Accurate Quantitation of RNA for Hybridization Blots Using the VersaFluor™ Fluorometer

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Introduction

Biologists frequently monitor the effect of environmental stimuli on living organisms by quantitating mRNA levels. The relative abundance of mRNA is typically determined with probes and hybridization blots, but these methods depend on accurate and sensitive methods for measuring total RNA. When fluorochromes bind to nucleic acids, fluorescence occurs, and changes can be monitored with a fluorometer. The following section describes the quantitation of total RNA with a VersaFluor fluorometer (Bio-Rad).

Materials and Methods

Total RNA was isolated from the strictly anaerobic ruminal bacterium, *Prevotella bryantii*, using an acid-phenol extraction procedure.[1] All solutions, tubes, and pipets were DEPC treated and autoclaved before use. *Escherichia coli* K12 total RNA was isolated using a commercially available RNA purification kit and was stored at -70 ˚C. RNA concentrations were determined using the VersaFluor fluorometer and RiboGreen™ RNA quantification kit (Molecular Probes, Inc). The excitation was at 490 nm (EX 490/10 filter), and the emission was monitored at 520 nm (EM 520/10 filter). A standard curve was generated with *E. coli* ribosomal RNA (supplied with the kit), and the range was 1 to 50 ng/ml.[2] The standard and all samples were diluted in TE buffer and mixed with RiboGreen (Molecular Probes, Inc.) that had been diluted 2,000-fold. Fluorescence emission intensity was measured using disposable cuvettes, and the gain and range settings were adjusted to medium and 10,000, respectively. Relative fluorescence units (RFU) were recorded and plotted as a function of RNA added (Figure 1, R^2=.988), and this linear relationship was used to determine the RNA concentration of samples.

When *E. coli* ribosomal RNA was treated with DNase-free RNase[3] for 1 hour at 37 ˚C, no RFU was detected. If samples were treated with DNase-free RNase, there was a 97% decrease in RFU. These results corroborated the idea that fluorescence emissions were being generated by RNA and not DNA.

RNA quantitation is a crucial feature of many molecular measurements. *Prevotella bryantii* endoglucanase mRNA was detected with slot-blots using a fluorochrome-labeled probe (Figure 2). Accurate quantitation of RNA was critical, and the VersaFluor fluorometer was used reliably in determining RNA concentrations.

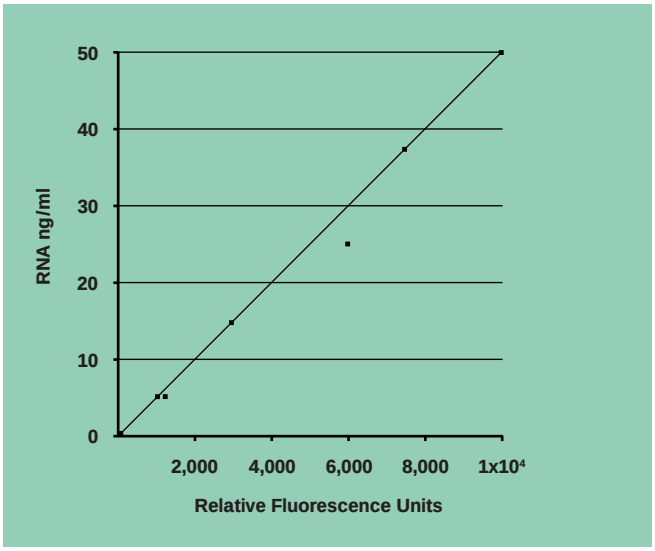
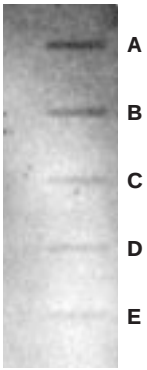


Fig. 1. Standard curve generated by plotting RNA concentration (ng/ml) as a function of relative fluorescence units (RFU).

Fig. 2. Decline in Prevotella bryantii *endoglucanase mRNA after exposure of cells to repressor for A) 0 generation, B) 0.24 generation, C) 1.22 generations, D) 1.92 generations, E) 3.13 generations. Equal amounts of total RNA were blotted to each slot.*



References

1 *Wen, Z. and Morrison, M.,* AEM, ***62****, 3826–3833 (1996).*
2 *Molecular Probes, Inc.* Product Information Sheet*, RiboGreen RNA quantitation reagent (1997).*
3 *Ausubel, F.,* et al*.,* Short Protocols in Molecular Biology*. A1-51 (1997).*

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