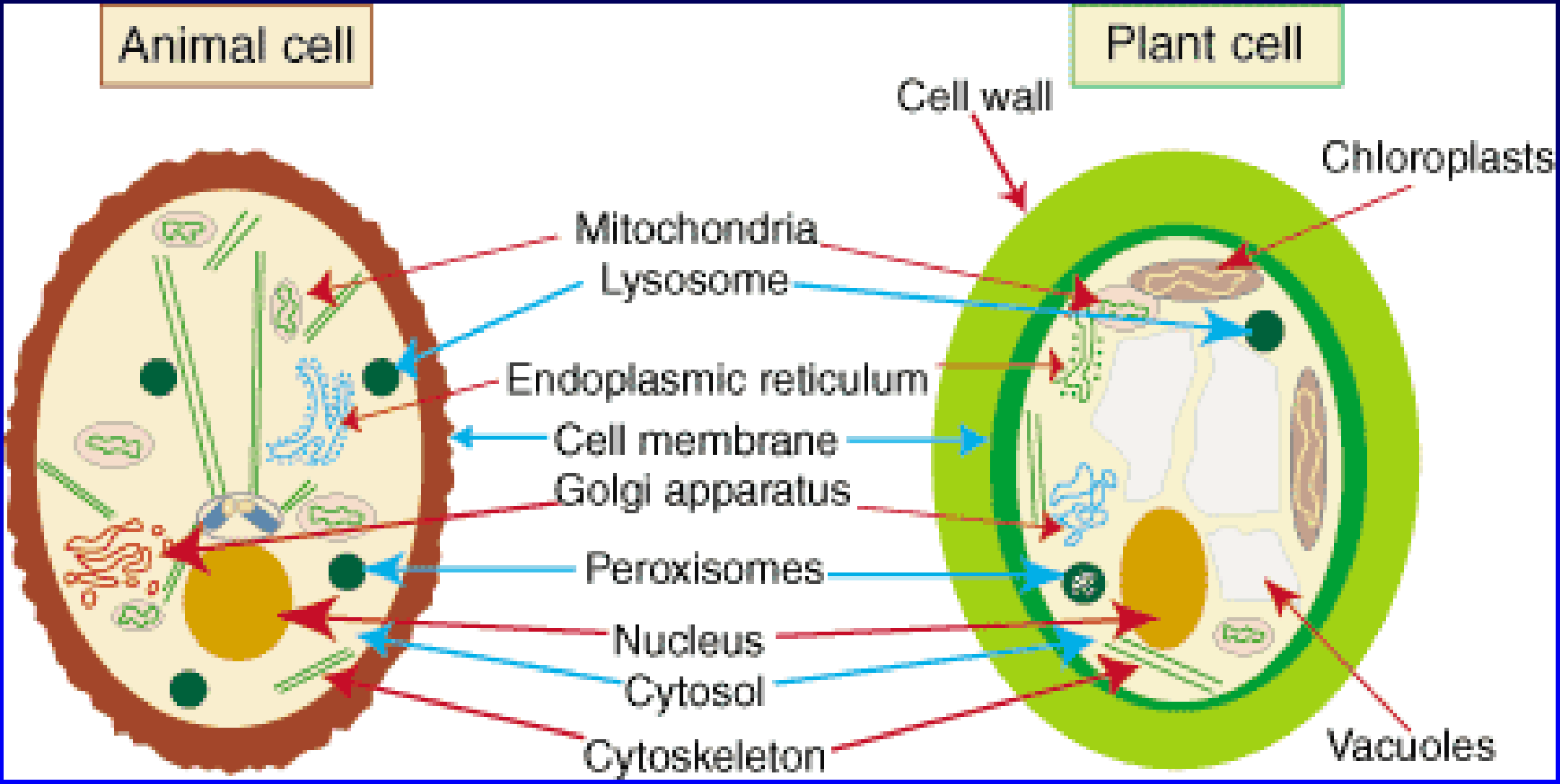

DNA
Extraction

DNA extraction

DNA Extraction is the removal of deoxyribonucleic acid (DNA) from the cells in which it is normally resides.

What are the essentials of the DNA extraction procedures?

1. **Maximize DNA recovery**
2. **Remove inhibitors**
3. **Remove or inhibit nucleases**
4. **Maximize the quality of DNA**


Animal cell
Plant cell
Cell wall
Chloroplasts
Mitochondria
Lysosome
Endoplasmic reticulum
Cell membrane
Golgi apparatus
Peroxisomes
Nucleus
Cytosol
Cytoskeleton
Vacuoles

Most DNA extraction protocols consist of two parts:

- A technique to lyse the cells gently and solubilize the DNA.
- Enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules

What are the Most Commonly used DNA Extraction Procedures?

- **Organic Extraction**

- **Silica Based (Kits)**

DNA extraction

- **Lysis and disruption of the cells**

- **NaCl** , neutralized DNA molecules ----grouping.
- **EDTA** - inhibit DNases.
- **Detergents** such as (SDS, Tris HCl, CTAB) used to brake cellular membranes and remove lipids

- Addition of **phenol: chlorophorm : isoamyl alcohol (25:24:1).**

Organic solvents, hydrophobic lysates keep trapped, e.g. membrane lipids, proteins or polysacharids. Besides denature proteins.

- **Centrifugation:**
  - **Watery phase:**

    hydrophilic compounds, nucleic acids, salts, sugars, DNA

  - **Organic phase:** hydrophobic compounds

- **DNA precipitation with alcohol –** usually pure and cold ethanol in the presence of salt (sodium acetate) **.**

  – Because DNA is non-soluble in alcohol, precipitate and form a pellet in the bottom of the tube after centrifugation. This step also remove alcohol soluble salts.

- **DNA cleans with 70% ethanol, dry and dilute in TE buffer (protect DNA from degradation) or sterile distilled water.**

Extraction kits

- Commercial DNA extraction kits employs spin columns, for the isolation of DNA.

- The spin columns contain a silica resin (silica membrane) that selectively binds DNA, depending on the salt conditions and other factors.

- **lysis buffer** containing a high concentration of chaotropic salt mainly: guanidine HCL, guanidine thiocyanate, urea and proteinase K

- **The chaotropic salts are critical for lysis, but also for binding the DNA to the column . Additionally, to enhance and influence the binding of nucleic acids to silica, alcohol is also added.**

- **Wash steps serve to remove these impurities chaotropic salts (low salt conc. then ethanol to remove the salts).**
- **A centrifugation step to dry the column to remove the ethanol then elute in 10mM Tris buffer or water If the column still has ethanol on it, then the nucleic acids cannot be fully rehydrated.**

- **10 mM Tris at a pH between 8-9 is typically used.**
- **DNA is more stable at a slightly basic pH and will dissolve faster in a buffer.**
- **Water tends to have a low pH, high molecular weight DNA may not completely rehydrate in the short time used for elution.**
- **Elution of DNA can be maximized by allowing the buffer to sit in the membrane for a few minutes before centrifugation.**

PCR

PCR

- **Reactive needed for a PCR reaction:**

- **Buffer solution :** provide a suitable chemical environment for optimum activity and stability of the DNA polymerase.

- **$MgCl_2$:** is a critical factor that can affect the success of the amplification. It´s cofactor for thermo-stable DNA polymerases.

- **Deoxynucleotide triphosphates** (**dNTP's:** dATP, dGTP, dCTP, dTTP): the building-blocks from which the DNA polymerase synthesizes a new DNA strand.

- **Primers:** Short sequences of 20-24 nucleotides in length, that are complementary to the 3′ ends of each of the sense and anti-sense strand of the DNA target.

- **Taq Polymerase**: DNA polymerase.
- **DNA template:** contains the DNA region (target) to be amplified.
- **Readdy Mix**
- **Master Mix**

Thank You

For your attention

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