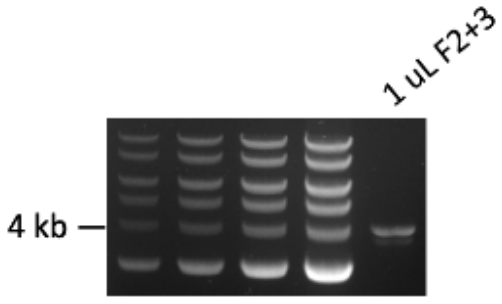
Gel Quantification

Materials

- Agarose
- 1x TAE Buffer
- Erlenmeyer flask
- Gel stain
- Gel cast system
- DNA ladder
- DNA loading dye
- Fiji or ImageJ software
- R or excel

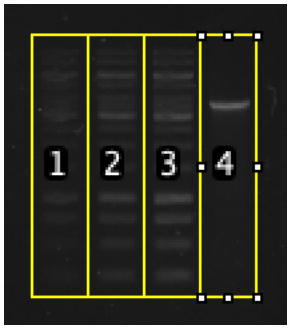
Protocol

1. Dissolve appropriate amount of agarose in 1X TAE buffer.
2. Heat the solution in a microwave and let it cool to about 50°C before adding gel stain.
3. Distribute the stain gently by shaking the flask.
4. Pour the gel into the tray and remove the bubbles. Add the comb and let gel cool at RT.
5. Add DNA loading dye to each sample and mix.
6. Load 3-5 different volumes of DNA ladder to make the standard curve for DNA quantification. Ensure that your standard curve covers the expected range of DNA concentration.
7. Run the agarose gel after loading the sample.
8. Take pictures of the gel, avoiding oversaturation.

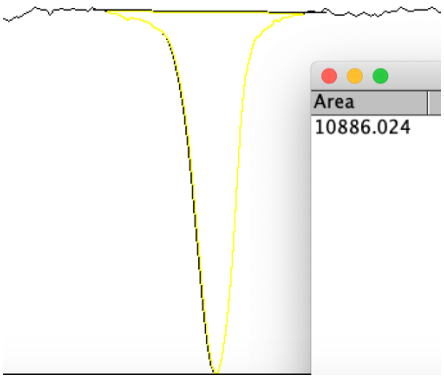


9. Analyze the concentration with ImageJ or Fiji
    a. Open the picture in ImageJ or Fiji
    b. Do a tight selection around the first lane with DNA ladder using the “Rectangular Selection” tool. Press “Ctrl+1” to designate the first lane.
    c. Grab the selection and drag it to select the second lane with DNA ladder. Press “Ctrl+2” to designate the second lane.

d. Continue to select all lanes including your DNA samples, pressing “Ctrl+2” each time.
e. Once done, press “Ctrl+3” to plot the lane grayscale density.



f. For the ladder choose the peaks belonging to the band closest in length to your DNA sample.
g. Determine the mass of DNA corresponding to the volume you used for this band from the ladder info sheet.
h. Close off the area of the peak with the “Straight” tool and use the wand (tracing) tool to measure the area.



10. Plot the calibration curve using the loaded ladder intensities and calculate the DNA concentration from the curve.