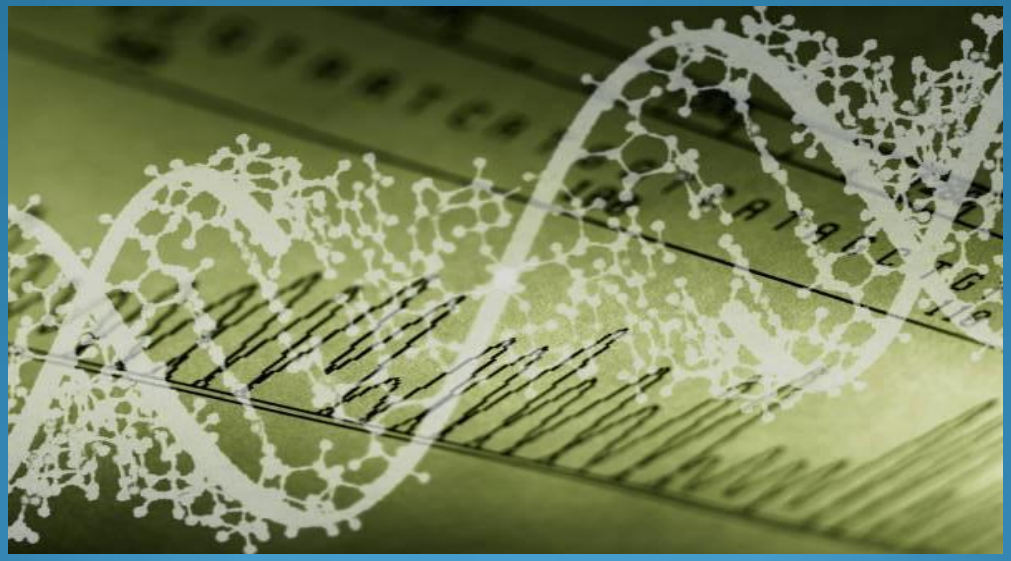
DNA EXTRACTION



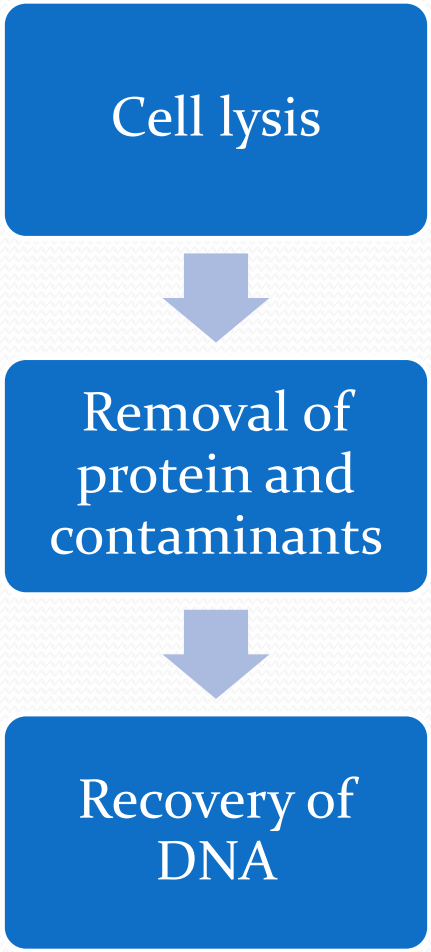
Purpose of DNA Extraction

To obtain DNA in a relatively purified form which can be used for further investigations, i.e. PCR, sequencing, etc

How Can We Recover DNA From a Variety of Sources of Biological Evidence?

- Blood
- Semen
- Saliva
- Urine
- Hair (w/Root & Shaft)
- Teeth
- Bone
- Tissue
- Cigarette Butts
- Envelope & Stamps
- Fingernail Clippings
- Chewing Gum
- Bite Marks
- Feces

Basic steps of DNA isolation



CELL LYSIS

- Cell lysis is a method or process for releasing biological molecules from inside a cell.

- It has two main methods;

1- Physical methods
2- Chemical methods

- **Physical methods** include;
- Freez thaw method
- Sonication
- Grinding
- Blending
- Beads method
- Boiling method
- **Chemical methods** involve;
- Enzymatic lysis
- Detegents method

PHYSICAL METHODS;

Freez-thaw method;

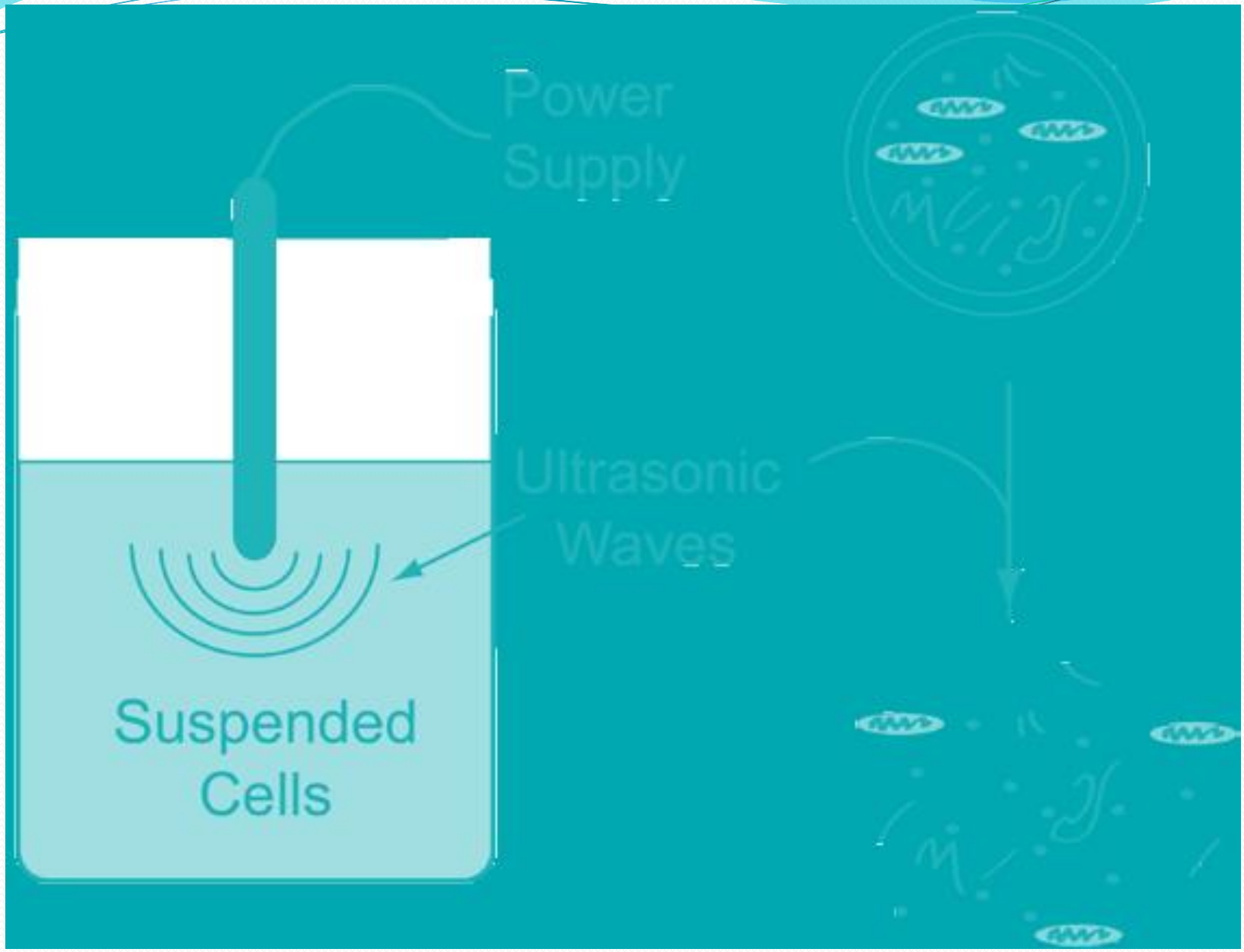
- cells are suspended in an appropriate experimental buffer and subjected to repeated freezing and thawing.

- Enzymatic lysis may be used to improve this method.
- ***Advantages****: No specialized equipment is necessary.*
- ***Disadvantages****: Very slow.*

Sonication

- In this process, high frequency waves (20-50kHz) are generated into the suspension of cells.This process generates a significant amount of heat which can lead to protein unfolding.

- ***Advantages:*** *Useful for most cell types. Easily applicable to large or small scale.*
- ***Disadvantages:*** *Requires specialized equipment. Loud - hearing protection required.*


Power Supply
Ultrasonic Waves
Suspended Cells

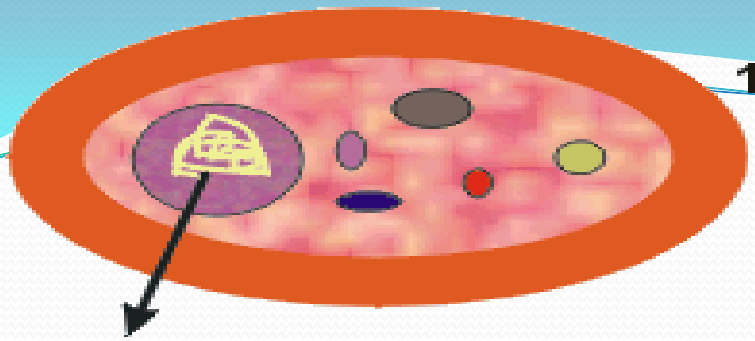
Beads Method;

- uses tiny glass, ceramic or steel beads mixed with a sample suspended in aqueous media.

- the sample and bead mix is subjected to high level agitation by stirring or shaking. Beads collide with the cellular sample, cracking open the cell to release intercellular components.

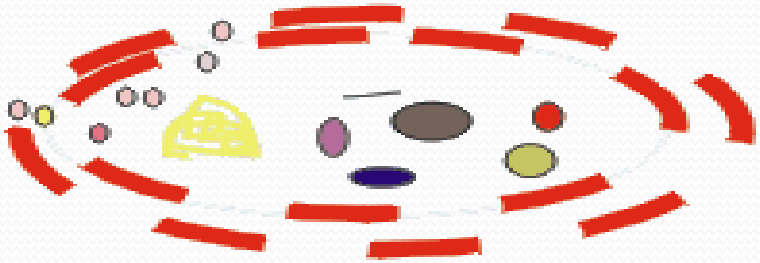
Chemical Lysis

- Resuspending the cells in a detergent containing media will dissolve the outer membrane and allow cytosolic proteins to be extracted.
- choice of detergent is particularly important. There is a number of commercially available optimized detergent containing solutions for this technique (e.g. B-PER from Pierce).
- ***Advantages:*** *Fast and cheap.*
- ***Disadvantages:*** *May influence protein structure or purification.*

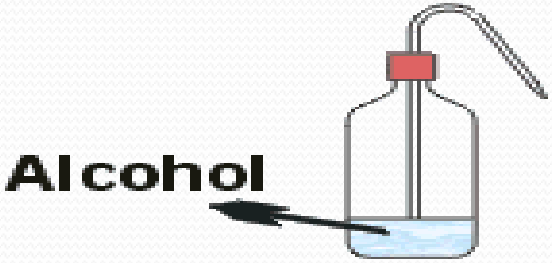


1. DNA is in the nucleus of the cell

2. Cell membrane is disrupted with a detergent



3. Alcohol is added to the tube to separate DNA from other cell components. DNA moves to the alcohol layer

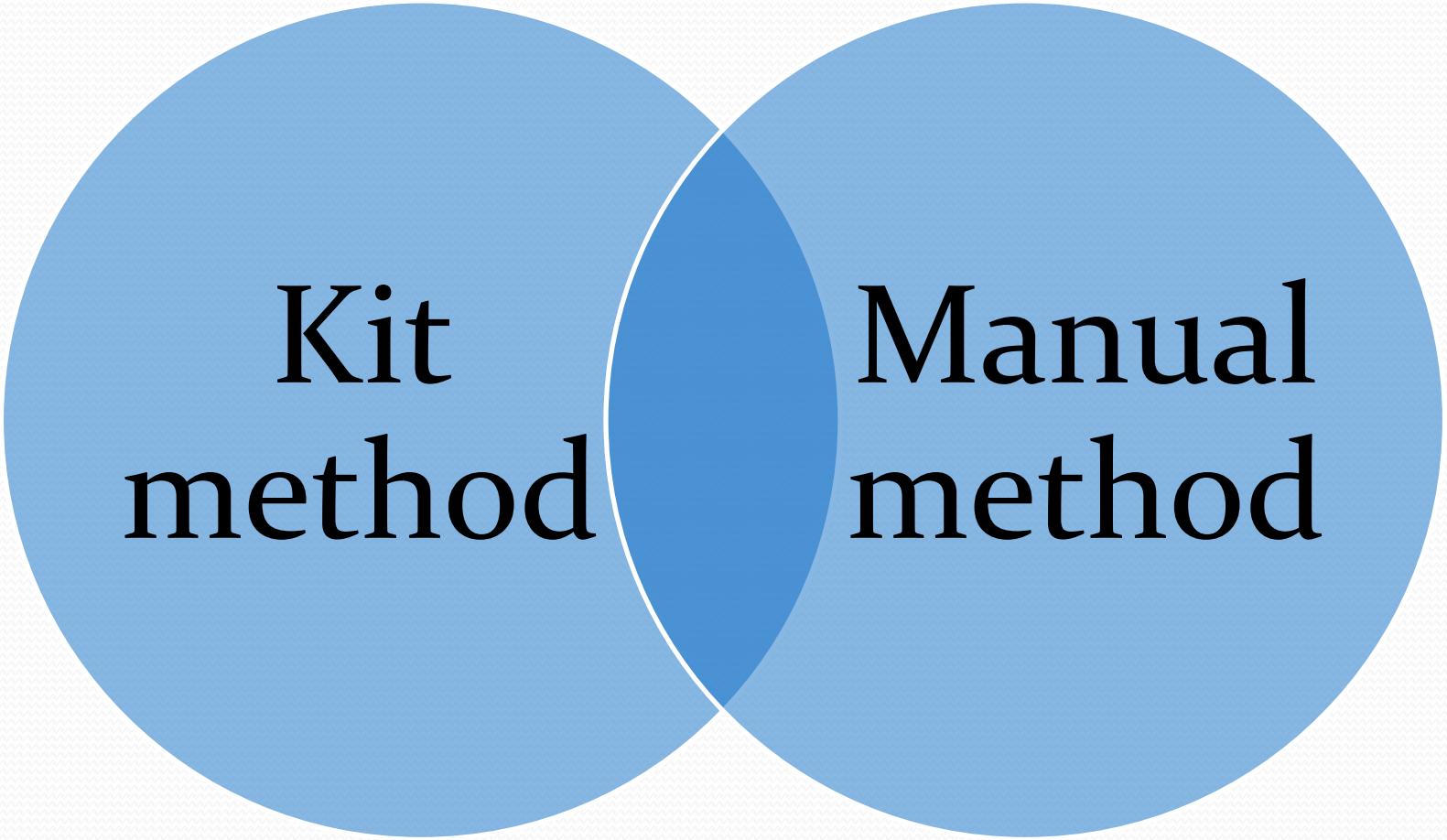


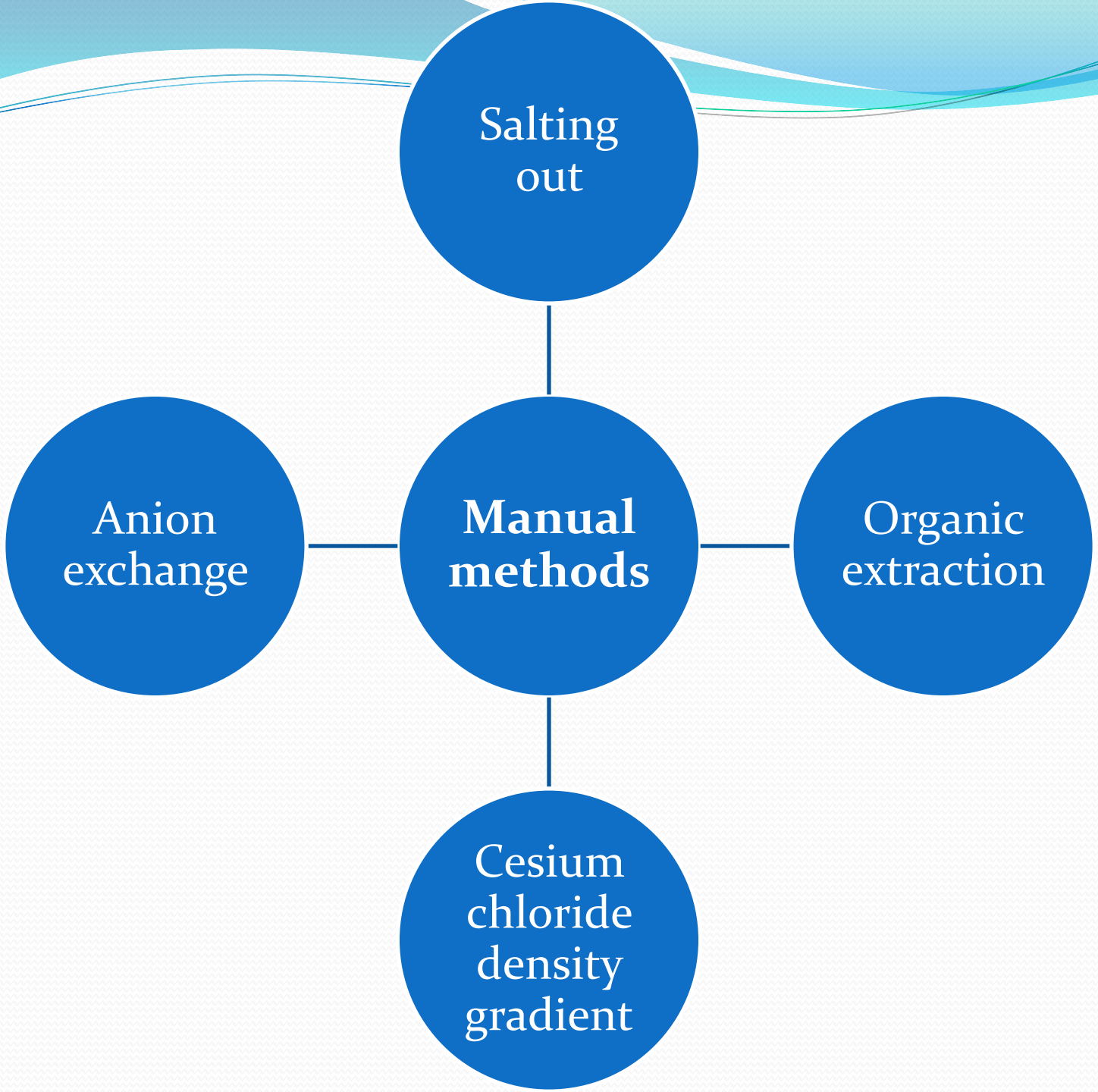
4. DNA is spooled onto a glass pipette

Enzymatic Lysis

- Although not physically able to destroy the cell membrane, enzymatic degradation of the cell wall is well established and widely used. If the protein of interest is secreted and trapped in the periplasm, enzymatic lysis is all that is necessary.
- lysozyme is the most commonly used.
- ***Advantages:*** *No specialized equipment necessary.*
- ***Disadvantages:*** *Not always reproducible. Enzyme Stability can be an issue. Can be expensive to scale up.*

DNA PURIFICATION:-




Salting out
Anion exchange
Manual methods
Organic extraction
Cesium chloride density gradient

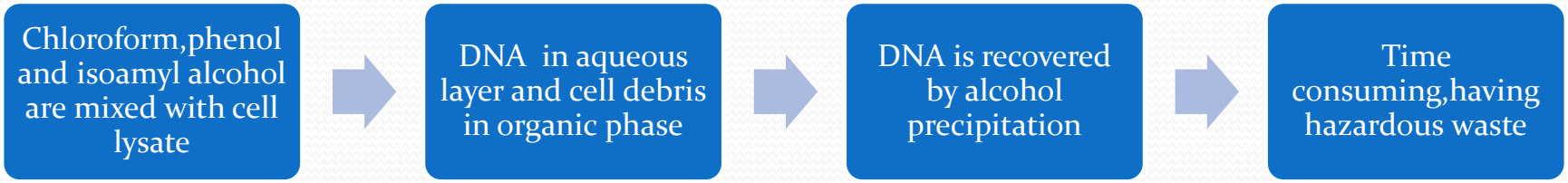
Salting out method;

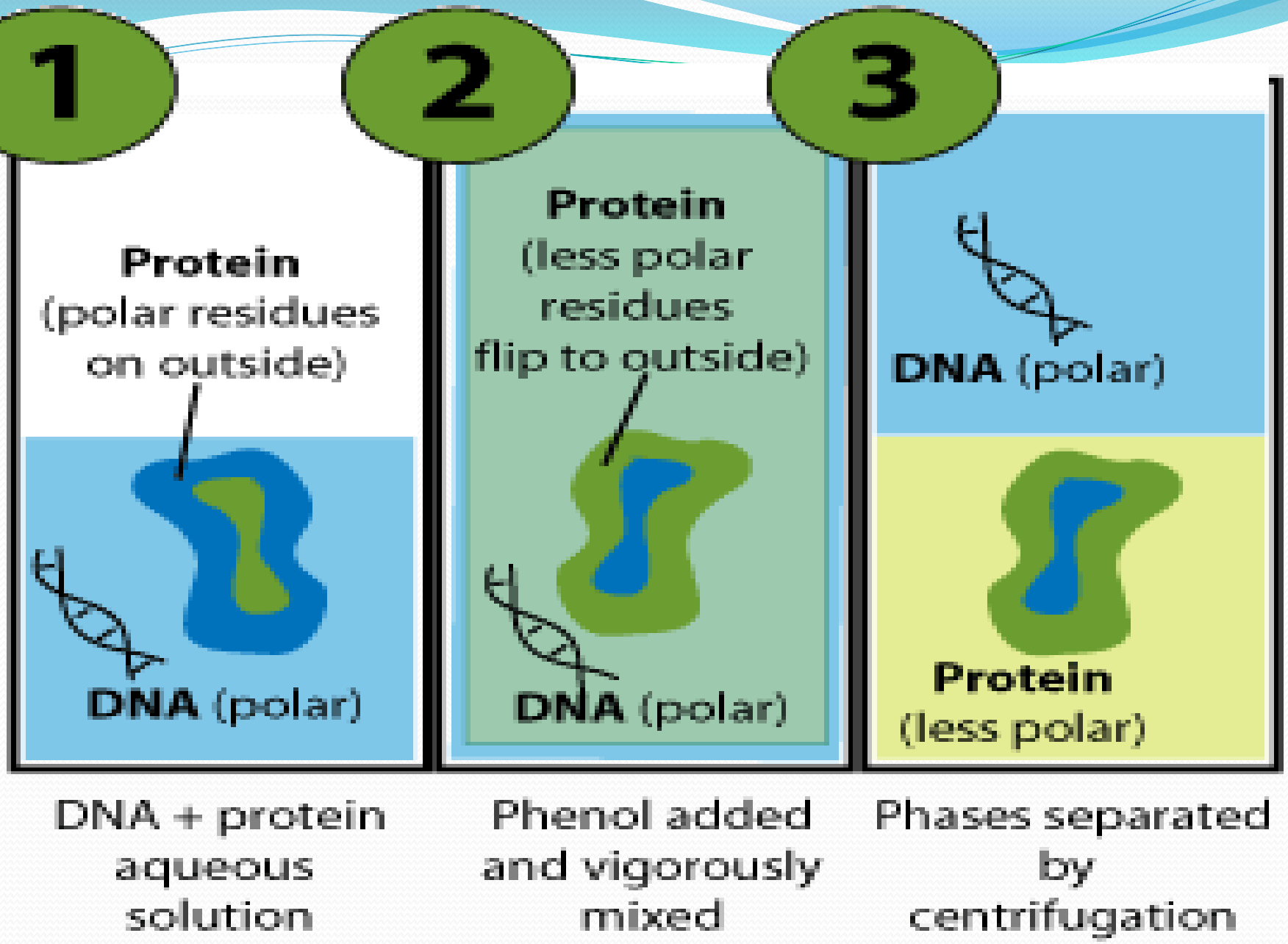
High salt conc. are used to precipitate proteins of cell lysate

PPt are removed by centrifugation and DNA by alcohol precipitation

Inefficient,DNA yield and purity is variable

Organic extraction methods




1
2
3
Protein
(polar residues
on outside)
DNA (polar)
Protein
(less polar
residues
flip to outside)
DNA (polar)
DNA (polar)
Protein
(less polar)
DNA + protein
aqueous
solution
Phenol added
and vigorously
mixed
Phases separated
by
centrifugation

Cesium chloride density gradient method;

- Cells are lysed using a detergent and the lysate is alcohol precipitated.
- Resuspended DNA is mixed with CsCl and ethidium bromide and centrifuged for several hours.
- The DNA band is collected from the centrifuge tube, extracted with isopropanol to remove the ethidium bromide, and then precipitated with ethanol to recover the DNA.
- This method allows the isolation of high-quality DNA, but is time consuming.

Anion exchange methods;

- Anion-exchange chromatography is based on the interaction between the negatively charged phosphates of the nucleic acid and positively charged surface molecules on the substrate.
- DNA binds to the substrate under low-salt conditions, impurities are washed away using medium-salt buffers, and high-quality DNA is eluted using a high-salt buffer.
- The eluted DNA is recovered by alcohol precipitation,
- Anion-exchange technology completely avoids the use of toxic substances.

Silica-based methods — DNeasy Tissue Kits;

- DNeasy Tissue technology provides a simple, reliable, fast, and inexpensive method for isolation of high-quality DNA.
- based on the selective adsorption of nucleic acids to a silica-gel membrane in the presence of high concentrations of salts.

Use of optimized buffers in the lysis procedure ensures that only DNA is adsorbed while cellular proteins, and metabolites remain in solution and are subsequently washed away.

This is simpler and more effective than other methods where precipitation or extraction is required.No alcohol precipitation is required, and resuspension of DNA.

- DNeasy Tissue Kits are designed for rapid isolation of pure total DNA (genomic, viral, and mitochondrial) from a wide variety of sample sources, including fresh and frozen animal cells and tissues,yeasts, and blood.

DNA purified using DNeasy Tissue Kits is free

from contamination and enzyme inhibitors and is highly suited for applications such as Southern blotting, PCR, real-time PCR.

DNeasy Tissue Kits are available in convenient spin-column or 96-well formats, suitable for a wide range.

- Genomic DNA isolated using DNeasy Tissue technology is up to50 kb in size, with an average length of 20–30 kb.
- DNA of this length is particularly suitable for PCR analysis as well as Southern

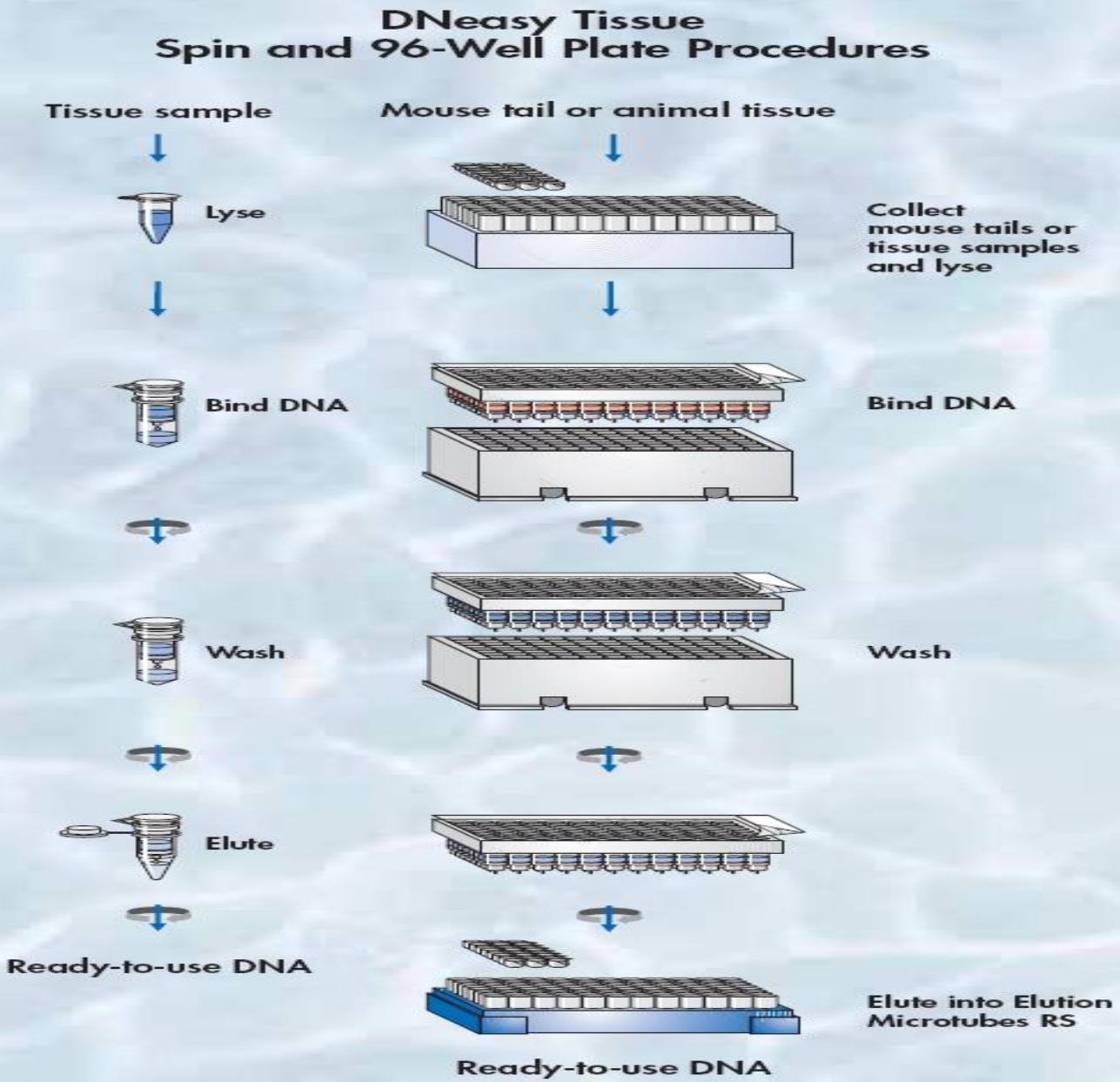


Figure 4. The DNeasy Tissue spin and 96-well plate procedures.

References:-

- *science.marshall.edu/murraye/…/samantha%20qiagin%20**method**.pdf*

- *gmo-crl.jrc.ec.europa.eu/doc/Genomic-**DNA**-**Extraction**-**Kit**-8--3.pdf*

- Lectures delivered in class


Thank You