Aug 12, 2022

DNA Purification (NEB)

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This protocol is published without a DOI.

Yeast ORFans CURE

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ABSTRACT

This protocol purifies DNA from an enzymatic reaction (like a PCR or a digestion with a restriction enzyme.) It is NOT an miniprep -- if you are trying to purify DNA from a culture of E. coli, you are in the wrong place!

PROTOCOL CITATION

Brian Teague 2022. DNA Purification (NEB). **protocols.io** https://protocols.io/view/dna-purification-neb-ce6sthee

KEYWORDS

pcr, purification, monarch, spin column

CREATED

Aug 11, 2022

LAST MODIFIED

Aug 12, 2022

PROTOCOL INTEGER ID

68530

MATERIALS TEXT

- Binding buffer
- Wash buffer
- Elution buffer
- One spin column and collection tube per cleanup
- Chemical waste container (50 ml conical)

The buffers and spin column are from the

Monarch PCR and DNA Cleanup Kit - 50 preps **New England Biolabs Catalog #T1030S**

SAFETY WARNINGS

The binding buffer may cause irritation to skin and eyes. Additionally, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats, gloves and safety glasses.

The flow-through (containing the binding buffer or wash buffer) cannot go down the drain. Dispose of it as chemical waste, per the directions of your instructor.

1 ⚠

Make sure you are using the "Monarch PCR & DNA Cleanup Kit", not the Monarch Plasmid Miniprep Kit. They come in identical boxes -- read the label!

2 Mix the ENTIRE DNA sample with 5 times its volume of Binding Buffer. Pipette up and down several times to mix the sample thoroughly.

Ie, if you had **200 µL** of sample, mix it with **1000 µL** of binding buffer.

3 Insert the column into the collection tube and load the sample onto the column.

4 Centrifuge **16000 x g, 00:01:00** . Discard the flow-through in the **chemical waste container.** 1m

5 Re-insert the column into the collection tube. Add **200 µL** of Wash Buffer.

6 Centrifuge **16000 x g, 00:01:00** . Discard the flow-through in the **chemical waste container.** 1m

7 Repeat steps 5 and 6 once.

8 Transfer the column to a clean 1.7 ml microcentrifuge tube.

9 Add **10 µL** of Elution Buffer to the center of the silica matrix. Wait **00:01:00** , then centrifuge **16000 x g, 00:01:00** 2m

10 Quantify your purified DNA on the Nanodrop.