DNA Quantification by Spot Fluorescence

1. Place a UV-transparent Plexiglas tray over UV Transilluminator (a gel tray is fine).
2. Spot several 3 µl drops of TE containing 2 mg/ml EtBr on tray.
3. Add 1 µl of a series of known DNA concentration (λ DNA at 0, 1, 2.5, 5, 10 & 20 ng/µl) standards to the EtBr drops. Mix by pipetting up and down several times.
4. Add 3 dilutions (1/2, 1/4, 1/8) of each of your unknown DNA samples to the ethidium bromide drops as follows:

   Mix 1 µl of DNA sample and 1 µl TE, pipet 1 µl into a 3 µl EtBr drop and mix.
   Add 1 µl of TE to that sample drop, pipet 1 µl into a 3 µl EtBr drop and mix.
   Add 1 µl of TE to that sample drop, pipet 1 µl into a 3 µl EtBr drop and mix.
5. Photograph the spots using UV illumination so that the lowest concentration standard is barely visible (this is essential for precise quantification).
6. Estimate the concentration (in ng/µl) of the DNA sample by comparing the intensity of the fluorescence in the samples with the standard drops.

   NOTE: The DNA standards should be similar in structure to the sample you wish to measure. Work very fast to avoid any evaporation from the drops before the photograph is made.