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1. Isolation and purification of nucleic acids

The basic step for further work with DNA and RNA

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FaF MU

Useful links

- BioTechniques
  - The International journal of Life Science Methods
  - http://www.biotechniques.com/
- Reseach Gate
  - http://www.researchgate.net/
- BitesizeBio
  - Brainfood for biolologists
  - http://bitesizebio.com/
- Journal of Visualized Experiments (JoVE)
  - http://www.jove.com/

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The Aim of NA isolation and purification

Is to obtain the NAs in native state from a natural material in a sufficient amount and quality for further analysis

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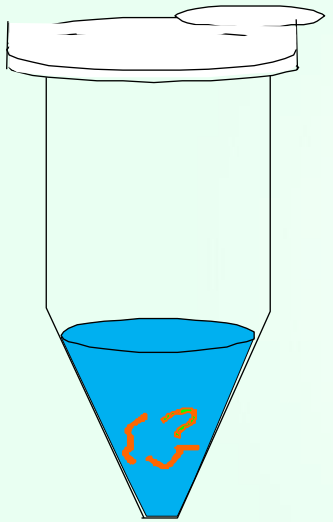

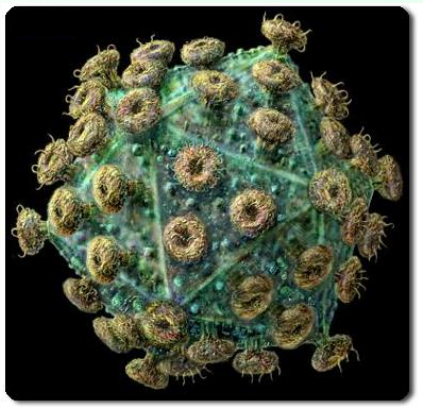

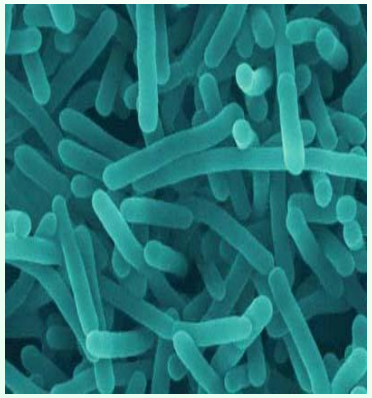

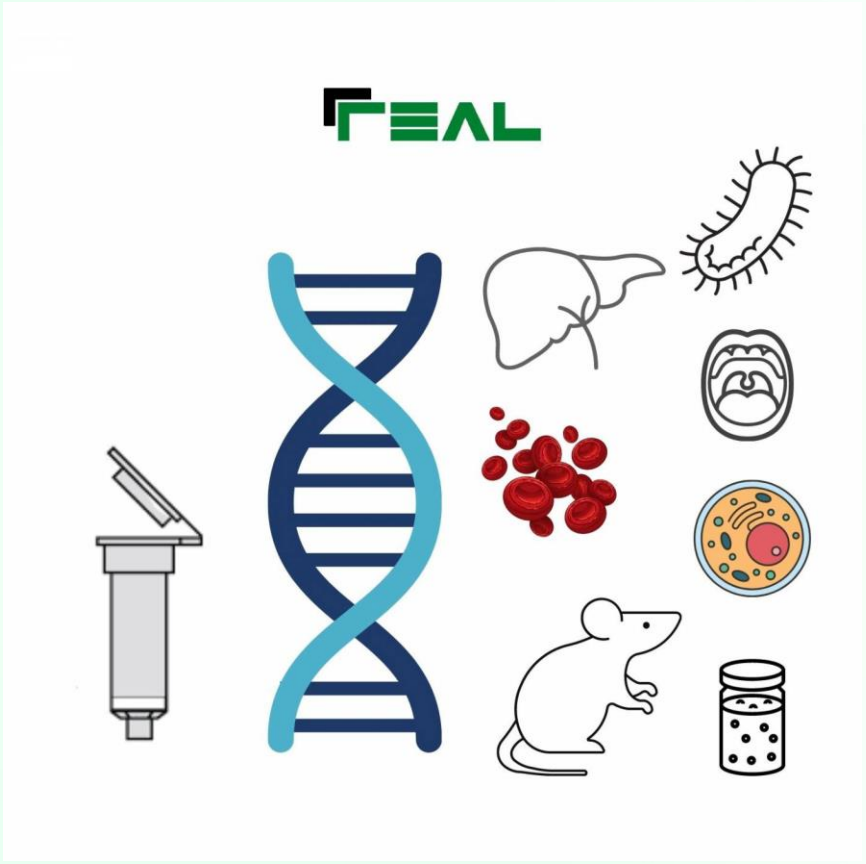

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NAs isolation general steps

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Lysis of cells and tissues

Extraction of NAs

Purification of NAs

Characterisation of the isolated NA

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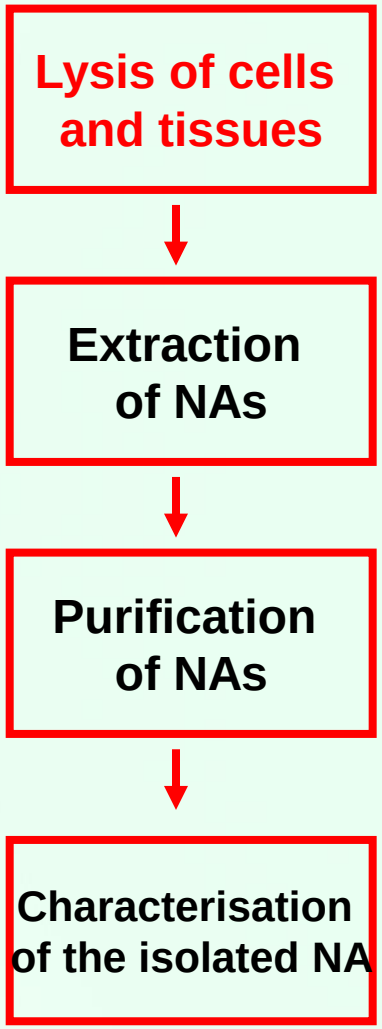
Lysis of cells and tissues
releasing the internal contents of cells



- **Biochemical lysis**
  using enzymes, which degrade cell wall (lysosyme, proteinase K, cellulase …)
- **Chemical lysis**
  detergents (e.g. sodium lauryl sulphate), chelating solutions (EDTA), guanidine salts
- **Physical lysis**
  boiling, freezing, grinding, sonication

Mostly combination of several methods

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Lysis of cells and tissues

releasing internal contents of the cells

Lysis of cells and tissues

↓

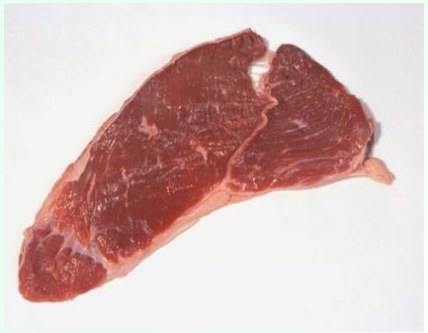
Extraction of NAs

↓

Purification of NAs

↓

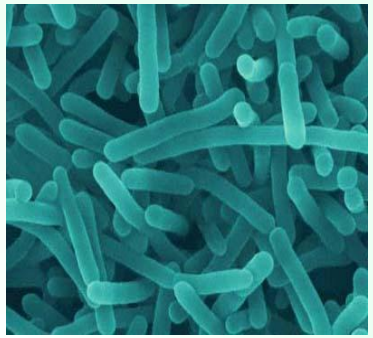
Characterisation of the isolated NA



Homogenisation by grinding

Proteinase K
Lysozyme

Detergents



Boiling

Sonication

Freezing by liquid nitrogen

Homogenisation by grinding

Cellulase

Detergents

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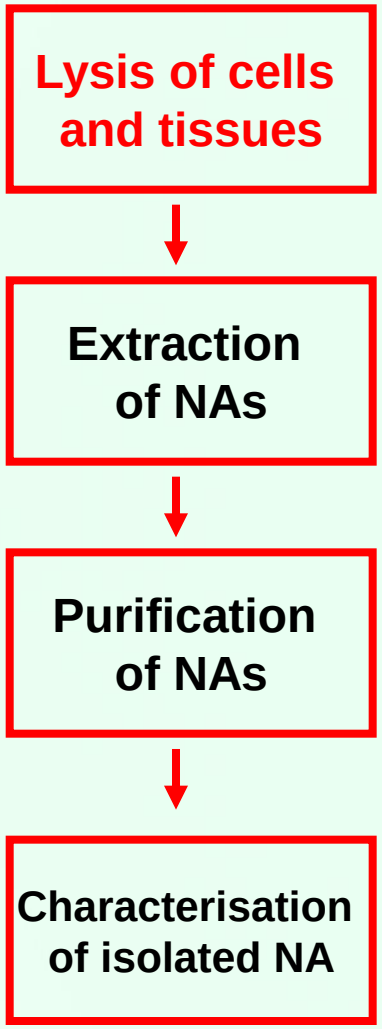
After the cell lysis



Complex mixture

DNA, **RNA**, **lipids**, **proteins**,
saccharides, carbohydrates
and other low-molecular compounds

Next steps:

separate DNA and/or RNA
from the other components

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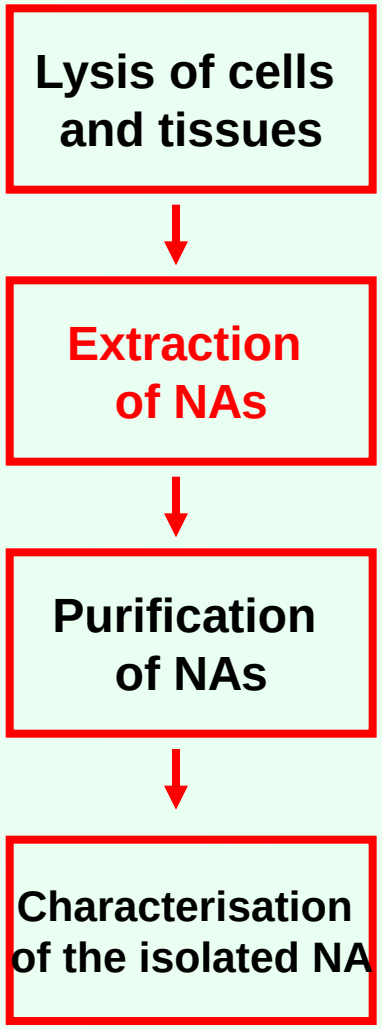
Extraction



Extraction is a purification and separation process, in which one substance passes from the mixture of compounds in liquid or solid phase to another liquid phase, i.e. solvent.

Extraction is suitable for isolation of temperature sensitive substances because it can be carried out at room temperature or under cold conditions.

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Extraction of the phenol - chloroform mixtures

Lysis of cells and tissues

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Extraction of NAs

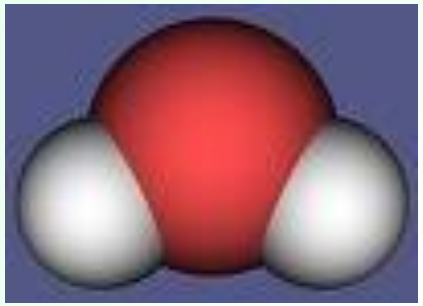
↓

Purification of NAs

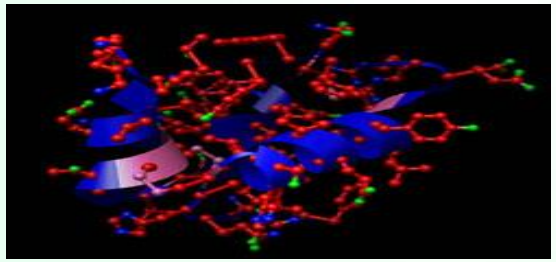
↓

Characterisation of the isolated NA

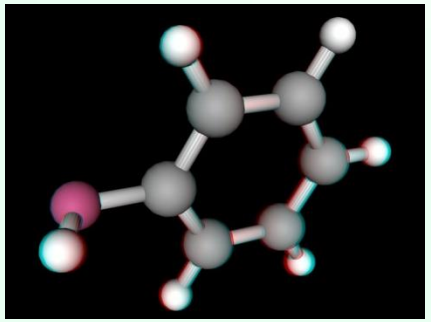
Separation of proteins and NA based on



light water



protein denaturation at the interphase



heavier organic phases

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General steps in phenol extraction

Lysis of cells and tissues

↓

Extraction of NAs

↓

Purification of NAs

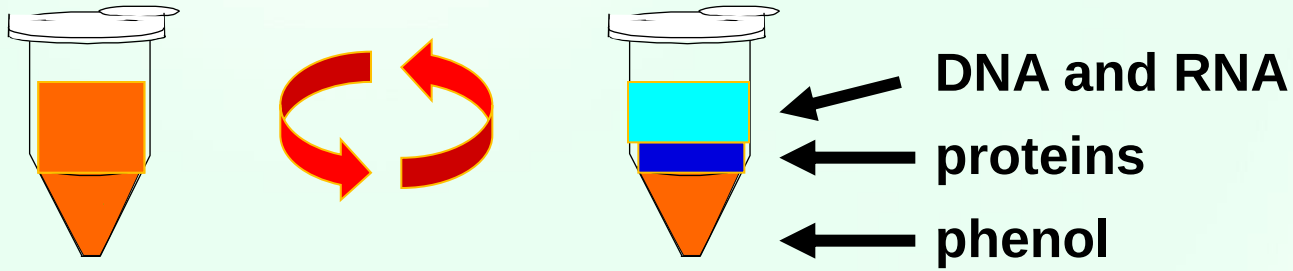
↓

Characterisation of the isolated NA

1) Disruption of cell membranes

2) Denaturation of proteins and lipids – phenol, chlorophorm

3) Separation of individual phases by centrifugation – organic layer (phenol), interphase (proteins and rest of the cells), water (NA)



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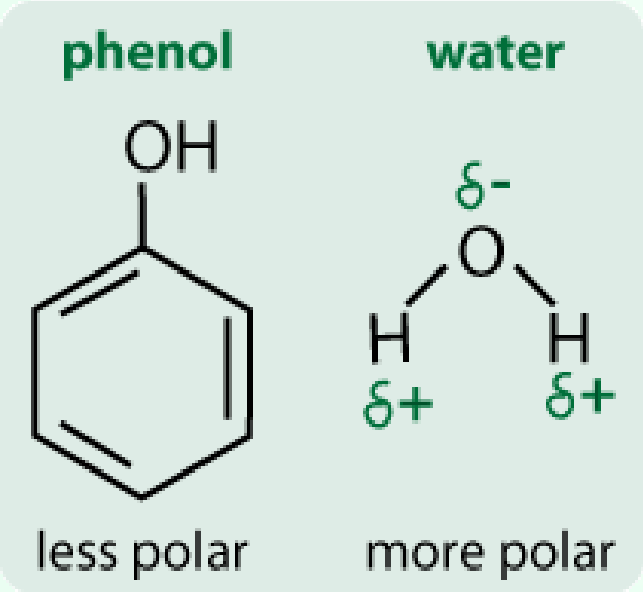
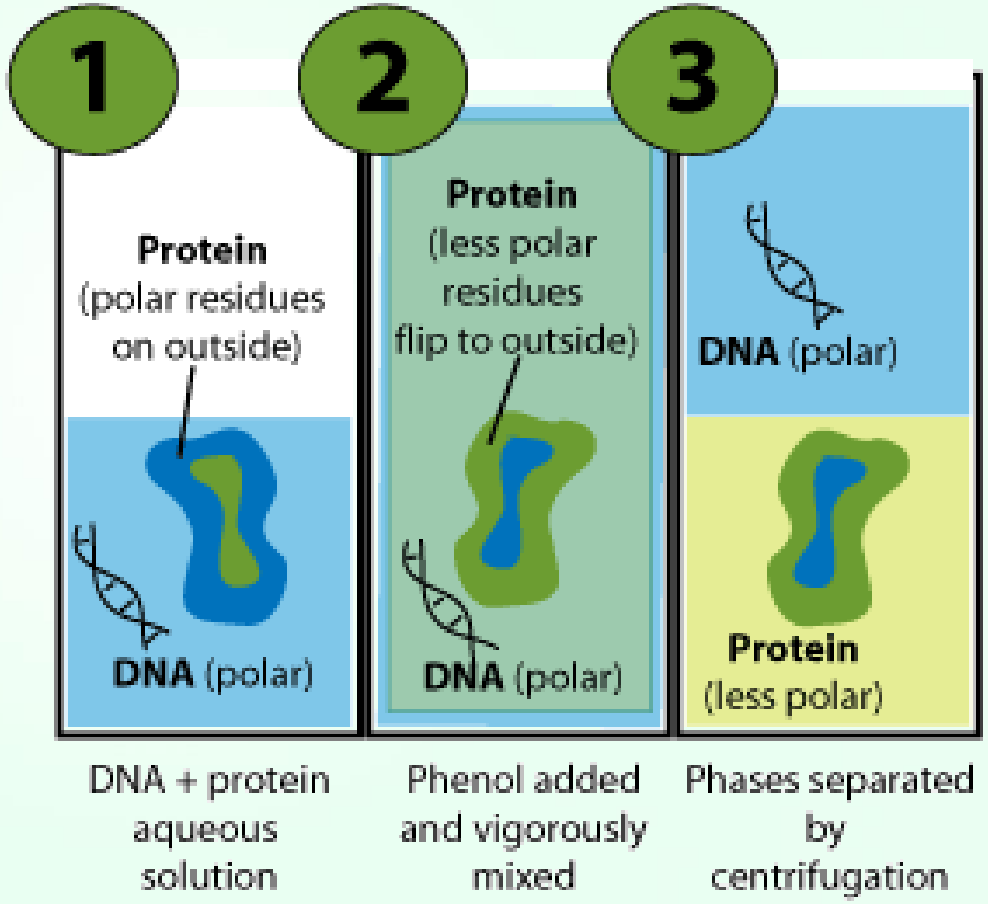
Principle of phenol extraction





http://bitesizebio.com/

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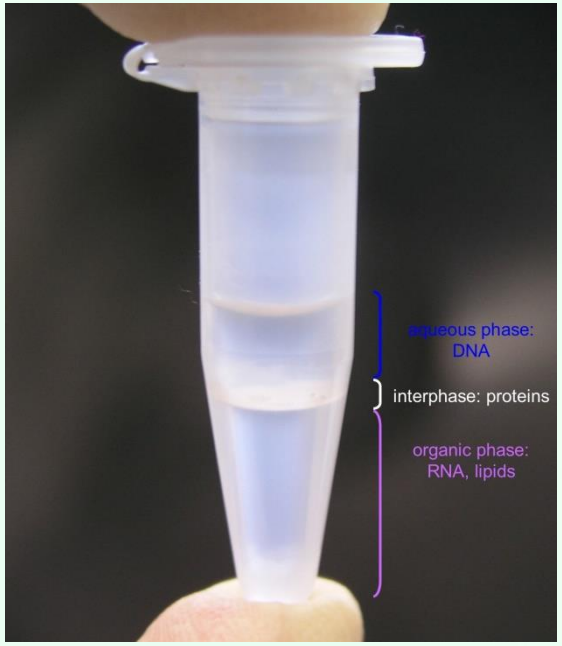
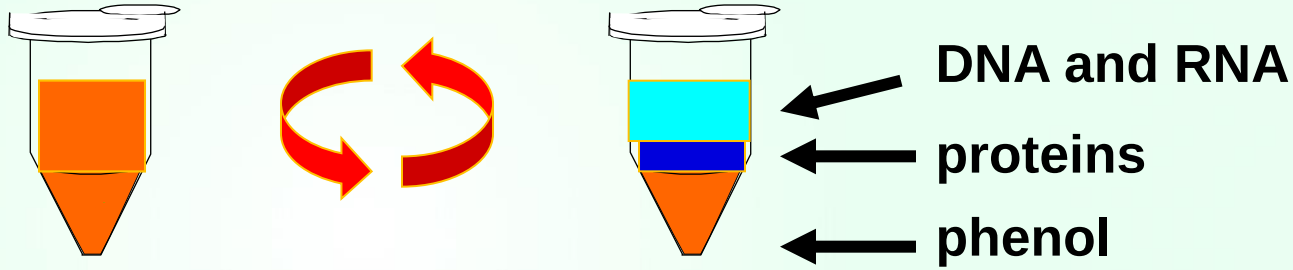
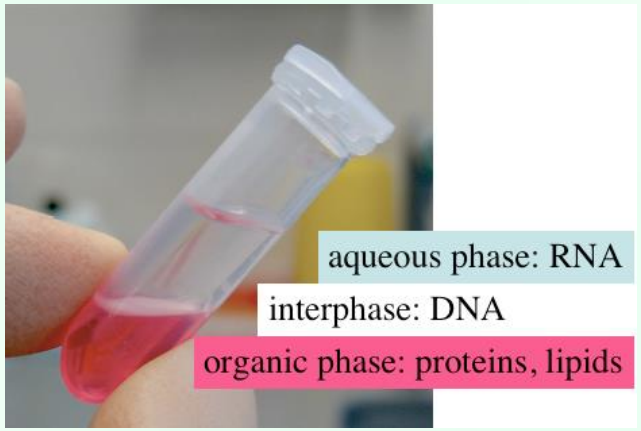
Phenol extraction modifications

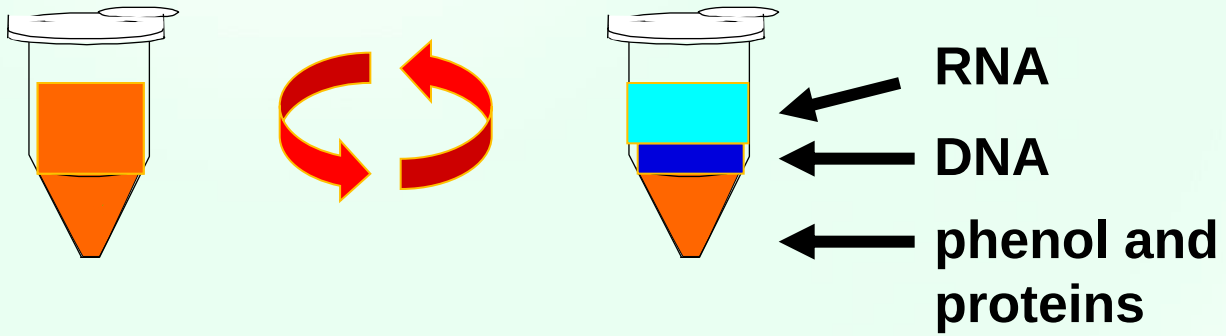


Phenol equilibrated with neutral or alkaline buffer





The use of acid phenol



http://openwetware.org/

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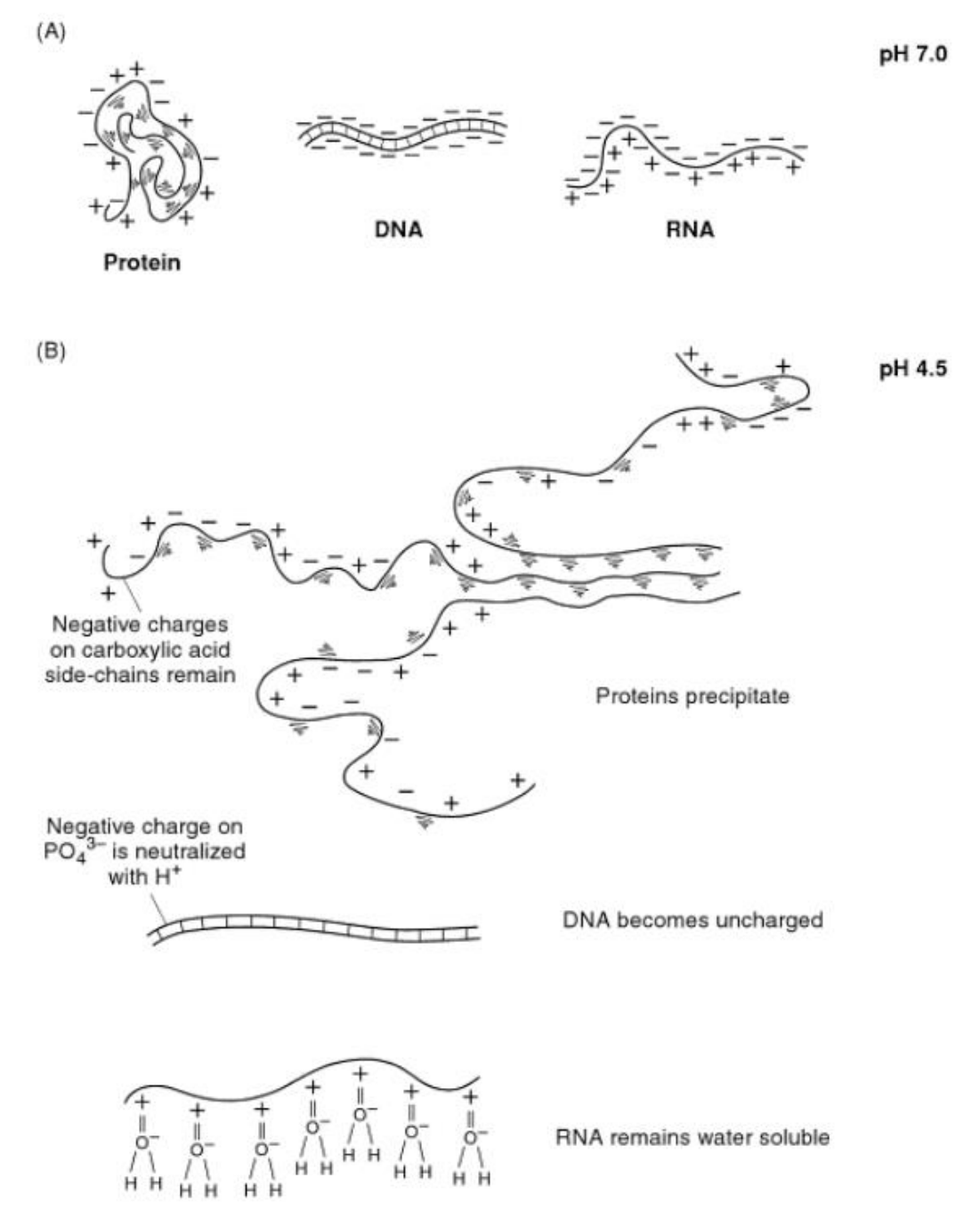
Application of acid phenol



P. Zumbo, WEILL CORNELL MEDICAL COLLEGE

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After extraction

Lysis of cells and tissues

↓

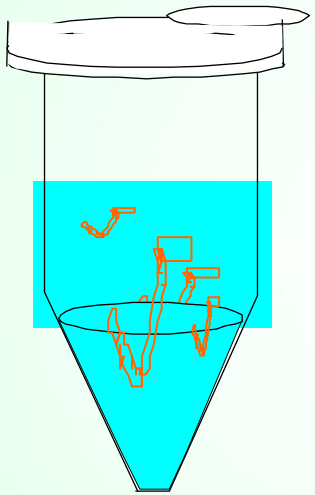
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



Strongly diluted DNA, RNA

Traces of chloroform

Traces of phenol

Next steps:

Concentration and purification of NAs

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Purification of NAs by precipitation

Lysis of cells and tissues

↓

Extraction of NAs

↓

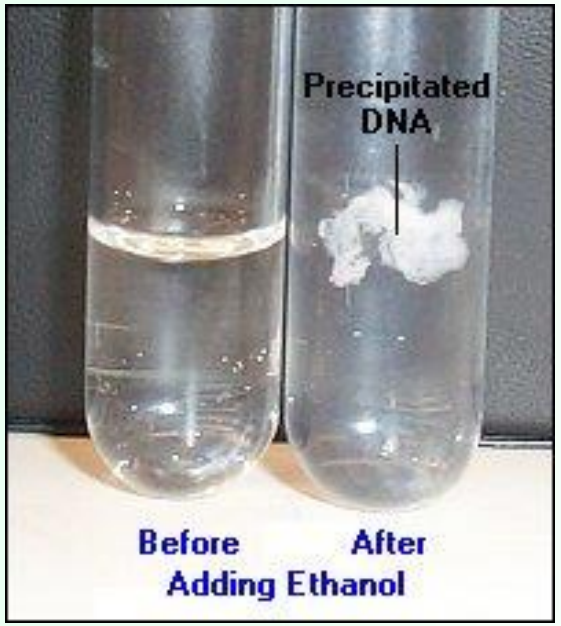
Purification of NAs

↓

Characterisation of the isolated NA

Precipitation is one of the basic methods for isolation and concentration of biological macromolecules

- **A certain amount of the precipitating agent (ammonium sulphate, ethanol, acetone, etc.) is added to the solution containing the desired macromolecule. Macromolecules are precipitated without denaturation**
- **<u>Later they can be dissolved again and used in their natural, biologically active state</u>**



http://www.vivo.colostate.edu

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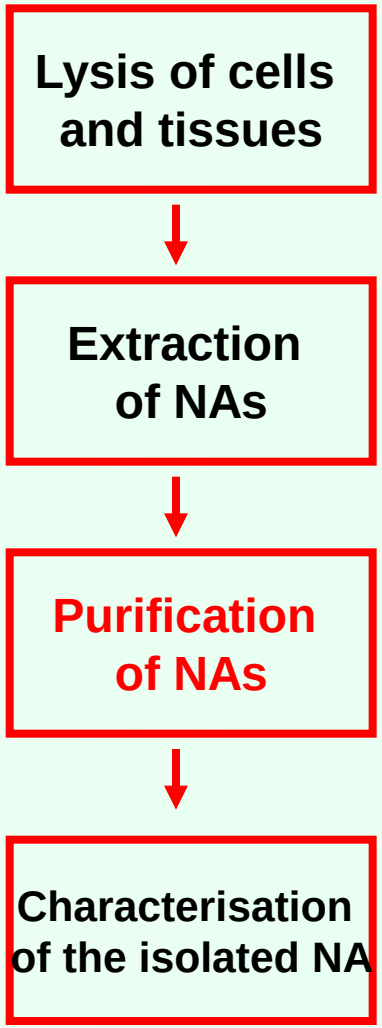
General steps in the purification of ethanol



1) Adding ethanol or isopropanol

2) Adding monovalent ions (K^+, Na^+…)

3) Sample concentration by centrifugation (sample is cooled down to -70ºC)

4) Sediment (NAs) washing by 70% ethanol

5) NAs solution in water

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Solubility of DNA in water

Lysis of cells and tissues

↓

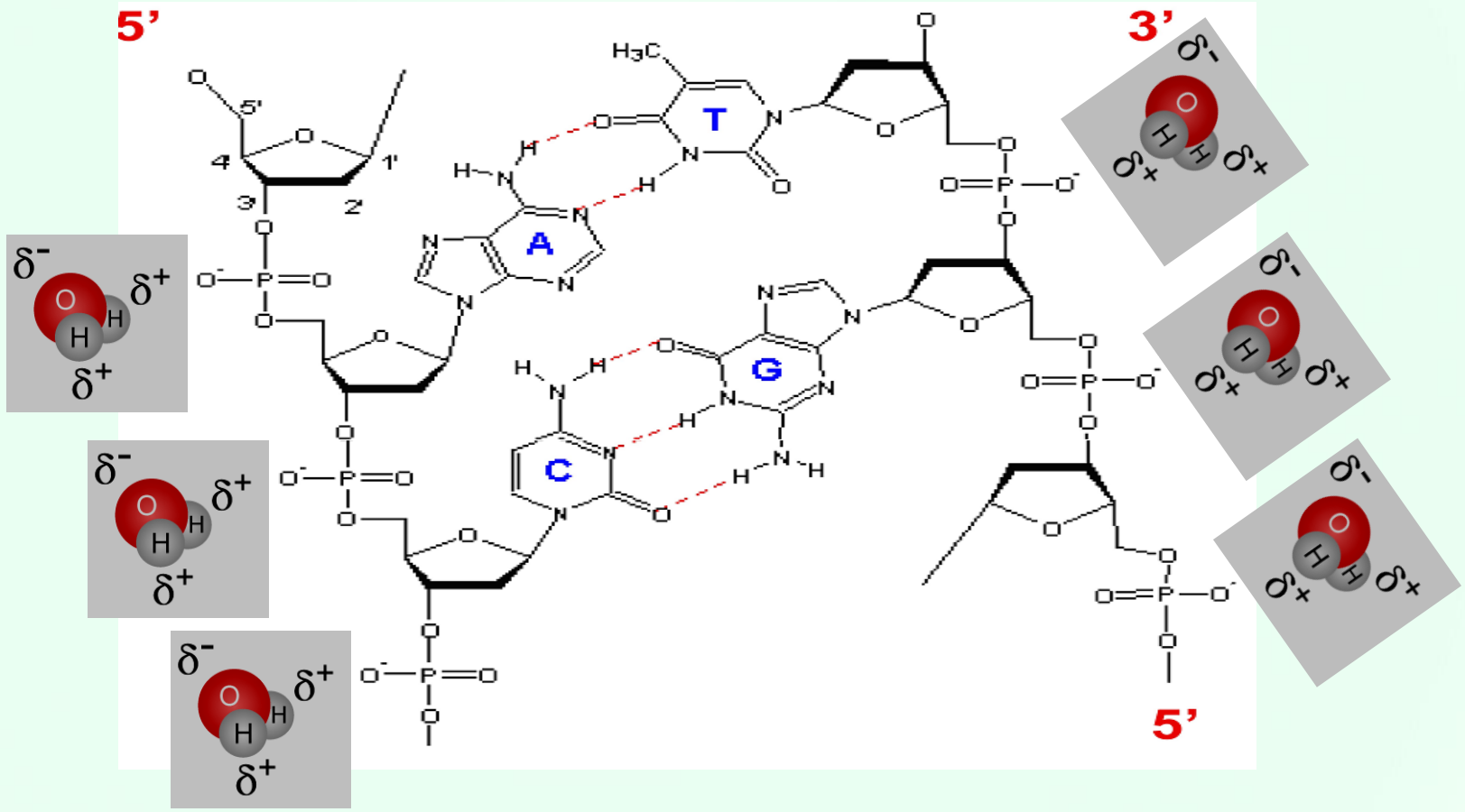
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



DNA IS HYDROPHILIC

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General steps in the purification of ethanol

adding of NaCl + C_2H_5OH

Lysis of cells and tissues

↓

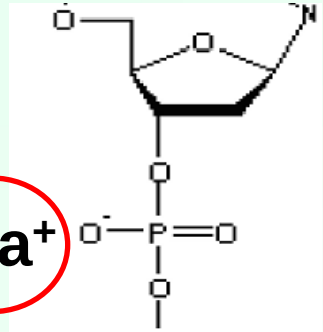
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



→ Lowering the DNA hydrophility

↓

PRECIPITATION OF DNA in water solution

Neutralisation of PO3-

↑

Na^+

↑

Cl^- ← **NaCl** → C_2H_5OH

Low dielectric constant
Low solubility

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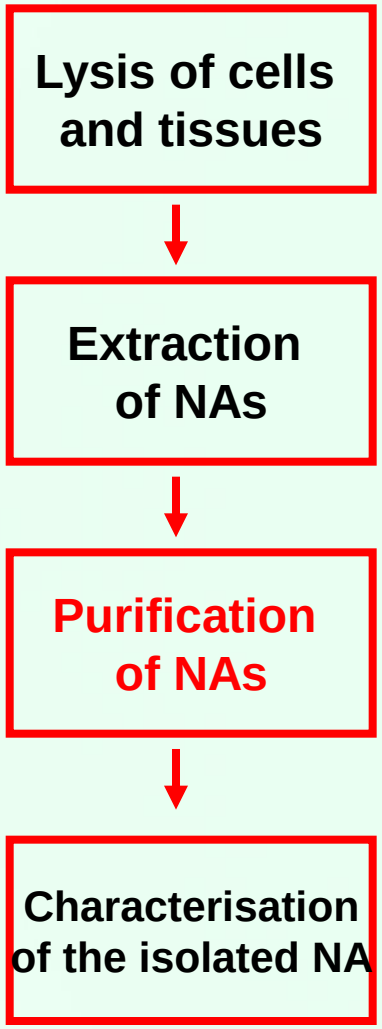
General steps in precipitation - FINISHING



3) Sample concentration by centrifugation (sample is cooled down to -70ºC)

4) Washing the sedimented DNA to eliminate the traces of salts by 70% ethanol and evaporation of the ethanol by warming

5) Dilution of NA in water (adding the EDTA or Tris-HCl)

Native NA concentrated in a small volume of water solution

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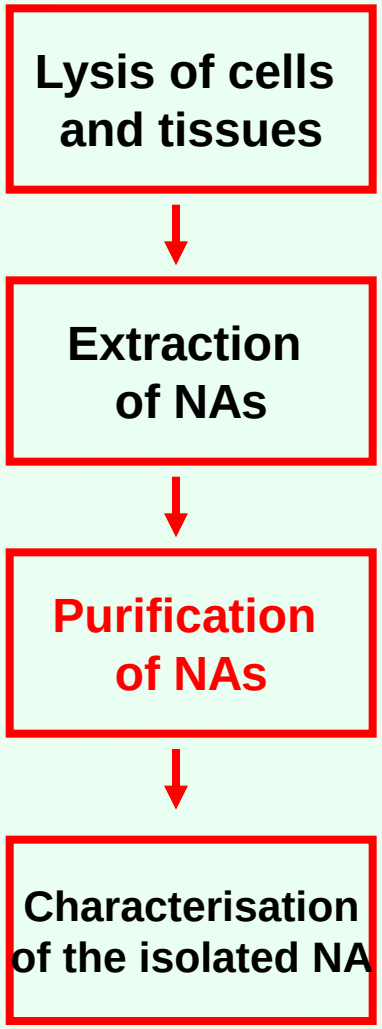
Purification of NA by chromatography



- Preparative affinity column chromatography
- Column contains a sorbent (matrix) that is able to specifically bind NAs
- It is used to prepare greater amounts of purified molecules

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Purification of NA by chromatography

Lysis of cells and tissues

↓

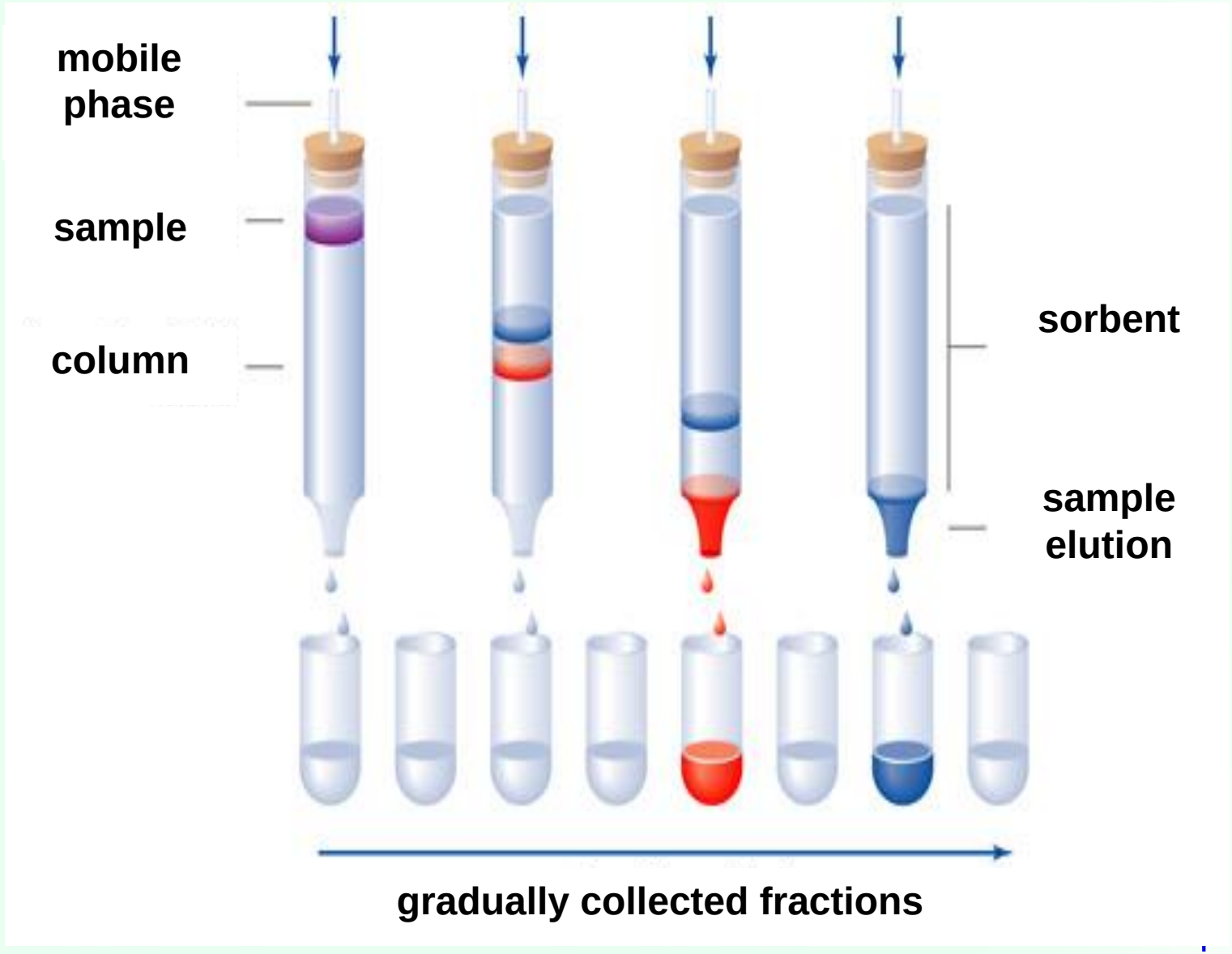
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



Commercial chromatography columns for NAs isolation - spin columns

Lysis of cells and tissues

↓

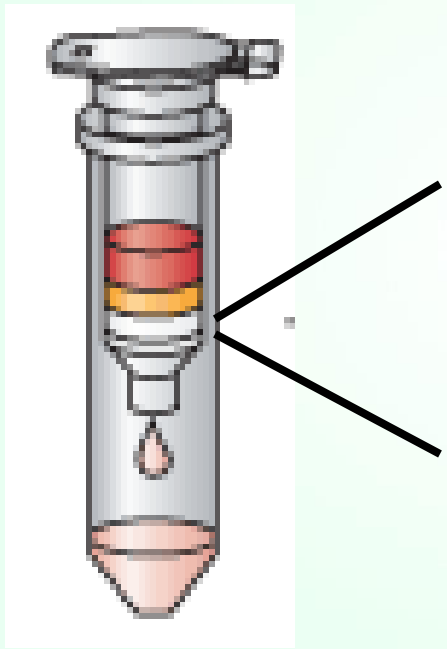
Extraction of NAs

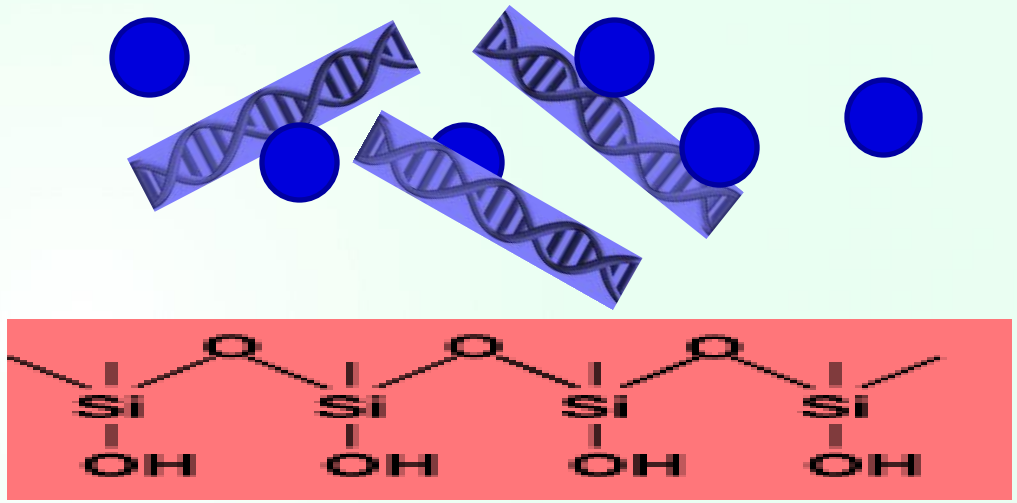
↓

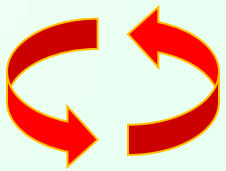
Purification of NAs

↓

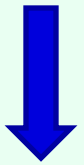
Characterisation of the isolated NA







silica columns



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Commercial chromatography columns for NAs isolation - spin columns

Lysis of cells and tissues

↓

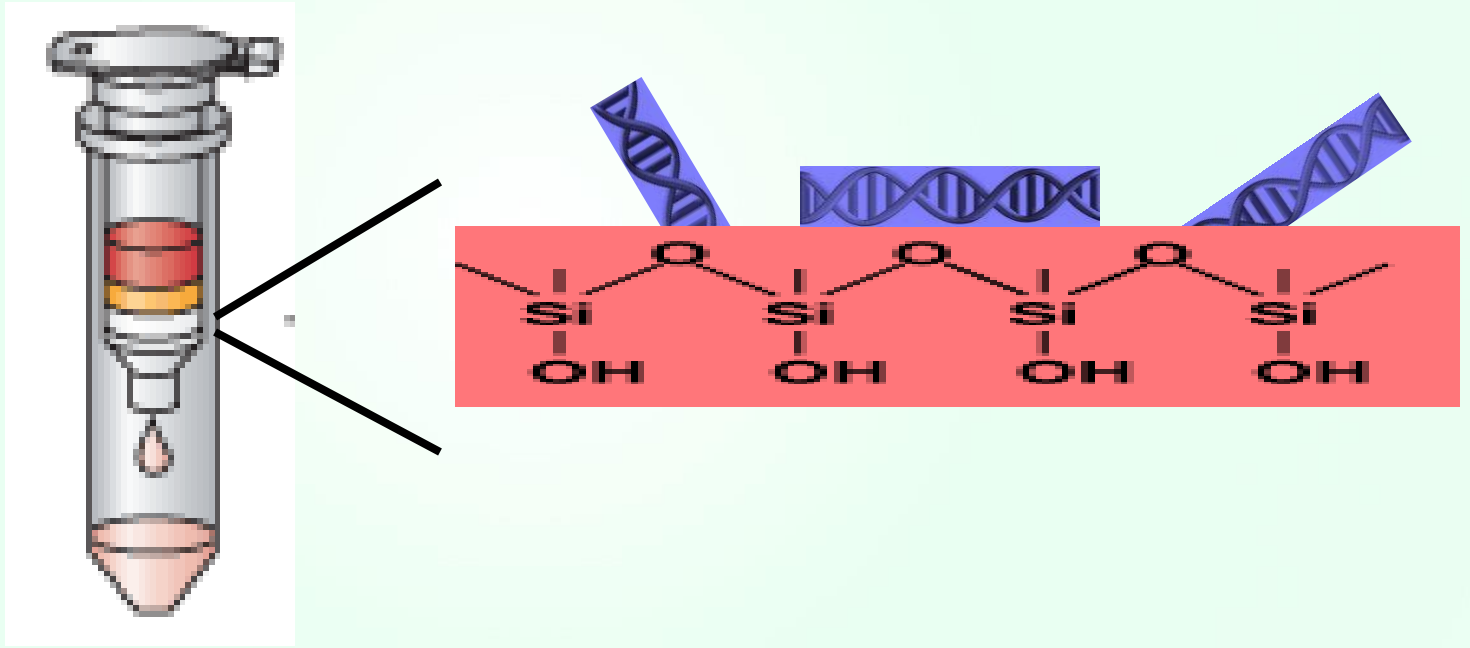
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



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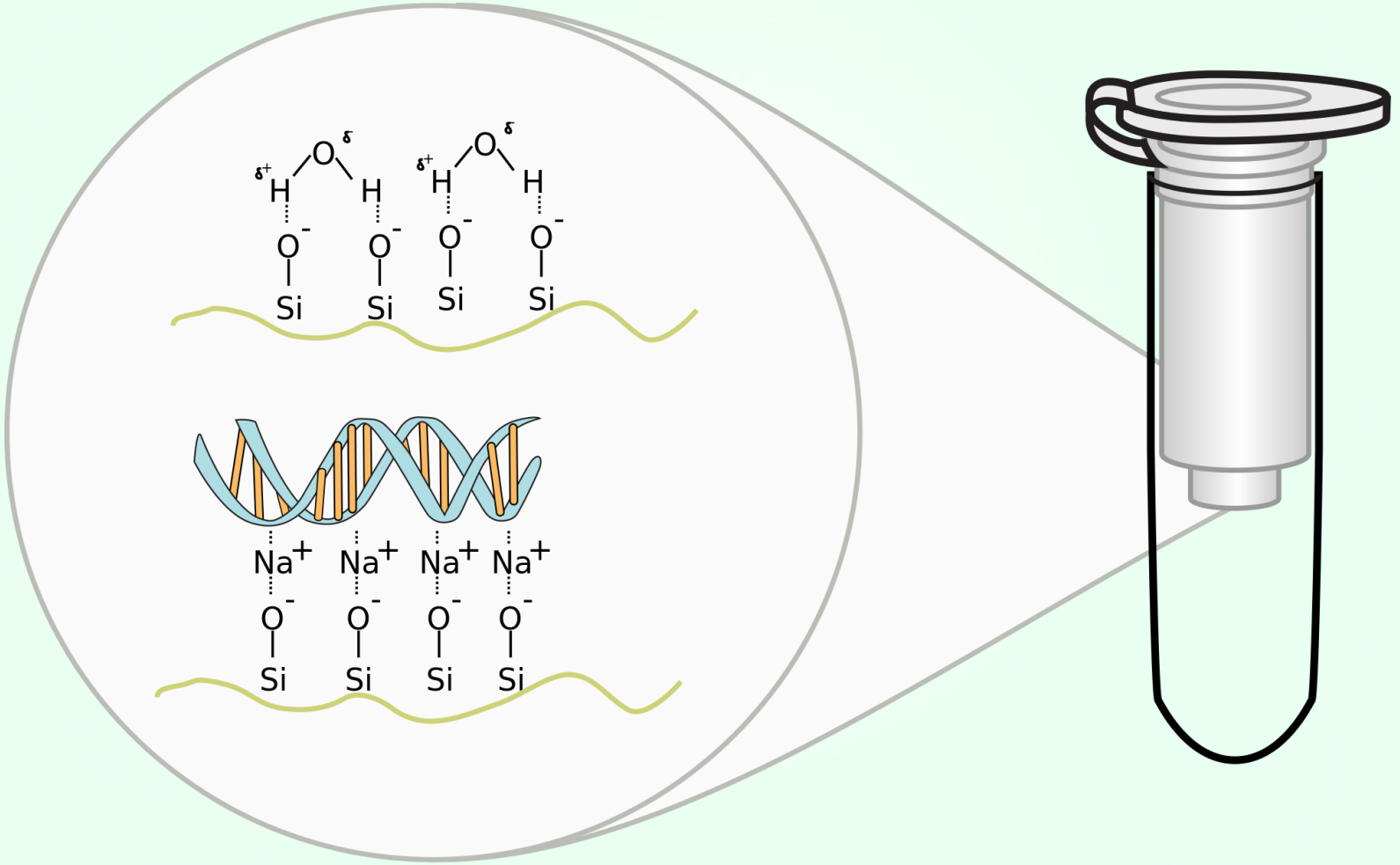
How DNA is bound on membrane



en.wikipedia.org

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Commercial chromatography columns for NAs isolation - example

Lysis of cells and tissues

↓

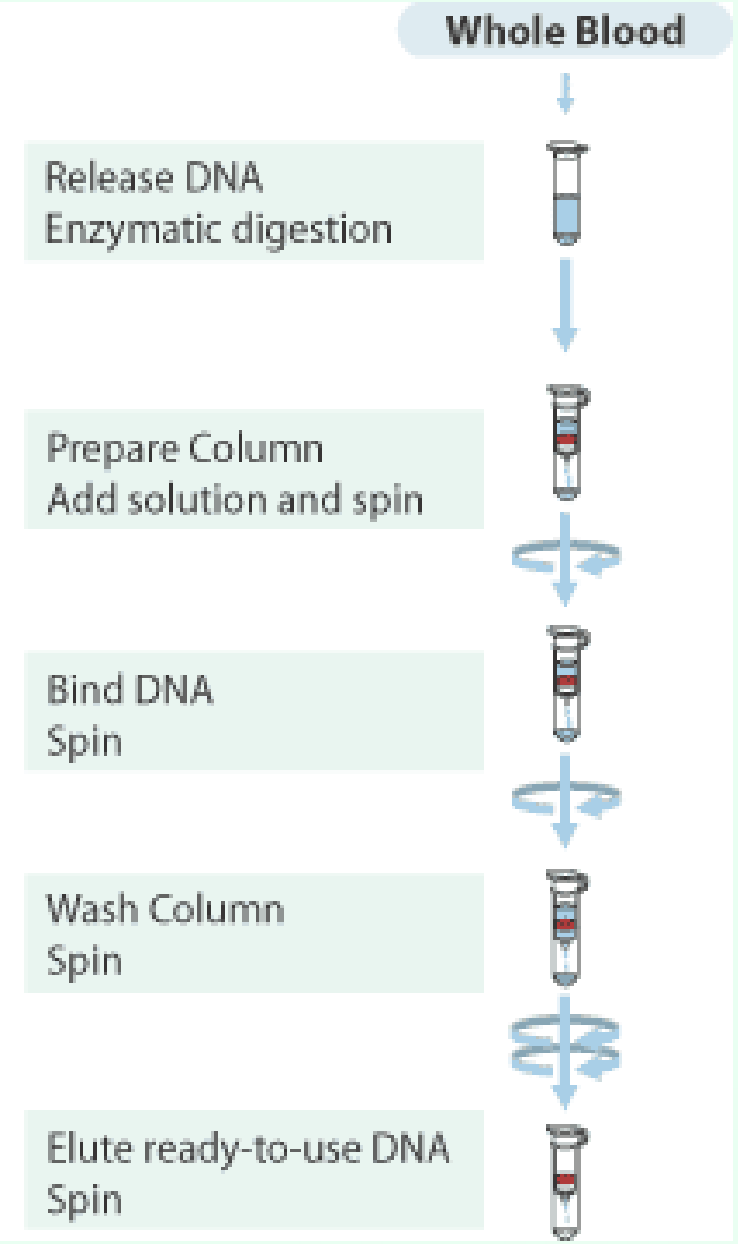
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



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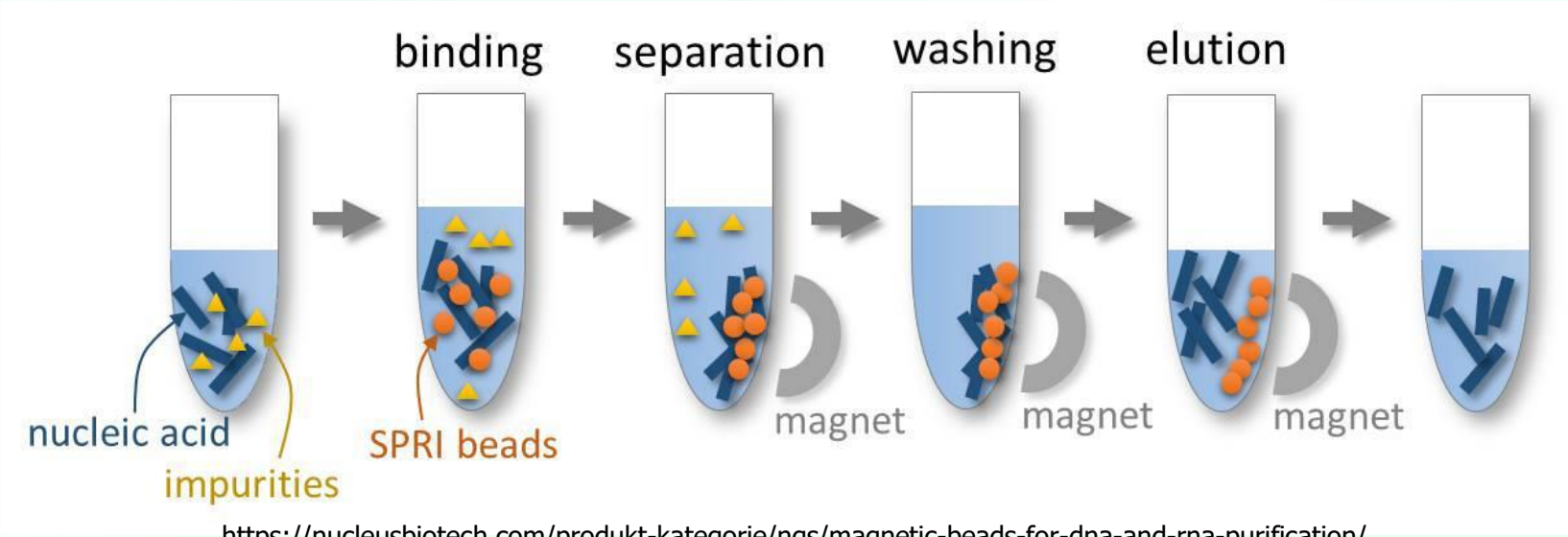
DNA isolation by magnetic beads

- DNA is bound on the surface of magnetic beads
- The surface of beads is coated by:
  - Ion-exchange polymer, e.g. Diethylaminoethyl (DEAE)
  - Silica



https://nucleusbiotech.com/produkt-kategorie/ngs/magnetic-beads-for-dna-and-rna-purification/

http://www.diagenode.com/

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Principle and Procedure

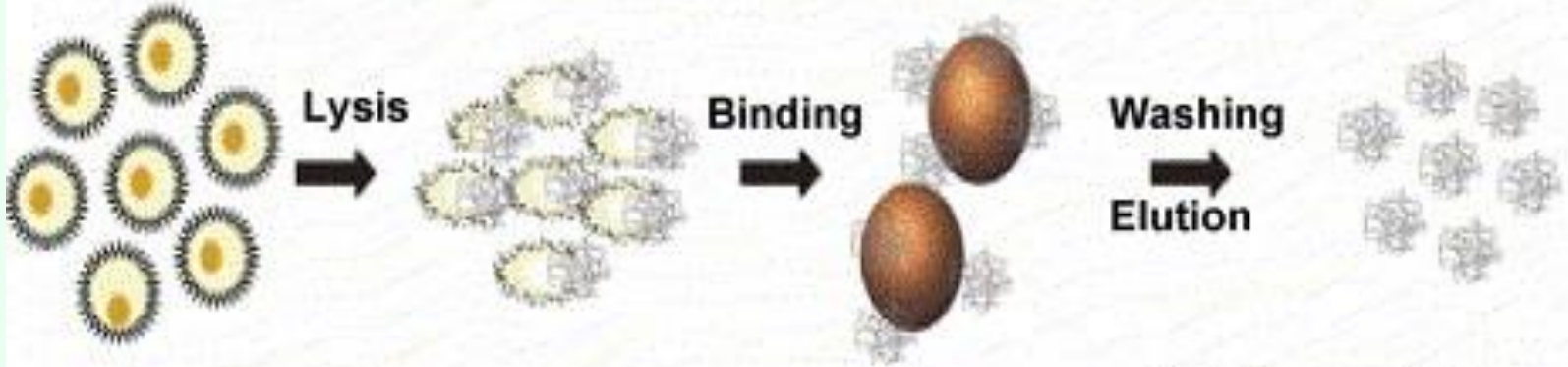
Addition of Lysis and Binding Solution

Addition of Magnetic Beads

DNA-binding to silica in the presence of chaotropic salt

Elution of DNA in Water

Collection of beads by applying a magnetic force



DNA is „ready for use“

Purification of DNA by magnetic separation

- **+ Chaotropic solution (NaJ/guanidinium hydrochloride/guanidinium thiocyanate) → - removed unbound components**
- **+ ethanol → - removed chaotropic reagents**
- **+ water → - removed ethanol**

http://www.fsijournal.org/

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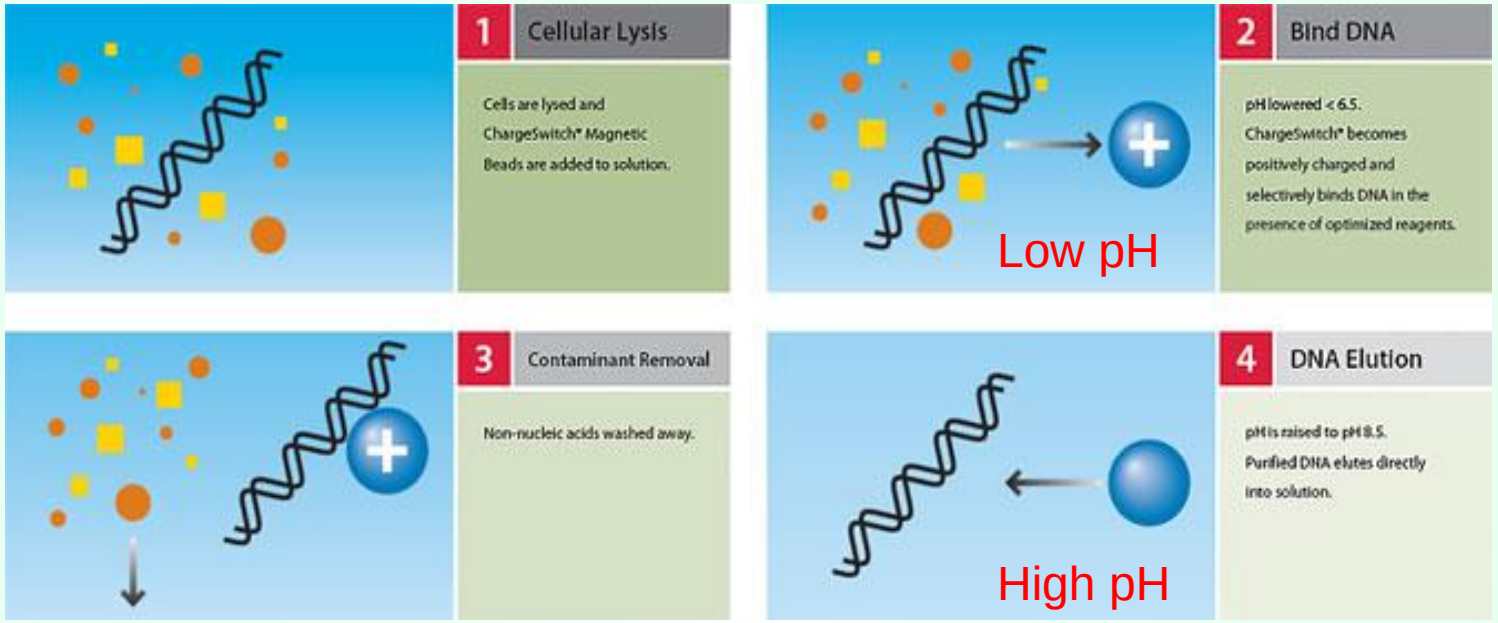
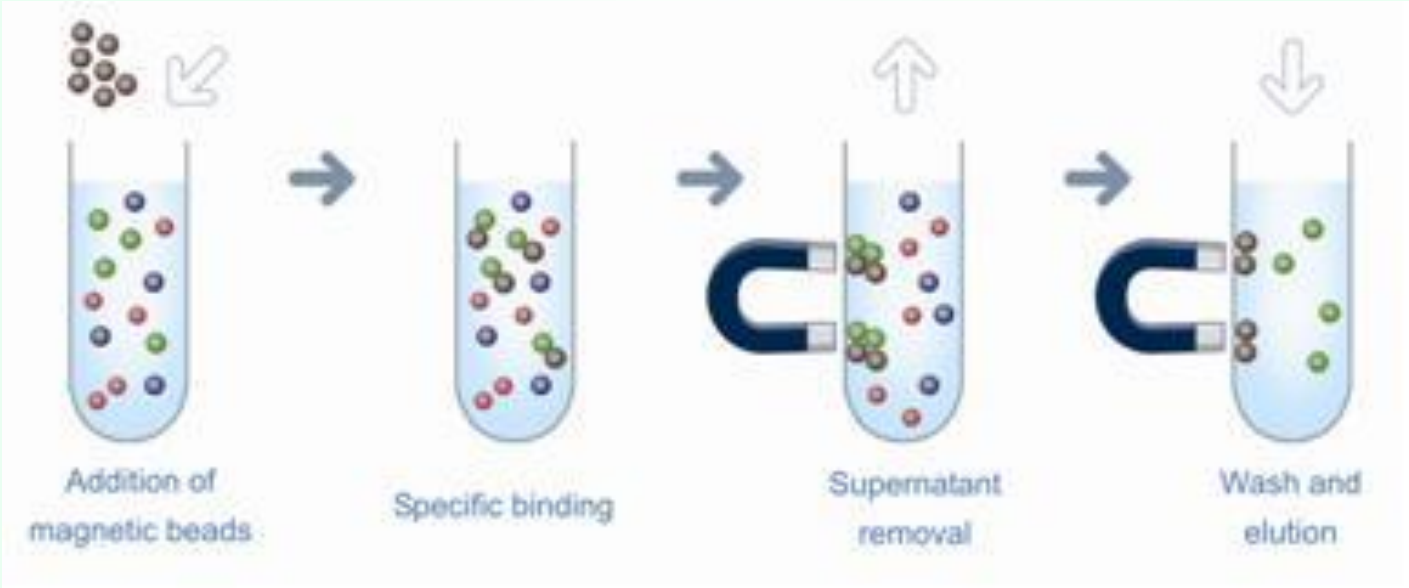
Magnetic beads - protocol



http://bitesizebio.s3.amazonaws.com/



http://blog.labplanet.com/

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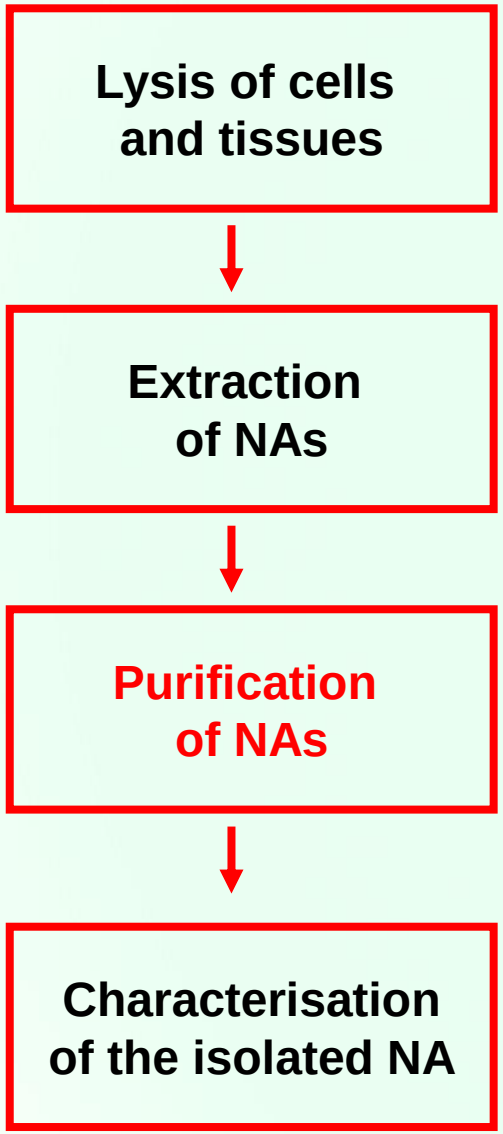
Plasmid isolation by alkaline denaturation



- One of the method for separation of plasmid molecules from the chromosomal DNA in bacterial cells extracts
- It uses different sensitivity of DNA strands for denaturation in high alkaline pH solutions according to conformation of the strands and their state
- For plasmid isolation can also be used the commercial "spin column" processes

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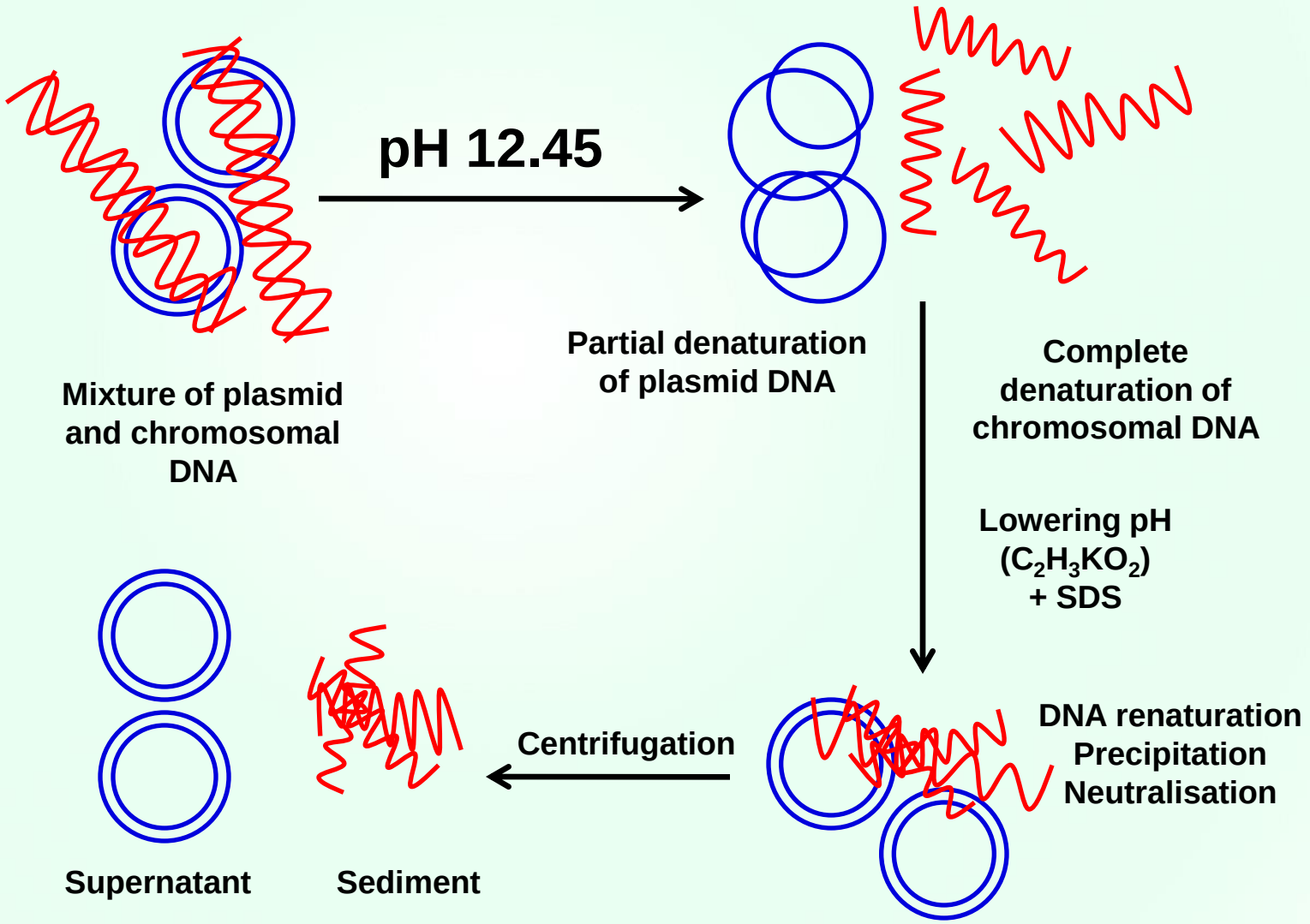
Plasmid isolation by alkaline denaturation the principle of the method

Lysis of cells and tissues

Extraction of NAs

Purification of NAs

Characterisation of the isolated NA



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Characterisation of isolated DNA

Lysis of cells and tissues

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Extraction of NAs

↓

Purification of NAs

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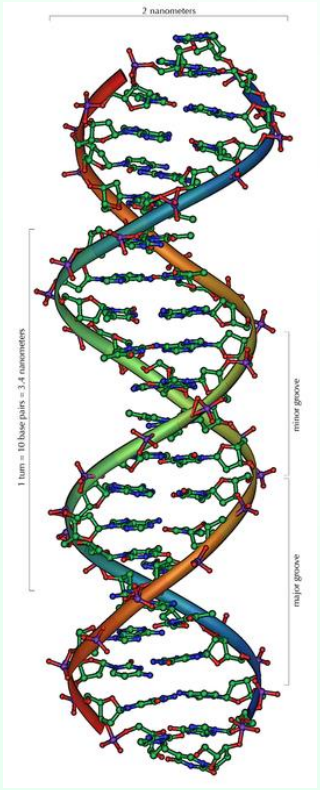
Characterisation of the isolated NA

The two of important characteristics of the isolated NAs are



Concentration

Purity

Characterisation of NAs by spectrophotometry

Lysis of cells and tissues

↓

Extraction of NAs

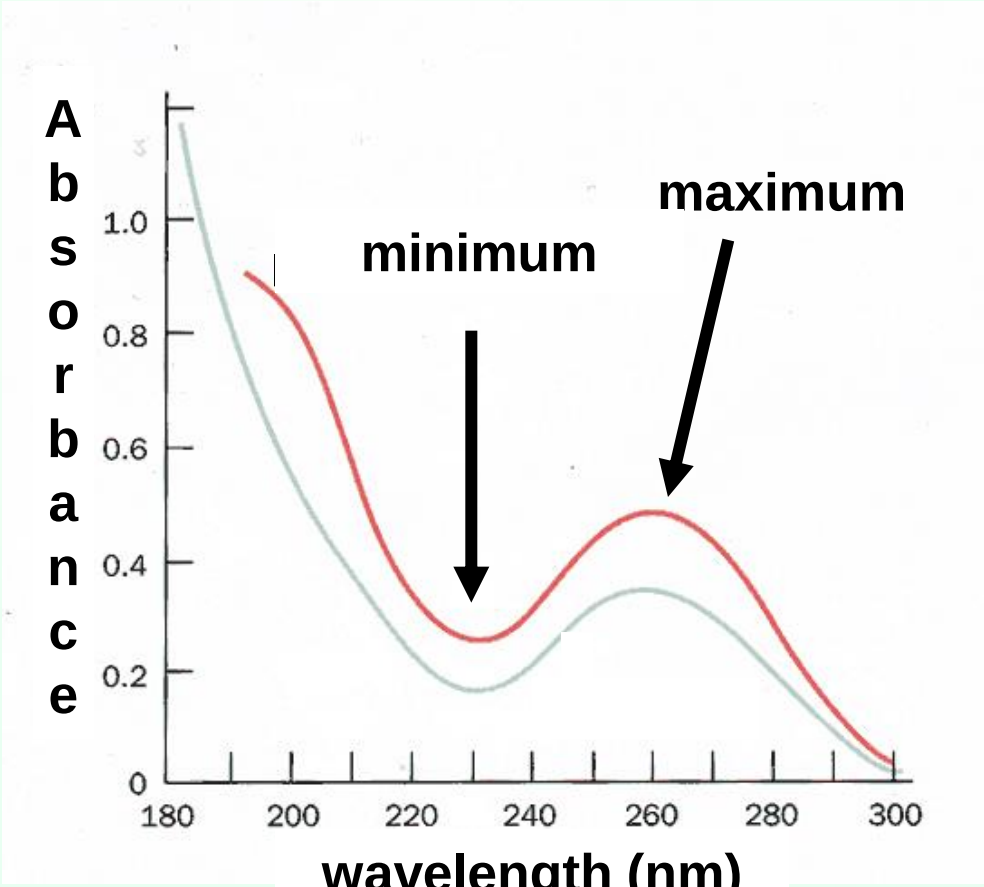
↓

Purification of NAs

↓

Characterisation of the isolated NA

- NAs absorb UV light with maximum at 260 nm



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Characterization of NAs by spectrophotometry

Lysis of cells and tissues

↓

Extraction of NAs

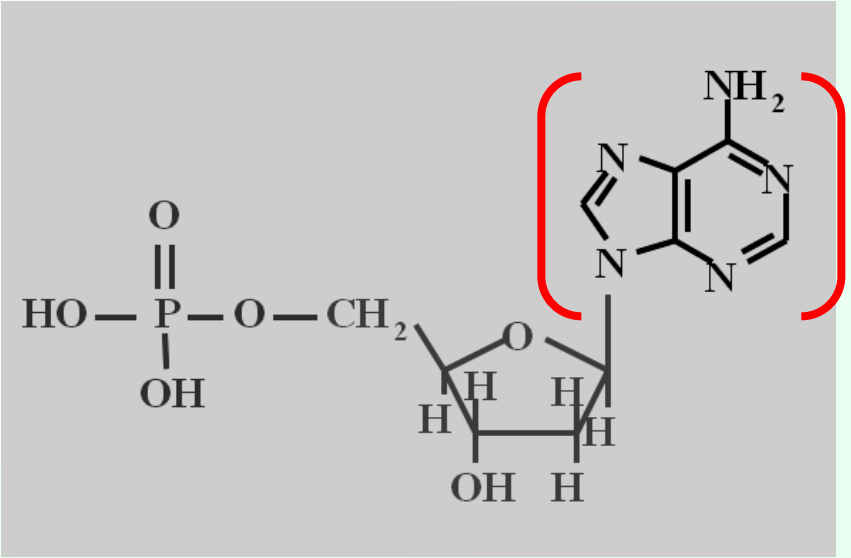
↓

Purification of NAs

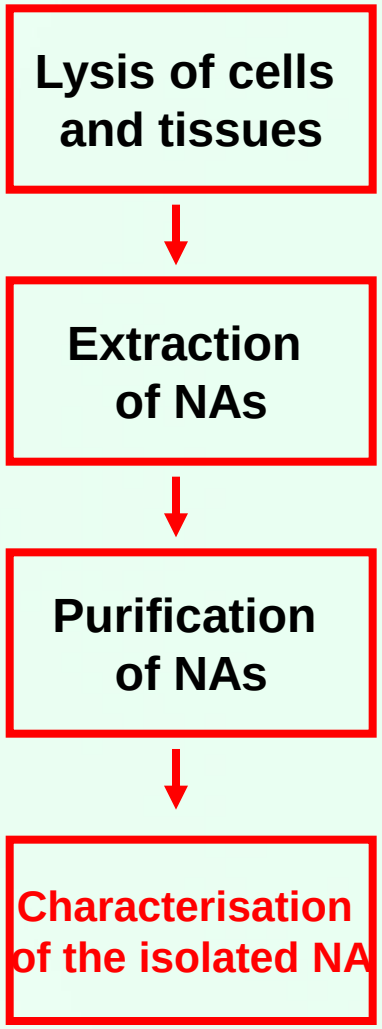
↓

Characterisation of the isolated NA

By which part the DNA absorbs the UV light?



Characterization of NAs by spectrophotometry



- Optical density corresponds to concentration
- Absorbation is measured at different wavelengths (230 – 320 nm)
- Ratio of absorbance = purity of sample

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Optical density corresponds to concentration

Lysis of cells and tissues

↓

Extraction of NAs

↓

Purification of NAs

↓

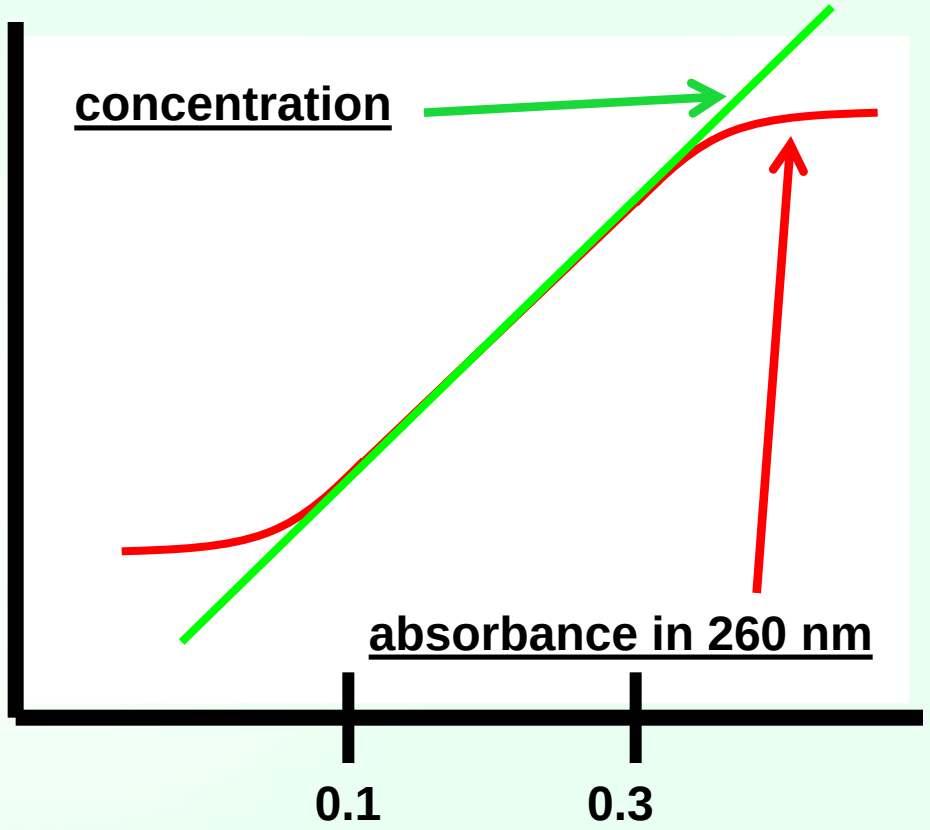
Characterisation of the isolated NA

A_{260} = 1.0 (in 1 cm cuvette)

dsDNA ~ 50 µg/ml

ssDNA ~ 33 µg/ml

ssRNA ~ 40 µg/ml



Dependence of absorption on concentration is linear in A_{260} = 0.1 – 0.3

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Low concentration of DNA

Lysis of cells and tissues

↓

Extraction of NAs

↓

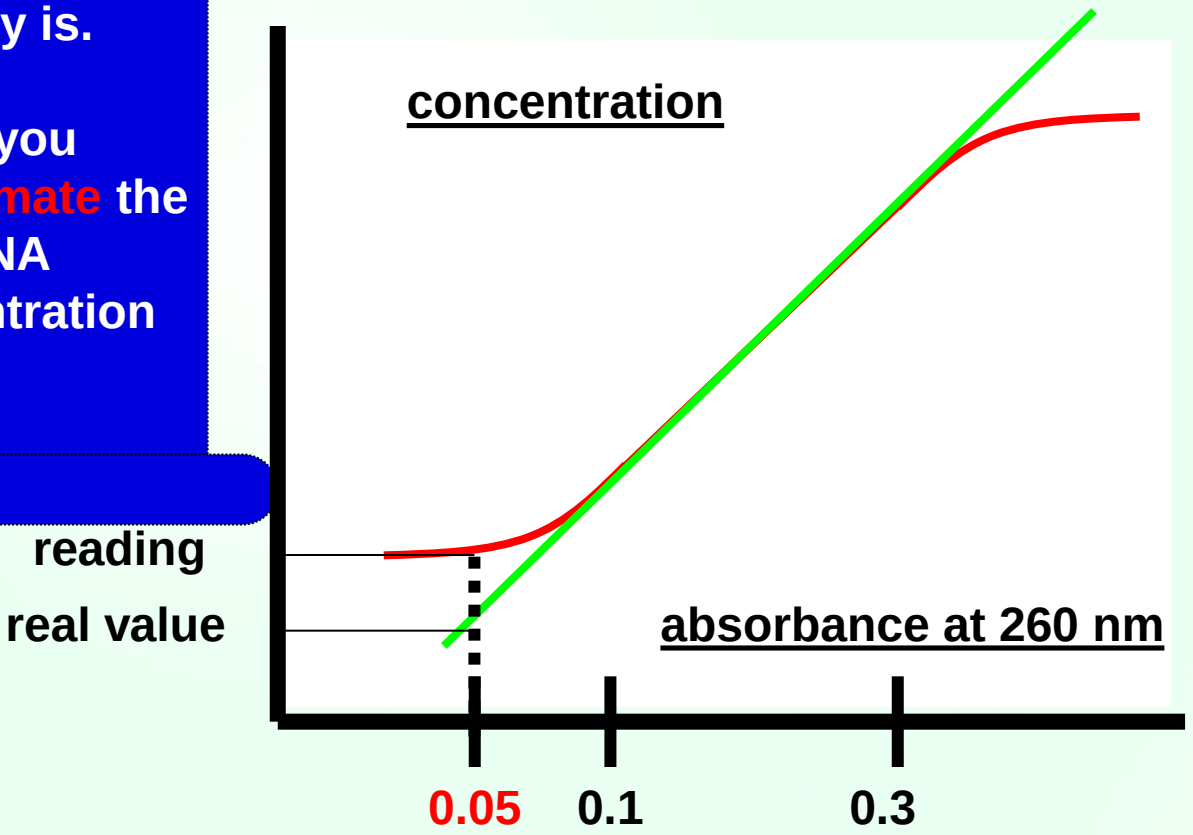
Purification of NAs

↓

Characterisation of the isolated NA

If the absorbance is LOW (below 0.1), you will read the concentration HIGHER than it really is.

So you overestimate the DNA concentration



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High concentration of DNA

Lysis of cells and tissues

↓

Extraction of NAs

↓

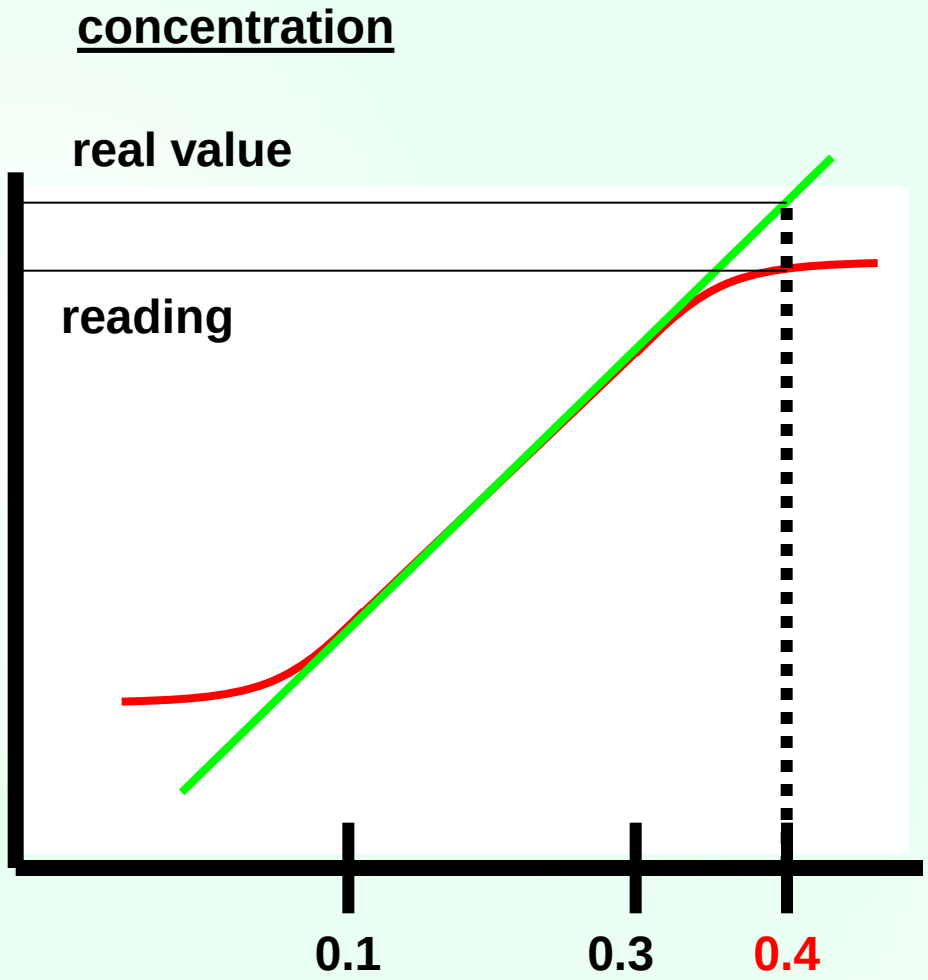
Purification of NAs

↓

Characterisation of the isolated NA

If the absorbance is HIGH (over 0.3), you will read the concentration LOWER than it really is.

So you **underestimate** the DNA concentration



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Purity of DNA

Lysis of cells and tissues

↓

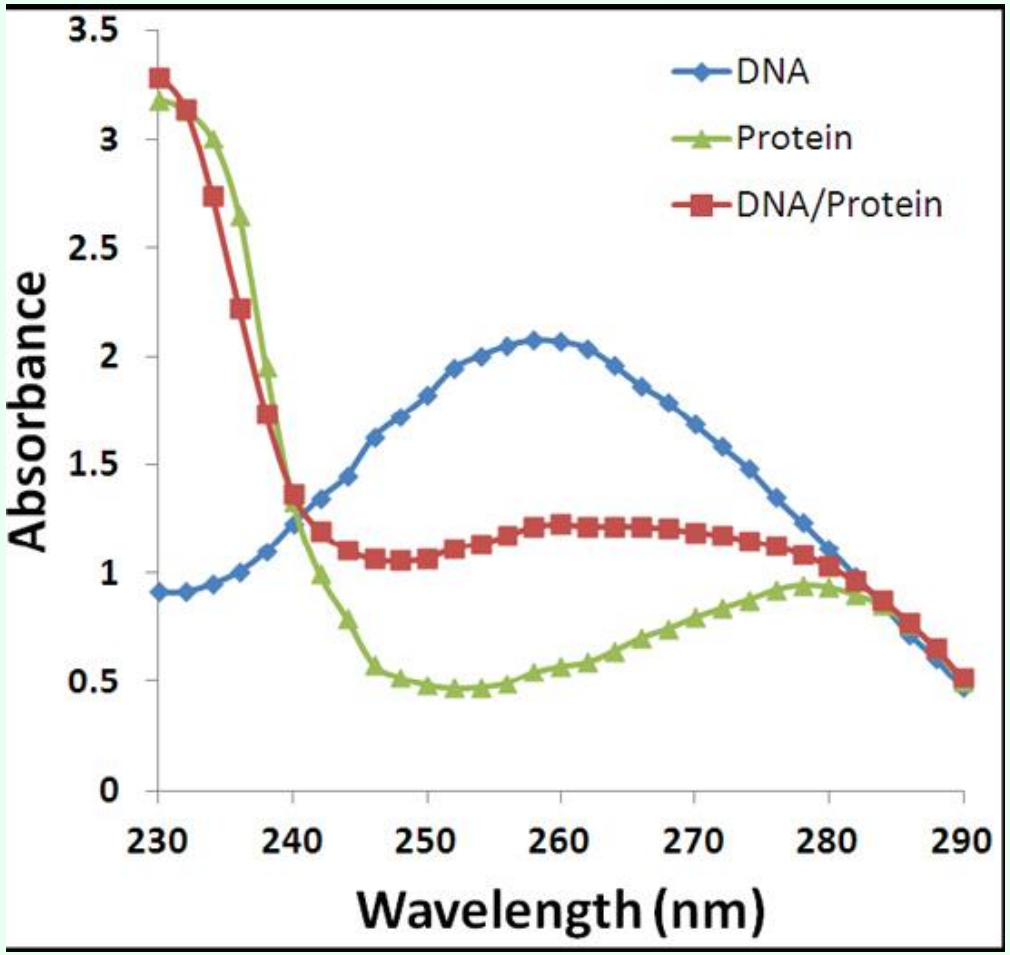
Extraction of NAs

↓

Purification of NAs

↓

Characterisation of the isolated NA



http://www.biotek.com

Is determined according to the ratio of absorptions at different wavelengths

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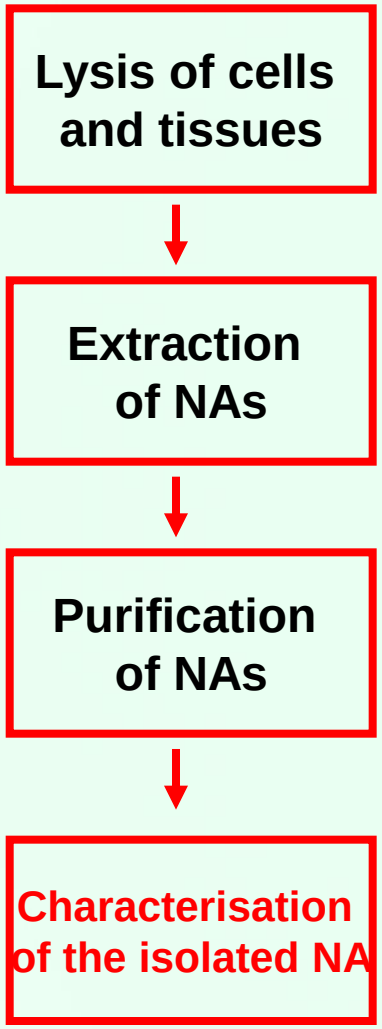
Purity of DNA



$A_{260/A280} = 1.8$

< 1.8 = contamination by proteins

> 1.8 = contamination by RNA

$A_{260/A230} > 2.0$

< 2.0 = contamination by compounds included in the commercial kits

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Congratulation, you have just learnt one of the most important steps in the molecular biology

Isolation of nucleic acids

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