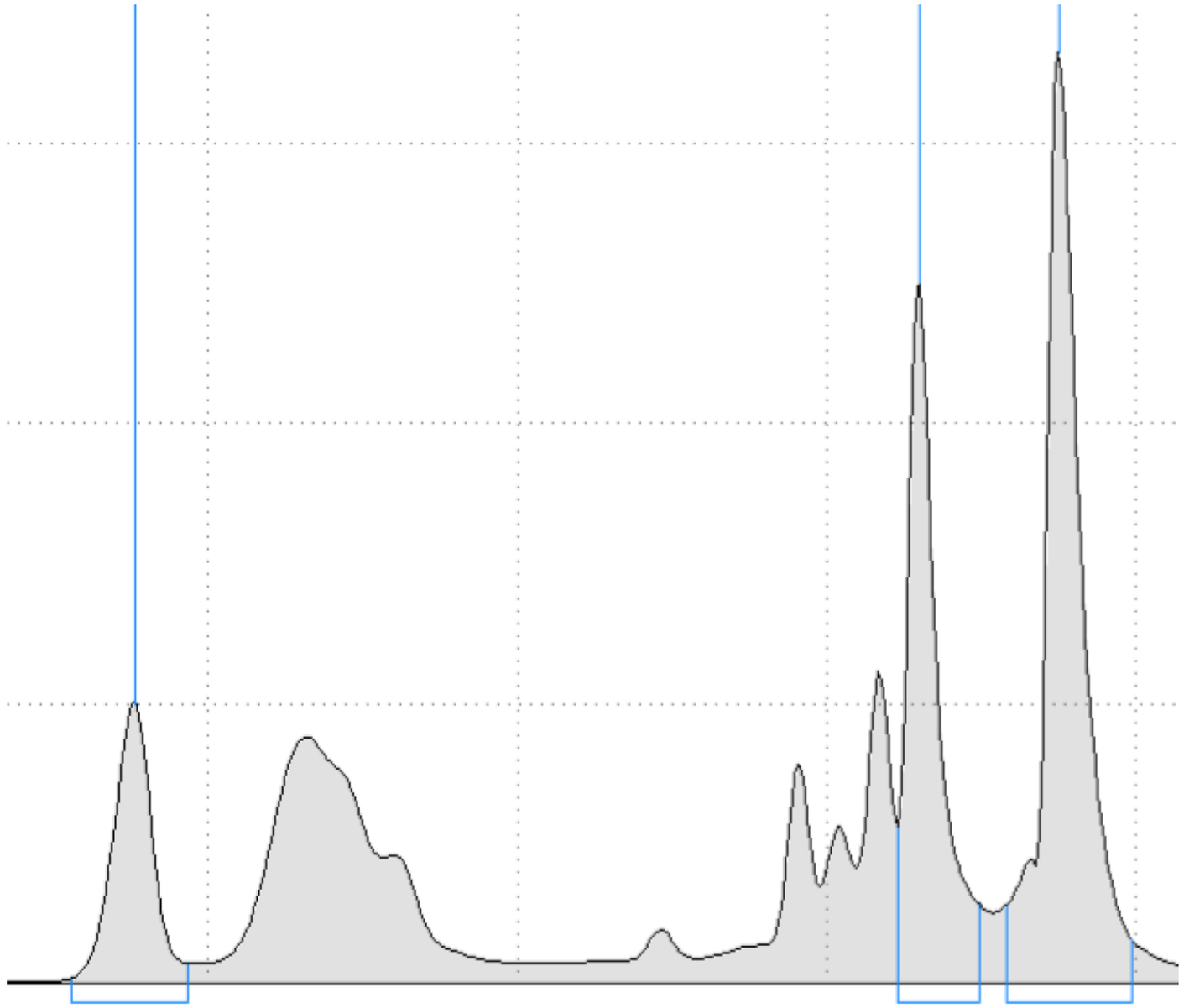
Nucleic Acid Quality Control



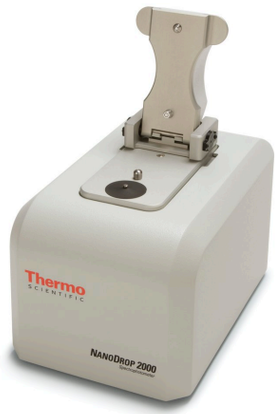
Three Dimensions of DNA and RNA Sample Quality Control

- **Quantity**. How much total DNA/RNA is in the sample, and what is the sample concentration?
- **Integrity**. What is the size distribution of my DNA/RNA molecules? Is the sample degraded?
- **Purity**. Is the sample contaminated with other chemicals or types of nucleic acids?

Tools for DNA and RNA Quality Control

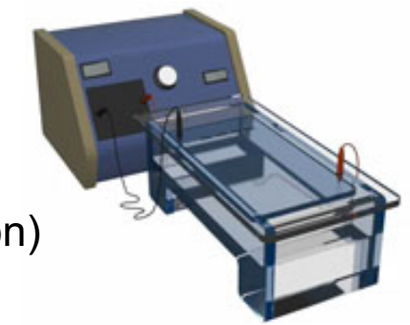
1. Fluorometry (Qubit)

- Quantity



2. Spectrophotometry (NanoDrop)

- Quantity
- Purity (non-nucleic-acid sources of contamination only)



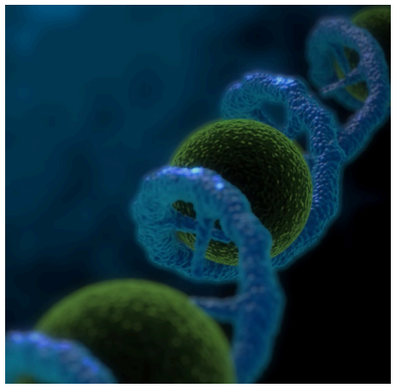
3. Conventional Gel Electrophoresis

- Integrity (especially for genomic DNA samples)
- Purity (DNA or RNA contamination only; not other sources of contamination)

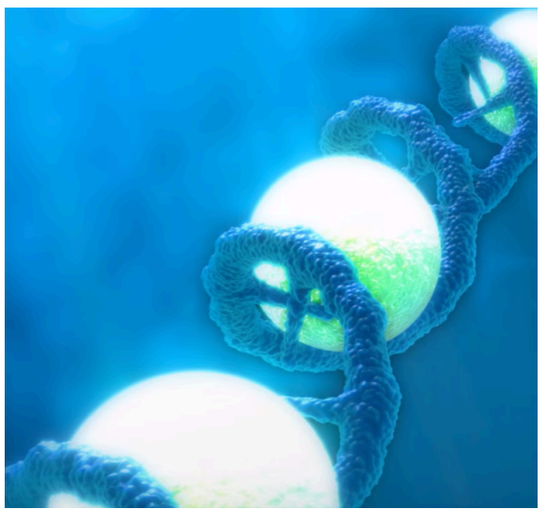
4. Agilent TapeStation or Bioanalyzer Electrophoresis

- Quantity
- Integrity (especially for RNA samples)
- Purity (DNA or RNA contamination only; not other sources of contamination)

Measuring DNA or RNA Quantity with Fluorometry (Qubit)



Dyes that specifically bind to DNA or RNA and fluoresce when bound



Advantages

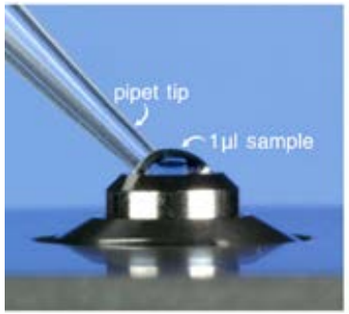
- Accurate measurement of DNA or RNA concentrations even in samples contaminated with chemicals or other types of nucleic acids
- High sensitivity

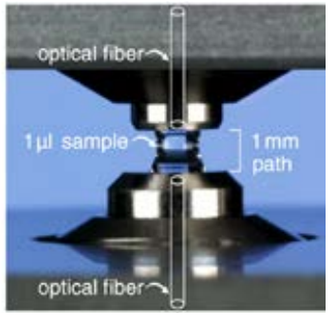
Disadvantages

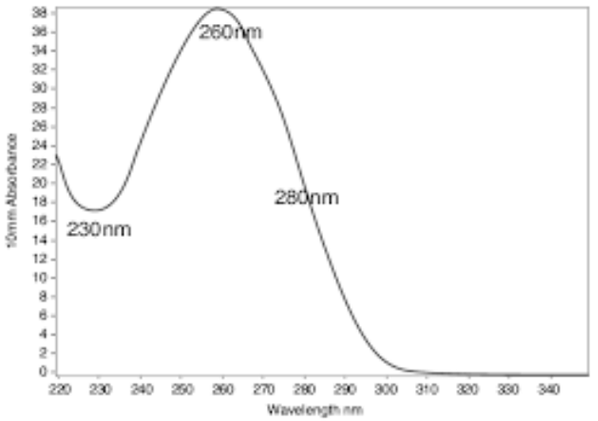
- Little to no information about sample integrity or purity

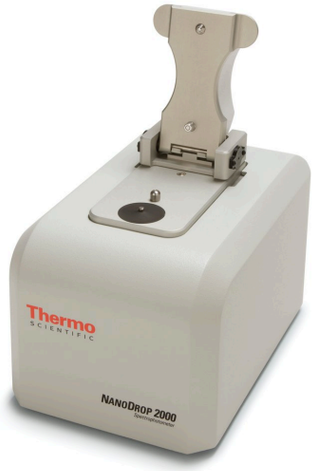
Measuring DNA or RNA Quantity and Purity with NanoDrop

Quantifies UV absorbance of sample





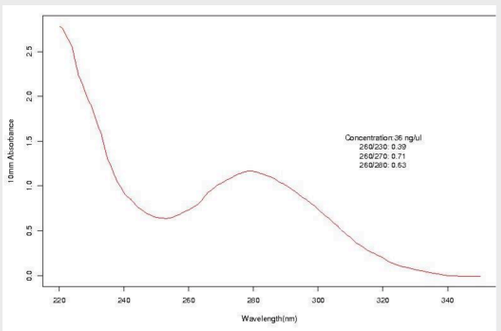




Advantages

- Can detect the presence of chemical contamination from sources such as phenol, polysaccharides, salts, and protein.

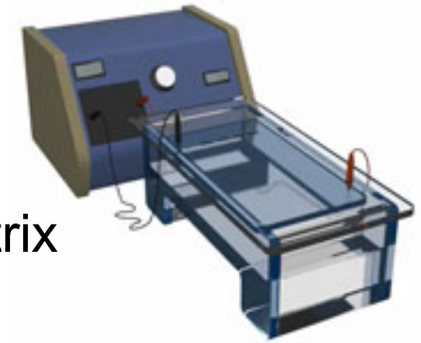
Example of phenol contamination



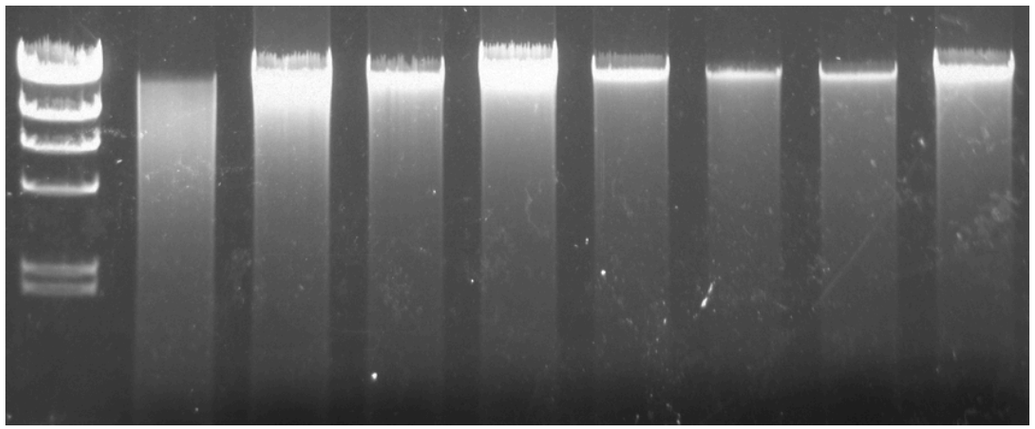
Disadvantages

- Quantification is (highly) overestimated when contaminants are present.
- Limited sensitivity (unreliable below 20 ng/ul)
- Cannot distinguish well between DNA and RNA
- No information about nucleic acid size or degradation

Assessing DNA or RNA Integrity with Gel Electrophoresis



Separate nucleic acids by size by applying current across a porous gel matrix



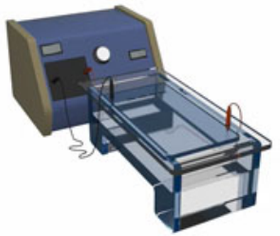
Advantages

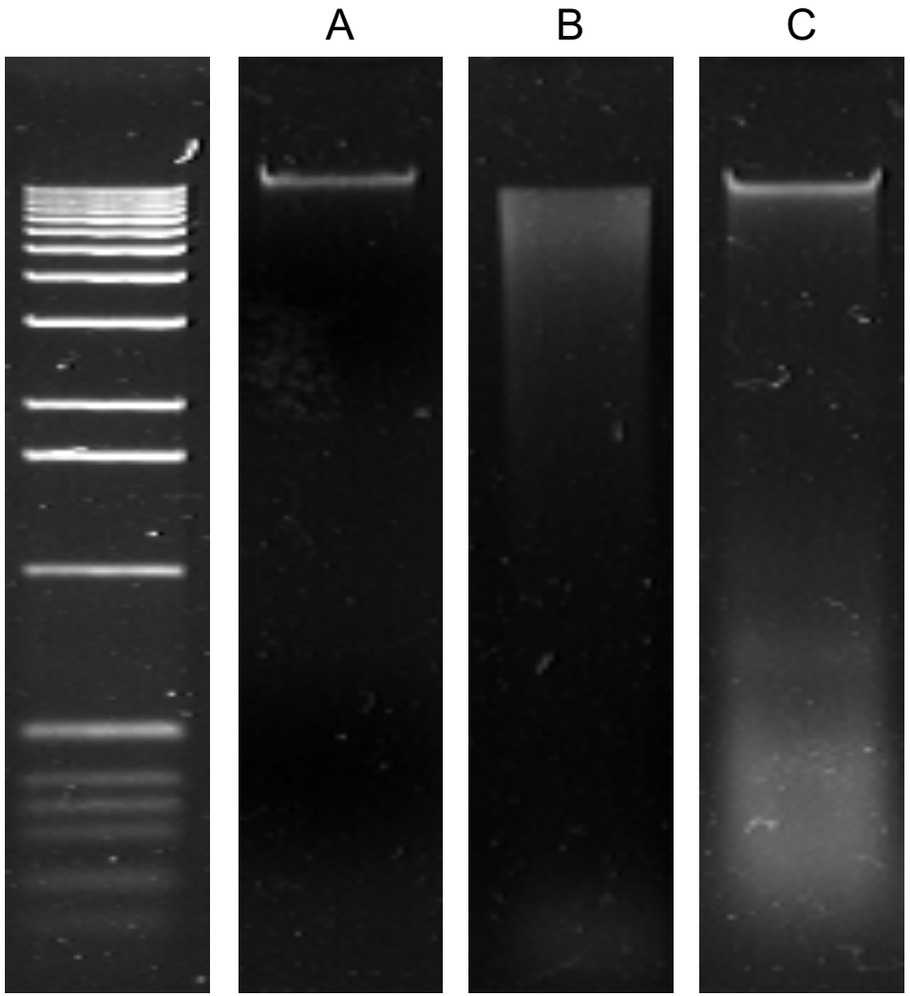
- Can identify size distribution of nucleic acid molecules and detect degradation
- Can identify contaminating RNA in DNA samples

Disadvantages

- Only provides a rough sense of nucleic acid quantity based on intensity of fluorescence
- Uninformative with respect to chemical contamination in sample

Agarose Gel Electrophoresis – Genomic DNA Samples

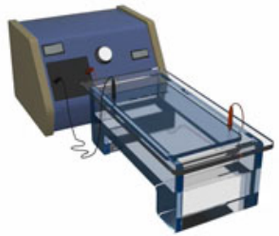


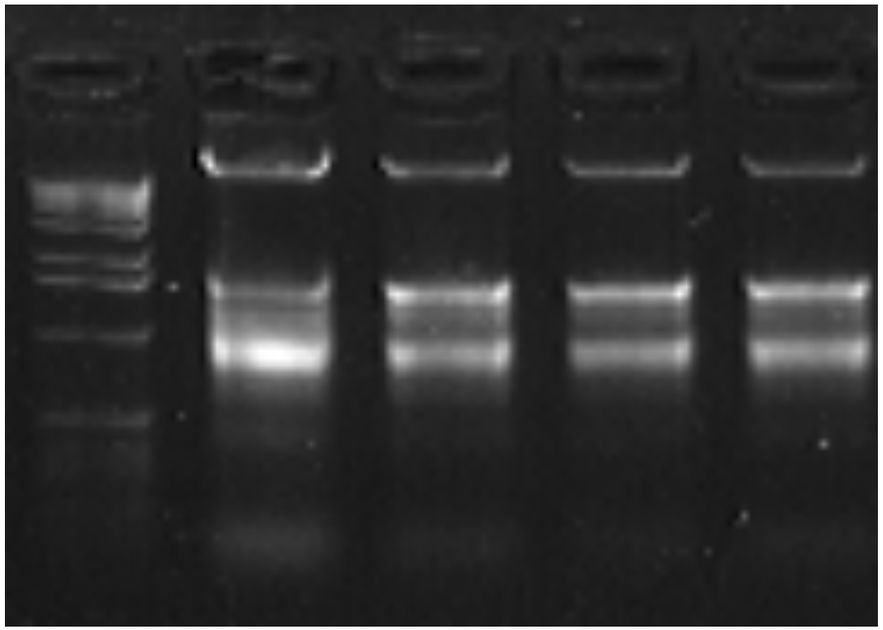


For each of the three genomic DNA samples, answer the following questions.

- **Is there evidence of DNA degradation in the sample?**
- **Is there evidence of contamination in the DNA sample?**
- **Would you expect a DNA Qubit test and NanoDrop analysis to produce similar estimates for the concentration of the DNA sample? Or would the Qubit and NanoDrop estimates differ greatly from each other?**

Agarose Gel Electrophoresis – Genomic DNA Samples



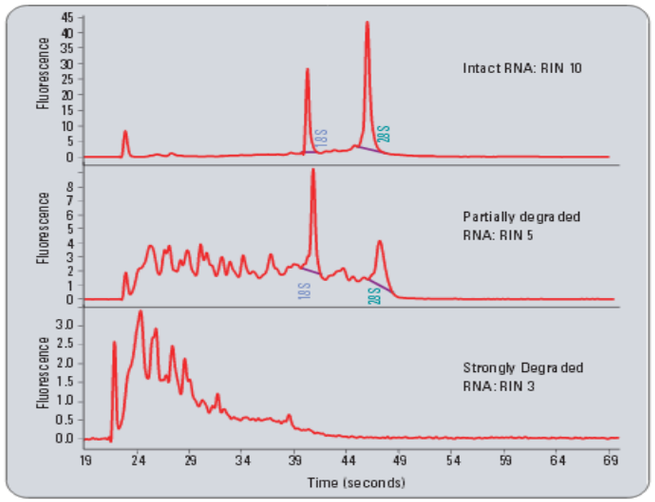


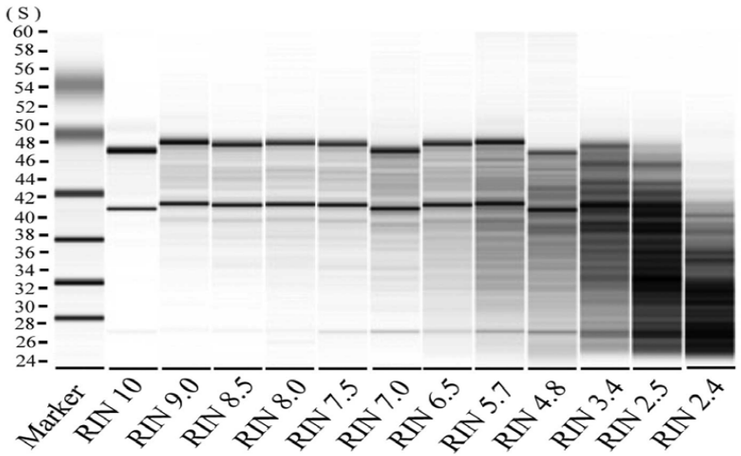
Genomic DNA

RNA (large and small subunit rRNA)

Agilent Bioanalyzer and TapeStation Electrophoresis

Scoring RNA integrity





Advantages

- Higher sensitivity and better size resolution than conventional electrophoresis

Disadvantages

- Uninformative with respect to sample contamination
- More expensive than the other described tools

Which is the Best Quality Control Tool?

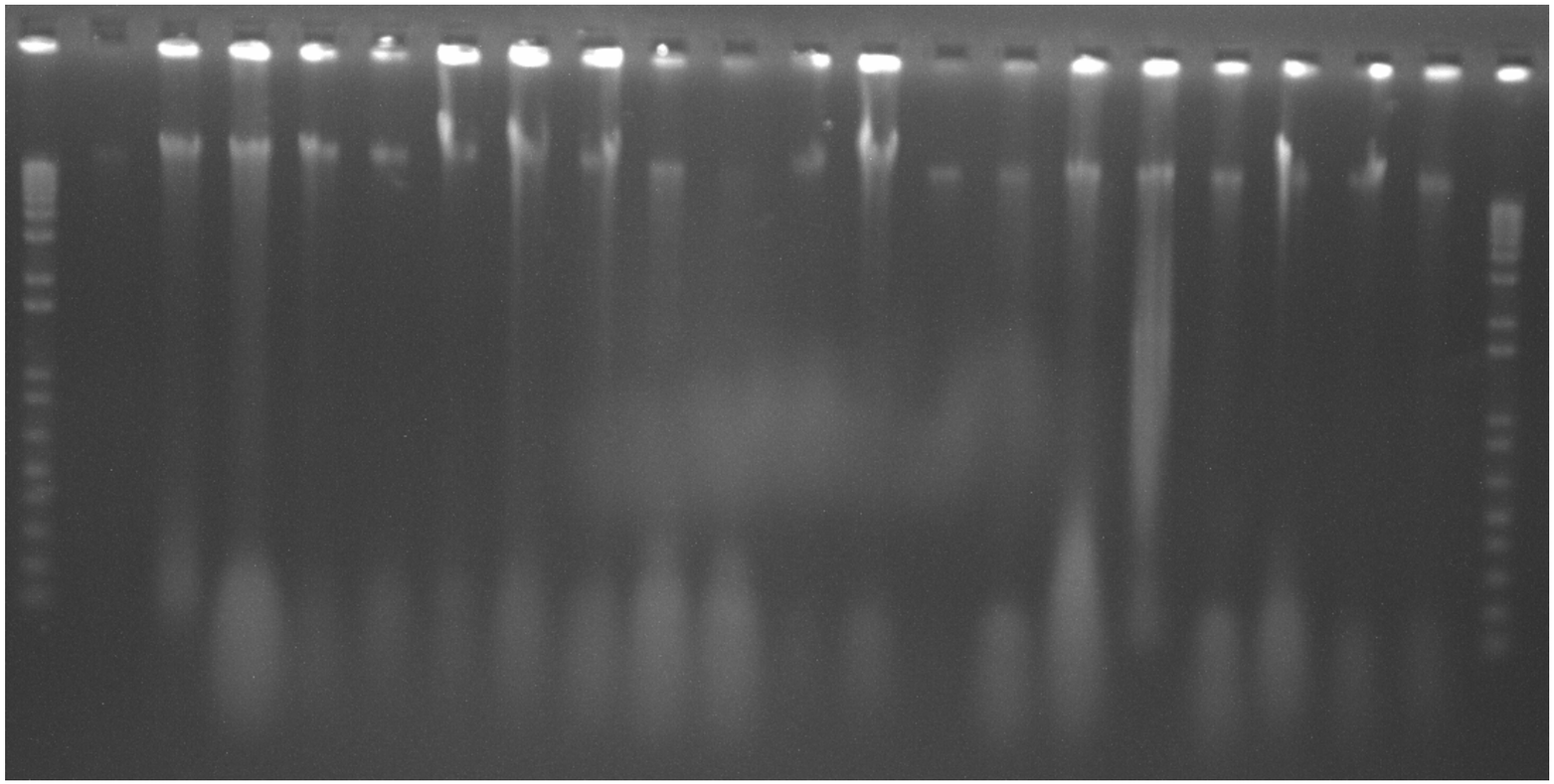
- You have been having difficulties with protein contamination in your DNA extractions and want to know if your new extraction method is fully removing this contaminant from your DNA preps.
- You are going to perform long-read nanopore sequencing and want to make sure your genomic DNA sample is not degraded (fragmented).
- You are going to perform mRNA-seq to measure gene expression and want to make sure that your RNA sample is not degraded.
- You have completed a DNA extraction and want to know whether it is contaminated with RNA.

Methods Summary

	Qubit	NanoDrop	Gel Electrophoresis	TapeStation
Quantify Concentration	Yes	Yes	Very Roughly	Yes
Assess Integrity	No	No	Yes	Yes
Detect Chemical Contaminants	No	Yes	No	No
Distinguish RNA and DNA	Yes	No	Yes	Yes
Equipment Cost	$	$$	$	$$$
Per Sample Cost	$$	$	$$	$$$

Many Sequencing Applications Do Not Require Perfect DNA

"… le mieux est l'ennemi du bien." -- *La Bégueule,* Voltaire



>20 year old insect DNA samples that were degraded, contaminated, and visibly yellow in color… still worked fine for Illumina sequencing! So be pragmatic and use common sense.