

Annealing 3G Adapters

Overview

This protocol covers the resuspension and subsequent annealing of dried (lyophilized) oligos that are to be used as 3G adapters. Single stranded oligos are resuspended, and then annealed to each other before being diluted. In this protocol un-annealed stock (100 μ M), duplex stock (5 μ M) and duplex working (50nM) solutions are made.

Materials

- Lyophilized Oligos (IDT)
 - o Each oligo should have a corresponding specification sheet, which should be stored in the specification sheet drawer.
- Labeled 1.7mL centrifuge tubes (2 per sample)
 - o Tubes should be labeled with adapter name and 5 μ M, dates and initials should be included on the side. A second tube should be labeled with 50nM.
- .2mL Tubes
- IDT duplex Buffer
 - o Stored at 4C
 - o Use the falcon tube aliquot, not the stock!
- Nuclease Free Water (NFW)

Procedure

1. Briefly spin down oligo tubes.
 - o Use green benchtop centrifuge
2. Determine correct amount of IDT duplex buffer to add to each tube for a 100 μ M solution. This number can be found on the specification sheet. Alternatively, it can be determined by multiplying the number of nanomoles of DNA provided by 10 and then using that many μ L of duplex buffer. Remember that each tube will have a different amount of duplex buffer to add.
3. Add correct amount of duplex buffer to each tube. Heat at 55C for 2 minutes. Vortex. Briefly spin down.
4. Add 5 μ L of each the two corresponding oligos to a .2mL tube.
 - o Each oligo is now at a concentration of 50 μ M
5. Then add 90 μ L of IDT duplex buffer.
 - o Each oligo is now at 5 μ M concentration
6. Program thermocycler to heat at 94C for 2 minutes, and then descend by 1C per minute until 20C is reached. This can easily be done using the gradient thermocycler's "delta" feature.
7. Once annealing is finished, transfer 5 μ M duplex to duplex stock tube
8. Next make a 50nM working solution by 1:100 diluting duplex stock in NFW (eg. 1 μ L duplex to 99 μ L NFW).

9. Store stock and working duplexes in duplex box. Log on inventory sheet.