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New approach to complete automation in sizing and quantitation of DNA and proteins by the Automated Lab-on-a-Chip Platform from Agilent Technologies

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The newly introduced Agilent 5100 Automated Lab-on-a-Chip Platform (ALP) offers a high-throughput system to overcome the limitations of slab gel analysis in protein and DNA sizing and quantitation. Agilent, well established in the classical analytical business providing gas and liquid chromatography as well as mass spectrometry instrumentation, has now moved further into the life sciences with its microarray and microfluidic 'Lab-on-a-Chip' solutions.

Lab-on-a-Chip technology

Lab-on-a-Chip is a microfluidic technology that uses circuits of tiny channels and wells etched into glass or molded into plastic substrates. Pressure or electrokinetic forces move small volumes of fluids through selected pathways on the chip in a controlled manner. These mini-laboratories often include or emulate elements such as pumps, valves, mixing and reaction chambers and separation channels. They can be used to measure the purity, size and quantity of DNA, RNA and protein samples. Others can also be used to analyze cells based on their physical or chemical characteristics ('flow cytometry').

Lab-on-a-Chip technology offers an alternative to one of the most widely used techniques in the life sciences: gel electrophoresis. In gel electrophoresis, nucleic acids or proteins are loaded into a gel matrix that separates molecules by size, charge and/or shape when an electric field is applied. By miniaturizing and automating the process, Lab-on-a-Chip technology confers several key advantages over this cumbersome and time-consuming technique. Firstly, it requires only a small amount of material, conserving both reagents and samples. The former saves costs and the latter is critical for many types of analyses in the life sciences, where samples are often radically limited. Secondly, the small scale of the chip devices significantly increases the speed with which samples can be processed and analyzed. Thirdly, because these platforms eliminate most of the user interaction associated with conventional techniques, results are more accurate and reproducible.

Finally, recent advances in Lab-on-a-Chip technology enable numerous samples to be run out of standard well plates in parallel and completely unattended, including during data analysis. Such a level of automated high-throughput operations—which have become increasingly important in the 'post-genome' era—is currently impossible using the classic gel electrophoresis approach.

Agilent and Lab-on-a-Chip

Agilent Technologies has been instrumental in bringing microfluidics into the life sciences laboratory. In 1999, the company intro-

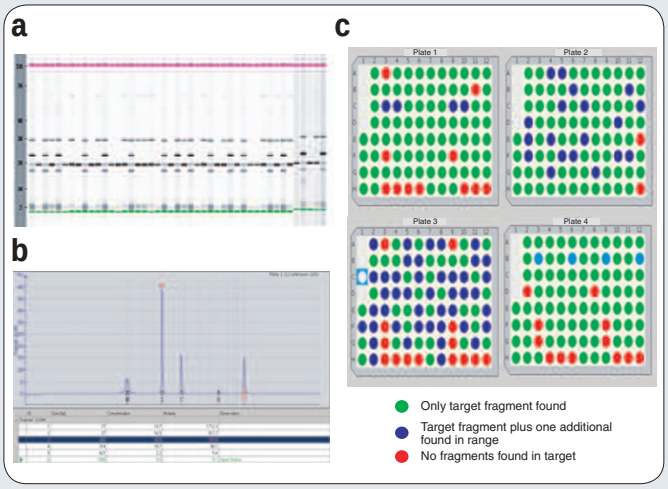

Figure 1 | Analysis of mPCR using the Automated Lab-on-Chip platform. Altogether, 384 unattended measurements were carried out within one job. (**a**) The gel-like images show the microfluidic separation of 45 mPCR samples. (**b**) The results of a DNA analysis can also be displayed as an electropherogram. The calculated sizes and concentrations of all fragments separated appear in the peak table of the software. (**c**) Result flagging allows quick screening of results based on user-defined rules. Each color represents the occurrence of a certain result: for example, the presence of a DNA fragment in the selected size range. Here, result flagging was applied to 4×96 samples.

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duced the first commercial Lab-on-a-Chip system, the Agilent 2100 bioanalyzer, which enables the analysis of DNA, RNA and protein via microfluidics-based electrophoresis. In addition, it is the only system of its kind that also supports flow cytometry.

With the 2100 bioanalyzer, microfluidic and Lab-on-a-Chip technology has become established in the scientific community, and today almost 1,000 literature references can be found citing the system. The bioanalyzer integrates sample handling, separation, detection and data analysis into a single platform. Depending on the application, up to 12 samples can be processed and analyzed in 30 min. For some protein analyses, the 2100 Bioanalyzer can reduce total turnaround time 20-fold in comparison with standard gel electrophoresis.

The next step has been to develop a more integrated instrument that combines these types of analytical measurements with complete automation and other enhancements to offer cost-effective high throughput. This project led to a completely new instrument, the Agilent 5100 Automated Lab-on-a-Chip Platform (ALP). In developing the Automated Lab-on-a-Chip Platform, the aim was to ensure that all resources, from samples to chips to reagents, are available for the system and can be moved, loaded, run and replaced without the need for any operator intervention.

Another requirement for high-throughput analysis is the availability of a powerful data analysis and data storage system that is able to store, archive and retrieve large sets of data. Additionally, data analysis has to offer filter and search capability to compare the actual highly reproducible data. The best tool for such a task is a database, which can offer these capabilities more easily than can single file–based data sets.

ALP enables fully automated sizing and quantitation

The heart of the new system is a reusable microfluidic glass chip that performs the tasks of loading the samples out of the well plates, injecting and separating up to four samples plus their internal standards in parallel, and enabling analysis with a scanning laser-induced fluorescence detector. Chips are equipped with capillaries for the sample transfer from plate to chip and are mounted automatically into a chip environment that supplies the needed connections to high voltage, pressure and vacuum, plus overall temperature controls.

A repository inside the benchtop-sized instrument offers storage space for up to ten standard microtiter plates (96- or 384-well format), the chip and the well plates for reagents. Because the reagents degrade during electrophoresis, they have to be exchanged if multiple well plates have to be processed in one sequence. This is achieved by an internal liquid-handling device that unloads used reagents, rinses the chip and adds aliquots of fresh reagents for further use in the chip channels. All movements of sample plates and resources are performed by an integrated robotic well-plate handler.

ALP for quality control of PCR fragments

In many laboratories, a large number of PCR or multiplex PCR (mPCR) samples need to be qualified via gel electrophoresis prior to use for sequencing, spotting to microarrays or target validation. The Automated Lab-on-a-Chip Platform system completely automates this process. **Figure 1** shows typical results as visualized by the system software. Results were obtained during an investigation of alternative splicing that can generate multiple transcripts encoding potentially different proteins. To optimize the throughput of RT-PCR, scientists from RZPD Heidelberg used an Agilent 5100 Automated Lab-on-a-Chip Platform to analyze 136 transcripts per clusters (292 alternative exons) on chromosomes 21 and 22, at Xq28 and in apoptosis genes[1].

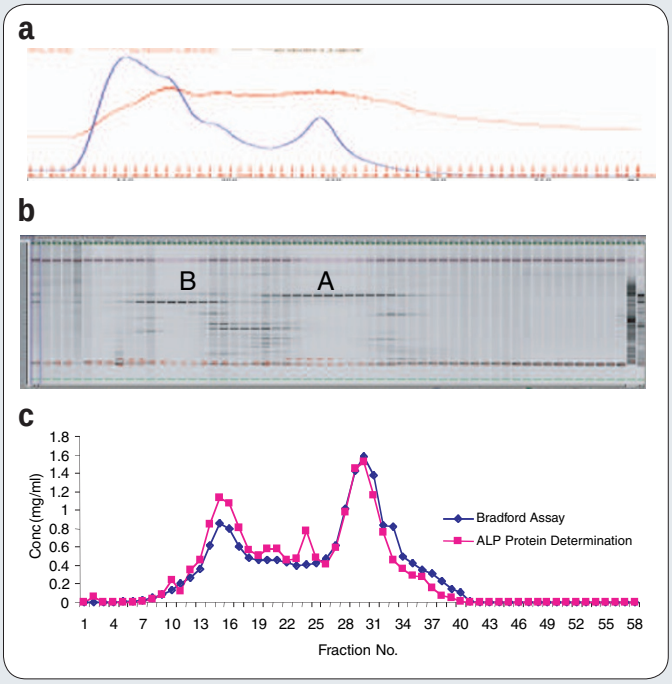

Figure 2 | (**a**) Elution profile after separation of a protein mixture by conventional fast-performance liquid chromatography (FPLC). Protein abundance was monitored by two-wavelength detection (280 nm, 254 nm). (**b**) Gel-like image of the FPLC fractions after electrophoresis using Agilent 5100 Automated Lab-on-a-Chip Platform. (**c**) Comparison of quantitative measurements of the FPLC fractions by conventional Bradford protein assay versus the total protein concentration determined by Agilent 5100 Automated Lab-on-a-Chip Platform.

The range of sizing for the assay used was from 25 to 1,000 bp of double-stranded DNA and quantitation was linear in a range from 0.5 to 50 ng/µl of DNA. The capacity of the system, with maximum loading of ten sample well plates allows, either 960 or 3,840 samples to be analyzed at a time, depending on what type of well plate is used. Owing to the higher resolution as compared to agarose gels, the separation of more complex mPCR reactions is improved and the quantitation of each individual fragment provides complete information to researchers who need to improve their throughput by multiplexing.

Results can be interpreted in several views. In a typical gel view (**Fig. 1a**), the data can be easily contrasted with the results of traditional gels, allowing easy pattern comparison. The actual measured signal can also be viewed (**Fig. 1b**): this is the electropherogram that results from the gel electrophoresis performed in the microchannels of the glass chip. Detection is undertaken using a laser-induced fluorescence (LIF) detector scanning four sample lanes in parallel with a red laser beam. When passing the detection window, the DNA fragments show fluorescence as a result of intercalating dyes dissolved in the gel matrix. This creates a peak that is integrated by the software and whose area is then used for direct quantitation of the fragment. Because the areas of all peaks or fragments are determined and calibrated based on internal standards in each sample,

the resulting data consist not just of the total amount of DNA, but the concentration for each specific fragment, plus its purity or relative abundance as compared to other peaks. The resulting detailed quantitative measurements are far superior to standard total UV measurements, which reflect anything that absorbs light at the detection wavelength. In addition, the qualitative and the quantitative measurements are completed in one step using the microfluidic system.

Also available is a software screenshot of the well plates (**Fig. 1c**), in which individual wells are represented with their results filtered and color-coded according to simple rules defined by the researchers. This provides an overview of a whole set of measurements based on queries such as "sample contains a specific fragment," "sample contains more than one fragment," "fragment concentration is over/under a defined threshold," and so on.

Protein purification fractions controlled by ALP instead of SDS-PAGE

The versatility of microfluidic devices offers solutions to a whole range of applications where separation and quantitation of complex mixtures is needed. Protein analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) is currently a major bottleneck for many researchers working with recombinant proteins looking for protein targets on a larger scale.

The new 5100 Automated Lab-on-a-Chip Platform system allows analysis of up to ten well plates in a single batch with a protein assay for proteins between 14 kDa and 200 kDa. The protein chip used has two capillaries and two separation channels allowing for parallel sample measurements. Sensitivity tests show comparable sensitivity to data from Coomassie Blue–stained gels for bovine serum albumin (BSA), with detection limits between 20 and 40 μg/ml, depending on buffer composition.

Figure 2 illustrates a typical workflow, where **Figure 2a** illustrates the eluent profile of a protein purification by affinity chromatography. The fractions are collected and analyzed qualitatively and quantitatively by using the 5100 Automated Lab-on-a-Chip Platform. **Figure 2b** shows part of the fractions depicted as a gel-like image: the major components, protein B and protein A, along with some impurities, can be easily seen as they are eluted from the column. In **Figure 2c**, the protein concentrations determined by the Automated Lab-on-a-Chip Platform are compared to those from a classical Bradford protein assay performed in parallel. The comparison shows a perfect match between the UV absorbance measurement from the Bradford assay performed in parallel to the total protein concentration determined by the new approach with its integrated technology.

Results obtained with the Automated Lab-on-a-Chip Platform are comparable in sensitivity, sizing accuracy and reproducibility to those for SDS-PAGE gels stained with Coomassie Blue. However, the resolution and linear dynamic range, as well as the quantitation accuracy and reproducibility, for the 5100 Automated Lab-on-a-Chip Platform are superior to those of SDS-PAGE. In addition, the qualitative and quantitative results are obtained in a single step during an unattended run.

Other major advantages of the microfluidic system are its parallel approach for separation and the high reproducibility that is achieved by using internal standards in each sample, which allows for normalization of the results and ensures independence from day-to-day measurement or operator influences.

The results stored in the database allow the same querying and filtering that was described for the PCR samples: so, for example, proteins specified by their size can be tracked for abundance or purity or any other parameter throughout the whole protein data set.

Conclusion

Using the new Automated Lab-on-a-Chip Platform, sizing, quantitation and purity of DNA and Protein samples can be undertaken accurately and simultaneously in a high-throughput mode. Compared to agarose gel electrophoresis or SDS-PAGE, this technology offers, for the first time, fast and unattended runs with high throughput. Resolution and reproducibility are better than those found with classical slab gels; furthermore, the sensitivity for DNA fragments is clearly superior to that of ethidium bromide staining, whereas the sensitivity for proteins is comparable to that of Coomassie stains. The normalized data stored in the database are easy to search and filter for specific information and show sufficient reproducibility to enable the comparison of data between different laboratories.

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