Increasing Sample Throughput on a Standard 36-Lane Model 377 DNA Sequencer to 48 or 64 Samples Per Run

During the last decade, automated DNA sequencing systems have started to gradually replace conventional methods of DNA sequencing and fragment analysis. Automated systems offer greater speed and ease of use, as they do not require a separate fragment detection step following electrophoresis. In addition, automated DNA sequencing and fragment analysis systems also offer substantial quality improvements over conventional manual systems; precision of fragment sizing, relative signal strength determination of different fragments, detection of mobility shift of fragments and data management are all improved. These benefits of automated DNA electrophoresis systems have led to ever-increasing demands on their usage. For the Model 377 DNA Sequencers (PE Biosystems, Foster City, CA, USA), optional upgrades of the basic 36-lane model to 48–64-lane or to 72–96-lane capacity can ease these pressures and increase throughput. However, upgrading a 36-lane Model 377 sequencer to a 48–64-tooth comb costs NZ $34800 (ca. US $18800), a sum many smaller institutions or laboratories cannot easily afford.

Because the 48–64-tooth upgrade involves only insubstantial changes to the detection electronics and control and analysis software, we reasoned that it might be possible, with minor alterations in work practice, to achieve 48–64-sample capacity using standard 36-lane hardware and software.

This article describes our method, enabling the user of a 36-lane Model 377 sequencer to run 48 or 64 samples on a single gel without upgrading to a 48–64-lane model. Our laboratory uses both DNA sequencing and GENESCAN™ (PE Biosystems) fragment analysis, and we have found the method highly reliable and satisfactory for both applications.

For sequencing reactions, samples were polymerase chain reaction (PCR)-amplified from DNA isolated from fresh human blood, using primers specific to the HLA-A gene. PCR products were purified by Exonuclease I and Shrimp Alkaline Phosphatase (both from Amersham Pharmacia Biotech, Uppsala, Sweden) digestion at 37°C for 30 min (2). Purified PCR products were cycle-sequenced using BigDye™ (PE Biosystems) chemistry according to the manufacturer’s protocol (1). Sequencing reactions were ethanol-precipitated in PCR microplates, resuspended in formamide/loading dye and denatured at 95°C before gel loading (1).

For GENESCAN fragment analysis, chromosome 10q23 microsatellite markers (PTEN/MMAC1 locus) were PCR-amplified from DNA derived from microdissected archival renal cell carcinomas using dye-labeled primers. PCR products were diluted between 1:2 and 1:16, combined with size-standard and deionized formamide/loading dye, denatured at 95°C for 5 min and quenched on ice before gel loading.

Depending on whether a 48- or a 64-tooth comb was used, either 48 or 64 sequencing or GENESCAN samples were loaded in 3 or 4 batches, using an 8-channel multipipettor (SGE International, Ringwood, VIC, Australia). Samples were named 1–36 in the sample sheet. After run completion, lanes on the gel were tracked manually from 1–36 from the left side of the gel and extracted. Actual sample names were then entered in the sample manager.
window corresponding to the loaded lane. For 48-lane gels, the remaining lanes were tracked by moving the tracking line No. 25 to the lane 37, tracking line No. 26 to lane 38 and so on. Once tracked, the lanes were extracted, making sure that the overwrite original sample files option in the Extract lanes dialog box was deselected. This resulted in the creation of new sample files named 25.1–36.1. Actual sample names were then typed into the sample manager. Sample file names 25.1–36.1 were renamed to 37–48 for easier identification. The procedure for 64-lane gels was analogous. Automation of the process was possible using AppleScript® (Apple Computer, Cupertino, CA, USA) and Microsoft® Excel® software (Microsoft, Redmond, WA, USA). Specific 48- or 64-lane scripts are available from the authors.

Alternatively, the run folder was duplicated before analysis, using the gel file in one run folder for the analysis of the first 36 lanes and the gel file in the duplicated folder for the remaining lanes.

For read lengths of approximately 500 bp, most commonly used in our laboratory, both sequencing and fragment analysis results obtained for either 48 or 64 lanes were comparable to those achieved using the standard 36-lane comb. Figure 1 shows comparative sequence electropherograms for the same HLA-A sequence of the same sample, obtained with 36-lane (bottom panel), 48-lane (middle panel) and 64-lane (top panel) shark’s-tooth combs. Sequence quality was virtually identical between the samples. Similar results (data not shown) were achieved for microsatellite fragment analysis.

In addition to improving per-run throughput without any perceptible loss of data quality, the use of 48- or 64-lane shark’s-tooth combs allowed the use of microplate-compatible PCR plates and multichannel gel-loading pipettors or syringes, which greatly simplified and sped gel loading. By contrast, the tooth spacing of the standard 36-lane shark’s-tooth comb used for the Model 377 sequencer is not microplate-format compatible, necessitating individual and laborious lane-by-lane loading.

There are some minor problems with our procedure. Unlike the official NZ $34800 (ca. US $18 800) 48–64-lane upgrade, our procedure uses existing hardware and software and therefore, does not increase the sampling frequency of the detector system. However, by analyzing the electropherograms in detail, it was apparent that most samples loaded onto 48-lane combs were nonetheless collected in 3 channels, with the occasional sample having only 2 channels. For 64-lane combs, most lanes also had 3 channels, but a significant proportion was reduced to 2 channels, and between 1 and 4 lanes per gel were reduced to a single channel. If the sequence quality on that particular channel is not good, then there is no option to re-track to improve the sequence quality. Further, our method does not allow automatic tracking for 48–64 lanes, which is a software upgrade feature of the official PE Biosystems 48–64-lane upgrade. Manually tracking 64 lanes can be challenging, for example, if the sequencing reaction of the first sample failed. To avoid this problem, we always focus on the primer peaks (blobs) at the beginning of the gel, where it is possible to follow a pattern of loading, with the first sample on the gel having the lowest position of the primer peak on the gel. For GENESCAN applications, tracking poses no significant problem, as all lanes contain size-standard.

In conclusion, with only minor inconveniences and some modified usage of the standard 36-lane collection and analysis software, the loading, running and high-quality analysis of 48 or 64 sequencing or GENESCAN samples is quite possible on an unmodified, standard 36-lane Model 377 DNA sequencer. The necessary 48- or 64-lane, 0.2-mm shark’s-tooth combs are sold by PE Biosystems for use with 48–64-lane Model 377 sequencers. Both types of combs are microplate compatible, enabling multichannel syringe sample loading, increasing the loading speed at least threefold. We think our protocol will prove to be particularly useful for smaller, cash-strapped laboratories.

REFERENCES


Address correspondence to Dr. Stefan Grebe. Department of Pathology, Wellington School of Medicine, P.O. Box 7343, Wellington South, Wellington, New Zealand. Internet: grebs@wnmeds.ac.nz

Received 13 April 1999; accepted 15 July 1999.

Zlatibor M. Velickovic, Marija B. Velickovic and Stefan K.G. Grebe
Wellington School of Medicine Wellington, New Zealand

Vol. 27, No. 4 (1999)