Apr 03, 2021

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

Nina Alperovich[1], Jane Romantseva[1], Olga Vasilyeva[1], David Ross[1]

[1]National Institute of Standards and Technology

1 *Works for me* dx.doi.org/10.17504/protocols.io.bjjvkkn6

David Ross

ABSTRACT

Here, we describe a protocol for automated extraction of plasmid DNA from 24 E. coli cultures. The protocol uses an automated liquid handler and a positive pressure filter press. It is based on the QIAprep Spin Miniprep Kit (Qiagen, 27106).

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*.

EXTERNAL LINK

https://doi.org/10.15252/msb.202010179

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Tack DS, Tonner PD, Pressman A, Olson ND, Levy SF, Romantseva EF, Alperovich N, Vasilyeva O, Ross D, The genotype-phenotype landscape of an allosteric protein. Molecular Systems Biology 17(3). doi: 10.15252/msb.202010179

DOI

dx.doi.org/10.17504/protocols.io.bjjvkkn6

EXTERNAL LINK

https://doi.org/10.15252/msb.202010179

PROTOCOL CITATION

Nina Alperovich, Jane Romantseva, Olga Vasilyeva, David Ross 2021. Automation Protocol for Plasmid DNA Extraction from E. coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjjvkkn6

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Tack DS, Tonner PD, Pressman A, Olson ND, Levy SF, Romantseva EF, Alperovich N, Vasilyeva O, Ross D, The genotype-phenotype landscape of an allosteric protein. Molecular Systems Biology 17(3). doi: 10.15252/msb.202010179

KEYWORDS

laboratory automation, bacterial culture, plasmid DNA extraction

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Citation: Nina Alperovich, Jane Romantseva, Olga Vasilyeva, David Ross (04/03/2021). Automation Protocol for Plasmid DNA Extraction from E. coli. https://dx.doi.org/10.17504/protocols.io.bjjvkkn6

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Aug 11, 2020

LAST MODIFIED

Apr 03, 2021

PROTOCOL INTEGER ID

40277

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 TEXT

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.

2 Remove supernatant from the wells containing each cell pellet.

3 Resuspend each cell pellet in in 200 µL of Resuspension Buffer; mix 10x by repeated aspiration and dispense.

4 Transfer resuspended cell samples to a new 96-well plate (Abgene, AB-1127) located on an automated microplate shaker.

Citation: Nina Alperovich, Jane Romantseva, Olga Vasilyeva, David Ross (04/03/2021). Automation Protocol for Plasmid DNA Extraction from E. coli. https://dx.doi.org/10.17504/protocols.io.bjjvkkn6

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

5 Add 250 µL Lysate Buffer to each sample and mix by shaking the plate at 90 rpm for 2 minutes.

6 Add 350 µL cold (4 °C) Neutralization Buffer (Qiagen, 19064) to each sample and mix by shaking at 90 rpm for 2 minutes.

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.

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 180 s followed by 65 psi for 30 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.

10 Add 900 µL Binding Buffer (Qiagen, 19066) to each well and use the filter press to push buffer through the binding plate at 40 psi for 60 s.

11 Add 900 µL Wash Buffer to each well and use the filter press to push buffer through the binding plate at 40 psi for 60 s.

12 Use the filter press to dry the binding plate by applying 65 psi for 7 minutes.

13 Add 100 µL nuclease-free water (Thermo Scientific, AM9938) warmed to 60 °C to each well; wait for 5 minutes.

14 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.

Citation: Nina Alperovich, Jane Romantseva, Olga Vasilyeva, David Ross (04/03/2021). Automation Protocol for Plasmid DNA Extraction from E. coli. https://dx.doi.org/10.17504/protocols.io.bjjvkkn6

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited