

Article Reprinted from the ©May 2001 issue.

# Lab-on-a-Chip Technology — Applications for Life Sciences



**Agilent Technologies**  
Innovating the HP Way



# Lab-on-a-Chip Technology — Applications for Life Sciences

image Agilent

**The determination of size, quality and concentration of biomolecules such as DNA, RNA and proteins is one of the fundamental steps in life science research. Traditional methods for this type of analysis, such as gel electrophoresis or capillary electrophoresis, can now be complemented by an analytical technique, Lab-on-a-Chip technology. This technology enables downscaling and integration of several experimental steps into one process, in combination with automated data analysis. Lab-on-a-Chip technology has several advantages compared with conventional techniques, such as minimal sample requirement, rapid analysis times, ease-of-use, minimized exposure to hazardous materials and reduced waste generation.**

## **Meike Kuschel**

is an applications chemist,  
Agilent Technologies  
Deutschland GmbH, D-76337  
Waldbronn, Germany.  
Tel. +49 7243 602 454  
Fax +49 7243 602 501  
meike\_kuschel@agilent.com

The enormous task of deciphering the human genome and developing new targeted therapeutics increasingly demands analytical tools that allow a much faster and more automated analysis of biomolecules. Most analytical techniques are time-consuming and laborious, and expend a considerable amount of sample. Gel electrophoresis is the most commonly used method for analysis of DNA, RNA or proteins. During the past few decades this technique has proven to be a versatile tool for sizing and analysis of sample quality and purity. Now a more recent development, 'Lab-on-a-Chip' or microfluidics technology,<sup>1-6</sup> enables the handling of fluids in very small volumes via microchannels etched into glass or plastic chips. This technology permits the integration of several sequential experimental steps into one single automated process. Many laboratory tasks, such as sample handling, electrophoretic

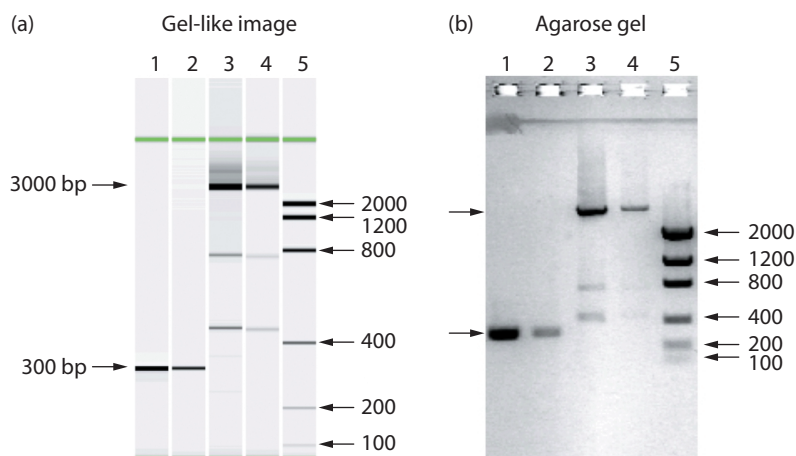
separation, staining/destaining and detection, can now be performed within the confines of small microfabricated chips. In combination with software, which automates data analysis and data archiving, a Lab-on-a-Chip analysis system profits from minimized manual intervention, increased speed of analysis, increased precision of data, significant cost reduction, less sample consumption and minimized operator exposure to hazardous materials.

Much progress within the field of Lab-on-a-Chip technology has been made by Caliper Technologies (Mountain View, California, USA) and has led to the development, in collaboration with Agilent Technologies, of the 2100 Bioanalyzer.

## **Lab-on-a-Chip analysis system**

The Bioanalyzer consists of a bench-top device (chip reader) that communicates with a PC. The chip

**Figure 1** Comparison of PCR fragments amplified from adenovirus 2 DNA analysed (a) with the DNA 7500 LabChip kit (gel-like image) and (b) on an agarose gel. The quantitation of the DNA fragments of a size standard (low mass ladder) obtained with the chip system is compared with the concentration given by the manufacturer. (Lane 1: 300 bp PCR fragment; Lane 2: 300 bp PCR fragment — fourfold dilution; Lane 3: 2966 bp PCR fragment; Lane 4: 2966 bp PCR fragment — fourfold dilution; Lane 5: low mass ladder (Gibco BRL); green bands, internal standard.)



exported to spreadsheet programs for further analysis.

### Applications for Lab-on-a-Chip technology

The chip technology will perform a variety of laboratory tasks, such as sizing and quantitation of DNA fragments (for example, polymerase chain reaction [PCR] products or restriction digests), quality and integrity assessment of total or messenger RNA (mRNA) samples and analysis of a variety of protein samples (for example, cell lysates and column fractions). Specific application examples for this technology are described in more detail in the following sections. All experiments were performed using the Agilent LabChip Kits and the 2100 Bioanalyzer.

### Sizing and quantitation of PCR products

Two PCR fragments of 300 bp and 2966 bp, amplified from adenovirus 2 DNA, were analysed with a chip system and the results compared with those obtained using agarose gel electrophoresis (Figure 1). In the gel-like image of the Lab-on-a-Chip system, the bands appear sharper than on the agarose gel. The sizing resolution above 1500 bp is slightly reduced when compared with the agarose gel; however, below 1500 bp it is significantly increased.

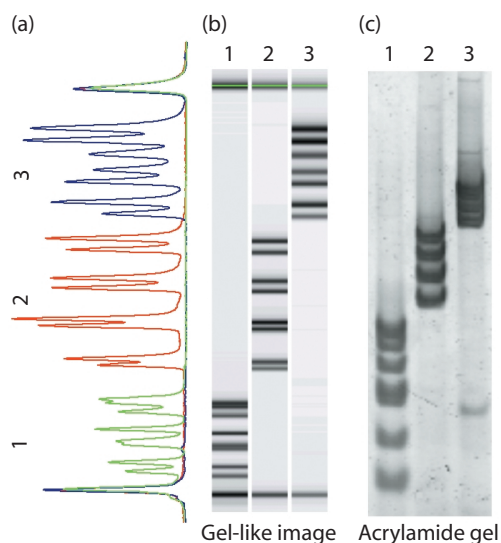
As a result of the higher sensitivity and sharpness of bands, the Bioanalyzer is able to detect DNA products that are not visible on the agarose gel (for example, by-products of PCR amplifications and very diluted DNA fragments; compare lanes 3 and 4). The quantitation is automatically performed by the software. To accurately determine the DNA fragment size and concentration, a sizing (low mass) ladder is run in the first well of the chip and internal standards are run in each sample. The use of internal standards, which bracket the sample, allows correction for small drifts and ensures accurate sizing.

### Analysis of small DNA fragments

Figure 2 shows the analysis of three mixtures of small PCR products, comparing the chip system with a conventional 4–20% polyacrylamide

## The chip technology will perform a variety of laboratory tasks, such as sizing and quantitation of DNA fragments ...

**Figure 2** Separation of mixtures of small PCR products. The electropherogram of each sample (a) is shown alongside the gel-like image of the DNA 500 LabChip kit (b). For comparison, the same samples were separated on a 4–20% gradient polyacrylamide gel stained with SybrGold (c). The fragment sizes of sample 1 are 25, 35, 50, 53, 70, 90 and 105 bp; sample 2: 150, 158, 200, 210, 250, 263, 300 and 315 bp; and sample 3: 350, 368, 400, 420, 450, 478 and 500 bp. The first and the last bands in the electropherogram and the gel-like image are internal standards.



reader contains programmable high voltage power supplies, each of which is connected to a platinum electrode. Small interconnected channels are etched into the glass chip, which can simultaneously analyse 10 protein samples. The chip, loaded with samples, is placed in the instrument, where it is connected to platinum electrodes by closing the lid. These electrodes enable the instrument to perform multiple injections and other fluid manipulations in specific sample wells on the chip. Injection and electrophoretic separation of the sample, detection of the fluorescent signal, as well as the data analysis, are fully automated by software control. Each sample is separated in less than 2 min (for example, 90 s for a DNA sample), so that a complete run with 10–12 samples, including the warm-up phase and the calibration of the instrument, is finished within 25–30 min. The software has data collection, presentation and interpretation functions. Data are displayed as a gel-like image and electropherogram. In addition, the data are also displayed in tabular format and can be

gel. By using the Bioanalyzer, PCR products are separated with a comparable or better resolution for the size range from 25–500 bp. The resolution is particularly improved for the larger PCR products. The sharpness of the bands and the ability to display the data as electropherograms enable easy identification of incompletely separated PCR products, which is not possible on the acrylamide gel.

**Quality control of total RNA**

The analysis of intact total RNA from cultured Jurkat cells is shown in

Figure 3. The 18S and 28S ribosomal RNA bands are automatically detected by the software and dominate the electropherogram. The amount of low molecular RNA (left-hand peak; Figure 3a), which consists of 5S, 5.8S or transfer RNA (tRNA), strongly depends on the preparation method that was used to isolate the RNA. Degradation of total RNA because of RNase contamination results in a shift of the RNA size distribution to smaller fragments and a decrease in signal intensity. The 18S and the 28S RNA bands can no longer be identified with certainty.

**Lab-on-a-Chip technology looks set to revolutionize the methodology used in biochemistry and molecular biology.**

With progressive degradation, only small fragments will be detected and the signal intensity further decreases.

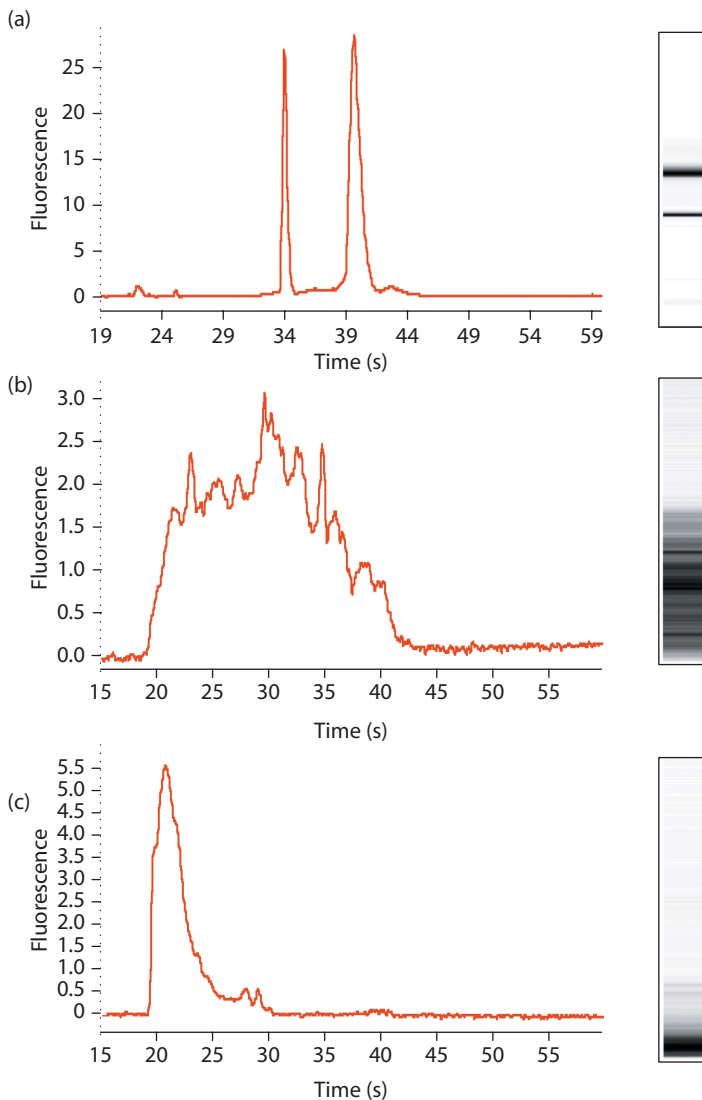
**Sizing and analysis of proteins**

A specific chip design also allows the analysis of up to 10 protein samples in the 14–200 kDa size range in less than 30 min when using the chip system.<sup>7</sup> Using SDS-PAGE, even with pre-cast gels, it would take approximately 3 h to obtain the same data. This is a significant time-saving compared with the conventional technique. Lab-on-a-Chip technology enables the integration and automation of many of the manual and time-consuming steps, such as staining and destaining of the proteins. A variety of different samples can be analysed, such as cell lysates, column fractions, antibodies or purified proteins. Furthermore, the sensitivity that can be achieved with a chip system, utilizing laser-induced fluorescence detection, is comparable with non-colloidal Coomassie gel staining. Figure 4 shows the analysis of a protein mixture with the Bioanalyzer and a 4–20% gradient polyacrylamide gel. The resolution that can be obtained with the chip system is comparable or better than the conventional technique. For example, carbonic anhydrase I and II, which only differ by less than 10% in molecular weight (31 and 29 kDa, respectively), are baseline separated using the chip system.

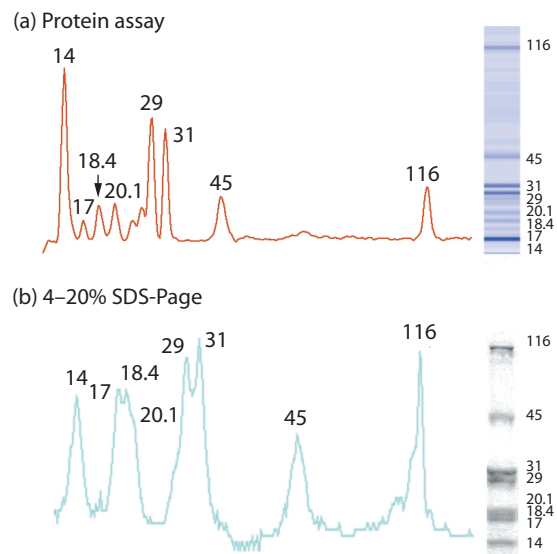
**Conclusion**

Lab-on-a-Chip technology looks set to revolutionize the methodology used in biochemistry and molecular biology. Currently, this technology enables rapid, reliable and reproducible sizing and quantitation of nucleic acid and protein samples. Furthermore, because these chips can be simply and safely handled, they could replace a large number of analytical gels, which must be

**Figure 3** Total RNA isolated from Jurkat cells (100 ng/mL) cultured at room temperature with a diluted RNase ( $2 \times 10^{-6}$  and  $1 \times 10^{-5}$  mg/mL). The results from the RNA 6000 LabChip kit are displayed as an electropherogram (left) and a gel-like image (right). (a) Intact total RNA; (b,c) progressive RNA degradation resulting from RNase contamination.



**Figure 4 Comparison of the analysis of a protein mixture on (a) the Protein 200 LabChip kit and (b) a 4–20% gradient polyacrylamide gel (SDS-Page). The gel-like image and polyacrylamide gel are both shown on the right; electropherograms are on the left.**



stained, destained, imaged and analysed in a number of manual, laborious and tedious steps. In addition, digitized data output by the new technology permit easy and convenient data archiving in a database and easy sharing with fellow researchers — across the bench or around the world.

In the future, microchips will cover an even wider array of analytical applications within many biochemistry and molecular biology laboratories, allowing the performance on a single chip of further complex experimental steps, such as sample purification, extraction and biochemical reactions. Furthermore, the chip technology can provide the tools to increase experimental throughput significantly.

#### References

1. C.S. Effenhauser *et al.*, "High-Speed Separation of Antisense Oligonucleotides on a Micromachined Capillary Electrophoresis Device," *Anal. Chem.* **66**, 2949–2953 (1994).
2. A.T. Woolley and R.A. Mathies, "Ultra-High-Speed DNA Fragment Separation Using Microfabricated Capillary Array Electrophoresis Chips," *Proc. Natl Acad. Sci. USA* **91**, 11348–11352 (1994).
3. A.T. Woolley and R.A. Mathies, "Ultra-High-Speed DNA Sequencing Using Capillary Electrophoresis Chips," *Anal. Chem.* **67**, 3676–3680 (1995).
4. M. Ogura *et al.*, "RNA Chip: Quality Assessment of RNA by Microchannel Linear Gel Electrophoresis in Injection-Molded Plastic Chips," *Clin. Chem.* **44**(11), 2249–2255 (1998).
5. M.A. Burns *et al.*, "Microfabricated Structures for Integrated DNA Analysis," *Proc. Natl Acad. Sci. USA* **93**, 5556–5561 (1996).
6. O. Müller *et al.*, "A Microfluidic System for High-Speed Reproducible DNA Sizing and Quantitation," *Electrophoresis* **21**(1), 128–134 (2000).
7. L. Bousse *et al.*, "Protein Sizing on a Microchip," *Anal. Chem.* **73**(6), 1207–1212 (2001). ■

Reprinted from:

**Pharmaceutical  
Technology**  
EUROPE

AN ADVANSTAR  PUBLICATION

©May 2001 issue.

PDF Reprint No: 0468

Agilent Technologies  
Publication Number: 5988-3035EN