Protocol for Annealing Oligonucleotides


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

1. Re-suspend both complementary sequencing adapter oligonucleotides (P5 and P7) at the same molar concentration (20 uM), using Annealing Buffer. Keep at -20 °C for long-term storage.

2. Annealing the Oligonucleotides:
   a. Mix equal volumes of both complementary oligos (at equimolar concentration) in a 1.5 ml Lowbind microfuge tube: (final concentration) 20uM for whole genome sequencing or 0.5 uM for RNA sequencing
   b. Place tube in a standard heat block at 95 °C for 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.

3. Check annealed adapters on a DNA PAGE gel
   * > 80-90 % oligos should be annealed
   * Proper adapters will be located at around ~400 bp in the gel; higher than their actual length since they migrate slowly due to their Y-shaped form.

![DNA PAGE gel](image)