



Tips for Estimating the Amount of DNA in Agarose Gels

- Please make sure to quantitatively dissolve the comparative DNA (e.g. the marker) in buffer, in order to obtain the full DNA-concentration.
- Allow the gel to run far enough so that the bands have adequate space, the DNA can then disperse in the bands and stain well.
- Stain the gel thoroughly - we recommend staining after the gel run. Staining the DNA by adding ethidium bromide to the agarose will not achieve an even distribution in the gel and is frequently not quantitative.
- Apply several lanes of comparative-DNA with different quantities so that various bands can be compared. A calibration curve may also be plotted to determine the exact concentration.
- For comparison it is best to use bands of similar size to your DNA (maximum $\pm 50\%$ of the fragment length).
- Ethidium bromide is integrated much more effectively between the base pair stacks in linear DNA than in cyclic DNA. Therefore, please make sure that the conformation of the DNA whose concentration you wish to estimate is identical to that of the comparative DNA. In the event of cyclic DNA, e.g. isolated plasmids, cyclic DNA should also be used, e.g. plasmid-DNA pUC19 (Art. No. X911.1) or pBR322 (Art. No. X912.1). If you are using a DNA-marker as a comparative DNA and wish to measure cyclic DNA, you should initially linearise an aliquot of the DNA through restriction digestion and apply the preparation to the gel.
- In the event of a Lambda/*Hind*III marker: Do not use the 4.4 kb band of the marker for concentration analysis, as it is often not quantitatively present but coupled to the large fragment via the *cos*-site. Should you require the 4.4 kb band, please take care to heat the marker prior to applying (5 minutes at 65 °C).
- Only use the large marker fragments of a Lambda marker for concentration estimation when your fragments are in the same size range. Many visual systems do not depict the staining of the large bands proportionally to the DNA-amount.

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