

# Protocol for Annealing Oligonucleotides

## 1 Materials

**Annealing buffer, 10×:** 100 mmol l<sup>-1</sup> Tris, pH 7.5–8, 500 mmol l<sup>-1</sup> NaCl, 10 mmol l<sup>-1</sup> EDTA

**Complementary oligonucleotides:** diluted in water or TE to the same concentration (usually 200 μmol l<sup>-1</sup>).

## 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<sup>-1</sup> 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  $-20\text{ }^{\circ}\text{C}$  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  $5\text{ nmol l}^{-1}$ . 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  $16\text{ }^{\circ}\text{C}$  may not work well.

**Note:** Oligos may also be resuspended in either  $1\times$  ligase buffer or  $1\times$  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.