

几种 DNA 定量方法

Quantitation of DNA

Fluorescent quantitation

Wear gloves and goggles protecting from EtBr and UV light

With an EDP (dilute mode) pick up 9 μl TE and 1 μl vortexed DNA sample. Spot this on a piece of scrap parafilm, pull it up and down one time, and make a second 10 fold dilution with 9 more μl TE and 1 μl of the first dilution. Spot 5 μl of these dilution on different parts of an EtBr agarous (in TBE with EtBr same as a gel) plate (label the bottom of the plate), if the concentration of the original DNA sample is suspected to be less than 20 $\mu\text{g/ml}$, then spot 5 μl of the undiluted sample also. Also spot on the plate 5 μl of at least the 2, 5, 10, 15, and 20 $\mu\text{g/ml}$ DNA standard. Let the liquid soak in and invert the plate on the transilluminator and compare, interpolate, and record the unknown spots for fluorescent intensity to the standard. Use the dilution that gives between 2 and 20 $\mu\text{g/ml}$ reading. Back calculate the amount of DNA by the dilution factor. It is best if the sample spots are coded and some one else reads them.

DPN Assay quantitation

Care of handling perchloric acid and DPN

In a fume hood, place the following reagent in a MFT

Stock Volume (μl)	
H ₂ O	140
DNA	10
3N perchloric acid	150
DPA solution	180

Cap the tubes and mix by inversion.

Incubate in the dark overnight at room temperature. Note the incubation time for use in the future when repeating the procedure. A calibration curve then will not be necessary each time.

Read the optical density at 600 nm and compare with a calibration curve made from measurements with a standardized DNA solution. The curve is only linear between the concentrations of 5 - 50 $\mu\text{g/ml}$: dilution may be necessary for more concentrated

solutions.

3 N perchloric acid: dilute 49.2 ml perchloric acid up to 200 ml with H₂O in a hood.

DPA (diphenylamine) solution: In a hood, add 20 μ l paraldehyde to 198 ml of glacial acetic acid in 250 ml Erlenmyer flask. With gloves, carefully weigh out 8 g of DPA and add to the above solution, quickly cover with foil, and shake until dissolved. Store in the dark in a fume hood. This solution is stable for 6 weeks.