Pipet Tips
Ideas to Streamline Your Research

Annealing oligonucleotides

It is sometimes necessary to make double-stranded DNA from single-stranded, complementary oligonucleotides. While the anne procedure is fairly straightforward, attention to a few details can greatly reduce the presence of undesired single-stranded mater

Protocol

1. **Resuspend.** After briefly spinning down each oligonucleotide pellet, dissolve in Duplex Buffer (http://www.idtdna.com/pages/products/reagents/buffers-and-solutions) (100 mM Potassium Acetate; 30 mM HEPES, pH available from IDT)—this provides a buffering environment and the salt is necessary for oligonucleotide hybridization. Diss each oligo at high concentration (1–10 OD_{260}/100 µL); see Table 1 for guidelines on resuspension volumes. Heating (up to 94°C) and vortexing will facilitate resuspension.

Oligo Amount	*Volume for 100 µM*	*Volume for 50 µM*	*Volume for 20 µM*	*Volume for 10 µM*
10 nanomoles	100 µL	200 µL	500 µL	1 mL
25 nanomoles	250 µL	500 µL	1.25 mL	2.5 mL
100 nanomoles	1 mL	2 mL	5 mL	10 mL

2. **Mix.** Add the 2 oligo strands together in equal molar amounts. This step is critical to avoid residual single-stranded materi
3. **Anneal.** Heat the mixed oligonucleotides to 94°C for 2 minutes and gradually cool. For many oligos "cooling" can be as si as transferring samples from the heat block or water bath to the bench-top at room temperature. For sequences with significant secondary structure, a more gradual cooling/annealing step is beneficial. This is easily done by placing the olig solution in a water bath or heat block and unplugging/turning off the machine.
4. **(Optional) Dilute.** if needed, dilute the annealed oligonucleotides using Nuclease-Free Duplex Buffer (http://www.idtdna.com/pages/products/reagents/buffers-and-solutions) or 1X IDTE Buffer (http://www.idtdna.com/pages/products/reagents/buffers-and-solutions) (Part #11-01-02-05).
5. **Store.** The resulting product will be in a stable, double-stranded form and can be stored at 4°C or frozen.

Things to consider:

Avoiding contamination and degradation—If you plan to use the duplex on multiple occasions, divide it into smaller alic and store at –20°C. Nuclease-free Duplex Buffer is available from IDT and is certified nuclease-free by testing with IDT RNaseAlert™ and DNaseAlert™ Kits. Find out more about these products at www.idtdna.com.

Secondary Structure—Low yields of the expected, annealed product can be caused by secondary structure. Use the OligoAnalyzer® program (www.idtdna.com/scitools) to determine whether there is significant secondary structure in the oligonucleotides. Problematic annealing can often be resolved by slow cooling, as described in Step 3, above.

Ligation—If the double-stranded oligonucleotide product will be used in a ligation reaction, you may need to add 5'-phosphates to the strand ends. These can be added at the time of oligo synthesis (chemical phosphorylation; done by rec or anytime thereafter (before or after annealing) using polynucleotide kinase (enzymatic phosphorylation).

Cloning—If the resulting double-stranded DNA fragment will be relatively long (>60 bp), or will be used in cloning, we recommend starting with PAGE-purified oligos (IDT can provide this service).

Annealing RNA—The IDT Research team also uses this protocol to create siRNA duplexes from single-stranded, complementary R oligos.

Let us anneal your oligos for you!
For a small fee, IDT will anneal your oligos for you, so that you can proceed with your experiments as soon as your oligos arrive. request this Duplex Service, go to the Duplex Entry page (http://www.idtdna.com/site/order/duplexentry).

For additional information, please contact the IDT Technical Support Group at applicationsupport@idtdna.com (mailto:applicationsupport@idtdna.com).

Product focus—useful buffers and solutions

IDT provides solutions for resuspension and dilution of oligos. These solutions are guaranteed to be DNase- and RNase-free. Each lot is tested using our RNaseAlert® and DNaseAlert™ reagents to document the absence of any detectable nuclease activity. Individual lots are also screened for endotoxins using a Limulis Abocyte Lysate (LAL) assay.

- IDTE (10 mM Tris, pH 7.5 or 8.0, 0.1 mM EDTA)
- Nuclease Decontamination Solution
- Nuclease-free Water
- Duplex Buffer (30 mM HEPES, pH 7.5, 100 mM Potassium Acetate)

Learn more about these products on our Buffers and Solutions webpage (http://www.idtdna.com/pages/products/reagents/buffers-and-solutions) .

Additional reading

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