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Nucleic Acid Preparation

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CLINICAL CHEMISTRY TRAINEE COUNCIL

Basic Nucleic Acid (NA) Preparation Steps

Release NA
Lyse cell/organism

Separate NA
From other cell or sample material, including proteins

Purify NA
Wash away unwanted material

Concentrate (optional)
Any time in the process, increase the concentration of target organism or NA

Figure courtesy of Stephanie Thatcher

Nucleic Acid Preparation Considerations

- Input
  - Sample type
  - Sample volume
  - Cell or organism type
- Output
  - Sensitivity desired
  - DNA vs. RNA
  - Extent of purity
  - Batch size
  - Turn-around time
  - Complexity of procedure

Sample Factors to Consider

- Sample type (extent of purification, will inhibitors need to be removed?)
  - Protein removal may be required
  - Nucleic acid isolation efficiency may vary from sample type to sample type
- Sample volume available (consider for sensitivity)
- Sample flexibility (will multiple sample types need to be compatible with the method?)

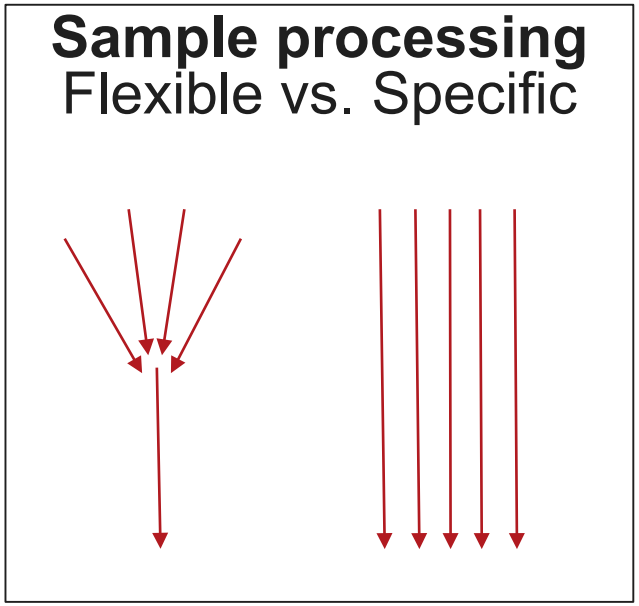


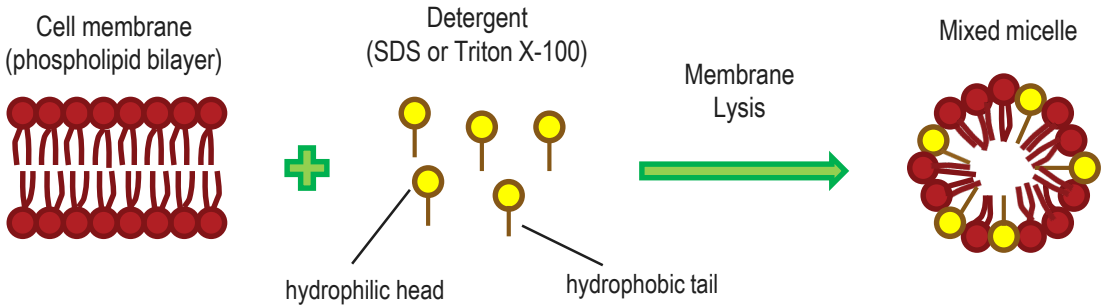
Figure courtesy of Stephanie Thatcher

Note on Solid Samples: Pre-processing

- Solid samples include stool, tissues and swabs
  - Can clog filters
  - Can interfere with nucleic acid isolation
  - Hard to lyse
- Some pre-processing may be necessary
  - Pre-filtration
  - Chemical or enzymatic digestion
  - Dilution in liquid
  - Freeze-thaw
  - Physical grinding or other physical method
- Formalin fixed paraffin embedded tissue- paraffin removal (xylene, ethanol wash)

Lysis

- Chemical methods are common, usually salts, chaotropic salts, or detergents



- Enzymatic methods are also common using lysozyme or proteases, effective for some organisms and cells
- Mechanical or physical methods can be more effective for some sample types
  - E.g. physical shearing, bead milling, freezing or pressure

Figure courtesy of Linnea M. Baudhuin, Ph.D.

Sample Inhibition and Protein Removal

- Many samples contain unwanted proteins, such as:
  - Nucleases
  - Hemoglobin
  - Chromosomal proteins
- Large numbers of proteins can interfere with nucleic acid isolation
- Proteases (e.g. proteinase K), chaotropic salts (e.g. guanidine isothiocyanate) and detergents aid in the removal of proteins in combination with affinity methods
- Inhibitors from a sample (like stool, blood or sputum) may interfere with nucleic acid isolation or with downstream analyses
- Most procedures will easily remove most inhibitors
- To test for presence of inhibitors, dilute a sample and re-analyze. If results improve with the dilution, then there is likely inhibition
  - A more extensive preparation procedure may be required

Which Method Should be Used

- Downstream analysis of nucleic acid
- Extent of nucleic acid purity required
- Required yield of DNA/RNA
- Batch size/Throughput
- Processing speed
- Ease of operation
- Cost
- Hazardous reagents

Nucleic Acid Isolation Methods

- Liquid phase
  - Organic
  - Inorganic
- Solid phase
  - Size exclusion by gel filtration
  - Ion exchange chromatography
  - Affinity chromatography

Liquid Phase Inorganic Extraction

- Inorganic chemicals are used
  - Detergents
  - EDTA
  - Acetic acid
  - Salt (salting out)
- Cell membranes lysed with SDS
- Proteins precipitated with salt solution
- DNA is precipitated with alcohol and rehydrated
- Advantages: fast, easy, non-hazardous reagents, produces high-quality DNA

Liquid Phase Organic Extraction

- Nucleic acids have differential solubility in immiscible liquids
- Phenol:chloroform/isoamyl alcohol
- Using immiscible liquids, two layers are formed:
  - Nucleic acids in top aqueous layer
  - Proteins/lipids in interface and organic phase
- Advantages: very pure product
- Disadvantages: Manual, labor-intensive, fume hood required, hazardous reagents and waste, low throughput

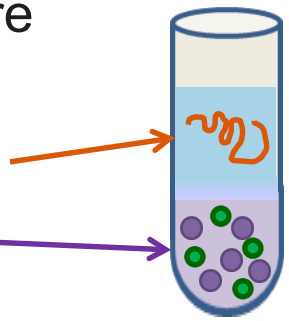
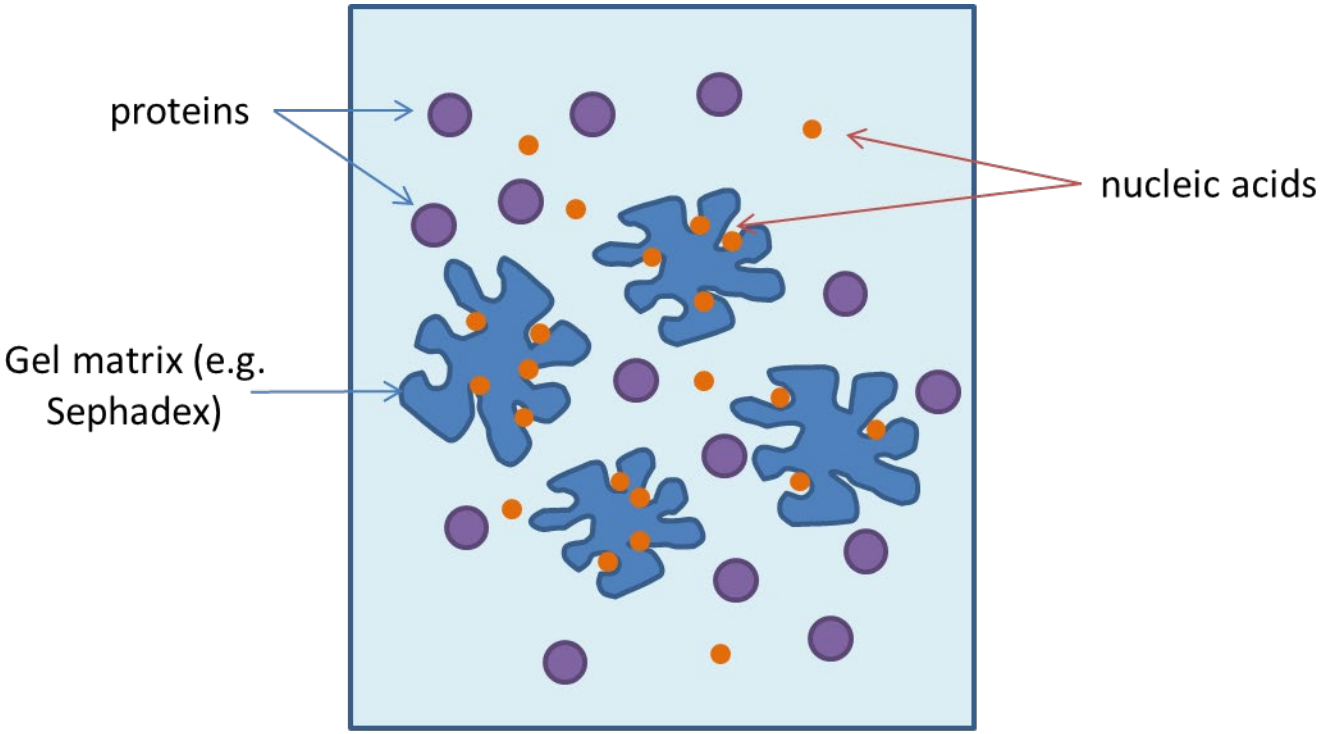


Figure courtesy of Linnea M. Baudhuin, Ph.D.

Solid Phase Extraction

- Most common method
  - Utilizes minimal hazardous reagents
  - Automatable
  - Fast/high-throughput

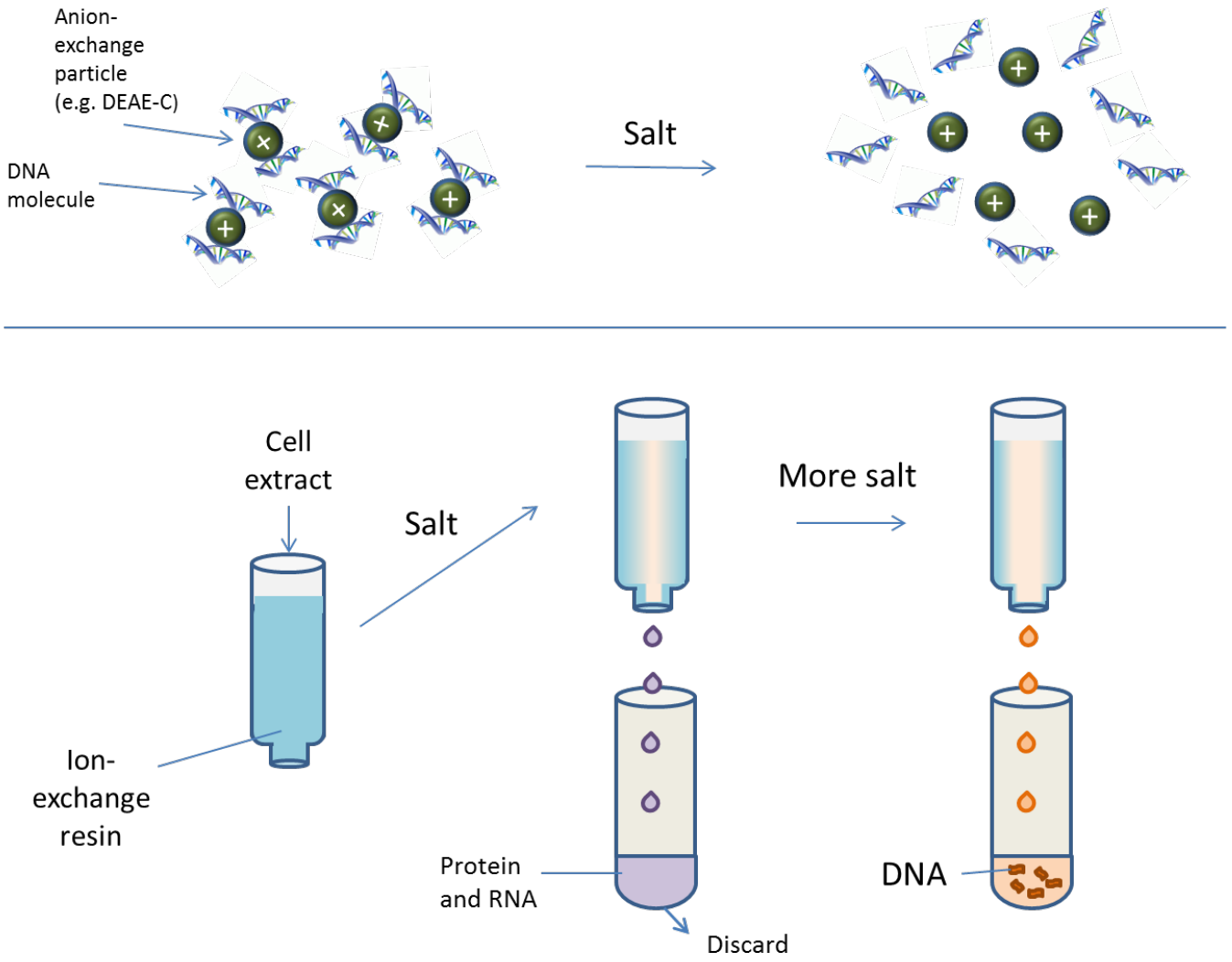
Solid Phase Extraction: Gel Filtration



- Small molecules are retained in pores of gel matrix
- Large molecules pass through column

Figure courtesy of Linnea M. Baudhuin, Ph.D.

Solid Phase Extraction: Ion Exchange Chromatography



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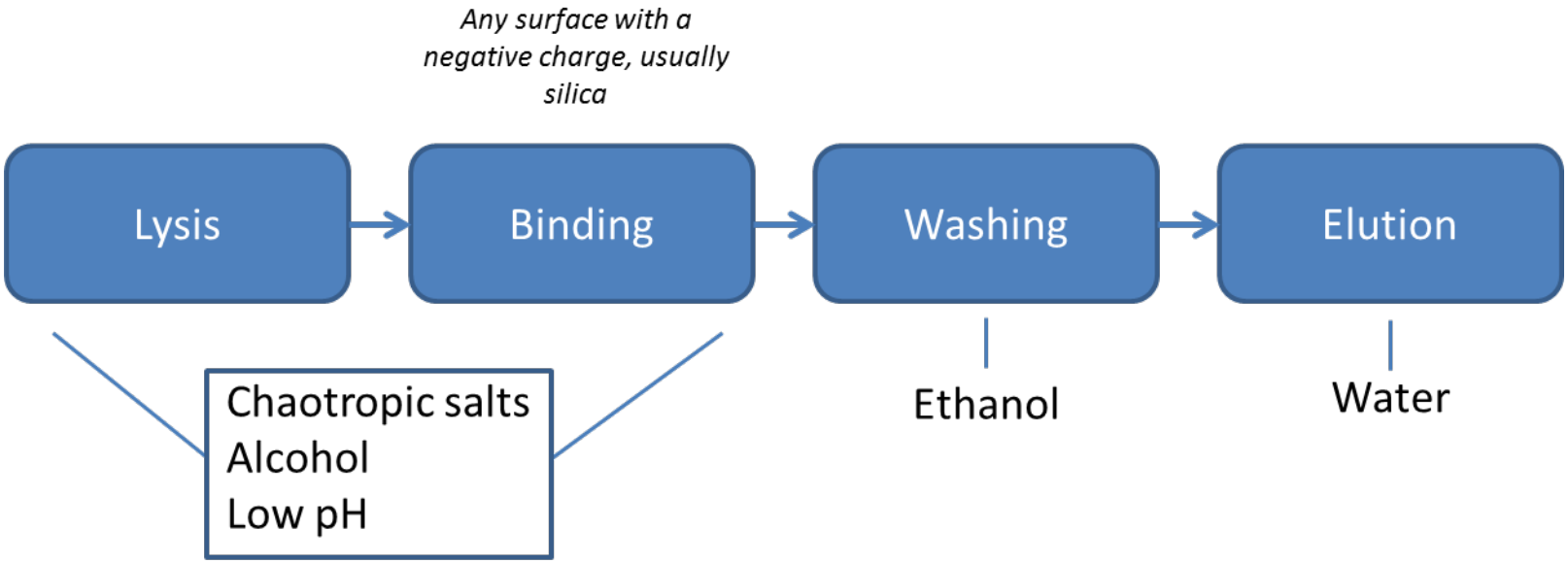
Solid Phase Extraction: Affinity Chromatography



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Spectrophotometric Measurement of Nucleic Acid Quantity and Quality

- Nucleic acids absorb light at 260 nm
- Protein absorbs light at 280 nm
- Beer-Lambert Law for nucleic acid quantity
  - **DNA**: A260 x **50** x dilution factor = ug/mL DNA
  - **RNA**: A260 x **40** x dilution factor = ug/mL RNA
- Purity of DNA:
  - A260/280 ratio should be 1.6-2.0
  - <1.6 indicates protein contamination
  - >2.0 indicates RNA contamination
- Purity of RNA:
  - A260/280 ratio should be 2.0-2.3
  - <1.7 indicates protein contamination

Common mistakes

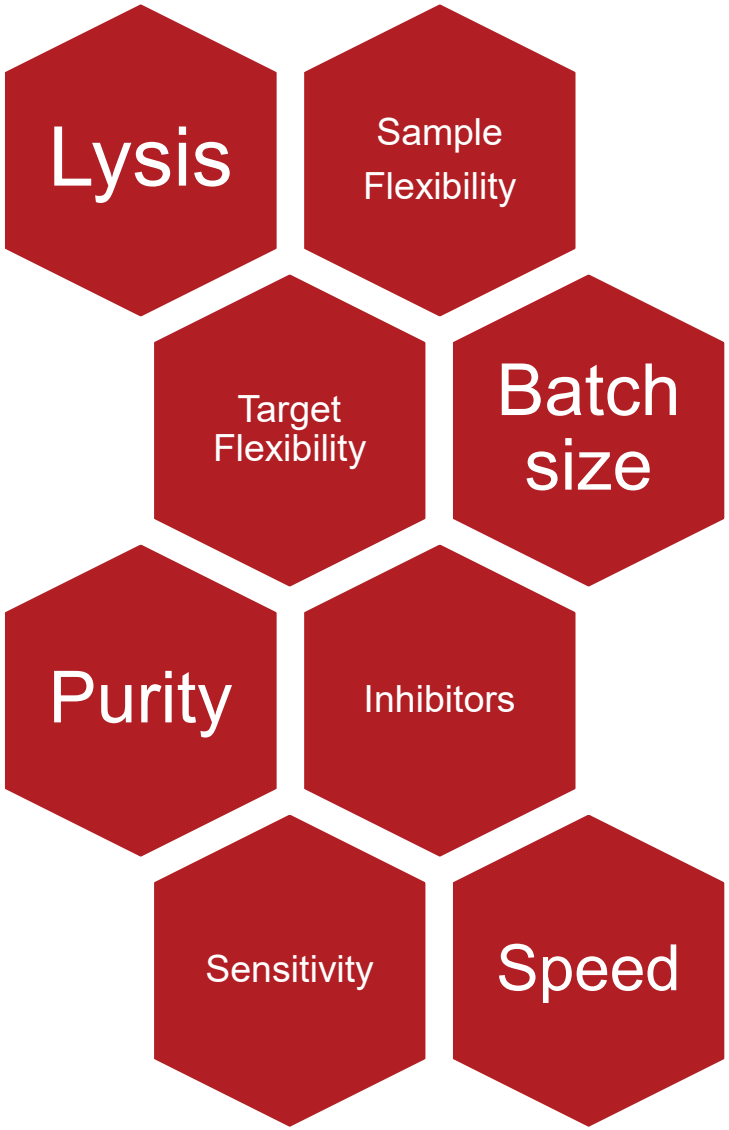
- No or low nucleic acid yield
  - Repeat isolation (or process additional sample)
  - Ensure ample time allowed for sample re-suspension
  - Concentrate nucleic acid with ethanol precipitation
- Inadequate lysis for the target
- Method may be too complicated for downstream application, using unnecessary time
- Method may be inadequate, inhibitors hindering the downstream application
- Large batch sizes can require longer wait times and longer pre-processing times

Best Practices for Nucleic Acid Isolation

- Optimize the input sample volume
- Optimize the output sample volume
- Use the appropriate part of the sample for the target
- Look for user-developed protocols or ask the manufacturer how to process your sample type, they may have additional information
- Test for inhibition and extraction
- Test for cross contamination

Summary

Nucleic acid procedures can meet any need with careful consideration of the factors important for any application



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