Cost-effective automation of sample preparation for Sanger and next-generation sequencing

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Overview

DNA sequencing using either next-generation (NGS) or standard Sanger sequencing methods is one of the predominant applications in molecular biology and diagnostics. While the sequencing reactions themselves are fairly simple and straightforward, the numerous steps in sample preparation, especially at higher throughput, are tedious and error-prone when performed manually.

The 4titude 4LAB™ (Figure 1) is a cost-effective, compact pipetting robot. It is designed to perform PCR fragment clean-up, primer and dye terminator removal, as well as concentration adjustment of your samples. This system, with dedicated reagents available in kit format from LGC Genomics GmbH, offers one of the best cost / throughput ratios for these applications available.

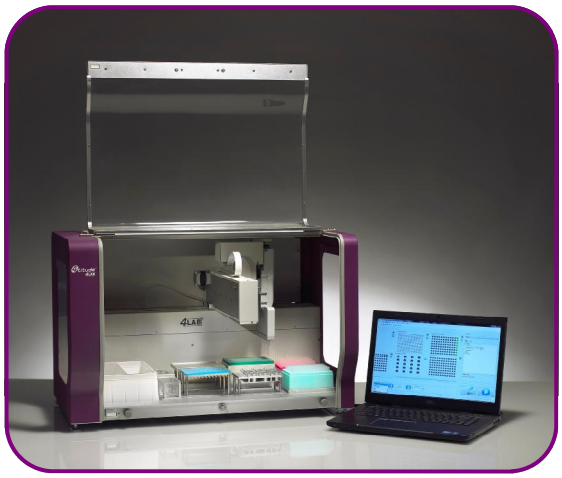

Figure 1 : 4LAB compact liquid handling robot

4LAB automated liquid handling unit

The 4LAB is an automated, high precision, pipetting system specifically designed for low-volume liquid handling. Available with 6 or 8 workstation positions and different modules like UV lamp, HEPA filter and active heating and cooling, the 4LAB's simple and intuitive software allows easy programming and features a dedicated sample normalization application.

Concentration Adjustment

Using the import function of the 4LAB, concentration measurement data from a Nanodrop™ [1] instrument is imported as csv files. A dedicated normalisation function then carries out the concentration adjustment of either up to 20 samples, in 1.5 ml tubes, or up to 96 samples in SBS plate format.

Figure 2 shows an example of the result file that is automatically generated by the 4LAB software, giving details of the dilution and illustrating the adjustments made. In this example, the resulting dilutions were again measured on Nanodrop; the concentrations are shown in Table 1. The final, normalised concentration was set to 50 ng / µl; Table 1 shows a typical variation of final concentration by a factor of maximum 1.5 with two exceptions (sample F3, A4). The reduction of a concentration range of 20 to a factor of 1-2 is acceptable for the majority of protocols where a concentration adjustment is needed.

	1	2	3	4
A	A-1 (V), 80.1µl (50ng/µl) Contains: ddH20 (V)43.7µl Sample 1 (V)36.4µl [110ng/µl] DNA	A-2 (V), 79.9µl (50ng/µl) Contains: ddH20 (V)46.6µl Sample 6 (V)33.3µl [120ng/µl] DNA	A-3 (V), 80.1µl (50ng/µl) Contains: ddH20 (V)49.3µl Sample 11 (V)30.8µl [130ng/µl] DNA	A-4 (V), 80.1µl (50ng/µl) Contains: ddH20 (V)51.5µl Sample 16 (V)28.6µl [140ng/µl] DNA
B	B-1 (V), 79.8µl (50ng/µl) Contains: ddH20 (V)60.8µl Sample 2 (V)19µl [210ng/µl] DNA	B-2 (V), 80.1µl (50ng/µl) Contains: ddH20 (V)61.9µl Sample 7 (V)18.2µl [220ng/µl] DNA	B-3 (V), 80µl (50ng/µl) Contains: ddH20 (V)62.2µl Sample 12 (V)17.4µl [230ng/µl] DNA	B-4 (V), 80.2µl (50ng/µl) Contains: ddH20 (V)63.5µl Sample 17 (V)16.7µl [240ng/µl] DNA
C	C-1 (V), 80µl (50ng/µl) Contains: ddH20 (V)67.1µl Sample 3 (V)12.9µl [310ng/µl] DNA	C-2 (V), 80µl (50ng/µl) Contains: ddH20 (V)67.5µl Sample 8 (V)12.5µl [320ng/µl] DNA	C-3 (V), 79.9µl (50ng/µl) Contains: ddH20 (V)67.8µl Sample 13 (V)12.1µl [330ng/µl] DNA	C-4 (V), 80.2µl (50ng/µl) Contains: ddH20 (V)68.4µl Sample 18 (V)11.8µl [340ng/µl] DNA
D	D-1 (V), 80.4µl (50ng/µl) Contains: ddH20 (V)70.6µl Sample 4 (V)9.8µl [410ng/µl] DNA	D-2 (V), 79.8µl (50ng/µl) Contains: ddH20 (V)70.3µl Sample 9 (V)9.5µl [420ng/µl] DNA	D-3 (V), 80µl (50ng/µl) Contains: ddH20 (V)70.7µl Sample 14 (V)9.3µl [430ng/µl] DNA	D-4 (V), 80.1µl (50ng/µl) Contains: ddH20 (V)71µl Sample 19 (V)9.1µl [440ng/µl] DNA

Figure 2 : ***Example of the Normalisation log file generated by the 4LAB software.*** *Information generated by the software includes:*

[1] Nanodrop™ is a trademark of Thermo Fisher Scientific Inc

Well	Conc. (ng/µl)	Well	Conc. (ng/µl)	Well	Conc. (ng/µl)
A1	44,7	A3	47,2	A5	50,0
B1	34,3	B3	45,0	B5	50,7
C1	44,1	C3	41,2	C5	46,2
D1	42,9	D3	47,4	D5	67,0
E1	37,6	E3	43,6	E5	48,8
F1	48,4	F3	219,5	F5	10,3
G1	30,6	G3	52,1	G5	50,2
H1	46,8	H3	50,2	H5	56,7
A2	43,7	A4	100,8	A6	49,9
B2	43,1	B4	49,9	B6	56,8
C2	42,7	C4	45,4	C6	56,6
D2	41,6	D4	48,5	D6	61,3
E2	43,5	E4	52,2	E6	53,6
F2	51,6	F4	52,7	F6	51,0
G2	45,4	G4	51,4	G6	62,5
H2	49,1	H4	50,6	H6	54,9

Table 1 : Final concentrations measured using Nanodrop (target concentration is 50 ng / µl).

PCR Clean Up With Primer and Dye-Terminator Removal

Using a commercially available NeodymIronBor magnet separation plate (Figure 3), a magnetic bead purification procedure has been developed to process up to 48 samples at one time. Currently the protocol is semi-automated, as the separation plate must be manually moved to and from the magnet station; design of a full, walk-away automation is underway.

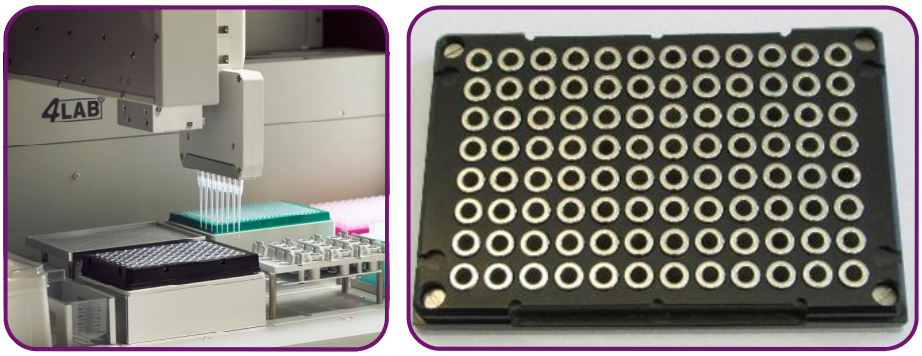

Figure 3 : The NeodymIronBor magnetic separation plate used by 4LAB for PCR product clean-up and dye terminator removal.

Figure 4 shows the results produced from samples prepared using 4LAB's clean-up function. pUC plasmid samples were prepared for use in Sanger sequencing reaction mixes for capillary electrophoresis using a protocol with just three quick steps - binding, washing and elution.
The purified sequencing reactions were run on an Applied Biosystems® [2] 3730xl sequencer; results are shown in Figure 4, illustrating that read lengths and signal intensities are indistinguishable from LGC Genomics' standard clean-up process.

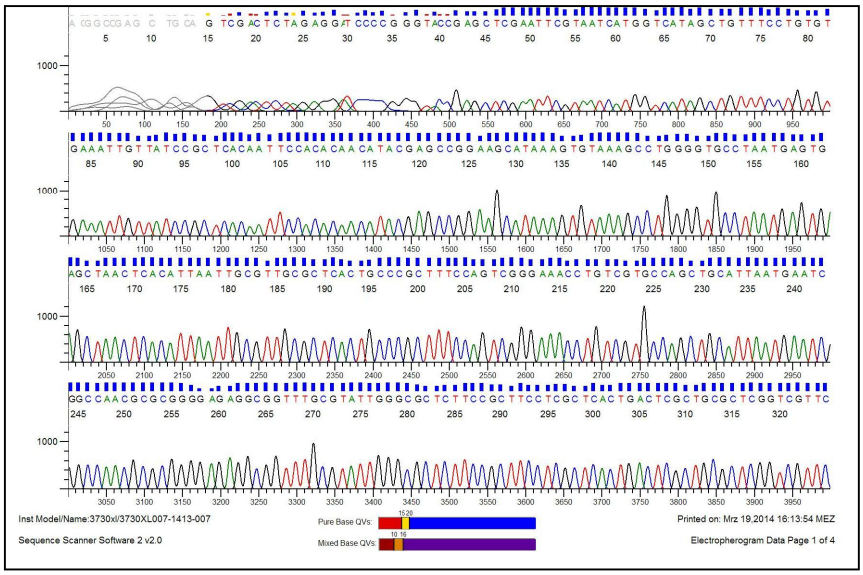

Figure 4 : Standard Sanger sequencing reaction with pUC plasmid DNA purified with LGC Genomics' Dye Terminator removal chemistry on 4LAB.

Summary

Using the 4LAB as a small and cost-effective liquid handling device, we have shown that with a combination of specific application programs and LGC Genomics' proprietary kit reagents, an accurate and reliable system for the automation of the fundamental but labour-intensive steps in both NGS and Sanger sequencing protocols has been established.
The use of reagents specifically developed for their respective purposes, for example sizing of DNA fragments for NGS purposes, will greatly expand the scope of this combined instrument / application system in the future.
In terms of the cost / throughput ratio, the 4LAB instrument is now at the leading edge of systems available in the market. Currently this applies especially to the semi-automated protocol and will soon also be true for a full walk-away solution which is in development. It is intended to use selected features of this system in LGC Genomics' service labs.
With dedicated applications, the 4LAB is an attractive alternative when a high-throughput automated system is not yet required and therefore not as cost effective. Where a significant number of samples need to be processed, the 4LAB instrument offers the quality improvement of automated sample processing compared to traditional labour-intensive and error-prone methods.

[2] Applied Biosystems® is a registered brand of Life Technologies