DNA Quantitation Laboratory (SXHL288)

You may find it helpful to print this document and have it at hand as you work onscreen in the DNA Quantitation laboratory during your study of Investigation 1 of Topic 3.

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1 Introduction

The DNA Quantitation Laboratory consists of three different activities that you carry out as you study Investigation 1. You can navigate between the three activities by using the navigation arrows on the bottom right hand side of the screen to move between pages 1-4. You should visit each activity only when directed to do so from the module text. For each activity there is a set of help pages you can consult and these are reproduced in the appendices at the end of this guidance document for easy reference.

2 Guidance for Activity 2

In Activity 2 you are tasked with determining whether any of 10 different genomic DNA samples are contaminated with co-purified protein by testing their absorbance at two different wavelengths using a UV spectrophotometer. When you open Activity 2, you will see an interactive spectrophotometer on the screen (Figure 1, left).

Figure 1 Left: Activity 2 screenshot showing the spectrophotometer and the cuvettes containing the positive control dichromate, water blank and experimental sample cuvettes. Right: The Activity 2 ‘Samples’ table, allows you select a sample and also stores the most recent absorbance value collected from each sample but will not store any data.
This instrument functions in a similar way to the spectrophotometer you used in the animal physiology laboratory, except that it is designed to provide data in the UV wavelength range of 201-340 nm. If you need to remind yourself how to use the spectrophotometer at any point you can either read the Show Help pages as you work onscreen or review these in Appendix 1. You should ensure that you set the correct wavelengths required as no warning will be given if incorrect values are selected.

You should plan your experiment before starting and have a suitable table in your notebook ready to record your data.

To operate the instrument:

- You should recall how to switch the instrument on and set the wavelength from your study of Topic 2.
  - Switch the instrument on
  - Adjust the wavelength to the required setting
- To set the reference value, select the Water Blank cuvette. This places the water cuvette into the spectrophotometer.
- Set the absorbance of the water sample as the reference value by selecting ‘R’. You can test that this has worked by reselecting the water blank cuvette and testing its absorbance by pressing ‘T’. This will give a value of approximately zero.

Setting the reference value on the water blank should always be done after switching the instrument on. You can determine the absorbance of the Dichromate sample by selecting it when required.

To access any of the 10 DNA samples you are tasked with assessing:

- Select the ‘Samples’ button to see a table of samples to analyse, as shown in Figure 1 (right).
- Select a DNA sample using the check-box in the right-hand column. This places that DNA into the ‘sample’ cuvette.
- Close the table and select the sample cuvette to place it into the spectrophotometer.
- Determine its absorbance, ensuring that you collect duplicate readings for each sample.

You should collect absorbance values of all the samples at the desired wavelengths by returning to the samples table and/or adjusting the wavelength as required. Note that only the last absorbance value determined for any individual sample will be presented in the sample table.

When you complete your data collection, return to the module text to complete analysis and discussion of your findings in Activity 2.
3 Guidance for Activity 3

Activity 3 is found on page 2 of the DNA Quantitation Laboratory. In Activity 3, you can practise the calculations required to make dilutions of genomic DNA. The activity is presented on a single screen as shown in Figure 2.

To use this table:

- Note the target DNA dilution you are asked to make (e.g. 1 in 2 etc.)
- Note the final total volume of diluted DNA required (e.g. 20 µl)
- Enter the volumes of DNA (µl) and water (µl) you would use to achieve the desired dilution

The Check Table button will check which of your calculations is correct. You should record the correct values in your laboratory notebook as you will be carrying out dilutions in the next activity and these volumes might be useful in guiding you to the correct volumes to use.

When you have completed the activity, return to the module text to complete discussion of Activity 3.
4 Guidance for Activity 4

Activity 4 consists of two different interactive laboratories. In the first, you prepare experimental samples of DNA. In the second you measure the absorbance of your experimental samples in the same spectrophotometer you used in Activity 2.

On Page 3, you start Activity 4/1 with an onscreen view of a micropipette and several sets of small tubes, as is shown in Figure 3.

Figure 3: Activity 4/1 screenshot showing an interactive micropipette in the centre. Five tubes of genomic DNA (labelled 1-5) and one tube of water are used to select the individual components of each dilution you make. The tube on the right hand side of the micropipette represents the active experimental tube.

The first screen of the laboratory has five small tubes that represent the genomic DNAs you are testing. Start by selecting ‘Show Table’ to reveal the table you use to label each of your experiment tubes (Figure 4). To activate a tube, select the relevant number button. Enter a written label for the tube you will be using in the text box, ensuring that you have also recorded this in your laboratory notebook. This label will appear whenever that particular tube is selected as the active experimental tube. You have up to 20 experimental tubes you can use and selecting the R button removes that tubes contents and the written label.

Figure 4: The ‘Sample’ table allows you to record a ‘label’ for each of the experimental tubes; in this case, a label for the experimental tube of 200 µl of undiluted genomic DNA 1 has been entered and this tube is selected as the active tube, as indicated by the green highlighting of the tube number.
Once a tube has been selected and labelled, return to the first screen by selecting ‘Hide Table’. At this stage, the label entered into the table appears under the image of the experimental tube on the right hand side.

You now need to add genomic DNA to the labelled experimental tube. As an example, for Tube 1 shown in Figure 4, the tube is to contain 200 µl of undiluted genomic DNA 1.

In order to transfer 200 µl DNA 1 into this experimental tube:

- From the five DNA sample tubes on the left hand side, select the DNA 1 tube (a tick will confirm your selection)
- Select the interactive micropipette you want to use to measure the volume of DNA you require.
- In this case, in order to transfer 200 µl, from the pull-down menu, the selection is Set P200.
- Set the volume to 200 µl using the arrows either side of the pipette numerical display to scroll to the appropriate setting 200, as shown in Figure 5.

![Figure 5: Selection of the P200 micropipette, adjusted using the two arrows to add 200 µl DNA 1. The DNA is transferred into the labelled experimental tube using the Transfer Liquid option.](image)

Once you have adjusted the numerical display on the micropipette to the correct volume required:

- In the pull down menu, select Transfer liquid to transfer the 200 µl of DNA 1 into the labelled experimental tube 1.
  - The actual volume transferred will be reported on screen
  - Record the transferred volume in your notebook. This value is to allow you to record any slight variation that arises in this micropipetting stage for subsequent calculations.
- Note that once a volume has been chosen for any micropipette, this setting will remain fixed for all subsequent transfer action until a new micropipette is selected from the pull down menu or a new volume is input.
- To create experimental tubes containing the other genomic DNAs, repeat these steps, starting by returning to the sample table and selecting the next available unused experimental tube, so that you eventually have 5 different experimental tubes each containing 200 µl each of DNAs 1-5.

When each of the 5 experiment tubes for your pilot experiment is ready, navigate to page 4, which is the UV spectrophotometer-based Activity 4/2. You now need to collect data for absorbance for the DNAs in each of the experimental tubes.

- Ensure that you have set the appropriate wavelength.
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- Use the *Water Blank* to set the reference value.
- Open the sample table. The labels you assigned to each tube will be listed.
- When you select one of the tubes, its contents are transferred into a cuvette.
- Close the table.
- Record duplicate absorbance readings from the DNA in the cuvette, then return to the sample table to test each of the other experimental tubes, ensuring that you record all data in your laboratory notebook.

From your pilot data, plan your full experiment.

- For each DNA in your pilot study with an \( A_{260} \) in the linear range, plan to collect data from two independent experimental tubes, each containing 200 \( \mu l \) of the required DNA.
- For each DNA sample in your pilot study with an \( A_{260} \) higher that the linear range, plan to make a suitable dilution of that DNA by mixing appropriate volumes of water and DNA.
  - Aim to for dilutions using a total volume of 200 \( \mu l \).
  - Draw up a table with the appropriate volumes of DNA and water.
  - Write a suitable informative label for each dilution (e.g. ‘DNA 1, 1 in 4 dilution’).
    - As a guide to choosing which dilution to create, review the pilot \( A_{260} \) value and start by using a dilution that would reduce the \( A_{260} \) to below 2.0. For example, a sample with \( A_{260} \) of 4.0 would require at least a dilution of 1 in 2 to bring it into the linear range, so a good estimate would be make a 1 in 3 or 1 in 4 dilution.
    - You practiced making dilutions in Activity 3 and recorded the correct volumes used in your notebook.
    - Remember to create two independent experimental tubes carrying dilutions of each diluted DNA.

Return to the laboratory; create each of your experimental samples and record their absorbance values in duplicate for both \( A_{260} \) and \( A_{280} \). In order to use the various pipettes, you might find the details shown in Figure 6 helpful.

**Figure 6:** Appearance of the dial for setting volumes of the micropipettes. Left: P20 set for 9.0 \( \mu l \), Middle: P200 set for 120 \( \mu l \), and Right: P1000 set for 100 \( \mu l \) or 0.10 ml. Note that the red digit indicates the decimal for the P20 micropipette.

You should work methodically to ensure that you transfer the correct DNA to each of your experimental tubes.

- For each dilution you make: Add the DNA to tube first, then add the required amount of water.
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Once you have prepared your experimental tubes, carry the samples forward to the UV spectrophotometer in Activity 4/2 by opening page 4 and collect absorbance data. Return to the module text to complete discussion of Activity 4.
Appendix 1: Help pages for Activity 2

Activity 2
In this activity you will be measuring the absorbance of samples of freshly prepared genomic DNA at 260nm and 280nm. Decide if the absorbance at 260nm lies within the linear range, where the absorbance value reliably relates to the DNA concentration. Then check whether any of the 10 samples are contaminated with protein, as judged by the $A_{260}/A_{280}$ ratio. Select the samples to analyse using the Samples button.

Start by switching on the spectrophotometer. On screen you can see a UV spectrophotometer. You can control its functions by selecting the various buttons as detailed below.

Click on the button to switch on. The wavelength will appear on the digital display. To adjust the wavelength, click on the button, and adjust up or down to the required wavelength, then confirm this by clicking on .

Clicking on a cuvette inserts it into the spectrophotometer. Click on to measure the absorbance. This will be displayed after a few seconds.

To determine the absorbance of a genomic DNA sample, click on Samples.
Select one sample by clicking on the numbered button and then on Close to return to the spectrophotometer.
Clicking on the cuvette containing your selected DNA sample inserts it into the spectrophotometer.
To process additional samples, return to the table.
For this activity the absorbance values are stored temporarily in the table and so you should record these in your laboratory notebook.

Storing a reference value.
You can additionally subtract a reference value from the absorbance of a sample.
For example measuring the water blank in the spectrophotometer will give a small value due to the absorption of pure water.
Clicking stores this in memory, and this value is subtracted automatically from all subsequent readings.

A positive control (called a Dichromate standard) is provided that has a known average absorbance value at both 260nm (1.135) and 280nm (0.8665).

Appendix 2: Help Pages for Activity 3

Activity 3
In this activity you are asked to enter the values (in µl) that would be used to dilute genomic DNA samples. You are asked to enter these values in the table for both water and DNA, aiming for the stated dilution and in the final volume specified.

You can check your entries at any stage by clicking on Check Table. You can reattempt incorrect entries (marked with ×) as many times as you like.

You should remember to note down the volumes you use in your laboratory notebook.

Remember that the dilution volume is the sum of the volume of the DNA you added and the volume of the water with which you dilute it.

For example suppose we want to set up a dilution of 1 in 3, with a final volume of 60 µl.
We would choose a volume for the DNA of 20 µl, and add 40 µl of water.
This would give us a total volume of (20+40) µl, and a dilution of:

\[
\begin{array}{c}
20 \mu l \\
60 \mu l \\
(20+40) \mu l \\
\end{array} \quad \frac{20 \mu l}{60 \mu l} = \frac{1}{3} \quad or \quad 1 \text{ in } 3
\]
Appendix 3: Help Pages for Activity 4

**Activity 4/1**

In this activity you will be using micropipettes to make dilutions of the 5 control DNA samples.

_Tube labelling:_ Click on Show Table to enter labels for each of your experimental tubes that will contain diluted DNA samples. To select a tube to make a dilution, click on a tube number. One tube needs to be selected in this table to activate the micropipettes.

You can use the R button at any stage to erase the label and remove the tube’s contents.

The five small tubes on the left contain DNAs 1-5. These can be selected individually by clicking. The selected tube will be highlighted with a tick.

The large central tube contains water and is selected by clicking. The tube on the right is the active tube selected in the table into which you will dispense DNA and water. To activate the pipette, a tube must be selected in the data table window.

To create your dilution, you should first select a control DNA to add. Clicking on the micropipette reveals a pull down menu to allow you to chose a pipette type to set, and once this is done, to dispense the liquid.

**Activity 4/2**

Your diluted DNA samples are transferred from the DNA dilution laboratory and can be selected by clicking on **Samples**.

Switch on the UV spectrophotometer, set the wavelengths and use the water blank as required. You should record all absorbance values in your laboratory notebook as these will not be stored.

Remember to perform sufficient duplicate readings of your samples.

Use the navigation bar to return to the DNA dilution laboratory. You can remake dilutions by first clearing a tubes contents using the 'R' button.

To subtract a reference value from the absorbance of a sample, use the $R$ button.

Measuring the water blank in the spectrophotometer will give a small value due to the absorption of pure water.

Clicking $R$ stores this in memory, and this value is subtracted automatically from all subsequent readings.