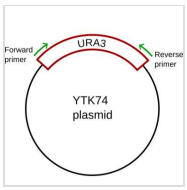


Aug 12, 2022

URA3 PCR

Brian Teague[1]

[1]University of Wisconsin - Stout

1 *Works for me* Share

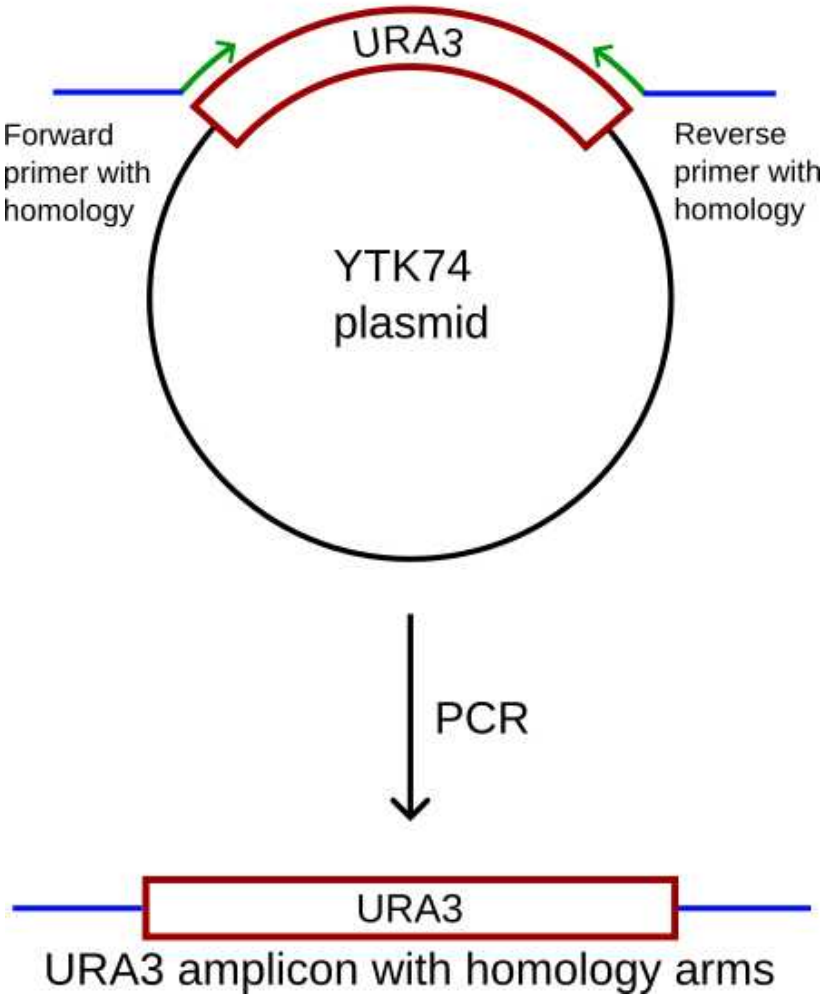
This protocol is published without a DOI.

Yeast ORFans CURE

Brian Teague
University of Wisconsin - Stout

ABSTRACT

The polymerase chaine reaction (PCR) amplifies linear DNA using a DNA polymerase enzyme and a pair of short single-stranded DNA "primers." This protocol is for amplifying the URA3 gene from the YTK74 plasmid and adding the homology sequences needed for homology-directed repair.



PROTOCOL CITATION

Brian Teague 2022. URA3 PCR. **protocols.io** https://protocols.io/view/ura3-pcr-ce6tthen

CREATED

Aug 11, 2022

LAST MODIFIED

Aug 12, 2022

PROTOCOL INTEGER ID

68531

MATERIALS TEXT

- YTK74 PCR template, 1 ng/ul
- YFG-URA3 F forward primer, [M]**10 micromolar (µM)** concentration
- YFG-URA3 R reverse primer, [M]**10 micromolar (µM)** concentration
- Q5 Hot Start High-Fidelity 2X Master Mix - 100 rxns **New England Biolabs Catalog #M0494S** Step 4
- Nuclease free water **Contributed by users** Step 4
- A **200 µL** PCR tube

SAFETY WARNINGS

None of the materials used in this lab are hazardous.

HOWEVER, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats and gloves to keep your samples nuclease-free.

1 Check that the thermocycler is programmed and holding at 98°C. The thermocycler program we're using is the following:

A	B
98°C for 30 seconds	
Repeat 35 times:	
	98°C for 5 seconds
	60°C for 15 seconds
	72°C for 30 seconds
72°C for 2 minutes	
Hold at 8°C	

Do not skip this step -- you don't want to wait for a thermocycler to warm up!

2 Grab an ice bucket and fill it with ice. If you don't have an ice bucket, a beaker will work in a pinch.

3 If necessary, dilute the primers to a concentration of **10 micromolar (µM)** in TE Buffer **Contributed by users** . (Make 100 µl of each dilution.)

Remember, by convention the blue-capped tubes from IDT have a concentration of **100 micromolar (µM)** .

4 Mix the following in a PCR tube **on ice, in this order:**

- **7 µL** Nuclease free water **Contributed by users**
- **1 µL** forward primer
- **1 µL** reverse primer
- **1 µL** template DNA
- **10 µL** Q5 Hot Start High-Fidelity 2X Master Mix - 100 rxns **New England Biolabs Catalog #M0494S**

5 Mix the reaction by gently flick the tube several times, then spin down in a microfuge.

Do this quickly and return the tube to the ice bucket ASAP. There aren't enough thermocycler blocks for every group -- you may need to wait to share a thermocycler with other groups.

6 Transfer the tube from ice to a pre-heated thermocycler holding at 98°C. Start the PCR program.

7 After the PCR program has run, store the tube at -20°C.