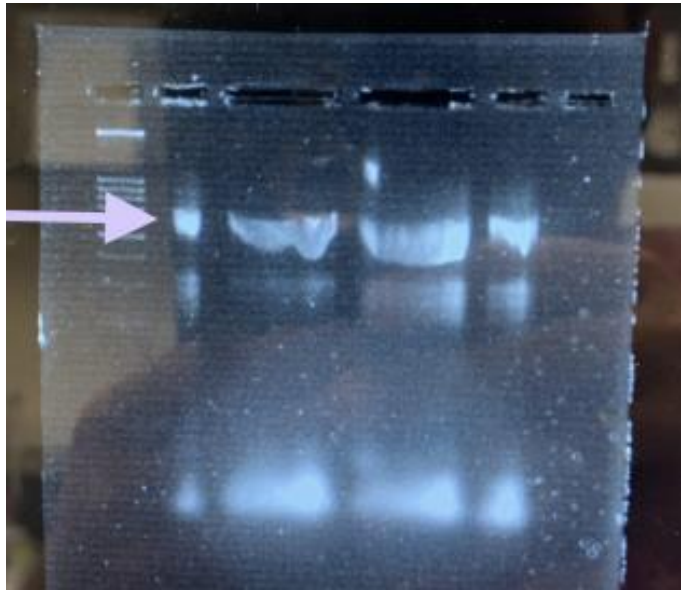
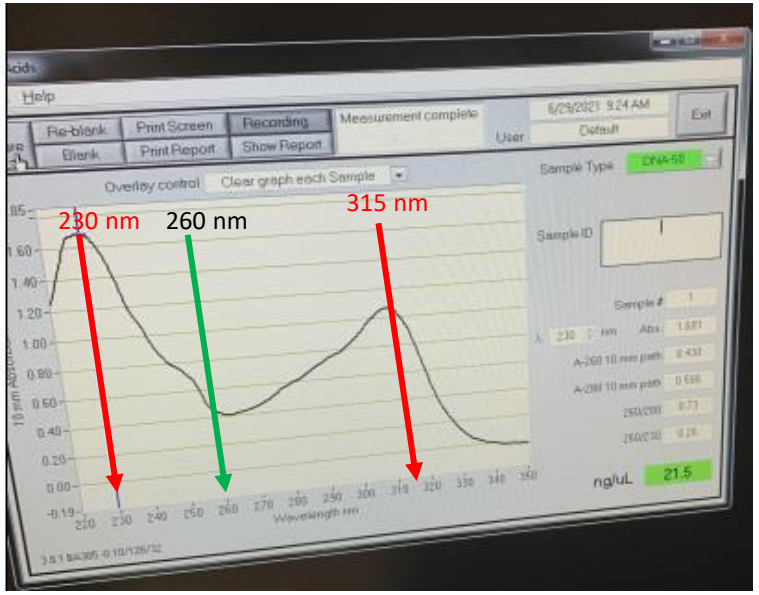


Client comment: This is not the picture of the gel that I used to prepare this sample, but it looked something like this. I loaded 40 ul of PCR product in two lanes. So, total of 80 ul of PCR product was used to run the gel. Two tubes were made for gel dissolving and at the end, everything was mixed to get the final product.



Client comment: Quantification of concentration – 21.5 ng/ul – was done at the end using Nanodrop (230, 260 and 315 nm *arrows added during analysis by Genomics Facility*).

Please review at least the following topics in the Science Aid Center of the Genomics Facility website. In particular, spend time reviewing the Nanodrop Guide for Nucleic Acids.

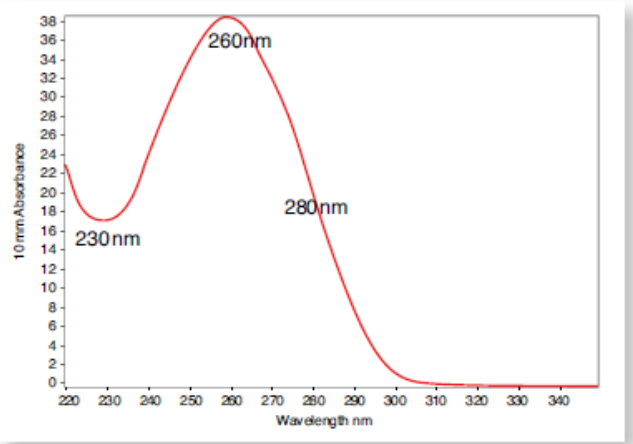
- Is it essential to clean PCR products for sequencing?
- How to clean DNA template for sequencing?
- How much DNA to use in a sequencing reaction?
- Why are Spectrophotometers "Bad", and what to do instead?
- How to interpret 260/230 & 260/280 OD ratios?
- Should I use a Nanodrop to quantitate DNA?
- Nanodrop Guide for Nucleic Acids (ThermoFisher)?

Analysis by Genomics Facility:

1. Clearly, you have DNA in the gel; however, gel quantitation is not possible due to over-saturation from too much DNA.
2. Nanodrop profile shows that the 'ng/ul' value is completely untrustworthy. There are two peaks, one at ~230 nm and one at ~315 nm... both over twice the height of the reading at 260 nm... and the value at 260 might merely represent the transition point between whatever is creating the other two peaks... which don't even match the profiles of common contaminates.
3. Nanodrop profile clearly indicates presence of at least two 'contaminates', presumably from the gel extraction process. These contaminates, even if there were sufficient template in the sequencing reaction, might prevent the sequencing reaction from happening... especially since the reactions included 4 ul of template.
4. If I saw this Nanodrop profile, I would not even attempt to sequence the product until I achieved a more normal profile for the samples.

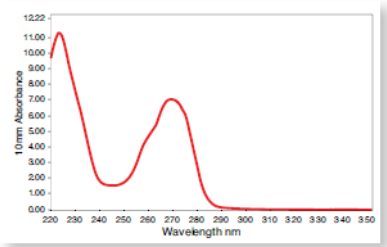
5. Review spectral image to assess sample quality.

Images from Nanodrop Guide to Nucleic Acids

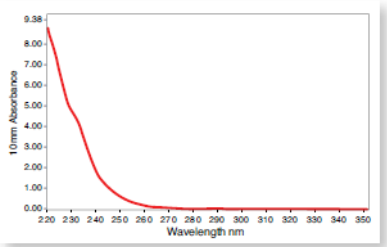


Typical Nucleic Acid Spectrum

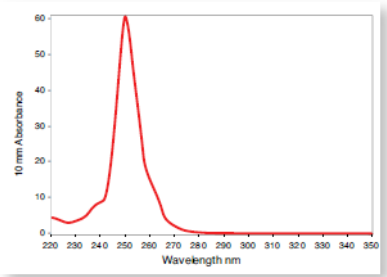
Below are several examples of reagents commonly used with nucleic acids that have absorbance in the 220 – 240 nm range. Note: Phenol also exhibits significant absorbance between 260 – 270 nm which may shift the peak and result in an overestimation of the nucleic acid concentration.



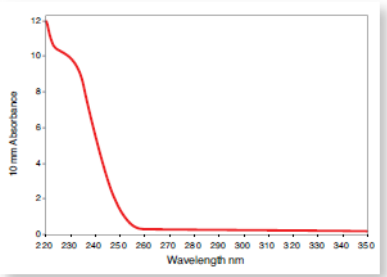
Phenol



EDTA



Guanidine Isothiocyanate



Guanidine HCl