

Protocol DNA Quantitation & Gel Analysis



INTRODUCTION- DNA quantitation is done one of two ways, using either absorbance at 260 nm or using a fluorescent dye based assay and a standard curve. The latter takes a little more time but is much more sensitive and accurate than the former method. See page 289 of *At the Bench* for additional background.

DNA Quant - ABSORBANCE METHOD: It is IMPORTANT to use Tris EDTA (TE) buffer for diluting and blanking your samples. Water or other buffers will give a much different and inaccurate reading due to differences in absorbances at various pH levels. ALSO use the correct cuvette. A glass or plastic cuvette will absorb at the low absorbance and interfere with your results. If you don't know, look for the Q on the cuvette and if all else fails, blank against air and then read with just water. There should only be a slight absorbance. Anything more than 0.3 absorbance units (AU aka optical density OD) is likely to be due to glass. Our cuvettes are quartz.

- Prepare two microfuge tubes. In one tube, place 1.0 ml TE. In the second place 995 μ l of TE and 5.0 μ l (exactly) of DNA.
- Using the TE only tube, blank at 260 nm and 280nm and determine the absorbance at 260 and 280 nm of the DNA sample.
- The concentration of DNA will be 10 times the absorbance at 260 nm. The units will be mg/ml.
- If the absorbance is lower than 0.02 the reading is too low. Repeat using more sample and less buffer. Keep the volume the same. Adjust your final concentration based on your changes in sample concentration.
- The actual purity of the sample can be determined by taking the ratio of absorbance at 260 to 280 nm. If the ratio is greater than 1.8 the absorption is due to nucleic acids. A high quality prep should have a ratio of 2.0 to 1.8. If the ratio is below 1.6 there may be proteins or other organic contaminants and the DNA can be extracted by chloroform phenol extraction for a final clean up.

Analyze the sample by agarose minigel (see Pages 373 and 380 of *At the Bench* for background and additional details): These gels are useful for separating small amounts of DNA. They can be used to monitor the progress of enzyme action on DNA or the purity of the DNA preparation. Add 5 μ l of DNA Sample buffer to your saved samples (5-10 μ l of your pure DNA) and load the whole sample + dye mixture. In your notebook indicate the loading order. Include 5 μ l of the DNA ladder. Don't forget to write down the masses for the bands in the ladder.

- Assemble the gel apparatus using tape or the black dams.
- Melt 0.8 g of agarose in 100 ml 1 x Borate buffer (10 mM NaOH pH adjusted to 8.5 with boric acid) in a 250 ml flask. Use the microwave. Save unused agarose for later.
- After the agarose is warm to the touch (50 – 60 °C) pour the gel into the gel cassette and add 2 μ l of 10 mg/ml ethidium bromide. Mix with a pipet tip.
- Immediately insert the comb. Act quickly to remove any air bubbles by touching them with a pasture pipet.
- Allow the gel to solidify at room temperature and gently remove the comb.
- Remove the tape or damn. If using the black rubber dams, it is better to first use a pipet tip to release the gel from the rubber. This step helps avoid the gel from tearing at the comb.
- Fill the buffer reservoirs with 1x Borate buffer containing ethidium bromide (0.5 μ g/ml final conc) until it just covers the gel.
- Load the sample.
- Run the gel at 180 volts for 10-15 min. Remember DNA is negatively charge so it will "run to red" (the positive electrode)
- Visualize and take the picture.