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 We use this protocol and it's working

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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 .

GUIDELINES

Lab coat and gloves must be worn at all times.

MATERIALS

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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

BEFORE START INSTRUCTIONS

Spray work area with 70% EtOH solution.

Culture bacteria

- 1 Culture *E. coli* K12 in BHI broth overnight.
Add 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 $13,000\text{--}16,000 \times g$ 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 $\times g$ 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 containing 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 $\times g$ 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 $\times g$ 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 .