FLUOROMETER PROCEDURES

1. Place 2mL of fluorometer buffer (see Recipes section) into a small borosilicate glass test tube. There are two buffer pHs. pH 8 is for measuring DNA and RNA; pH 11 is for measuring only DNA.
2. Add 1ul of transcript or DNA solution to the buffer. If you plan to take your time, protect the tubes from light since the buffers contain ethidium bromide which is light sensitive. Ethidium bromide is also considered a mutagen since it binds to DNA so wear gloves and lab coat when handling. Read the MSDS.
3. Turn on the fluorometer and let it warm up for approx. 15 min. Read a blank (2mL of fluorometer buffer) and set the zero to zero.
4. Use a solution of 0.1mg/ml DNA (any DNA of known concentration is suitable) as the DNA standard. You may set up a standard curve if you wish using 0.1, 0.2, 0.3, 0.4, and 0.5ug of standard in 2mL of fluorometer buffer. Set the span to 150 using the 0.5ug standard. Read the values for the remaining standards. If the span cannot be set to 150 using the 0.5ug standard, then the gain must be increased to improve the sensitivity.
5. For transcripts, read unknown samples, add 5ul of RNase A, then read samples again. The first reading will be RNA and DNA, the second reading will be DNA alone, thus the difference will correspond to the amount of RNA in the sample. If you are interested in absolute amounts, remember that RNA fluoresces approx. half as much as DNA.
6. For DNA, simply read the unknowns and determine the quantity from the standard curve.