

Why Do DNA Samples Melt at Different Temperatures?

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Target Audience: This activity is designed for undergraduate biology students who are learning the structure of DNA and the factors that influence its melting temperature.

Estimated Time to Complete: 10 – 20 minutes.

Inquiry Question: How does base pair composition affect DNA melting temperature?

Learner Activity

1. Read

Different samples of double stranded DNA are heated to separate its strands. The melting temperature (T_m) is the temperature at which 50% of the structure has been denatured. In this activity, each sample is the same length but composed of different ratios of A-T and G-C base pairs. Examine how DNA base pair composition affects T_m .

2. Predict

Before looking at Table 1, what base pair do you predict will cause a higher T_m and why?

3. Examine the Evidence

Compare the T_m values of each sample to determine how the base pairs A-T and G-C can cause an increase or decrease in the melting temperature.

Table 1. Effect of base pair composition and salt concentration on the T_m of double stranded DNA.

DNA Sample	A-T base pairs (%)	G-C base pairs (%)	[Na ⁺] (mM)	Melting Temperature T_m (°C)
A	30	70	50	66
B	50	50	50	55
C	70	30	50	44
D	60	40	100	53
E	50	50	100	59

Note. Melting temperatures were calculated using BioToolsKit (2026). Primer concentration remained constant at 250nM while Na⁺ concentration (mM) was changed to simulate low and high salt. The length of each sample remained constant at 20 base pairs.

4. Explain

Using the data from Table 1, identify what base pair causes the T_m to increase. What sample comparisons did you make to support your answer? If you were only given samples C and E, would you still be able to confidently determine which base pair increased T_m ? Why or why not? Use data from Table 1 to support your conclusion.

5. Reflect and Extend

Using Table 1, identify another factor that can cause T_m to increase. What samples did you compare to determine your answer? Use the data from Table 1 to support your conclusion.

Instructor/Teacher Guide

1. Purpose

The purpose of this activity is for students to identify how the melting temperature of double stranded DNA is affected by base pair composition and salt concentration. Students will interpret and analyze information in a given dataset to determine the answer and will explain their results using the given data.

2. Materials

A printed worksheet with pens or pencils or an available digital pdf with electronic devices is required.

3. Facilitation Notes

This activity should be introduced after students learn about DNA structure and the bonds involved in base pairing, but before they learn what factors affect T_m . Encourage students to reflect on what they know about DNA structure and how it interacts with its environment when making predictions.

4. Expected Responses

Students should conclude that higher G-C content increases T_m . Comparing samples A and C would be most useful because both samples have the same salt concentration, so this will determine which base pair increases T_m . Samples A and C show that as G-C composition increases from 30% to 70%, T_m increases from 44°C to 66°C. If only samples C and E were given, you would not be able to confidently determine what base pair increases T_m because those samples have different salt concentrations and base pair compositions.

Another factor influencing T_m is the salt concentration. Using samples B and E would be most useful to compare because they have the same percent composition of the base pairs (50:50). The salt concentration of sample E at 100mM produces a larger T_m at 59°C than sample B at 50mM with a T_m of 55°C.

5. Safety Notes

No chemicals, laboratory equipment, or physical experiments are required for this activity.

6. Accessibility Notes

This activity can be made inclusive by providing large-print data tables that are clear and easy to read. If printed worksheets or using electronic devices is not available, the instructor can project the data table and questions on a large screen for everyone to see and have students discuss in groups.

SDG 4 Connection

This activity supports SDG 4 by helping students develop scientific literacy through making predictions, comparing and interpreting data, and using evidence to support their conclusions. This activity also encourages active learning by getting students to discover how DNA composition affects its stability rather than simply memorizing the relationships.

References

DNA melting temperature calculator (2026). *BioToolsKit*. <https://biotoolskit.com/dna-tools/dna-melting-temperature-calculator>

What is Tm in biochemistry: DNA melting temperature. (2026). ScienceInsights. <https://scienceinsights.org/what-is-tm-in-biochemistry-dna-melting-temperature/>

Yakovchuk, P., Protozanova, E., & Frank-Kamenetskii, M. D. (2006). Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. *Nucleic acids research*, 34(2), 564–574. <https://doi.org/10.1093/nar/gkj454>

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