



Jun 03, 2022

Working Reverse Primer Plate(s)

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protocol .



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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, 10uM is used for this protocol.

Allyson Hirsch, George Testo 2022. Working Reverse Primer Plate(s).

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Reagents

- 1 x Molecular grade water
- 1 x Reconstituted Reverse Primer

Supplies

- 6 x 96-well skirted plates
- Green temporary seal(s)
- 50mL conical
- Foil seal(s)
- Sharpie

Equipment

- Conical flip rack
- 200uL pipette & tips
- 10uL Multi-channel pipette & 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 PCR Hood

31m

- 1 Decontaminate a PCR hood with DNA Away and Ethanol.
- 2 Place materials inside of the PCR Hood (refer to materials section).

3



30m

Turn on the UV setting for at least 00:30:00 .

Preparing Skirted Plates

31m

4

Temporary seal 6 96-well skirted plates and label each "Reverse Primer Plate ____ Initials/Date."

5



Add 90 μL of molecular grade water (poured into a 50 mL conical) to each well with a 200uL single-channel pipette. Make sure to get a new tip for each subsequent well (this techelps prevent contamination).

6

After completing 3 primer plates, retrieve the 100 micromolar (μM) stock from the freezer and let it thaw.

7

Finish the remaining 3 primer plates.

7.1 Temporary seal each primer plate after loading with molecular grade water.

8

Once the EMP 100 micromolar (μM) stock has thawed, spin each plate down for 00:01:00 at full speed.

1m

Adding EMP Primer(s)

31m

9



Add 10 μL from each well from the EMP 100 micromolar (μM) primer plate using a 10uL multichannel pipette, and pipetting to corresponding well(s) on the

[M]**10 micromolar (μM)** primer plate loaded with water.

Note: Use temporary seals when necessary to help reduce contamination.

- 10 Once the [M]**10 micromolar (μM)** primer plate stock has been made, seal with a foil freezer seal and store in the $\text{♾ -20 }^{\circ}\text{C}$ freezer.