WHAT YOU NEED
DNA ladder that contains fragments of known concentration appropriate to unknown sample.
Agarose or hPAGE gel after electrophoresis with unknown sample(s) and dilutions of DNA ladder.

1. Preparation of DNA ladder and samples

1. For an hPAGE gel with a 55 well gel comb, each well can be loaded with a maximum volume of 4.5 µL. Load 1 µL, 2 µL and 4 µL of an

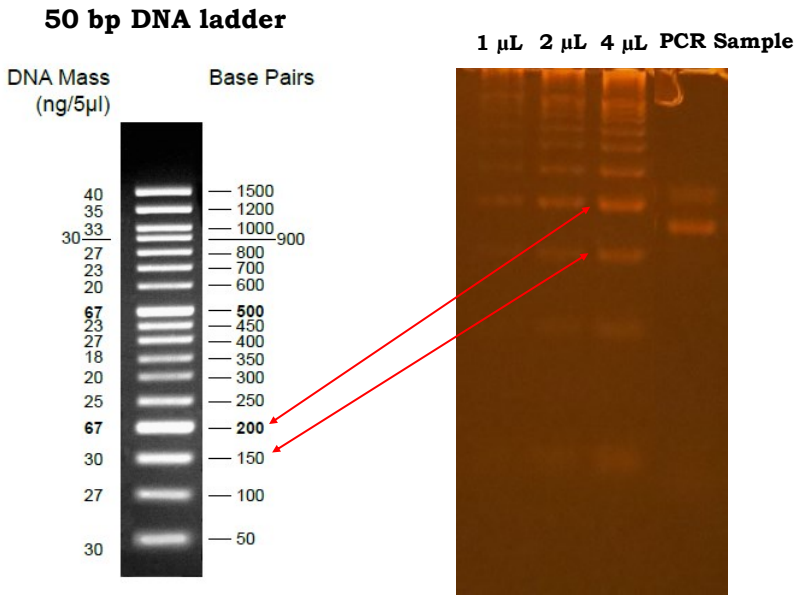
2. The unknown sample (either from a genomic DNA extraction or PCR product) will require the addition of loading buffer. Note the volume of sample and loading buffer added for final calculation.

2. Determining the approximate DNA concentration post-electrophoresis:

1. After ethidium bromide staining, the DNA concentration of the unknown sample can be estimated by visualisation and comparison with the intensity of the unknown 'band' and the intensity of 'bands' of either DNA ladder dilutions (e.g. 50 bp,

2. DNA ladders contain fragments of known concentrations listed in the datasheet e.g. 67 ng/5 µL. Recalculate the fragment concentration for the volume that has been loaded onto the gel.

3. After recalculation, the approximate concentration of the unknown sample can be estimated. If the sample has been diluted when loaded, this will need to be taken into account. See the following example opposite.



A PCR sample (4 µL) was run on a 6 % gel (hPAGE) next to 4 µL, 2 µL and 1 µL dilutions of a 50 bp ladder. The gel was post-stained with EtBr.

To estimate the concentration of the unknown PCR sample, visually compare the intensity of the band with the 50 bp DNA ladder dilutions as follows: Looking at the 4 µL ladder lane, the intensity of the unknown is less than the 200 bp band but greater than the 150 bp band.

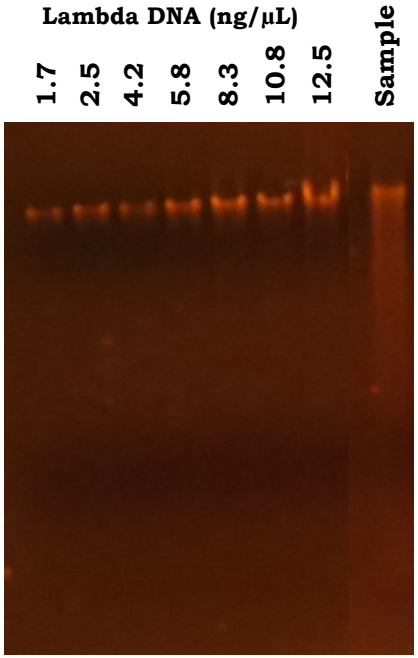
The 200 bp and the 150 bp bands have a concentration of 67 and 30 ng/5 µL respectively. Since 4 µL of ladder was loaded onto the gel, the concentrations of these bands can be calculated to be about 53 ng and 24 ng/4 µL respectively. As the intensity of the unknown sample is closer to that of the 53 ng/4 µL it is estimated that the concentration of the unknown PCR sample is 44 ng/4 µL or 11 ng/µL.

3. Estimation of genomic DNA

1. The amount of genomic DNA obtained from a CTAB extraction can be estimated by comparison with a standard solution of DNA Lambda[1]. DNA Lambda will show one band on an agarose or hPAGE gel.

2. Prepare dilutions of DNA Lambda and the unknown sample(s) as for a DNA ladder (Section 2).

3. The approximate concentration of the unknown sample can be estimated. If the sample has been diluted when loaded, this will need to be taken into account. See the example opposite:



A unknown genomic DNA sample (diluted 1:10) was run on a 0.8% agarose gel next to various dilutions of lambda DNA. The gel post-stained with EtBr.

To estimate the concentration of the unknown genomic DNA sample, visually compare the intensity of the band with the lambda DNA dilutions.

The intensity of the unknown genomic DNA sample appeared brighter than the lambda DNA band for 5.8 ng/µL , but less than the band for 8.3 ng/µL

The unknown genomic DNA sample can be estimated as follows:

The two corresponding lambda DNA bands have a concentration of 5.8 and 8.3 ng/µL respectively. Therefore, the approximate concentration of the diluted unknown genomic DNA sample is 7 ng/µL or undiluted sample of 70 ng/µL.

Notes:

[1] DNA Lambda (uncut) is supplied in a solution and as a consequence must be shipped at -20°C. If this standard is required please contact Kirkhouse Trust.