Extraction and Quantification of Nucleic Acids

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CLEVELAND CLINIC CENTER FOR PATHOLOGY EDUCATION

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Objectives

- Discuss preanalytic considerations for specimens common to molecular diagnostic labs
- Compare and contrast nucleic acid extraction methodologies
  - Liquid Phase vs. Solid Phase
  - Organic vs. Inorganic
  - Spin Column vs. Magnetic Bead
- Compare and contrast nucleic acid quantification/qualification methodologies
  - Electrophoresis
  - Spectrophotometry
  - Fluorometry
  - Microfluidics

References and Additional Resources

- Buckingham, Lela. *Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications.* 3rd ed., F.A. Davis, 2019.
  - Chapter 3: Nucleic Acid Extraction Methods

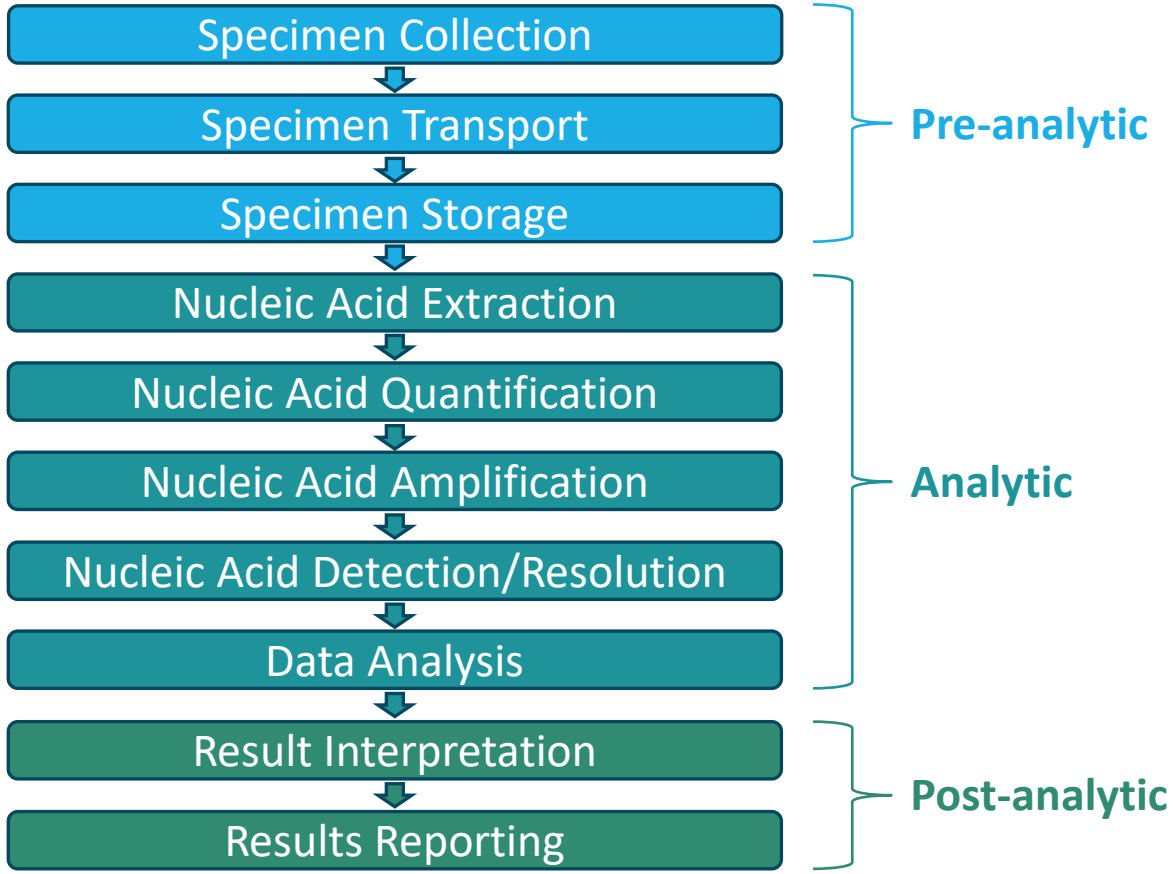
Specimen Collection and Processing

Nucleic acid extraction is the first analytic step for any molecular diagnostic procedure.

Specimen type, handling, and processing will vary by desired nucleic acid, test methodology, and disease type.

- DNA vs RNA
- NGS vs. qPCR
- Genetic vs. Somatic vs. Infectious disease

A good extraction begins with controlling pre-analytic variables that can affect extraction.



Specimen Types

HUMAN SPECIMENS

Peripheral blood (EDTA preferred, ACD also acceptable)

Bone marrow (EDTA preferred)

Tissue biopsy (fresh or frozen)

Formalin fixed paraffin embedded tissue (FFPET)

Buccal swabs

Saliva

Dried Blood Spot Cards

Pre-natal specimens

MICROBIOLOGY SPECIMENS

Peripheral blood/plasma/serum (EDTA)

Upper respiratory
- Nasal swabs
- Nasopharyngeal swabs
- Oropharyngeal swabs

Lower respiratory
- Sputum
- Bronchoalveolar lavage (BAL)

Stool

Urine

Urogenital swabs

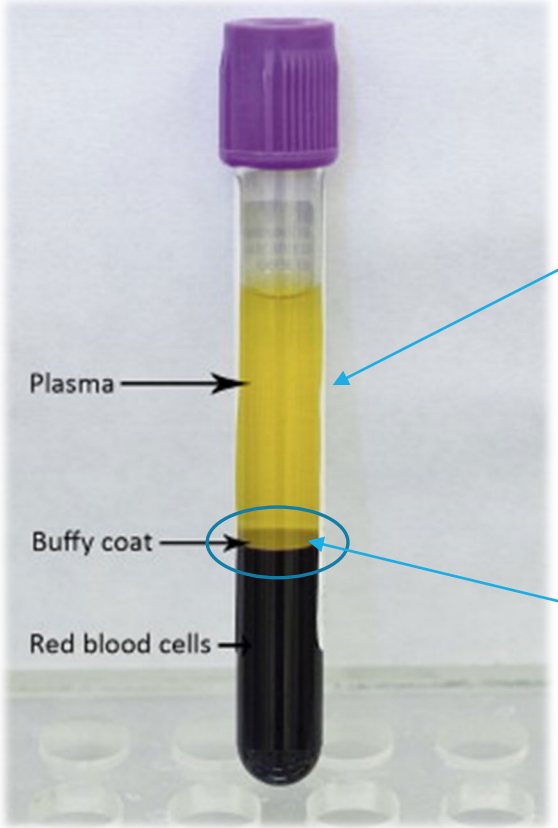
Anorectal swabs

Cultured bacteria or virus

Why EDTA preserved PB/BM?

Ethylenediaminetetraacetic acid (**EDTA**) functions by chelating metal cations (calcium, magnesium, iron, etc.) found in blood and bone marrow.

- **Calcium chelation**: prevents blood from clotting, ensuring that nucleated WBCs will be accessible for extraction
- **Magnesium chelation**: Magnesium is an important cofactor for nucleases (DNase, RNase), so removal reduces their activity and slows degradation of nucleic acids



Plasma contains cell-free NAs (cfDNA, cfRNA), useful for viral load testing

Buffy coat contains nucleated cells, useful for genomic testing

Formalin-Fixed Paraffin-Embedded Tissue (FFPET)

Formalin fixation: formalin, a solution of formaldehyde and water, is used to preserve the protein and organelle structure of tissue

Paraffin-embedding: fixed tissue is immersed in liquid paraffin wax, which cools to create a paraffin block containing the fixed tissue

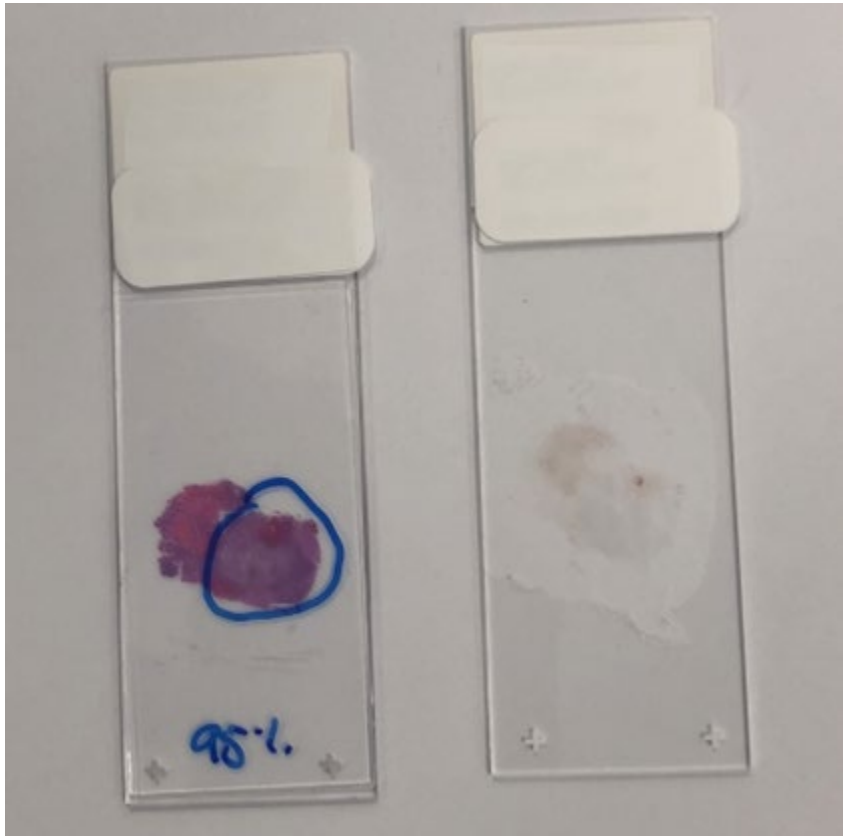
Microtomy: FFPET block is cut by microtome instrument to create thin (4 – 10 µm) sections of the tissue that are attached to slides for further analysis

- DNA/RNA completely stable at room temperature for years when fixed this way

Formalin-Fixed Paraffin-Embedded Tissue (FFPET)

Basic pre-extraction processing steps:

- Pathologist circles area of interest (tumor) on H&E stained slide area
  - Avoids non-tumor, necrotic tissue
- Technologist compares H&E to unstained slides, identifies comparable region for DNA/RNA extraction
- **Deparaffanization** process using a xylene/alcohol series to remove paraffin wax
- Enzymatic digestion of deparaffinized tissue
  - Proteinase K
- Proceed with extraction methodology

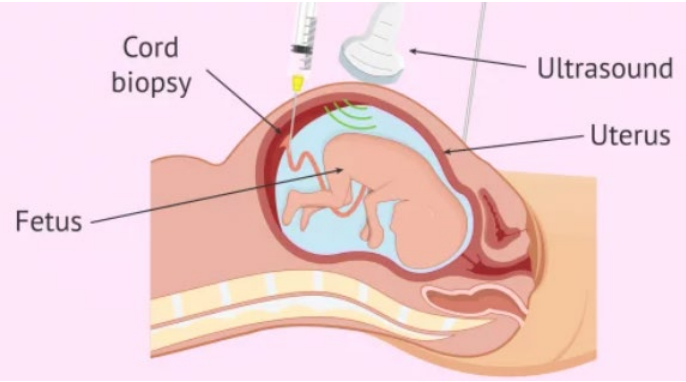


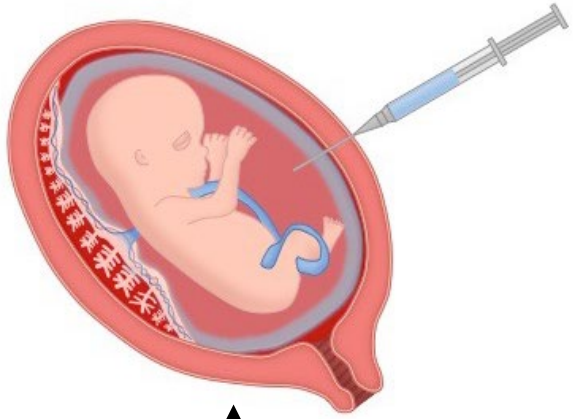
Pre-Natal Specimens

Various specimens collected pre-delivery or post-miscarriage to identify constitutional diseases of the fetus:

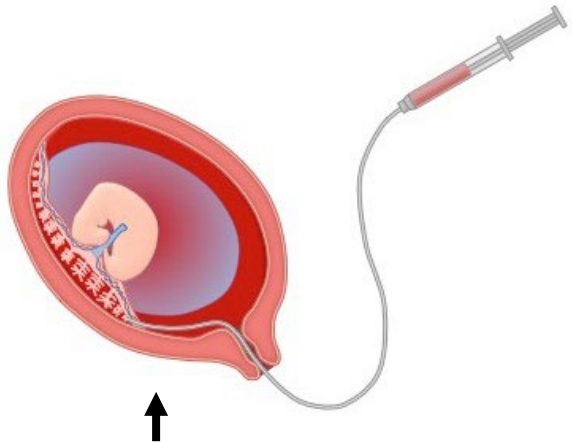
- **Cell Free DNA** (cfDNA) = fetal DNA circulating in the mother's peripheral blood
- **Amniotic fluid** = fluid taken from the amniotic sac through a procedure called amniocentesis
- **Percutaneous umbilical blood sample** (PUBS) = blood sample taken from fetal umbilical cord through a procedure called cordocentesis
- **Chorionic villus sample** (CVS) = placental tissue sample taken by a procedure of the same name
- **Products of conception** (POC) = any tissue developed during pregnancy (fetus, placenta, etc.)

Cordocentesis for PUBS





Amniocentesis for amniotic fluid



Chorionic villus sampling (CVS)

Dried Blood Spot Cards

Dried blood spot cards, or Guthrie Cards, are collected by pricking a newborn's heel and collecting enough blood to fill a series of circles on an absorbent paper card.

Collected for newborn screening of various inherited diseases.

Saliva and Buccal Swabs

Saliva and buccal (cheek swab) samples are non-invasive sources of genomic DNA.

- Option for self-collection
  - Less control over preanalytic variables

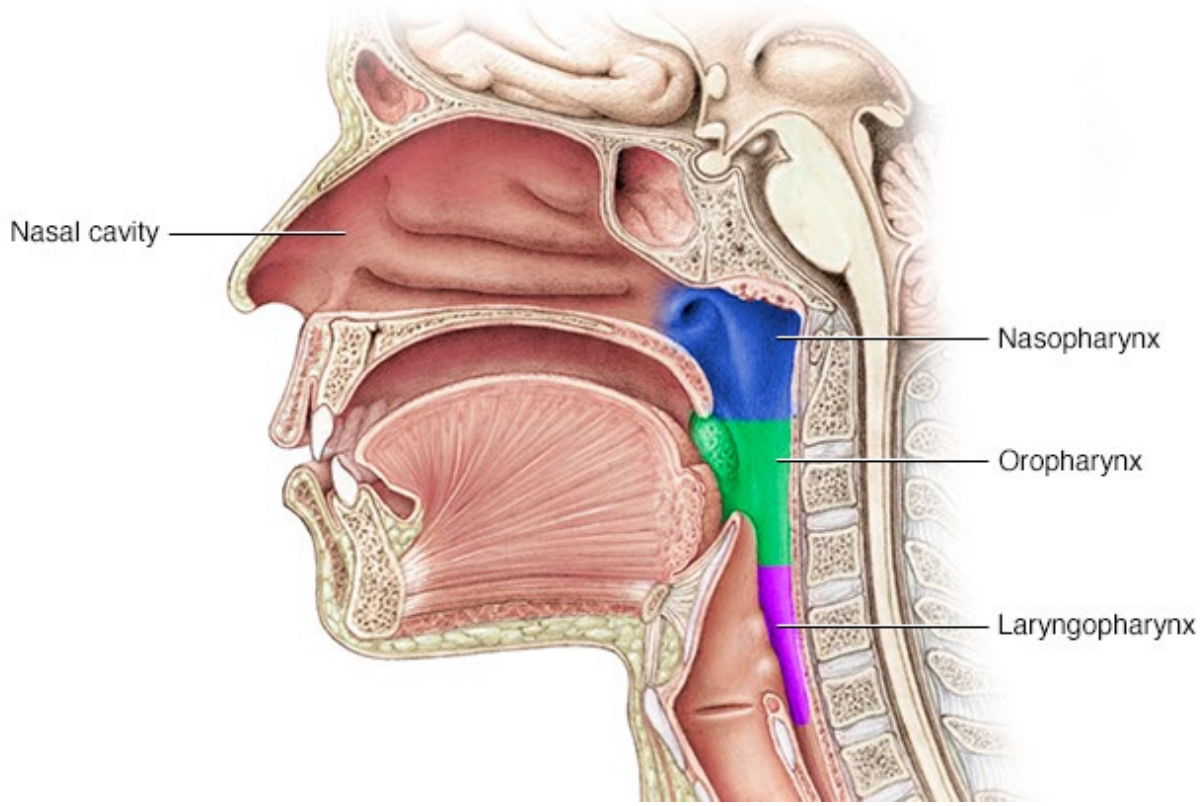
Upper Respiratory Tract Specimens

Specimens derived from the upper respiratory system:

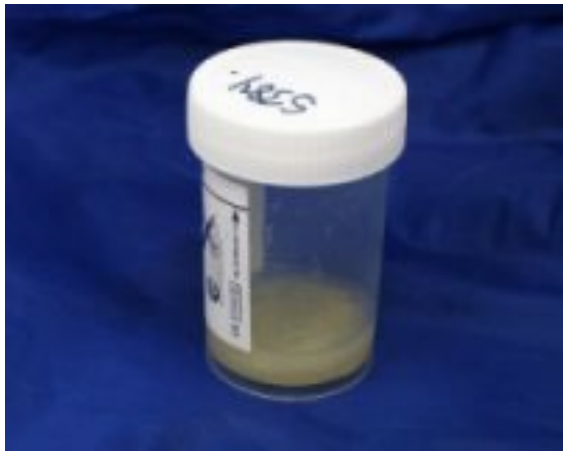
- Nasal Swab (NS)
- Nasopharyngeal Swab (NPS)
- Oropharyngeal Swab (OPS)

Not necessarily interchangeable specimens.

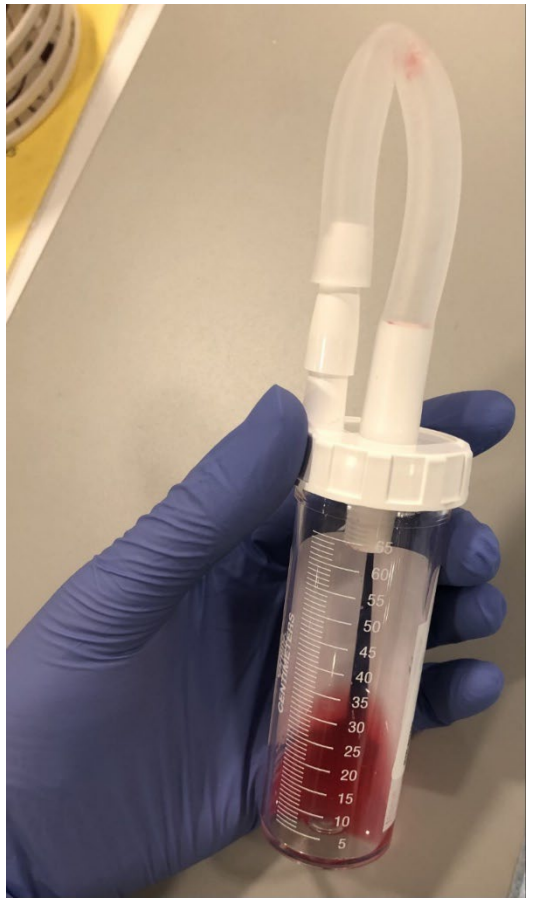
Flocked, nasopharyngeal swabs transported in Universal Transport Medium (UTM) or Viral Transport Medium (VTM) are preferred for most respiratory infectious disease testing.



Lower Respiratory Tract Specimens



← **Sputum** (SPUT): a mixture of saliva and mucus coughed up from the lower airways (trachea, bronchi)



Bronchoalveolar Lavage (**BAL**): specimen derived from the procedure of the same name. During bronchoscopy, a saline solution is pushed through the scope to wash the airways and collect a fluid specimen. →

Both generally require additional processing (sputolysin, DTT) to break down mucinous material prior to nucleic acid extraction.

Bacterial and Fungal Isolates

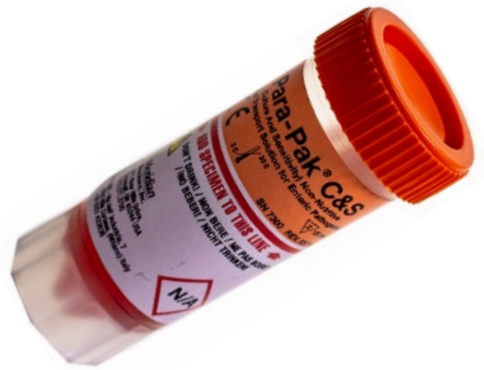
Thanks, Clinical Micro!

Colonies are pulled from a variety of growth mediums for infectious disease testing.

Stool, Urine, Urogenital and Anorectal Swabs

…thanks (?), Clinical Micro…

Collected for a variety of infectious disease testing.



Transport and Storage of Specimens

Endogenous and exogenous nucleases (DNase, RNase) are ubiquitous. Degradation of DNA/RNA can be slowed by storing specimens cold (2-8°C).

Blood specimens for DNA extraction can tolerate ambient temperatures and longer time before extraction (5-7 days).

- Still store refrigerated (2-8°C) ASAP to slow specimen degradation

Blood specimens for RNA extraction must be kept on ice during transport, must be extracted within 48-72 hours.

- Store refrigerated (2-8°C) upon receipt

Processing of Specimens

Lower respiratory specimens may require additional enzymatic digestion prior to extraction.

Tissue biopsies may require mechanical processing (razor, scalpel, grinder) and enzymatic digestion.

FFPET will require deparaffinization and enzymatic digestion prior to extraction.

Fungal and bacterial isolates often have thick cell walls that will require manual/enzymatic lysing prior to extraction.

DNA Extraction Methods

All methodologies begin with an initial cell lysis that releases nucleic acid (DNA and/or RNA) into solution.

Methodologies differ in how they isolate nucleic acid from proteins, lipids, carbohydrates, and other cellular debris.

Four major methods, divided into two groups:

- Liquid-Phase Isolation
  - Organic Isolation Method
  - Inorganic Isolation Method, also called “salting-out”
- Solid-Phase Isolation
  - Spin Column Method
  - Magnetic Bead Method

Organic Isolation Method

Mostly historical methodology, as far as medical labs are concerned

- Still used in academic, research, and forensic settings

Utilizes very toxic chemicals (**phenol, chloroform**)

Highest nucleic acid yield and purity of all extraction methods

Not routinely performed at CCF

- Potential benefits for DNA extraction do not outweigh the occupational health risks

Phenol

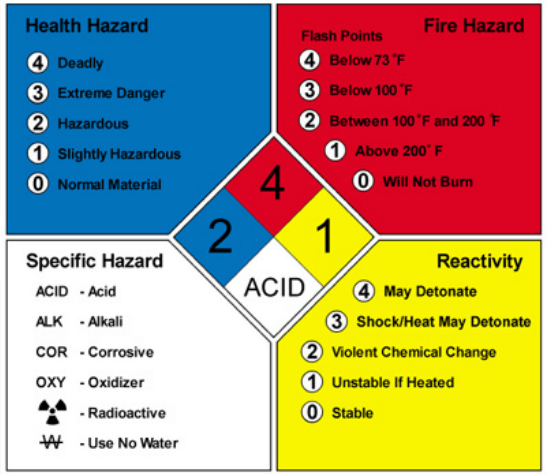
White, crystalline solid; medicinal odor. Poison! Corrosive, causes severe burns to the eyes (blindness)/skin/respiratory tract. Also causes: severe neurological effects (shock and coma), liver and kidney damage. Absorbed through the skin. Combustible.

CAS No. 108-95-2

Chloroform

Colorless, volatile liquid; ethereal odor. Irritating to eyes/skin/ respiratory tract. Toxic. Also causes: dizziness, liver/kidney damage. Chronic: CNS effects. Possible cancer hazard. May cause birth defects based on animal data.

CAS No. 67-66-3



Organic Isolation Method

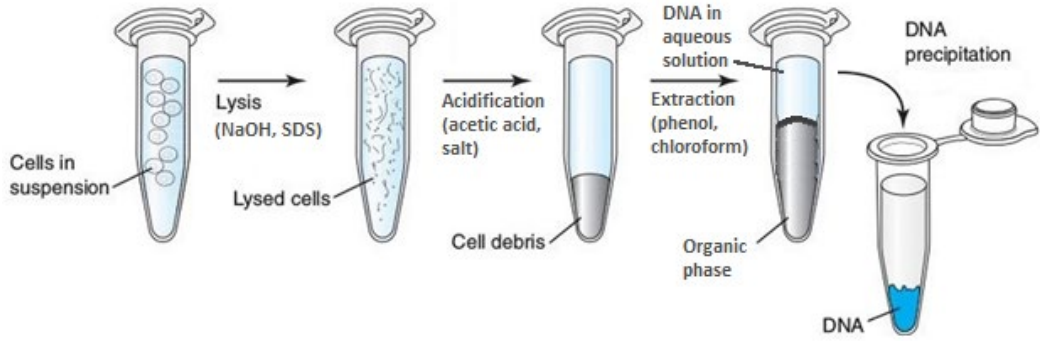
Step 1: Cell lysis and DNA release

- Sodium dodecyl sulfate (SDS): anionic detergent that destabilizes cell membranes/walls

Step 2: Protein precipitation

- Low-pH and high-salt environment reduces solubility of proteins
- Centrifuge to pellet protein and cellular debris

Step 3: **Optional RNase step** to remove contaminating RNAs for DNA extraction.



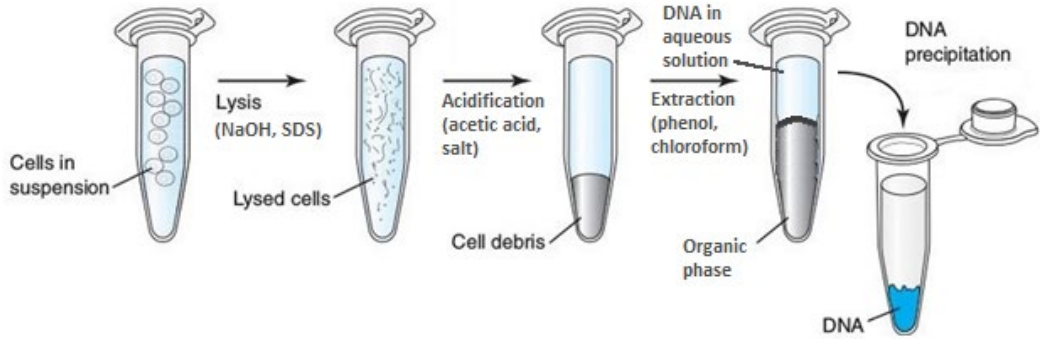
Organic Isolation Method

Step 4: Extraction

- Supernatant from protein precipitation step is added to phenol-chloroform
- Centrifugation creates two layers
  - **Upper: aqueous, hydrophilic layer (DNA)**
  - Lower: organic, hydrophobic layer (lipids, proteins)

Step 5: DNA precipitation

- Aqueous supernatant added to ethanol/isopropanol to precipitate DNA
- Centrifuge to pellet DNA
- Remove supernatant



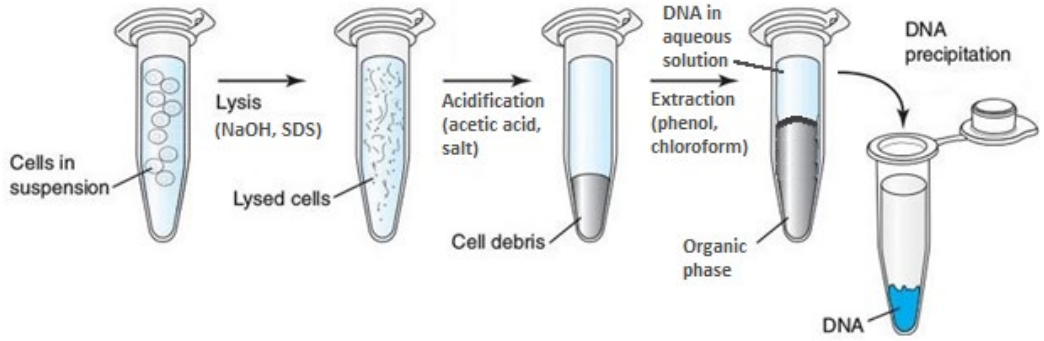
Organic Isolation Method

Step 6: Ethanol wash

- Pelleted DNA is washed several times with ethanol to further purify, remove contaminants

Step 7: Hydration

- Pelleted DNA is hydrated in Tris-EDTA (TE) buffer or nuclease-free water
  - More on this later



Inorganic Isolation Method

Safer methodology than organic

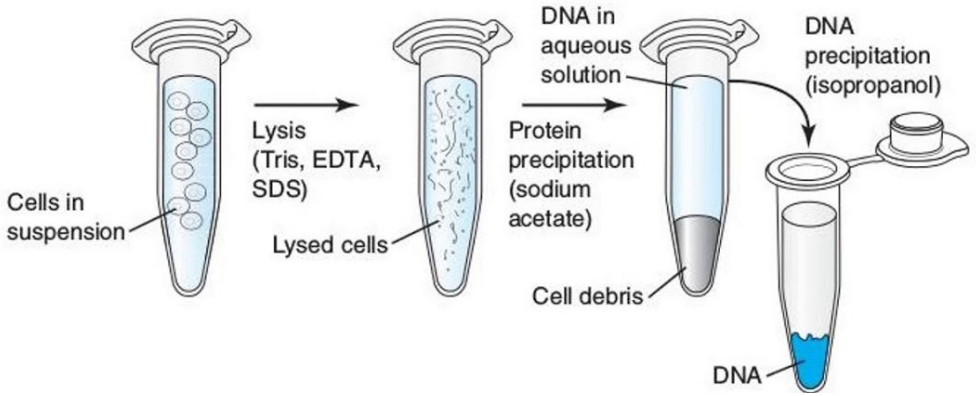
- Does not use phenol/chloroform

Often called the “salting out” method, due to its use of high-salt/low pH protein precipitation step

Fewer steps, but did not initially provide as high quantity/quality yields

- Modern kits have optimized this process, comparable yields to organic method

Preferred manual extraction method at CCF



Inorganic Isolation Method

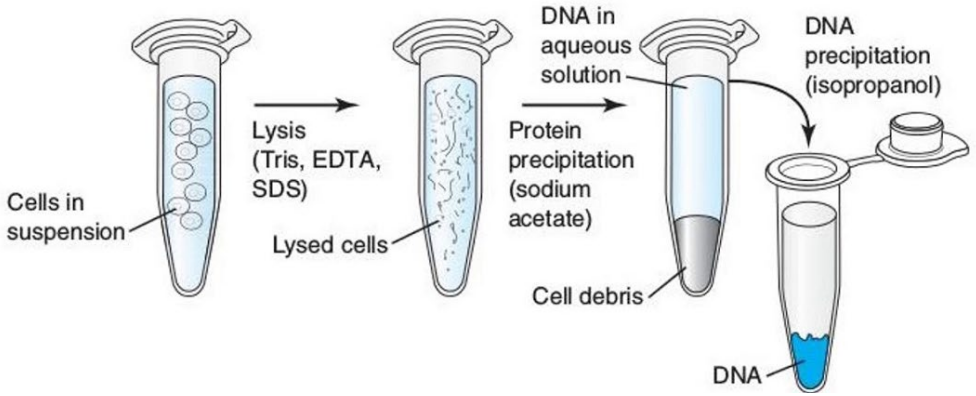
Step 1: Cell lysis

- Sodium dodecyl sulfate (SDS): anionic detergent that destabilizes cell membranes/walls

Step 2: Protein precipitation

- Low-pH and high-salt environment reduces solubility of proteins
- Centrifuge to pellet protein and cellular debris

Step 3: **Optional RNase step** to remove contaminating RNAs to remove contaminating RNAs for DNA extraction.



Inorganic Isolation Method

Step 4: DNA precipitation

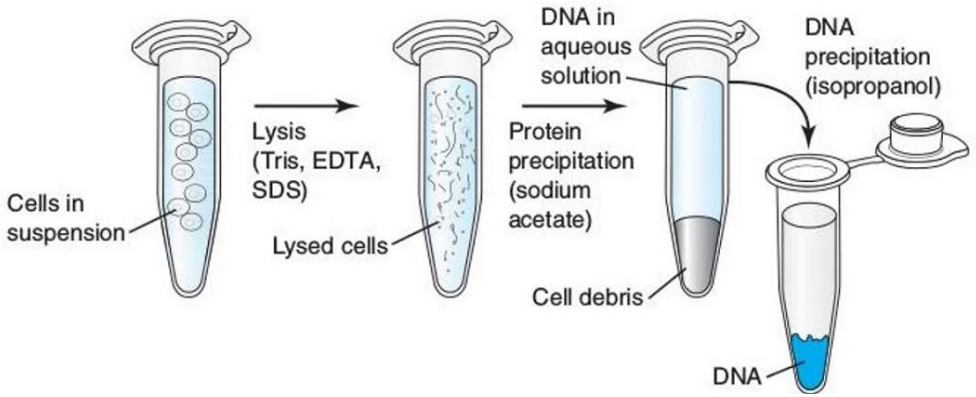
- Supernatant from protein precipitation step added directly to isopropanol
- Inversion of mixture will cause visible precipitation of DNA
- Centrifugation pellets DNA, remove supernatant

Step 5: Ethanol wash

- Pelleted DNA is washed several times with ethanol to further purify, remove contaminants

Step 6: Hydration

- Pelleted DNA is hydrated in Tris-EDTA (TE) buffer or nuclease-free water



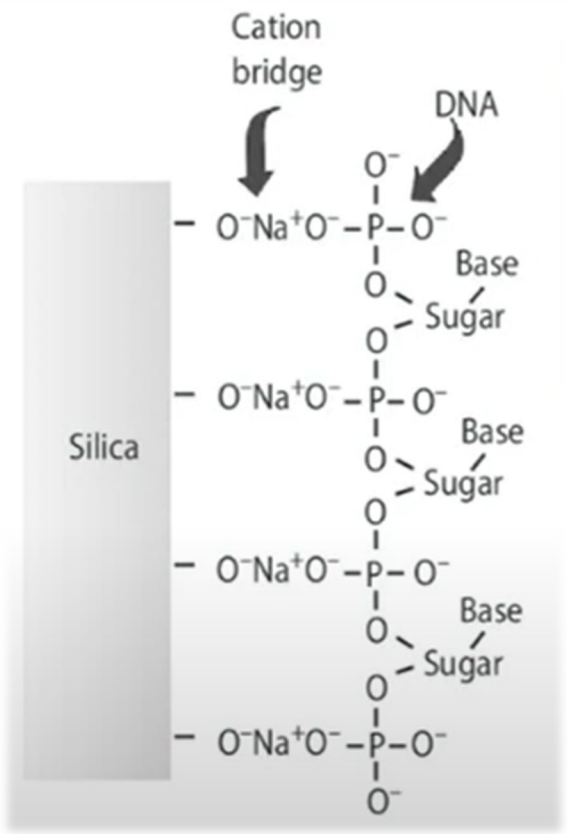
Solid-Phase Isolation

Nucleic acid binds to silica at low-pH levels and high-salt conditions

- Formation of **cation bridges** between negatively charged silica and negatively charged sugar-phosphate backbone

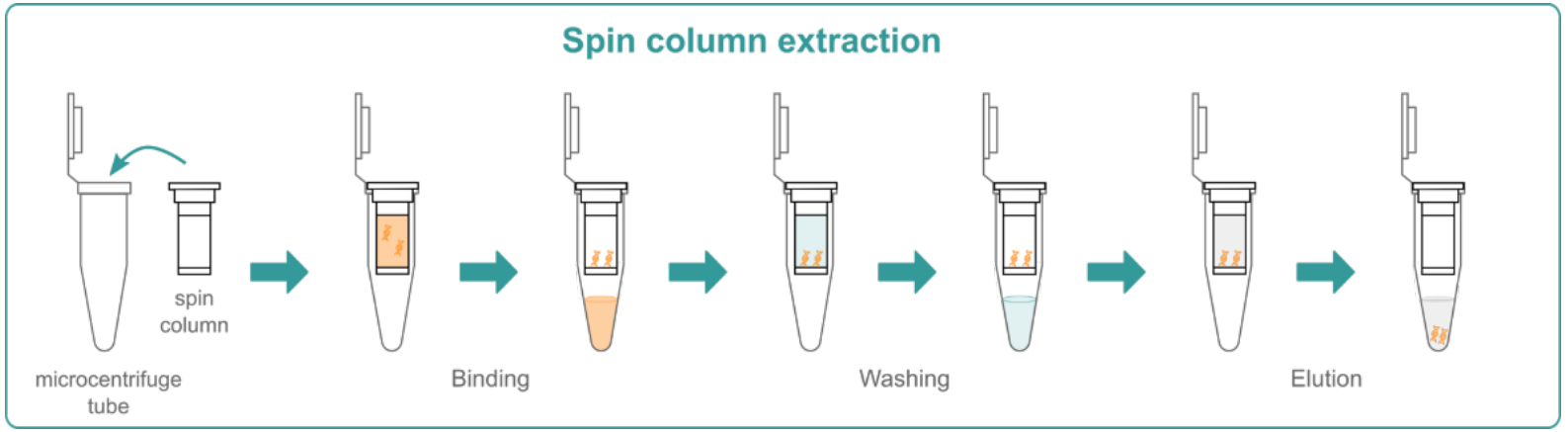
Two types:

- Spin columns: lysed sample added to spin column containing silica filter that binds DNA/RNA
- Magnetic beads: magnetic beads covered in silica are added to lysed sample, magnet is used to retain nucleic



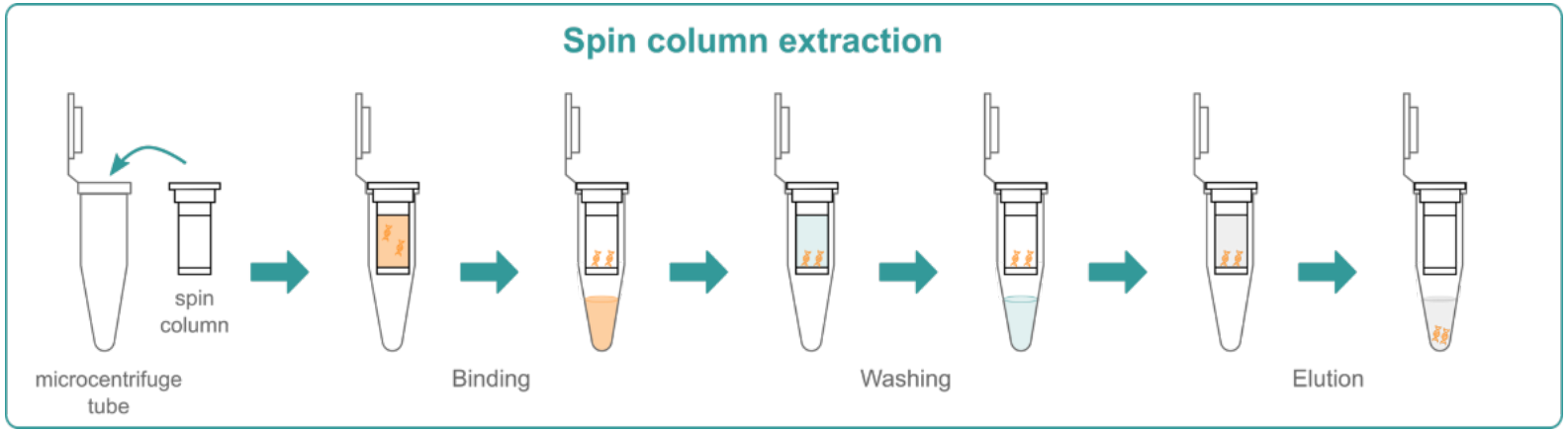
Spin Column Method

- Step 1: Sample lysis
- Step 2: Lysate added to spin column in collection tube, DNA/RNA binds to silica filter in high salt conditions (binding buffer).
- Step 3: Centrifugation, DNA/RNA is retained by the silica filter in the spin column, flowthrough containing proteins and cell debris is discarded.



Spin Column Method

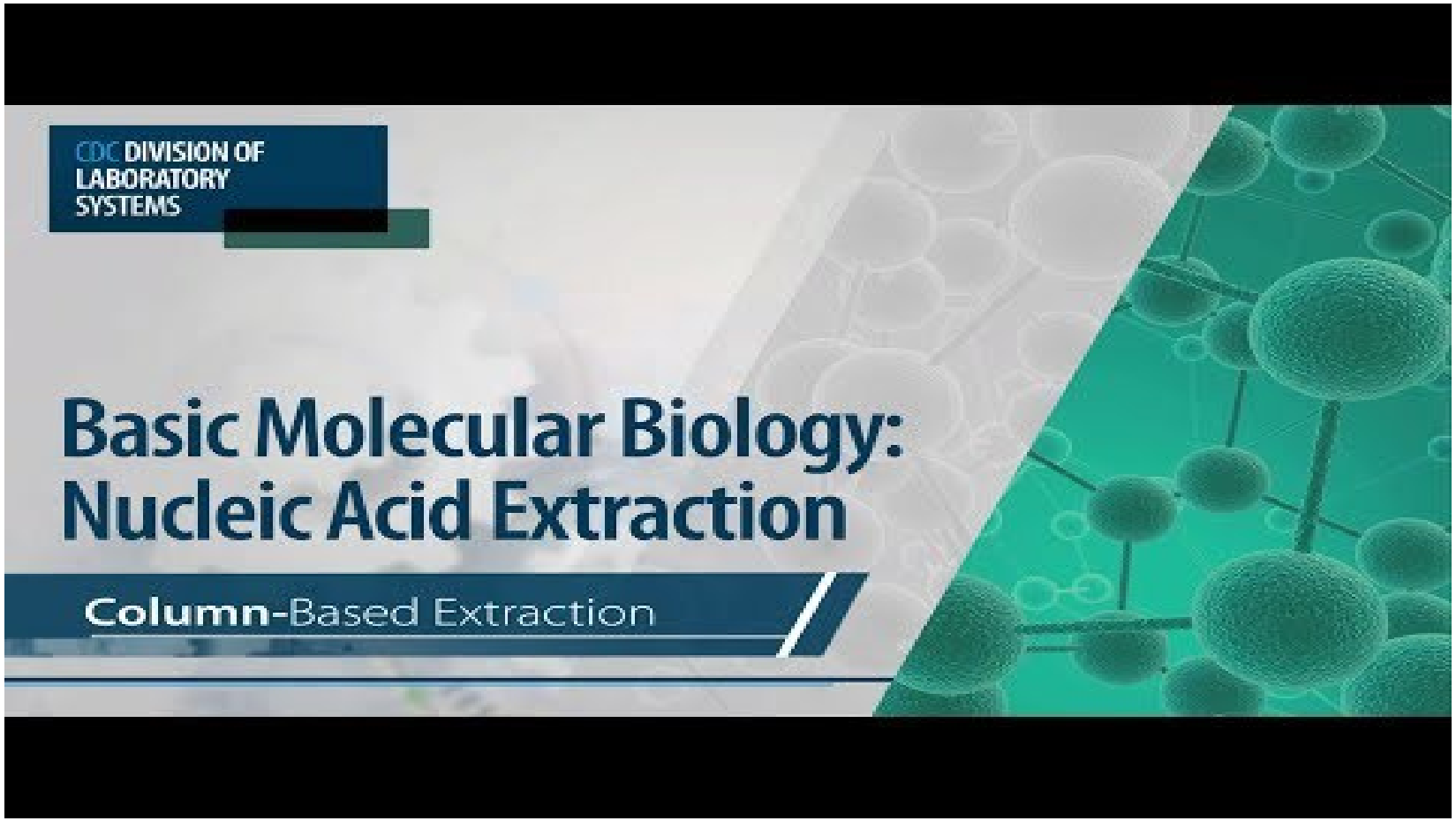
- Step 4: Wash buffer (usually ethanol-based) added to spin column
- Step 5: Centrifugation, wash buffer flowthrough tube discarded
- Step 6: Elution buffer (low salt) added to spin column, DNA/RNA is released from silica
- Step 7: Centrifugation, elution buffer containing DNA/RNA is collected in final tube.



Colmun-Based Extraction

This video is part of CDC's free online educational series, CDC OneLab™.

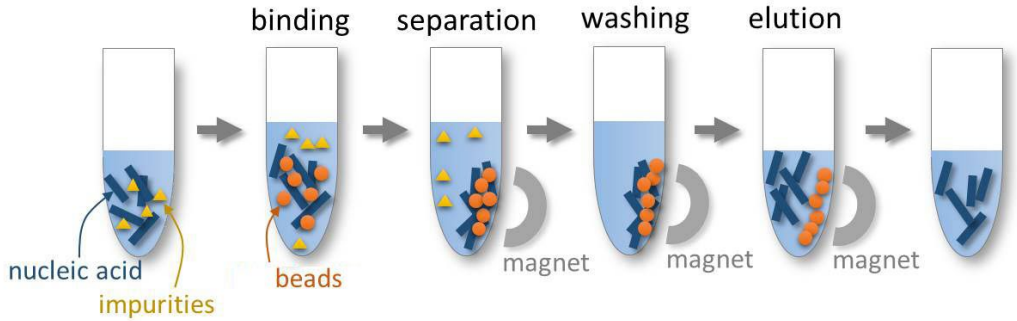
YouTube:
https://youtu.be/ZN1bZ6Q_mG4



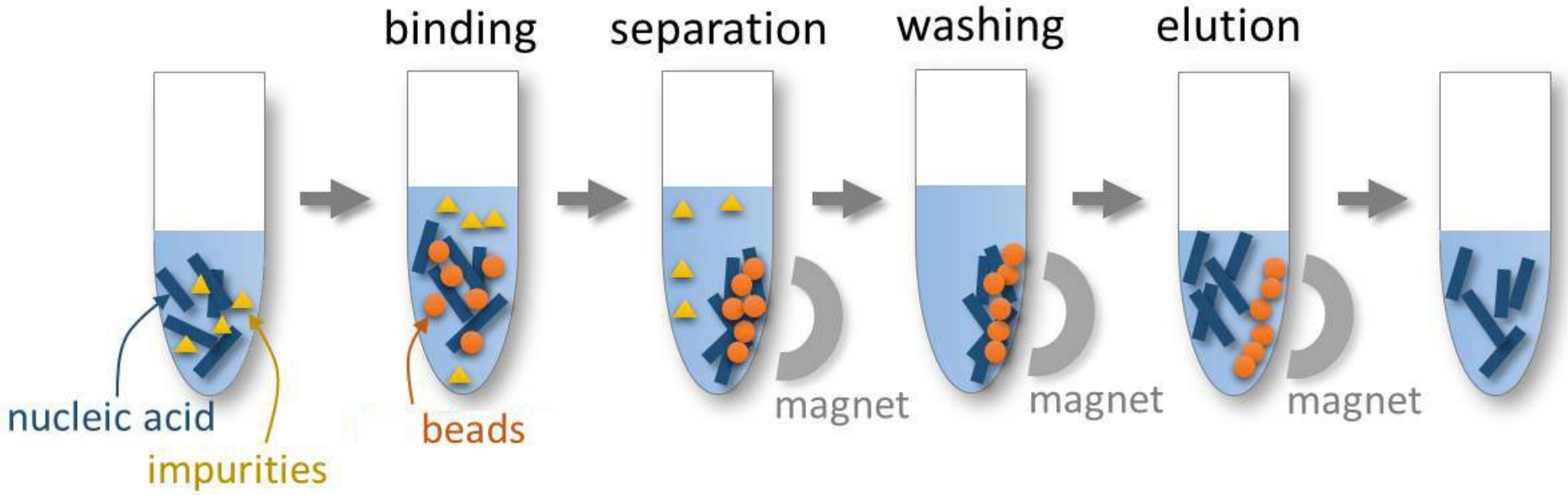
Magnetic Bead Method

Magnetic bead method

- Step 1: Sample lysis
- Step 2: Magnetic beads (silica coating) added to lysate along with binding buffer. DNA/RNA binds to beads in high salt conditions.
- Step 3: Magnet retains beads while proteins and other cellular debris are washed away
- Step 4: Elution buffer (low salt) is added and causes DNA/RNA to denature from beads
- Step 5: Magnet removes beads, leaving purified DNA/RNA behind



Magnetic Bead Method



Magnetic Bead Extraction

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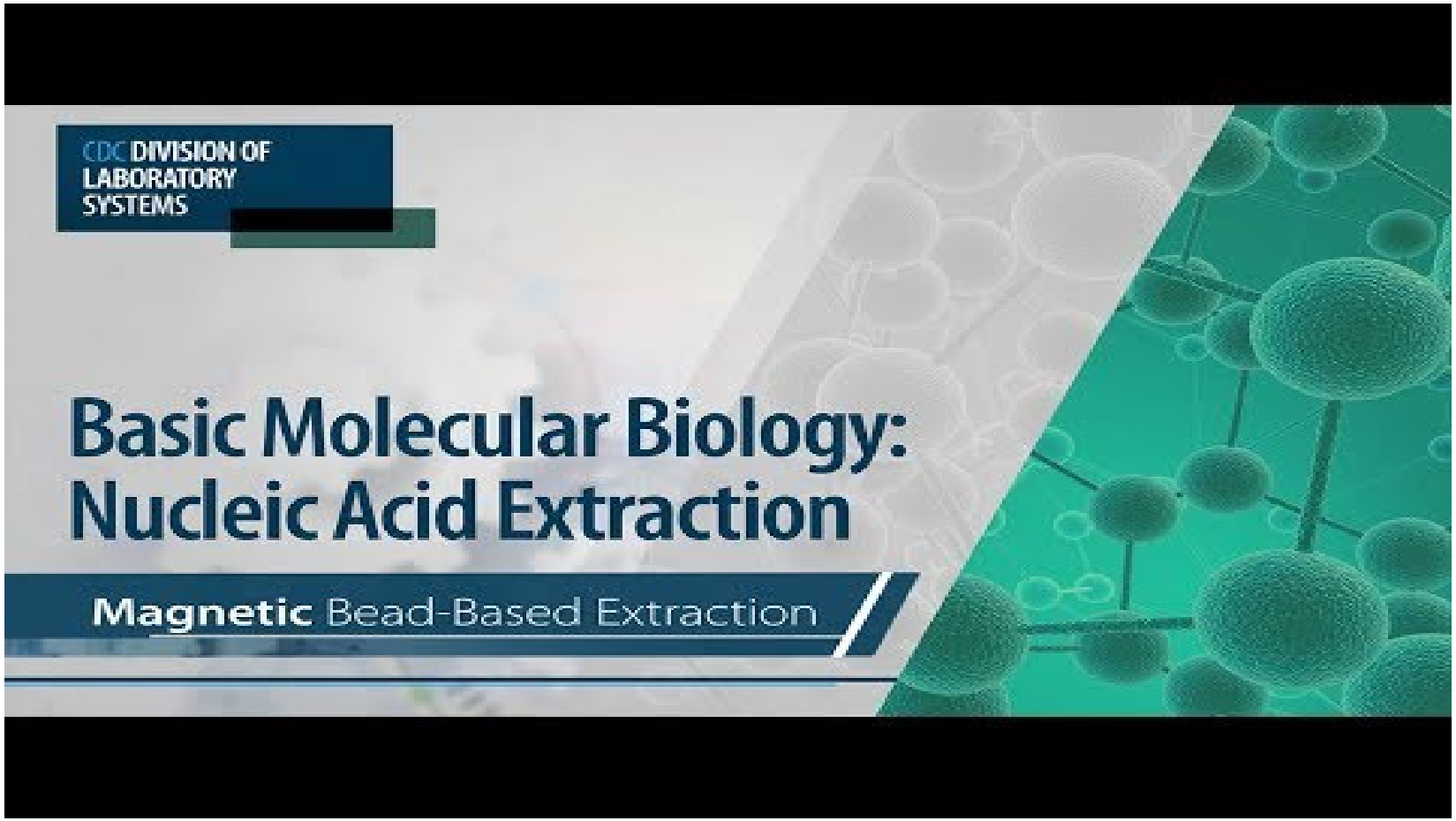


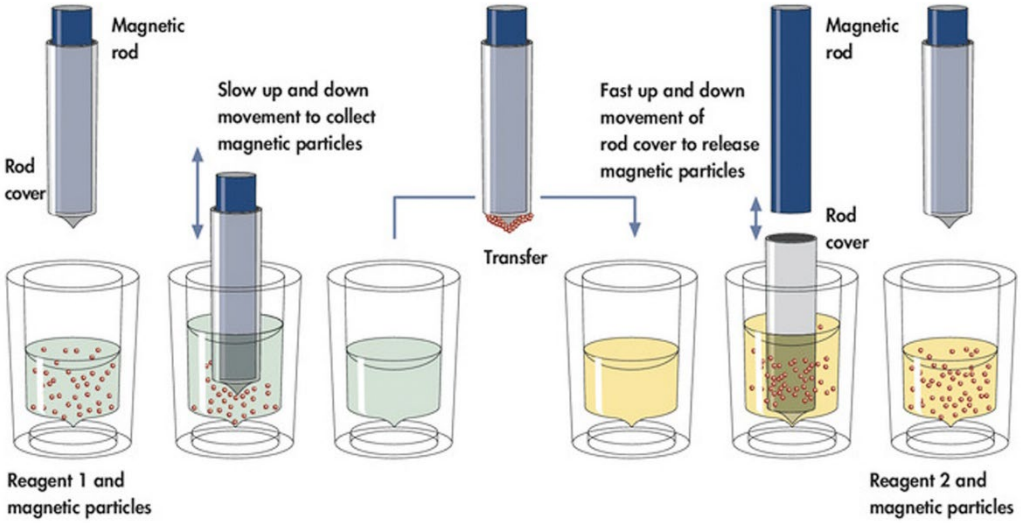
Automated Systems (Magnetic Bead)

There are many different brands of automated nucleic acid extraction instruments.

- QiaSymphony, MagnaPure, Maxwell, Hamilton, Kingfisher, etc.

Most operate using a magnetic rod-magnetic bead technology.

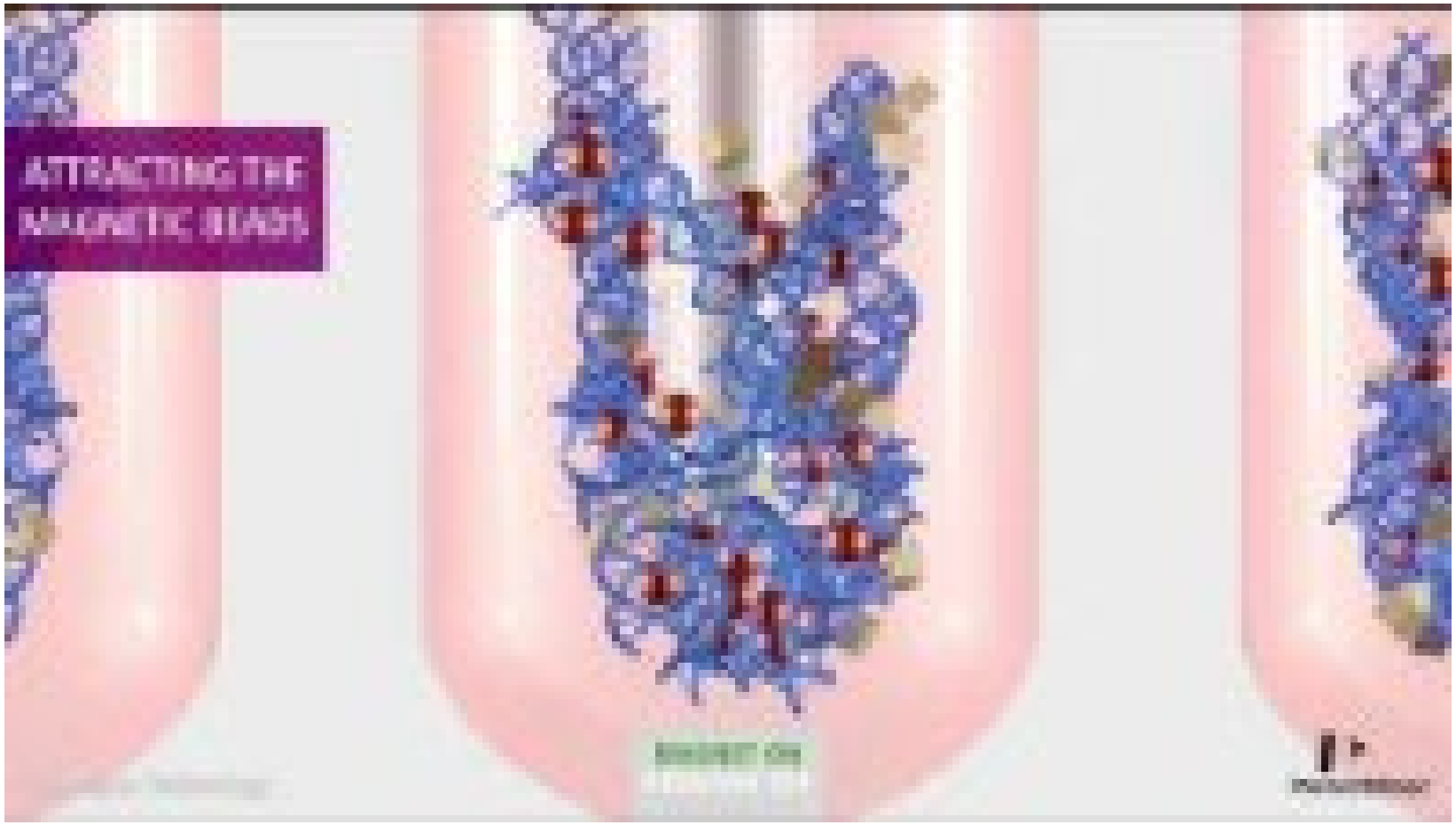
- Sample lysate is combined with magnetic beads that bind nucleic acid
- Magnetic rods transfer beads through a series of wash steps to remove contaminants
- Beads are transferred to well/tube containing elution buffer where nucleic acid is released
- Beads are removed by magnetic rod



Automated Systems (Magnetic Bead)

PerkinElmer Demo video.

YouTube: https://www.youtube.com/watch?v=deVufNDJ4GI



RNA Extraction Methods

RNA extraction methods are very similar to the previously described DNA extraction methods (organic, inorganic, spin column, magnetic bead)

Some additional considerations to prevent RNA degradation:

- Work in a separate, dedicated RNase-free area to prevent introduction of environmental RNase
- Work over ice to prevent thermo-labile RNA molecule from degrading
- Incorporate chemical denaturant of RNases
  - Guanidine isothiocyanate (**GITC**), more commonly known by the brand name **TRIzol**
  - β-mercaptoethanol (**BME**)
  - Dithiothreitol (**DTT**)
  - Diethyl pyrocarbonate (**DEPC**)
- DNase step to remove contaminating DNA

Storage of Nucleic Acids

Regardless of extraction method, final step involves elution/hydration of isolated nucleic acid in solution.

Tris-EDTA Buffer, aka **TE**

- Preferred for long-term storage
- Prevents nucleic acid degradation by EDTA-chelation of cationic cofactors of nucleases
  - Be careful with the concentration , Mg^{2+} is an important cofactor for PCR

Nuclease-free water

- Acceptable for short-term storage
- Preferred for low-yield specimens

Storage and Handling of Nucleic Acids

DNA

Can be worked with at room temp

Very short-term storage (days/weeks) at room temp is acceptable

Short-term storage of several years at 2-8°C

Longer-term storage should be frozen at -20°C or lower

Avoid repeated freeze-thaws to prevent mechanical degradation

RNA

Never store or work with at room temperature

- Work with over ice

Short- and long-term storage at -70°C

- RNases are still functional at -20°C

Avoid repeated freeze-thaws to prevent mechanical degradation

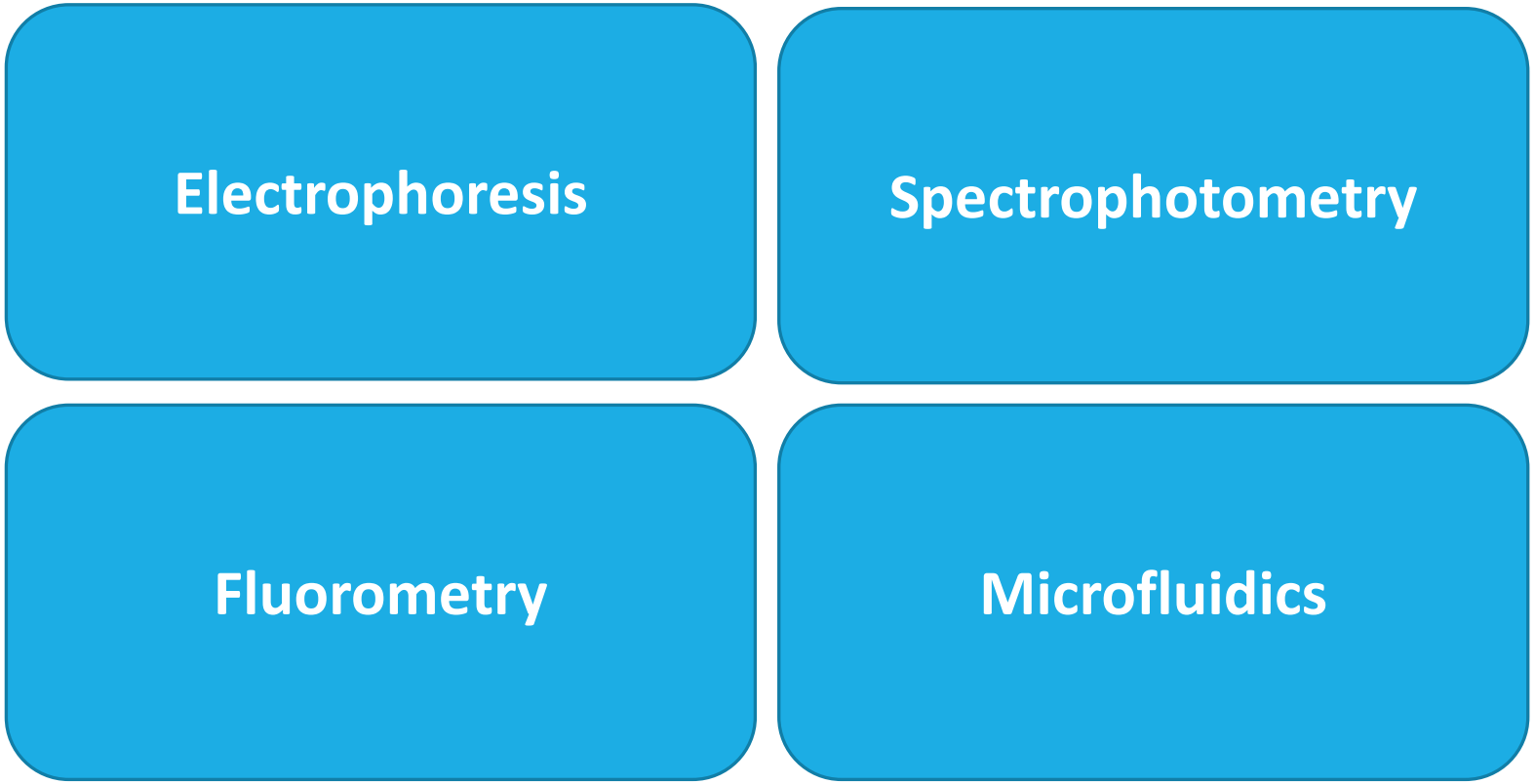
Quantification and Qualification

Extracted nucleic acid samples must meet standards of quantity and quality prior to being tested.

QUANTITY		QUALITY	
Total Yield (µg)	**Concentration (µg/mL)**	**Purity**	**Molecular Weight**
Is there enough nucleic acid to perform all required testing?	Is nucleic acid present at a high enough concentration to perform testing?	Are there contaminating, interfering or inhibiting substances present?	Is nucleic acid too degraded or fragmented to be used for testing?
e.g., Total loading volume for Carrier Screen Panel (FX, SMA, CF, AJPNL) is 0.5µg.	*e.g.*, Loading for FX is 5µL at 20ng/µL, while loading for SMA is 2µL at 10ng/µL.	*e.g.*, residual protein from original specimen, alcohols or phenols from extraction	*e.g.*, old specimen, harsh specimen processing

Quantification and Qualification (cont.)

There are four primary methods of quantification and qualification:



Electrophoresis

Agarose gel electrophoresis can be used to roughly assess quantity and quality of DNA and RNA samples

Negatively-charged nucleic acids migrate towards the positively-charged pole (anode)

Specimen is run alongside a sizing ladder with fragments of known base pair size

Fluorescent dyes allow for UV visualization of DNA/RNA as it migrates through agarose gel matrix

- Ethidium bromide, SybrGreen

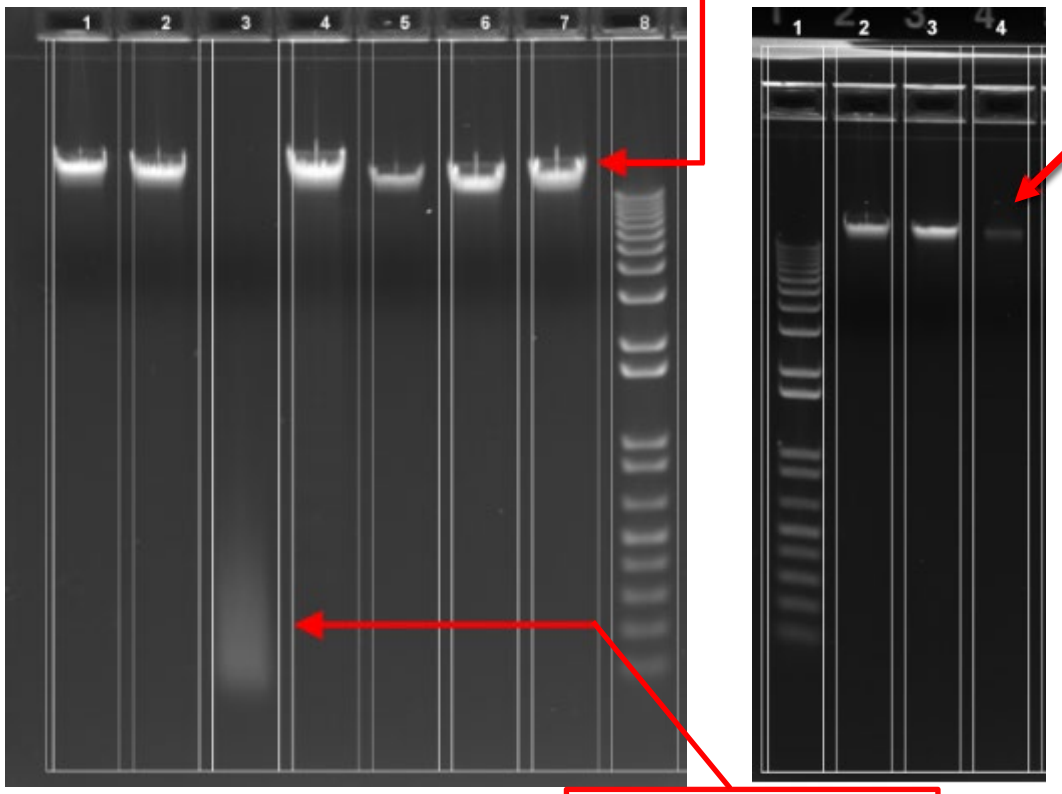
Electrophoresis

Size of DNA/RNA molecules affects mobility

- Larger, dsDNA and ssRNA migrates slowly and will remain close to the loading well
- ssDNA and highly fragmented DNA and RNA will migrate further from the loading well and smear

Faint, smeared, or highly mobile bands indicate sample may be unsatisfactory for testing

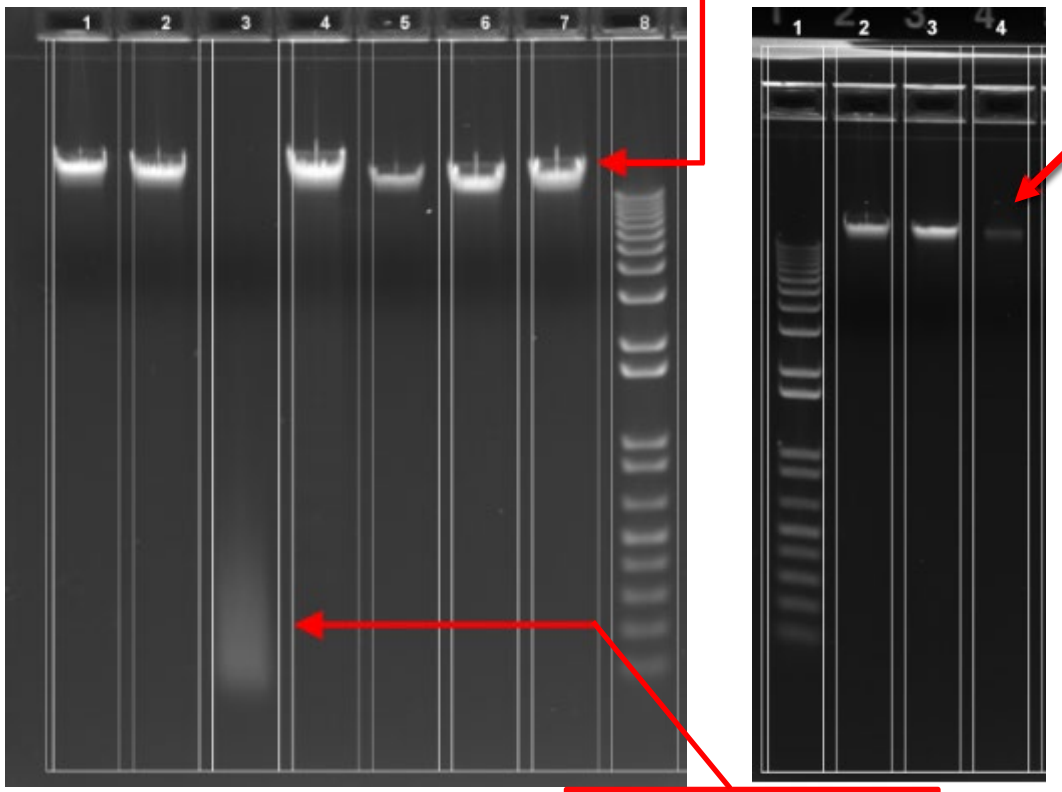
Gel electrophoresis is often used as a post-nucleic acid amplification quality check.



Electrophoresis

Summary of banding patterns and their meanings for nucleic acid quant/quality:

- Intense band = high quant
- Faint band = low quant
- Single, solid band = unfragmented
- Smeared band = fragmented
- Low mobility = high molecular weight
- High mobility = low molecular weight



Spectrophotometry

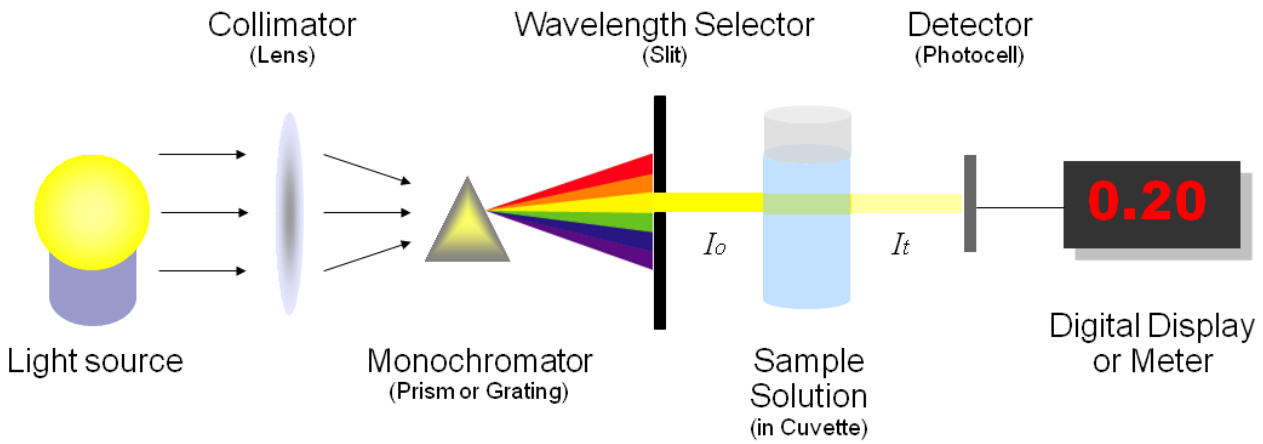
Nucleic acids, specifically the nitrogenous bases they contain, absorb light at wavelength of 260nm.

Concentration can be calculated using a modified version of the Beer-Lambert equation ($A=\epsilon bc$) where…

Nucleic Acid Concentration = ($Absorbance_{260}$) x (Absorptivity Constant) x (Sample Dilution)

The absorptivity constant will differ for the measurement of DNA vs. RNA

- DNA = 50 µg/mL per absorbance unit
- RNA = 40 µg/mL per absorbance unit



Spectrophotometry

The spectrophotometric quantification method, in practice, is very user-friendly.

NanoDrop spectrophotometers are a commonly used quantification/qualification instrument.

- Adjust for absorptivity constant
- Blank with hydration/elution solution
- Apply DNA/RNA sample
- Measure
  - No need to generate a standard curve, as in traditional spectrophotometry

NanoDrop measures absorbance and auto-calculates sample nucleic acid concentration.

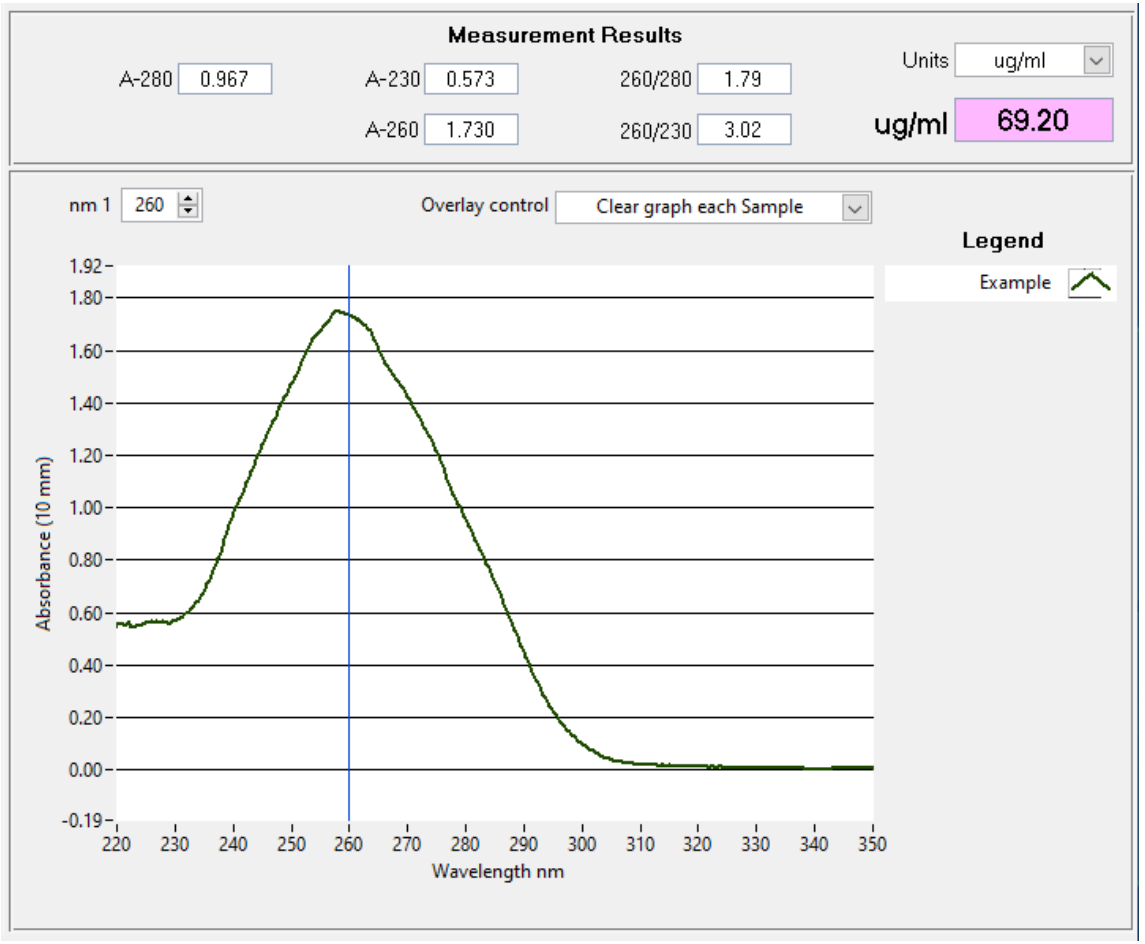
NanoDrop 8000 Spectrophotometer

Spectrophotometry

Image to the right shows a good NanoDrop Spectrophotometer result for an RNA extraction, with high absorbance at 260nm.

To manually calculate the concentration of the measured sample, multiply absorbance at 260nm by the absorptivity constant:

1.730 x 40 µg/mL = 69.200



Spectrophotometry

Practice Problem #1: an undiluted DNA specimen

A **DNA** specimen was eluted with **0.5mL** of elution buffer. Absorbance of the **undiluted** specimen at 260nm was **0.300**.

To calculate concentration, multiply absorbance at 260nm by absorptivity constant for DNA:

(0.300)(50 µg/mL per absorbance unit) = 15 µg/mL

To calculate total yield, multiply concentration by total volume of the specimen:

(15 µg/mL)(0.5mL) = 7.5 µg

Spectrophotometry

Practice Problem #2: a diluted RNA specimen

A **1:5 dilution** of an **RNA** specimen had a measured absorbance of **0.100** at 260nm. The original sample was rehydrated in **2.0 mL** of hydration solution.

To calculate the concentration of the original undiluted sample, multiply absorbance at 260nm by absorptivity constant for RNA by dilution factor:

(0.100)(40 µg/mL per absorbance unit)(5) = <u>20 µg/mL</u>

To calculate total yield, multiply measured concentration by total volume of the specimen:

(20 µg/mL)(2.0 mL) = <u>40 µg</u>

Note: ng/µL is preferred concentration unit for MDx, but it will be the same number as ug/mL. (1000 ng in a µg, and 1000µL in a mL)

Spectrophotometry

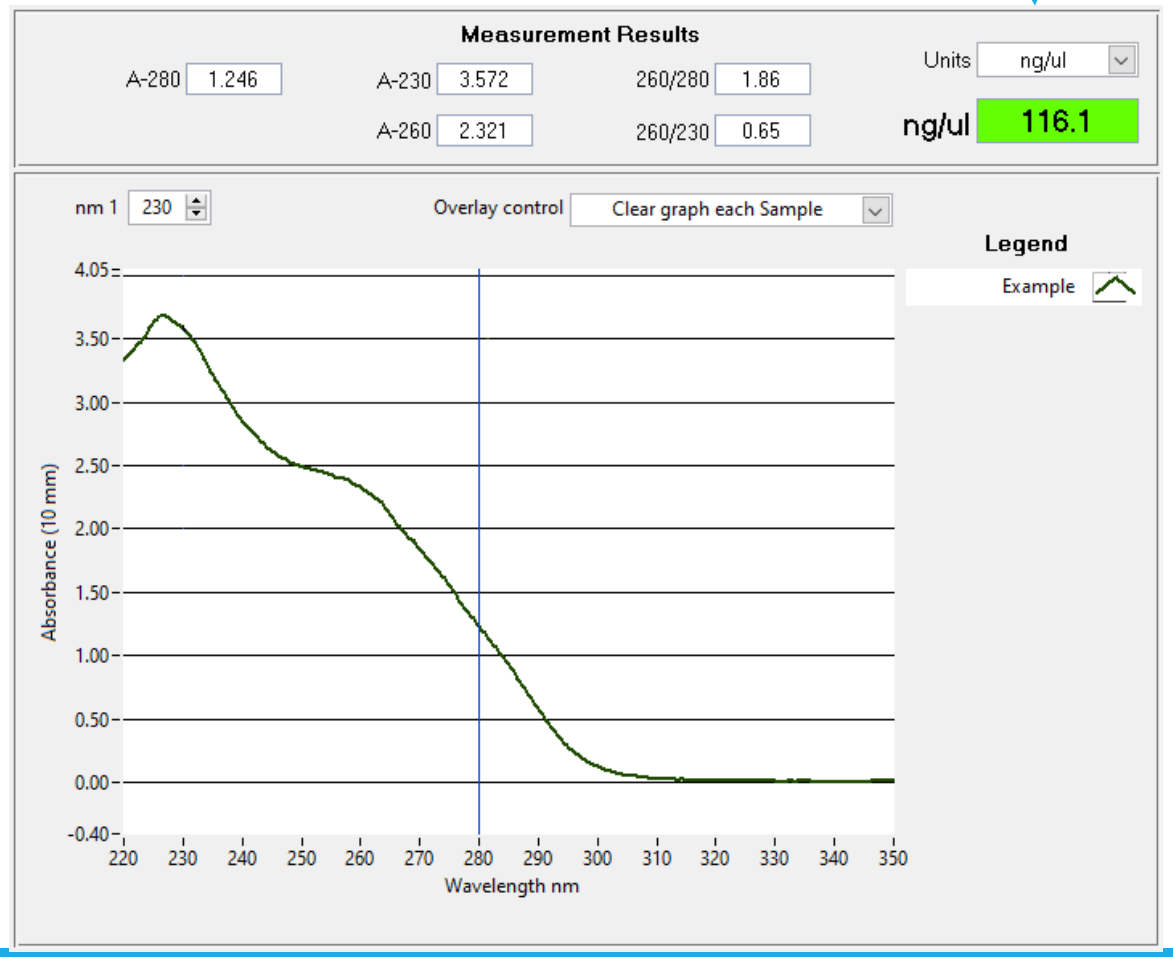
Quality can be assessed with reads at other wavelengths, specifically 280nm and 230nm

260/280 ratio

- For DNA, absorbance at 260nm should be 1.6 – 2.0x the absorbance at 280nm.
- For RNA, absorbance at 260nm should be 2.0 – 2.3x the absorbance at 280nm.
  - DNA measurement to the right:

    260 = 2.321 and 280 = 1.246

    So 260/280 = 2.321 ÷ 1.246 = 1.863 (acceptable)
- Low ratio (<1.6 for DNA, <2.0 for RNA) may indicate contamination with **proteins**.



Note: ng/µL is preferred concentration unit for MDx, but it will be the same number as ug/mL. (1000 ng in a µg, and 1000µL in a mL)

Spectrophotometry

Quality can be assessed with reads at other wavelengths, specifically 280nm and 230nm

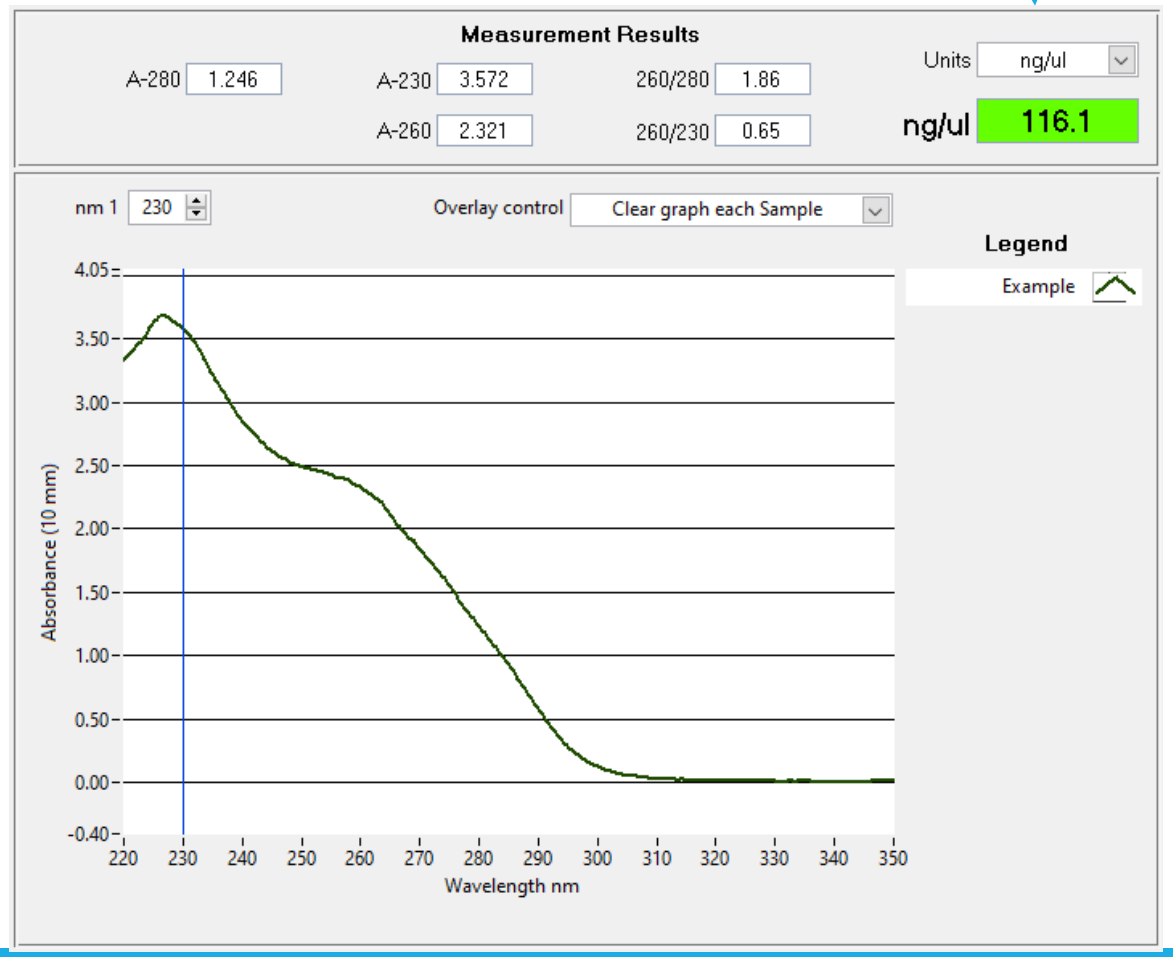
260/230 ratio

- Absorbance at 260nm should be 2.0 - 2.2x the absorbance at 230nm for both DNA and RNA.
  - DNA measurement to the right:

    260 = 2.321 and 230 = 3.572

    So 260/230 = 2.321 ÷ 3.572 = 0.650 (unacceptable)
- Low ratio (<2.0) may indicate contamination with **alcohols, organic compounds, and chaotropic salts**.

  examples: ethanol, isopropanol, phenol, glycogen, guanidine HCL, carbohydrates, EDTA

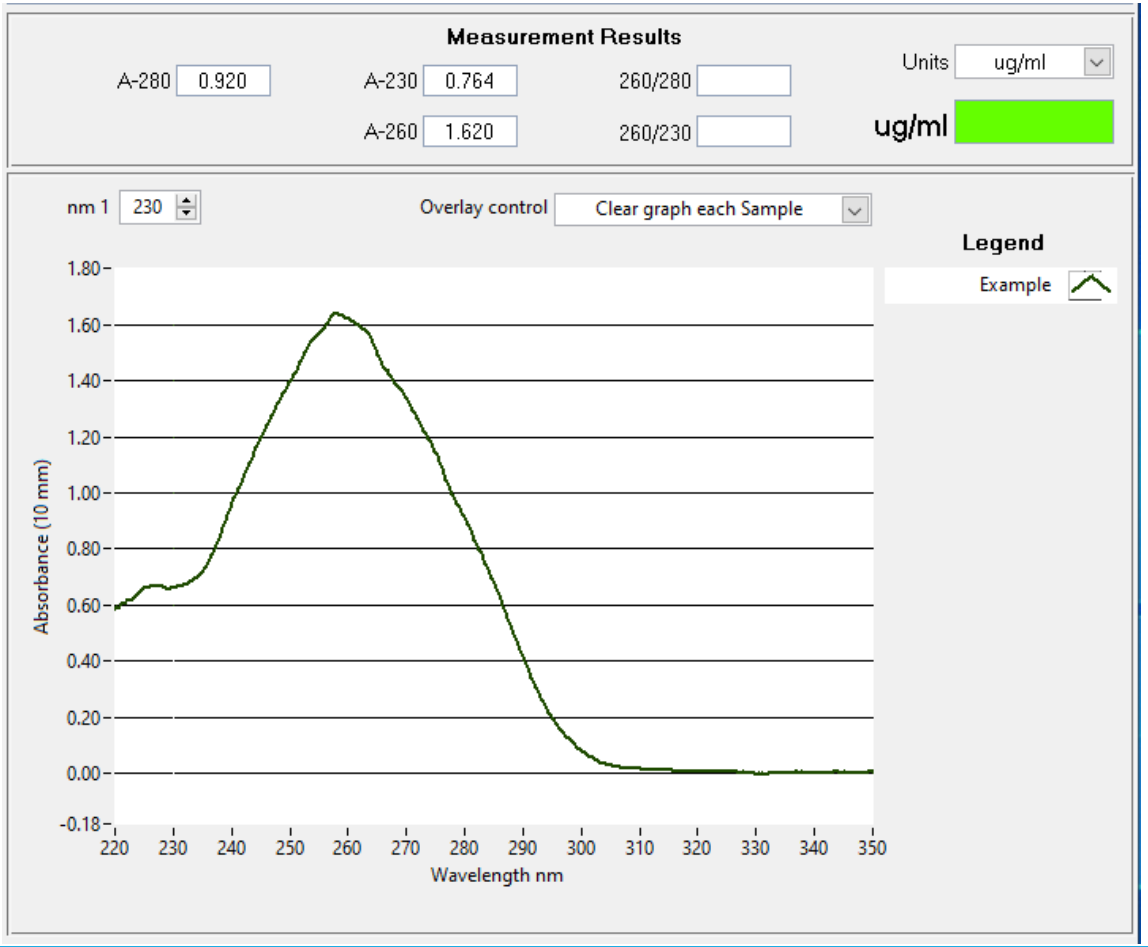


Let's Practice

Consider the NanoDrop spectrophotometer result to the right for a **DNA** sample.

Answer the following questions:

- What is the 260/280 value? Is this result acceptable?
- What is the 260/230 value? Is this result acceptable?
- What is the concentration of the measured sample?
- Suppose the measured sample had been diluted 1:20. What is the concentration of the original sample?
- Suppose the original sample was rehydrated in 0.50 mL of TE. What is the total DNA yield in µg?



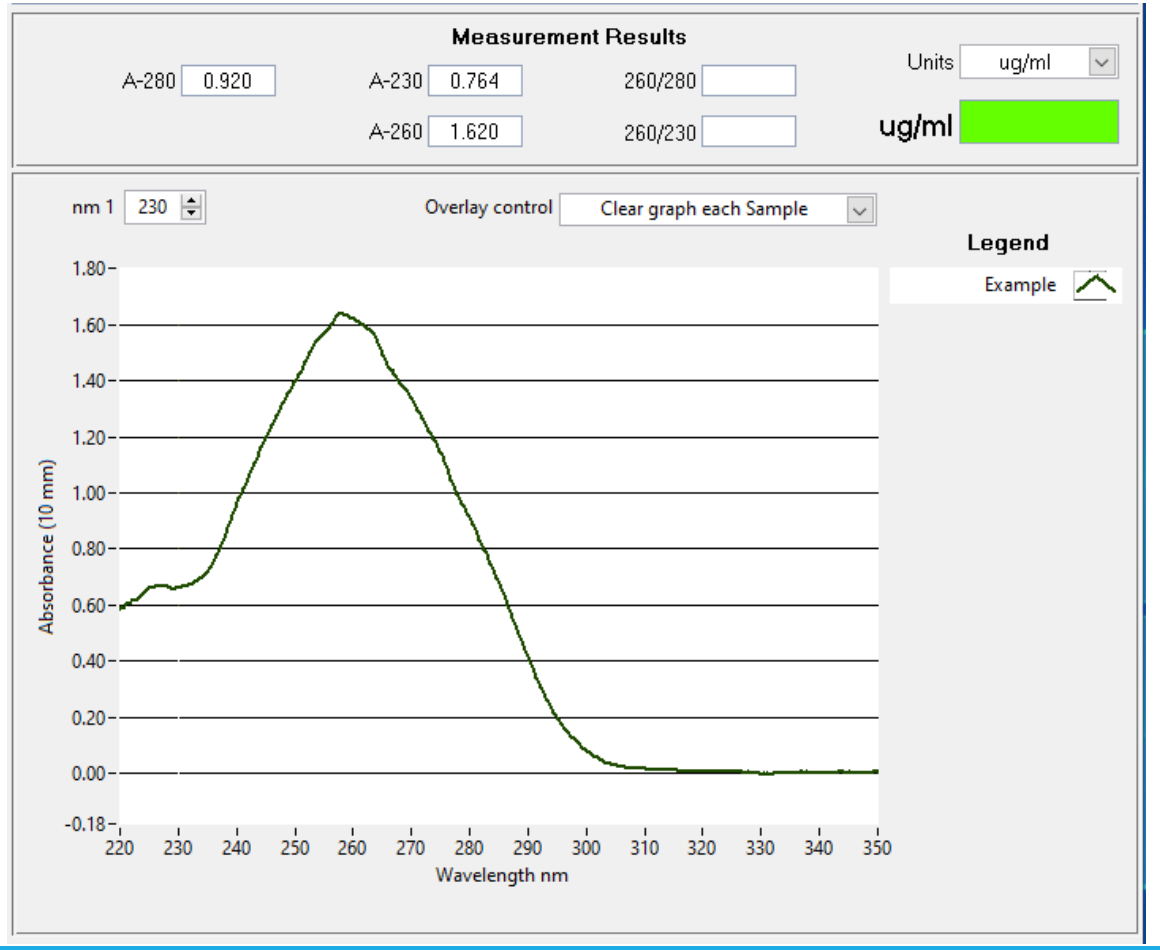
Let's Practice: 260/280

Consider the NanoDrop spectrophotometer result to the right for a **DNA** sample.

- What is the 260/280 value? Is this result acceptable?

  260/280 = 1.620 ÷ 0.920 = **1.761**

  This is an **acceptable** (1.6 – 2.0 for DNA) 260/280 result.



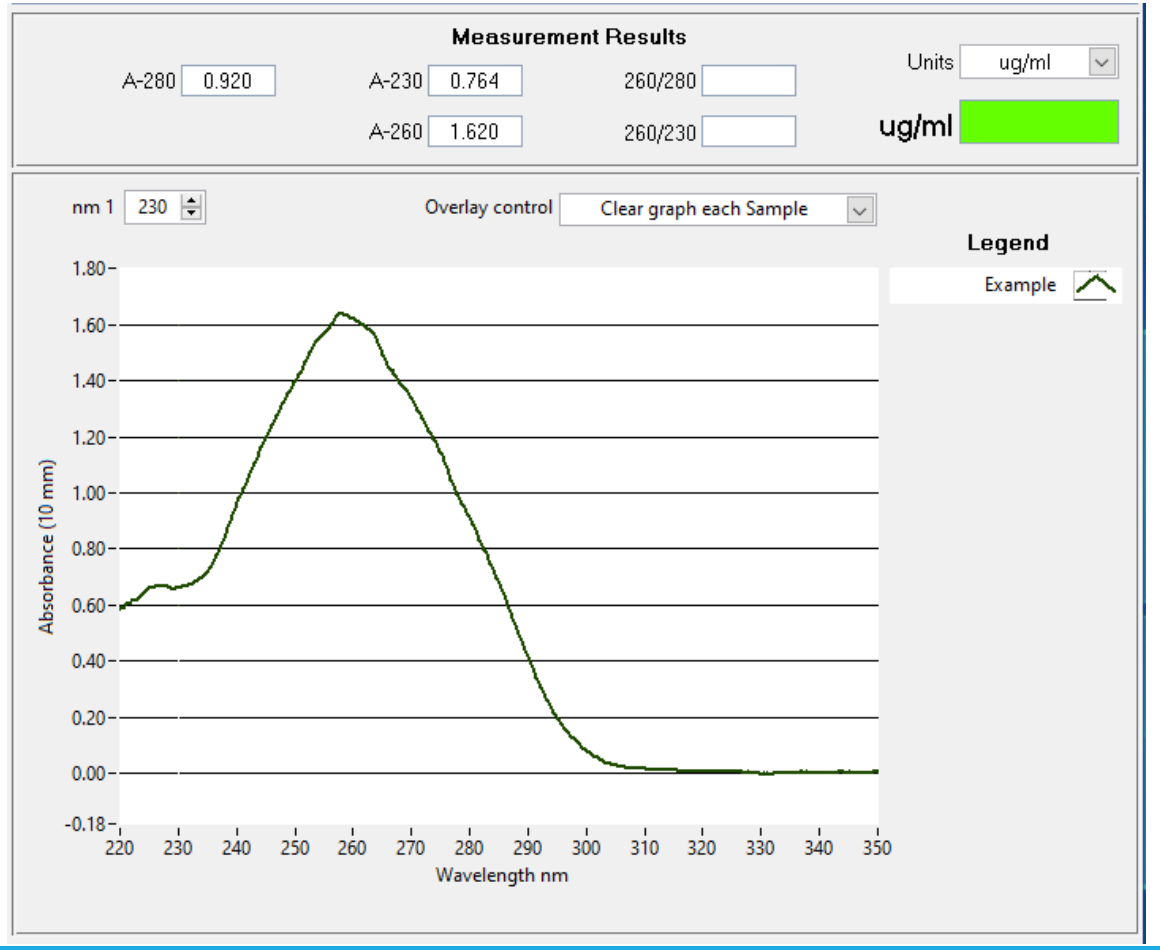
Let’s Practice: 260/230

Consider the NanoDrop spectrophotometer result to the right for a **DNA** sample.

- What is the 260/230 value? Is this result acceptable?

  260/230 = 1.620 ÷ 0.764 = **2.120**

  This is an **acceptable** (2.0 - 2.2) 260/230 result.



Let's Practice: Concentration

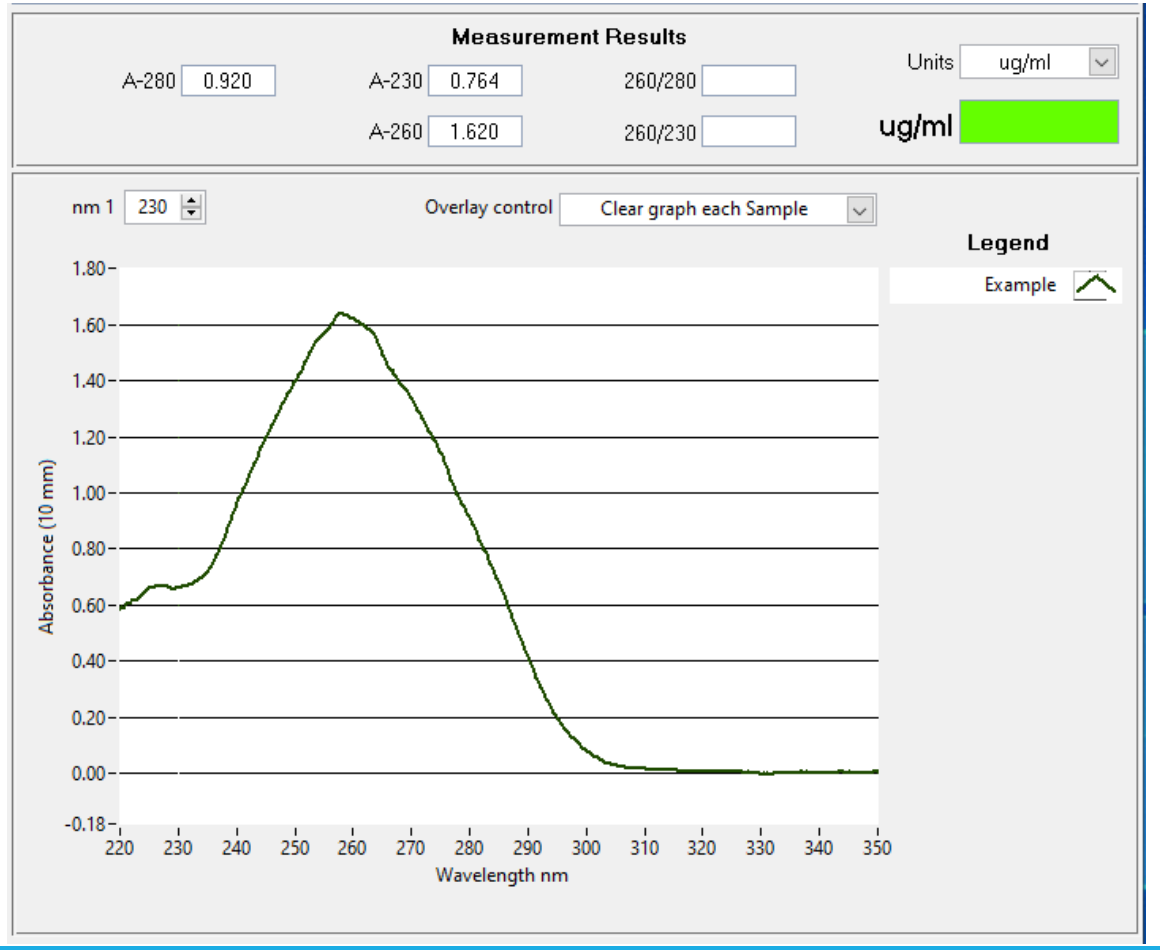
Consider the NanoDrop spectrophotometer result to the right for a **DNA** sample.

- What is the concentration of the measured sample?

  Measured concentration = (1.620)(50 µg/mL) = **81.00 µg/mL**

- Suppose the measured sample had been diluted 1:20. What is the concentration of the original sample?

  Original concentration = (81.00 µg/mL)(20) = **1620 µg/mL**

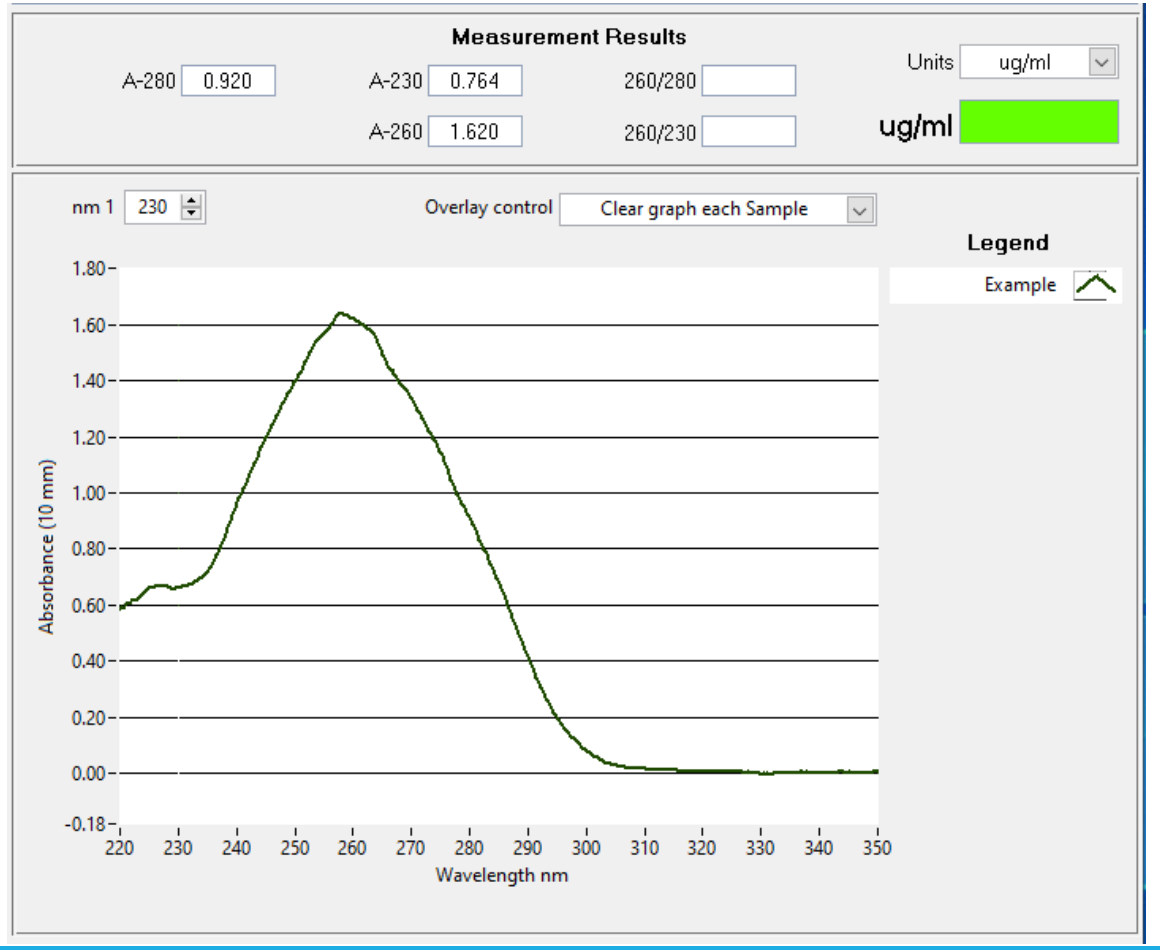


Let's Practice: Total Yield

Consider the NanoDrop spectrophotometer result to the right for a **DNA** sample.

- Suppose the original sample was rehydrated in 0.50 mL of TE. What is the total DNA yield in µg?

  Total Yield = (1620 µg/mL)(0.50 mL) = **810 µg**



Limitations of Spectrophotometry

Note that spectrophotometry measures **ALL** nitrogenous bases, not just analyzable ones.

- Bases absorb equally among high molecular weight DNA/RNA, fragmented DNA/RNA, and free-floating nucleotides.

This makes spectrophotometry a relatively inaccurate method for true, absolute quantification of analyzable nucleic acids.

- Generally okay to use for robust assays with high-yield specimens.
- May not be a sufficient measurement for some molecular methods that require high molecular weight nucleic acid molecules (*e.g.*, sequencing) or for low yield specimens that are prone to nucleic acid fragmentation during processing (*e.g.*, FFPET).

Fluorometry

Uses **intercalating** fluorescent dyes to detect specifically dsDNA and high molecular weight ssDNA/ssRNA

- Intercalation = dye inserts between successive bases of nucleic acid
- Hoechst 33258, PicoGreen, OligoGreen

Exclusion of highly fragmented DNA/RNA and free nucleotides in measurement gives a more accurate quantification of *useable* nucleic acid than spectrophotometric method.

Preferred for many procedures, particularly sequencing.

Qubit 4 Fluorometer

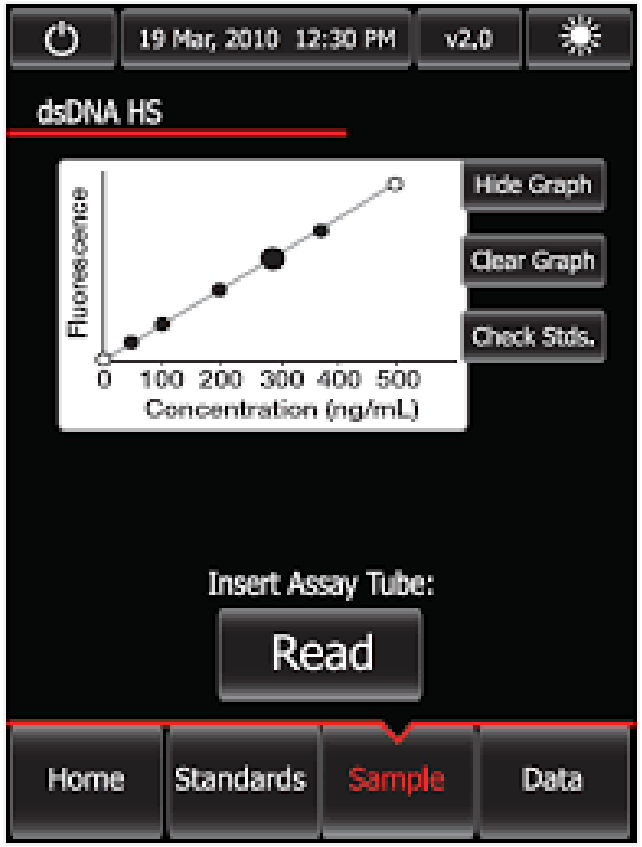
Fluorometry

The process:

- Standard curve is generated by measuring low and high standards of known concentration.
- Patient samples are plotted along standard curve to calculate concentration.
  - Auto-calculated by Qubit

Limitations:

- Does not provide additional quality indicators, like 260/230 or 260/280 ratios
- Often used in tandem with spectrophotometric results.



Qubit 4 Fluorometer

Demo video from Thermo Fisher Scientific.

YouTube: https://www.youtube.com/watch?v=gtSLkbaLlMU

Comparing Spectro/Fluorometer Results

Consider a solid-phase (magnetic bead) extraction of RNA from bone marrow:

- NanoDrop results are as follows:

  Concentration = 122.00 µg/mL

  260/280 = 1.80

  260/230 = 2.25

- Qubit results are as follows:

  Concentration = 120.00 µg/mL

Consistent results indicate that most nitrogenous bases detected by spec are part of hmw-RNA molecules.

- How would this look on an E-gel?

Consider a solid-phase (magnetic bead) extraction of RNA from FFPET:

- NanoDrop results are as follows:

  Concentration = 79.84 µg/mL

  260/280 = 1.88

  260/230 = 2.02

- Qubit results are as follows:

  Concentration = 36.10 µg/mL

Disparate results indicate many nitrogenous bases detected by spec are in lmw-RNA molecules or are free-floating nucleotides.

- How would this look on an E-gel?

Microfluidics

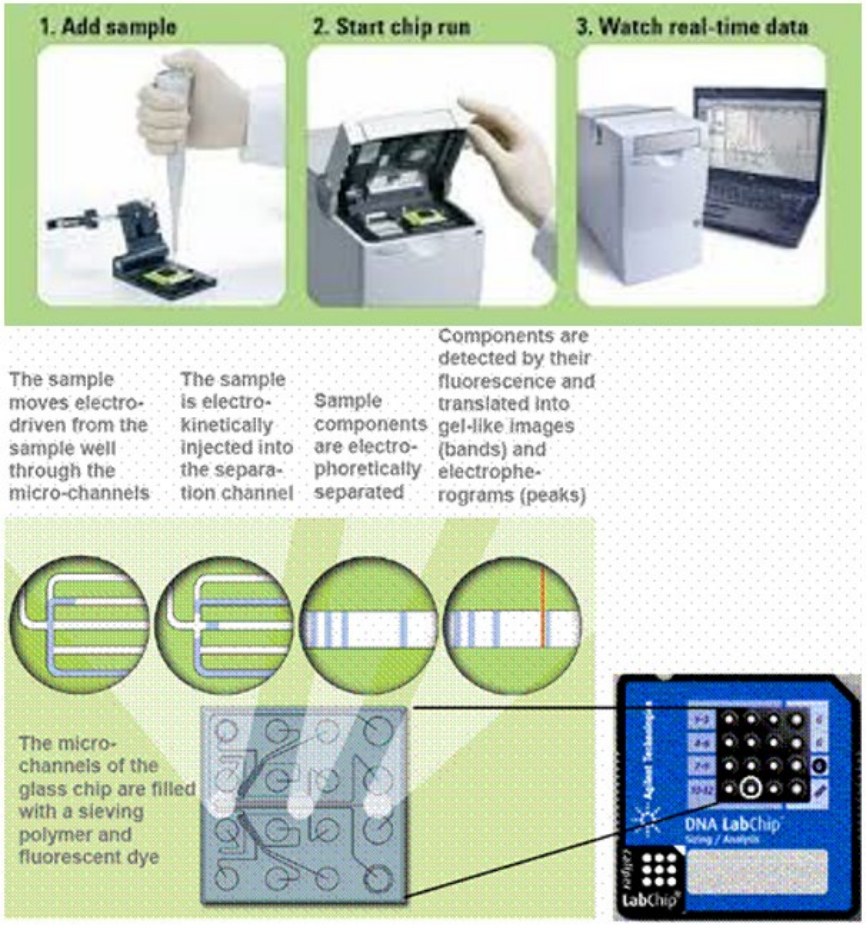
Lab-on-a-chip technology mimics electrophoresis, but on much smaller scale

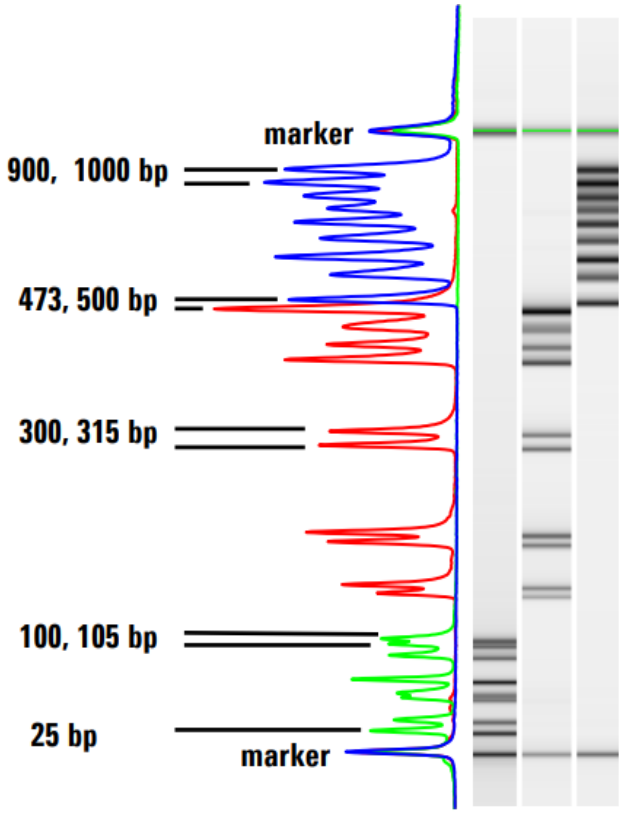
- DNA/RNA applied to sample wells alongside sizing standard
- Electro-driven mobility of samples through sieve like polymer containing intercalating fluorescent dye
- Detection unit measures fluorescence
- Electropherogram or gel-like read-out, comparable to electrophoresis

Pros: automated and uses very minimal amount of sample (1µL)

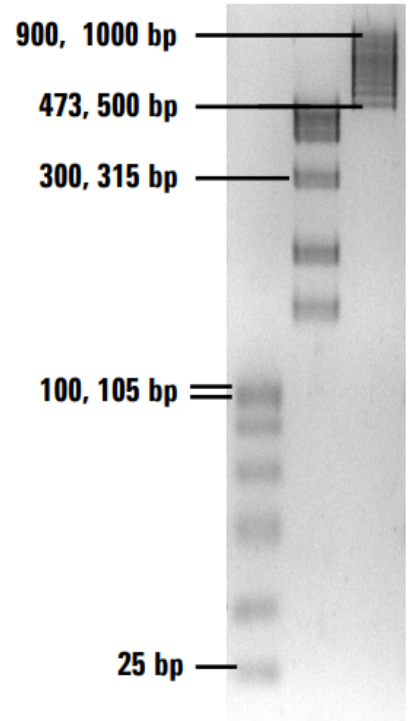
Often used as a post-nucleic acid amplification quality check.

Microfluidics





2100 bioanalyzer data
Gel-like image



2 % agarose gel stained
with Ethidiumbromide

Questions?

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This concludes the presentation.

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