APPLICATION INFORMATION

CEQ™ 2000

IMPROVED SEQUENCING OF PLASMIDS ON THE CEQ™ 2000 BY A SIMPLE TEMPLATE PREHEATING PROCEDURE

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Introduction

Recent advances in automated sequencing technology have centered around capillary-based systems. Such systems are highly automated and simple to run. One potential drawback, however, concerns the ability of such systems to sequence large or otherwise difficult plasmid DNA samples. Here we report a simple, cost-effective and convenient solution to overcome problems associated with sequencing plasmid DNA on the CEQ™ 2000 Automated DNA Analysis System from Beckman Coulter.

Methods

Two different plasmid DNA templates were used in this study: a GC-rich cDNA cloned into pUC18 (3.5 Kb), and large DNA plasmid (>12 Kb).

Plasmid DNA templates were prepared using commercially available kits from either QIAGEN (QIAprep*) or Promega (Wizard**). Plasmids were prepared according to manufacturers' instructions.

Templates were quantitated by running restriction digested aliquots on agarose gels and comparing ethidium bromide staining intensities to known standards.

Samples were diluted in deionized H_2O, the tubes were closed and then heated for 1 minute at 96°C in an MJ Research PTC. After 1 minute at 96°C, the temperature was ramped down immediately to 25°C, and the samples were used in sequencing reactions.

Sequencing reactions were set up as described in the Beckman Coulter DTCS sequencing protocol. Cycling conditions were as described: 96°C for 20 seconds, 50°C for 20 seconds, 60°C for 4 minutes.

Post-reaction clean up was by ethanol precipitation as described in the Beckman Coulter DTCS Sequencing Protocol (608019-AC).

The sequencing reaction products were run on the CEQ 2000. The capillary temperature was set to 40°C and the separation voltage to 8.2 kV; otherwise all parameters were as for the default DTCS - 2 method. Data analysis was carried out using CEQ software version 1.1.

Results

The CEQ 2000 DNA sequencing system allows the user to monitor the raw data and the current in each individual capillary. These two features allow the user to quickly diagnose problems associated with sequencing templates on the CEQ 2000 system.

Low signals can lead to inaccuracies in base calling as well as failure to automatically call bases by the CEQ 2000. In the example shown in Figure 1a, low signal strength led to no sequence being called. A simple incubation of the plasmid sample resuspended in water just before adding the cycle sequencing reaction mix (described in the Methods section) results in a substantial increase in the raw data signal. Figure 1b shows the signal

* QIAprep is a registered trademark of QIAGEN, Inc.
**Wizard is a registered trademark of Promega Corporation.

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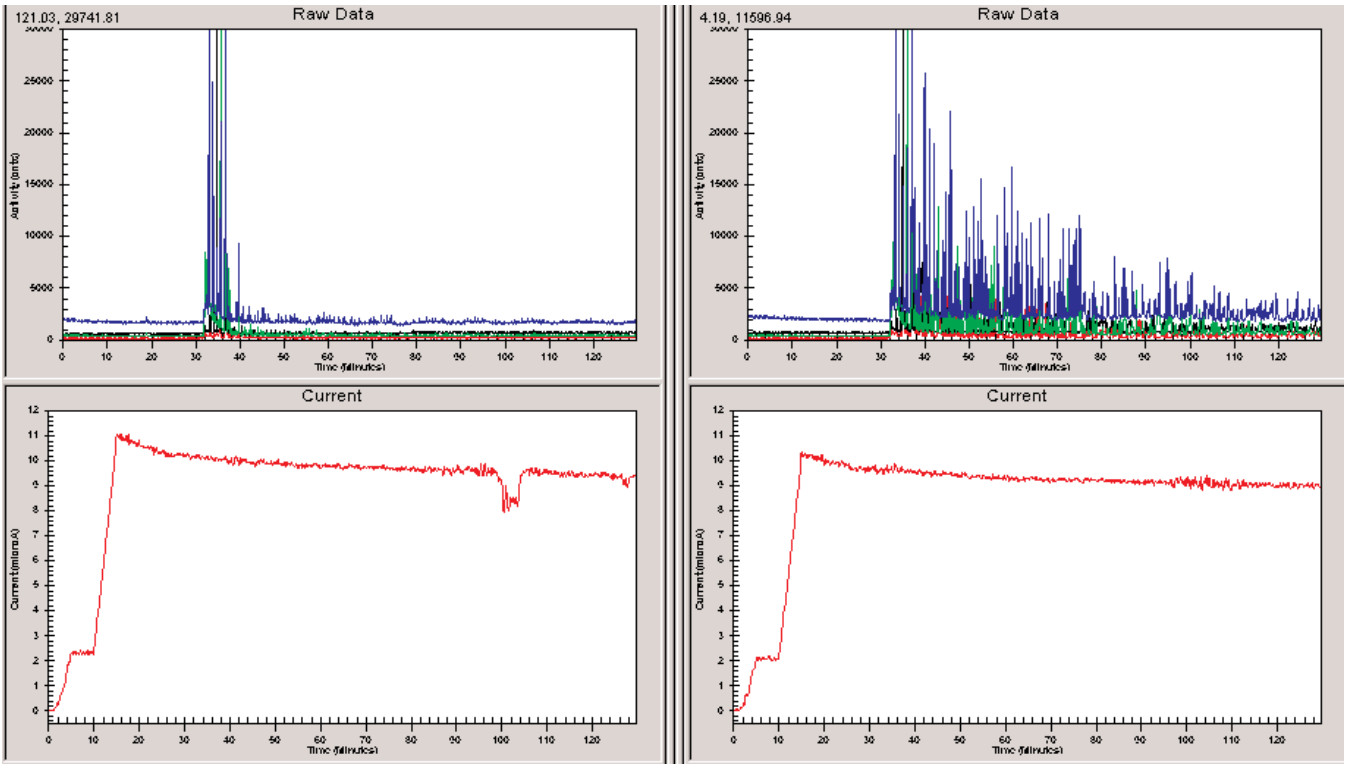

Figure 1a (left). *Raw data and current profiles for GC-rich DNA prepared using QIAGEN QIAprep column. 100 fmol (230 ng) of DNA was used in the sequencing reaction. Raw data is scaled to 30,000 counts and the current profile is scaled to 12 µA.*

Figure 1b (right). *Raw data and current profiles for GC-rich DNA prepared using QIAGEN QIAprep column and pre-treated for 1 minute at 96°C. 100 fmol (230 ng) of DNA was used in the sequencing reaction. Raw data is scaled to 30,000 counts and the current profile is scaled to 12 µA.*

improvement when the same plasmid as used in the experiment from Figure 1a is treated at 96°C for 1 minute prior to sequencing. This treatment improved the sequence call from no bases to 704 bases with the first 637 bases being called at 98% accuracy.

The data shown in Figure 2 compares the same GC-rich plasmid prepared by a different method (Promega Wizard). In this case, the preheating treatment improves the signal strength and the current stability. Unstable currents can lead to anomalous peak spacing and hence errors in the sequence call. In the example shown here, the pretreated sample called 536 bases and the sequence was 99.3% accurate. The untreated sample was only 65% accurate at 550 bases.

Analysis of the data from a large plasmid template (shown in Figure 3) showed that the treated sample called a total of 579 bases, whereas before treatment only 461 bases were called. Although both sequences were accurate to at least 98% over the length called, the more stable current led to a longer sequence being called.

Conclusions

We have demonstrated a simple, cost-effective, and convenient method for improving automated sequencing of plasmid DNA templates on a capillary-based system. The preheating procedure is carried out with the samples diluted in deionized H_2O prior to adding the remaining sequencing

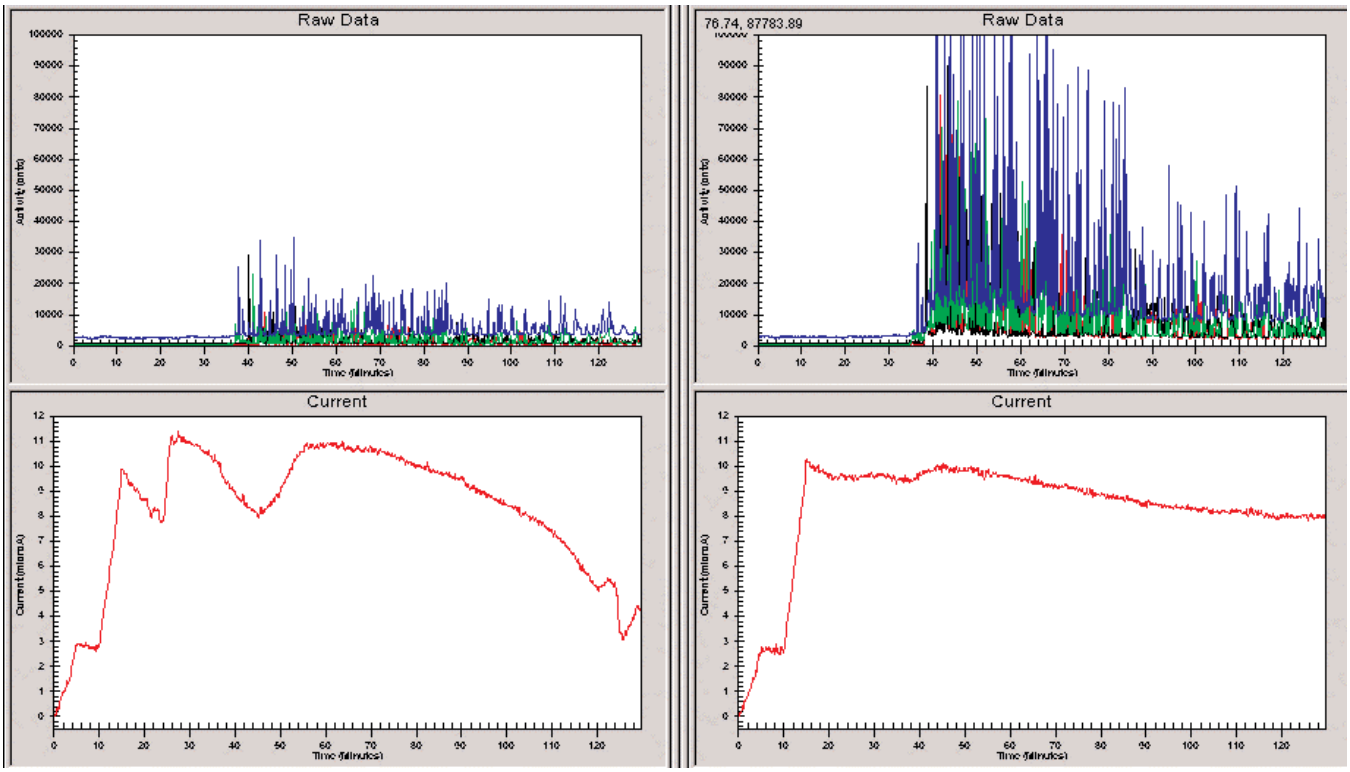

Figure 2a (left). *Raw data and current profiles for GC-rich DNA prepared using Promega Wizard column. 100 fmol (230 ng) of DNA was used in the sequencing reaction. Raw data is scaled to 30,000 counts and the current profile is scaled to 12 µA.*

Figure 2b (right). *Raw data and current profiles for GC-rich DNA prepared using Promega Wizard column and pre-treated for 1 minute at 96°C. 100 fmol (230 ng) of DNA was used in the sequencing reaction. Raw data is scaled to 30,000 counts and the current profile is scaled to 12 µA.*

reaction components. In a few cases, a 1-minute treatment at 96°C can lead to decreased signal strength for longer sequencing fragments. In these cases, preheating the template at 86°C for 5 minutes produced results similar to the ones shown here (data not shown). Still other templates required preheating at 96°C for 3 minutes to stabilize current profiles. It appears that 96°C for 1 minute is a good compromise treatment. In certain cases, the treatment may have to be altered as described above in order to maximize the observed signal and current benefit. Future work will investigate the mechanism of action in this simple procedure.

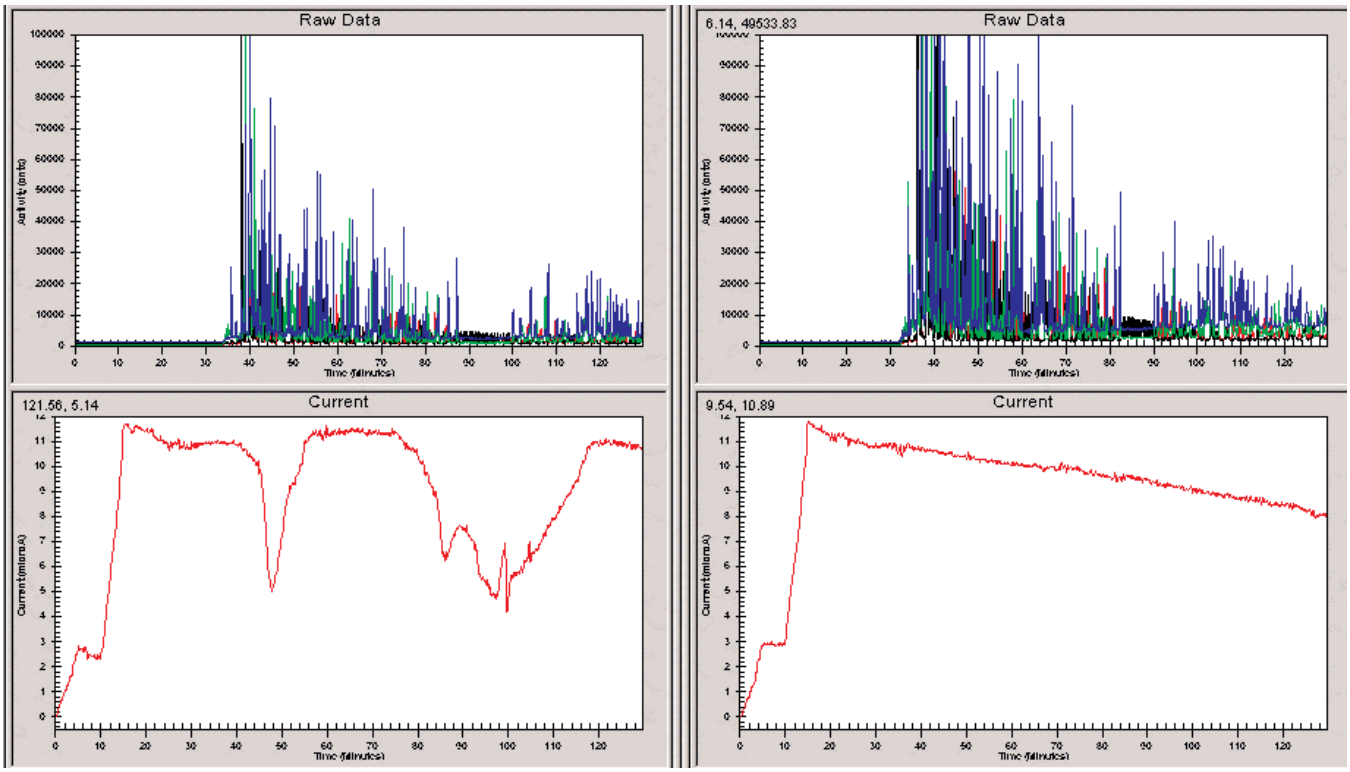

Figure 3a (left). *Raw data and current profiles for a large (12 Kb) plasmid prepared using QIAGEN QIAprep column. 100 fmol (800 ng) of DNA was used in the sequencing reaction. Raw data is scaled to 100,000 counts and the current profile is scaled to 12 µA.*

Figure 3b (right). *Raw data and current profiles for a large (12 Kb) plasmid prepared using QIAGEN QIAprep column and pretreated for 1 minute at 96°C. 100 fmol (800 ng) of DNA was used in the sequencing reaction. Raw data is scaled to 100,000 counts and the current profile is scaled to 12 µA.*

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