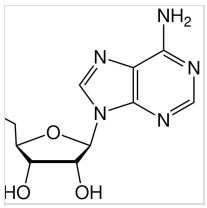

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Annealing Oligonucleotides

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Yeast ORFans CURE

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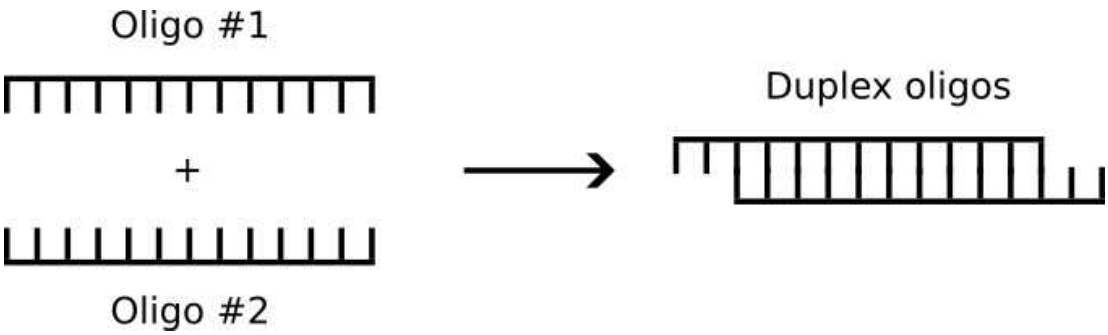
ABSTRACT

While most biologically-synthesized DNA molecules are double-stranded, chemically-synthesized DNA molecules are single-stranded. In order to use them in a biological system, we need to make them double-stranded by "sticking" them to their complementary molecules:



We "stick" them together by mixing them in equimolar amounts, then heating the mixture and cooling them slowly. As the image shows, the duplexed oligos are not a perfect match. Instead, they have four-base "overhangs" on each end -- this will be important in the next protocol.

PROTOCOL CITATION

Brian P P Teague 2022. Annealing Oligonucleotides. **protocols.io** https://protocols.io/view/annealing-oligonucleotides-cebvtan6

KEYWORDS

anneal, oligonucleotides

IMAGE ATTRIBUTION

By Smokefoot - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=117155213

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PARENT PROTOCOLS

In steps of

Annealing Oligonucleotides (Instructor Protocol)

GUIDELINES

Make sure you take care in your pipetting and mixing. The volumes are small!

MATERIALS TEXT

- Nuclease-free TE buffer

We resuspend and dilute oligonucleotides in TE, not water. The Tris keeps the pH at 8.0, which is good for stability, and the EDTA chelates ions and prevents nuclease degredation.

- Guide and Guide RC oligonucleotides, dry or resuspended at a concentration of 100 uM.
- Two microcentrifuge tubes
- One thin-walled 200 ul PCR tube

Equipment

- Microfuge with a PCR tube rotor (optional)
- Thermocycler programmed with a program for annealing oligos

SAFETY WARNINGS

TE is not hazardous; neither are synthetic DNA oligonucleotides.

HOWEVER, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats and gloves to keep your samples nuclease-free.

BEFORE STARTING

Make sure that the thermocycler is programmed with the the annealing program.

Resuspending oligos

1 **If the oligonucleotides have already been resuspended, skip to step 5.**

Look carefully at the label of the oligo that you're resuspending. Find the line that looks like "14.5 OD = 26.1 nmol = 0.46 mg". (This is an example; the numbers on your tube will be different.) These are three different measurements of exactly how much DNA is in this tube.

2 Take the number of nmol and move the decimal point *one place to the right*. Add this number of microliters of TE to the blue-capped tube with the dried oligo.

For example, on the tube above, I take "26.1 nmol" and move the decimal to the right to get "261". Then I add 261 µl of TE to the tube.

3 Vortex for 15 seconds to fully resuspend the oligo.

4 The resulting oligos are at a concentration of **100 micromolar (µM)** .

Diluting oligos

5 Using the TE and the microcentrifuge tubes, dilute the oligonucleotides to **10 micromolar (µM)** . Make 100 µl of each. Record your dilution calculations in the table below. Ask an instructor or TA to double-check your math before proceeding.

A	B	C	D	E
	Volume of resuspended oligo (100 µM concentration)	Volume of TE	Total volume	Final concentratinon
Guide oligo			100 µl	10 µM
Guide RC oligo			100 µl	10 µM

If you are diluting previously resuspended oligos that have been frozen, make sure that the oligos are completely thawed before diluting them.

Annealing the oligos

6 In a thin-walled PCR tube, mix:

- **5 µL** of oligo #1
- **5 µL** of oligo #2

Flick the tube several times to mix well, then use the microfuge to spin down the sample.

7 Label the tube and record how you labeled it in your lab notebook.

8 Find an instructor or TA to help you load the tube into the thermocycler and run the annealing program.