AUC Multi-wavelength Experiment: DNA+BSA

1. using two spectrophotometers, we will first blank each spectrophotometer with water and then measure the absorbance of our buffer

2. After blanking with our buffer, we will make a 0.8, 0.6, 0.4, and 0.2 dilution of a 1 OD260 nm DNA sample and a 1 OD280 nm protein sample. All measurements will be recorded.

3. Next, we will show you how to assemble and disassemble an AUC cell. You will get to do your own cell.

4. Once each of you has an assembled AUC cell, we will load both channels with water (for practice).

5. One of the cells will be loaded with a 1:3 ratio of protein:DNA in one channel, and a 3:1 ratio of protein:DNA in the other channel.

All lab work will be assisted by Sophia, Zach, Liam and Reece.

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Dilution Protocol:

You will start with 250 ul at 1.0 OD. The cuvette holds approximately 1 ml.

The first dilution will be:

C1 V1 = C2 V2

(1 OD * 250 ul)/0.8 OD = V2 = 312.5 ul (total, so add 62.5 ul of buffer)

For the second dilution:
(0.8 OD * 312.5)/0.6 OD = V2 = 416.7 ul (total, so add:
416.7-312.5 = 104.17 ul of buffer)
...
Etc. until you reach 0.2 OD. If the volume gets to be too much, remove a Known amount and adjust your calculations accordingly. Save all dilutions or excess material in a separate Eppendorf tube.

All lab work will be assisted by Sophia, Zach, Liam and Reece.

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Groups:

Sophia: Fatimah and Matt

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