

**Please, review the class work
and think of questions**

Studies in molecular biology could be performed either

- ***in vivo* - in a living cell or in a living organism**
- ***in vitro* – in a test tube with isolated biological molecules**

***in vitro* methods in studies of
nucleic acids**

The methods include:

- Isolation of nucleic acids
- Site-specific fragmentation and fractionation of nucleic acids
- Amplification of unique nucleic acid molecules by cloning or PCR
- Detection and identification of unique nucleic acids by hybridization
- Determining of the primary structure of nucleic acids (sequencing)

Isolation of nucleic acids

In order to isolate nucleic acids from cells various methods of extraction and purification are used. Depending on the goal of experiment a researcher might want to isolate:

- **Genomic DNA**
- **mRNA or total RNA**
- **Viral DNA**
- **Episomal (plasmid) DNA**

Traditional method of genomic DNA isolation

- Cells are lysed with detergents
- Proteins are digested with proteases (Proteinase K is most commonly used)
- RNA is digested with DNase-free RNase enzyme
- DNA is extracted with organic solvents (phenol+chlorophorm)
- DNA is precipitated with alcohol, dried and rehydrated

Traditional methods of total RNA isolation

- RNA is extremely susceptible to degradation by RNase enzyme. Therefore special precautions are used to prevent possible contamination with RNase and to inactivate all RNase activity.
- Various methods to RNA is separate RNA form DNA are used.

Viral DNA

- Viral particles are isolated from the host cells and purified
- DNA is extracted with with organic solvents (phenol+chlorophorm)

Plasmid DNA

- Plasmids are episomes – relatively small DNA molecules that can replicate in a bacterial cell independently from the chromosome.
- Plasmids are widely used for DNA cloning and manipulation with DNA sequence
- Plasmid isolation methods usually include an alkaline extraction step

Alkaline-lysis method

