

Experiment 8

Verification of Purified Plasmids

Before embarking on a series of experiments with a newly prepared plasmid stock, it is prudent to verify that you indeed isolated the DNA that you thought you had. It is also important to make sure that your DNA is not contaminated with RNA or exonuclease. Therefore, you need to do a restriction digestion and run a gel.

The concentration of DNA is an important variable and will have an important impact on experiments. Too much DNA on a gel can lead to bands so thick that it is impossible to determine an accurate fragment size. Too little DNA can cause you to miss small bands.

Gel Analysis

1. Prepare three *Hind*III digests of each of your plasmids, one using 1 μ l of DNA, one using 5 μ l and one using 10 μ l. As controls, prepare *Hind*III digests of your rapid-plasmid preps and pUC9.
2. Run your samples on a 1% “piggy-back” gel. One row should have your cut plasmids and molecular weight standards. The other should contain uncut samples.

DNA Concentration

1. Dilute your samples 1/20 in TE buffer to give a final volume of 1 ml in a microfuge tube.
2. Transfer your sample to a quartz cuvette and determine the optical densities of your samples at 260 nm.

The peak absorption of DNA is at 260 nm and there is a simple relationship between the amount of light absorbed and the concentration of DNA:

$$1 \text{ OD}_{260} \text{ unit} = 50 \text{ } \mu\text{g/ml DNA}$$

3. The purity of DNA can be determined by measuring the optical density at 280 nm. DNA absorbs almost twice as much light at 260 nm than at 280 nm. Therefore, you want to determine the ratio of the two readings:

$$\text{OD}_{260} / \text{OD}_{280} = 1.8$$

A ratio of 1.8 or better means that your DNA is sufficiently pure.

A ratio lower than 1.8 means that there is something else in your sample that is absorbing 280 nm light - i.e. proteins.

Contaminating protein may or may not have an effect on restriction digestion and gels. You have to try it to find out. What it does mean, however, is that your estimation of DNA concentration is inaccurate.

A ratio significantly greater than 1.8 means that there may have been some degradation of your DNA.