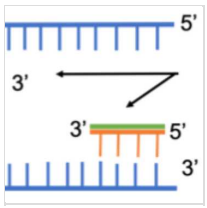

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Working Reverse Primer Plate(s)

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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, 10uM is used for this protocol.

Allyson Hirsch, George Testo 2022. Working Reverse Primer Plate(s). **protocols.io** https://protocols.io/view/working-reverse-primer-plate-s-cakdscs6

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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

:

- 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).

30m

3 ⚠

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 **[M]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.

1m

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

Adding EMP Primer(s) 31m

9 

Add **10 µL** from each well from the EMP **[M]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 **-20 °C** freezer.