DNA Quantification

Stephanie Mouanud

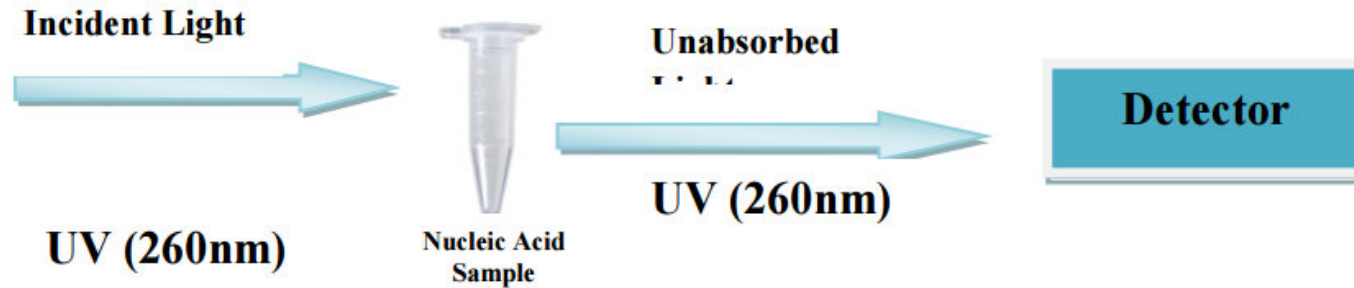
Why?

- Assess quality DNA/RNA
- Determine amounts needed for downstream applications (Sequencing, PCR, Cloning)
- Confirm experiment / extraction success

Methods

- UV Spectrophotometry
  - Nanodrop
- Ethidium Bromide Staining
  - Gel Electrophoresis Analysis
- Fluorometric Quantification
  - PicoGreen
  - Qubit
  - Hoechst 33258 dye
- Real time PCR
- Bioanalyzer

UV Spectrophotometry

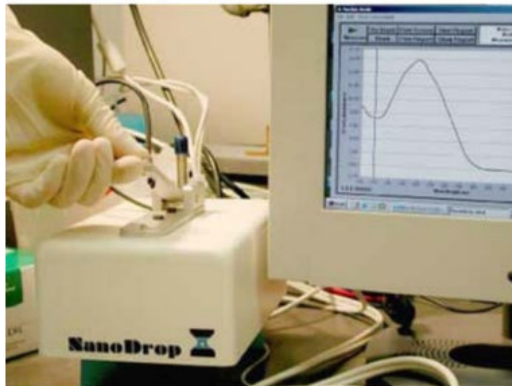


- Biomolecules absorb light in UV range.
  - Allows us to estimate amount of DNA by its absorbance
- DNA: 260nm and 280nm
- Proteins: between 215-230nm and 280nm
- *Both proteins and DNA absorb light at 280nm. If sample is mixed, this can interfere with one another

UV Spectrophotometry: Nanodrop

Advantages:

- Uses small microvolumes (1-2 µl)
- Rapid results for quick assessments
- Graph gives indication of quality
- Widely used



Disadvantages:

- Not species specific
- Bad resolution for low concentration samples (lower limit of 2ng/ul)
- Does not distinguish between ds or ssDNA
- Contaminating samples leads to falsely high quantitation readings

UV Spectrophotometry: Nanodrop

Nucleic Acid Purity determined by:

- Absorbance of solution at two wavelengths (260nm and 280nm)
- Calculate ratio A260/A280

Pure RNA: 2.0
Pure DNA: 1.8
Prue Protein: 0.6

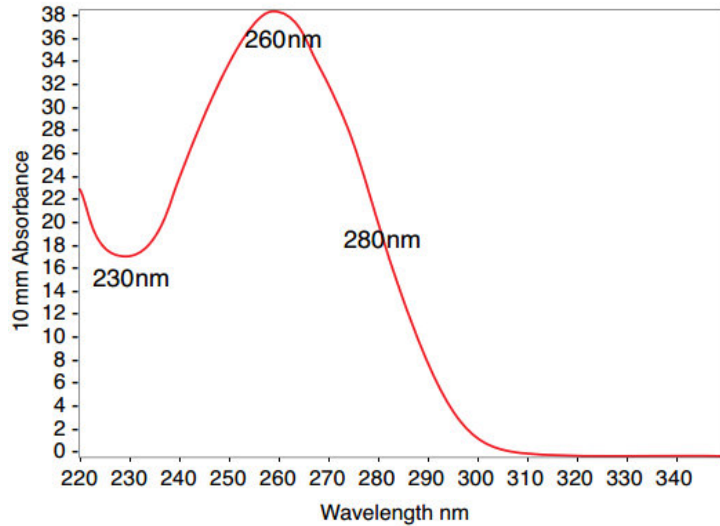
- Ratio of less than 1.8 signifies that sample is contaminated with protein or phenol. Indications poor extraction.
- *dependent on pH and ionic strength of buffer

- Absorbance of solution at two wavelengths (260nm and 230nm)
- Calculate ratio A260/A230

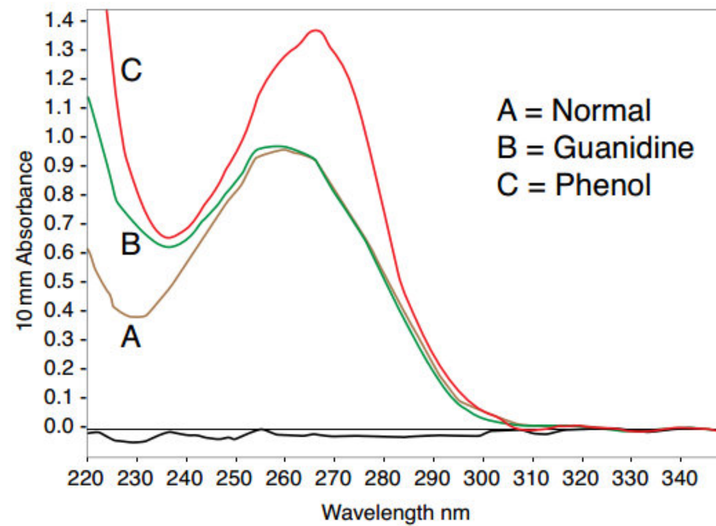
Pure RNA/DNA: 2.2-1.8

- If ratio varies, may indicate presence of residual phenol, magnetic beads, carbohydrates.

UV Spectrophotometry: Nanodrop



Typical nucleic acid sample spectrum



Comparison of nucleic acid sample spectra with and without 2 common contaminants

Plots | Report

Test type: Nucleic Acid

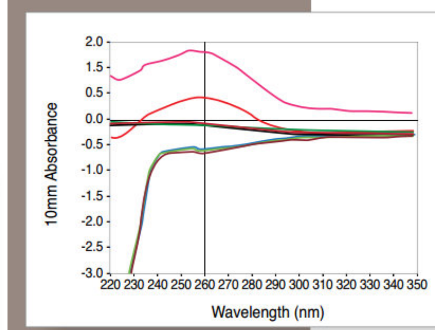
3/22/2017 5:04 PM

Exit

Report Name

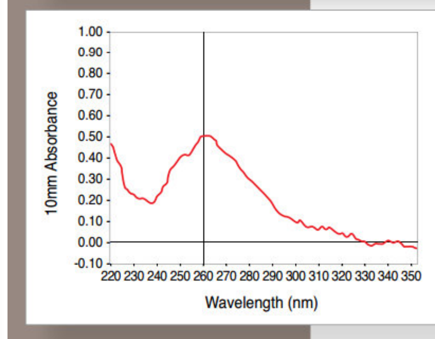
Report Full Mode: Ignore

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
1a	Default	3/22/2017	5:01 PM	2.49	0.050	0.040	1.26	0.12	50.00	230	0.409	-0.012
1b	Default	3/22/2017	5:02 PM	2.41	0.048	0.056	0.86	0.13	50.00	230	0.371	-0.013
2a	Default	3/22/2017	5:02 PM	1.73	0.035	0.030	1.16	0.02	50.00	230	1.568	-0.018
2b	Default	3/22/2017	5:03 PM	3.10	0.062	0.052	1.18	0.17	50.00	230	0.372	-0.011
3a	Default	3/22/2017	5:03 PM	5.81	0.116	0.081	1.43	0.18	50.00	230	0.628	-0.009
3b	Default	3/22/2017	5:04 PM	3.91	0.078	0.044	1.80	0.38	50.00	230	0.204	0.027



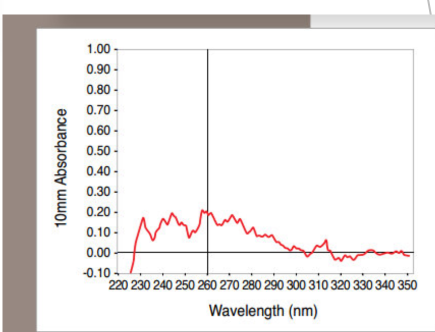
1

Negative values: dirty pedestals or incorrect blank



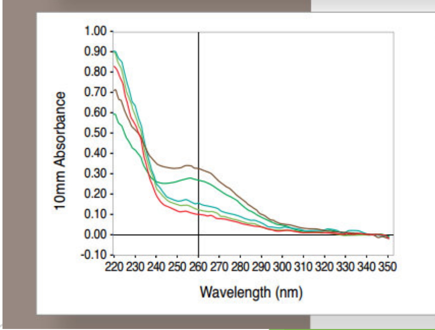
2

Ragged line: Bad blank



3

Jagged line: Broken read or low volume

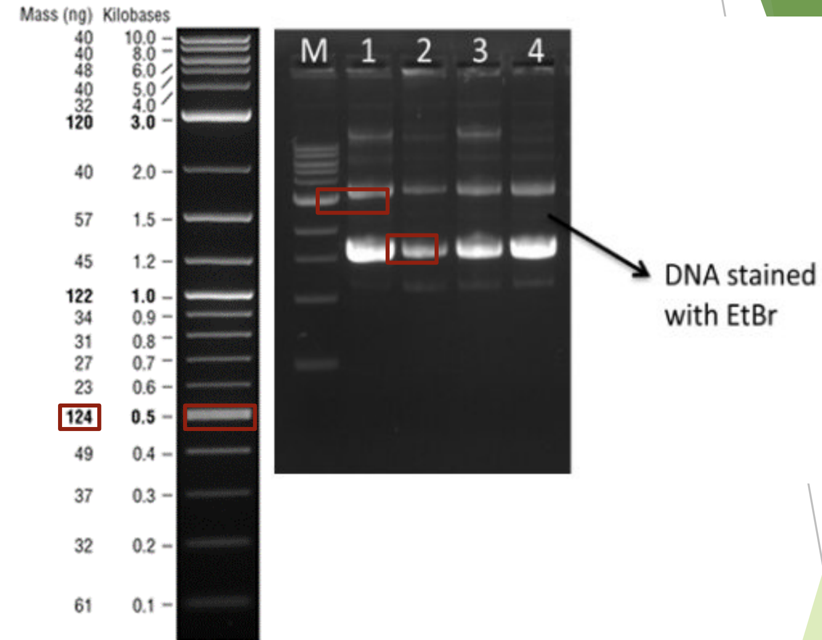


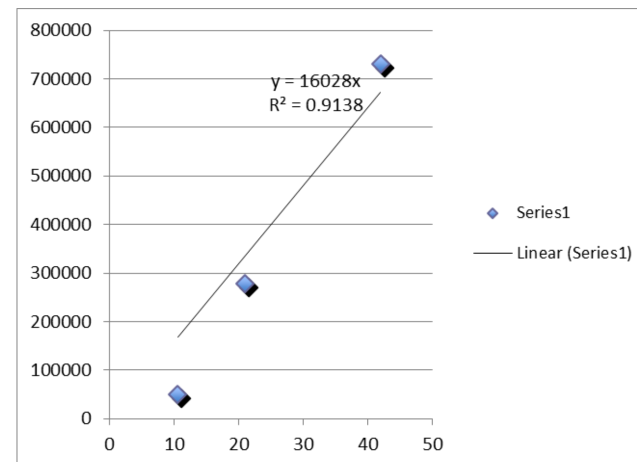
4

High 230nm: Contaminates; carbohydrates, phenols, guanidine isothiocyanate

Other Methods

- **Ethidium Bromide Staining:** Binds to Nucleic Acid and gives orange fluorescence.
  - Gel Electrophoresis Analysis;
  - Calculate band size using software from imager. Compare fluorescence intensities of ladder and sample to estimate DNA concentration
  - Create graph with linear trendline to calculate mass wrt intensity numbers





Advantages:
- Specific bands
- Not pure samples

Disadvantages:
- Need lots of DNA
- Not very accurate

Other Methods

- **Fluorometric Quantification**; uses fluorescent dye
  - PicoGreen; binds dsDNA
    - Measure fluorescent intensity of PicoGreen dye with spec.
    - DNA quantified by comparing sample to set of standards

Advantages:

- High throughput
- Increased sensitivity
- Less prone to contaminants

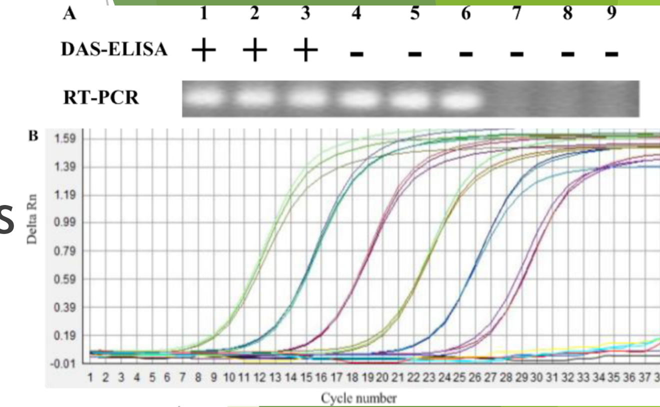
Disadvantages:

- Need special equipment and reagents/kit
- Longer prep time

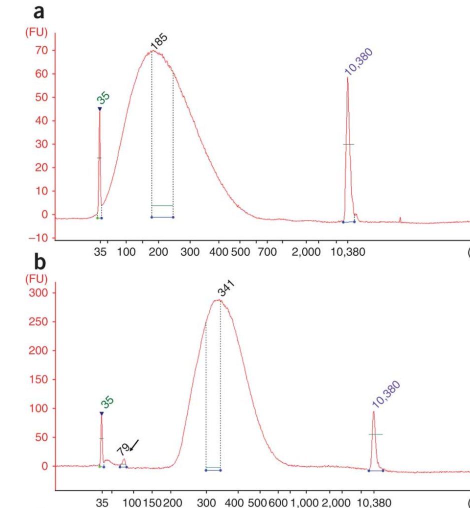
  - Qubit; binds DNA, RNA or protein depending on kit
    - Similar Advantages and Disadvantages to PicoGreen
  - Hoechst 33258 dye; specific to DNA
    - Good for both large and small amounts of DNA

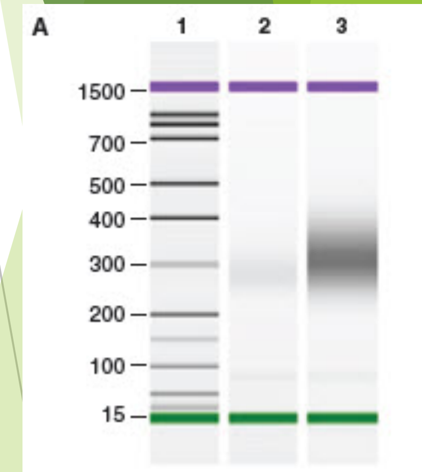
Other Methods

- **Real time PCR**
  - Fluorescent dye binding to dsDNA as it accumulates during PCR process
  - Targets specific region of DNA template
  - Used for sequencing prep, to verify quality and quantity of DNA libraries



- **Bioanalyzer;** automated electrophoresis
  - Size, quantitation and purity assessments
  - Small volume of sample needed
  - Multiple platforms (RNA, DNA)
  - Both low and high concentration samples
  - Easy to use, but expensive





- **Hybridization-Based Techniques;** Southern or Northern Blotting
  - “Probe sequence” based
  - Higher resolution (down to actual nucleotide sequence)