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Measure DNA concentration

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[1]iGEM_NTHU_Taiwan

iGEM NTHU

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We use this protocol and it's working

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Abstract

Measure the DNA concentration and purity of the purified PCR product (SpyCatcher segment) to ensure it meets Sanger sequencing submission requirements, and dilute or concentrate as needed.

Materials

- Purified PCR product (product purified in the previous step using a DNA purification kit)
- Nuclease-free water
- 70% ethanol and Kimwipes
- NanoDrop spectrophotometer
- Pipettes and sterile tips

Starting and Calibrating the Nanodrop

1 Turn on the Nanodrop device and launch the software.

2 Select **Nucleic Acid → dsDNA mode**.

3 Pipette 2 µL of nuclease-free water onto the lower measurement pedestal.

4 Close the arm and click **Blank** to calibrate the background.

Measuring DNA Sample Concentration

5 Using a clean pipette tip, pipette 2 µL of the purified PCR sample onto the lower pedestal.

6 Close the arm and click **Measure**.

7 ecord the following data:

- Concentration (ng/µL) — typically 10–20 ng/µL is required for sequencing.
- 260/280 ratio (purity) — should be approximately 1.8–2.0.

8 Clean both the upper and lower pedestals with 70% ethanol and a Kimwipe.

Diluting DNA Concentration if Necessary

9 If the DNA concentration is too high (>100 ng/µL), dilute to 10–20 ng/µL using the following formula:

$$V1 \times C1 = V2 \times C2$$

10 Add nuclease-free water to dilute.

11 Mix thoroughly and re-measure with the Nanodrop to confirm the correct concentration.