hogeschool Leiden

Introduction to PCR
isolation of nucleic acids

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Willem van Leeuwen

TopLab

content

- DNA isolation
  - Diverse methods such as phenol-extraction, silica, magnestic bead purification
  - Determination of purity and yield
- RNA isolation
  - mRNA isolation
  - cDNA synthesis

DNA and RNA isolation

Extraction of nucleic acids:

1. Cell lysis
   - Mechanical disruption (grinding, hypotonic lysis)
   - Chemical treatment (detergent lysis)
   - Enzymatic digestion (proteinase K)

2. Inactivation with nucleases and proteases

3. Separation of the nucleic acid from the cell debris
   - Extraction/precipitation
   - Centrifugation

DNA isolation: many different targets and matrices

- Genomic DNA:
  - Clinical samples
  - Cell cultures
  - Micro organism
  - Plant material
- Plasmid DNA
- DNA fragments:
  - PCR
  - Restriction enzyme digestion

The methods…

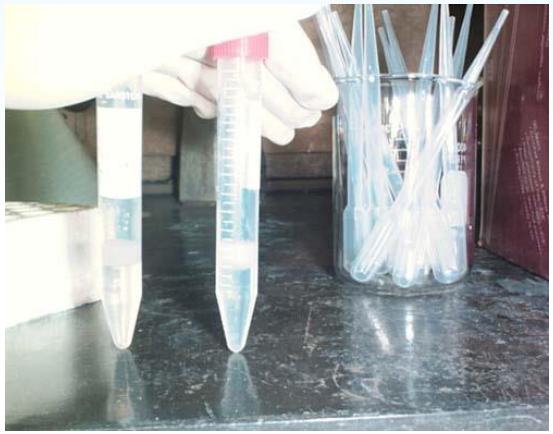
- phenol/chloroform extraction and ethanol precipitation
- silica membrane columns
- anion exchange resins (ion exchange)
- (para)magnetic beads

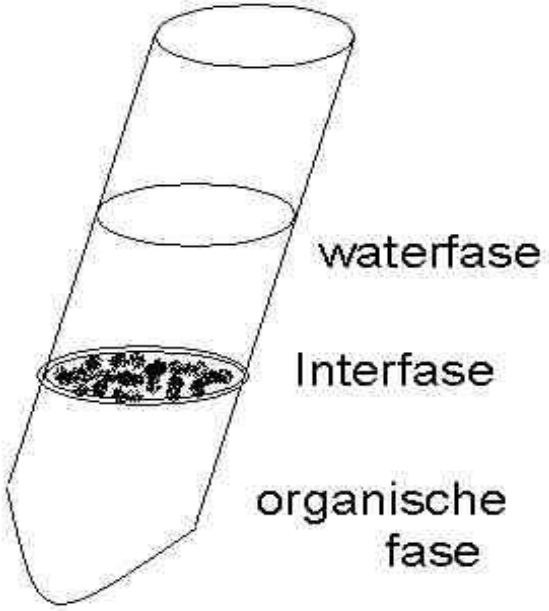
DNA isolation phenol chloroform

- homogenizing sample: lysis, proteinase K (enzymatic), chemical or physical
- extraction

  phenol: chloroform: isoamylalcohol 25:24:1

- centrifugation



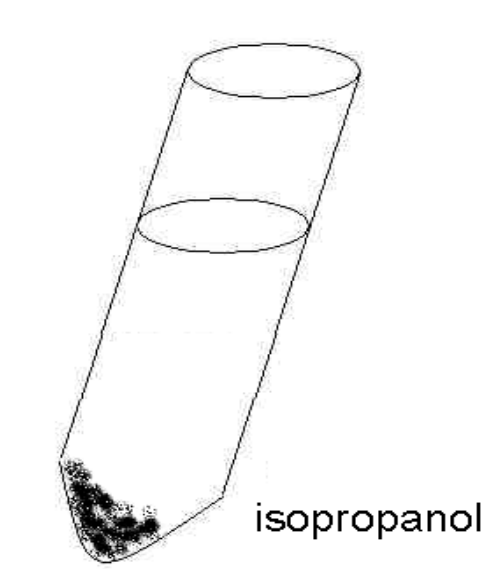


DNA isolation phenol chloroform: precipitation and washing

Precipitation

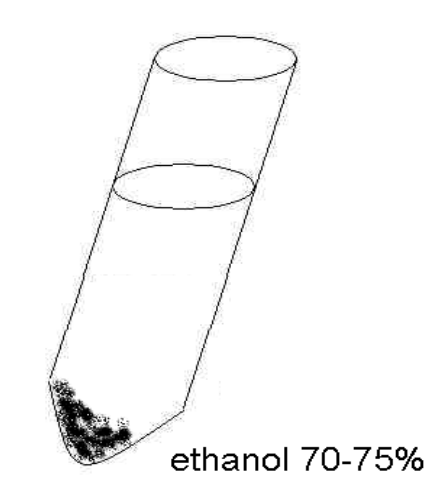
- isopropanol, ethanol
- ammoniumacetaat

cold: -20°C to 4°C



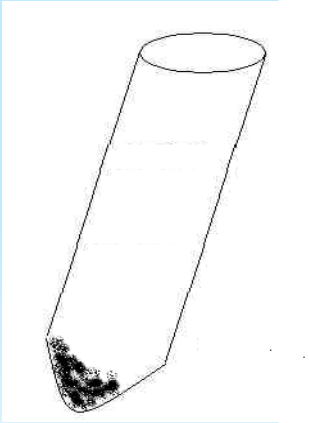
Wash

ethanol 70-75% bij RT/-4°C



DNA isolation phenol chloroform: dry and dissolve

Dry (air or speedvac)

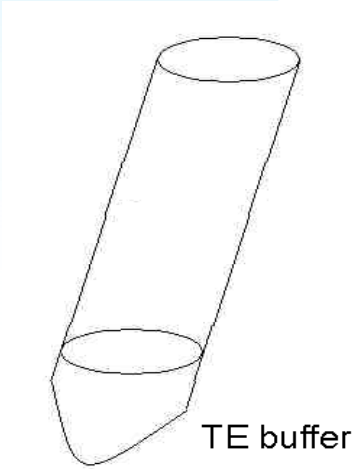


Dissolve DNA into

H_2O or 10 mM Tris 1mM EDTA ($T_{10}E_1$) pH 7,4 - 7,6

NB. Dissolve RNA into H_2O

Vortex!!!



DNA isolation: silica membrane (spin column)

- Homogenize sample (lysate with salt)
- Centrifugation
-Eluate

Roche,
Fermentas

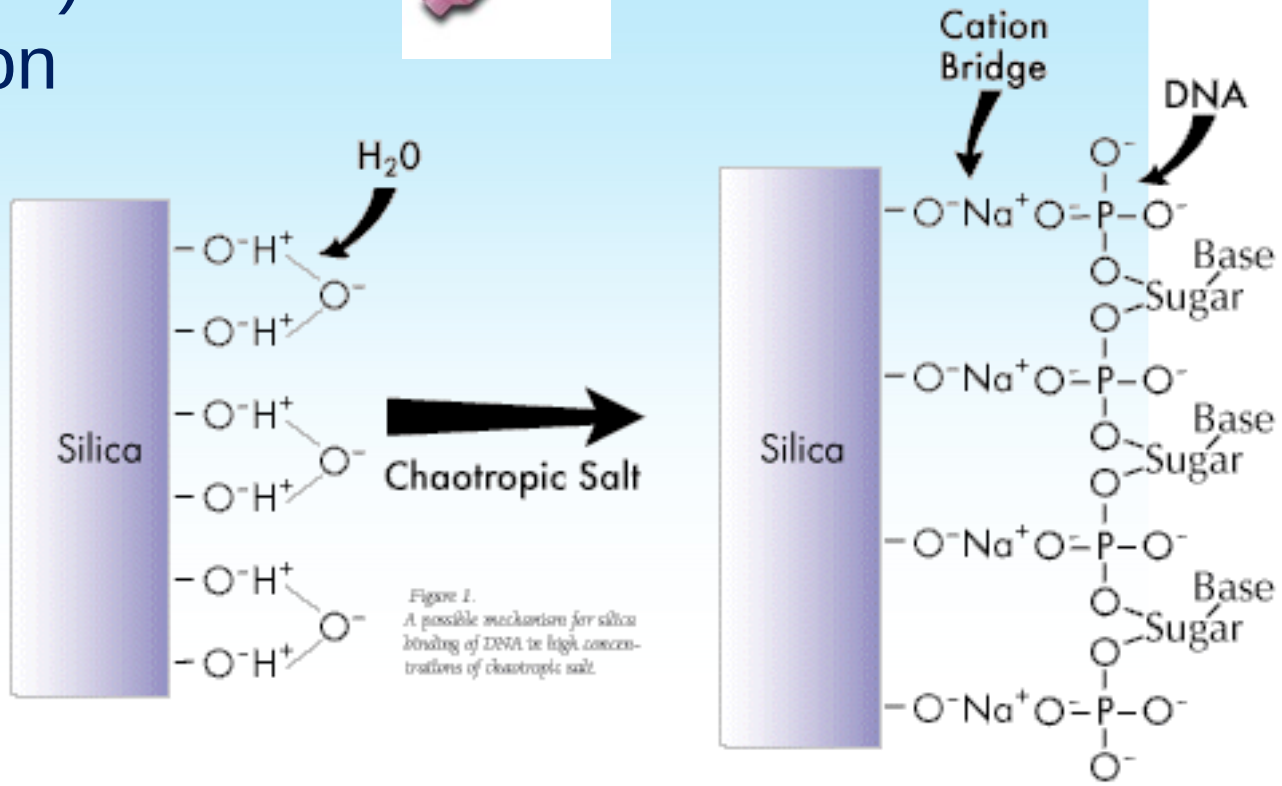
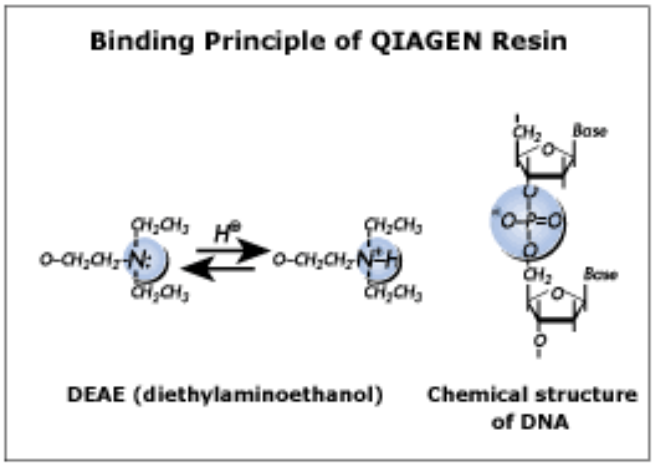


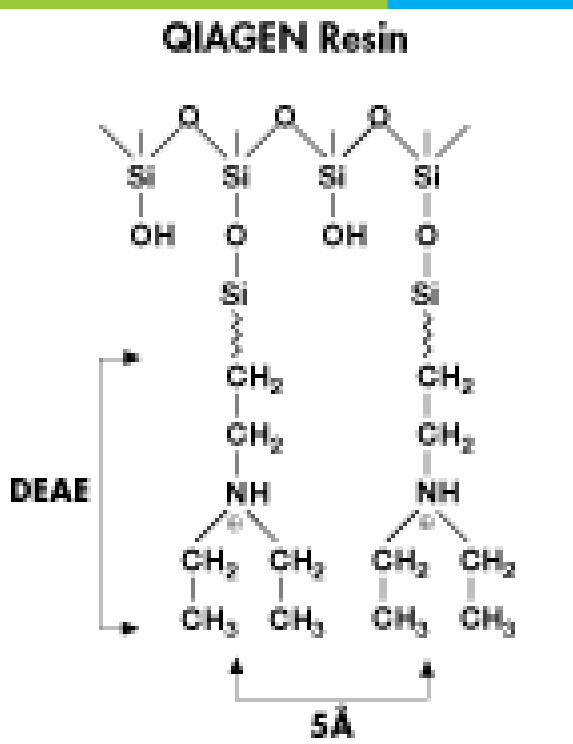
Figure 1.
A possible mechanism for silica binding of DNA in high concentrations of chaotropic salt.

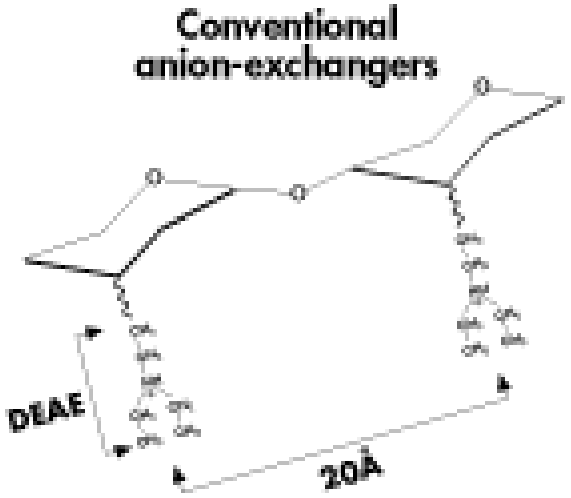
Anion-exchange resin

- Homogenize sample
  - Lyserate without salt
- Centrifugate
- Eluate (pH and salt concentration)

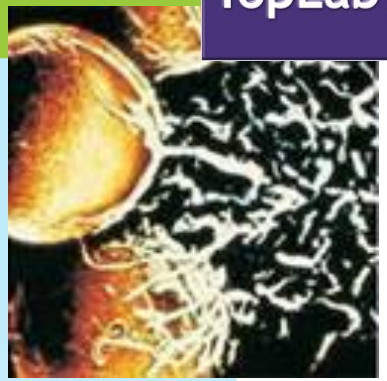
Qiagen kits



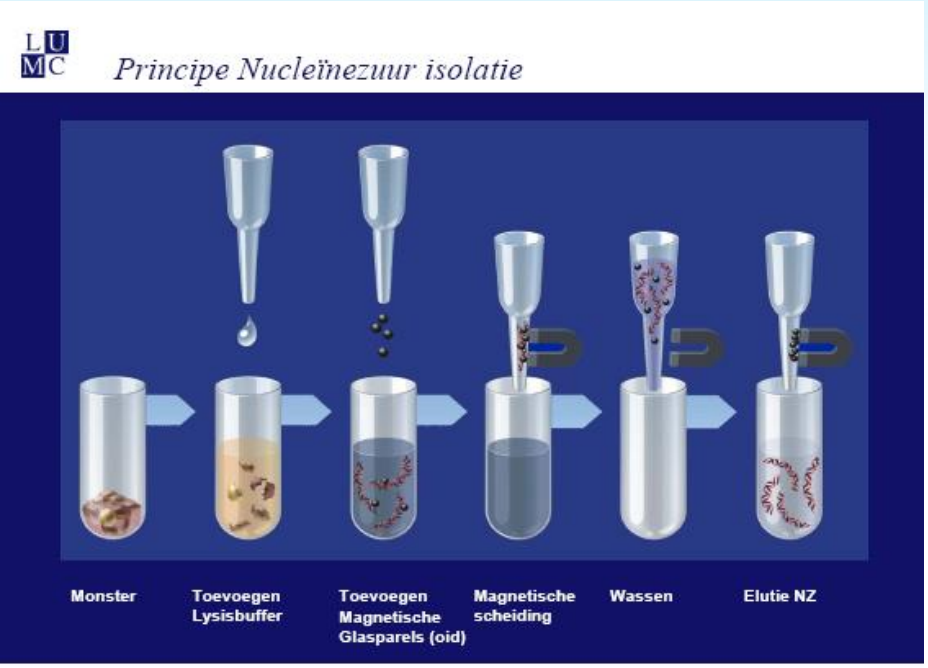




Magnetic particle/beads



- Homogenize with beads
- Magnetization
- Wash
- “Eluate” from the beads



EZ1 Biorobot, Qiagen

EasyMag, bioMérieux

Magnapure, Roche

Qiacube, BioRobot, Qiagen

Cobas Ampliprep

Determination of nucleic acid purity and yield

- **(Target) DNA: genomic, plasmid, DNA fragments**
  - UV spectroscopy
  - Agarose gels; high molecular DNA
  - Bioanalyzer
  - qPCR test

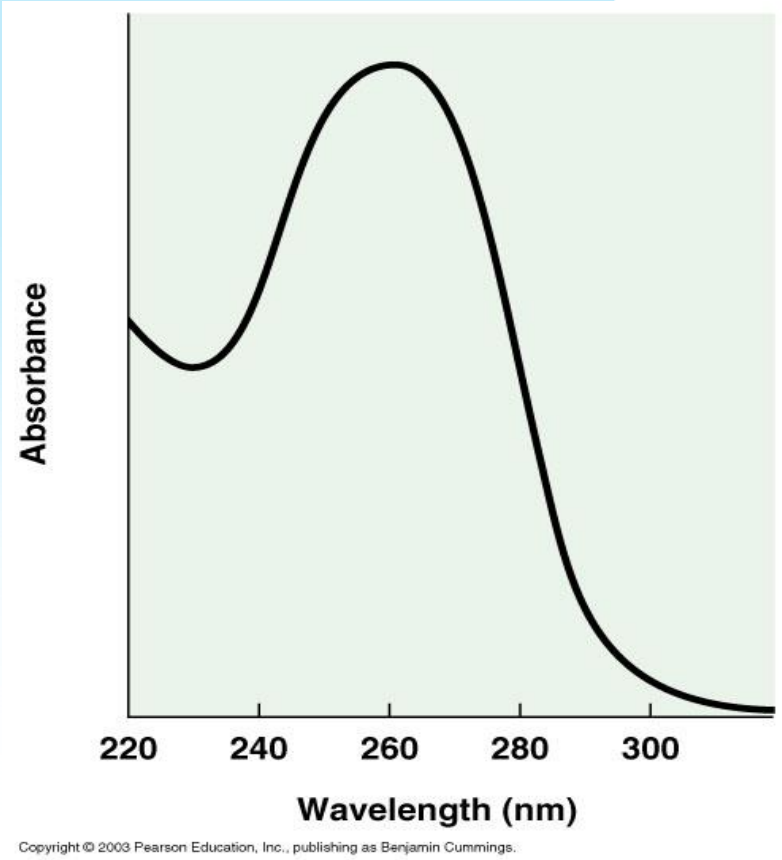
- **(Target) RNA: total RNA, mRNA (poly A^+ fraction)**
  - UV spectroscopy
  - Agarose gels: Ribosomal RNA
  - Bioanalyzer

Determination of nucleic acid purity and yield

- Ultraviolet Absorbance Spectrum of DNA

- Aopt 260 nm by pyrimidin and purin bases.

  (proteins at 280 nm
  DNA/RNA at 260nm
  ratio 260/280 ≈ 2)



Determination of nucleic acid purity and yield

Law of Lambert-Beer: $A=\varepsilon bc$

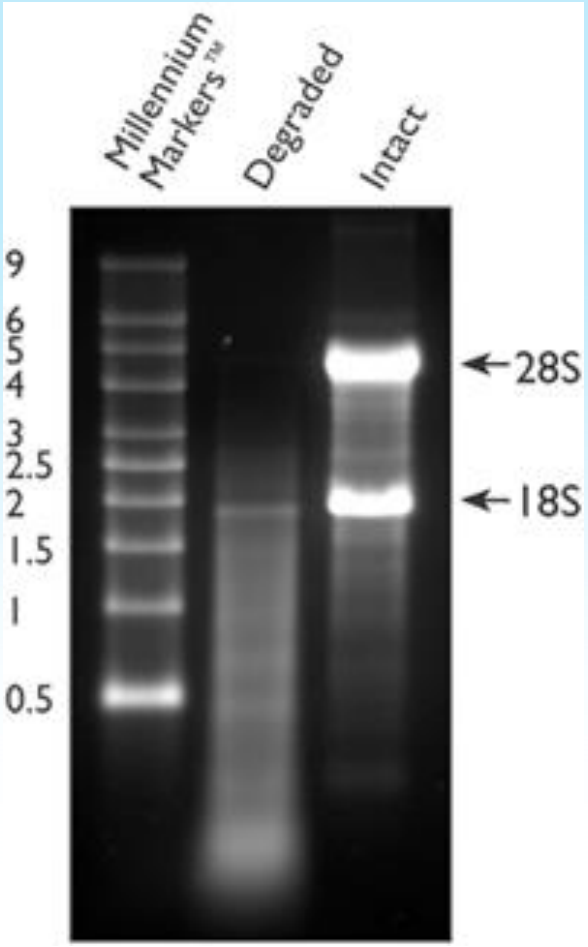
- A= absorbance
- ε= absorbance coefficient (ε=0.025 for RNA and ε=0.02 for DNA)
- b= cuvet length (=1)
- c= concentration

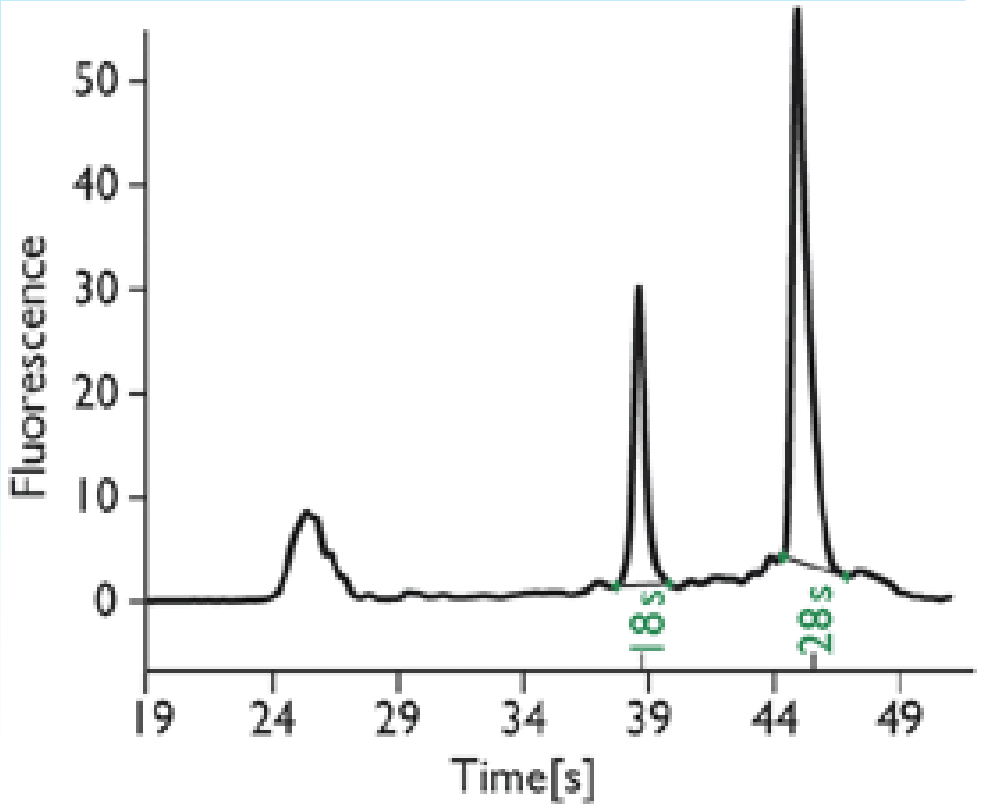
[dsDNA] = A260/ 0.02 (ug/ml) => A260 x 50 (ug/ml)

[RNA] = A260/ 0.025 (ug/ml) => A260 x 40 (ug/ml)

- **A260/A280** ≥ 1.8 (DNA) or ≥ 2.0 (RNA) (lower value indicates protein or phenol contamination)

RNA quality





Agilent 2100 Bioanalyzer Data. Electropherogram of a high quality, eukaryotic, total RNA sample. The 18S and 28S peaks are clearly visible at 39 and 46 seconds, respectively. The microchannels of the Bioanalyzer are filled with a sieving polymer and fluorescence dye.

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TopLab

Why much more trouble with RNase than with DNase in your lab?

DNases

- Degrate DNA
- Present everywhere, especially on hands!

Preparation to work with DNA

- Wear gloves
- Always use sterile, DNase-free disposables
- DNase inactivation by autoclaving or overnight backing at 250 ºC
- Use DNase-free water

RNases

- Degrate RNA
- Present everywhere, especially on hands, bacteria and in dust
- Very stabile enzymes
- Difficult to inactivate
- Rnase do not need a cofactor like DNase

<u>Preperation to work with RNA</u>

- Wear gloves
- Always use sterile, RNase-free disposables
- Rnase-free lab reagent/-material by DEPC treatment or overnight backing at 250 ºC
- Use Rnase-free water

In conclusion

- DNA and RNA isolation for many purposes
- Different DNA/ RNA isolation methods (phenol-extraction, silica, ion exchanger, magnetic beads)
- RNases and DNases