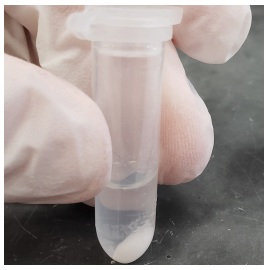
Mar 05, 2019

E. coli K12 DNA Extraction



Forked from E. coli K12 DNA Extraction

DOI
dx.doi.org/10.17504/protocols.io.yujfwun

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DOI: https://dx.doi.org/10.17504/protocols.io.yujfwun

Protocol Citation: Kenneth Schackart, Kattika Kaarj 2019. E. coli K12 DNA Extraction. **protocols.io https://dx.doi.org/10.17504/protocols.io.yujfwun**

Protocol status: Working
We use this protocol and it's working

Created: March 05, 2019

Last Modified: March 05, 2019

Protocol Integer ID: 21099

Keywords: k12 dna extraction, dna extraction, extraction, dna

Abstract

How to extract DNA from *E. coli* K12 using Wizard® Genomic DNA Purification Kit by Promega®.

I do not claim any credit for the development of this protocol. It has been adapted from the protocol detailed in: Wizard Genomic DNA Purification....

Guidelines

Lab coat and gloves must be worn at all times.

Materials

MATERIALS

Wizard(R) Genomic DNA Purification Kit **Promega Catalog #**A1620

Reagents in kit:

- Nuclei Lysis Solution
- RNAse Solution
- Protein Precipitation Solution
- DNA Rehydration Solution

Additional Reagents:

- Isopropanol
- 70% Ethanol

Additional Materials:

- 2 mL centrifuge tubes
- Pipettes and tips
- Heating block
- Centrifuge

Troubleshooting

Before start

Spray work area with 70% EtOH solution.

Culture bacteria

1 Culture *E. coli* K12 in BHI broth overnight.
2 mg lyophilized *E. coli* K12 in 10 mL BHI broth.

Pellet the cells

2 Add 1 mL cell suspension to 2 mL microcentrifuge tube.

3 Label centrifuge tube with your group number.

4 Centrifuge at 13,000-16,000 × *g* for 00:02:00 .

5 Remove supernatant.

Lyse nuclei

6 Add 600 µL of Nuclei Lysis Solution.

> Note
>
> Nuclei Lysis Solution is marked "NL"

7 Gently pipet until the cells are resuspended.

8 Incubate at 80 °C on heating block for 00:05:00 to lyse the cells.

9 Cool to room temperature.

Degrade RNA

10 Add 3 µL RNase Solution to the cell lysate.

> Note
>
> RNAse solution is marked "R"

11 Invert 2-5 times to mix.

12 Incubate at 37 °C for 00:15:00 to 01:00:00 .

13 Cool to room temperature.

Precipitate proteins

14 Add 200 µL of Protein Precipitation Solution to the RNase-treated cell lysate.

> Note
>
> Protein Precipitation solution is marked "P"

15 Vortex vigorously at high speed for 00:00:20 .

16 Incubate on ice for 00:05:00

17 Centrifuge at 13,000-16,000 × *g* for 00:03:00 .

Harvest DNA

18 Transfer the supernatant containing the DNA to a clean 1.5 mL microcentrifuge tube containing 600 µL isopropanol.

Note

Some supernatant may remain in the original tube conatining the protein pellet. Leave this residual to avoid contaminating the DNA solution with the precipitated protein.

Note

Isopropanol is marked "IPA"

19 Label centrifuge tube with your group number.

20 Gently mix by inversion until the thread-like strands of DNA form a visible mass.

Wash and dry DNA

21 Centrifuge at 13,000-16,000 × *g* for 00:02:00 .

22 Carefully pour off the supernatant and drain the tube on clean absorbent paper.

23 Add 600 µL of 70% ethanol and gently invert the tube several times to wash the DNA pellet.

Note

70% ethanol solution is marked "Et"

24 Centrifuge at 13,000-16,000 × *g* for 00:02:00 .

25 Carefully pour off the ethanol.

26 Drain the tube on clean absorbent paper and allow to air-dry for 10-15 minutes.

Rehydrate DNA

27 Add 100 µL of DNA rehydration solution to the tube.

> Note
>
> DNA Rehydration solution is marked "DR"

28 Rehydrate by incubating the solution overnight at room temperature or 4 °C .

29 Store DNA at 2 °C to 8 °C .