Quantifying DNA in Agarose Gels

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DNA and RNA can be quantified with a Spectrophotometric method, however such analysis does not allow you to visually see any degradation which may be providing false information for future use of the nucleic acids.

Visualisation is particularly important when performing ligations which are sensitive to contaminants, high levels of DNA and degraded DNA.

Running an analytical gel allows you to

1. Check that the quantification from an absorbance reading is correct.
2. Visually examine the quality of your DNA.

Make an agarose gel and load sufficient DNA to be able to see clearly any issues of degradation, contamination concentration.

Generally 10% is sufficient – if you cannot see 10% of your DNA then you may not have enough for your experiments.

If you do not see 10% it is recommended to load ½ your DNA – if you cannot see this then your entire product has most likely been lost and you should prepare more.

Quantifying.

1. Use a standard of known concentration alongside samples under analysis – DNA ladders usually have one band which is qualified by concentration if a known volume is loaded.
2. Load your samples close together.
3. Run the gel sufficiently to detect
    a. contaminating DNA
    b. degraded DNA
    c. contaminants such as protein in the wells
    d. salt contamination which will make the DNA migrate in a warped fashion different from the expected size
4. Check the live image is represented faithfully in the printed image.
5. Estimate the concentration of each of your samples against the standard.
6. Estimate the concentration of each of your samples relative to eachother – particularly the vector vs insert material.
7. If the difference in estimated values is greater than 100 fold or you estimate that there is greater than 1ug of DNA in a loaded sample you should dilute the samples accordingly and re-run them; Concentrations such as these are difficult to estimate correctly.
8. Use your estimations alongside any data from absorbance readings to make an informed choice on how much DNA to use in your experiment.