



Chemical Education

A CHIMIA Column

Topics for Teaching: DNA Chemistry

A Brief History and Practical Applications in DNA Extraction

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Abstract: In the late 1860s, DNA was first identified by the Swiss physician and biochemist Friedrich Miescher. Since this time, we have solved its structure, learned how DNA divides in our cells, and elucidated molecular mechanisms for the transmission of our hereditary information. Fundamental to all these discoveries is the ability to extract our DNA in high purity. In laboratories today, DNA extraction is a routine practice performed from readily available commercial kits. However, in the late 1800s, DNA extraction was an emerging method that required tedious laboratory approaches.

Keywords: Chemistry education · DNA extraction · Double helix · Nucleic acids · Practical classwork

Friedrich Miescher (Fig. 1) graduated from the University Medical School in Basel in 1868. Afterwards, he moved to the University of Tübingen to study the chemical composition of cells. He employed lymphocytes as his first model system, which proved to be difficult to isolate and purify in sufficient quantities. His fortuitous shift to leukocytes, from the pus of surgical bandages at the local clinic, provided an optimal cellular system that was comparatively purer and yielded higher amounts of material for his analysis. To enable the chemical analysis, Miescher needed to develop new methods that would allow him to segregate nuclear and cytoplasmic components. This was labor intensive and very time consuming to implement. Once he optimized the conditions to separate these components, he worked to characterize their biochemical composition. Miescher detected a substance with unknown properties in the nucleus. This mysterious substance could be precipitated in acid and re-dissolved with the addition of an alkaline solution. Unbeknownst to Miescher, he had extracted, for the first time, a crude precipitate of DNA.



Fig. 1. Johannes Friedrich Miescher (source: Wikimedia Commons, https://en.wikipedia.org/wiki/File:Friedrich_Miescher.jpg#file).

Further analysis showed that this material was resistant to proteases and had a high concentration of phosphorus. He later showed that this material lacked sulfur, which, taken together with the other evidence, excluded the possibility that it was protein in origin. Miescher termed this new substance *nuclein*. Years later, his student Richard Altmann would modify the name to *Nucleinsäure* (nucleic acid); a name that is still present in the acronym of DNA. Miescher's first isolates of *nuclein* were not of high purity and in order to learn more about the chemical composition and function of this material, new protocols to increase its purity were necessary. Miescher worked to optimize his leukocyte purification from the surgical bandages without damaging the cells. He would filter the cell suspension and then allow the cells to sediment for 1–2 hours, since no centrifuges were available to him in the late 1800s. Next, isolating the nuclei required painstaking rinsing steps of 1:1,000 dilute hydrochloric acid over several weeks. This was done in the winter months to allow the temperature in the lab to remain cold enough so as to not cause damage by DNases. To reduce contamination from the cytoplasm, Miescher employed a recently reported method using proteases to further digest proteins. To do this, Miescher would isolate pepsin from pig stomachs using hydrochloric acid solution. Miescher published his seminal work in 1871, titled 'Ueber die chemische Zusammensetzung der Eiterzellen' (On the chemical composition of pus cells).^[1]

The unique presence of *nuclein* in the nuclear compartment signaled to Miescher its chemical importance, but its function remained a mystery. Miescher suggested that perhaps *nuclein* may be needed for phosphorus storage in the cell. It would be decades later before the function of *nuclein* (DNA) as the storage of genetic material and its chemical composition would be discovered (Fig. 2). Since the discovery of *nuclein*, the protocols for extracting DNA have advanced significantly and it has become a routine laboratory practice that can be performed in an afternoon, a significant advance in comparison to the long and tedious experiments performed by Miescher.

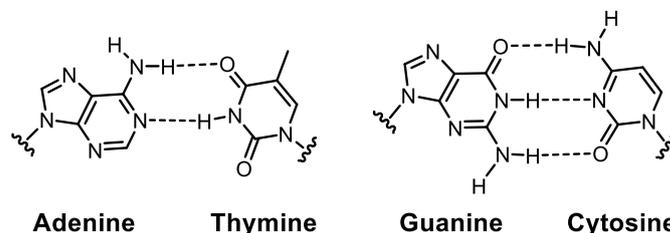


Fig. 2. The monomeric building blocks of DNA. A:T base pairing (left) with two hydrogen bonds and G:C base pairing (right) with three hydrogen bonds.

After many decades of further optimization, routine laboratory approaches were developed to extract DNA using density gradient centrifugation. For example, the famous experiments

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of Meselson and Stahl utilized cesium chloride (CsCl) density gradient centrifugation to demonstrate semiconservative DNA replication.^[2] Today, CsCl gradient centrifugation is not very common, as it is time and labor intensive and more complex than other approaches.

Currently, there are various techniques available to isolate nucleic acids, both DNA and RNA, from cells in high purity. The two main approaches employ solution-based and column-based methods and most are available as commercial kits. Depending on the desired target material for DNA extraction, for example genomic DNA from mammalian cells or plasmid DNA from bacteria, different approaches can lead to optimal recovery and purity (Table 1). However, each method generally starts with cellular lysis to generate a cell lysate, inactivation of cellular nucleases to protect the DNA, and separation of the DNA from cell debris.^[3]

Table 1. Various extraction methods to isolate DNA and the target material for each approach.

Extraction method	Target material
Phenol-Chloroform	Genomic DNA
CsCl gradient centrifugation	Genomic DNA
Solid-phase extraction	Genomic DNA
Magnetic beads	Genomic DNA
Alkaline method	Plasmid DNA
CTAB method	Plant / Gram-negative bacteria

There are various approaches available to isolate DNA (Table 1). The phenol-chloroform extraction is a routine approach to isolate DNA. The general approach of this method relies on a mixture of phenol:chloroform:isoamyl alcohol (25:24:1). Cell debris, as well as protein, lipids, and carbohydrates are removed during the phase separation steps. Following centrifugation, DNA can be precipitated by the addition of ethanol. This is a general method used to extract genomic DNA. Magnetic bead-based methods can also be employed to isolate DNA and several commercial suppliers offer extraction kits based on this method. Most readily available commercial kits rely on the solid-phase extraction method. It is generally a rapid and easily reproducible approach that relies on four basic steps: cell lysis, DNA adsorption, washing and elution. Meanwhile, plasmid and bacterial DNA can rely on the alkaline extraction method. Here, bacterial cultures are suspended in the detergent, sodium dodecyl sulfate (SDS). Using alkaline conditions, high molecular weight DNA is denatured, while circular plasmid DNA remains intact for subsequent recovery by centrifugation. The cetyltrimethylammonium bromide (CTAB) extraction method can be used to isolate DNA from plants and Gram-negative bacteria. In low ionic strength solutions, CTAB is a non-ionic detergent that precipitates DNA. CsCl gradient centrifugation, as mentioned above, is not ideal for the isolation of bacterial DNA as it requires large quantities of bacterial culture. Overall, several extraction approaches are available and deciding which method to apply depends on various factors such as the type of genetic material to be isolated and the purity desired.

Using the basic chemical principles of extraction, DNA from various food items can be isolated. Below is an experiment to isolate DNA from strawberries, with a supplies list and a detailed protocol provided. This is an easy experiment to do in the classroom to teach students about the chemical basis of DNA extraction.

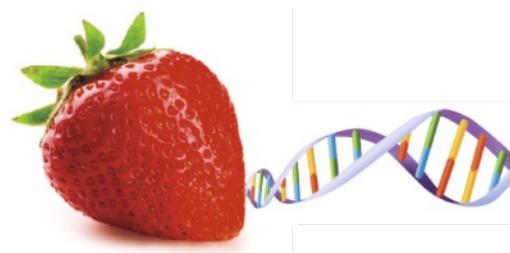


Fig. 3. DNA can be extracted from strawberries with common household ingredients.

Supplies needed

3–4 strawberries, isopropyl alcohol, water, dishwashing liquid soap, salt, a small bowl, basic kitchen scales, cheesecloth, funnel, tall drinking glass, re-sealable plastic sandwich bag, small glass jar, tweezers.

Protocol

1. Chill isopropyl alcohol in the freezer, for use later.
2. Prepare extraction mix by combining 3 grams salt, 80 milliliter tap water and 7 grams dishwashing liquid in a small bowl.
3. Next, line a funnel with cheesecloth and insert the funnel into an empty drinking glass.
4. Prepare the strawberries by cutting away and discarding the green tops and place the strawberries into a re-sealable plastic sandwich bag. Seal the bag and remove the extra air. (Note: fresh or frozen strawberries that have been thawed can be used).
5. For approximately 1–2 minutes, squeeze the strawberries with your fingers.
6. To the squeezed strawberries, add 40 grams of the extraction mixture (from step 2) and reseal the bag.
7. Now, squeeze the strawberry extraction mixture for one minute.
8. Pour the contents of the strawberry mixture into the funnel and let it drip, by gravity, through the cheesecloth. The liquid collected in the drinking glass is taken further, while the contents in the cheesecloth can be discarded.
9. Take the strawberry isolate from the drinking glass and pour into a narrow neck small glass jar. To the strawberry isolate, add approximately 50–60 milliliters of cold isopropyl alcohol very slowly down the side of the glass container so it forms a layer on top of the strawberry isolate. It may help to tilt the glass container while adding the isopropyl alcohol, as the goal is to not let the strawberry liquid and alcohol mix.
10. Observe the mixture inside the glass jar. The DNA of the strawberries will slowly appear as a white stringy material at the water–alcohol interface.
11. Dip the tweezers into the jar where the strawberry liquid and alcohol layers meet and pull out the white DNA material.

From the arduous experimentation of Miescher in the 1860s, to the commercial kits we buy today, we have come a long way in developing efficient extraction approaches for nucleic acids. This has streamlined the time and reproducibility of results in the lab. We can only wonder what Miescher would think today, being able to extract DNA from strawberries with items found readily in our homes.

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