DNA Extraction

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outlines:

- Definition of DNA extraction .
- Clinical Applications of DNA extraction
- Samples of choice.
- Criteria of DNA extraction methods
- Good DNA quality
- Concentration and purity measurements and calculations .
- DNA extraction steps.

DNA Extraction:

DNA Extraction is the removal of DNA from the cells.

Clinical applications:

- Diagnosis of genetic disease .
- Forensic analysis.
- Detection of bacteria and viruses in the environment.

Kind of samples :

- All living cells can be used to extract DNA.
- Whole blood and blood spots as a first choice.
- Fluid and chorionic villus sampling (CVS) for prenatal diagnosis (PND).
- Buccal swab and hair follicles in forensics.

Choice of sample depends on:

- Amount of DNA needed for analysis.
- The conditions and resources for collecting sample.

DNA extraction Methods Criteria:

- Safe
- Simple
- Inexpensive
- Yield good DNA quality

What does it mean to yield a good DNA quality?

Good DNA quality

- Concentration of DNA is sufficient for analysis.
- Purity; no contamination, lipids, proteins and RNA.
- Integrity; no DNA degradation

DNA concentration:

- Spectrophotometer method.
- Nanodrop method.

DNA concentration by Spectrophotometer:

- Concentration (µg/ml)= (A260 reading – A320 reading) × dilution factor × 50µg/ml
- N.B. turbidity is estimated at 320 nm and excluded .

DNA Yield :

DNA Yield (µg) = DNA Concentration × Total Sample Volume (ml)

When using a quartz rectangular standard cuvette the optical density at 260 nm (OD260) equals 1.0 for the following solutions:

- ❑ a 50 µg/mL solution of dsDNA
- ❑ a 33 µg/mL solution of ssDNA

Example of Calculation:

A sample of dsDNA was diluted 50X. The diluted sample gave a reading of 0.7 on a spectrophotometer at OD260. determine the concentration of DNA in the original sample .

Example of Calculation

- dsDNA concentration = 50 µg/mL × OD260 × dilution factor
- dsDNA concentration = 50 µg/mL × 0.7 × 50
- dsDNA concentration = 1.75 mg/mL

Purity of DNA:

Pure DNA preparations have an A260/A280 ratio of greater than or equal to 1.8.

Purity of DNA:

- For pure DNA and RNA the ratio is approximately 1.8 and 2.0 respectively.
- If DNA is contaminated with proteins then the ratio will be < 1.8
- If DNA is contaminated with RNA then the ratio will be > 2.0

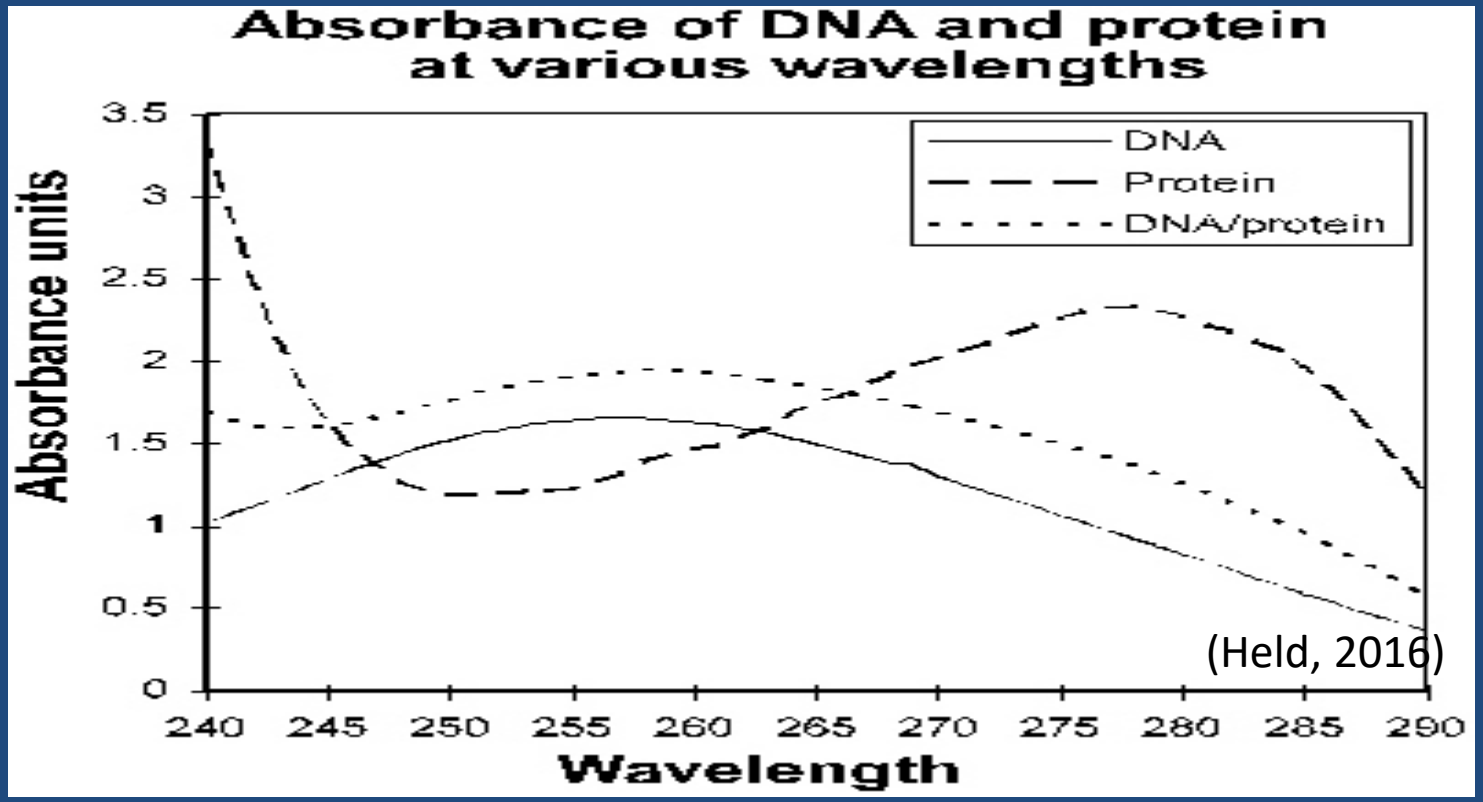
Purity of DNA:

- Spectrophotometer method.
- Nanodrop method.

Purity of DNA:

DNA Purity (A260/A280) = (A260 reading – A320 reading) ÷ (A280 reading – A320 reading)

Purity of DNA:



(Held, 2016)

DNA concentration an purity by Nanodrop:

Automatic measuring depending on spectrophotometer principle .

DNA extraction methods

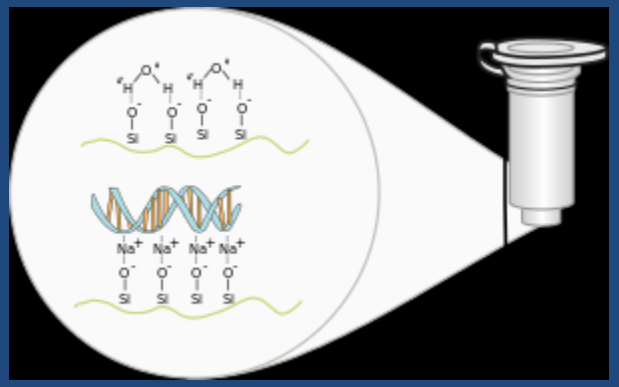
A- Manual DNA extraction steps :

- Cell lysis :breaking the cells and to expose the DNA within.
- Removing membrane lipids by adding a detergent.
- Removing proteins by adding a protease
- Precipitating the DNA with an alcohol usually ice-cold ethanol or isopropanol.

Wash and : DNA is washed in ethanol

Resuspend (rehydration): resuspended in H20 or TE buffer.

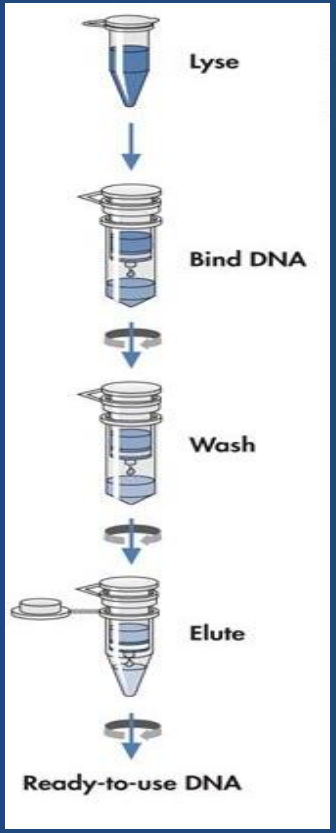
B-spin column based DNA extraction:

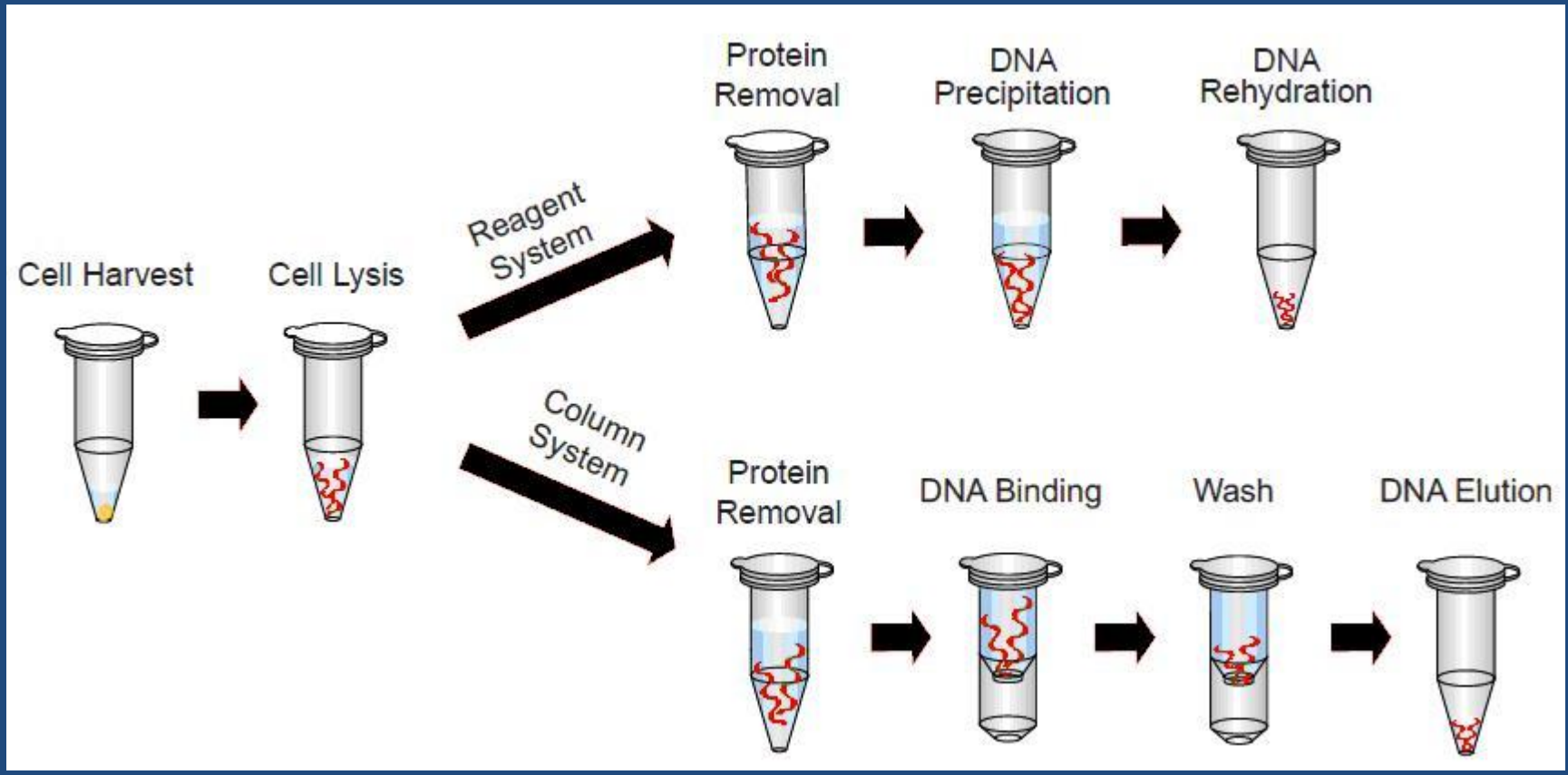


- is a solid phase extraction method to quickly purify nucleic acids.
- relies on the fact that nucleic acid will attached to the solid phase of silica under certain conditions.

B-spin column based DNA extraction steps

- Cell lysis :breaking the cells and to expose the DNA within.
- DNA binds to the silica membrane in presence of ethanol or isopropanol (binding solution).
- Wash of all impurites using washing buffers to force them through the silica membrane
- Elution of DNA using the elution buffer to remove the nucleic acid from the membrane and the nucleic acid is collected from the bottom of the column.
- N.B. use centrfuge after each step .




Cell Harvest
Cell Lysis
Reagent System
Protein Removal
DNA Precipitation
DNA Rehydration
Column System
Protein Removal
DNA Binding
Wash
DNA Elution

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In-text citations