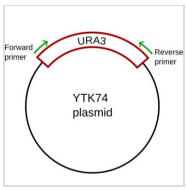


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# PCR (Instructor Protocol)

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Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for the CURE's two PCRs,

URA3 PCR
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PREVIEW RUN

and

Knockout PCR
**by Brian Teague,**
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PREVIEW RUN

The URA3 PCR is preparative, to make the URA3 cassette for the gene knockout. The knockout PCR is diagnostic, to see whether the knockout succeeded.

PROTOCOL CITATION

Brian Teague 2022. PCR (Instructor Protocol). **protocols.io** https://protocols.io/view/pcr-instructor-protocol-cfaptidn

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MATERIALS TEXT

- Q5 Hot Start High-Fidelity 2X Master Mix - 100 rxns **New England Biolabs Catalog #M0494S** Step 1
- E. coli harboring the YTK74 plasmid
  Monarch® Plasmid Miniprep Kit **New England Biolabs Catalog #T1010** Step 3
- Nuclease free water **Contributed by users** Step 2

SAFETY WARNINGS

The solutions in the Monarch® Plasmid Miniprep Kit **New England Biolabs Catalog #T1010** have safety implications (particularly the lysis solution, which is quite caustic.) Wear appropriate PPE.

## Setup

1 Aliquot Q5 Hot Start High-Fidelity 2X Master Mix - 100 rxns **New England Biolabs Catalog #M0494S** into **100 µL** aliquots, 1 per 4 students.

2 If not done already, aliquot Nuclease free water **Contributed by users** into **1 mL** aliquots, 1 per 4 students.

3 For the URA3 PCR: grow and miniprep the E. coli hosting YTK74, using Monarch® Plasmid Miniprep Kit **New England Biolabs Catalog #T1010** or similar. Dilute to 1 ng/ul in elution buffer and make **20 µL** aliquots, 1 tube for every 4 students.

## Instructor Tips & Common Student Errors

4 **Instructor Tips**

- Students often struggle with PCR. It's tetchy - I still struggle with it occasionally! Several choices make success more likely:

- Hot-start Q5 master mix. The Q5 master mix is really robust, and the hot-start formulation keeps the enzyme from chewing up primers in the wait between mixing the reaction and starting it in the thermocycler.
- For the URA3 preparative PCR - purified plasmid template. No nuclease contamination from suboptimal genomic DNA preps.

- The idea that PCR works by an exponential DNA amplification is another thing that students often struggle with. I firmly believe that students should understand *why* they're doing what they're doing instead of just following a protocol. How best to motivate and evaluate student understanding is something I'm still struggling with.

- Related to the above, the fact that there are three sets of oligos -- and that they're used in different ways, for different protocols -- is another thing to help students with. Why can't you just reuse the targeting oligos for PCR?

- There is a LOT of pipetting of small volumes here. A reminder of good small-volume pipetting technique would not go amiss.

- Interpreting the PCR gel often helps students solidify their understanding of what's going on here. Why is it important to estimate the PCR amplicon's size, not just see whether there's DNA there or not? For the knockout PCR, what does a long amplicon vs a short amplicon tell us?

## 5 Common student errors

- Used wrong primers or template
- Pipetting errors!