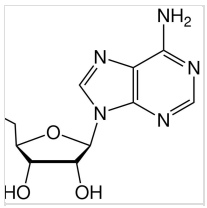

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Annealing Oligonucleotides (Instructor Protocol)

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Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for

Annealing Oligonucleotides
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PREVIEW RUN

PROTOCOL CITATION

Brian Teague 2022. Annealing Oligonucleotides (Instructor Protocol). **protocols.io** https://protocols.io/view/annealing-oligonucleotides-instructor-protocol-cebwtape

IMAGE ATTRIBUTION

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MATERIALS TEXT

- TE Buffer **Contributed by users** Step 1

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.

I do not trust my ability to prepare nuclease-free TE buffer, so we usually order it from a manufacturer (it's not expensive.) However, if you do prepare your own, make sure to autoclave it to destroy DNAses!

- 15 ml conical tubes **Contributed by users**

Equipment:
- Microfuge with PCR tube rotor
- Thermocycler

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.

Lab Setup

1 Prepare 15 ml conicals with **5 mL** TE Buffer **Contributed by users** in each. Prepare one tube for each 4 students (2 groups of 2).

2 Program a thermocycler with the following program:

- Step 1: 95°C for two minutes
- Step 2: 95°C for 45 seconds, decreasing one degree each cycle
- Step 3: Go to step 2 70 times

- Step 4: Store at 8°C

Instructor Tips & Common Student Errors

3 Instructor Tips

- This is the first "real" lab. I generally start the class explaining what annealing is and why we're doing it, point out where the materials are, and then sit down. My approach is deliberately hands-off.
- Each semester, I am amazed that this protocol takes students an entire two-hour lab period. It probably has to do with my hands-off approach, as mentioned above.
- Students often struggle with the dilution math. Remind them of c1 * v1 = c2 * v2 and remind them that they've already done this once. Suggest that they review the dilutions and pipetting lab and that they ask their colleagues for help. Only if students are really stuck will I review the procedure with them.
- We've only got one thermocycler -- but this reaction is fine to wait until the entire class has loaded their samples to start. (As opposed to the PCR, which needs to stay on ice until it's time to start.) So I ask students to load their samples into the thermocycler as they finish.
- As students load their samples, double-check that they look like about 10 µl of liquid. Significantly more may be a sign of a pipetting error.
- As students load their samples, double-check that they're labeled. Remind them that their tube will be one of many and ask if they'll be able to pick their tube out from all the others?
- Remind students to record their tube label in their notebook.

4 Common Student Errors

- Difficulty doing the dilution math.
- Didn't mix the sample well
- Didn't label their tubes