Protocol: Plasmid Concentration Measurement Using Micro-Volume Spectrophotometer (Nanodrop or 70 µL cuvette)

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Note: Protocol was taken from last year's iGEM Team, with a few modifications.

Materials

- Spectrophotometer with cuvette for microvolume DNA measurement



- Eluted plasmid DNA sample
- NanoDrop lid (check cap factor)
- dH_2O
- Lint-free tissue

Instructions

Using the NanoDrop:

1. Prepare the NanoDrop
    - Turn on the machine.
    - Remove the cuvette and insert it with the writing “Facing you” toward you.
    - Ensure the two optical “windows” are facing left and right.
    - Push it in completely; the top should only stick out ~1.5 cm.
2. Select the measurement method:
    - Method type: NanoDrop: **Option 1**
    - Measurement type: Nucleic Acid: **Option 1**
    - DNA type: dsDNA: **Option 1** (if measuring plasmid DNA)
3. Choose the correct lid and set the correct lid factor
    - Read the cap factor on the lid (e.g., 10 = 1 mm path length).
    - Set **Lid Factor** so that it matches the cap factor.
4. Select remaining parameter values
    - **Dilution factor** 1.000 will give you the concentration in the cuvette.
    - Set **Factor** to 50.0 (should be set as default).
    - Set **Background** to on (should be set as default).
5. Clean the pedestal
    - Wipe both surfaces with a soft tissue dampened with dH_2O.
    - Repeat before and after each use.
6. Blank the instrument
    - Pipette 2 µL of the eluent (liquid you used to elute the DNA from the spin column) onto the measurement pedestal.
    - Align the lid with the pedestal holes and tap the lid twice gently.
    - Press Blank.
    - After blanking, wipe the pedestal clean with a damp tissue.
7. Measure the sample
    - Apply 2 µL of your plasmid DNA sample onto the pedestal.
    - Align the lid and tap twice as before.
    - Press Measure.
8. Record the results
    - Note down:
        - DNA concentration (e.g., ng/µL)
        - 260/280 ratio (should be ~1.8–2.0)
        - 260/230 ratio (should be ~1.8)
9. Clean up
    - Wipe the pedestal with a clean tissue dampened with dH_2O.
    - Close the lid and shut down the instrument if no further use is planned.

Using the 70 µL cuvette:

1. Turn on the machine.
2. Select measurement method
    - Method type: Cuvette applications → Option 2
    - Measurement type: Nucleic Acid → Option 1

    - DNA type: dsDNA → Option 1 (if measuring plasmid DNA)
3. Select parameter values
    - **Dilution factor** 1.000 will give you the concentration in the cuvette.
    - Set **Factor** to 10 (should be set as default).
    - Set **Background** to on (should be set as default).
    - Set **Path Length** to 10 mm.
4. Insert the blank.
5. Click **Blank** (the blue button on the spectrophotometer).
6. Take out the blank.
7. Insert sample.
8. Click **Sample** (the green button on the spectrophotometer) to measure the concentration.
9. Record the results.
    - Note down:
        i. DNA concentration (e.g., ng/μL)
        ii. 260/280 ratio (should be ~1.8–2.0)
        iii. 260/230 ratio (should be ~1.8)
10. Take out the sample.
