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Note

An automated, pressure-driven sampling device for harvesting from liquid cultures for genomic and biochemical analyses

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Abstract

Here we describe an automated, pressure-driven, sampling device for harvesting 10 to 30 ml samples, in replicate, with intervals as short as 10 s. Correlation between biological replicate time courses measured by microarrays was extremely high. The sampler enables sampling at intervals within the range of many important biological processes.

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Routine, sterile sampling of intact cells from liquid cultures, in volumes needed for biochemical and genomic analyses and with time points ranging from seconds to minutes, presents a significant challenge for systems biology. Several methods have been developed to rapidly harvest cells in liquid cultures (Lange et al., 2001; Mashego et al., 2003; Theobald et al., 1993). However, these methods are limited either by the collection of relatively small volumes of cells or by sampling intervals in the range of minutes. Smaller sample sizes do not allow isolation of sufficient quantities of RNA for microarray analysis without amplification or sufficient quantities of proteins for biochemical analyses. Finally, biological processes that occur in time

frames faster than the sampling rate cannot be detected or analyzed with long sampling times. To address these issues, we have developed a simple, automated sampler that can be used in any laboratory.

Protocols, parts lists, tools required for assembly, wiring diagrams, contact information for the microcontroller program, and supplier contact information is available at http://biology.unm.edu/biology/maggieww/Public_Html/aragon/RS.htm. Maximal dead volume was approximately 1 ml for both 2- and 4-l flasks holding 1- and 2-l of media, respectively. To obtain constant volumes over time as the culture volume decreased, small increases in pressurization time (0.0118 s) were determined empirically, by measuring the volume of sample obtained with different pressurization times and programmed into the microcontroller. A VWR Traceable Expanded Range Thermometer (VWR, West Chester, PA) was used to monitor cooling times.

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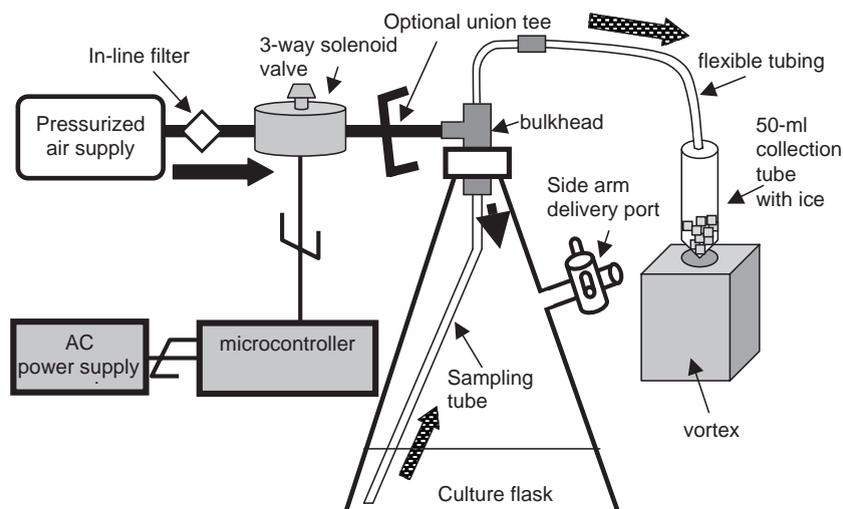


Fig. 1. Diagram of the automated-sampling device. Solid arrows show the direction of air flow from the breathing air supply (typically using 5 to 15 psi) through solenoid valve to the bulkhead into the flask. The valve is controlled by a microcontroller programmed to regulate: 1) the duration of valve on-time (pressurization time); 2) the time interval between sampling; and 3) the increase in the on-time of the valve during sampling to compensate for increases in air volume (a function of decreases in liquid volume from sampling) within the cell culture flask. Hatched arrows show the direction of fluid flow from the culture through the sampling tube, out the bulkhead, and into the flexible tubing for sampling. For simultaneous biological replicates, additional flask assemblies are coupled to the union tee. After sample collection the pressure in the flask is returned to atmospheric pressure through the 3-way valve. At 15 psi and an initial sampling time of 1 s, the mean volume for 20 samples was 17.3 ± 0.19 ml. At 15 psi, flow rates were 23.3 ± 0.41 ml/s and 16.7 ± 0.18 ml/s for 2- and 4-l flasks, respectively. All liquid-handling components are autoclavable.

MAT α S288c yeast cells were grown as described (Martinez et al., 2004). Menadione (500 μ M in 100% EtOH) was added to a final concentration of 50 μ M. Samples were harvested into 50-ml conical tubes containing 20 g of ice, mixed by vortexing, and held on ice until centrifugation. For larger samples, holes were drilled into the lid of each conical tube to allow simultaneous sample collection and vortexing. Cell pellets were obtained by centrifugation at 4 $^{\circ}$ C, washed once in ddH₂O at <4 $^{\circ}$ C, pelleted, and frozen at -70 $^{\circ}$ C in 1.5 ml tubes.

Total RNA was isolated using a modified version of the Genra Purescript RNA Purification Kit protocol (Genra, Minneapolis, MN), described elsewhere (Martinez et al., 2004). cDNA was synthesized and fluorescently labeled with Cy3-dCTP or Cy5-dCTP (Amersham Pharmacia Biotech, NJ), slides were pre-hybridized and hybridized, and data analyzed using GenePix Pro 6.0 (Axon Instruments, Union City, CA) as previously described (Martinez et al., 2004). In-house generated scripts for MATLAB 6.5 (The MathWorks Inc., Natick, MA) were used to determine correlation in gene expression ratios between two hybridizations, and produce correlation plots (R^2) and histograms showing deviations from the regression line and are available by request.

A diagram and parameters of the sampling device are presented in Fig. 1. For our purposes, samples were

harvested in to ice to keep cells cold but not frozen to prevent membrane damage and release of RNases until cell pellets could be frozen and to cool cells rapidly to decrease sample heterogeneity. For 10 ml of culture, cooling time for culture without vortexing was >3 s and with vortexing was <0.1 s (Table 1).

Reproducibility between sampling methods, i.e., pipetting vs. automated sampling, and time courses was tested using microarray analysis. Replicates obtained by the same sampling method had R^2 values greater than 0.98 (Fig. 2, A and C). When samples obtained by automated sampling and pipetting were compared, their R^2 values were 0.97 (Fig. 2, E). The small deviations (\log_2 ratios <0.5) for all three comparisons (Fig. 2 B, D, and F) supported our conclusion that measured gene expression was not affected by the sampling method.

Table 1

Cooling rate of samples taken with automated sampler with and without vortexing

Sample volume (ml ^a)	Time to reach 4 $^{\circ}$ C	
	w/vortexing	wo/vortexing
10	<0.1 s	>3 s
20	<0.1 s	>3 s
30	<0.1 s	>30 s ^b

^a All samples were harvested into 20 g of ice.

^b Sample was at 12 $^{\circ}$ C when timing was stopped at 30 s.

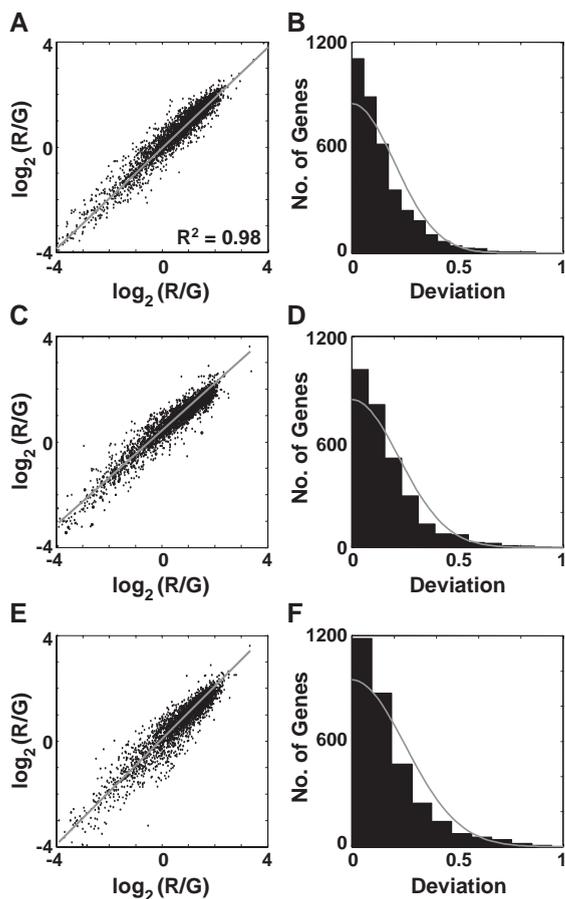


Fig. 2. Comparison of gene expression data (\log_2 R/G) for samples obtained by pipetting or with the automated sampler. A) Correlation of gene expression ratios between biological replicates harvested with a pipette. B) Deviation from the regression line for samples in A, in \log_2 units. C) Correlation of gene expression ratios between biological replicates harvested with the automated-sampling device. D) Deviation from the regression line for samples in C, in \log_2 units. E) Correlation of gene expression ratios between samples harvested by pipette and the automated-sampling device. F) Deviation from the regression line for samples in E, in \log_2 units.

The reproducibility of time-course data was evaluated by comparing gene-expression ratios for biological replicates collected 0, 1, and 5 min after yeast cells in stationary-phase cultures were exposed to oxidative stress (50 μ M menadione) (Fig. 3). Samples harvested at 1 min (Fig. 3A and B) or 5 min (Fig. 3C and D) were highly correlated with biological replicates sampled with the same time intervals, with an R^2 of 0.98 and deviations from the regression line generally less than 0.5 \log_2 units (Fig. 3, B and D). Samples obtained at 1 and 5 min after exposure to oxidative stress, when compared with each other (Fig. 3E and F), were much less correlated (R^2 of 0.92), with a large percentage of the deviations from the regression line concen-

trated above the 0.5 \log_2 unit threshold. $T=0$ and 1 min samples were even less correlated ($R^2=0.78$) (Fig. 3G), with deviations extending beyond 2 \log_2 units (Fig. 3H). We concluded that highly reproducible time

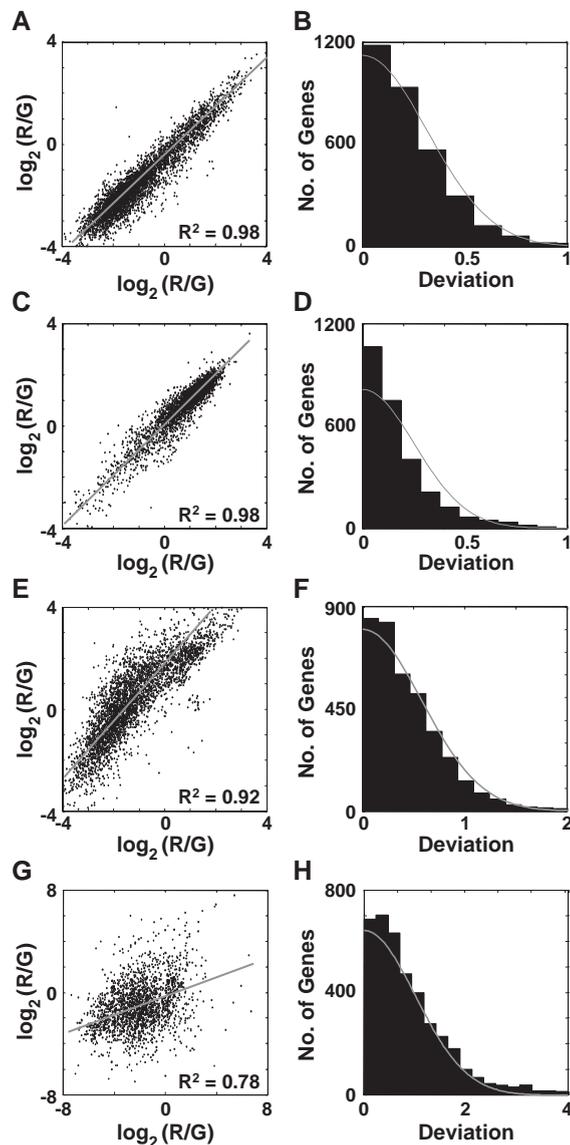


Fig. 3. Comparison of gene expression data (\log_2 R/G) for samples at 0, 1, and 5 min after exposure to 50 μ M menadione obtained using the automated sampler. A) Correlation of gene expression ratios between two biological replicates harvested 1 min after exposure. B) Deviation from the regression line for samples in A, in \log_2 units. C) Correlation between two biological replicates harvested 5 min after exposure. D) Deviation from the regression line for samples in C, in \log_2 units. E) Correlation between samples harvested 1 min and 5 min after exposure. F) Deviation from the regression line for samples in E, in \log_2 units. G) Correlation between samples harvested prior to (0 min) and 1 min after exposure. H) Deviation from the regression line for samples in G, in \log_2 units.

course data could be obtained using the automated sampler.

The sampling device we have developed is relatively simple to build and operate, easily adaptable, and permits researchers to obtain accurate, reproducible time-course data in replicate from cells under a variety of conditions. Manual sampling at time intervals below 5 min has been shown to introduce significant variability (Mashego et al., 2003) and this variability decreases quality and increases costs of high-throughput, functional genomic data. Thus, the automated sampler fills an important gap in this area.

The automated sampler maintains sterility, allows sampling times as short as 10 s for 10-ml samples, and is compatible with a wide range of sample volumes and intervals as well as harvesting techniques. The adaptability of the device presented here makes modifications easy to imagine. For example, it has recently been shown that high yields of RNA are obtained from yeast by filtration (Belinchon et al., 2004), thus, a system for fast, cold filtration allowing sampling intervals of 5 s or less would be a powerful add-on to this sampler. Meanwhile, the ability to obtain reproducible samples with 10 s time points will open up important biological processes to real-time genomic analyses and, hopefully, will facilitate the discovery of new biology.

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