High resolution DNA analysis with the DNA 500 and DNA 1000 LabChip® kits

Application

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Introduction

Slab gel analysis is the traditional technique used for DNA analysis. By comparing the separated bands of an unknown sample to a known standard (size or mass ladder), the size and quantitation of each fragment can be estimated. The Agilent 2100 bioanalyzer is an instrument, which utilizes LabChip® technology to achieve DNA and RNA analysis on micro-fabricated chips. This approach entails significant advantages including increased speed of analysis, improved data precision, and ease of use.

In contrast to slab gel electrophoresis, the Agilent 2100 bioanalyzer in conjunction with LabChip® kits performs chip-based separations under standardized conditions, resulting in more reproducible results. Using prepackaged reagents and an assay specific software script that defines all instrumental parameters, a user can always obtain equivalent results for a given sample. This approach simplifies sample preparation, minimizes user errors, and facilitates the exchange of data between different users and laboratories.

In the context of LabChip Technology, several DNA sizing kits are available to cover a wide range of DNA fragment sizes. In addition to the DNA 500 and DNA 1000 kits, two other DNA LabChip kits are available: the DNA 7500 and DNA 12000 LabChip kits. They allow separation of DNA fragments up to 12000 base pairs. The DNA 500 LabChip kit for analysis of DNA fragments, ranging in size from 25 to 500 base pairs, and the DNA 1000 LabChip kit for analysis of DNA fragments, ranging in size from 25 to 1000 base pairs, supplement these two kits. The size range of the DNA 500 and DNA 1000 kits is especially useful for small to medium sized PCR and RT-PCR products and can resolve primer dimers. Other relevant applications include competitive PCR and cleavage based applications such as RFLP. Also, the resolution in the lower range, i.e. below 100 bp, is significantly increased compared to the other two DNA LabChip kits, making it possible to distinguish between fragments that are very similar in size. Since the DNA 500 and the DNA 1000 LabChip kit show similar performance and are used for similar applications, they are presented jointly in this application note.

DNA 500 LabChip kit

The performance of the DNA 500 assay is demonstrated by the analysis of several mixtures of PCR products (figure 1). All chip-based separations were performed on the Agilent 2100 bioanalyzer according to the instructions provided with each kit. The conditions for the acrylamide gel separations are listed in the appropriate figure caption. Figure 1 shows the overlay of 3 different electropherograms acquired on the Agilent 2100 bioanalyzer that are mixtures of PCR samples ranging from 25 to 500 base pairs in size. The two closest eluting bands (50 bp and 53 bp) are partially separated and identified by the software as two separate peaks. The DNA 500 assay achieves a resolution of 5 base pairs from 25 to 100 base pairs and a 5 % resolution from 100 to 500 base pairs where the sizing error is less than 10 % over the entire size range. The digital data format allows accurate quantitation of the separated bands based on peak areas.
A DNA mass ladder was used to verify quantitation accuracy. The ladder contains 3 fragments in the specified size range, with concentrations guaranteed by the supplier (Low DNA Mass™ ladder, Life Technologies, USA). Table 1 lists the quantitative results obtained on the Agilent 2100 bioanalyzer. All separated bands were well within the specified margin of 70 % accuracy, with a standard deviation of 10 % or less.
The DNA 500 assay relies, like the other DNA assays, on an intercalating dye for fluorescence detection. The dye incorporation depends on the basepair composi-

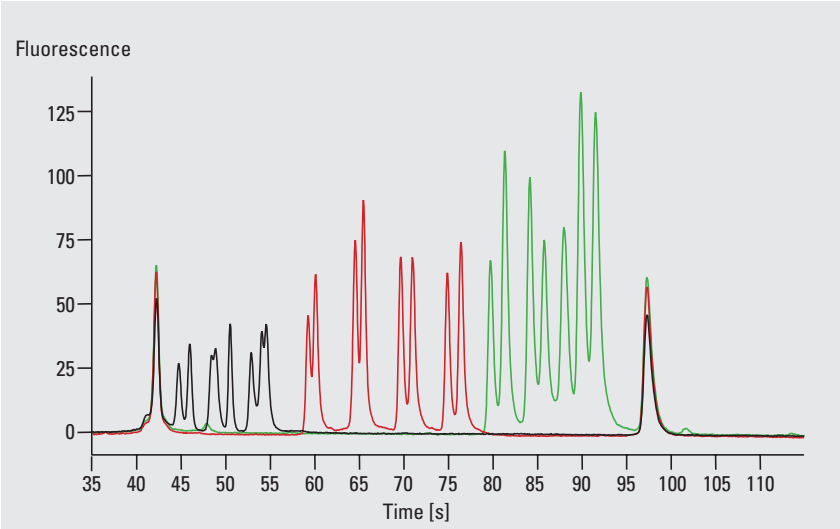

Figure 1
Overlay of three different samples which span the size range of the DNA 500 assay. The fragment sizes are: 25, 35, 50, 53, 70, 90, 100, 105, 150, 158, 200, 210, 250, 263, 300, 315, 350, 368, 400, 420, 450, 478, 500 (Note: the first and last peak in the electropherogram are marker peaks of 15 and 600 base pairs). The samples were separated according to the manufacturer's protocol using the DNA 500 LabChip kit in conjunction with the DNA 500 assay.

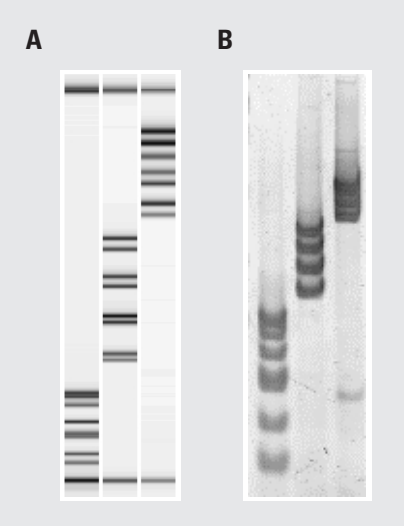

Figure 2
Comparison of chip based separations with results obtained on slab gels. Sample from Figure 1 separated with:
A) Agilent 2100 bioanalyzer using the DNA 500 LabChip kit together with the DNA 500 assay. Data is represented as gel-like image.
B) Novex precast polyacrylamide 4 - 20 % gradient gel run using the XCell™ Mini-Cell gel box (Novex, USA). 5 ml of all samples were analyzed, stained for 12 minutes with SybrGold nucleic acid stain (Molecular Probes) and then de-stained with TBE to remove any background fluorescence. The gel was imaged using the FluorImager™ 595 with a 488-nm filter.

	100 bp	200 bp	400 bp
Average [ng/µl]	0.73	1.53	3.02
Target [ng/µl]	0.80	1.60	3.20
% error	-8.28	-4.56	-5.62
STDV	0.08	0.11	0.20
RSD	10.73	7.46	6.73

Table 1
Quantitation accuracy of the Agilent 2100 bioanalyzer. A relative standard deviation of less than 15 % was obtained for all separated bands in the selected samples.

tion and the secondary structure of the DNA fragments. For larger fragments such sequence differences are usually statistically evened out. For shorter fragments, however, the sequence can make a difference in dye incorporation. Therefore, deviations from the sizing and quantitative specifications can occur for individual fragments below 70 bp. The same samples shown in the chip-based electropherograms in figure 1 were compared to a slab gel separation using a commercially available 4-20 % gradient gel (Novex, USA). As shown in figure 2, the Agilent 2100 bioanalyzer performs with equal or better resolution than the slab gel, delivering a clearer image of the sample.

DNA 1000 LabChip kit

In the size range below 500 bp, the DNA 1000 LabChip kit shows a performance that is comparable to the DNA 500 LabChip kit. The operating range was extended to 1000 bp, to better match the size range, in which most PCR fragments fall. From 500 to 1000 bp the resolution of the DNA 1000 assay is 10 %, while the maximum error for quantitation remains at 30 % (this corresponds to a 95 % confidence level).

The DNA 1000 kit was used to optimize PCR conditions for a competitive RT-PCR reaction of two genes: GAPDH and the heat shock protein hsp72[1] . The goal of the experiment was to achieve a co-amplification of GAPDH (housekeeping gene) and hsp72. The hsp72 was the gene of interest, the expression of which can be influenced by various factors. For the experiment, RNA was isolated from HepG2-cells and reverse transcribed into cDNA. The cDNA was amplified using a specific set of primers to result in a 439 bp fragment for the amplification of GAPDH and a 650 bp fragment for the amplification of hsp72. A primer dropping method was employed because the two genes are expressed at different levels within the cell. In the present case this means that hsp72

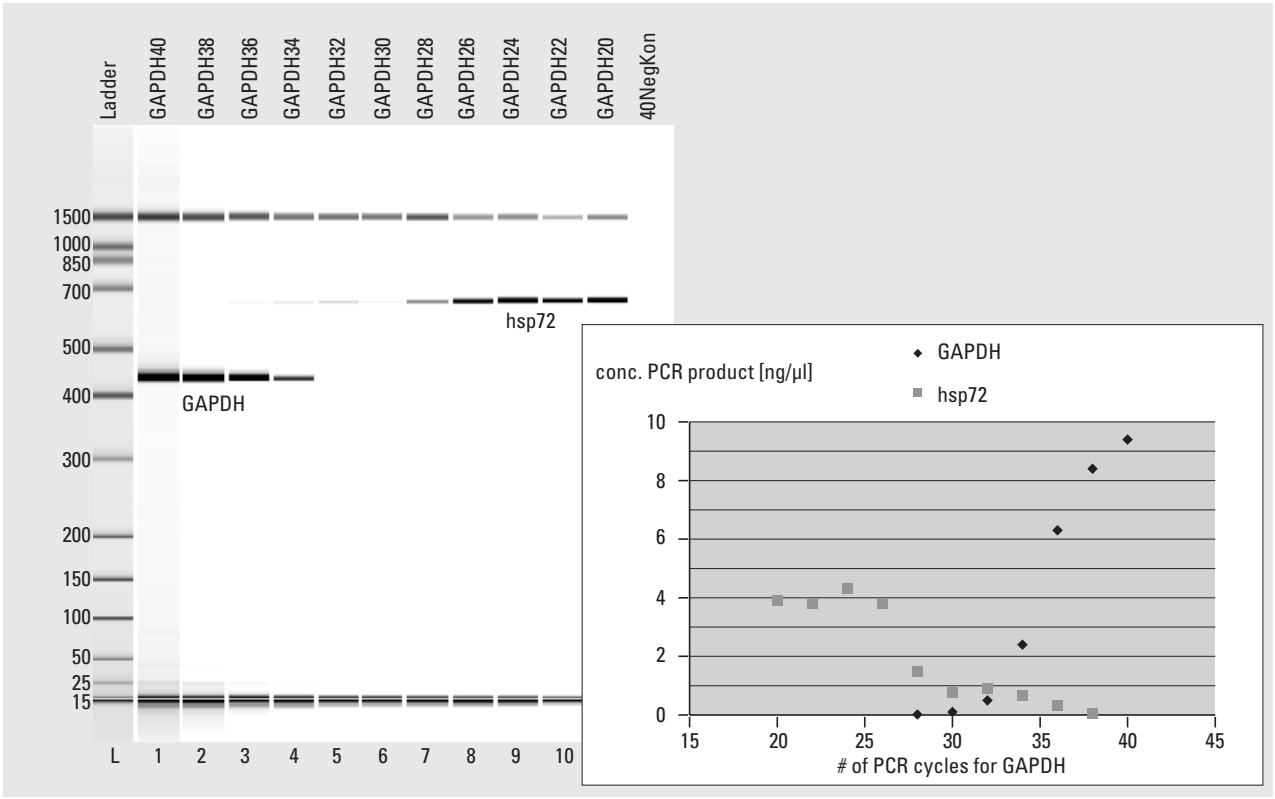

Figure 3
Determination of optimum PCR conditions for competitive PCR. For details see text

[1] Data kindly provided by Dr. Eric Gottwald, Forschungszentrum Karlsruhe, Germany

was amplified for 40 cycles and the primers for the amplification of GAPDH were added at the beginning, after 2 cycles, 4 cycles and so on, resulting in 20 to 40 amplification cycles. Since the PCR reaction was carried out under competitive conditions, the PCR product of hsp72, though at a constant amplification of 40 cycles, disappears with increasing cycle number of GAPDH (figure 3). Figure 3 shows the separation of the two PCR products as a gel-like image and also shows a plot of the concentrations that were calculated by the bioanalyzer for the different amplification conditions. From the results it was concluded that a primer dropping method with 40 cycles for hsp72 and 36 cycles GAPDH would be preferable. Under these conditions a large GAPDH and a small hsp72 peak are visible. This allows monitoring up-regulation of the hsp72 gene in future experiments. For other experiments, a different number of cycles might be preferable.

The advantage of using the DNA 1000 assay for the analysis of competitive PCR products lies in the accurate absolute and relative quantitation of each amplified product. Small differences in the amplified amount, that cannot be detected using slab gel analysis, are easily analyzed with the DNA 1000 assay. This allows not only the optimization of PCR conditions but also detection of changes in gene expression via RT-PCR methods.

Conclusion

The DNA 500 and DNA 1000 assay perform DNA separations reliably in the range of 25 to 500 base pairs and 25 to 1000 bp, respectively. Both assays are optimized to give accurate sizing and quantitation of PCR products, which is needed for numerous PCR-based methods, such as multiplex PCR, RT-PCR, competitive PCR, and so on. They supplement the two other DNA assay kits to allow analysis of a wide range of samples. LabChip technology allows the molecular biologist to not only perform nucleic acid analyses in a more accurate way and with greater ease of use but also requires less time and sample. With additional automated data analysis and storage capabilities, a complete package is offered enabling precise and well-documented experiments. Considering the advantages of Lab-on-a-Chip technology, the chip-based approach outperforms slab gels and represents an indispensable tool for molecular biologists.

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