Annealing Oligos

Introduction

Anealing Oligos

Materials

- Nuclease-free TE
  - We resuspend in TE to help supress nuclease activity that would degrade the primers.
- 1M NaCl solution (in water)
- Dehydrated oligos from IDT
- One thin-walled 200 ul PCR tube
- One epi tube (required), plus another (optional)

Procedure

Preparing oligos

1. Resuspend the oligos at 100 uM, as described in "Resuspending Primers"

2. Set up a thermocycler program for the following annealing program:

   - Heat to 95° for 2 minutes
   - Every 1'30", decrease the temperature by 1° to a final temperature of 25°
   - Hold at 4°

Procedure

3. In the thin-walled PCR tube, mix:

   - 75 μl nuclease-free TE
   - 5 μl 1M NaCl
   - 10 μl oligo #1
   - 10 μl oligo #2
   -(This is 10uM)

4. Place tube in the thermocycler and run the annealing program

5. Transfer annealed oligo to the epi tube. Label with name and concentration (10 μM) and store at -20°C.

6. For setting up Golden Gate reactions: 1 μM is 1000 fmol/μl. If your desired input to a GoldenGate reaction is 50 fmol, then:

   - Add 199 μl of TE to the second epi tube
   - Add 1 μl of the 10 μM duplex

- Mix well, label with name and concentration (50 fmol/μl) and store at -20°.