Gene expression study of *Saccharomyces cerevisiae* with the Agilent 2100 bioanalyser

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ABSTRACT

This study explores the restriction display-polymerase chain reaction (RD-PCR) application of a new chip-based nucleic acid analysis system (Agilent 2100 bioanalyser) in a gene differential expression study. Total RNAs is extracted from *Saccharomyces cerevisiae*, double-stranded complementary DNA (cDNA) is synthesised by reverse transcription from the purified messenger RNA (mRNA), RD-PCR conducted to obtain the cDNA fragments and bioanalyser and agarose gel electrophoresis compared for the analysis of RD-PCR products. The bioanalyser proved to be faster and more sensitive in separating and detecting gene fragments, and was also able to compare different gene fragments quantitatively. Using this technology, comparison of several differential gene fragments is performed.

KEY WORDS: Electrophoresis, agar gel. Gene expression profiling. Polymerase chain reaction. Saccharomyces cerevisiae.

Introduction

DNA analysis is now a fundamental aspect of life science research. At present, agarose gel electrophoresis is a widely used technique gel electrophoresis; however, although it is comparatively inexpensive and easy to use, accuracy of results is limited and it is usually combined with fluorimetry or ultraviolet (UV) spectrophotometry to achieve more accurate concentration information. Such instruments cannot provide information about sample constituents or potential contaminants, only data on the concentration of the total sample, and both are time- and sample-consuming techniques.

The Agilent 2100 bioanalyser is the first commercially available 'lab on a chip'-based nucleic acid analysis system, which overcomes the limitations of traditional gel electrophoresis. Several DNA kits are available to size a broad range of DNA fragments, with the DNA 7500 LabChip kit being most suitable for DNA fragments of 100 – 7500 bp.

Here, the DNA 7500 Labchip kit is used with the bioanalyser to study the gene expression profile of normal and heat shock-induced *Saccharomyces cerevisiae* cells in order to explore the application of this new analytical system in differential gene expression studies.

Materials and methods

Materials

Saccharomyces cerevisiae was provided by the medical laboratory of Guangzhou General Hospital of Guangzhou Command (China); Superscript II (reverse transcriptase) was purchased from Life Technologies (USA); QuickPrep mRNA (mRNA purification kit) was purchased from Amersham Pharmacia (USA); Sau3A I, T4 DNA ligase, and *Escherichia coli* DNA polymerase I were obtained from TaKaRa (Japan); ethidium bromide and agarose were from Sangon (China); and the 7500 LabChip kit was ordered from Agilent Technologies (Germany).

Instrumentation and software

All chip-based separations were performed on the Agilent 2100 (Agilent Technologies, Palo Alto, CA, USA) according to the manufacturer's instructions. The instrument was operated by software running on a PC. The bioanalyser software package included data collection, presentation and interpretation functions. Data can be displayed as gel-like images (bands) and/or electropherograms (peaks), and can exported easily to various spreadsheet programmes. Other instrumentation used included the GeneAmp PCR system 9700 (PerkinElmer, USA) and UVP acquisition and analysis software (Ultra-Violet Products, USA).

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Sample preparation

Normal yeast cells were grown[1] to logarithmic phase (about 24 h) in 2 mL YPD liquid medium at 30°C with shaking at 220 rpm. Heat shock-treated yeast cells were cultivated initially at 37°C for 1 h, then the temperature was reduced to 30°C and allowed to grow to logarithmic phase.

Total RNA extraction was conducted according to a method described previously.[2] Messenger RNA (mRNA) was prepared using the QuickPrep mRNA purification kit (Amersham Pharmacia), following to the manufacturer's instructions.

Complementary DNA (cDNA) was synthesised using the Superscript II kit (Life Technologies), following the manufacturer's instructions.

Restriction display-PCR
Restriction display-polymerase chain reaction (RD-PCR)[3-5] was performed according to a method described previously.[3] Double-stranded cDNAs were digested with Sau 3A I, T4 DNA ligase was used to link gene fragments with universal adapters (SIP: 5'pGATC™CACACCAGCCAAACCCA3'; SIR: 5'GGTTTGGCTGGTGTG3',PCR primers were designed to match the universal adapters, including the restriction site sequence, but with one base overhang at the 3'-terminal. PCR reactions were divided into 10 subgroups. Reactions were performed in a GeneAmp PCR 9700 system with an initial denaturation of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C, and a final extension of 7 min at 72°C.

Chip preparation, detection and analysis
All chips were prepared according to the instructions provided with the DNA 7500 LabChip kit. In addition to 25 chips, gel matrix, dye concentrate, DNA markers, DNA sizing ladder, syringe and spin filters were provided. The gel/dye mix was prepared by mixing 400 μL of the gel matrix with 20 μL of dye concentrate and the mixture was filtered through a spin filter. The separation chip was filled with the gel matrix/dye mixture and 5 μL of the markers were added to each sample well. After adding 12 samples (1 μL each) to the sample wells and the DNA sizing ladder (1 μL) to the assigned ladder well, the chip was vortex-mixed and run on the bioanalyser.

Agarose electrophoresis
PCR products (2 μL) were separated by agarose (2%) electrophoresis at 60 V for 1h, then visualised by staining with ethidium bromide (5 μL of 10 mg/mL ethidium bromide, 50 mL of 1x TBE) for 15 min, after which the gel was destained to remove background fluorescence by rinsing with 50 mL of 1X TBE (x2). Destaining may be unnecessary when low ethidium bromide concentrations are used. Finally, the gel was scanned and analysed using the UVP software.

Results

Speed of analysis
Compared with the traditional gel electrophoresis, the speed of Bioanalyzer is dramatically improved; it can complete the analysis of 12 DNA samples consecutively in about 35 min, which is about two to seven times faster than gel electrophoresis (Table 1).

Differential gene expression
By comparing the results obtained by gel electrophoresis (Figure 1) and bioanalyser (Figure 2) it was possible to see that the bands obtained using the latter were sharper, making it possible to distinguish between fragments of similar sizes. In addition, the bioanalyser was able to detect the fragments of less than 100 bp in size and lower than 0.5 ng/μL in concentration.

Overall, bioanalyser sensitivity was approximately 25 times higher than that of gel electrophoresis, and it detected DNA fragments as low as 0.02 ng/μL in concentration. Thus, it proved easier to detect and compare differentially expressed gene fragments on the bioanalyser's gel-like images.

Table 1. Comparison of the time needed to run chips on the bioanalyser versus electrophoresis

	Time	
Procedure	**Chip system**	**Agarose gel system**
Preparation	5 min	30 min
Separation	30 min	30-180 min
Staining/destaining		0-15 min
Scanning/analysis		20 min
Total	35 min	80-245 min

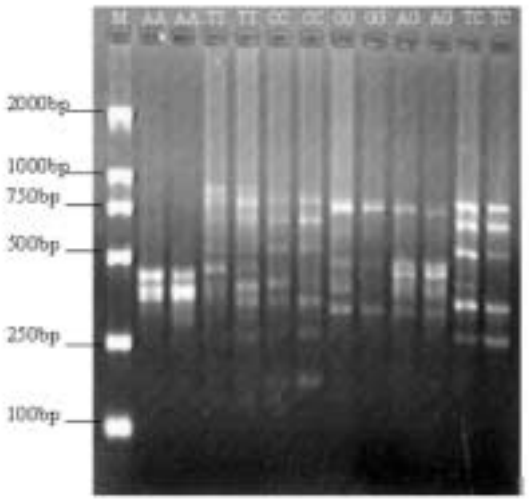

Fig. 1. Electrophoretic analysis on 2% agarose gel of the gene fragments amplified by RD-PCR.
M: DNA marker DL2000; lanes 1-12: PCR subgroups (lanes 1,3,5,7,9,11: normal yeast cells; lanes 2,4,6,8,10,12: yeast cells after heat-shock treatment).

Odd numbers in Figure 2 represent normal yeast cells, even numbers represent yeast cells after heat-shock treatment, and AA-TT represent individual RD-PCR subgroups. By comparing each pair of RD-PCR subgroups, we found that after heat-shock treatment, the expression of some genes in the TT subgroup was up-regulated, while the expression of some genes in the GG and TC subgroups was down-regulated – information that is hard to obtain from a gel electrophoresis image. The pairs of arrows in Figure 2 indicate some of the differentially expressed gene fragments.

Quantitative analysis
In addition to providing gel-like images, the bioanalyser provided electropherograms and could overlay two or more examples from different DNA fragments (Figure 3) and provide concentration information. This made comparison of differentially expressed gene fragments easier and more accurate.

For example, the quantity of peak 5 obtained from heat shock-treated yeast is approximately four times lower than

Table 2. Quantitative comparison of differential RD-PCR fragments in subgroup TC

Normal yeast (lane 11)			Yeast after heat-shock (lane 12)			Ratio
Peak	Size (bp)	Concentration (ng/μL)	Peak	Size (bp)	Concentration (ng/μL)	
2	93	2.60	2	93	2.40	1.08
3	261	1.10	3	260	1.50	0.73
4	341	4.70	4	340	3.00	1.57
6	513	3.00	5	513	0.71	4.23
7	661	2.30	6	660	1.50	1.53
9	788	4.90	7	788	2.50	1.96

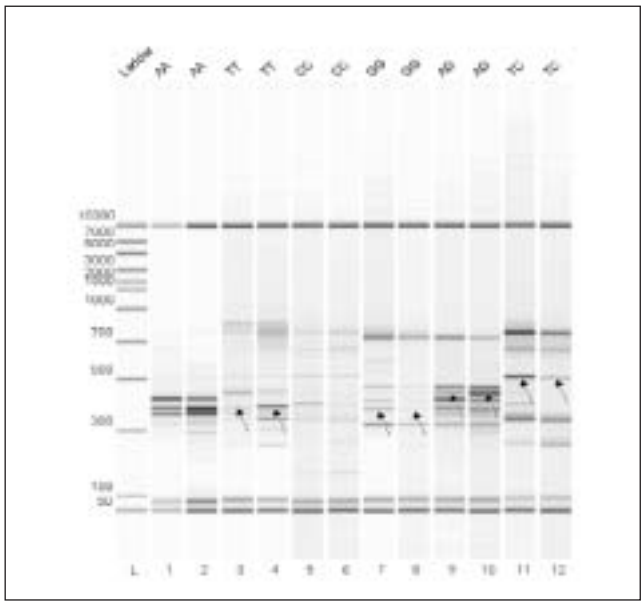

Fig. 2. Gel-like image of RD-PCR products of normal and yeast cells after heat-shock treatment run on the bioanalyser. Lanes 1,3,5,7,9,11: normal yeast cells; lanes 2,4,6,8,10,12: yeast cells after heat-shock treatment. Differential genes (indicated by arrows) are clearly displayed in pairs.

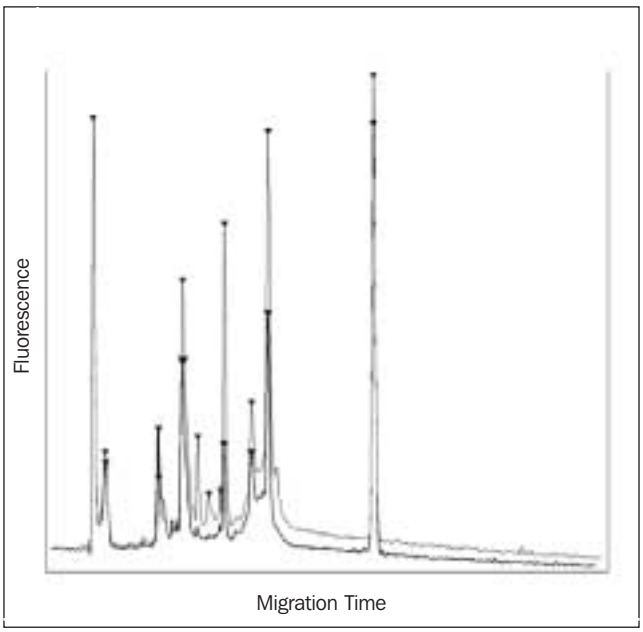

Fig. 3. Overlay of two electropherograms of RD-PCR products in subgroup TC analysed on the bioanalyser. Light grey line represents normal yeast while the darker line represent yeast after heat-shock treatment.

that of peak 6 obtained from normal yeast. The two peaks represented the same gene fragment, as determined by sizing information, indicating that it was down-regulated after heat shock treatment.

Discussion

As the frontiers of genetic, biological and biochemical research continue to expand, modern laboratories increasingly demand analytical tools that permit faster and more automated analysis of biomolecules. Through lab-on-a-chip technology, and the use of LabChip kits, the Agilent 2100 bioanalyser integrates sample handling, separation, detection and data analysis on one small chip measuring 5 cm^2. This integration streamlines the entire workflow process (sample loading, analysis and data viewing) and reduces the analysis time dramatically. In addition, it minimises exposure to hazardous materials, such as ethidium bromide, and reduces waste.

In gene expression studies, the quantity of nucleic acid obtained is limited so the sensitivity of detection is very important. With a high-sensitivity fluorescence detection system and the use of microfluidic technology, the bioanalyser is more sensitive than gel electrophoresis, and only 1 μL of DNA sample is needed to perform the analysis. Higher sensitivity, together with a broad linear dynamic range and sharpness of bands, permits detection of less-abundant gene fragments that are not visible by agarose gel electrophoresis.

Although poor band resolution may be improved by preparing agarose gels at concentrations higher than 2%, this would increase the time needed for electrophoresis. In addition, lower gel concentrations are often necessary to separate larger fragments (>1000 bp).

The main advantage of the bioanalyser proved to be its ability to automatically size and quantitate DNA fragments accurately and reproducibly. This was aided by the use of prepackaged reagents and kits, using standardised protocols, which resulted in more reproducible data,

minimal user errors, and facilitated the exchange of data between different chips, users and laboratories.

Both external standards (DNA ladders) and internal standards (mass and size markers) are used to calibrate the sizing and quantitation of each sample. This eliminates slight differences as samples are run sequentially through the separation channel. In contrast, internal standards are not run with samples separated by gel electrophoresis.

In gel electrophoresis, additional variability can occur when loading samples into the gel and during staining after separation – a process that is unnecessary when using the bioanalyser.

In the present study, when comparing the quantities of DNA fragments of each RD-PCR subgroup, we found several differences in differentially expressed genes which will provide important information for further gene cloning and function studies.

In addition to DNA analysis,[6-7] the Agilent 2100 bioanalyser can be used with a wide range of LabChip kits for the analysis of RNA,[8] proteins and cells. □

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