Quantitation of DNA for Automated Sequencing using the VersaFluor™ Fluorometer

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Introduction

DNA sequence analysis is an integral part of genetic research. Recently, the use of automated sequencers has become increasingly more common in small research laboratory settings. However, the success of automated long read sequencing reactions require precise picomolar concentrations of DNA. We have successfully employed the VersaFluor fluorometer (Bio-Rad) to accurately and routinely quantify DNA for sequencing with the LiCor 4200 automated sequencer.

Materials and Methods

A genomic clone for x*Hif* (*Xenopus hypoxia* inducible factor) was isolated by a homology screen of a *Xenopus* muscle genomic library using a ^{32}P-dCTP labeled mouse probe (a gift of Steven McKnight). Phage DNA was isolated using a standard protocol, digested with *EcoR*I and *Hind*III and run on a 1.2% agarose gel (Figure 1). Bands corresponding to insert DNA were excised and the DNA isolated on glass milk. The DNA fragments were subcloned into BlueScript SK+ (Stratagene) and transformed into DH5α cells. Plasmid DNA was isolated using anion exchange chromatography. A λZap (Stratagene) cDNA clone for *Xenopus* x*Hif* was also isolated using a homology screen of a tadpole library. Rescued plasmid DNA was obtained using the manufacturer's instructions and was subsequently transformed into DH5α cells. Plasmid DNA was isolated using anion exchange chromatography.

Quantitation of the DNA was achieved using the Fluorescent DNA Quantitation Kit (Bio-Rad) with a 360 nm excitation filter and a 460 nm emission filter. The standard curve was established for quantification of DNA concentrations ranging from 100–5,000 µg/ml by plotting seven points of varying DNA concentrations made by serial dilution of control calf thymus DNA. All dilutions were made in 2 ml of 1 µg/ml Hoechst 33258 dye and included 0 ng DNA as a blank and 10,000 ng DNA as the upper range. The values were recorded in relative fluorescence units (RFUs) and were plotted on a graph with the x axis = DNA concentration (ng) and the y axis = RFU (Figure 2). The slope of the line was determined to be 1.0.

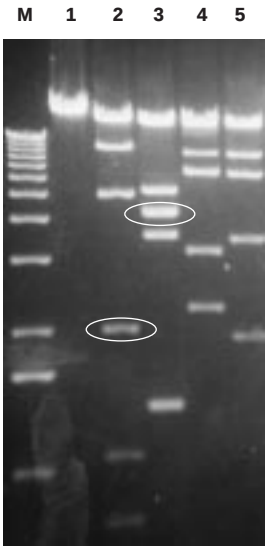

Fig.1. Genomic xHif DNA run on a 1.2% agarose gel and digested with BamHI, EcoRI, HindIII, SalI, and XhoI. DNA bands corresponding to EcoRI 2,000 base pair fragment and HindIII 4,000 base pair fragment (circled) were excised and the DNA isolated and sequenced.

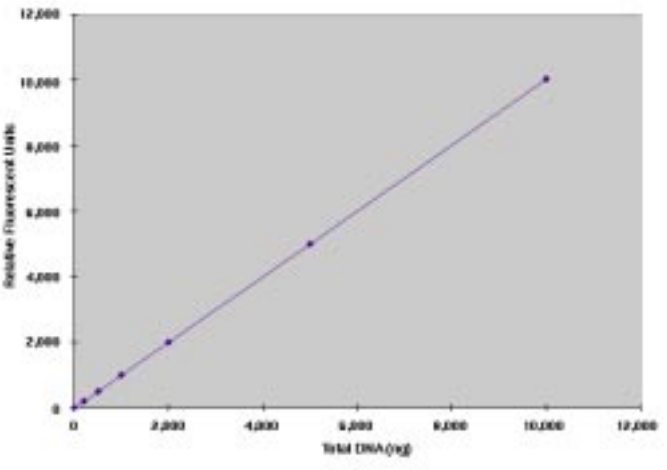

Fig. 2. Calibration curve generated by plotting seven points of known DNA concentrations against corresponding relative fluorescence units (RFUs). The x axis is DNA concentration in nanograms, the y axis is the corresponding RFU. The slope of the resulting line was determined to be 1.0.

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By employing the equation y = mx+b, we established the value of b to be equal to 0. Therefore, by setting the upper range value at 10,000 RFU using 10 µl of 1 mg/ml calf thymus DNA and using 2 µl of the sample DNA in 2 ml of the 1 µg/ml Hoechst dye, the RFU reading is equal to ng/ml for our sample. We are then able to calculate the volume of DNA needed to obtain a 250 femtamolar concentration per reaction.

Results and Discussion

Using the Excel II sequencing kit (Epicenter) and quantifying our DNA with the VersaFluor fluorometer, we have been able to achieve highly readable and reproducible sequencing runs of up to 1,200 bp with the LiCor 4200 automated sequencer. This has enabled us to sequence the entire 2.2 Kb cDNA for *Xenopus hypoxia* inducible factor, *xHIF*, in several runs, and will allow us to sequence our genomic clone easily and efficiently.

The correct concentration of DNA in each reaction is the key to obtaining good reads on the LiCor 4200. This entails precise quantitation of the DNA. The VersaFluor fluorometer has proven to be easy to calibrate and provides excellent and reproducible results.

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