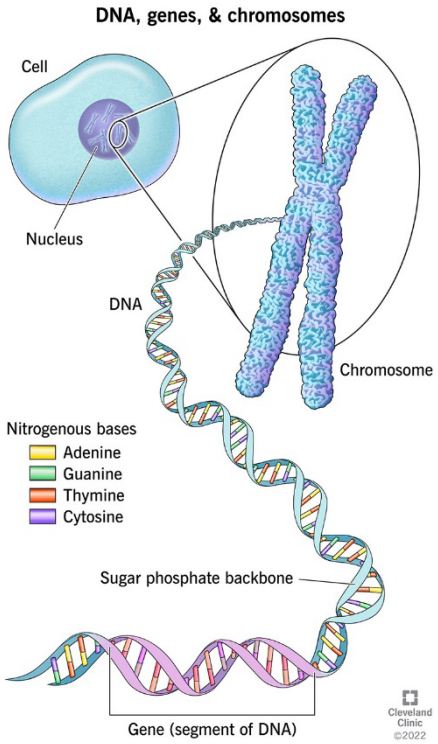
Lab 1

DNA Extraction

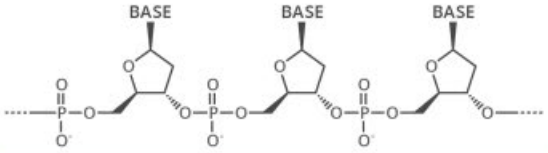
- Biological information is stored in DNA through its linear sequence of polynucleotides.
- It is translated into mRNA and subsequently transcribed into a sequence of amino acids that determines the three-dimensional structure of the protein, which in turn determines its biological function.
- DNA provides the instructions to maintain biological function and is considered to be the blueprint of life



What makes up the chemical structure of DNA?

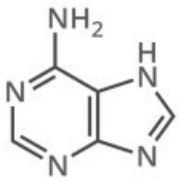
COMPOUND INTEREST

The sugar-phosphate backbone

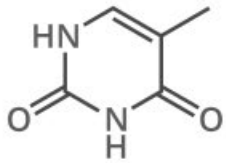


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

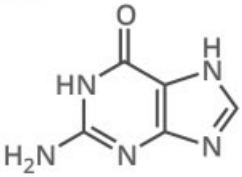
A Adenine



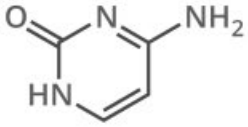
T Thymine



G Guanine

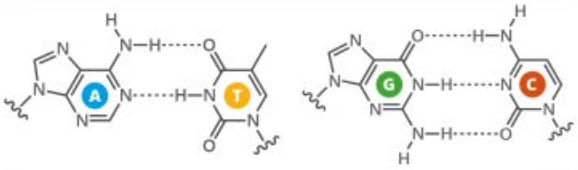


C Cytosine



What holds DNA strands together?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



From DNA to proteins

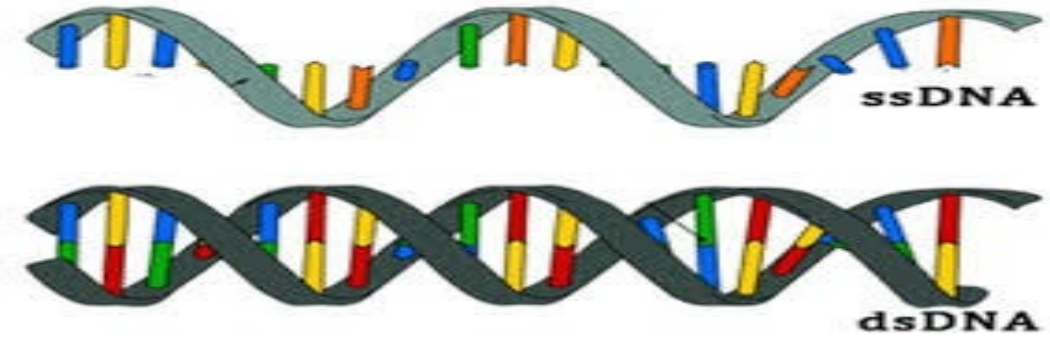
The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.

DNA → (Transcription) → RNA → (Translation) → Protein

An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.

- While double-stranded DNA is present in almost all organisms, single-stranded DNA is found in very few pathogenic viruses but is also present in bacteria and cells of higher organisms



DNA extraction

- Since the first DNA extraction performed by Friedrich Miescher in 1869, scientists have made extraordinary progress in designing extraction methods that are more reliable, easier and faster to perform, more cost-effective, and produce a higher yield.

The principle behind DNA extraction

Consists of the following steps:

(1) disruption of cytoplasmic and nuclear membranes

(2) separation and purification of DNA from other components of the cell lysate such as lipids, proteins, and other nucleic acids

(3) concentration and purification of DNA

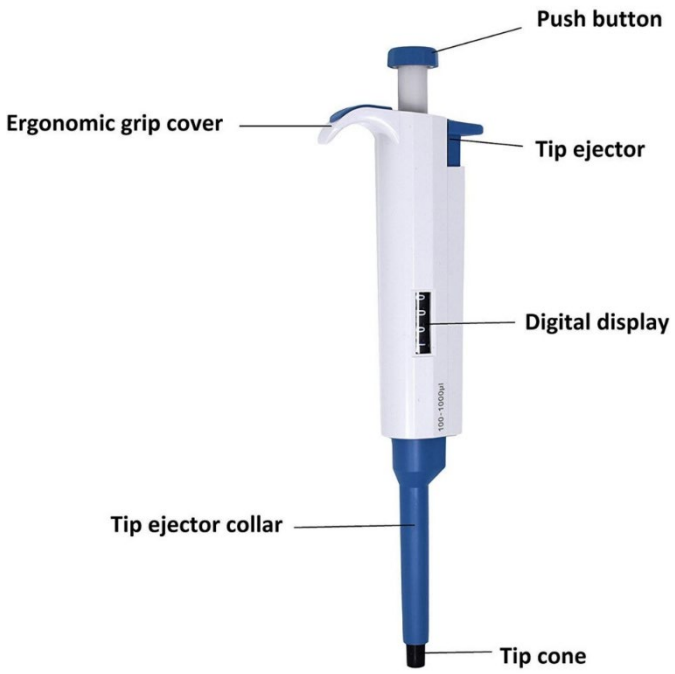
The Experiment

DNA extraction from bacteria

Materials:

Micropipette

A molecular instrument used to measure and transfer small amounts of liquids with a volume range between 1 and 1000µl.



Micropipette tips

DNA Extraction Kit

Eppendorf tubes

Staphylococcus + *E.coli* 24h cultures

Vortex

Centrifuge

d.d.H_2O

100% - 96% Ethanol

Methods:

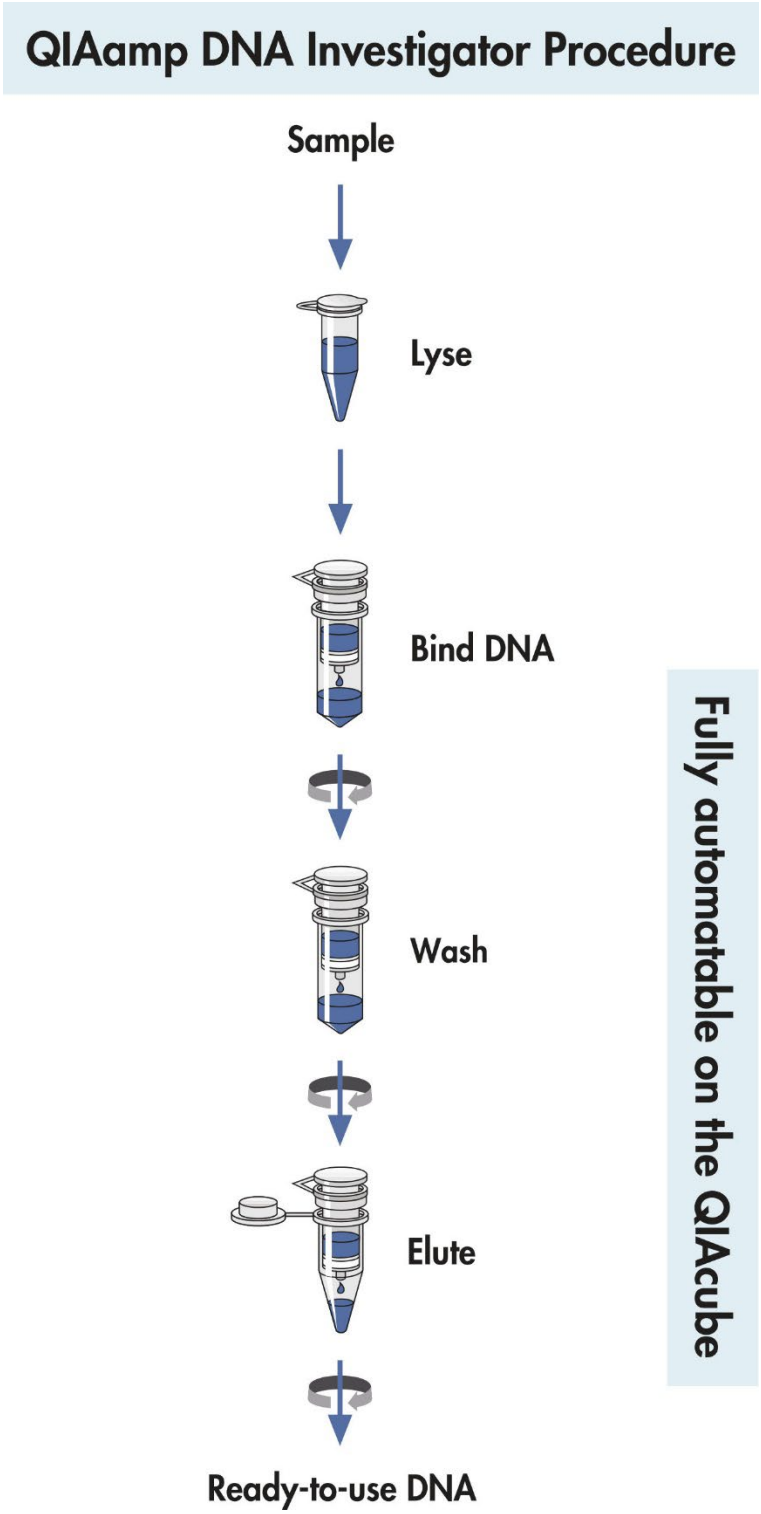
The following procedure was used for DNA extraction from *Staphylococcus* and *E.coli* according to the manufacturer's instructions:

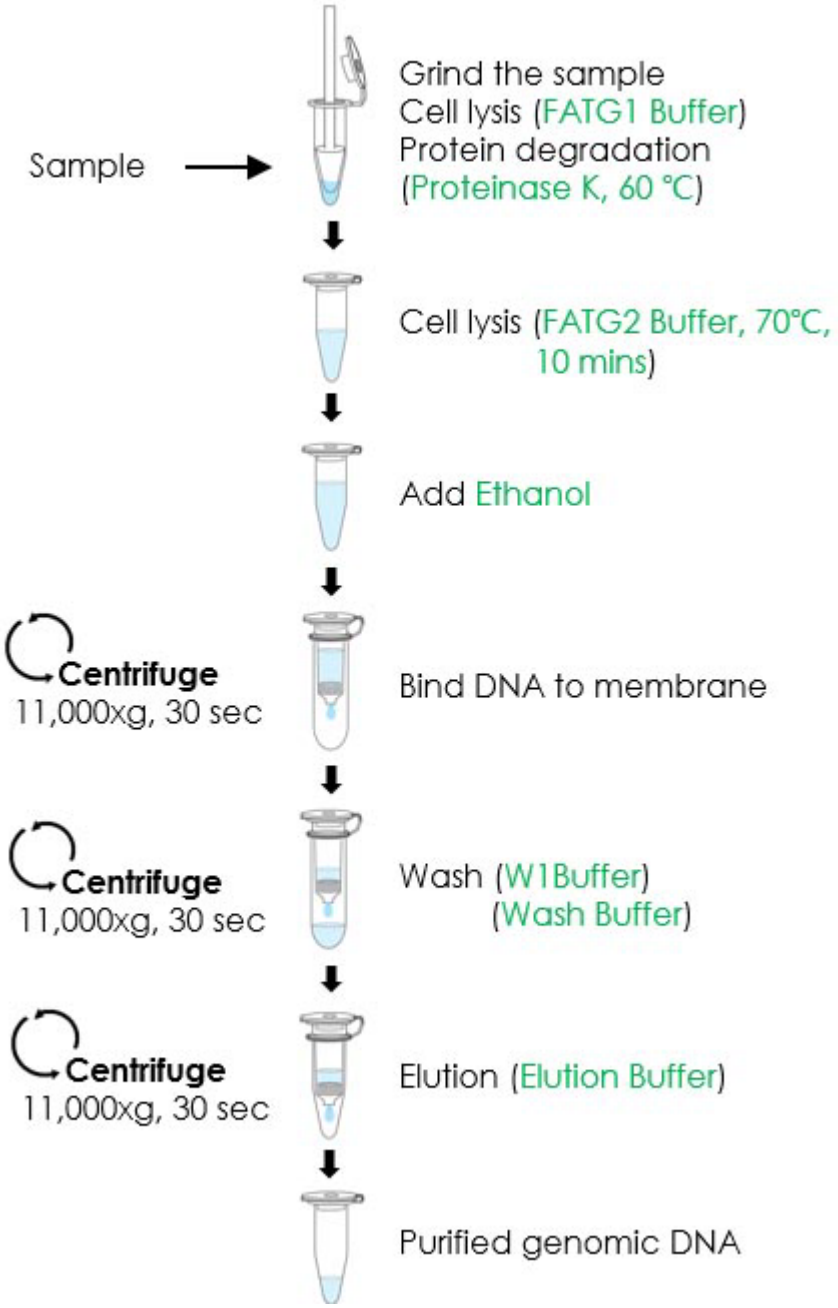
1. Inoculate the bacteria into Luria-Bertani (LB) broth and incubate overnight at 37 °C.
2. The appropriate number of cells (maximum 5 x 10^6) were centrifuged for 5 min at 300 x*g* (190rpm). The pellet was resuspended in 200 µl PBS. 20 µl Proteinase K was Added.
3. 200 µl Buffer AL (without added ethanol) was Added. Mixed thoroughly by vortexing, and incubated at 56°C for 10 min.
4. 200 µl ethanol (96–100%) was added to the sample and mixed thoroughly by vortexing.
5. Pipeted the mixture from step 3 into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Flowthrough was discarded and the collection tube.
6. The DNeasy Mini spin column was placed in a new 2 ml collection tube, 500 µl Buffer AW1 was added, and centrifuged for 1 min at

≥6000 x *g* (8000 rpm). Flowthrough was discarded and the collection tube.

7. The DNeasy Mini spin column was placed in a new 2 ml collection tube, 500 µl Buffer AW2 was added, and centrifuged for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Flowthrough was discarded and the collection tube.
8. The DNeasy Mini spin column was placed in a clean 1.5 ml or 2 ml microcentrifuge tube.
9. The DNA was eluted by adding 200 ul BufferAE to the center of the spin column membrane. Incubated for 1 min at room temperature (15-25^{O}C). Centrifuge for 1 minute at >6000x *g*.
10. Repeated step 8 for more increased DNA yield.

Protocol Steps




Sample
Grind the sample
Cell lysis (FATG1 Buffer)
Protein degradation
(Proteinase K, 60 °C)
Cell lysis (FATG2 Buffer, 70°C,
10 mins)
Add Ethanol
Centrifuge
11,000xg, 30 sec
Bind DNA to membrane
Centrifuge
11,000xg, 30 sec
Wash (W1Buffer)
(Wash Buffer)
Centrifuge
11,000xg, 30 sec
Elution (Elution Buffer)
Purified genomic DNA