

Genetic Analysis

INCREASED THROUGHPUT AND SAMPLE TRACKING TECHNOLOGY WITH THE CEQ™ 8800 GENETIC ANALYSIS SYSTEM

Han-Chang Chi
Beckman Coulter, Inc.

Introduction

As a valuable tool for the genome era, capillary electrophoresis- (CE) based automatic analyzers have assisted institutions and laboratories in carrying out DNA sequencing and genetic analysis. Since its inception, Beckman Coulter's multi-capillary CEQ™ system has been a successful and powerful CE-based automatic analyzer⁽¹⁾ in supporting life science research laboratories. Moving forward into the post-genome era, the demand for laboratory automation to create unattended, continuous workflow has been increasing. When different methods of sample identification are used in sample preparation and data evaluation by multiple researchers and laboratories, sample and data tracking become essential in order to ensure the integrity of information flow through the different processes.

Building on more than a decade of experience in innovative CE and laboratory automation technologies, Beckman Coulter has developed the CEQ 8800 system with a new level of capacity in genetic analysis.⁽²⁾ The system utilizes dual plates and sample tracking technology to provide an advanced, industry-leading, genetic analysis solution. This results in a fully automated, high-resolution system that adapts well to daily workflow changes in sample type and complexity. The CEQ 8800, as part of Beckman Coulter's new GenomeLab family of products, also offers a bar coding link with the Biomek® series of liquid handlers for increased automation. This enables sample tracking between the Biomek for automated CEQ sample setup and the CEQ 8800 for sample analysis.

The CEQ 8800 system has many advanced features, including:

- 192 sample capability: two 96-well sample microplates
- Unattended, continuous operation of 2 × 96 samples results in extended throughput
- Integrated bar code reader enables accurate sample tracking and reporting
- Bar code interface to link CEQ 8800 with automated liquid handlers such as the Biomek series
- Single setup facilitates both DNA sequencing and fragment analysis (such as STR, SNP, AFLP®, LOH and quantitation capabilities)

Materials and Methods

Sample Preparation

The pUC18 Control Template, the M13-47 Sequencing Primer, and the sequencing chemistry used in this study were from Beckman Coulter (DTCS Quick Start Kit, PN 608120). Sequencing reactions were performed using the Quick-Start chemistry in accordance with the standard thermal cycling and preparation protocol.⁽³⁾ CEQ Sequencing Test Samples (PN 608070) were used in accordance with the provided protocol⁽³⁾ as an additional validation of DNA sequence analysis.



CEQ Fragment Analysis Test Samples (PN 608105) and CEQ DNA Size Standard-600 (PN 608095) were used in accordance with the provided protocol⁽⁴⁾ except that 2 μ L of Size Standard-600 were used for the preparation of the test samples for eight wells.

The bar codes used to label the sample plate (PN 609801) were the Code 128 format or the Code 39 format.⁽⁵⁾

Sample Separation

All samples were separated on the CEQ™ 8800 using the standard CEQ consumable materials: 33 cm \times 75 μ m capillary array (PN 608087), CEQ Separation Buffer (PN 608012), and the 20-mL LPA gel (PN 391438). The sequencing samples (pUC18 and CEQ Sequencing Test Samples) were separated with the default Seq-Test or LFR-1 method, respectively. The CEQ Fragment Analysis Test Sample/Size Standard-600 samples (the FA samples) were analyzed with the Frag-Test method.

Sample Analysis

The DNA sequences were analyzed using CEQ 8800 version 7.0 software with the following analysis settings altered from the Default Sequencing Analysis Parameters:⁽²⁾

Alignment Preference:

Perform Alignment (pUC18dG) = “activated”
Cutoff Accuracy = 98%

The FA samples were analyzed using CEQ 8800 version 7.0 software with the following analysis settings altered from the Default Fragment Analysis Parameters:⁽²⁾

Analysis Method:

Size standard = Size Standard-600
Model = Quartic (Migration Variable
= Migration time)

Advanced:

Dye Mobility Calibration = AE ver.1

Results

Bar Code Sample Plate Setup and Tracking

A sample plate file containing sample plate information with a bar code generated by the Biomek® FX system and saved as a preformatted, CEQ-compatible .TXT file can be imported easily into the CEQ 8800 under the Sample Setup module.⁽⁶⁾

The imported and opened sample plate file can be saved into the CEQ database as the CQ* format. Alternatively, a customized plate name and plate information, such as the sample name, run method,

note, and analysis parameters, etc., can be input manually under Sample Setup. The actual bar code can be keyed in to the Barcode field manually (Figure 1).

The bar code from each plate is assigned to each sample entered into the sample table. From this point forward, the original plate bar code remains associated with the raw sample data (each well of the sample plate), including all future analysis export, import, replication, and printed report activities derived from the sample. Full traceability to the sample plate of origin, regardless of the number of analysis steps that have been performed or the database in which the data ultimately resides, is summarized in Table 1.

Sample Plate Loading

The user has the option of having the CEQ instrument automatically scan the bar code on the plate by pressing the Load Plates... button in the Sample Plate Run Confirmation window. The sample plate with the matching bar code, if present, is then selected automatically from the database and configured to run (Figure 2). When using this auto load-configuration feature, the CEQ system searches the database for the sample plate file having the bar code that matches the physically scanned bar code. This mechanism guarantees the correct configurations and minimizes errors.

Users can choose to physically load and configure two plates simultaneously and perform the walk-away operation on two plates, or to load and run one plate first and the second plate subsequently using the pause-to-load feature (Figure 3).

Throughput

Unattended operation of two continuous 96-well plates of samples on a CEQ 8800 for DNA sequencing using either the default Seq-Test or LFR-1 run method took an average of 39.1 hours. In this paper we report the convenience of performing three sequencing runs, or six 96-well plates of samples, with minimum intervention on a CEQ 8800. Thus the throughput of one CEQ 8800 unit for DNA sequencing in an average period of approximately five days is 576 samples or 499,392 bases (calculated based on the average read length from this study; see Tables 2 and 3).

Unattended operation of two continuous 96-well plates on a CEQ 8800 using the Frag-Test method took an average of 26.2 hours. In three working days, three FA runs, or six 96-well plates of samples, were run and analyzed (Table 2).

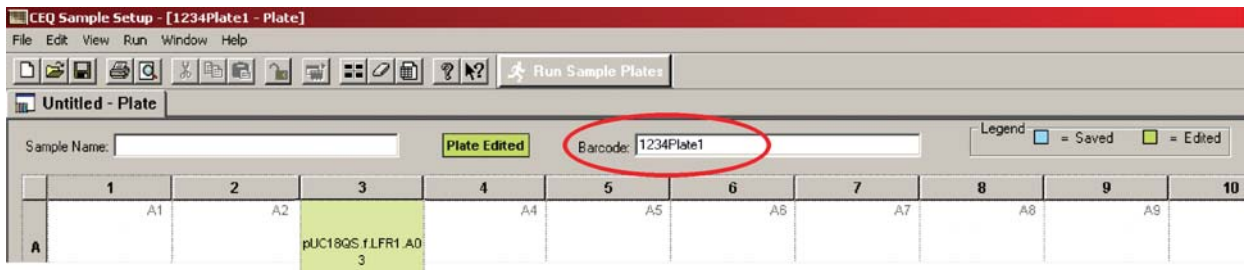


Figure 1. New Barcode field in the CEQ™ Sample Setup for sample plate bar code entry.

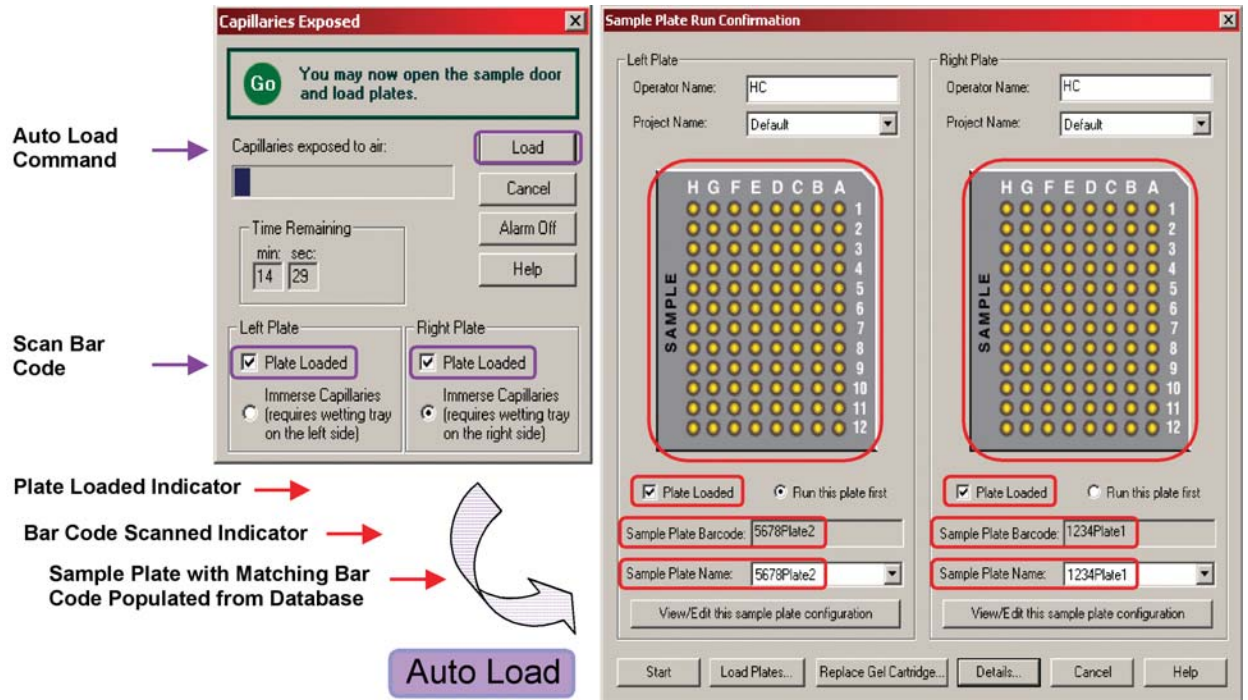


Figure 2. Auto load configuration of sample plates.

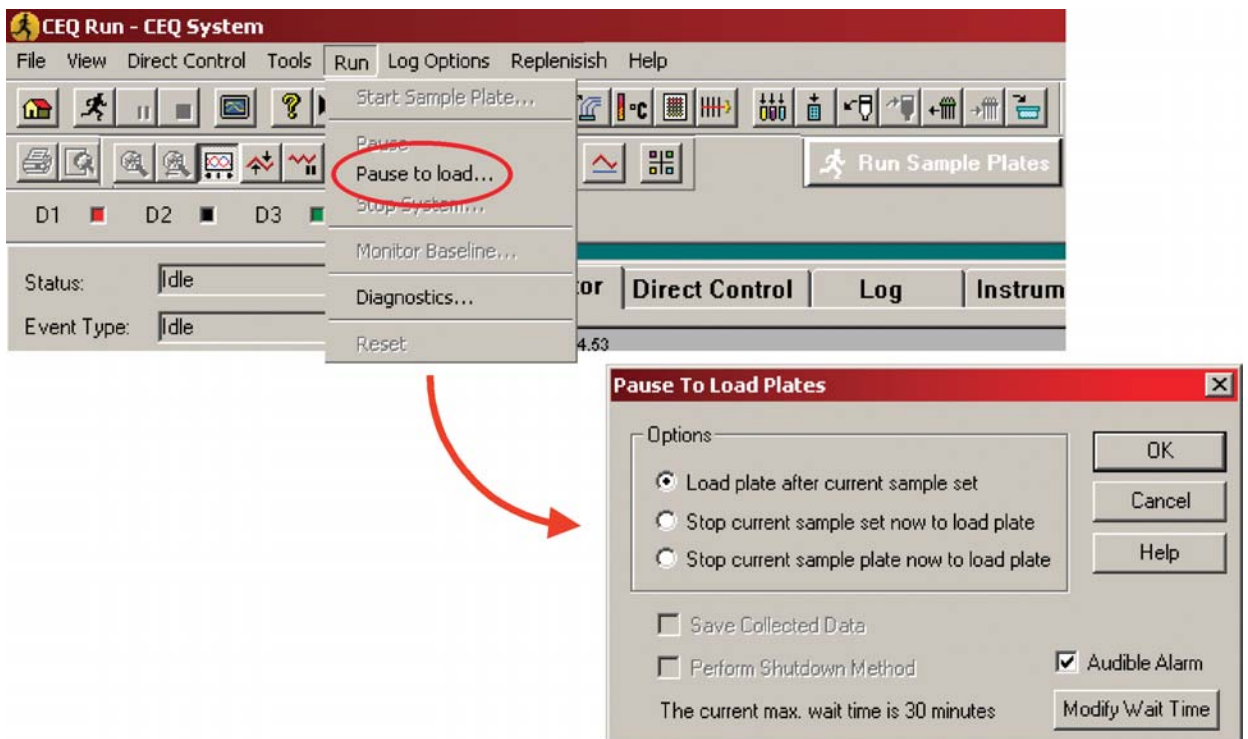


Figure 3. Pause to load.

Table 1. Bar Code Traceability in the CEQ™ Software System

	<i>Export</i>	<i>Import</i>	<i>Report Printing</i>	<i>View Property</i>	<i>Copy/Paste</i>
<i>Sample Plate</i>	CQ*	CQ*	✓	✓	✓
	TXT	TXT			
<i>Sample Plate Result</i>	CQ*	CQ*	N/A	✓	✓
	TXT				
<i>Sample Data</i>	CQ*	CQ*	✓	✓	✓
	TXT				
	SCF				
<i>Sample Result</i>	CQ*	CQ*	✓	✓	✓
	TXT				
	SCF	SCF			
<i>Fragment Result</i>	CQ*	CQ*	✓	✓	✓
	TXT				

Table 2. CEQ 8800 Throughput

<i>Sequencing</i>							<i>Fragment Analysis</i>					
<i>LFR-1/Seq-Test</i>			<i>Short PCR</i>				<i>Frag-4/ Frag-Test</i>		<i>Frag-1</i>		<i>SNP-1</i>	
<i>Num Plates</i>	<i>Num Samples</i>	<i>Num nt. (kb)</i>	<i>Num Plates</i>	<i>Num Samples</i>	<i>Num nt. (kb)</i>	<i>Num Plates</i>	<i>Num Samples</i>	<i>Num Plates</i>	<i>Num Samples</i>	<i>Num Plates</i>	<i>Num Samples</i>	
<i>Per 24 Hours</i>	1	96	83.2	3	288	57.6	2	192	3	288	4	384
<i>Per 5 Days</i>	6	576	499.4	15	1,440	288	10	960	15	1,440	20	1,920

Table 3. Sequencing Performance

Total (Num Samples = 768, Num Nucleotides = 665,856)

	<i>Success* Rate (%)</i>	<i>Average</i>	<i>Stdev</i>	<i>%CV</i>
<i>Read Length</i>	97.7	867	61	7.04%
<i>98% Cutoff</i>	95.7	773	44	5.69%

* Success: > 700 for Read Length and 98% Cutoff.

Table 4. Fragment Analysis Performance

<i>Marker</i>	<i>Total</i>				
	<i>Frag. Counts</i>	<i>Estimated Fragment Size</i>			
		<i>Average</i>	<i>% Sizing Error</i>	<i>Stdev</i>	<i>%CV</i>
<i>D4-109</i>	767	108.63	-0.34%	0.04	0.04%
<i>D4-110</i>	767	109.96	-0.03%	0.04	0.04%
<i>D3-389</i>	764	389.05	0.01%	0.06	0.01%
<i>D3-390</i>	764	390.06	0.02%	0.06	0.01%
<i>D2-588</i>	765	589.27	0.22%	0.08	0.01%
<i>D2-590</i>	765	590.78	0.13%	0.08	0.01%

Data Quality

In this study, eight 96-well plates (total 768) of sequencing samples and eight 96-well plates (total 768) FA samples were analyzed. Of the sequencing samples analyzed on the CEQ™ 8800, 97.7% had a read length longer than 700 nucleotides, and the average read length of all the samples was 867 nucleotides (Table 3). All data obtained from the sequencing run was analyzed and aligned/compared to the pUC18dG reference (Materials and Methods) to validate the sequencing accuracy. The sequence data was analyzed against the Med-High Quality-based trimming stringency (Materials and Methods) to verify the quality of the sequences.

Of the 768 FA samples that were run on the CEQ 8800 using the Frag-Test method, 99.5% analyzed all three pairs of the marker fragments and gave appropriate size estimates (Table 4). The estimated size of each analyzed fragment was compared to the corresponding reference size to verify the accuracy, quality, and reproducibility of the system. All successfully analyzed FA samples have high-quality size estimation for the six fragments of the three pairs of the Fragment Analysis Test Sample⁽⁴⁾ (Table 4). The system performed at a high reproducibility from sample to sample, plate

to plate, and run to run, where the overall sizing deviation for the six marker fragments was between 0.01-0.06% (Tables 4 and 6).

To validate the robustness and stability of the system, the data sets of the samples run on Rows 1 and 2 (Row 1 and Row 2 of Plate 1) and the samples run on Rows 23 and 24 (Row 11 and Row 12 of Plate 2) were compared. Note that the average sequencing run-completion time for Row 1 is at 112 minutes; for Row 2, it is at 209 minutes; for Row 23, it is at 2,249 minutes; for Row 24, it is at 2,346 minutes. The results show that the data quality of the 64 sequencing samples of Rows 1 and 2 is equivalent to that of Rows 23 and 24 (Figure 4A). For all of the indicators we looked at for sequencing (the average read length, 98%-Cutoff), the scores for the two sets of results are comparable (Table 5). Correspondingly, the average accuracy and sizing deviation of the 64 FA samples for Rows 1 and 2 are equivalent to those for Rows 23 and 24 (Figure 4B and Table 6). The fragment analysis and sequencing results of Rows 1 and 2 and Rows 23 and 24, with elapsed times on the CEQ of 23.9 and 35.6 hours, respectively, show that the samples on the two plates and the 20-mL gel are robust and stable through the time it takes to completely run the two plates.

Table 5. Sequencing Performance Comparisons of Rows 1 & 2 and Rows 23 & 24, and Plate 1 and Plate 2

	<i>Ave Row 1+2 (N=64)</i>	<i>Ave Row 23+24 (N=64)</i>	<i>Dev*</i>	<i>%Dev</i>	<i>Ave Plate 1 (N=384)</i>	<i>Ave Plate 2 (N=384)</i>	<i>Dev†</i>	<i>%Dev</i>
Read Length	871	867	-4	-0.46%	870	864	-6	-0.69%
98% Cutoff	773	777	4	0.52%	773	773	0	0.00%

Note: * Dev = Ave Rows 23+24 - Ave Rows 1+2.

† Dev = Ave Plate 1 - Ave Plate 2.

Table 6. Comparison of the Fragment Analysis Performance of Rows 1 & 2 and Rows 23 & 24

<i>Marker (N=64)</i>	<i>Rows 1 & 2</i>				<i>Rows 23 & 24</i>			
	<i>Estimated Fragment Size</i>				<i>Estimated Fragment Size</i>			
	<i>Average</i>	<i>% Sizing Error</i>	<i>Stdev</i>	<i>%CV</i>	<i>Average</i>	<i>% Sizing Error</i>	<i>Stdev</i>	<i>%CV</i>
D4-109	108.61	-0.36%	0.06	0.05%	108.64	-0.33%	0.03	0.03%
D4-110	109.93	-0.06%	0.06	0.05%	109.98	-0.02%	0.03	0.02%
D3-389	389.02	0.01%	0.11	0.03%	389.05	0.01%	0.03	0.01%
D3-390	390.03	0.01%	0.11	0.03%	390.06	0.02%	0.03	0.01%
D2-588	589.34	0.23%	0.26	0.04%	589.23	0.21%	0.13	0.02%
D2-590	590.72	0.12%	0.35	0.06%	590.79	0.13%	0.15	0.03%

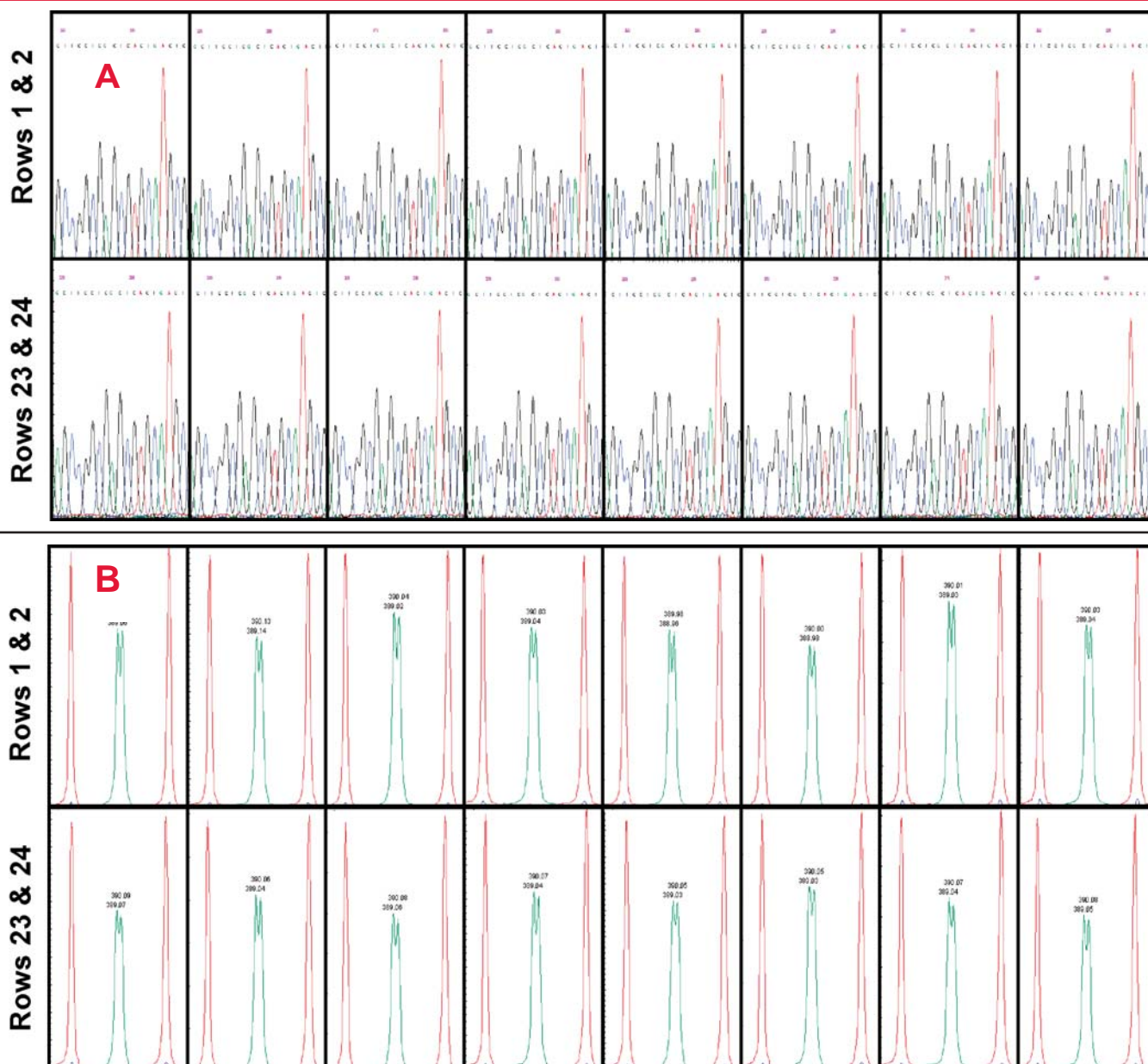


Figure 4. Data Comparison of Rows 1 & 2 and Rows 23 & 24. **A:** Analyzed sequencing sample electropherograms. Eight randomly selected traces in the vicinity of 325 nt are shown. **B:** Analyzed FA sample electropherograms. Eight randomly selected traces displaying the 389/390-nt test fragments bracketed by the 380- and 400-nt size standards are shown.

Discussion

The CEQ™ 8800 system provides a 20-mL gel cartridge to separate 24 rows of samples. During the normal operation of the system, fresh gel is pumped through the manifold and through the capillaries prior to each run of eight capillaries. The electrophoretic separations are programmed into the run method when the Run Sample Plates is configured, so that, once the run starts, the separation and replenishment cycles proceed automatically without any human intervention until the completion of the two-plate run. The quality of the data, even for the

long sequencing and Fragment Analysis (Tables 5 and 6, and Figure 4), demonstrates the robustness of the CEQ gel and the chemistries.

The two-plate capability of the CEQ 8800 system extends the overall throughput (Table 2) from the single-plate systems: CEQ 2000XL and CEQ 8000. This feature is especially useful for the short-cycle runs such as short fast sequencing for PCR products and Fragment Analysis. With the CEQ 8800, the user can now perform unattended, walk-away runs continuously for two short-run 96-well plates of samples overnight or two long-run

96-well plates over the weekend (Table 2). In addition, with the CEQ™ 8800, two runs, or four 96-well plates of samples (384 samples), can be performed for the extremely short runs such as SNP analysis in a normal working day (Table 2). Moreover, the dual-plated CEQ 8800 system provides additional flexibility because one sample plate can be set to run first, and, during the run of the first plate, the system can be paused for the loading of the second plate. Following this, the separation can be resumed until the completion of both plates (Figure 3). This pause-to-load feature grants the user the possibility of managing the preparation of the samples on the second plate while the first plate is running on the CEQ, and then later to load the second plate while the run of the first plate is proceeding.

Further, the CEQ 8800 system retains all of the outstanding features of the CEQ family in addition to the same benchtop design. With one gel, one array, and one software, users can perform sequencing, STR, AFLP®, and SNP analyses on one system without having to switch gels or arrays. With the eight-capillary array, the CEQ 8800 system provides flexibility for users in that they can choose to carry out small- (8 samples) to large- (192 samples) scale analyses at a time. The system also provides the versatility of performing different applications on the same sample plate which can be set to run and analyze automatically with the CEQ 8800 software.

The labeled bar code of Code 39 or Code 128 format is compatible with both the Biomek® FX and CEQ systems. Two additional bar code formats, Codabar and Interleaved 2 of 5,⁽⁶⁾ are compatible with the CEQ system as well. The bar code reading feature provides the user with a flexible range of options that are widely used in the environments of high throughput and lab automation. The bar code can be entered early during sample preparation on the Biomek system, where the DNA purification, quantitation and normalization of samples, sequencing reaction setup, and sequencing reaction cleanup can all be conducted automatically on the same platform.⁽⁶⁾ The bar code system links the automation system and the CEQ. This delivers a continuous sample tracking functionality from the start at the isolation of DNA from the cell or tissue samples to the final results (sequencing or fragment analysis). Bar code tracking ability is vital for researchers in the biomedical and pharmaceutical industries where accurate sample tracking is critical.

As part of the GenomeLab family of products, the CEQ 8800 Genetic Analysis System with its dual plates and internal bar code scanner was designed to simplify and automate genomics processes. It is a turnkey system that provides high-capacity, high-performance, and high-quality genetic analyses. This GenomeLab solution helps accelerate the identification of new markers with biological, diagnostic and therapeutic interest and advance the discoveries that will reshape tomorrow.

Acknowledgements

Thanks to the CEQ 8800 Program Team, the Biomedical Research Applications Center, Kevin Cheung, Ryan Le, Roger Winer, and Gerald Kowalski for their development, communications, data analysis, and support.

References

1. Galvin, N., Clark, M. W., Reddy, M. P. CEQ™ 2000 DNA Analysis System performance. Beckman Coulter, Inc., *Technical Information Bulletin T-1854A* (1998)
2. CEQ™ 8800 Genetic Analysis System Manuals and CEQ™ 8800 Genetic Analysis System User's Guide. Beckman Coulter, Inc., 390186-AA (2003).
3. CEQ™ Dye Terminator Cycle Sequencing with Quick Start Kit, Beckman Coulter, Inc., 608118-AB, and CEQ™ 8000 Series Genetic Analysis System Dye Terminator Cycle Sequencing Chemistry Protocol, Beckman Coulter, Inc., 390003-AC (2003).
4. CEQ™ Fragment Analysis system. Beckman Coulter, Inc., 608113-AE (2002).
5. Barcode specifications. Adams Communications, BarCode 1 <http://www.adams1.com/pub/russadam/info.html> (2003).
6. CEQ™ 8800 Genetic Analysis System and Biomek® FX Liquid Handling Workstation. Beckman Coulter, Inc., *Software Connectivity*, A12801AA (2003).

* The PCR process is covered by patents owned by Roche Molecular Systems and F Hoffmann-La Roche, Ltd. AFLP is a registered trademark of Keygene N.V. All other trademarks are the property of their respective owners.



Developing innovative solutions in genetic analysis, drug discovery, and instrument systems.

Innovate **Automate**
SIMPLIFY

Beckman Coulter, Inc. • 4300 N. Harbor Boulevard, Box 3100 • Fullerton, California 92834-3100

Sales: 1-800-742-2345 • Service: 1-800-551-1150 • Telex: 678413 • Fax: 1-800-643-4366 • www.beckmancoulter.com

Worldwide Biomedical Research Division Offices:

Australia (61) 2 9844-6000 **Canada** (905) 819-1234 **China** (86) 10 6515 6028 **Eastern Europe, Middle East, North Africa** (41) 22 994 07 07
France 01 49 90 90 00 **Germany** 49 21 513335 **Hong Kong** (852) 2814 7431 / 2814 0481 **Italy** 02-953921 **Japan** 03-5404-8359
Mexico 525-605-77-70 **Netherlands** 0297-230630 **Singapore** (65) 6339 3633 **South Africa/Sub-Saharan Africa** (27) 11-805-2014/5 **Spain** 91 3836080
Sweden 08-564 85 900 **Switzerland** 0800 850 810 **Taiwan** (886) 2 2378 3456 **Turkey** 90 216 309 1900 **U.K.** 01494 441181 **U.S.A.** 1-800-742-2345