How to use the DNA Quantitation Laboratory

This laboratory consists of three activities that take you through the stages involved in checking the purity of a genomic DNA sample and estimating its concentration.

Activity 2: Familarisation with the interactive spectrophotometer, collecting absorbance values for 260nm and 280nm in order to assess possible contamination with co-purified proteins.

Activity 3: Practise in making up dilutions of DNA in water, with differing target dilutions and final volumes.

Activity 4: Estimating the concentration of genomic DNA in 5 control DNA samples. You have a free choice of pipettes, tubes, water and DNA stocks to make dilutions that you can test in the UV spectrophotometer.

Activity 2 Checking Absorbance ratios to check for protein contamination

Important: The data table within the activity will display your data but will not store it, so ensure that you have recorded it in your notes before you end your session.

To help you with your planning, you will be provided with both a negative control (water) that should be used to set the spectrophotometer to read 'zero'. You are also provided with a positive control (called a Dichromate standard) that has a known average absorbance value at both 260nm (1.135) and 280nm (0.8665).

Start by clicking on 'help' for instructions. Use the link to the Table to select which DNA sample you want to select to place into the UV spectrophotometer.

Activity 3 Making up dilutions of genomic DNA

In this activity, you are presented with a table into which you should enter volumes of DNA and water (in microlitres) that you would measure and dispense using micropipettes if you were to make up specific dilutions of a DNA sample in specified final volume of liquid.

When you have entered the values, check that you have entered the correct values by clicking on 'Check Table'. Incorrect answers will be indicated by X; repeat your calculations, re-enter and re-check.

Activity 4 Diluting and measuring absorbance for control DNAs 1–5.

Instructions (read these before starting your experiment)

This activity involves you working in the two laboratory areas both to make up dilutions of DNA and to measure their absorbance. In the first area (Activity 4/1) you need to create your diluted DNAs. Follow the help link for instructions on using the laboratory.

- Open the data table. Note: the data table in this activity will not check your dilutions or calculations but the volumes transferred at each pipetting step will be confirmed on screen, so note these down.
- Enter an appropriate tube label for each of the dilutions you will make.
- Make up each dilution using the interactive micropipettes by choosing the appropriate micropipette, the appropriate control DNA, the volume of DNA to add and the volume of water to add. Note that to ensure that you use the minimal number of pipetting steps you can only pipette one volume of each.
- Once a volume has been chosen for a micropipette, the volume will remain fixed until a new micropipette is selected. You should consider how this will influence how you carry out your experiment before starting.
- As you perform each pipetting step, check that the volume that is confirmed as having been transferred into the dilution tube matches the volume in your experimental plan.

If you make a mistake and the volume transferred does not match your experimental plan, you can return to the table and discard that particular dilution using the Reset button and prepare it again.

- Remember to include a sample that contains only water to use to set the spectrophotometer to zero.

When each of your dilutions has been made, carry the set of dilutions you have made to the UV spectrophotometer in Activity 4/2.

- Open the table. The labels you assigned to each dilution will have been transferred. To select a sample to test in the spectrophotometer, click on the table.
- Ensure that you have set the appropriate wavelength, test each sample in the UV spectrophotometer and record each absorbance value in your notebook. Note that the programme will record your data on the table but will not store the data when you exit.
- If you do not have two dilutions that give suitable values for calculating the DNA concentration in the sample, clicking on Previous will allow you to repeat the experiment with new dilutions. Note that if your values were too high (above 2.0) then you need to remake your dilutions as values above 2.0 are outside of the acceptable linear range for estimating DNA concentrations.

By using the known relationship between DNA concentration (50ng/ul solution gives an absorbance of 1.0 at 260nm) and your dilution factors, determine the concentration of each of the 5 DNAs. To obtain robust values, you should ensure that your estimate is based upon the average of at least two independent dilutions and each of these is based upon the average of at least two absorbance readings.

Before you start this activity, you should have:

- An experimental plan with the calculated volumes required to create suitable dilutions of each of the control DNAs. Note that you might consider carrying out a 'pilot' study where you check the undiluted DNAs first. This would both allow you to become familiar with the laboratory functionality and also to provide you with an estimate of the undiluted concentrations. This may influence your dilution strategy.
- Created a table in your notebook on which to record your absorbance measurements (remembering to always take two measurements).