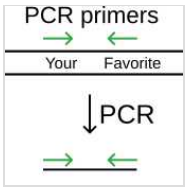

Aug 15, 2022

Knockout PCR

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Yeast ORFans CURE

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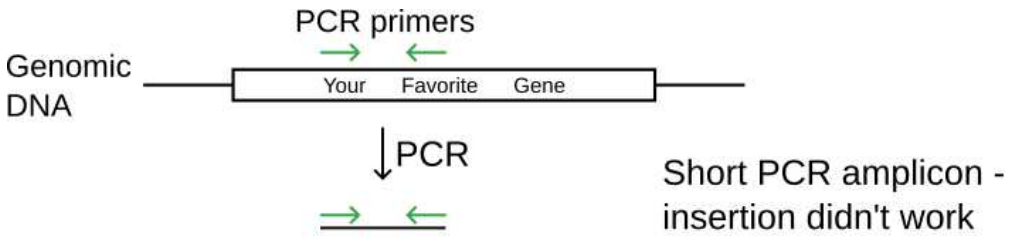
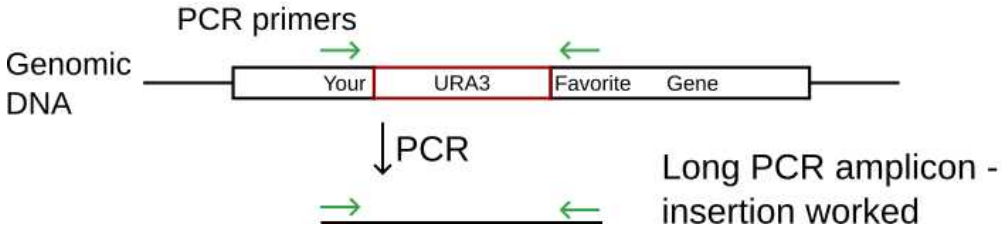
ABSTRACT

The polymerase chaine reaction (PCR) amplifies linear DNA using a DNA polymerase enzyme and a pair of short single-stranded DNA "primers." This protocol amplifies a region that spans the Cas9 cut site so you can verify whether or not the URA3 "patch" inserted correctly (and thus disabled your gene.)





PROTOCOL CITATION

Brian Teague 2022. Knockout PCR. **protocols.io** https://protocols.io/view/knockout-pcr-cfamtic6

KEYWORDS

genomic, pcr, knockout

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MATERIALS TEXT

- Q5 Hot Start High-Fidelity 2X Master Mix - 100 rxns **New England Biolabs Catalog #M0494S** Step 4
- Yeast genomic DNA
- "YFG KO F" Forward primer, 10 µM concentration
- "YFG KO R" Reverse primer, 10 µM concentration
- Nuclease free water **Contributed by users** Step 4
- TE Buffer **Contributed by users** Step 3

SAFETY WARNINGS

None of the materials in this protocol are particularly 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 with the following program and holding at **98 °C**

A	B
98° for 30 seconds	
Repeat 35 times:	
	98° for 5 seconds
	60° for 15 seconds
	72° for 1 minute
72° for 2 minutes	
Hold at 8°	

Do not skip this step!

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, the blue-capped tubes 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 flicking the tube several times, then spin down in the 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**