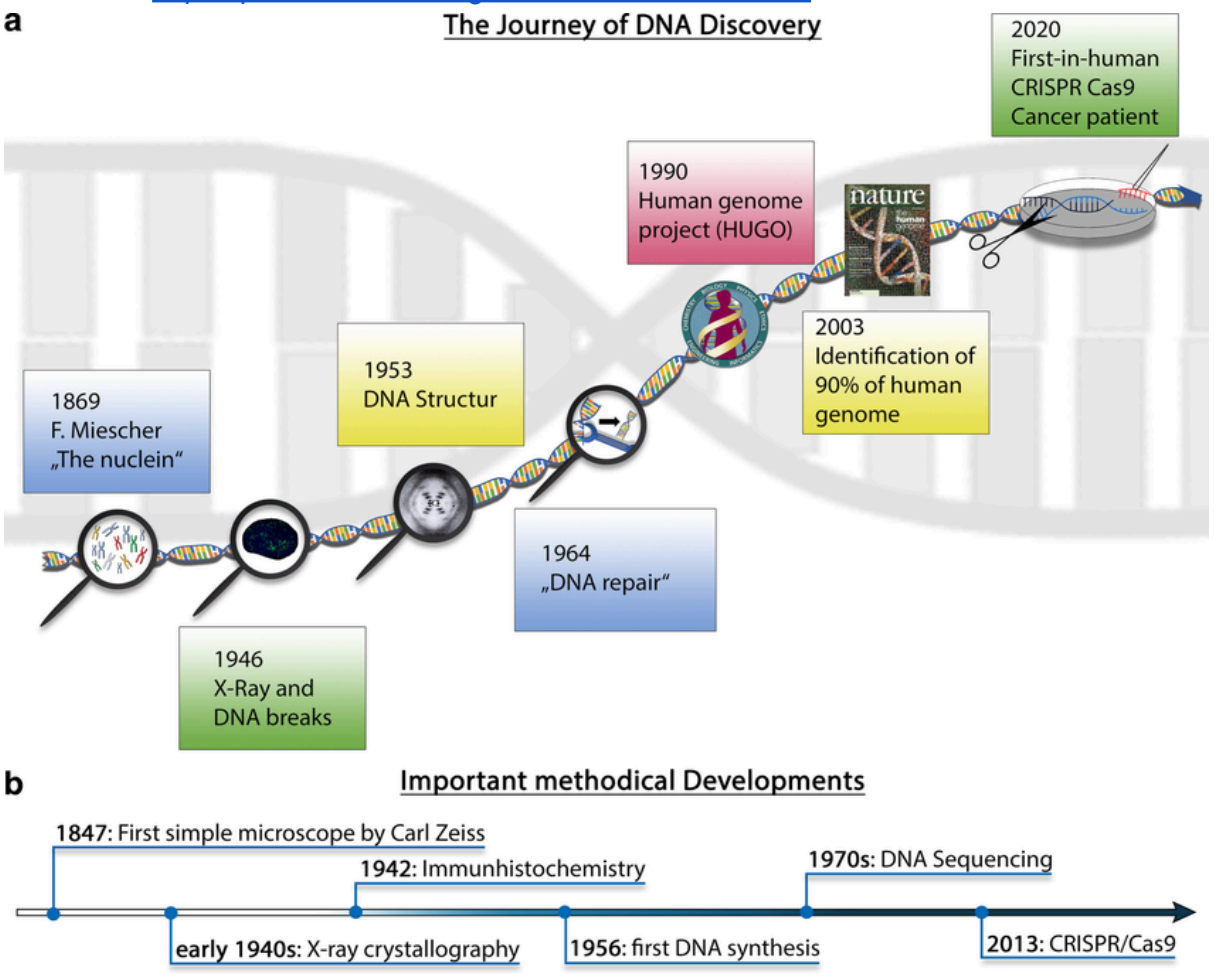
DNA Extraction

Reference: https://pmc.ncbi.nlm.nih.gov/articles/PMC6425773/



The methods used for DNA extraction to ensure **high-quality DNA** in the right amount.
PCR is then used to amplify DNA, **DNA extraction** is the process of **purifying DNA** from a sample by separating it from **cell membranes, proteins, and other cellular components** using **physical or chemical methods.**
The first DNA isolation was done by Friedrich Miescher in 1869.
Swiss physician Friedrich Miescher (1844-1895) first isolated DNA in 1869. He called the substance "**nuclein**" because he found it in the nuclei of cells.

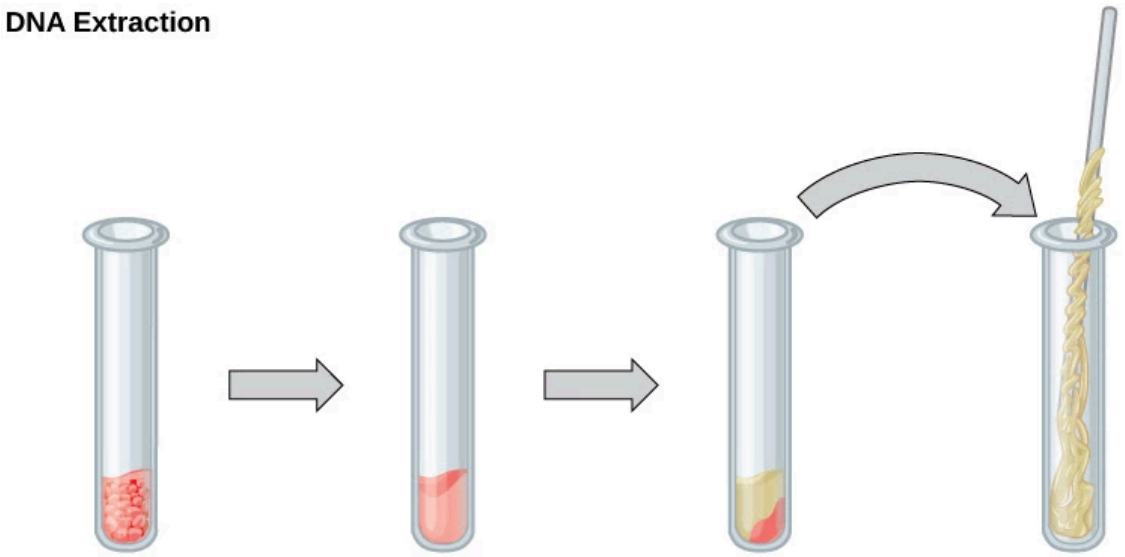
DNA timeline

1866: Gregor Mendel discovers the basic principles of genetics
1869: Friedrich Miescher isolates 'nuclein'/DNA
1944: Oswald Avery identifies DNA as the 'transforming principle'
1950: Erwin Chargaff discovers that DNA composition is species specific
1952: Rosalind Franklin photographs crystallized DNA fibers
1953: James Watson and Francis Crick discover the double helix structure of DNA
1977: Frederic Sanger develops rapid sequencing techniques
1982: Human insulin, the first drug based on recombinant DNA, enters the market
1983: Kary Mullis invents polymerase chain reaction (PCR)
1983: First genetic disease mapped (Huntington's disease)
1990: Sequencing of human genome begins
1995: First bacterium (*Haemophilus influenza)* sequenced
1996: Dolly the sheep is cloned
1999: First human chromosome (22) is decoded
2003: Human Genome project is completed
2012: Discovery of the CRISPR/Cas9 technology
2013: First proof that twins have differences in their genetic make-up

… and the timeline goes on

Friedrich Miescher made groundbreaking discoveries but was hesitant to promote his work. A perfectionist, he repeated experiments and published only nine papers, with many ideas shared through letters. In 1871, Miescher published his discovery of DNA, calling it "the chemical composition of the pus cells," unaware of its significance. **It took 75 years for scientists to grasp its importance.** Today, Miescher's legacy is honored by institutions like the Max Planck Society and the Friedrich Miescher Institute. Researchers there continue exploring DNA, epigenetics, and personalized medicine, advancing our understanding of Miescher's pioneering work.

DNA Extraction

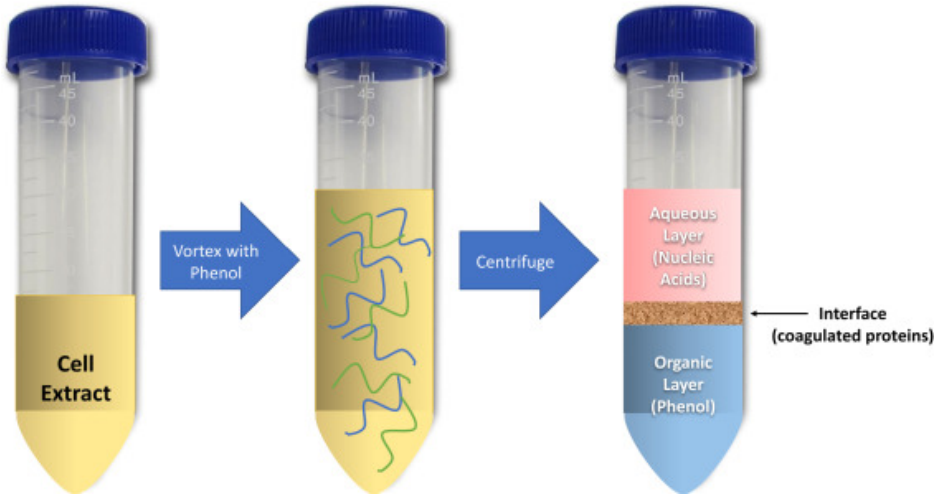


Cells are lysed using a detergent that disrupts the plasma membrane.

Cell contents are treated with protease to destroy protein, and RNAase to destroy RNA.

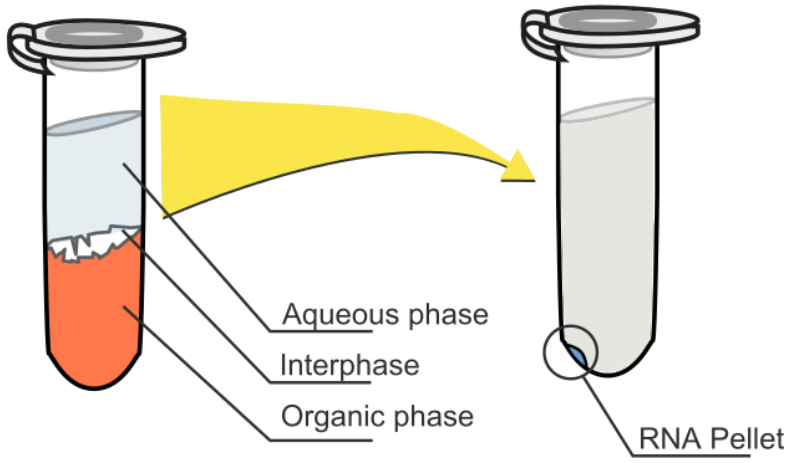
Cell debris is pelleted in a centrifuge. The supernatant (liquid) containing the DNA is transferred to a clean tube.

The DNA is precipitated with ethanol. It forms viscous strands that can be spooled on a glass rod.



https://www.youtube.com/watch?v=_J_tB9PKNGI

Phase separation Isopropanol precipitation



DNA is isolated to study its structure, function, and genetic elements. This process is called DNA extraction.

The goal is to obtain high-quality, pure DNA without contaminants like RNA or proteins. DNA can be extracted from various tissues using manual methods or commercial kits. The process includes lysing cells, solubilizing DNA, and using chemical or enzymatic techniques to remove unwanted molecules. Methods include organic extraction (phenol-chloroform), nonorganic methods (salting out, proteinase K), and adsorption (silica-gel membranes).

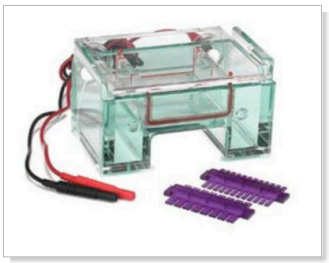
Organic Extraction (Step-by-Step):

Basic DNA Extraction Outline:

1. **Break Open Cells or Virus: Use sonicating or bead beating, or vortex with heated phenol to break down cellular walls or viral capsids. Add SDS detergent to remove lipid membranes.**
    a. **Remove Debris:** Centrifuge to remove cell debris.
2. **Degrade Proteins: Add a protease to degrade DNA-associated proteins. Use salt (e.g., ammonium or sodium acetate) to help precipitate proteins.**
    a. **Protease Treatment:** Denature proteins.
3. **Separate Proteins and DNA: Vortex with phenol-chloroform and centrifuge. Proteins stay in the organic phase, while DNA remains at the interface.**
    a. **Centrifuge and Wash**
    b. **RNA Removal:** Treat with RNase to remove RNA.
    c. Remove denatured proteins by centrifugation and washing.
4. **Precipitate DNA: Add cold ethanol or isopropanol, then centrifuge to precipitate DNA.**
5. **Wash DNA: Wash the DNA pellet with cold alcohol and centrifuge again.**
6. **Dry and Resuspend DNA: Pour off the alcohol, dry the pellet, and resuspend DNA in a buffer like Tris or TE.**
    a. Recover nucleic acid precipitate.
    b. **Centrifuge: Resuspend:** Dissolve the DNA in TE buffer or water.
7. **Confirm DNA Presence: Check the DNA on an agarose gel with UV light and a fluorescent dye (e.g., ethidium bromide).**

This ***bead beater*** is used in the breaking apart or "lysing" of cells in the early steps of extraction in order to make the DNA accessible. Glass beads are added to an eppendorph tube containing a sample of interest and the bead beater vigorously vibrates the solution causing the glass beads to physically break apart the cells. Other methods used for lysing cells include a french press and a sonication device.

A ***centrifuge*** such as this can spin at up to 15,000 rpm to facilitate separation of the different phases of the extraction. It is also used to precipitate the DNA after the salts are washed away with ethanol and or isopropanol.



A ***gel box*** is used to separate DNA in an agarose gel with an electrical charge. When the red and black leads are plugged into a power supply the DNA migrates through the gel toward the positive charge due to the net negative charge of the molecule. Different sized pieces of DNA move at different rates, with the larger pieces moving more slowly through the porus medium, thereby creating a size separation that can be differentiated in a gel.

https://serc.carleton.edu/microbelife/research_methods/genomics/dnaext.html

https://www.youtube.com/watch?v=vdyNebSxX6I

Alternative Methods:

- Silica-based technology
- Magnetic separation
- Anion exchange technology
- Salting out
- Cesium chloride density gradients

Quality and Yield Assessment:

https://iastate.pressbooks.pub/genagbiotech/chapter/pcr-and-gel-electrophoresis/

- The A260/A280 ratio for pure dsDNA is **~1.8**. A ratio below 1.7 suggests protein contamination.

The A260/A280 ratio is used to assess the purity of DNA.

- A lower ratio indicates the presence of contaminants, such as proteins or phenol.
- The ratio can be corrected for turbidity (not clear) (absorbance at 320 nm).
- The ratio can also provide information about the type of nucleic acid present (dsDNA or RNA).
- A ratio of around **2.0** is generally accepted as “pure” for RNA.
- A ratio of **1.6** does not necessarily make the DNA unsuitable for use, but lower ratios indicate more contaminants.
- The ratio limits that anticipate sample functionality in downstream applications should be empirically determined.
- Software such as the DS-11 Series EasyApps can generate automatic alerts when samples exceed standard values.

Brand	Blue-Ray Biotech	DeNovix®	Thermo Fisher®	Thermo Fisher®
Micro-Volume Model	EzDrop 1000	DS-11	NanoDrop™ One	NanoDrop™ 2000
Minimum Sample Volume	1 µL	0.5 µL	1 µL	0.5 µL
Wavelength Range	190 - 1000 nm	190 - 840 nm	190 - 850 nm	190 - 840 nm
Pathlength	0.5 mm / 0.05 mm	0.5 mm (auto ranging to 0.02 mm)	0.030 to 1.0 mm auto ranging	1.0 mm (auto ranging to 0.05 mm)
Detection Range	0.06 mg/mL BSA; 2 ng/µL dsDNA	0.04 mg/mL BSA; 0.75 ng/µL dsDNA	0.06 mg/mL BSA; 2 ng/µL dsDNA	0.1 mg/mL BSA; 2 ng/µL dsDNA
	600 mg/mL BSA; 20000 ng/µL dsDNA	1125 mg/mL BSA; 37500 ng/µL dsDNA	820 mg/mL BSA; 27500 ng/µL dsDNA	400 mg/mL BSA; 15000 ng/µL dsDNA
Light Source	Pulsed Xenon flash lamp	Pulsed Xenon flash lamp	Xenon flash lamp	Xenon flash lamp
Detector Type	2048 element CMOS	2048 element CCD	2048 element CMOS linear image sensor	2048 element linear silicon CCD array
Wavelength Accuracy	1.0 nm	0.5 nm	±1.0 nm	1.0 nm
Spectral Resolution	1.5 nm (FWHM at Hg 253.7 nm)	1.5 nm (FWHM at Hg 253.65 nm)	≤1.8 nm (FWHM at Hg 254 nm)	≤1.8 nm (FWHM at Hg 253.7 nm)
Absorbance Precision	0.0015 A (0.5 mm) or 1%, whichever is greater	0.015 A (10mm equivalent) or 1%, whichever is greater	0.002 A (1 mm) or 1% CV, whichever is greater	0.002 A (1 mm)
Absorbance Accuracy	3.0% at 0.75 A at 300 nm	1.5% at 0.75 A at 260 nm	3.0% at 0.97 A at 302 nm	2.0% at 0.76 A at 257 nm
Absorbance Range (10 mm equivalent)	0 (0.04) - 400 A	0.015 - 750 A	0 - 550 A	0.02 - 300 A
Touch Panel	Yes	Yes	Yes	No
Detection Time	3s	2s	8s	< 5s

https://www.youtube.com/watch?v=-et7jDXOLB4

The **quality and yield of DNA** are assessed by **spectrophotometry or by gel electrophoresis.**

Spectrophotometry involves estimation of the **DNA concentration by measuring the amount of light absorbed by the sample at specific wavelengths.**

Absorption peak for nucleic acids is at ~260 nm.

The A260/A280 ratio is ~1.8 for dsDNA. **A ration of less than 1.7 indicates protein contamination.**

The A260/A280 ratio is a spectrophotometric measurement used to assess the purity of nucleic acids (DNA and RNA). It compares the absorbance of a sample at 260 nm (where

nucleic acids strongly absorb UV light) to its absorbance at 280 nm (where proteins absorb UV light). A ratio of approximately 1.8 for DNA and 2.0 for RNA is generally considered pure, indicating minimal protein or other contaminant contamination.