Bootcamp Part II

DNA Extraction-

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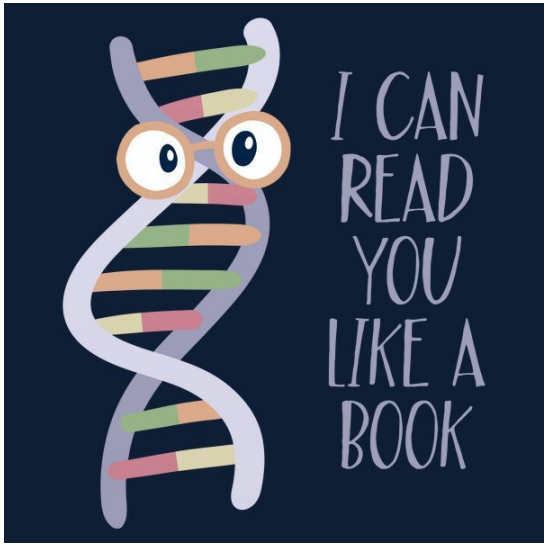
What is DNA?

DNA = **D**eoxyribo**N**ucleic **A**cid

It's a molecule…

which carries the
genetic instructions!

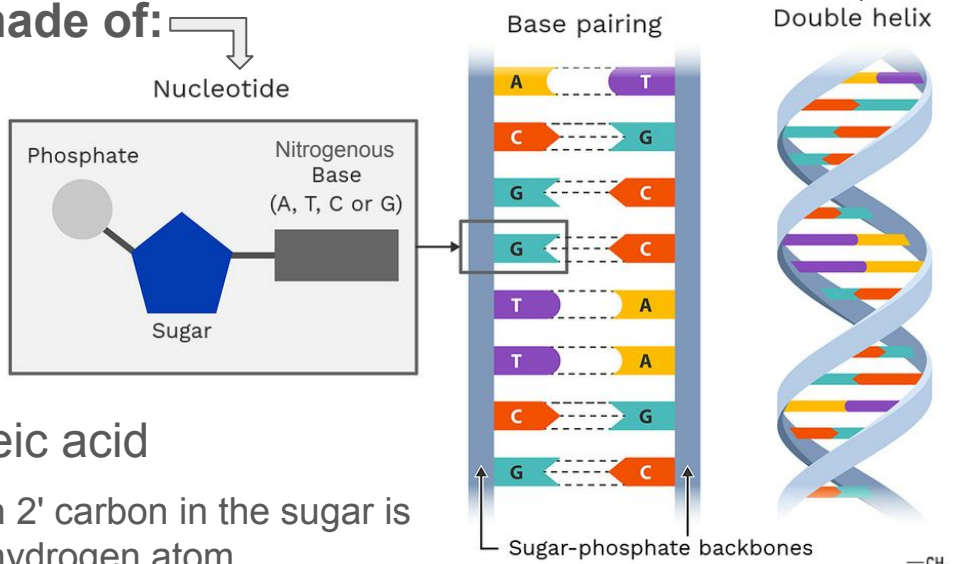


Instructions that are written only with **4 letters**:

- Adenine (A)
- Thymine (T)
- Guanine (G)
- Cytosine (C)

What is DNA components?

Strands are made of:

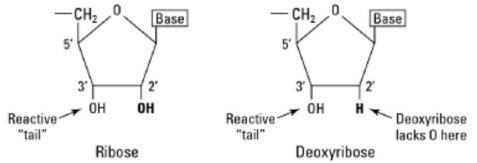


DNA is composed of ***two long strands that coil around each other*** to form a double helix structure.

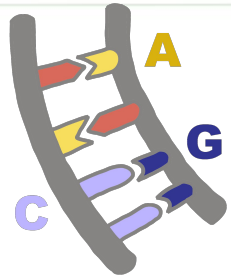
deoxy - ribonucleic acid

Why? The oxygen in 2' carbon in the sugar is replace by a hydrogen atom.

FUN FACT: RNA (ribonucleic acid),it DOES have the 2' carbon oxygen.



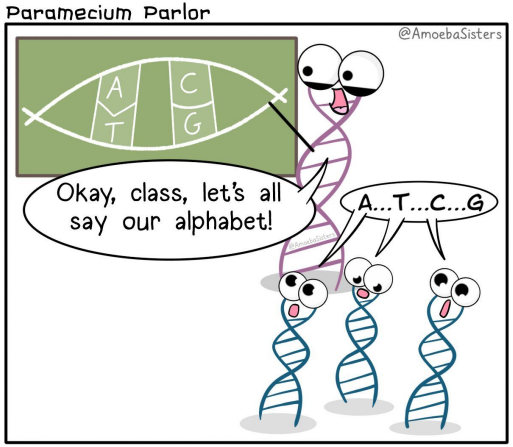
DNA features



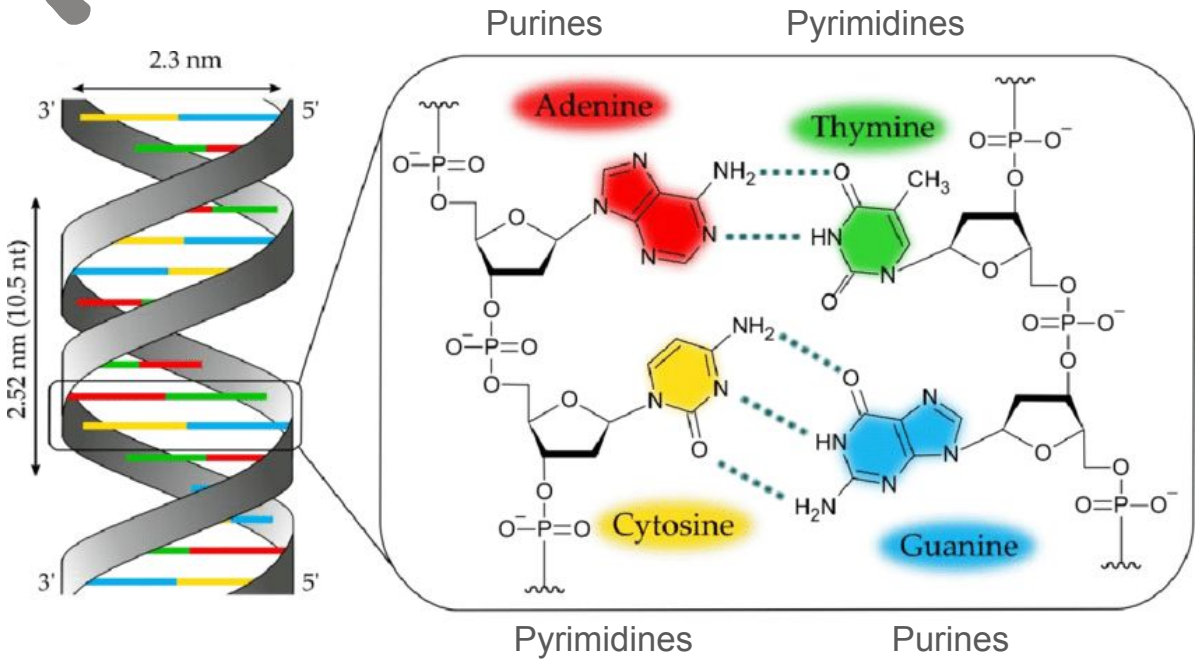
1. Base pairing:

Always **A** with **T**

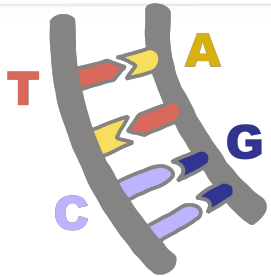
Always **G** with **C**



Pure **A**s **G**old

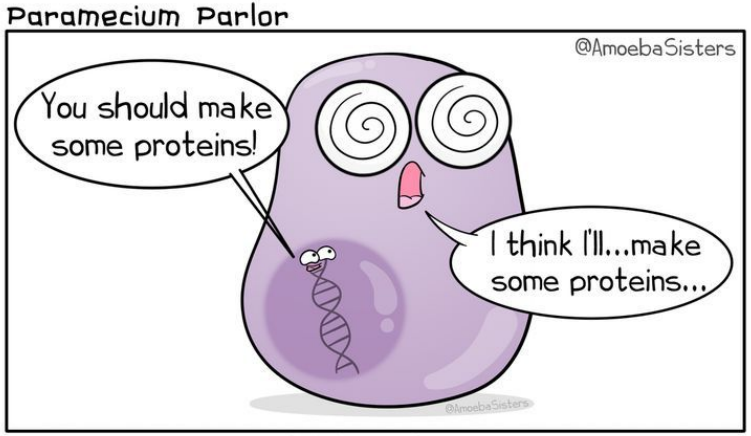


DNA features

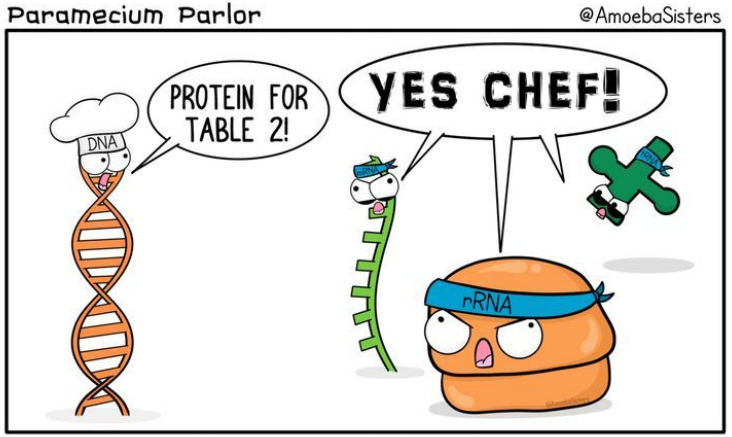


2. Genetic Code:

DNA sequences are organized into genes, which are instructions for synthesizing proteins.



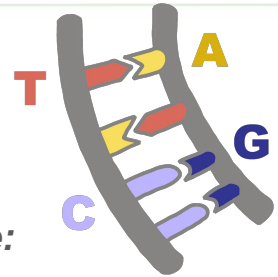
DNA to Protein



RNA: If you can't help make proteins, get out of the kitchen.

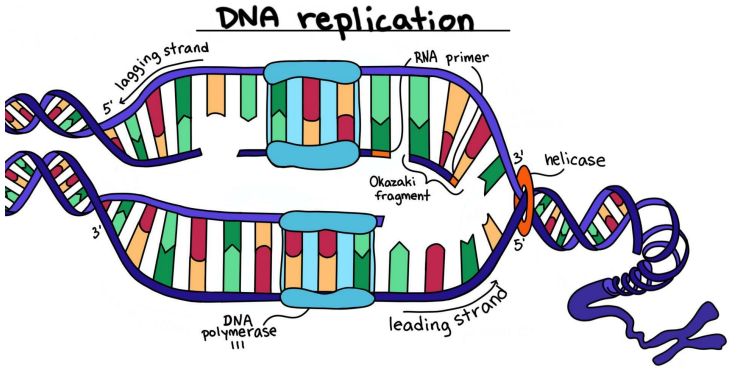
…Therefore, it has **essential** information that we aim to study – which is why we need to ***extract it***!

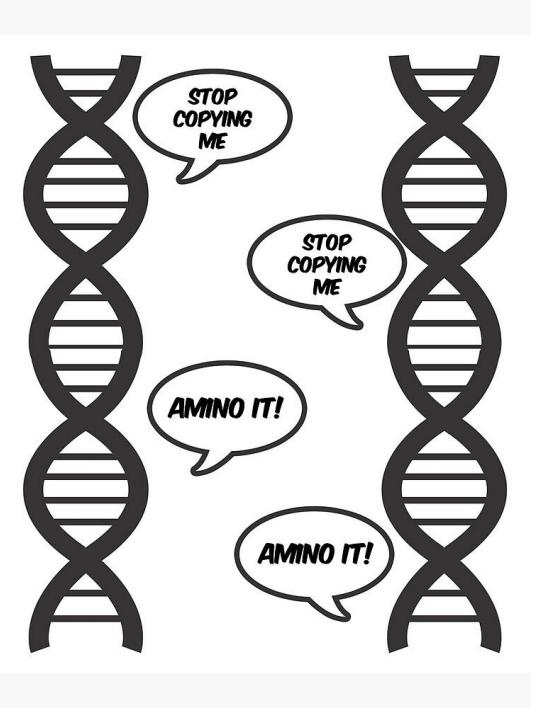
DNA features



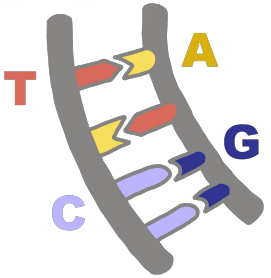
3. Replication and storage:

DNA can replicate itself, ensuring genetic information is passed from cell to cell and from generation to generation.

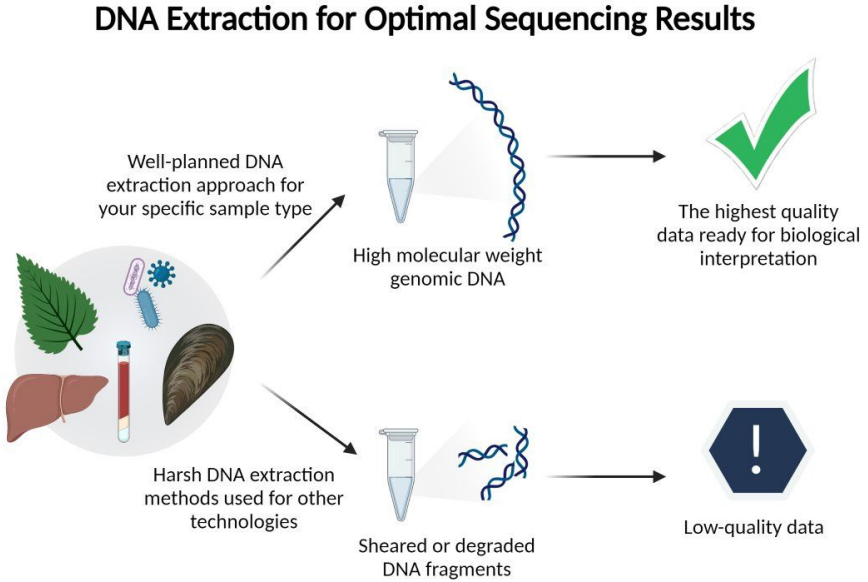




DNA Extraction purpose



It seeks to ***isolate*** and ***purify*** DNA from cells or tissues for further analysis and experimentation

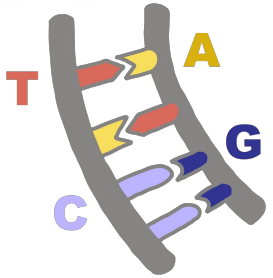


This is a foundational step in many molecular biology, biotechnology, and genetic studies

Meaning, it's crucial to make a good DNA extraction process

…that would set the quality of DNA we would be working on

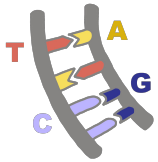
Things to consider first!



DEFINE ABOUT YOUR EXPERIMENT:

1. *Type of DNA needed*
2. *Chemical and enzymatic stability*
3. *Cellular concentration*
4. *Source*

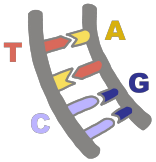
Things to consider first!



1. ***TYPE OF DNA***
   - *Genomic DNA (gDNA)*
   - *Plasmid DNA*
   - *Mitochondrial DNA (mtDNA)*
   - *Viral DNA*
   - *Chloroplast DNA (cpDNA)*

Even if you want DNA from different sources inside the same sample (e.g. bacterial DNA from oral swabs)

Things to consider first!

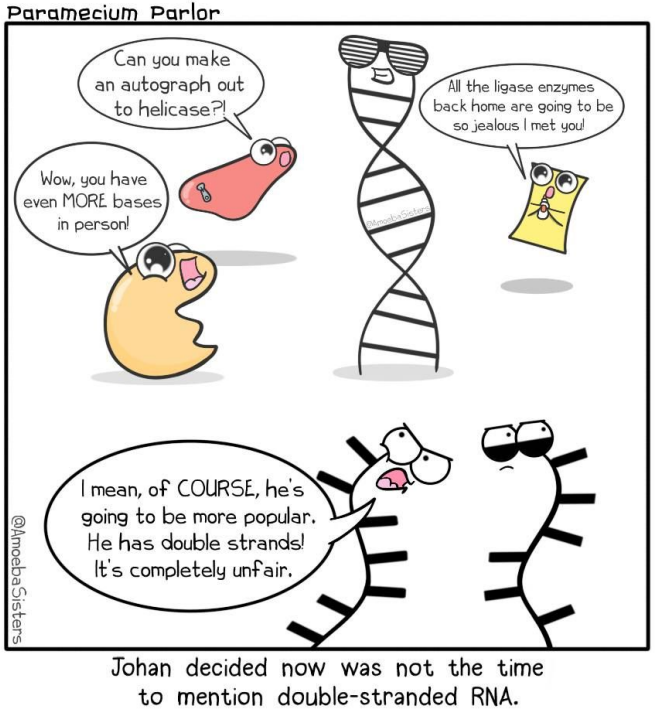


2. *Chemical and enzymatic stability*

DNA vs RNA

DNA is more stable than RNA

… due to its chemical structure



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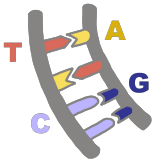


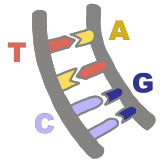
3. Cellular concentration

For example

RNAr vs RNAm

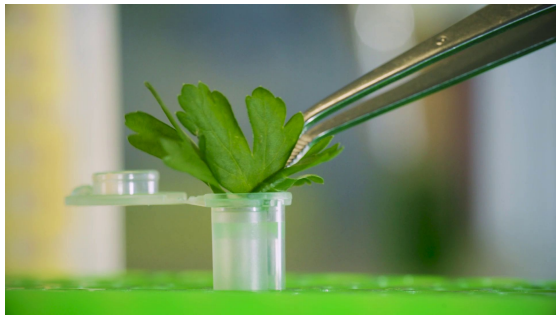
There is more RNAr (ribosomal) 80% than RNAm (messenger) 3-7% in a cell.

Things to consider first!

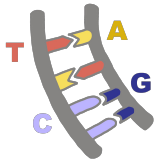


4. Source (which is your sample)

- *Liquid cell cultures*
- *Whole organism: e.g. insects*
- *Suspension with virus*
- *Vegetal tissue*
- *Blood sample*
- *Oral swabs*
- *etc*

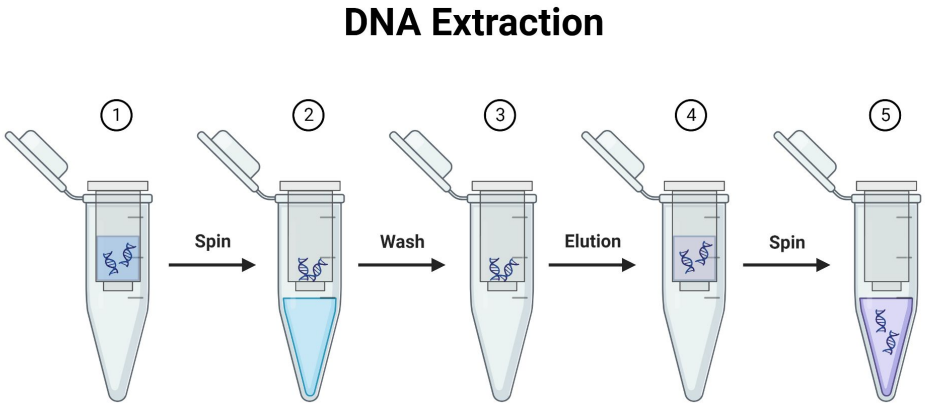


DNA Extraction STEPS

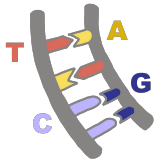


OVERVIEW

1. *Cell lysis*
2. *Removal of contaminants*
3. *DNA precipitation*
4. *DNA Washing*
5. *DNA resuspension*
6. *DNA quality and quantity check*



DNA Extraction STEPS



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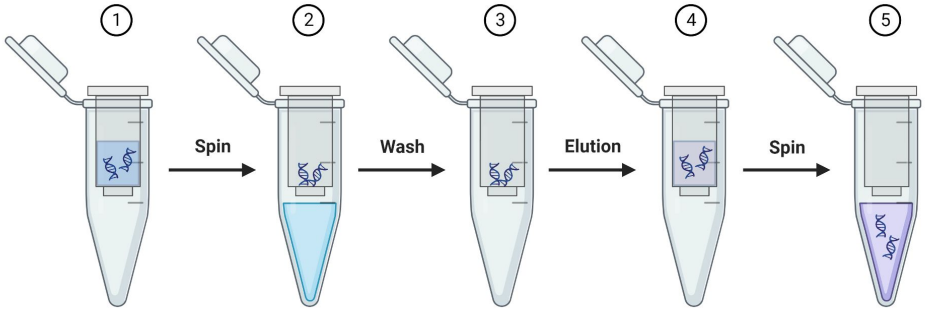
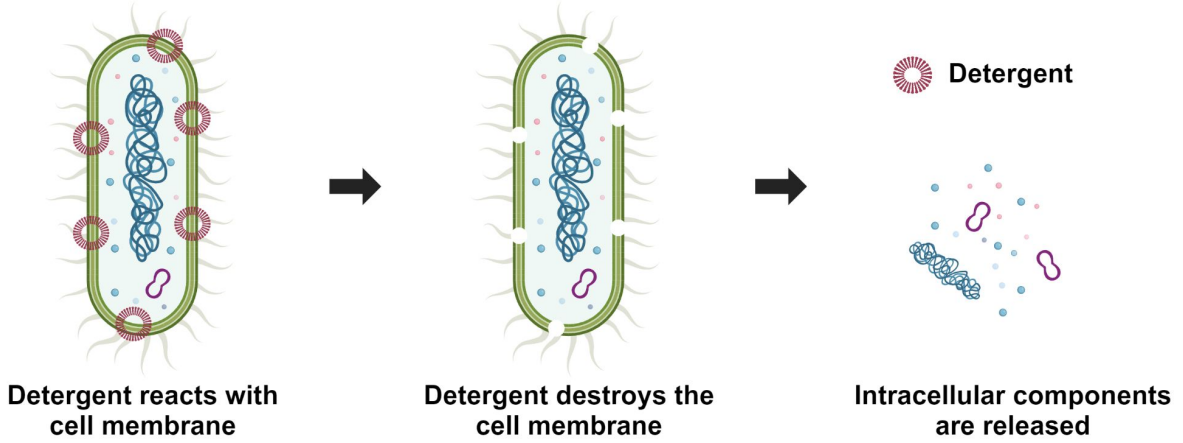


IMPORTANT: each step would have variations according to the protocol used. Some steps are optional or change according to the sample and experiment need.

DNA Extraction STEPS



1. *Cell lysis*

Goal: Break open the cells to release the DNA

- *Add lysis buffer, which contains detergents (like SDS).*

  This breaks down cell and nuclear membranes

- *Usually is important to add **proteinase K** to digest proteins and protect DNA from degradation*

DNA Extraction STEPS

2. Removal of contaminants

<u>Goal:</u> Remove proteins, lipids, and other cellular debris

- *Treat sample with salt solution (like NaCl).*

  This neutralizes negative charges of DNA and promotes protein and lipid separation.

- *Usually we use centrifugation to separate layers and get rid of proteins and other contaminants that stay in the organic phase*

IMPORTANT: for some type of samples (like blood or bacterial culture) you can skip this step because are less complex samples to work with

DNA Extraction STEPS

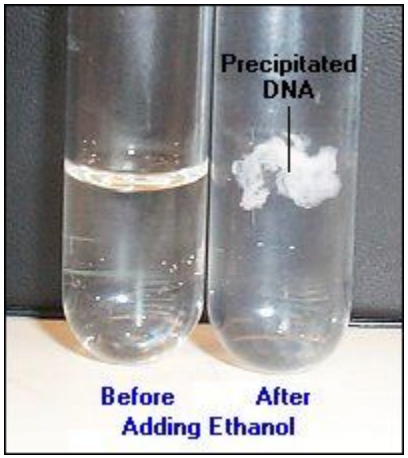
3. DNA Precipitation

Goal: Precipitate and concentrate the DNA

- *Add alcohol (e.g. ethanol or isopropanol) to DNA*

  This causes DNA to precipitate out of solution

- *The solution is well mixed and -on some occasions- left on ice to help precipitate*



DNA Extraction STEPS

4. DNA Washing

Goal: Wash the DNA to remove residual contaminants

- *Add wash buffer (e.g. 70% ethanol) to DNA*

  This removes residual from DNA solution

- *It needs to centrifuge and probably repetitive steps, and to let it dry to remove remaining ethanol*

DNA Extraction STEPS

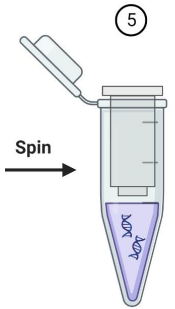
5. DNA Resuspension

Goal: Resuspend the purified DNA

- *Use a resuspension buffer (e.g. TE buffer or water) to dissolve the DNA*

  This provides an aqueous medium for DNA to be preserve

- *The extracted DNA should be stored at -20°C or -80°C, if needed for long-term storage.*



DNA Extraction STEPS

6. DNA Quality and Quantity check

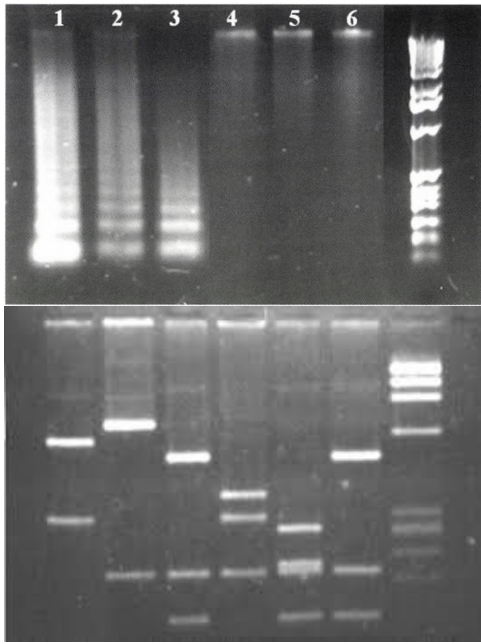
Goal: Confirm the integrity and quantity of extracted DNA

For DNA concentration:

Any instrument that uses absorbance (e.g. 260 nm) can be used or fluorometer.

For DNA purity check:

An absorbance ratio (e.g. 260/280 nm) with a result of 1.8-2 is a good result.



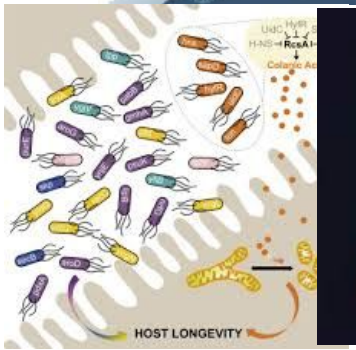
Running the DNA sample in an agarose gel to check DNA integrity is also important, that helps see if it is fragmented or degraded

NOW IT'S READY TO USE!!

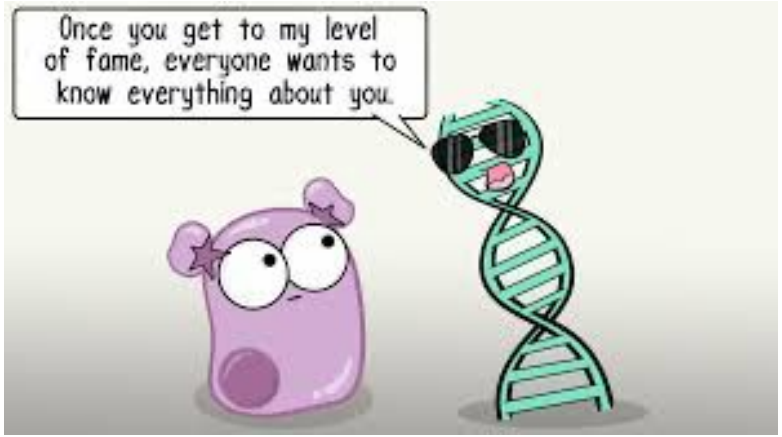
Extracted DNA could be used for:

Molecular techniques (e.g.PCRs and Sequencing) to:

- *Diagnose genetic diseases*
- *Study microbiome composition*
- *Microbial diversity and evolution*
- *Genetic engineering*
- *Synthetic biology*
- *Etc*



DNA EXTRACTION VIDEO



https://www.youtube.com/watch?v=gmNw6CWtN5k

IF YOU WANT, I LEAVE YOU WITH AN EXAMPLE OF A BLOOD EXTRACTION PROTOCOL (FOR A COMMERCIAL KIT) FOR YOU TO READ AND IDENTIFY THE DIFFERENT DNA EXTRACTION STEPS

QIAamp® Blood Mini Kit

The QIAamp Blood Mini Kit (cat. nos. 51104 and 51106) can be stored at room temperature (15–25°C) for up to 12 months. Reconstituted QIAGEN Protease is stable for 12 months when stored at 2–8°C.

Further information

- *QIAamp DNA Mini and Blood Mini Handbook*: **www.qiagen.com/HB-0329**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Dissolve any precipitates in Buffer AL by warming at 56°C until the precipitate has dissolved.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates, as indicated on the bottle.
- Add Protease Solvent to lyophilized QIAGEN Protease, as indicated on the label.
- Equilibrate samples to room temperature (15–25°C).
- Preheat a water bath or heating block to 56°C.

Procedure

1. Pipet 20 µl QIAGEN Protease into a 1.5 ml microcentrifuge tube. Add 200 µl sample. If the sample volume is less than 200 µl, add the appropriate volume of PBS.
2. Add 200 µl Buffer AL. Mix thoroughly by vortexing.
3. Incubate at 56°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the lid.
4. Add 200 µl ethanol (96–100%). Mix thoroughly by vortexing. Briefly centrifuge the tube to remove drops from the lid.
5. Pipet the mixture onto the QIAamp Mini spin column (in a 2 ml collection tube) and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and collection tube.

   Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.
6. Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500 µl Buffer AW1. Centrifuge at 6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and collection tube.
7. Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500 µl Buffer AW2. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min. Discard the flow-through and collection tube.
8. **Recommended**: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and centrifuge at full speed for 1 min. This eliminates the chance of possible Buffer AW2 carryover.
9. Place the QIAamp Mini spin column in a new 1.5 ml microcentrifuge tube (not provided), add 200 µl Buffer AE or distilled water and incubate at room temperature (15–25°C) for 1 min. Centrifuge at 6000 x *g* (8000 rpm) for 1 min to elute the DNA.

Source:
https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-dna-blood-kits