cat. no. 30603303)

1 Pellet cell cultures in 96-well deep-well plate by centrifugation at 3878 g (4500 rpm) for 10 minutes at 23 °C.
- ~1.8 mL culture per well (after cultures have been combined from growth plate)

2 Remove supernatant from the wells containing each cell pellet.
- Using 8-channel pipetting head
- Take care to avoid disturbing the cell pellet.

3 Resuspend each cell pellet in in 200 µL of Resuspension Buffer (P1); mix 10x by repeated aspiration and dispense.
- 8 samples at a time with the 8-channel head

4 Transfer resuspended cell samples to a new 96-well plate (Abgene, AB-1127) located on an automated microplate shaker.
- 8 samples at a time with the 8-channel head

5 Add 250 µL Lysate Buffer (P2) to each sample and mix by shaking the plate at 90 rpm for 2 minutes.
- 24 samples at a time with the 96-channel head

6 Add 350 µL cold (4 °C) Neutralization Buffer (N3, Qiagen, 19064) to each sample and mix by shaking at 90 rpm for 2 minutes.
- 24 samples at a time with the 96-channel head

7 Using wide bore tips (3.2 mm tip diameter), gently mixed the lysate samples by 3 repeated cycles of aspiration and dispensing, then transfer samples to a 96-well filter plate (Agilent, 201702-100) and allowed to settle in the filter plate for 2 minutes.
- 24 samples at a time with the 96-channel head

8 Use the filter press to push the lysate solutions through the filter plate into a new 96-well deep well plate (Eppendorf, 951033588) at 20 psi for 240 s followed by 65 psi for 60 s.

9 Transfer the cleared lysate solutions to a 96-well glass fiber binding plate (Nunc, 278010) and use the filter press to push the solutions through the binding plate at 40 psi for 60 s.
- 24 samples at a time with the 96-channel head

10 Add 900 µL Binding Buffer (PB, Qiagen, 19066) to each well.
- 24 samples at a time with the 96-channel head

11 Use the filter press to push buffer through the binding plate at 40 psi for 60 s.

12 Add 900 µL Wash Buffer (PE) to each well.
- 24 samples at a time with the 96-channel head

13 Use the filter press to push buffer through the binding plate at 40 psi for 60 s, then increase the pressure to 65 psi for 420 s to dry the binding plate.

14 Place the binding plate on top of the Elution plate (96-well low-binding plate 350 uL per well, Eppendorf, 30603303)

15 Add 130 µL nuclease-free water (Thermo Scientific, AM9938) warmed to 60 °C to each well.
- 8 samples at a time with 8 channel head
- Jet dispense onto binding plate filters

16 Perform the final elution following the subsequent steps. Choose the step case appropriate for the type of equipment you have, either: a filter press or a centrifuge.

STEP CASE

Use filter press for final elution 5 steps

The final elution can either be done with the MPE2 filter press or by centrifugation.
- Using the filter press requires less manual intervention: the entire plasmid extraction method can be run unattended. But, less eluent will typically be recovered.

17 Wait for 5 minutes

18 Elute DNA from binding plate into a 96-well low-binding elution plate (Eppendorf, 30603303) using the filter press at 65 psi for 7 minutes.

19 Remove the binding plate from the elution plate, and put the binding plate in an appropriate storage location if it will be used again or throw it to waste.

20 Put a lid on the elution plate, and move it to pickup location for user.

21 Seal the elution plate with a clear adhesive seal (Fisherbrand 8408240), and store at 4C until it is used for the BarSeq library prep.