Lab 2

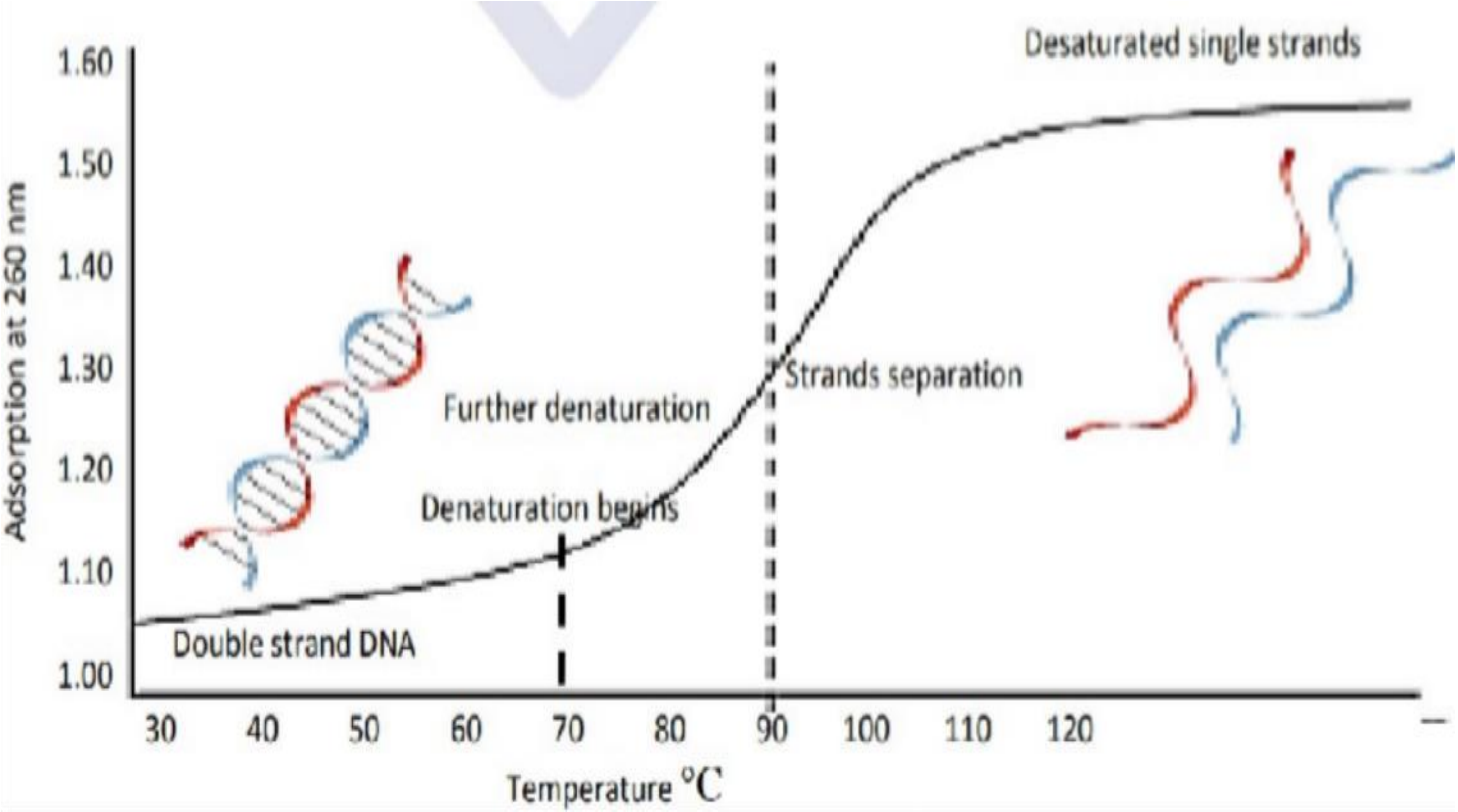
Determination of G+C percentage rate & Melting temperature

Although the ratio of G to C and A to T in an organism's DNA is fixed, the GC content (percentage of G+C) can vary considerably from one DNA to another. This character is very important in determination the genetic similarity among living organisms and widely used in molecular biology techniques and genetic analysis to estimate the genetic distance between two species, Ex: PCR, DNA/DNA and DNA/RNA hybridization.

As molecules, DNA is considered a thermo-stable molecule up to 70°C due to many structural factors although when a DNA solution is heated enough, the non-covalent forces (hydrogen bonds) that hold the two strands together weaken and finally break. When this happens, the two strands come apart in a process known as **DNA denaturation** or DNA melting. The temperature at which 50% of the DNA exists as a double and 50% is single strands (half) is called the **Melting temperature (Tm).**

On further increasing the temperature, both the strand becomes completely separated. However, this separation is not permanent and the complementary strands seal again as soon as the temperature is decreased, the condition called as

Renaturation. The melting point of a DNA sample hence gives information about temperature. The inflection points of the **sigmoid curve** gives the determination of melting point for tested DNAsample


Desaturated single strands
Adsorption at 260 nm
1.60
1.50
1.40
1.30
1.20
1.10
1.00
Strands separation
Further denaturation
Denaturation begins
Double strand DNA
30
40
50
60
70
80
90
100
110
120
Temperature °C

Methodology

1) Suspend DNA sample in TE buffer to obtain 20 μg/ml working concentration.

2) Reset (zero) the spectrophotometer device on 260 nm by TE buffer as blank.

3) Gradually heat the DNA sample in water bath and measure the absorbance of sample at 50,60, 70, 80, 90, 100 °C.

4) Draw the DNA melting curve to determination the Tm value.

- ❖Calculate the mol. G+C % by using the following formula:

Note: Tm in solution is a function of:

Length of DNA, GC content (%GC), salt concentration (M) and TE buffer concentration.For a aqueous solution of DNA (no salt used) the formula Tm is

$$Tm = 70 + (0.47 \times (\%GC))\ ^{\circ}C \quad \longrightarrow 1$$

$$G{+}C\ \%\ \text{of a DNA sample} = (Tm - 70) \times 2.5$$

- the optimum temperature of Renaturation (TOR) was calculated by using the followingformula:

$$TOR = 52 + (0.47 \times \%GC) \quad \text{———} 2$$

From equation 1 & equation 2; equation 3 is derived, as the following:

$$Tm = TOR + 18$$

There is another equation to calculate the renaturation is:

$$Renaturation(\%) = 100 - \left(\frac{R\_FinalA_{260} - R\_BlankA_{260}}{R\_InitialA_{260}} - 1\right) \times 200$$

Here is what each component represents in your experiment:

- $R\_InitialA_{260}$: The absorbance of your **native (double-stranded) DNA** before heating.
- R_FinalA_{260}: The absorbance of the DNA **after** you have allowed it to cool down (the renatured sample).
- R_BlankA_{260}: The absorbance of the buffer alone (to subtract background noise).
- **The "-1" and "x 200" Factor:** This mathematically scales the "hyperchromicity" (the increase in absorbance when DNA melts) to a percentage. Typically, fully denatured DNA shows about a **30-40%** increase in absorbance.

Problem: Calculate the Tm and TOR of a DNA sample contain 10% of A.

From all above The melting temperature (Tm) of a DNA sample depends on many factors, including:

1) Proportion of GC pairs, because the three hydrogen bonds between GC pairs are more stable than the two between AT pairs.

2) The hydrogen bonds within the structure.

Determination of DNA & RNA purity:

After a DNA purification procedure, the quantity (concentration) of DNA sample should be determined in order to evaluate its purity. Both quantity and quality can be determined by usingspectrophotometer. DNA absorbs light in the UV range A 260 nm, therefore when light of thiswavelength shines through a sample of DNA, the amount of light absorbed is proportional amount of DNA is the solute. The absorbance of light is called **optical density (O.D)** the concentration of DNA can be calculated using the following formula:

$O.D_{260}$ value of 1 unit = 50 µg/ml dsDNA

$O.D_{260}$ value of 1 unit = 37 µg/ml ssDNA

$O.D_{260}$ value of 1unit = 40 µg/ml ssRNA

Actually most interested in the amount of protein that might have been inadvertently purified with DNA. Proteins absorbs light at a different wave length 280 nm. A convenient measure of DNA purity is determined by measuring the **$O.D_{260}/O.D_{280}$ ratio.**

1. Measure the nucleic acid purity **(<u>Pure DNA</u>)** that mean free of protein contamination using **O.D $_{260}$/ O.D $_{280}$** ratio 1.8-2.0 in 10 mM Tris, pH 8.5
   - If phenol or protein contamination is present in the DNA preparations, the **O.D $_{260}$/ O.D $_{280}$ ratio will be less than 1.8.**
   - If RNA is present in the DNA preparation, **O.D $_{260}$/ O.D $_{280}$ ratio may be greater than 1.8.**
2. Use low salt buffers as they provide a more accurate measurement. Purity is influenced by pH and lower pH solutions lower the **O.D $_{260}$/ O.D $_{280}$** ratio and reduce the sensitivity to protein contamination.
3. Pure RNA has an **O.D $_{260}$/ O.D $_{280}$** ratio of 1.9-2.1 in 10 mM Tris, pH 7.5.

Example:

Measuring DNA concentration Volume of DNA = 50 µl

Dilution: 10 µl DNA sample + 490 µl distilled H_2O (1/50 dilution)A260 of diluted sample (1 cm length) = 0.75

DNA Concentration = 50 µg/ml x A260 x dilution factor

= 50 µg/ml x 0.75 x 50

= 1875 µg/ml

Total Amount of DNA = concentration x volume of sample in ml

= 1875 µg/ml x 0.050 ml

= 93.75 µg

also , can determine the amount of contaminated protein by using **Kalcar equation:**

Contaminated protein (µg/ml) = (1.55 x O.D 280) – (0.76 x O.D 260)

To detecting other contamination by:

1. Absorbance at 230 nm and 270 nm indicates the presence of phenol or urea
2. Absorbance at 325 nm indicates contamination by particulates and/or dirty cuvettes.

There are several factors that may affect **O.D $_{260}$/ O.D$_{280}$ratios.** The 260 nm measurements are made very near the peak of the absorbance spectrum for nucleic acids, while the 280 nm measurements is located in a portion of the spectrum that has a very steep slops; as a result, very small differences in the wavelength in and around 280 nm will effect greater changes in the **O.D $_{260}$/ O.D $_{280}$** ratio than small differences at 260 nm.

1. Different instruments
2. Concentration of nucleic sample
3. The type (s) of protein present in a mixture.

How to identify the oxidoreductase protein from E.coli the start codon and stop codon

THANK YOU