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DNA extraction & purification

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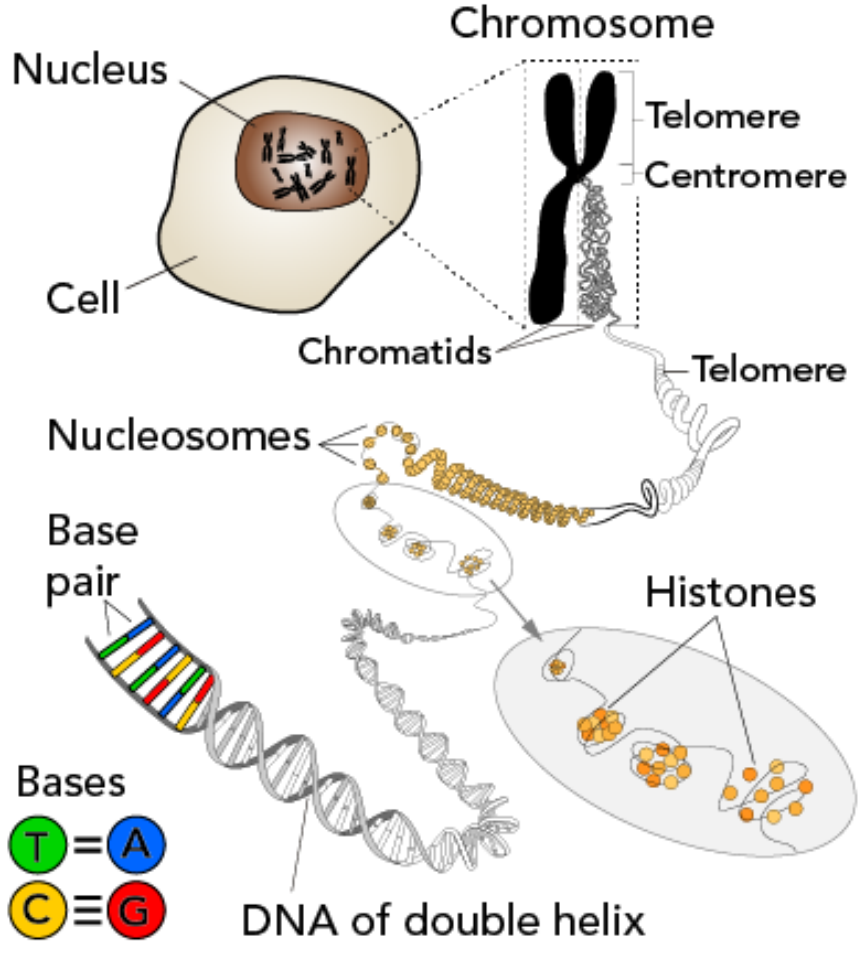
Aim

- **Learn the basics of how DNA can be extracted from cells.**



Background information

- DNA extraction is a method to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components. Friedrich Miescher in 1869 did DNA isolation for the first time.
- The sample can be plant or animal cells, blood, bacteria, parasite, fungi, and virus. The idea of extracting the DNA is quite basic: Disruption of the cell membrane (and cell wall in case of plant cells) to make the DNA exposed and then separate it from the rest of the cell debris.
- It is performed by many methods, but the choice of methods depends upon DNA properties (**size & conformation**) and behaviour of nucleic acid under different extracting & fractionating conditions.
- Physical features of genomic DNA (**rigidity**, **viscosity** & **length** to diameter) render DNA highly susceptible to pipetting, mixing & vortexing.

Why important DNA extraction is ?

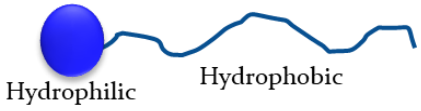
The ability to extract DNA is of primary importance to

1. Studying genetic causes of disease
2. For development of diagnostics and drugs
3. For performing forensic science and determine paternity
4. Sequencing genomes, Identifying organisms and microorganisms (bacteria, fungi and virus) in the environment via

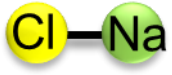
DNA extraction from banana

Samples, Materials, and reagents

1. Banana, Tomato, and Strawberries.
2. Water (100-250ml)
3. liquid soap (KOH) has two sides



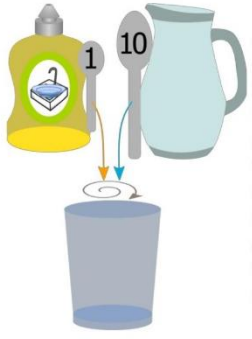
4. Table Salt (1 teaspoon)



5. 3 beakers or small glasses
6. Knife
7. Alcohol (as pure as you can find).
8. 1 Teaspoon and 1 tablespoon
9. Sieve/sifter (or coffee filter)
10. Test tube (or tall thin glass/jar)

1. Chop the banana
Cut half of a banana (ripe is better), and peel it. Then chop it finely. Transfer it to cup #1 where you will mash it. You may eat the other half ☺

2. Mash it very well
Use a pestle if you have one, otherwise a spoon will do. Spend a few minutes doing this. We need to make sure the tissues of the banana are broken down well.

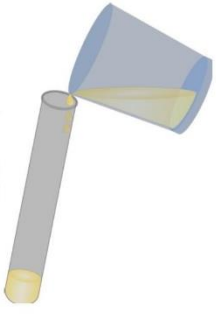


3. Make "lysis" solution
DNA is trapped deep inside the cells of the banana. We need to break it free (this is called "cell lysis", breaking the cell). In cup #2, mix about 1 teaspoon of washing up liquid (or liquid soap) and some 10 tablespoons of water (no need to be too exact here).

4. Add the lysis solution and mix very well
Pour the lysis solution onto the banana paste, and mix very well, for at least 5 minutes. You can use the pestle again if you have it, otherwise the spoon again

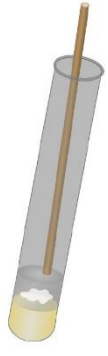
5. Filter the solution
Using a sieve (sifter/strainer) with a fine mesh, extract the liquid into cup #3. You can also use a coffee filter, or a funnel with a layer or two of toilet or kitchen tissue.

6. Transfer to a tall transparent container
The ideal container would be a big test tube, but if you don't have one, try to get the thinnest glass jar you can find.



7. Extract the DNA
Lean the container to around 45º and add alcohol at the top, letting it flow down the walls slowly. About a finger in height is enough. Almost immediately, you will see a white slime at the interface. That's DNA! It "precipitates" because the water in which it was dissolved goes with the alcohol instead.

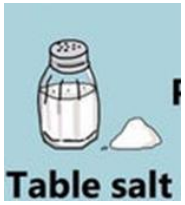
8. Fish the DNA out
Using a stick appropriate to the shape of your container, try to fish out the DNA. Note that this is not pure DNA, it has RNA, proteins, and a lot of other things. We would need to purify it with professional reagents if we wanted to analyse it. You can try a similar method with tomatoes, strawberries, and even saliva! (starting at step 3).
We recommend that you now do the *DNA & Genetics* activity.



Extraction of your own DNA

Samples, Materials, and reagents

1. Water (100ml)
2. Salt (dissolve 4 teaspoons of salt in the water in a glass and then stir it until the salt dissolved)
3. Saliva (Gargle ¼ (50ml) of salt water for 1min in a beaker)
4. Dish soap (add 2/3 drops to the gargled salty water)
5. Chilled alcohol (add a half of cup to the dissolved solution and then wait for 5min).



Add 4teaspoons

Gargle it

Add 2drops of soap

Add 150ml of chilled alcohol

Left for 5 min

Extraction of DNA

A. Cell lysis

- **Lysozyme:** used to disturb the cell wall of the cell
- **Chemical lysis:** is done by **nonionic detergent** (resuspending buffer& lysis buffer):

1. **Tris-HCl:** used to digest cell membrane, and rupture of other organelle, protects DNA from pH shifts, buffer pH of the cells at 8.0.
2. **EDTA:** binds divalent cations such as **Calcium** and **magnesium**. EDTA destabilizes the cell membrane and also inhibit the activation of DNA.

❖ **Note: Tris-EDTA:** protects the DNA and RNA from dissolving. The pH is usually adjusted to 7.5 for RNA and 8.0 for DNA.

3. **SDS:** causes the distribution of cell membrane and nuclear envelop. It also denatures the proteins present in the cell membrane.

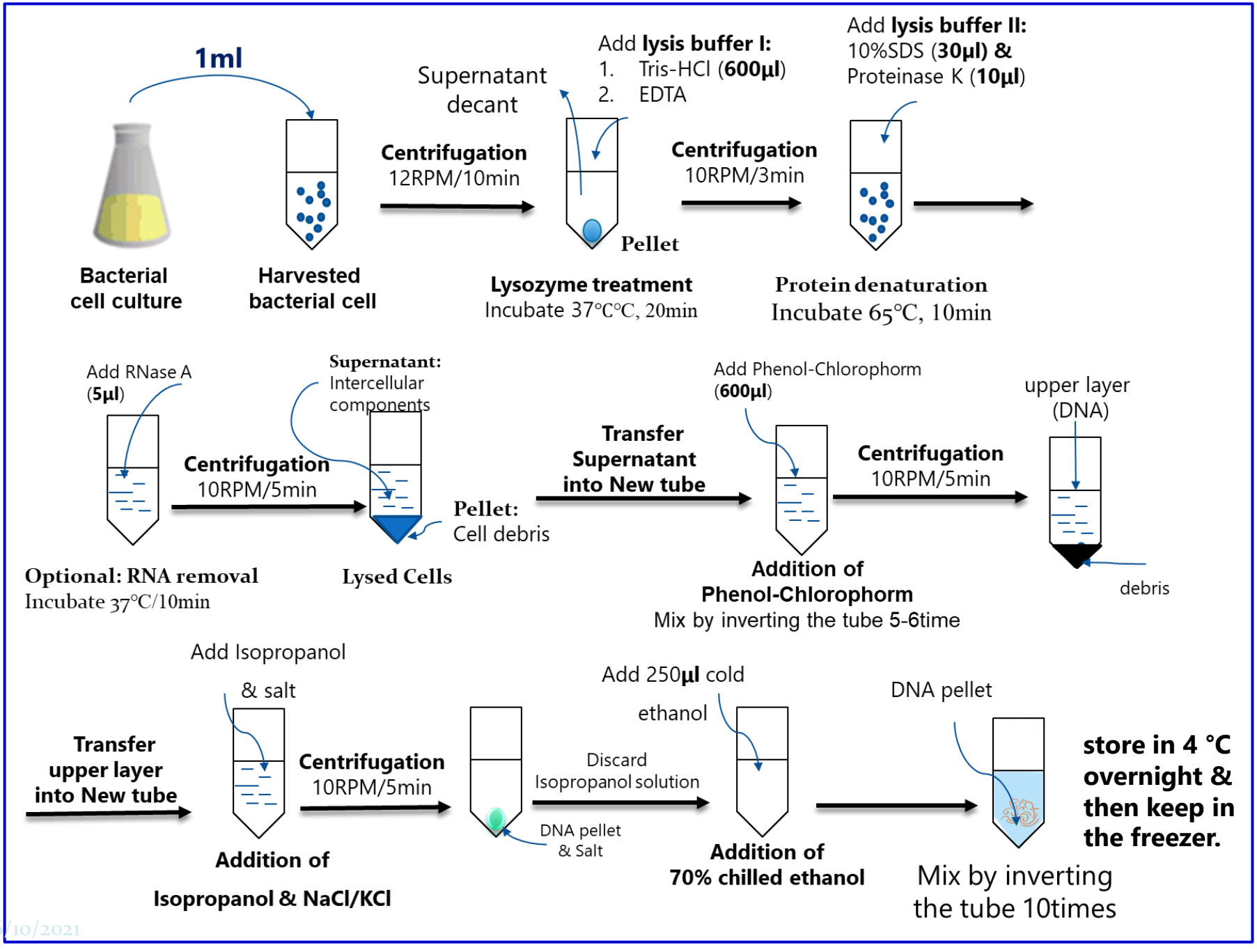
- **Proteinase K:** denatures proteins.

The function of used chemicals

B. DNA extraction materials

- **Phenol-Chlorophom (a mixture):** is organic solvents that denatures and precipitates cell membrane, lipids and proteins.
- **RNase A:** used for removal of unwanted RNAs.
- **Isopropanol/ethanol:** used for concentrating DNA.
- **Salt (NaCl & KCl):** nonorganic extraction and is positively charged and DNA is negatively charged. This salt reduces the hydrophilicity of DNA, It makes DNA precipitate more easily. **Cations Na+ & K+:** binds to negative phosphate groups of DNA and makes DNA more stable in aqueous solution.
- **70% cold alcohol:** remove salt and precipitate DNA.

Experimental protocol of DNA extraction from bacetria




Any Questions