

# **Methods of studying biological objects**

**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

