University of Belgrade
Institute of Chemistry, Technology and Metallurgy
National Institute of the Republic of Serbia

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Acronym: BIOLAWEB Boosting Institute of Chemistry, Technology and Metallurgy in Water Biomonitoring

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Duration: 36 months

eDNA Workshop

DNA extraction

Belgrade, October 2023

BIOLAWEB
presentation

Funded by
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DNA extraction

DNA extraction: a method to purify DNA by using physical and/or chemical methods

In a sample separating DNA from cell membranes, proteins, and other cellular components.

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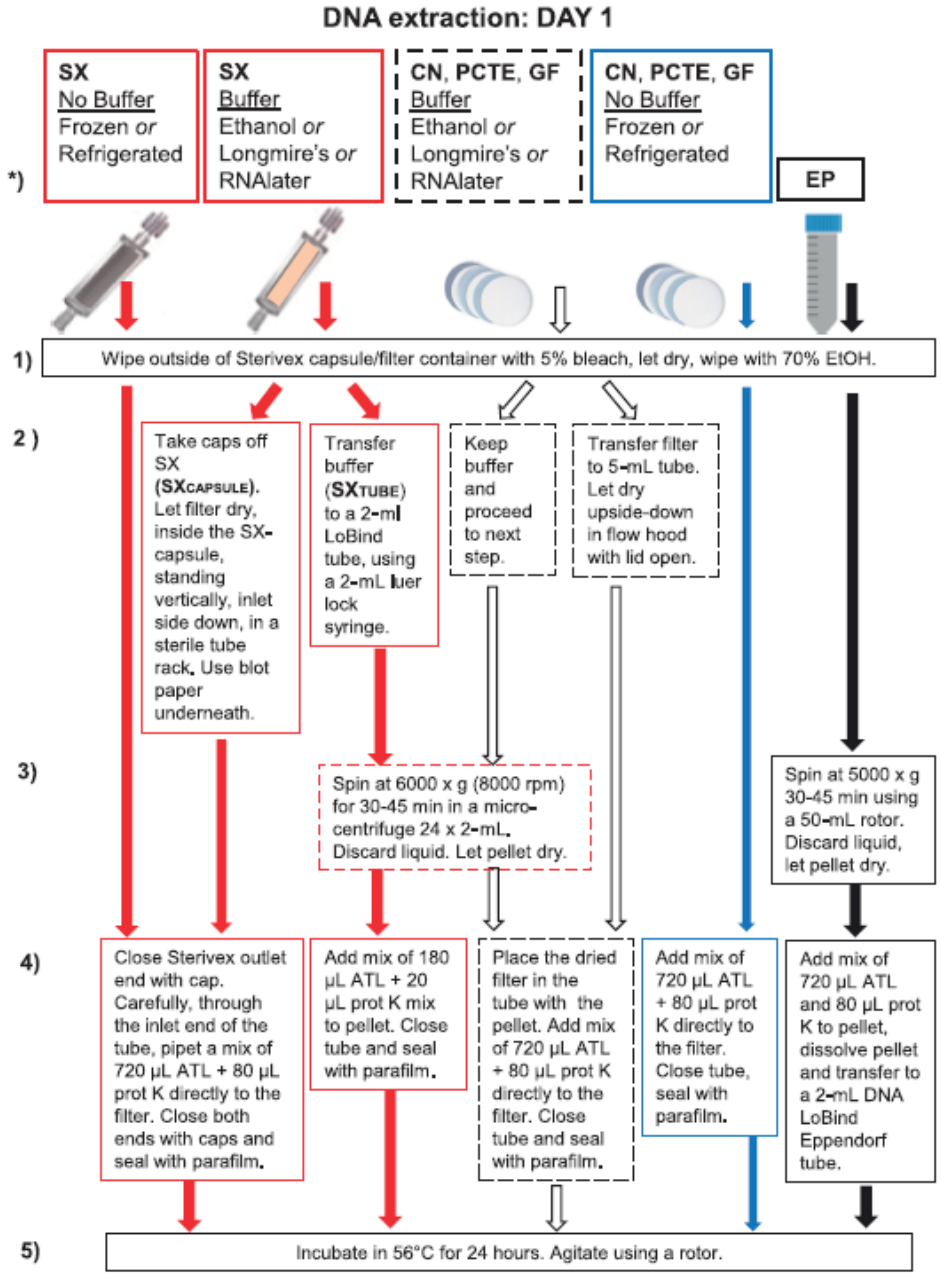


Fig. 1.

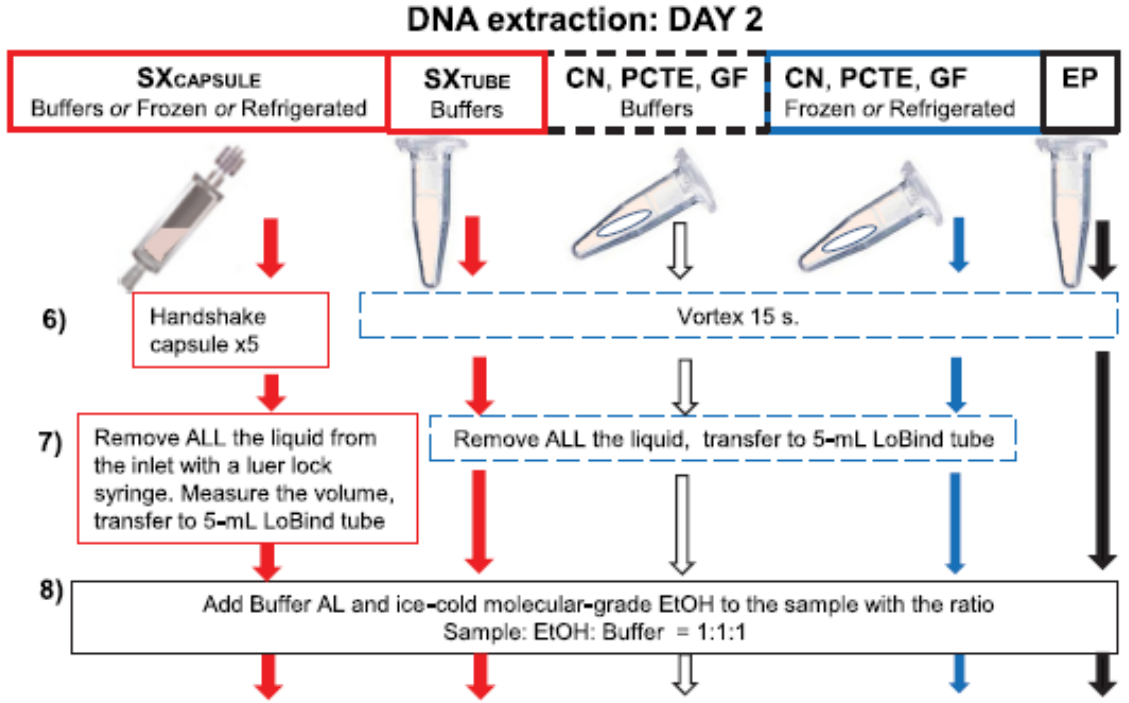


Fig. 1. Flow chart illustrating the modified environmental DNA (eDNA) extraction protocol based on DNeasy Blood & Tissue Kit (QIAGEN, Carlsbad, CA, USA). *) Capture: SX, Sterivex-GP polyethersulfone capsule filters, Note that $SX_{CAPSULE}$ and SX_{TUBE} are treated as separate samples from step 2. CN, cellulose nitrate; PCTE, polycarbonate track-etched; GF, glass fibre filters; EP, ethanol precipitation. Storage: Frozen at −20 °C, Refrigerated are samples stored at 8–10 °C and processed within 5 h. Steps 9–26 see Appendix S1.

Spens et al. 2017:
Methods in Ecology and Evolution, 8, 635–645

DNA extraction

Commercial kits

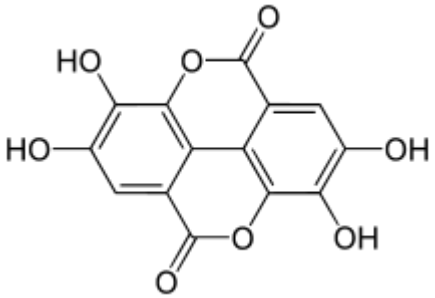
DNA extraction

eDNA sample

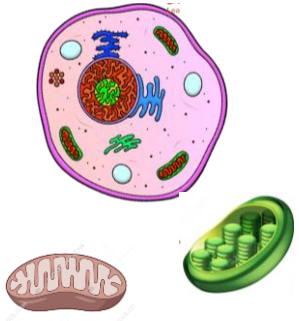
DNA extraction

In the sample the DNA is associated with different molecules/ compounds

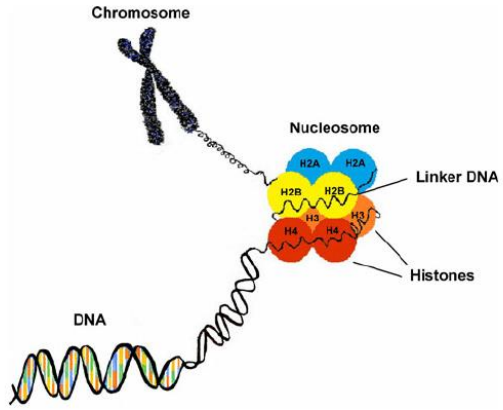
eDNA sample



Minerals, organic compounds



Cells, organelles



with proteins e.g. histones

DNA extraction

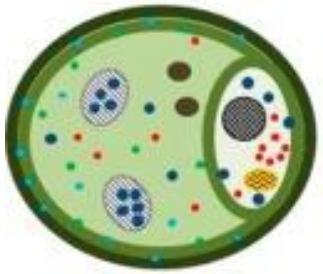
Possible inhibitors for PCR amplification in eDNA samples

Matrix	Contained inhibitors	References
Clinical specimens (e.g. blood; muscle tissues)	Antiviral substances (e.g. acyclovir),	Al-Soud and Rådström (2001); Burkardt (2000); Rådström et al. (2004); Yedidag et al. (1996)
	Haemoglobin, Heparin, Hormones, IgG, Lactoferrin, Myoglobin	
Stool	Complex polysaccharides, Bilesalts, Lipids, Urate	Kreader (1996); Monteiro et al. (1997); Rådström et al. (2004); Chaturvedi et al. (2008)
Seafood, bivalves, oysters	Algae, Glycogen, Polysaccharides	Atmar et al. (1993, 1995); Richards (1999)
Berries	Phenols, Polysaccharides	Seeram et al. (2006); Wei et al. (2008)
Plants	**Pectin, Polyphenols, Polysaccharides, Xylan**	Demeke and Adams (1992); Henson and French (1993); John (1992); Sipahioglu et al. (2006); Su and Gibor (1988); Wan and Wilkins (1994); Wei et al. (2008); Wilkins and Smart 1996
Cheese, milk	Proteases (e.g. plasmin), Calcium ions,	Bickley et al. (1996); Powell et al. (1994); Rossen et al. (1992)
Water, environment	**Debris, Fulmic acids, Humic acids, Humic material, Metal ions, Polyphenol**	Abbaszadegan et al. (1993); Ijzerman et al. (1997)
Palaeobiology, archaeology, forensic	Bone dust, Coprolite Peat extract, Clay-rich soil	Baar et al. (2011)

DNA extraction: main steps

Sample

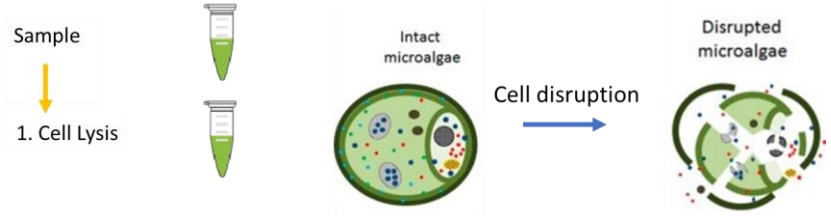
1. Cell Lysis



Cell disruption

DNA extraction: main steps

Cell Lysis



Physical
- Freezing/thawing cycles/boiling

- Ultrasonication

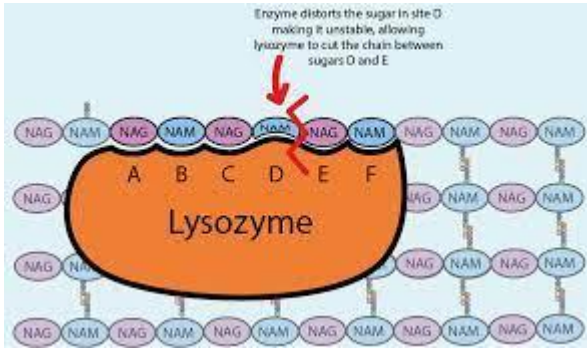
- Bead beating

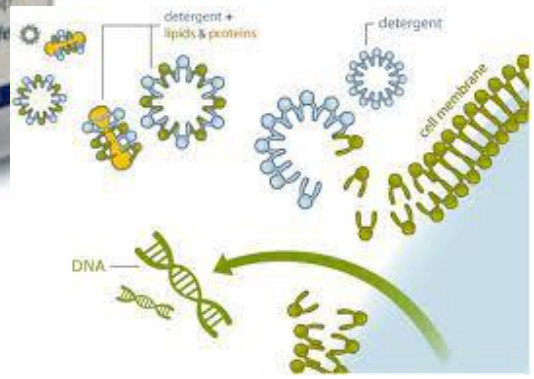
chemically
- detergents

- solvents (e.g., alcohols)

enzymatically
- lysozyme (bacteria)

- cellulase (plants



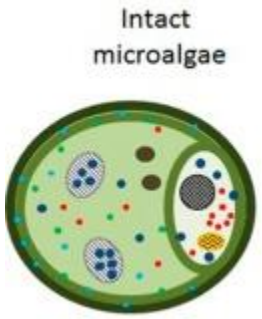


DNA extraction: main steps

Sample

1. Cell Lysis

2. Capture and cleaning of DNA



Cell disruption

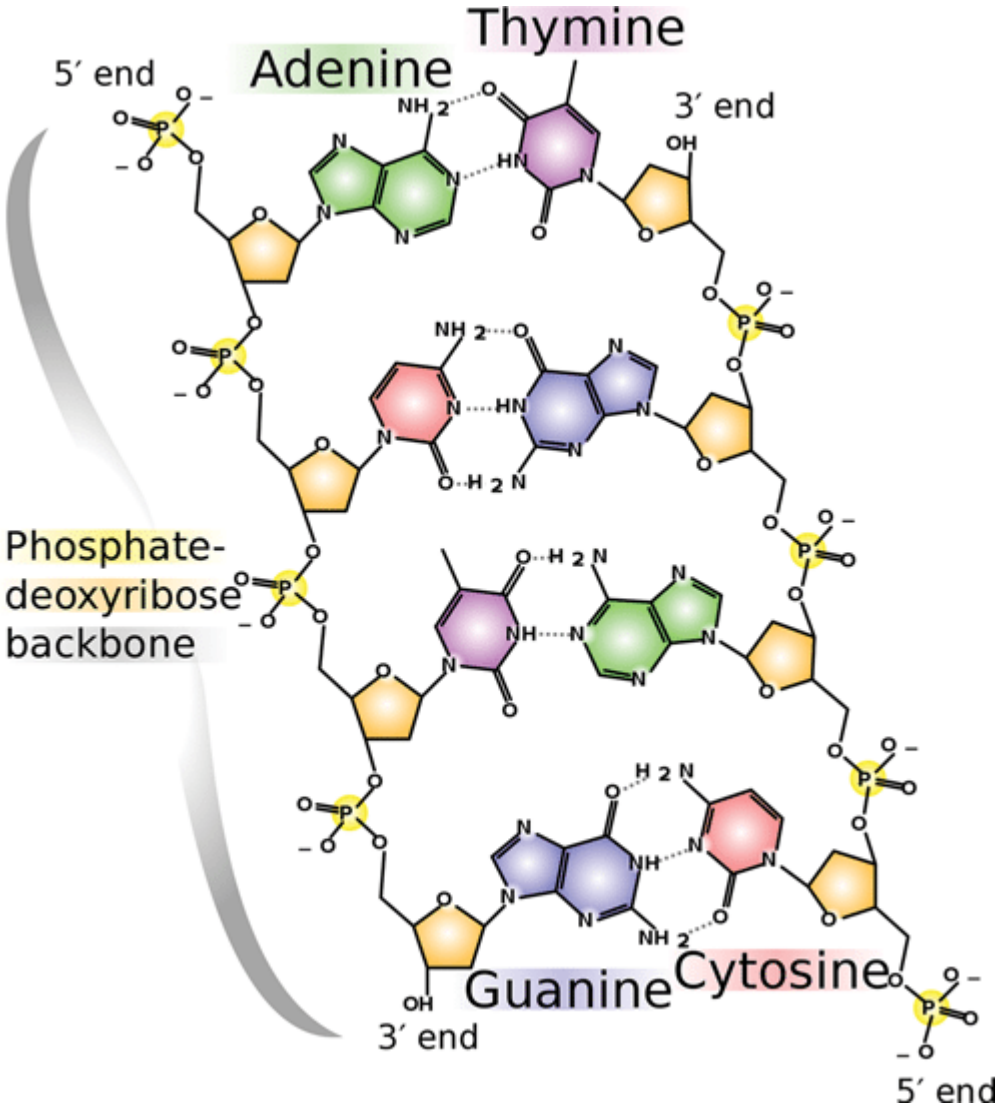
Bead beater
Lysis buffer

Separation of DNA - Cell debris, proteins, inhibitors

DNA extraction: main steps

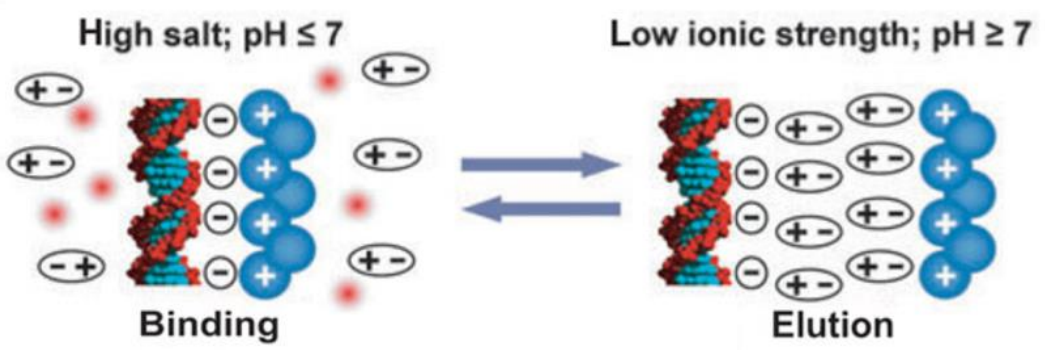
DNA: hydrosoluble

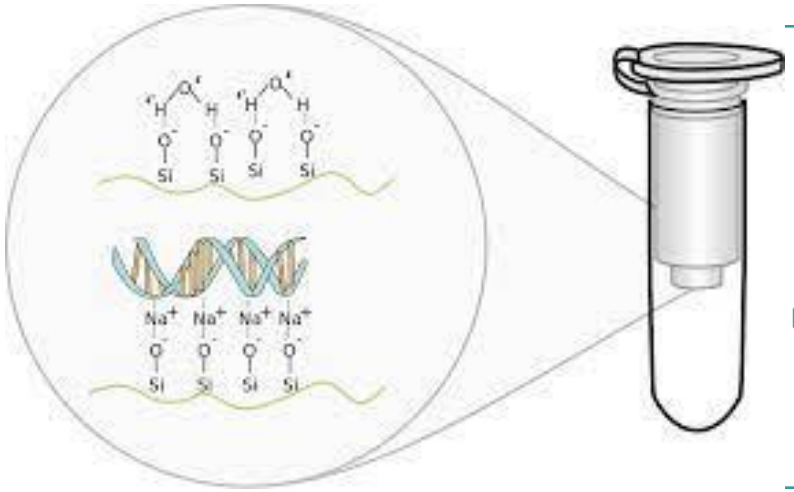
- polar molecule
- negatively charged

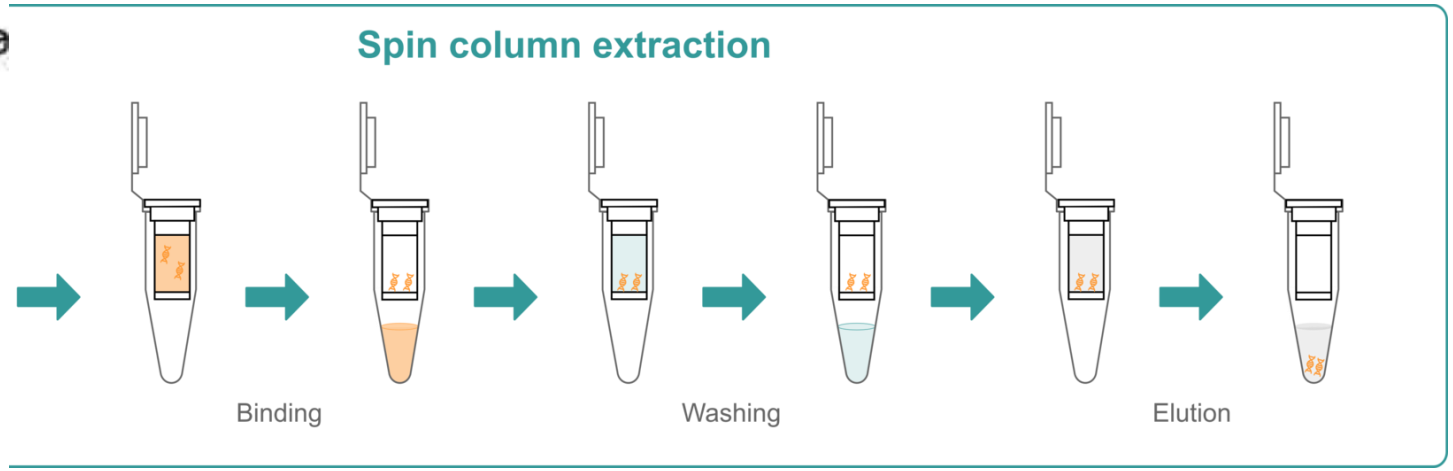


DNA extraction: main steps

DNA adsorbs to silica surface in the colum
- Low pH
- High salt concentration







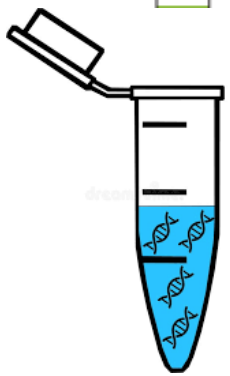
DNA extraction: main steps

Sample

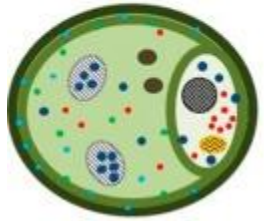
Cell Lysis

Capture and cleaning of DNA

Elution of DNA



Intact microalgae



Cell disruption

Bead beater
Lysis buffer

Disrupted microalgae

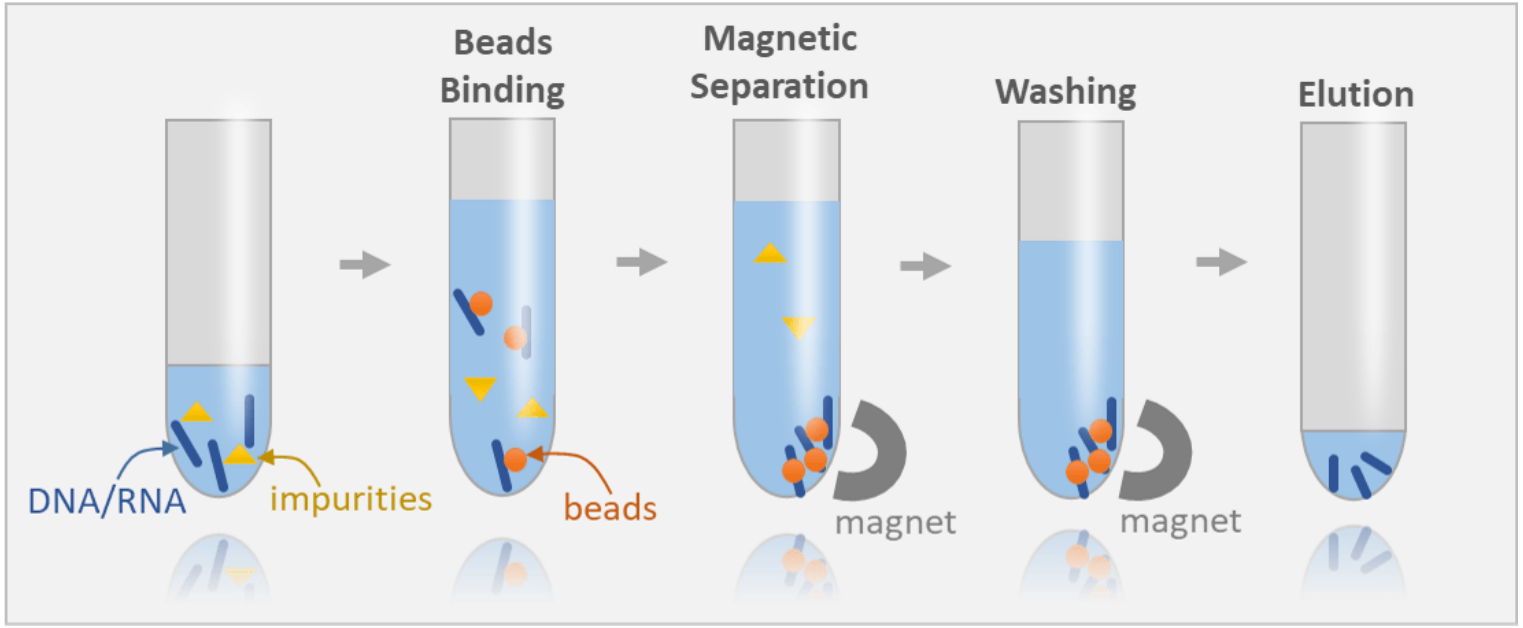
Centrifugation - Cell debris, proteins, inhibitors

DNA extraction: main steps

- magnetic beads (20 to 30 nm) iron oxide particles, such as particles of magnetite (Fe3O4).

- coated with a DNA-binding agent (Silica-coated) beads.

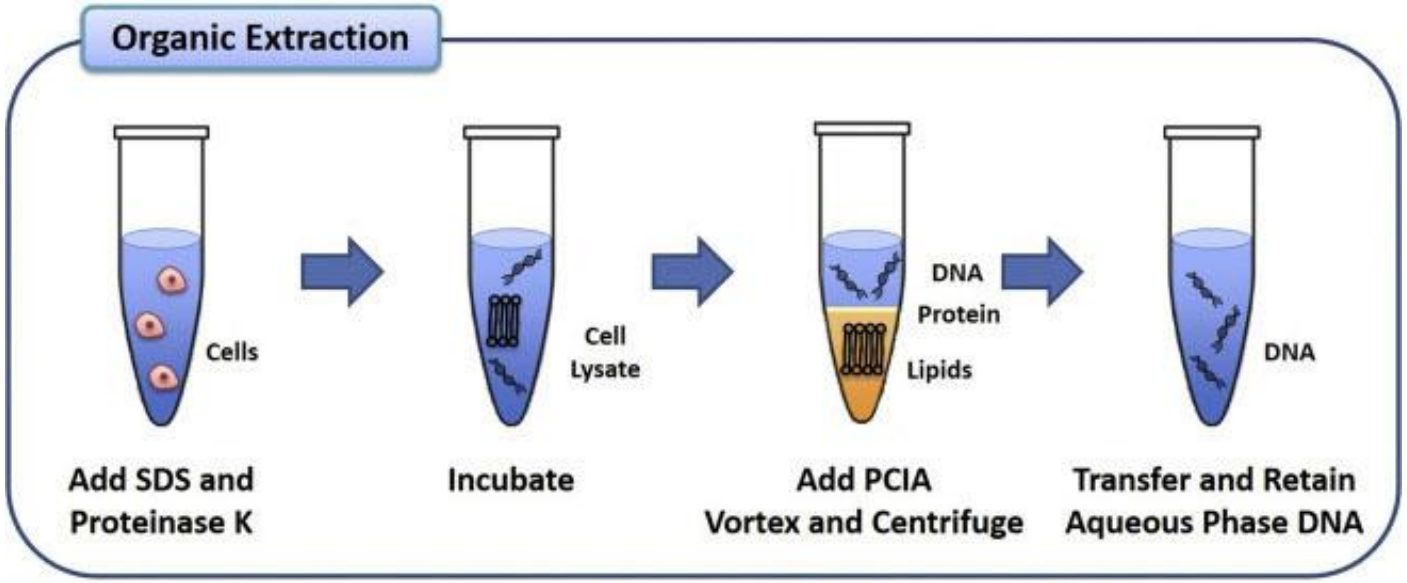
- reversibly binds nucleic acids based on salt concentration.

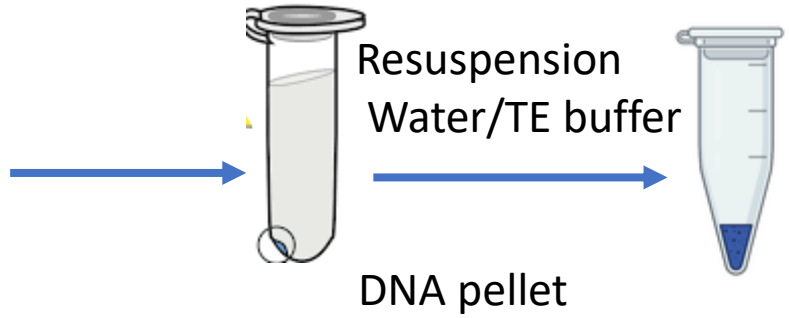


DNA extraction

No kit

Phenol/Chloroform

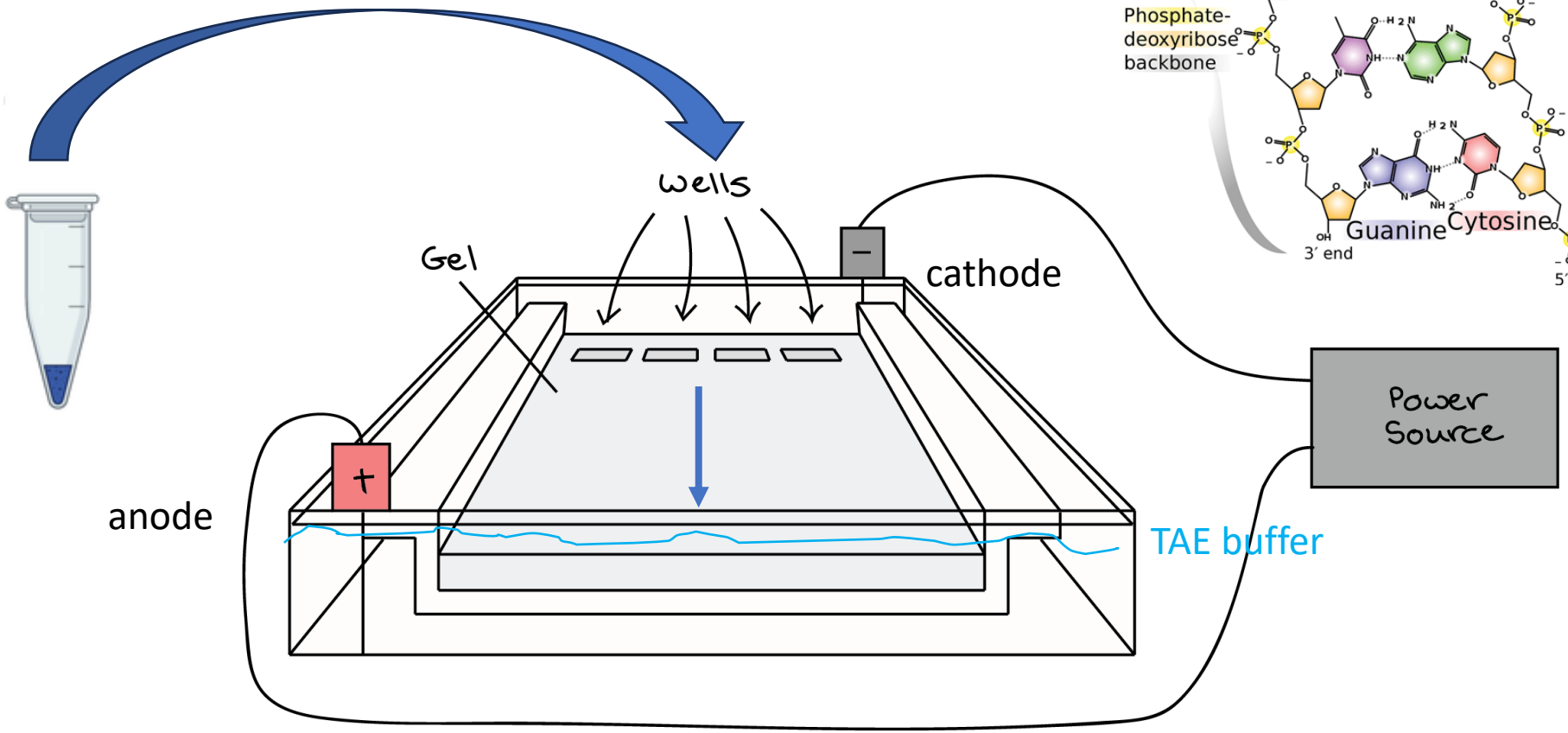


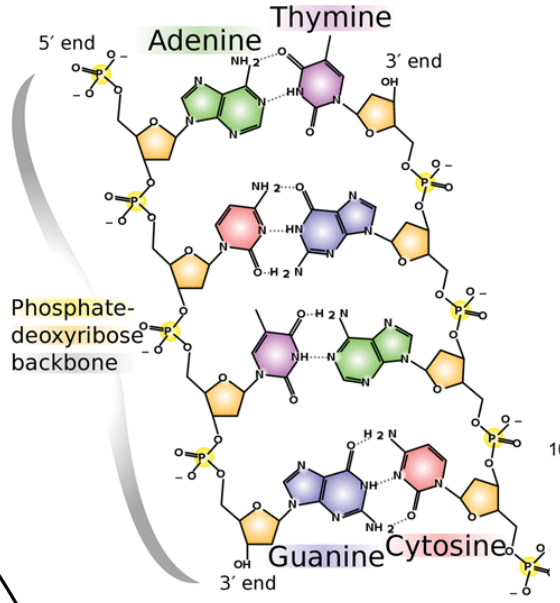


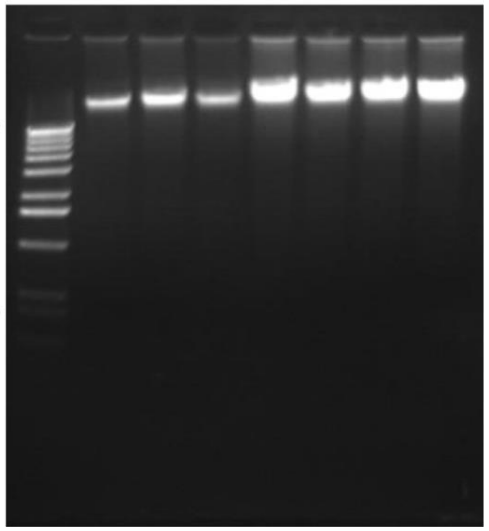
DNA precipitation using isopropanol and Na-acetate

DNA extraction

Electrophoresis



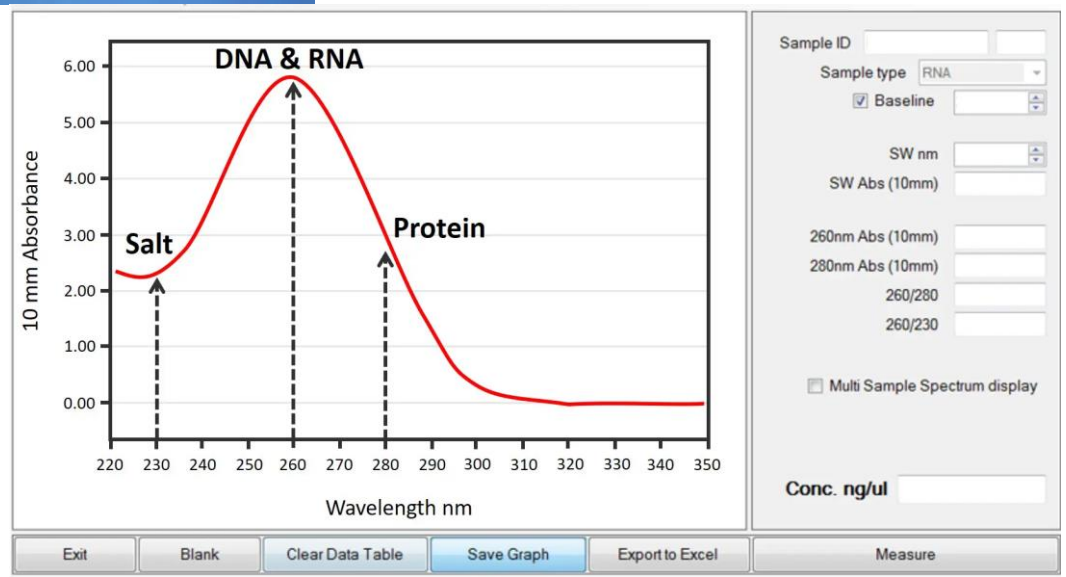




DNA extraction

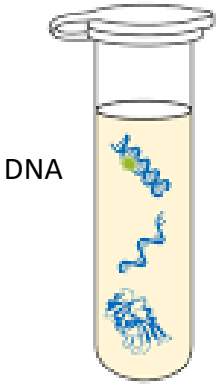
DNA quality check

Nanodrop: photometry

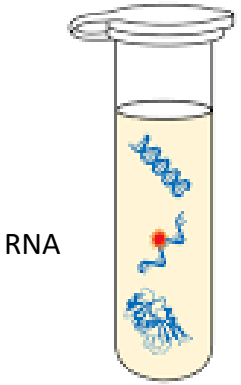


Qubit: fluorescence

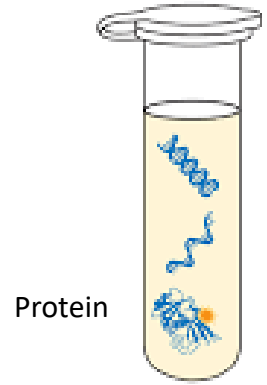
DNA dye selectivity



RNA dye selectivity



Protein dye selectivity



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Thank you for your attention!

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