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Miniprep (NEB Monarch) (Instructor protocol)

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Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for

Miniprep (NEB Monarch)
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PREVIEW RUN

PROTOCOL CITATION

Brian Teague 2022. Miniprep (NEB Monarch) (Instructor protocol). **protocols.io** https://protocols.io/view/miniprep-neb-monarch-instructor-protocol-ce56tg9e

IMAGE ATTRIBUTION

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GUIDELINES

The "pick colonies" part is in the instructor protocol because it needs to happen ~16-20 hours before the lab. (I suppose it would be possible to ask students to come in the previous day.) I do ask my students to interpret their transformation plates and choose which colonies they want me to pick -- this gives them an opportunity to think about what it means that some colonies are green and some are white, and which ones they want.

I prefer the NEB Monarch miniprep kits over the comparable products from Qiagen and Promega -- they produce less waste and the ergonomics (handling) are easier. But you can definitely use a different kit, if you have a strong preference or they are more readily available.

MATERIALS TEXT

Materials and Reagents

- BD Bacto™ Yeast Extract **BD Biosciences Catalog #212750** Step 2
- Tryptone **Fisher Scientific Catalog #BP1421-500** Step 2
- Sodium Chloride **Fisher Scientific Catalog #S271** Step 2
- Kanamycin **Research Products International (rpi) Catalog #K22000-25.0** Step 6
- Falcon™ Round-Bottom Polypropylene Test Tubes With Cap **Corning Catalog #352059**

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. Both should be disposed of appropriately. At my institution, we deactivate biological waste by either bleach or autoclaving, then dispose of down the train or in the trash. We consolidate the chemical waste in the "flammable" container (it has a bunch of ethanol

and isopropanol in it) and dispose of via our internal processes.

Prepare LB broth 30m

1 Fill a 1 liter screw-cap bottle with approximately **900 mL** of deionized water.

2 Add:

- **5 g** BD Bacto™ Yeast Extract **BD Biosciences Catalog #212750**
- **10 g** Tryptone **Fisher Scientific Catalog #BP1421-500**
- **10 g** Sodium Chloride **Fisher Scientific Catalog #S271**

3 Add water to a total volume of **1 L** (eyeballing is OK, no need to dirty a graduated cylinder). Cap and shake to mix.

> Make sure you get all of the powder off of the bottom of the bottle. It doesn't have to be completely dissolved, just resuspended.

4 Loosen the cap and autoclave at **121 °C** for **00:30:00** on a liquid cycle. 30m

Pick colonies 30m

5 For each group, plan to pick 2 colonies. Each will go into 5 ml of liquid culture. Compute how much media you'll need.

6 Measure out enough LB Broth **Contributed by users** in an appropriate container (a sterilized flask or a conical centrifuge tube.) Supplement with 1000X stock solution of

Kanamycin **Research Products International (rpi) Catalog #K22000-25.0** .

For example, if you are making 10 cultures, you'll need **50 mL** of LB broth and **50 µL** of kanamycin stock.

7 Label culture tubes (either disposable plastic test tubes or reusable glass ones) and aliquot 5 ml of LB + kanamycin into each.

8 Transfer one colony of E. coli into each.

Some labs will sterilize toothpicks for this. I like to use a micropipette tip on the end of a pair of forceps.

9 Grow **Overnight** in an incubating shaker, **200 rpm, 37°C** . 30m

Prepare waste containers 30m

10
- Label a set of 50 ml conicals "BIO", one per group. These will collect biological waste.
- Label a set of 50 ml conicals "CHEM", one per group. These will collect chemical waste.

Instructor Tips & Common Student Errors

11 **Instructor Tips**
- I always ask my students to interpret their transformation plates after the transformation and before the miniprep -- they tell me which colonies they want me to pick.
- The first few years, I aliquotted out small volumes of the buffers for each group. Nowadays, I have four miniprep kits (for a class of 24) and I simply require that they share reagents.
- Make doubly and triply sure that your wash buffer has been reconstituted with ethanol!
- There is often contention for the microcentrifuges in this lab. If you can borrow a few more, that's useful.
- Low(ish) DNA concentrations are fine, but ethanol contamination is not. Make sure to help

your students interpret their Nanodrop results, including the A230/A260 and A280/A260 values.

- This protocol generates BOTH biological AND chemical waste. I ask students to collect them in separate 50 ml conical tubes, one per group, and dispose of them appropriately at the end of the semester.

12 Common Student Errors

- Discarded the supernatant after the lysis
- Didn't move the spin column to a clean tube before elution (results in ethanol contamination)