Custom DNA Oligos

Protocol for Annealing Oligonucleotides

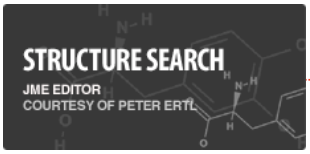
Annealing Buffer: 10 mM Tris, pH 7.5–8.0, 50 mM NaCl, 1 mM EDTA

1. Resuspending the Oligonucleotides: Resuspend both complementary oligonucleotides at the same molar concentration, using Annealing Buffer (see note below). For convenience, keep Annealing Buffer volume below 500 µl for each oligo. Annealing should perform well over a wide range of oligo concentrations. For larger scale oligo syntheses, it may be necessary to use larger volumes that can be aliquoted after resuspension.

2. Annealing the Oligonucleotides:
   a. Mix equal volumes of both complementary oligos (at equimolar concentration) in a 1.5 ml microfuge tube.
   b. Place tube in a standard heatblock at 90–95 °C for 3–5 minutes.
   c. Remove the heat block from the apparatus and allow to cool to room temperature (or at least below 30 °C) on the workbench. Slow cooling to room temperature should take 45–60 minutes.
   d. Store on ice or at 4 °C until ready to use.
   e. An alternative procedure for annealing involves the use of a thermal cycler. Dispense 100 µl aliquots of the mixed oligos into PCR tubes (500 µl size). Do not overlay the samples with oil. Place the tubes in a thermal cycler and set up a program to perform the following profile:
      i. Heat to 95 °C and remain at 95 °C for 2 minutes;
      ii. Ramp cool to 25 °C over a period of 45 minutes;
      iii. Proceed to a storage temperature of 4 °C. Briefly spin the tubes in a microfuge to draw all moisture from the lid. Pool samples into a larger tube, store on ice or at 4 °C until ready to use.

3. Long Term Storage: It may be necessary to aliquot and lyophilize the annealed sample. After drying, the sample may be stored at –20 °C in a desiccated container. Resuspend the annealed oligos at the desired concentration with sterile distilled water. The annealed pair of oligonucleotides is ready for use.

NOTE: Oligos may also be resuspended in either 1x Ligase Buffer or 1x Kinase Buffer instead of the above Annealing Buffer (prior to annealing).



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