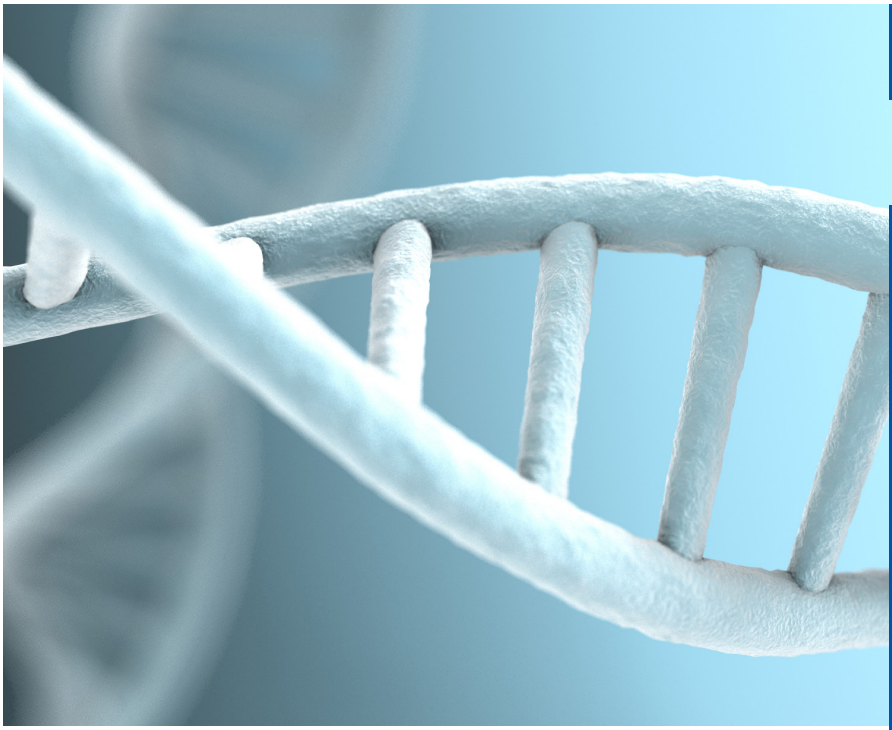

APPLICATION NOTE

UV/Visible Spectroscopy

Author:

Ian Robertson

PerkinElmer, Inc.
Seer Green, UK

Investigating the Thermal Denaturation of DNA Using a LAMBDA UV/Visible Spectrophotometer

Introduction

Thermal denaturation studies can yield a significant amount of information about the secondary structure of DNA molecules. The double helix structure is held together by the hydrogen bonds in base pairs of adenine-thymine (A-T) and guanine-cytosine (G-C). Since G-C base pairs have three hydrogen bonds compared to the 2 in A-T base pairs, a DNA sample with larger G-C content will require more energy to separate, leading to a higher DNA melt temperature. UV/Visible Spectrophotometry can be used to monitor the thermal denaturation of DNA as the sample is heated allowing determination of the DNA melt temperature, t_m, and ultimately the %G-C content of the molecule.

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DNA has a characteristic UV/Visible spectrum exhibiting an absorption peak at 260 nm as seen in Figure 1.

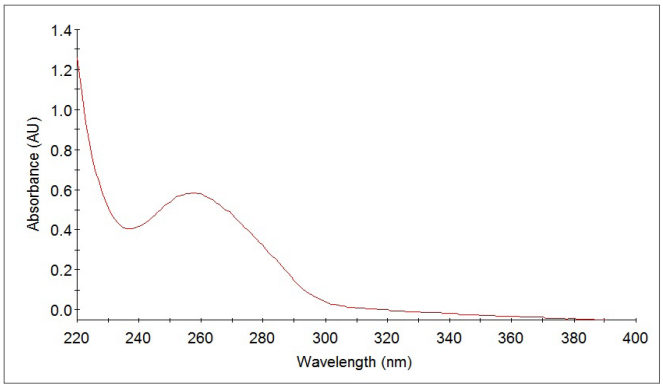

Figure 1. UV/Visible spectrum of a DNA sample.

During the thermal denaturation of the sample the intensity of this absorption increases significantly to a maximum value. Monitoring the intensity of this peak throughout the denaturation gives rise to a DNA melt curve from which it is possible to determine the actual melt temperature and hence, calculate the %G-C content according to the following formula:

$$\%G\text{-}C = 2.44 * (t_m - 81.5 - 16.6(\log(M)) + 500/K)$$

Where, M is the molarity of the solution in mol/L

K is the DNA base pair length (K has a negligible effect with samples containing more than 500 base pairs)

A solution of a given DNA type with a given number of base pairs will exhibit the same melt temperature under the same pH and ionic strength conditions. However, varying the conditions will have a significant effect on the melt temperature. For example, changing the ionic strength of the solution from 0.0165 M to 0.06 M will increase the melt temperature by approximately 10 °C.

A thermal denaturation experiment was performed on a sample of Calf Thymus DNA (Sigma D4522) diluted to give a final concentration of 40 ug/mL in a sodium chloride/sodium citrate buffer solution 0.06 M. The experimental details are shown in Figure 2.

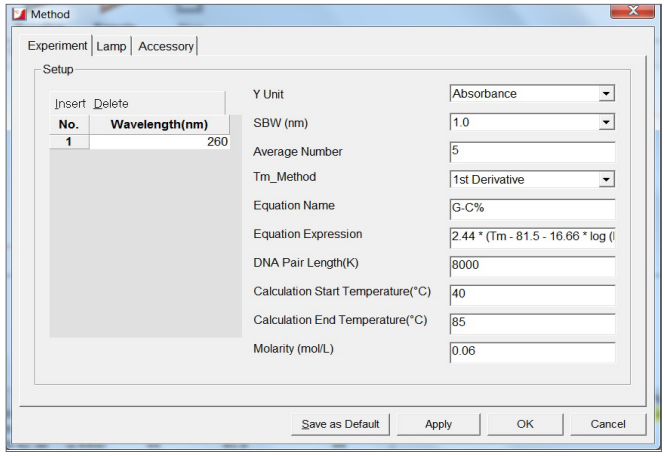

Figure 2. Method for the DNA melt experiment.

The single cell Peltier sample holder on the LAMBDA™ 365 was used for accurate temperature control of the experiment. The sample was measured in a 10 mm pathlength cuvette, using the buffer solution as a blank. Measurements were taken at 260 nm as the temperature, measured in the solution, was raised from 40-90 °C at ramp stages of 2 °C. The melting curve obtained is shown as Figure 3.

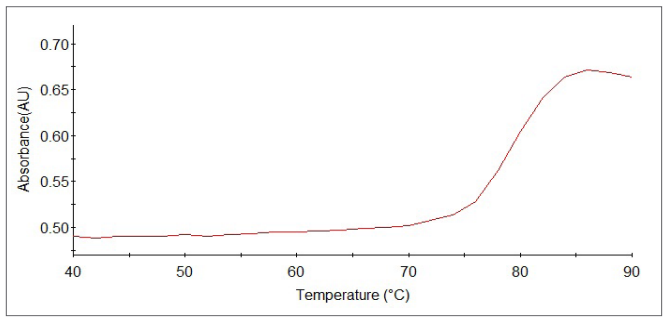

Figure 3. Calf Thymus DNA melt curve.

The t_m value was calculated using the 1st derivative algorithm, giving a result of 78.0 °C. The %G-C content was calculated according to the equation :

$$\%G\text{-}C = 2.44 * (t_m - 81.5 - 16.6(\log(M)) + 500/K)$$

which gave a value of 41.8%, very close to the reported value of 42%.

Summary

The LAMBDA 365 spectrophotometer has the versatility to allow both scanning and single wavelength measurements for a range of Bio applications. In this case, we have shown the capability of the instrument and UV Express software for measuring the DNA melt curve and calculating the melt temperature and %G-C content for a calf thymus DNA sample.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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