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Miniprep (NEB Monarch)

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Yeast ORFans CURE

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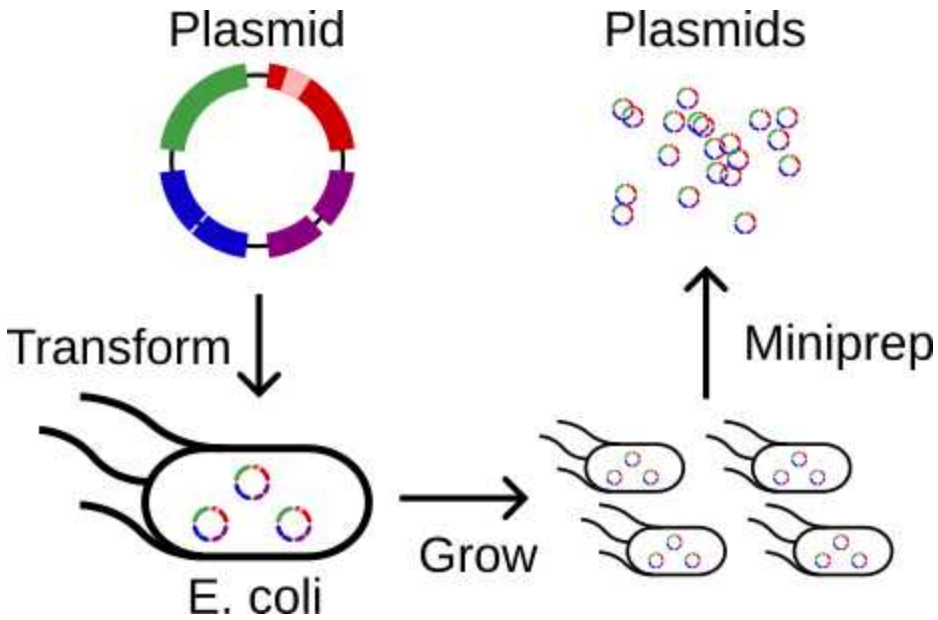
ABSTRACT

We transformed E. coli bacteria with our plasmid in order to make more of it -- to use the bacteria as highly accurate DNA copiers. Now that we've grown a bunch of E. coli, we need to get the plasmid DNA back out. That's the point of a miniprep.



PROTOCOL CITATION

Brian Teague 2022. Miniprep (NEB Monarch). **protocols.io** https://protocols.io/view/miniprep-neb-monarch-ce5btg2n

IMAGE ATTRIBUTION

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PARENT PROTOCOLS

In steps of

Miniprep (NEB Monarch) (Instructor protocol)

GUIDELINES

It is easy to get "into a rhythm" with this protocol and miss or mis-read important steps. Follow the directions very carefully -- there are lots of buffers and centrifugation stepsS

MATERIALS TEXT

Equipment

- Vortexer
- Microcentrifuge
- Nanodrop (or equivalent instrument for measuring DNA quantity and purity)

Materials and Reagents

- Microcentrifuge tubes
- A 5 ml culture of E. coli A 5 ml culture of E. coli containing your plasmid (one per miniprep)
- NEB spin column and collection tube (one per miniprep)
- Resuspension buffer (red)
- Lysis buffer (blue)
- Neutralization buffer (yellow)
- Wash buffer 1 (clear)
- Wash buffer 2 (clear)
- Elution buffer (clear)

(The spin columns and buffers come from the

Monarch Plasmid Miniprep Kit **NEB Catalog #T1010**)

- Biological waste container (50 ml conical)
- Chemical waste container (50 ml conical)

SAFETY WARNINGS

The chemicals in a miniprep are moderately hazardous, especially if you were to get them in your eyes. Wear appropriate PPE, including a lab coat, gloves and safety glasses.

Additionally, this protocol generates BOTH biological AND chemical waste. Follow your instructor's guidelines for disposing of these two waste streams.

Harvest the E.coli culture 1m

1 Transfer **1.5 mL** of bacterial culture to a microcentrifuge tube. Centrifuge 30s
16000 x g, 00:00:30

2 Remove the supernatant using a micropipettor and **discard in the biological waste container**. Try to get as much as you can without disturbing the pellet.

3 Transfer *another* 1.5 ml of bacterial culture *to the same microcentrifuge tube.* Centrifuge 30s
16000 x g, 00:00:30

4 Remove the supernatant using a micropipettor and **discard in the biological waste container**. Try to get as much as you can without disturbing the pellet.

Lyse the E.coli cells 8m

5 Add **200 µL** of **resuspension buffer**. Vortex to resuspend the pellet. Make sure it is completely resuspended -- there should be no visible clumps.

6 Add **200 µL** **lysis buffer**. Invert the tube 4-6 times to mix and incubate at 1m
Room temperature for **00:01:00** . **Do not vortex.**

7 Add **400 µL** **of neutralization buffer** and gently invert the tube until neutralized. Sample is neutralized when the color is uniformly yellow. **Do not vortex.**

8 Incubate at **Room temperature** for **00:02:00** 2m

9 Centrifuge lysate **16000 x g, 00:05:00** 5m

Bind and Wash 4m

10 Carefully transfer the supernatant (the liquid at the top, not the pellet at the bottom) to the spin column. Centrifuge **16000 x g, 00:01:00** . **Discard the flow-through in the chemical waste container.** 1m

11 Re-insert column in collection tube. Add **200 µL of wash buffer 1.** Centrifuge **16000 x g, 00:01:00** . **Discard the flow-through in the chemical waste container.** 1m

12 Add **400 µL of wash buffer 2.** Centrifuge **16000 x g, 00:01:00** . **Discard the flow-through in the chemical waste container.** 1m

13 Replace the spin column in the (now empty) collection tube. Centrifuge **16000 x g, 00:01:00** 1m

This clears out any left-over ethanol.

Elute 2m

14 Transfer the column to a clean 1.7 ml microcentrifuge tube. *Be careful: make sure the tip of the column doesn't come into contact with the flow-through.*

15 Add **30 µL of elution buffer** to the center of the silica matrix. Wait **00:01:00** , then centrifuge **16000 x g, 00:01:00** 2m

16 Measure the concentration and purity of your DNA on the NanoDrop.