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How to Make Blocker Aliquots from Individual Oligos

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When you receive the oligos (e.g., from IDT), there will be varying amounts in each tube. You will need to reconstitute each oligo with the appropriate volume of liquid to achieve the desired concentration (i.e., 1000 µM), then mix with the mate (1000 µL each -> 500 µM) & aliquot them. Note: We do not actually know that 500 µM is the optimal value, we just know that it works! We will be trying some slightly lower concentrations (e.g., 100 µM), but I would not use less than 500 µM unless you are willing to risk a crash & burn.

Liquid for reconstitution & annealing (TLE: 10 mM Tris pH 8, 0.1 mM EDTA):

For 50 mL of TLE, add the following to a 50mL conical:
40 mL dH_2O
500 µL 1M Tris pH 7.5 to 8
20 µL 0.5M EDTA pH 8
Fill with distilled water to 50mL mark.

Protocol:

1) Centrifuge the tubes to get all the primer to the bottom of the tubes.

2) To limit contamination, deal with tubes individually. Use barrier (filtered) pipette tips for each step.

3) Add TLE **equal to** the number of nmol of oligo (e.g., if you have 187.3 nmol; add 187.3 µL of TLE).
-Use the pipette tip to help scrape the bottom of the tube to dislodge any of the oligo that is stuck.
-Skloosh (pipette up & down) several times until sufficiently mixed.
-Wait a few minutes.
-Skloosh several more times until sufficiently mixed.
-Let the oligos sit in the liquid at room temperature for at least 5 minutes.

4) Mix equal volumes of each adapter pair (100 µL each oligo -> 500 µM blockers).

5) Place 50 µL aliquots of the blockers into new tubes.

6) Store the tubes of blockers at -20°C

7) Take blockers out to thaw shortly before use and **skloosh well before using!**