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

- Anion-exchange particle (e.g., DEAE-C)
- DNA molecule
- Salt

- Cell extract
- Ion-exchange resin
- Salt

- More salt

- Protein and RNA
- Discard

- DNA

Figure courtesy of Linnea M. Baudhuin, Ph.D.
Solid Phase Extraction: Affinity Chromatography

Any surface with a negative charge, usually silica

Lysis → Binding → Washing → Elution

Chaotropic salts
Alcohol
Low pH

Ethanol
Water

Figure courtesy of Linnea M. Baudhuin, Ph.D.
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**: $A_{260} \times 50 \times \text{dilution factor} = \mu g/mL \text{ DNA}$
  - **RNA**: $A_{260} \times 40 \times \text{dilution factor} = \mu g/mL \text{ RNA}$
- Purity of DNA:
  - $A_{260}/280$ ratio should be 1.6-2.0
  - $<1.6$ indicates protein contamination
  - $>2.0$ indicates RNA contamination
- Purity of RNA:
  - $A_{260}/280$ 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.
References


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