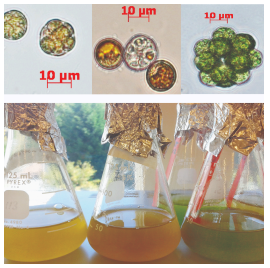
Jul 20, 2023

DNA quantification, Purity and Integrity



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We use this protocol and it's working

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Abstract

We will use three complementary methods to asses DNA.

Troubleshooting

Quantification.

1 Qubit system https://www.thermofisher.com/order/catalog/product/Q32850#/Q32850
This protocol assumes that you are preparing standards for calibrating the Qubit® Fluorometer. If you plan to use the last calibration performed on the instrument (see "Qubit® Fluorometer calibration" on page 2), you need fewer tubes (step 1.1) and less working solution (step 1.3).
1.1 Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® dsDNA HS Assay requires 2 standards.
Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit® assay tubes (Cat. no. Q32856) or Axygen® PCR-05-C tubes (part no. 10011-830).
1.2 Label the tube lids.
Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit® Fluorometer requires the standards to be inserted into the instrument in the right order.
1.3 Prepare the Qubit® working solution by diluting the Qubit® dsDNA HS Reagent 1:200 in Qubit® dsDNA HS Buffer. Use a clean plastic tube each time you prepare Qubit® working solution. **Do not mix the working solution in a glass container.**
Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit® working solution, and each sample tube requires anywhere from 180–199 µL. Prepare sufficient Qubit® working solution to accommodate all standards and samples. For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit® reagent plus 1990 µL of Qubit® buffer).
1.4 Add 190 µL of Qubit® working solution to each of the tubes used for standards.
1.5 Add 10 µL of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.
Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit® standard is added to 190 µL of Qubit® working solution.
1.6 Add Qubit® working solution to individual assay tubes so that the final volume in each tube after adding thesample is 200 µL.
Note: Your sample can be anywhere from 1–20 µL. Add a corresponding volume of Qubit® working solution to each assay tube: anywhere from 180–199 µL.
1.7 Add each sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.
1.8 Allow all tubes to incubate at room temperature for 2 minutes.
Read
2.1 On the Home screen of the Qubit® 3.0 Fluorometer, press **DNA**, then select **dsDNA High Sensitivity** as the assay type. The "Read standards" screen is displayed. Press **Read Standards** to proceed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 2.4. Otherwise, continue with step 2.2.

2.2 Insert the tube containing Standard #1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~3 seconds), remove Standard #1.

2.3 Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard #2.
The instrument displays the results on the Read standard screen. For information on interpreting the calibration results, refer to the Qubit® 3.0 Fluorometer User Guide.

2.4 Press **Run samples**.

2.5 On the assay screen, select the sample volume and units:

a. Press the **+** or **–** buttons on the wheel to select the sample volume added to the assay tube (from 1–20 µL).

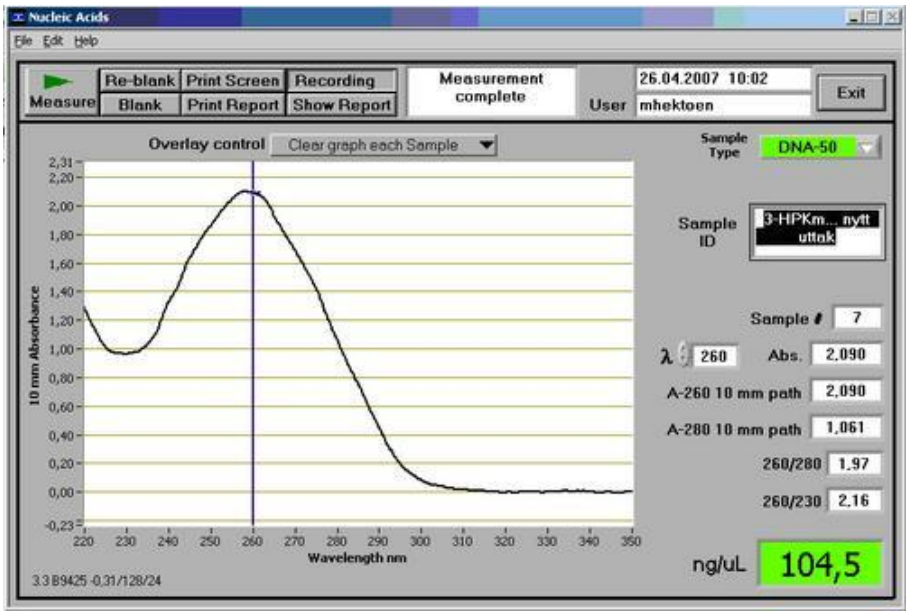
b. From the dropdown menu, select the units for the output sample concentration.

2.6 Insert a sample tube into the sample chamber, close the lid, then press the**Read tube**. When the reading is complete (~3 seconds), remove the sample tube.
The instrument displays the results on the assay screen. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration. For information on interpreting the sample results, refer to the Qubit® 3.0 Fluorometer User Guide.

2.7 Repeat step 2.6 until all samples have been read.

Nanodrop

2 The measure of quantity and quality using a **spectrophotometric method** is performed in a dedicated machine (Nanodrop, at JBP center) able to measure 1-2 ul of sample. The output provides information on the concentration and purity by measure of the absorbance ratio 260/280 nm. DNA absorbance peaks at 260.



A low A260/A280 ratio may be caused by:
• Residual phenol or other reagent associated with the extraction protocol
• A very low concentration(> 10 ng/ul).of nucleic acid
High 260/280 purity ratios are not indicative of an issue.
A low A260/A230 ratio may be the result of:
• Carbohydrate carryover (often a problem with plants).
• Residual phenol from nucleic acid extraction.
• Residual guanidine (often used in column-based kits).
• Glycogen used for precipitation.
A high A260/A230 ratio may be the result of:
• Making a Blank measurement on a dirty pedestal
• Using an inappropriate solution for the Blank measurement. The blank solution should be ofthe same pH and of similar ionic strength as the sample solution.

Agarose gels

3 Running the extracted DNA in an **agarose gel** will complement the information obtained in the nanodrop. Prepare a 0.7% agarose gel in 1x or 0.5x TBE buffer.

- Measure the appropriate volume of buffer.
- Add the agarose.
- Dissolve in the microwave.
- Allow it to cool, (around 60-70 C).
- Let it cool. Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5µg/mL (usually about 2-3µl of lab stock solution per 100mL gel). EtBr binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light.

- Cast the gel in the tray. Put the comb.
- Add 1x or 0.5x TBE buffer to the reservoirs of the electrophoresis apparatus, the agarose gel should be completely covered by buffer.
- Load your samples (1-2 ul). Use a loading dye to prevent your samples floating and visualize the advances of the electrophoresis.
- Use a high size marker (genomic DNA will run as 20kb). Optional, use a concentration marker (e.g. Lambda DNA of known concentration 50 ng/ul and 100 ng/ul)
- Connect the electrodes. Run.

Equipment

Electrophoresis apparatus

Power supply

Material

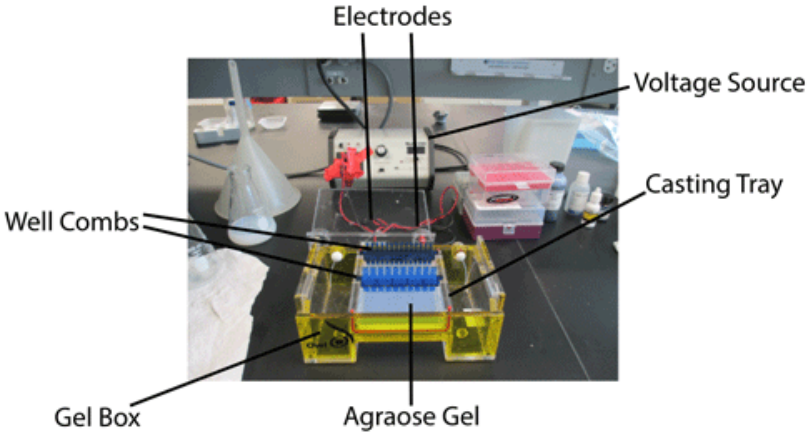
Agarose

SyBr safe

TBE buffer

Loading dye (bromophenol blue + glucose; bromophenol blue, xylene cyanol + formamide...)

High molecular weight size ladder



TBE electrophoresis buffer (10X)

	Reagent	Quantity (for 1 L)	Final concentration
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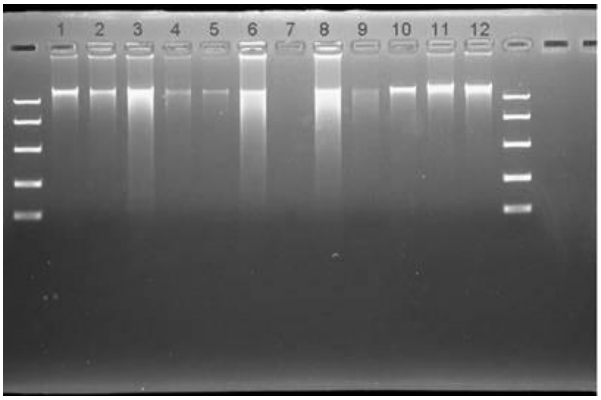
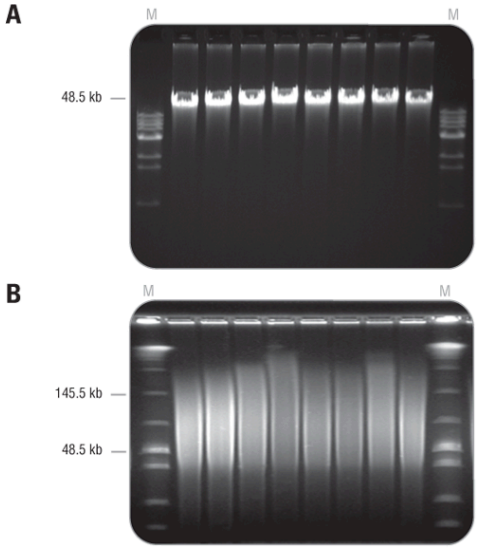
	Tris base	121.1 g	1 M

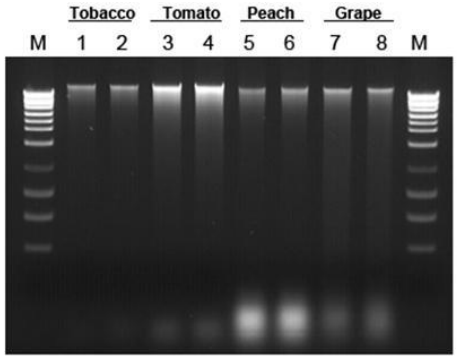
	Reagent	Quantity (for 1 L)	Final concentration
	Boric acid	61.8 g	1 M
	EDTA (disodium salt)	7.4 g	0.02 M
	Prepare with RNase-free H2O. Dilute 100 mL to 1 L to make gel running buffer. Store for up to 6 mo at room temperature .		



Lane 1 and 2 nice concentration. (Approx. 50-100 ng/ul). Lane 3. Too much. Shiny band close to the loading hole, dirt. **Lane 10, nice** and clean. Lanes 4, 5 and 9 too little (although enough for PCR).



Top, good DNA. Bottom degraded DNA.



Band in the bottom, approx. 50-150 bp is RNA.