Protocol for Annealing Oligonucleotides

1 Materials

Annealing buffer, 10×: 100 mmol l^{-1} Tris, pH 7.5–8, 500 mmol l^{-1} NaCl, 10 mmol l^{-1} EDTA
Complementary oligonucleotides: diluted in water or TE to the same concentration (usually 200 µmol l^{-1}).

2 Method

1. **Resuspending the Oligonucleotides:** Resuspend both oligos to the same molar concentration, using TE or water. 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:**

   - Mix equal volumes of both complementary oligos (at equimolar concentration) in a 1.5 ml microfuge tube along with an appropriate volume of 10× annealing buffer and water to make the final concentration of each oligo to equal 50 µmol l^{-1} and 1× annealing buffer.
   - Place tube in a standard heatblock at 90–95 °C for 3–5 minutes.
   - 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.
   - Store on ice or at 4 °C until ready to use.
   - 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:

   (a) Heat to 95 °C and remain at 95 °C for 2 minutes;
   (b) Ramp cool to 25 °C over a period of 45 minutes;
   (c) 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.

4. **Dilutions:**Dilute the annealed oligos to $5\,\mathrm{nmol\,l^{-1}}$. Do not allow dilutions to reach room temperature as oligos may melt. Diluted oligos can not be re-annealed as concentrations are too low.

5. **Ligations:** Annealed oligos can be ligated into the desired vector using at least a 15:1 molar ratio of insert:vector and T4 DNA ligase. According to Invitrogen, overnight ligations at 16 °C may not work well.

Note: Oligos may also be resuspended in either 1× ligase buffer or 1× kinase buffer instead of the above annealing buffer (prior to annealing).
Further Reading: This information is based on the "BLOCK-iT™Pol II miR RNAi Expression Vector Kits" manual from Invitrogen/Life Technologies.