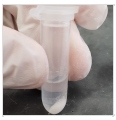

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Working

E. coli K12 DNA Extraction

Forked from E. coli K12 DNA Extraction

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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.pdf .

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Lab coat and gloves must be worn at all times.

MATERIALS

NAME	CATALOG #	VENDOR
Wizard(R) Genomic DNA Purification Kit	A1620	Promega

MATERIALS TEXT

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

BEFORE STARTING

Spray work area with 70% EtOH solution.

Culture bacteria

1 Culture *E. coli* K12 in BHI broth overnight.

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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.

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.

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.

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Precipitate proteins

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

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.

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.

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.

70% ethanol solution is marked "Et"

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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.

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** .

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