

Quantitation of DNA for Automated Sequencing using the VersaFluor™ Fluorometer

Wenda Ribeiro and Margaret Saha, Department of Biology, College of William and Mary, Williamsburg, Virginia 23187

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 *xHif* (*Xenopus hypoxia* inducible factor) was isolated by a homology screen of a *Xenopus* muscle genomic library using a ³²P-dCTP labeled mouse probe (a gift of Steven McKnight). Phage DNA was isolated using a standard protocol, digested with *EcoRI* and *HindIII* 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 xHif* 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.

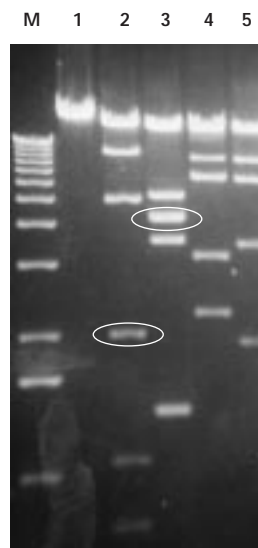


Fig. 1. Genomic *xHif* DNA run on a 1.2% agarose gel and digested with *BamHI*, *EcoRI*, *HindIII*, *Sall*, and *XhoI*. DNA bands corresponding to *EcoRI* 2,000 base pair fragment and *HindIII* 4,000 base pair fragment (circled) were excised and the DNA isolated and sequenced.

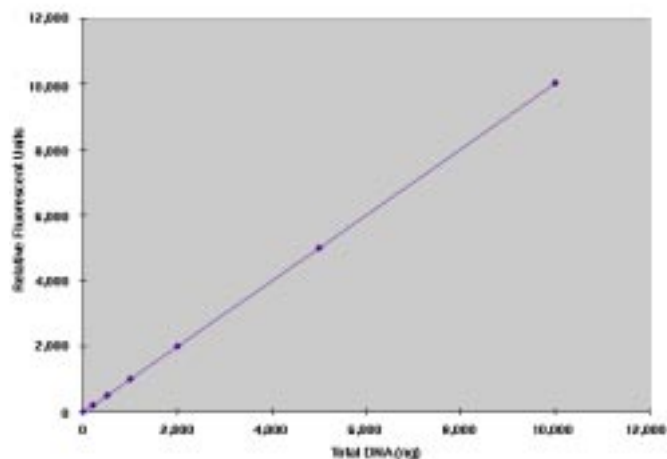


Fig. 2. Calibration curve generated by plotting seven points of known DNA concentrations against corresponding relative fluorescence units (RFUs). The x axis is DNA concentration in nanograms, the y axis is the corresponding RFU. The slope of the resulting line was determined to be 1.0.

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 μ l of 1 mg/ml calf thymus DNA and using 2 μ l of the sample DNA in 2 ml of the 1 μ g/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 femtomolar 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.

This work is supported by National Institute of Health Grant 1815HD32080 and National Science Foundation Grant 1BN9553176.

BIO-RAD

**Bio-Rad
Laboratories**

Life Science
Group

Website www.bio-rad.com U.S. (800) 4BIORAD Australia 02 9914 2800 Austria (01)-877 89 01 Belgium 09-385 55 11 Canada (905) 712-2771
China 86-10-62051850/51 Denmark 45 39 17 99 47 Finland 358 (0)9 804 2200 France 01 43 90 46 90 Germany 089 318 84-0
Hong Kong 852-2789-3300 India (91-11) 461-0103 Israel 03 951 4127 Italy 02-21609.1 Japan 03-5811-6270 Korea 82-2-3473-4460
The Netherlands 0318-540666 New Zealand 64-9-4152280 Russia 7-95-4585822 Singapore 65-2729877 Spain (91) 661 70 85
Sweden 46 (0)8 627 50 00 Switzerland 01-809 55 55 United Kingdom 0800-181134