

Primers dilution protocol

The primers arrive in an envelope. Each primer has an information page in the envelope (which we keep on our shelf in the students lab).

Prepare 100 μM solution and dilute it to 10 μM stock for each primer.

Use ultra pure water- free from DNA, RNA, enzymes, salts, contamination etc.

Procedure:

1. Prepare one ependorf for each primer you are going to dilute. On each ependorf place one of the label provided on the information page. Write on the label 10 μM .
2. Locate the information pages for the primers which you are going to dilute.
3. On the page, find the amount of primer in **nmoles** (in the "Amount of Oligo" section). **Multiply this number by 10**. This will be the amount of ultra pure water in μl which you will add to the primer in order to get the 100 μM stock.

Explanation:

Suppose the primer amount is 20 nmole, we are willing to prepare a 100 μM stock from it, so the volume of UPW we need to add is:

$$V_{(\mu\text{l})} = \frac{20\text{nmole} \times \frac{\mu\text{mole}}{10^3\text{nmole}}}{100 \frac{\mu\text{mole}}{\text{lit}} \times \frac{\text{lit}}{10^6\mu\text{l}}} = 200\mu\text{l}$$

4. Add the calculated amount of ultra pure water to the primer. **Mix using vortex**. This is now a 100 μM stock solution.
5. Dilute the 100 μM solution in the marked ependorf (the one you wrote 10 μM on) by adding 10 μl of 100 μM solution to 90 μl of ultra pure water. Mix using vortex. Now you have 100 μl of your 10 μM stock.
6. Store the primers in your primer's box at $-20\text{ }^\circ\text{C}$.