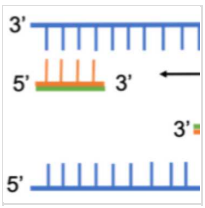

Jun 03, 2022

Forward Primer Reconstitution

Allyson Hirsch[1], George Testo[1]

[1]The Pathogen & Microbiome Institute, Northern Arizona University

1

protocol .

George Testo
The Pathogen & Microbiome Institute

Stock primers should not be directly used in a PCR because they are concentrated. Working from one tube is a bad idea, and thus it only takes a small amount of contamination to render your primers ineffective. For this reason, it is best practice to create working solutions that are of lower concentrations. The concentration of choice for a working primer solution is user-dependent. However, 100uM is used for this protocol.

Allyson Hirsch, George Testo 2022. Forward Primer Reconstitution. **protocols.io** https://protocols.io/view/forward-primer-reconstitution-cajmsck6

protocol ,

Jun 03, 2022

Jun 03, 2022

63821

Reagents

- 1 x PCR grade H2O
- 1 x solidified forward primer

Equipment

- 1000uL pipette, tip box, & tips

:

- Make sure you work in a DNA-free environment. Preferably in a PCR preparation hood. This is to avoid contamination of your stock and working primer solutions.
- Use PCR-grade water (DNase- and RNase-free) to reconstitute and dilute your primers.
- Use filter pipette tips to prevent contamination via pipetting.

Preparing Forward Primer 15m

1 

Vortex and spin down primer tube: this breaks up the solidified primer at the bottom of the tube and brings the primer debris up from the bottom of the tube.

2 Look for nM (nanoMoles) on the printed primer label and circle.

Ex: **779 µL**

3 Multiple nM by 10, then add that much PCR grade water to the tube.

Ex: **779 µL**

Incubating Reconstitution 15m

4 

Vortex new [M]**100 micromolar (µM)** tube.

5 Let sit for at least **00:15:00** (optimum: 1-2 hours). 15m

6 Label stock tube as “100uM” on cap.

7 Once the sitting period has elapsed, place new [M]**100 micromolar (µM)** stock in a **-20 °C** freezer.

7.1 **For Working Concentration of Forward Primer:**

100 µL of FP stock + **900 µL** molecular grade H2O