

Quantification of DNA

- several methods are available (only the two most commonly used are discussed here):

- **Spectrophotometry (light absorption at 260 nm wavelength):**

- DNA absorbs ultraviolet (UV) light with an absorption maximum at 260 nm (this is why the UV radiation of the sunlight causes DNA damage in our skin and sunburn; the ring systems of the nitrogenous bases are responsible for absorption)
- absorption or **optical density (OD) of 1 at 260 nm** corresponds to a **DNA concentration of 50 ng per microliter**
- method works only with pure DNA that is free of other compounds, in particular proteins, that absorb in the UV range as well
- purity (contamination with proteins) can be estimated by determining the **260 nm/280 nm absorption ratio**; proteins have an absorption maximum at 280 nm and the ratio will thus decrease with increasing protein contamination; the ratio should be close to 2.0 (ratio for pure, protein-free DNA) and a ratio of less than 1.9 indicates considerable contamination of the sample with protein (or other compounds that absorb strongly at 280 nm); note that this is only a **very crude measure of purity**, as the extinction coefficient of DNA at both 260 nm and 280 nm is much higher than that of protein (e.g. for solution containing 50% DNA and 50% protein, 260 nm/280 nm = 1.87)
- disadvantages:
 - does not work with heavily contaminated DNA (see above)
 - cannot be used if only small amounts of DNA are available; better suited for small amounts and more reliable in general is the

- **quantification of ethidium bromide-stained DNA after agarose minigel electrophoresis:**

- DNA is run on a minigel in parallel to serial dilutions (two or three are usually sufficient) of a size marker that contains DNA fragments of known quantity (requires that DNA is linear; this means that plasmid DNA will have to be cut with a single-cutting restriction enzyme first)
- fluorescence intensity of DNA bands of similar size is then compared visually; if, for example, a 900-bp DNA band of unknown quantity shows a fluorescence intensity that equals the fluorescence intensity of a 1-kb marker band containing 50 ng of DNA, we can conclude that the 900-bp band contains approximately the same amount; the precision of this kind of quantification is sufficient for most applications
- advantages:
 - can be done even if only small quantities of DNA are available
 - indicates at the same time whether restriction digests were complete
 - reveals possible contamination with RNA (stained by ethidium bromide as well)
- disadvantages:
 - more labor-intensive than OD method
 - does not provide information with regard to the purity of the DNA sample (except possible contamination with RNA; see above)