

Resuspending PCR primers and other oligos

Overview

Primers are often shipped and received in a lyophilized state. First create a master 100 μM stock (for each primer) and then dilute it to a 10 μM working stock. This reduces the number of freeze/thaw cycles that the master primer stock goes through and reduces the chances of contaminating the primary source for the primer.

Materials

Lyophilized primers

Sterile dH₂O

Procedure

1) *Spin Down Tubes*. Primers should **always be spun down before opening the tube** for the first time. The pellet can often come dislodged during shipping and may be in the cap!

2) *Prepare Master Stock, 100 μM*

$$100 \mu\text{M} = X \text{ nmoles lyophilized primer} + (X \times 10 \mu\text{l molecular grade H}_2\text{O})$$

To determine the amount of water to add to the lyophilized primer simply multiply the number of nmol of primer in the tube by 10. That will be the amount of water to add to make a 100 μM primer stock. For example, if there are 38.2 nmol of primer a 100 μM primer stock is created by adding 382 μl of water. The original primer tubes are used for this 100 μM stock.

Master stock primers newly suspended in water should be allowed to sit at room temperature for 10 minutes before they are used for working stock dilutions. Mix well before making working stock dilutions.

3) *Preparing Working Stock, 10 μM*

Dilute the primer master stock in a sterile microcentrifuge tube 1:10 with sterile molecular grade water.

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