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Forward Primer Reconstitution

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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.

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Reagents

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

Equipment

- 1000uL pipette, tip box, & tips

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- 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

4 

Vortex new **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 **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