



Volume 3, Issue 4 • Winter 1999

T³
update

Tips, Tricks and Techniques for users of
Beckman Coulter Automated Solutions
for Drug Discovery

The Microplate: Are Its Days Numbered?

The microplate continues to stand the test of time. To many in the HTS world, this is obvious, but in the past two to three years, we have witnessed our dear plastic buddy start to migrate up and down the process pipeline. Sequencers,

more than a little tired of pouring gels, have been asking for microplate-enabled instrumentation for years. Scientists downstream of the HTS labs are now starting to shift many of their tube-based assays to the microplate to keep up with the increased number of leads heading their way. And, in pockets of the clinical chemistry world, the microplate is being used for higher throughput assays. So what does the future have in store for the microplate?

For one thing, the microplate is finally being recognized globally by an entire committee of users and suppliers solely directed to define its exact dimensions. The need for increased sample throughput generates the need for more automation and increased sample density. To maintain the reliability of these systems, the Society for Biomolecular Screening (SBS) has set up the Standards in Automation and Instrumentation Discussion Group that is working on a Microplate Standard to define the exact physical future for our friend. The standard will also address the higher density formats that are now becoming more mainstream. A successful and adopted standard will enable the continued use of existing equipment, like robotics, incubators, and conveyor systems, while making it easier for suppliers to design new equipment without worrying about whether a new plate will fit or not. If you are interested in learning more about the current status of the SBS Plate Specification, please check out <http://www.sbsonline.com/sbs070.htm> for the latest news.

Two of the first microplate-based DNA sequencing systems, the CEQ™ 2000 from Beckman Coulter and the MegaBACE 1000, have started the trend in Genomics towards the microplate. This has started a huge shift from tube-based nucleic acid preparations to microplate-based procedures. As this trend continues, it should not be long before nearly all procedures from genetic discovery to a drug's submission to the clinic are carried out in microplates. But can the microplate hold on that long? Is the new threat from chips and arrays going to displace our friend from its ultimate goal of total domination of the sample-handling marketplace?

It is the view of this rather melodramatic author that the microplate, as we know it and in higher densities, will be around for a good long time. Yes, arrays and chips are going to prove valuable in Gene Discovery and Toxicological Screening in the relatively near future, but acceptance by the general user in lead screening and optimization is three to five years out. Microplates will have a hard time becoming the default standard in traditional diagnostics, but should make some strong inroads in the higher volume labs. The microplate has given researchers an immeasurable amount of automation in both physical procedures and the management of data, and has many good years left to offer us.

Best regards,

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Editor: Chris Neary

Strategic Marketing Manager
Beckman Coulter, Inc.

4300 N. Harbor Blvd., P.O. Box 3100

Fullerton, CA 92834-3100

(714) 773-6600 • (714) 773-8883

e-mail: cwneary@beckman.com

HTS of Protein Kinases: Searching for New Lead Structures for Tumor Therapy

CHRISTOPH SCHÄCHTELE

FRANK TOTZKE

KTB TUMORFORSCHUNGS GMBH

Protein kinases regulate a multitude of physiological processes by transfer of phosphate residues. This group of enzymes plays a central role in the control of growth, division, and differentiation of cells. According to today's knowledge in research, overexpression and loss of control of activity in different protein kinases are the reasons for the formation and development of tumors. Because of their function, protein kinases are considered to be a group of important targets for the development of novel therapeutic agents against cancer. The KTB Tumorforschungs GmbH (Germany) has recombinantly produced 44 human protein kinases and developed a novel kinase test for determination of kinase activity in microtiter plates. Two robot systems with a current capacity of 9000 measuring points per day are being used to detect new lead structures for tumor therapy in cooperation with various pharmaceutical companies.

INTRODUCTION

Despite exhaustive research efforts, the therapeutic treatment of tumor patients is still not very satisfying. However, advanced findings in molecular research open new strategies for tumor therapy. In the past years, a number of new structures, so-called targets, have been detected which have a high probability of being involved causally in the formation and development of tumors. According to today's knowledge in research, protein kinases play a key role here.

Protein kinases are enzymes which transfer phosphate residues onto protein molecules and thus trigger a change in the molecular conformation of the proteins. Adenosine triphosphate is the source for these phosphate residues (Figure 1). In many cases the phosphorylatable proteins display their own enzymatic activities which can be turned on or off via phosphorylation. Following this principle, about 2,000 different protein kinases in different cells of the body are participating in the regulation of a multitude of cell-specific reactions.

Among the 20 most often occurring amino acids, only serine, threonine, and tyrosine can be phosphorylated by protein kinases. Based on their substrate specificity, protein kinases are divided into two groups: the serine/threonine kinases and tyrosine kinases.

PROTEIN KINASES AS TARGET

According to today's research knowledge, protein kinases participate in tumor development in many ways. Mutations in the genes of certain protein kinases initially result in a growth advantage for one cell,

compared to other surrounding ones, which then divides more quickly or uncontrolled, resulting in a tumor cell clone. Mainly the tyrosine kinases of the growth factor receptors^[1] and the cyclin dependent kinases of the cell cycle^[2] participate in this transformation from normal cells to tumor cells.

Furthermore, protein kinases play a role in the regulation of the programmed cell death (apoptose)^[3]. By activation of certain protein kinases in tumor cells, apoptose signals can be turned off. This stops an important biological repair mechanism, mainly the elimination of tumor cells through apoptose. A third molecular mechanism contributing to tumor development under participation of protein kinases is the tumor angiogenesis^[4]. The rapid growth of solid tumors is made possible only if clones of tumor cells the size of pinheads, which are not yet vascularized, can connect to the already existing vascular blood system.

Due to the multiple roles protein kinases play in the initiation and progressive growth of tumors, it is expected that substances inhibiting

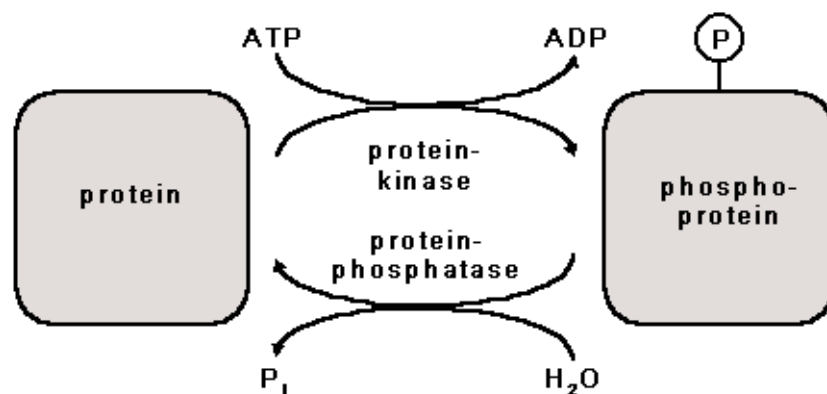


Figure 1. Reversible phosphorylation of proteins through protein kinases.

these enzymes can be used as remedies for treatment of cancer patients^[5]. Kinase inhibitors are desired which are capable of specific inhibition of tumor cell proliferation, of reversing the turning off of apoptosis, and of inhibiting the tumor angiogenesis.

PROVIDING PROTEIN KINASES

High-throughput screening of protein kinases has become possible due to the application of recombinant DNA technology. Up to now, 44 human protein kinases (Table 1) have been cloned at KTB Tumorforschungs GmbH (KTB) which were expressed using recombinant baculoviruses in Sf9 insect cells. To facilitate purification of kinases, they are being produced in form of "GST fusion proteins." This means that the enzyme glutathione-S-transferase (GST) is covalently bound to the N-terminal end of each kinase. The purification is done by binding the GST fusion proteins to glutathione-coupled sepharose with subsequent elution using soluble glutathione. The results of several experimental tests showed that it is not required to separate the GST part again in order to be able to measure the activity of the different kinases.

Most of the protein kinases contained in Table 1 are targets for the development of new tumor therapeutics. A couple of the kinases listed, however, were expressed to verify the selective effect of identified hits.

THE KINASE ASSAY

Until a few years ago, there were only time-consuming filtration assays available for determination of catalytic activity of protein kinases. In the meantime, different companies offered new technologies suitable for microtiter plates. Among them is the SPA method (scintillation proximity assay) developed by Amersham, for example. For tyrosine kinases, various

Table 1: Protein kinases available as recombinant proteins for high-throughput screening

Serine/Threonine Kinases	Receptor-Tyrosine Kinases	Soluble Tyrosine Kinases
PKC-alpha	PDGF-R-alpha	JAK1
PKC-beta-I	PDGF-R-beta	JAK2
PKC-beta-II		JAK3
PKC-gamma	EGF-R	Tyk2
PKC-delta	HER2 (ErbB2/neu)	
PKC-epsilon	HER4 (ErbB4)	FAK
PKC-zeta		
PKC-eta	FGF-R1	Abl
PKC-theta	FGF-R3	
PKC-iota	FGF-R4	
PKC-mu		
	VEGF-R1 (FLT-1)	
CDK1/CyclineB	VEGF-R2 (KDR)	
CDK2/CyclineA	VEGF-R3 (FLT-4)	
CDK2/CyclineE		
CDK4/CyclineD1	TIE-2 (Tek)	
CDK6/CyclineD1		
Raf-1	c-Met (HGF-R)	
PKB (AKT)	Ins-R	
SmMLCK	IGF1-R	
IKK-alpha		
p38		
S6 kinase		
Casein kinase II		

homogeneous, time-resolved fluorescence methods are available.

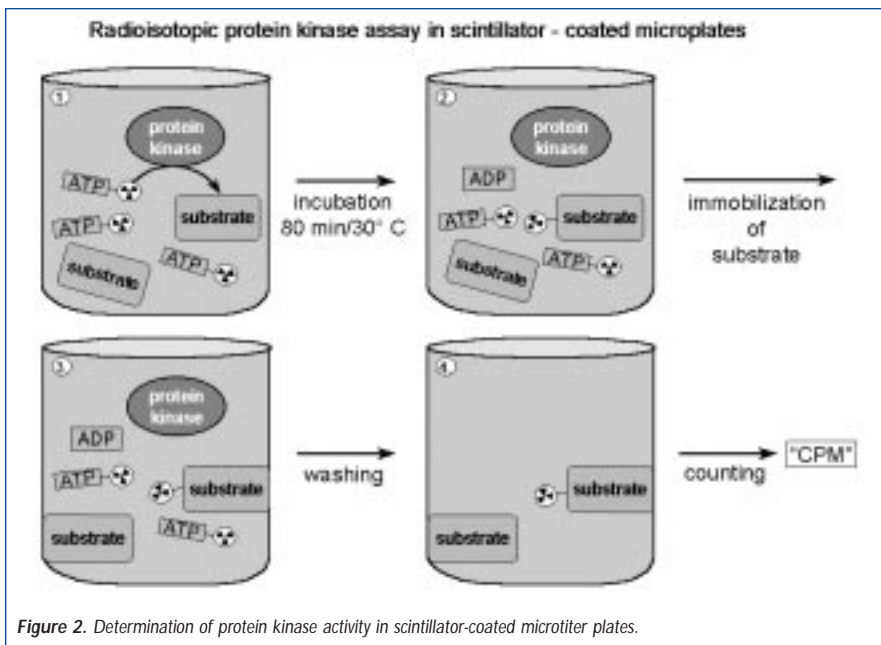
We at KTB developed our own method based on the principle of measuring the incorporation of ³³P-labeled phosphate into the relevant substrate. The specialty of this method is that the substrate, while still in solution during reaction and before the subsequent washing step, is being immobilized on the microtiter plate using a simple method. Then the incorporation of ³³P is determined by means of a scintillation counter suitable for use with microtiter plates (Figure 2). Scintillator-coated FlashPlates produced by NEN (NEN Life Science Products, Boston, USA) have been proven to be particularly useful for immobilization of different substrates. This method for immobilization of

substrates on FlashPlates, which is kept as a trade secret, can be applied to different substrates.

The standard reaction volume for the kinase assay in 96 well microtiter plates is 50 µL. The plates are incubated for 80 minutes at 30°C. The test is very simple, robust, and suitable for all kinases. The signal-to-noise-ratio for all kinases tested so far is above 100.

THE ROBOT SYSTEM

There are two robot systems available for high-throughput screening of protein kinases. The central unit of both systems is the Biomek® 2000 from Beckman Coulter which is used as an 8-channel pipetting unit. Plates and tips are transported in the larger unit linearly with the ORCA® system, and in the smaller unit in a half circle supported by a



sideloader from Beckman Coulter. Both units include a 96-channel washer for washing the plates. The determination of incorporated radioactivity is performed either with MicroBeta 1420 Plus or MicroBeta 1450 Trilux (Wallac Oy, Turku, Finland). Both systems are equipped with two incubators for storage of substance plates at 4°C and incubation of the reaction solutions at 30°C. Due to the different storage capacity for tips and plates as well as the different control software, the daily throughput of the smaller unit is 24 plates and 70 assay plates for the larger unit. The total capacity from both units currently is 9000 measuring points per workday.

FIRST SUCCESSES

The technological platform described here is used by KTB for detection of lead structures for tumor therapy in cooperation with pharmaceutical companies. For that purpose, comprehensive compound libraries are being screened with the target of choice (primary screening). Furthermore, comprehensive kinase panels are being used to test already

identified kinase inhibitors with other representatives from this enzyme class for selectivity of inhibitory action (profiling).

Different compound libraries are used for the different kinase tests. On the one hand compounds are being screened which have been produced using classical organic synthesis, on the other hand, also collections of natural substances are being tested. In addition, also substances from combinatorial chemistry are being

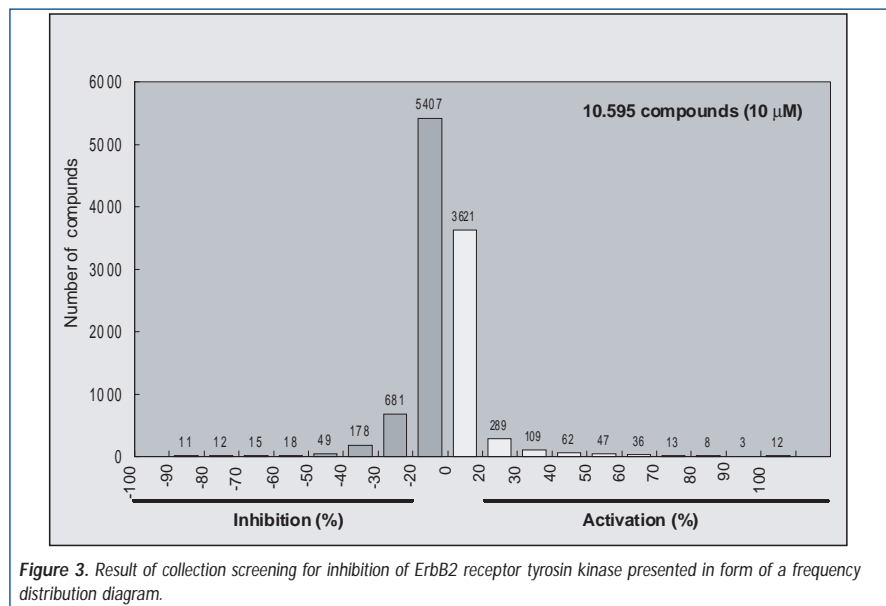
tested for inhibition of protein kinases. Figure 3 shows the results from such a project for which more than 10,000 compounds have been tested for inhibition of tyrosine kinase of the ErbB2 receptor. The number of compounds with more than 80% inhibition in this case was 23.

World-wide there are a number of protein kinase inhibitors currently under clinical testing^[6]. In the Klinik für Tumorbiologie in Freiburg, Germany, such a study started in the Fall of 1998. This substance, developed under significant cooperation of KTB, is an inhibitor of tumor angiogenesis^[7].

OUTLOOK

The detection of new protein kinases has not yet reached its end. Again and again there are reports of new protein kinases suitable as targets for tumor therapy. Therefore, the kinase panel at KTB will be continuously increased over the next years. As far as the test process is concerned, there will be a change to using microtiter plates with 384 wells.

It would also be desirable to determine in parallel the profile of kinase inhibitors for a multitude of protein kinases using chip technology—



if possible, even inside the cell. Preliminary tests which might make this possible have already been performed.

Whether protein kinases will play a role in HTS-supported development of active agents in ten years will depend mainly on the progress to be made in the field of marketability of kinase inhibitors, be it for tumor therapy or for treatment of other diseases.

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MultiScreen on Biomek® for DNA Purification

TAKAHIRO NAKAMURA
BECKMAN COULTER, JAPAN

Beckman Coulter K.K. (BCKK—Japan) recently succeeded in automating a protocol for plasmid purification by using Millipore's MultiScreen filtration plate and Biomek® 2000. The original protocol was developed by Millipore and modified by our scientist to fit onto Biomek 2000 by collaboration with our customer, Dr. Satoshi Nakagawa in Kyowa Hakko Kogyo Co. Ltd., a famous Japanese pharmaceutical company. Below are Dr. Nakagawa's comments.

BCKK: Thank you for your collaboration for developing the method for Biomek 2000. What is the current status of the instrument?

Dr. Nakagawa: Currently, only one plate per run is the maximum, but it automates the whole procedure successfully and it is very stable. Now, four or five plates can be processed per day.

BCKK: What is the biggest merit of this protocol?

Dr. Nakagawa: I have been looking for the best way to automate the DNA purification step for a long time. There were a lot of criteria

we had to consider, such as cost, purity, recovery of DNA, throughput and so forth. The Biomek + MultiScreen solution was the only one that satisfied those criteria. Even though the pre-sequencing technology was not as mature as the one for DNA sequencing, the Biomek + MultiScreen solution was a large progression.

BCKK: Was there any problem or difficulty when you developed the method?

Dr. Nakagawa: In the study of molecular biology, I hadn't had any ideas to use such a laboratory automation system. However, the Biomek has been stable all the time and I haven't encountered any big problems. The current issue is how fast I can learn to utilize the Biomek.

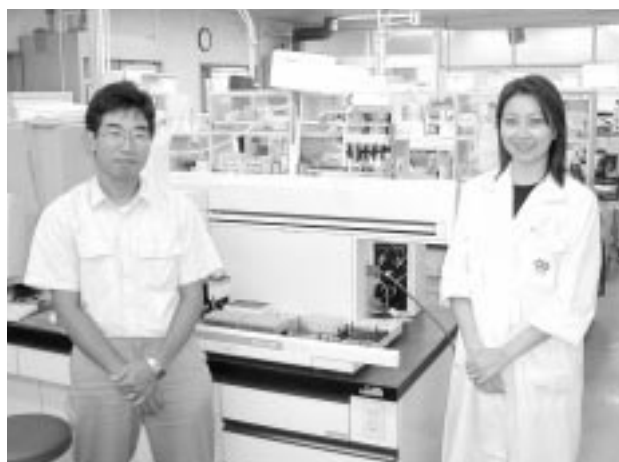
BCKK: Please let me know the benefits of using the Biomek 2000.

Dr. Nakagawa: The movement of the Biomek is humorous and I never

get tired of watching it. Sometimes I still have trouble making programs, but once I get it done, I feel so happy. I'll keep learning about B2K and try to make B2K my best partner.

BCKK: Please let me know if you have any requests to Beckman Coulter.

Dr. Nakagawa: 96-well format for this protocol improves the efficiency of the DNA analysis job very much and we'd like to ask Beckman Coulter to continue developing other applications for DNA analysis quickly. Also, 384-well format should be considered for the next step.



New Features in CEQ™ Sequencing Software Version 2.0

SUSAN STONE
BECKMAN COULTER, INC.

In conjunction with the introduction of software for Fragment Analysis on the CEQ™ 2000 system, the CEQ Sequencing Software has been improved and migrated to the Windows NT platform. The new release of software is version 2.0 and includes the changes briefly described below. Shipments of the new software are expected to begin in January 2000.

Transition to the Windows NT Platform

Windows NT is a more robust operating system with better security features than Windows 95 or 98, and is replacing those programs as the operating system of choice in most situations. With version 2.0, the CEQ 2000 controller now operates under Windows NT for all sequencing and genotyping functions.

New Parameters for Specifying Start of Data

The CEQ base-calling algorithm looks at the raw data

profile to determine when to start calling bases. Because the profile can differ depending on the post-sequencing reaction clean-up method used, the ability to adjust the algorithm for the type of data is beneficial. With CEQ Sequencing Software version 2.0, there are new "Start of Data" parameters (Figure 1) that can be set to optimize analyses for different data types. For example, the settings can be increased to start

later in the data to avoid early non-DNA peaks. The settings can be decreased to start closer to the primer when non-DNA peaks have been removed or for low signal data.

Pre-Run to Eliminate Early Peak Compression

On version 1.x CEQ systems, a small group of compressed peaks is sometimes observed in the early data. The compression is attributed to a salt front moving through the capillary.

A two-minute pre-run of the capillaries has been implemented in version 2.0 to remove this artifact from both sequencing and fragment analysis separations.

Reduced Gel Usage

The use of separation gel has been reduced so that even if 96 samples are not run all together but rather over several (up to 11) different runs, a single gel cartridge will still last for an entire plate.

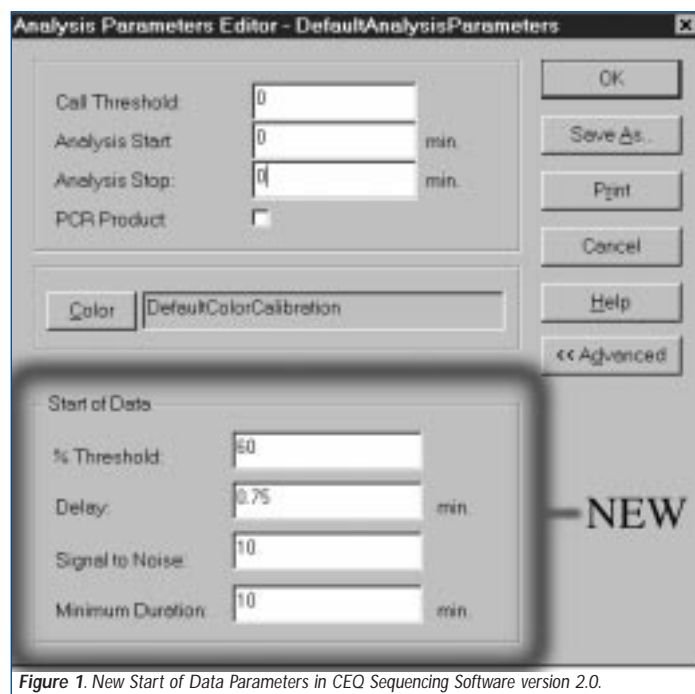


Figure 1. New Start of Data Parameters in CEQ Sequencing Software version 2.0.

Example Biomek® 2000 Integration: Automated Pick and Transfer Software for Hit Picking

MARK SPECTOR, PH.D.
BECKMAN COULTER, INC.

In the last issue of T3, Automated Pick and Transfer (APT) was introduced. Beckman Coulter, Inc., has since changed the web site.

The APT software can be found at the following link:
<http://www.beckmancoulter.com/beckman/biorsrch/prodinfo/biomek/apt.asp>

APT is an example of integrating new elements to a Biomek® 2000

system without requiring modifications to the BioWorks™ software. APT software is not an official Beckman Coulter product but is available to our customers on our web site as an example integration application. An extensive help file is provided with the APT software.

New AP96 Tips for Use with the Multimek™ 96

EDWIN DARIO & JOHN HICKS
BECKMAN COULTER, INC.

A variety of disposable tips are available on the market; however, there is no industry-wide standard for the dimensions of the pipette tip's hub. Originally, the Biomek® P20 and P250 tips were developed to work on the Biomek 1000 Workstation. The original mandrel design of the Multimek™ 96 is wider than the Biomek P20 and P200 tool's mandrel, preventing an effective seal of the Biomek tips.

A new tip rack has been developed that matches the industry standard of 9-mm center spacing used for 96-well microplates. The existing tip rack for the Biomek P20 and P250 tips has a column-to-column center spacing that is not 9 mm and is, therefore, incompatible with the Multimek head. The Biomek AP96 P20 and P250 products combine the original Biomek P20 and P250 disposable tips with this new 9-mm center-to-center spaced rack.

The availability of the Biomek AP96 tips and their direct application on the new AP96 dispense head will help meet the growing demand for disposable tips used on the Multimek 96. To demonstrate that the combination of the newly designed head and Biomek AP96 tips works reliably, a thorough, low-volume dispense study has been performed: see "Multimek 96: Pipetting with the AP96 Dispense Head" in this issue of *T³*.

Training Classes

CLASS	DATE	LOCATION
SAMI® and Core Systems User's Course	January 11-14	Indianapolis, IN
	February 8-11	Indianapolis, IN
	April 4-7	Indianapolis, IN
SAMI® and Core Systems Advanced User's Course	March 14-15	Indianapolis, IN
SILAS™ Developer's Course	Scheduled on demand	

Trade Shows—Q1 2000

SHOW	DATE	LOCATION
Plant and Animal Genome	January 9-12	San Diego, CA
Lab Automation ABRF	January 23-25	Palm Springs, CA
Pittcon	February 19-22	Bellevue, WA
Forum Labo, Paris	March 12-17	New Orleans, LA
	March 28-31	Paris, France

Current Software Version Information

PRODUCT	SOFTWARE	VERSION
Biomek® 2000	BioWorks™ BioScript™ Pro	3.1b 1.0 (works only with BioWorks 2.2x versions)
Multimek™ 96	Multimek Pro	1.4.4a
CEQ™ 2000	CEQ Controller Software	1.1
SAMI	SAMI	3.0 - <i>NEW!</i>

Current SAGIAN™ Core System Menu

TRANSPORTATION ROBOT

ORCA® 1-Meter ORCA 2-Meter ORCA 3-Meter

LIQUID HANDLING WORKSTATIONS

Biomek 2000 Multimek 96

SAGIAN™ STATIONS

Bar Code Reader	Plate Shaker
Hotel and Carousel	Plate Sealer
CS Incubator (4 and 8 position)	Filtration Station
Lid Removal/Storage Station	Tip Lift (Biomek & Multimek)
CO ₂ Incubator with Hotel Carousel	Lid Disposal Station
Bar Code Print & Apply	

CORE PARTNER STATIONS

EL-404R Microplate Washer (Bio-Tek)	Multidrop 384 (Titertek)
SPECTRAmax 190 (Molecular Devices)	SPECTRAmax 250 (Molecular Devices)
THERMOmax (Molecular Devices)	Microbeta Trilux (EG&G Wallac)
VICTOR ² (EG&G Wallac)	FLUOstar 97 (BMG Lab Technologies)
PTC-225 DNA Engine Tetrad Thermal Cycler (MJ Research)	LUMIstar (BMG Lab Technologies)
ELx405 UV Select (BioTek)— <i>NEW!</i>	Uninterrupted Power Supply (Best)

CORE COMPLIANT DEVICES

FLUOstar Galaxy (BMG Lab Technologies)—*NEW!*
POLARstar (BMG Lab Technologies)—*NEW!*
Multidrop Deepwell (Titertek)—*NEW!*



DNA Analysis

Searching for Base Sequences Using CEQ™ Sequence Analysis Software

SUSAN STONE
BECKMAN COULTER, INC.

In the CEQ™ Sequence Analysis Software, the Base Sequence dialog box is used to locate specific sequences in analyzed data (Figure 1). The box is displayed by checking Base Sequence on the list of tool bars under the View | Tool bars menu. Following are some tips for using the search feature:

- Use an asterisk to search for specified characters prior to the asterisk.
- Use ? to search for any character within the string of characters specified.
- Use brackets [] to search for any character within the brackets.
- To search for the text regardless of case, check the **Ignore Case** check box.
- To search for the **exact match** for text (*i.e.*, not IUB codes), check the Exact Match check box.
- To search for text within a range, highlight the range of interest in the base sequence pane and

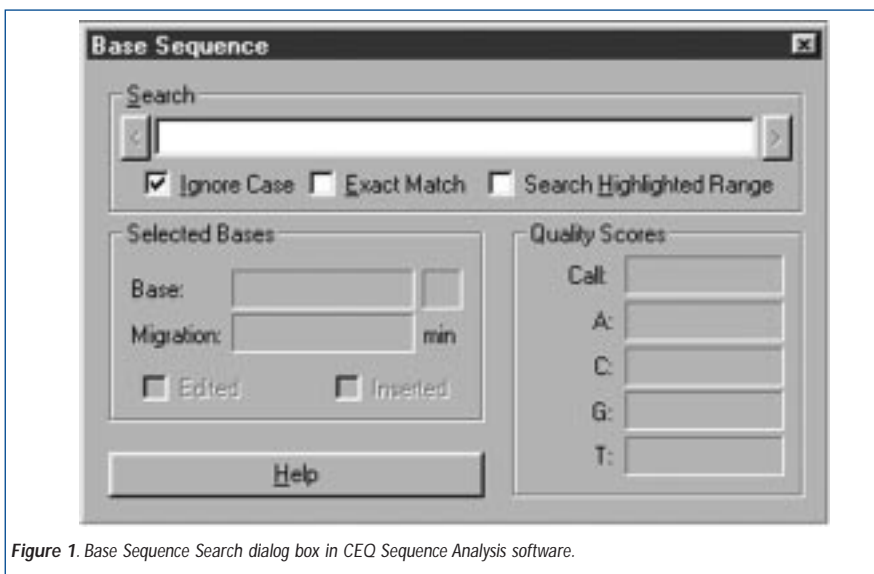
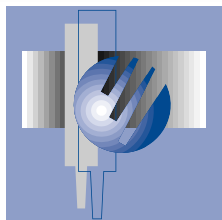


Figure 1. Base Sequence Search dialog box in CEQ Sequence Analysis software.

- check the **Search Highlighted Range** check box.
- Use the forward and back arrows to search backwards and forwards through the base sequence.
- IUB codes may be used when the Exact Match check box is not checked. The codes are as follows:
N = G, A, T, or C
V = G, A, or C

B = G, T, or C
H = A, T, or C
D = G, A, or T
K = G or T
S = G or C
W = A or T
M = A or C
Y = C or T
R = A or G





Liquid Handlers

Multimek™ 96: Pipetting with the AP96 Dispense Head

EDWIN DARIO & JOHN HICKS
BECKMAN COULTER, INC.

INTRODUCTION

A new dispense head for the Multimek™ 96 pipettor has been developed to accommodate the use of the existing Biomek® P20 and P250 disposable tips. Users of the Multimek product can now rely on the high standard of the tips historically used on the Biomek 1000 and 2000 workstations. The outside dimensions of the mandrels on the Multimek 96 dispense head were redesigned, allowing the disposable tips to be affixed to the mandrels at a precise height, ensuring a tight, reliable seal, and guaranteeing straightness.

A variety of disposable tips are available on the market; however, no industry-wide standard exists for the dimensions of the pipette tip's hub. Originally, the Biomek P20 and P250 tips were developed to work on the Biomek 1000 Workstation. The original mandrel design of the Multimek 96 is wider than the Biomek P20 and P200 tool's mandrel, preventing an effective seal of the Biomek tips.

To complete this new product offering, a tip rack has been developed that matches the industry standard of 9-mm center spacing used for 96-well microplates. The existing tip rack for the Biomek P20 and P250 tips has a column-to-column center spacing that is not 9 mm and is therefore incompatible with

the Multimek head. The Biomek AP96 P20 and P250 products combine the original Biomek P20 and P250 disposable tips with this new 9 mm center-to-center spaced rack.

The availability of the Biomek AP96 tips and their direct application on the new AP96 dispense head will help meet the growing demand for disposable tips used on the Multimek 96. To demonstrate that the combination of the newly designed head and Biomek AP96 tips works reliably, a thorough, low-volume dispense study was conducted.

Since the Multimek 96 liquid handler is employed in high-capacity, high-throughput applications, this instrument must pipette with a high degree of precision. Furthermore, the Multimek dispense head must be able to accomplish this with a variety of solutions. The following paragraphs describe the procedures used and the data obtained for analyzing the performance on each of the combinations listed below:

- Biomek AP96 P250 Tips with the Multimek AP96 200 µL Dispense Head
- Biomek AP96 P20 Tips with the Multimek AP96 200 µL Dispense Head
- Biomek AP96 P20 Tips with the Multimek AP96 200 µL Dispense Head

A Multimek 96 configured with a 200 µL AP96 dispense head and tip hardware was positioned on a SAGIAN™ Core System. An assay was

developed using SAMI® NT to schedule the plate manipulations between the following components:

- SAGIAN Microplate Carousel
- SAGIAN Microplate Tip Lift & Lid Removal (Biomek setup)
- SAGIAN Microplate Shaker
- Biomek Plate Reader

Eosin Y dye was added to three stock solutions of distilled water, 10% glycerol in water, and DMSO at a fixed concentration so that dispensing 2.5, 5.0 and 10.0 µL would give a similar absorbance reading at 490 nm (about 0.850 to 1.15 units). A total of ten plates at each volume for each stock solution were used. To maintain an absorbance value of 1.00 units, the following dye solutions were created:

2.5 µL required 2.70 grams of Eosin Y dye dissolved in one liter of each liquid

5.0 µL required 1.35 grams of dye/liter of specified liquid

10.0 µL required 0.675 grams of dye/liter of specified liquid

For this study, the total volume of liquid in each well was normalized to 200 µL; therefore, the method for 2.5 µL of dye/water was performed by bringing three assay plates to the deck and dispensing 197.5 µL of water to each plate without changing tips. The tip box was changed and a new rack of tips was used to dispense 2.5 µL of dye solution to one plate. The plate was transported to the SAGIAN Plate Shaker for a two-minute shake, then taken to the

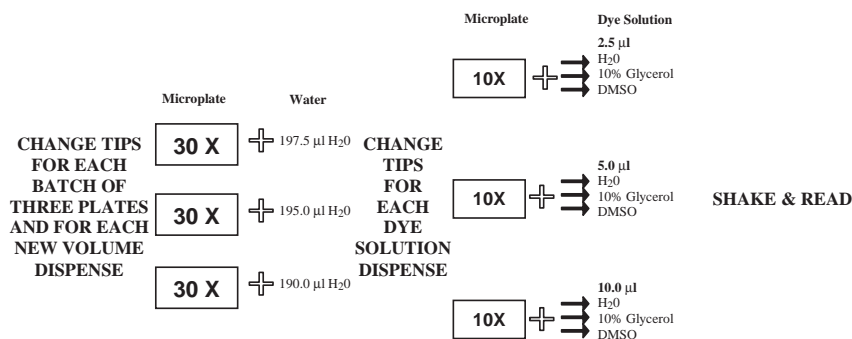


Figure 1. Protocol for measuring the pipetting precision of the Multimek AP96 200 µL Dispense Head. The microplates were processed in batches of three plates for each separate volume and solution. The remaining odd plate was processed by itself.

Biomek® Plate Reader for an immediate read at 490 nm and 650 nm (to account for background). A new rack of tips was used for each dye solution dispense operation. The SAGIAN™ Microplate Tip Lift was included in this particular study to enable the system to have an adequate amount of tip racks. Also, the new tip rack design was validated to work flawlessly with the existing Core System robotic motions.

The Multimek™ Pro method employed had the following settings:

- Single Aspirate/Dispense Method
- Pre-Aspirate Air-Gap 30 µL
- Aspiration Speed 10%
- Dispense Speed 20%
- Dispense height of 7 mm from the deck
- 5-Cycle Mix in the wells using 100 µL at 8 mm from the deck at 10% speed and Tip Touch

The average percent coefficient of variation (% C.V.) for two 200 µL AP96 dispense heads across all 96 wells within each plate is presented in Table 1.

Table 1. Biomek AP96 P250 Tip Study Using 200 µL Dispense Head

	Water	10% Glycerol	DMSO
2.5 µL	1.89%	1.40%	1.26%
5.0 µL	1.63%	1.07%	1.32%
10.0 µL	1.08%	0.83%	0.75%

Each 200 µL head is certified to dispense 200 µL at <1.00% C.V. As exhibited in the data, from 10 µL, the performance of the 200 µL head maintains a precision of under 1.00%.

THE MULTIMEK AP96 50 µL DISPENSE HEAD

The Multimek 96's 50 µL dispense head was originally designed for low-volume dispensing requirements. This dispense head's stainless steel pistons, which aspirate/dispense liquid by air displacement, were designed with a diameter which is one-half that of the 200 µL dispense head. More precise displacement of low-end volumes is obtained with the 50 µL head compared to the 200 µL dispense head. The higher precision in the 50 µL head is due to the mechanics, which use four times the number of motorsteps to aspirate and dispense the same volume of liquid. The corresponding validation study for the 50 µL AP96 head was performed with the same three liquids and at the following five volumes: 0.5, 1.0, 2.0, 25.0, and 50.0 µL. Three plates were used per assay. The Multimek was used as a standalone instrument. Plate manipulations into the Multimek 96, SAGIAN Microplate Shaker, and Biomek Plate Reader were performed manually.

Since the capacity of the low-volume dispense head is between 57–60 µL, the total assay volume for this study was set at 50 µL. In order to minimize the effect on assay results from liquid retained on the outside of tips, the following method was used.

The Multimek Pro method used for 0.5 µL volume is described below (see Figure 2).

1. Pre-aspirate air gap of 5 µL
2. Aspirate 49.5 µL of distilled water at 5% speed
3. Aspirate 0.5 µL of dye solution at 5% speed
4. Aspirate 1 µL of distilled water from a third reservoir at 5% speed to rinse the outside of the tips
5. Dispense completely at 20% speed at a height of 5 mm
6. 5-Cycle Mix of 50 µL with a 5 µL air gap at a height of 5 mm
7. Tip Touch

A new tip rack was used for each plate in the assay. The plates were mixed on a SAGIAN Plate Shaker for two minutes, then read on the Biomek Plate Reader after a 30-minute incubation period. The average percent coefficient of variation (% C.V.) for two 50 µL AP96

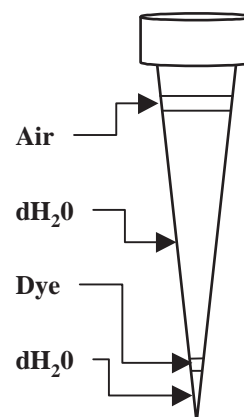


Figure 2. 50 µL AP96 head test method. A graphical cross-section of pipetted solutions prior to mixing. (Drawing not to scale.)

dispense heads across all 96 wells within each plate is presented in Table 2.

Table 2. Biomek® AP96 P20 Tip Study Using 50 µL Dispense Head

	Water	10% Glycerol	DMSO
0.5 µL	4.30%	4.38%	3.57%
1.0 µL	3.47%	3.49%	2.46%
2.0 µL	2.28%	2.82%	1.90%
25.0 µL	1.45%	1.45%	1.08%
50.0 µL	1.40%	0.96%	1.14%

It must be mentioned that the 50 µL dispense head is certified to have a % C.V. of under 1% at 20 µL. This is accomplished using a 200 µL dispense head to deliver 200 µL of water. Then a new 50 µL head is installed and 20 µL of dye solution is dispensed. For the purposes of this study, it was decided that the chosen method should use the low-volume dispense head exclusively. The method used in this study should simulate what most 50 µL dispense head customers can accomplish with their units.

BIOMEK AP96 P20 & P250 TIPS ARE INTERCHANGEABLE

The Multimek™ 96 will continue to be available with the two dispense head options. An advantage in using the Biomek AP96 tips with the Multimek AP96 head configuration is the ability to utilize either P20 or

Table 3. Biomek AP96 P20 Tips Using the 200 µL Dispense Head

	Study 3- P20 Tips with 200 µL Method (Table 1)	Study 4- P20 Tips with 50 µL Method (Table 2)
1.0 µL	2.97%	5.16%
2.0 µL	1.58%	3.01%
25.0 µL	0.53%	1.28%

P250 tips on each of the respective dispense heads. An abbreviated study was performed to obtain comparison data for the P20 tips loaded onto the 200 µL dispense head. Eosin Y dye was dissolved in distilled water and the methods used for the first two studies were performed with this configuration.

The 2 µL results of Study 3 compare favorably to the 2.5 and 5.0 µL results in Study 1 (Table 1). The P20 tip's 1.58% c.v. is slightly better than the 1.89% and 1.63% values for 2.5 and 5.0 µL using P250 tips, respectively. Study 4 used the same method that was used in the 50 µL head study (P20 tips and 50 µL assay volume). When comparing the same method of the P20 tips on the 50 µL AP96 heads, one can ascertain that the low-volume head performs measurably better at 1 and 2 µL:
1 µL @ 3.47% vs. 5.16%;
2 µL @ 2.28% vs. 3.01%.

BIOMEK AP96 P20 AND P250 DISPOSABLE TIPS—MAXIMUM CAPACITY

Finally, the volume capacities for each of the six Biomek AP96 tip varieties are listed in Table 4. Each Multimek 96 instrument will allow for a slightly different maximum stroke in the D-axis (Dispense). This is due to the positioning of the sensors in each instrument. In general, the maximum stroke on any 50 µL dispense head will translate to 55 µL, while the maximum stroke on any 200 µL dispense head will translate to 240 µL.

Due to mechanical constraints of maximum stroke with the Biomek P20 and MP20 tools, the volume of the P20 tips was restricted to 20 µL when used on the Biomek 2000 Workstation. The 100 µL maximum capacity of the Biomek AP96 P20 tips can be used fully when seated

Table 4. Biomek AP96 Disposable Tips Volume Capacities

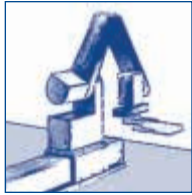
Part No.	Description	Max. Capacity
717251	Biomek AP96 P250 Disposable Tips—Nonsterile	240 µL
717252	Biomek AP96 P250 Disposable Tips—Sterile	240 µL
717253	Biomek AP96 P250 Disposable Tips—Barrier	160 µL
717254	Biomek AP96 P20 Disposable Tips—Nonsterile	100 µL
717255	Biomek AP96 P20 Disposable Tips—Sterile	100 µL
717256	Biomek AP96 P20 Disposable Tips—Barrier	50 µL

onto the Multimek 96 200 µL dispense head. Whenever the 50 µL dispense head is installed, caution should be taken to avoid flooding the mandrels.

CONCLUSIONS

The performance of the newly designed Multimek AP96 dispense heads was studied exhaustively. The data generated compares favorably to an earlier-reported study regarding Multimek disposable tip dispense (See Beckman Coulter Technical Bulletin T-1844A). The AP96 head and tip design allows for the precise delivery of small quantities of liquid to common labware.

The combination of a wide variety of disposable tips, along with the interchangeable nature of the P20 and P250 tips on the respective dispense heads, gives the AP96 product offering clear competitive and performance advantages.



Core Systems

NEW—Core System Continuum!

(or “How do I know my Core System will continue to be productive?”)

JAN ALLEN PURSLEY
BECKMAN COULTER, INC.

Beckman Coulter has long been an innovator of laboratory automation and liquid handling systems. In 1996, Beckman acquired SAGIAN™, an innovator of laboratory robotics integration and software. Prior to this acquisition the two created the concept of a Core System. The Core System approach automates individual lab processes by selecting and combining devices from a menu of choices. A typical Core System includes an ORCA® robot, a Multimek™ 96 or Biomek® 2000 liquid handler, Core Partner Stations, and SAGIAN Stations, all working together under the control of SAMI® software. Simply stated, it was an immediate hit.

While still the model of the industry, the Core System approach has suffered some growing pains. For example, our customers' detector technology demands have evolved more rapidly than our ability to add detectors to the Core Partner list. As a result, more and more customers are requesting custom additions to our Core systems. Customization adds cost for Beckman Coulter and our customers, and requires additional time to quote and deliver. Therefore, we decided a fundamental change to the Core System approach was necessary to provide more choices to our customers, without

compromising the integrity of the program: no easy task! The result ... the Core System Continuum.

The Continuum consists of four mechanisms for adding devices to a Core System:

CORE PARTNER STATIONS

Core Partner Stations are the most thoroughly evaluated devices available for use on any automated system. Beckman Coulter has extensively tested these devices to assure flexible robotics accessibility, rugged communication protocols, hardware reliability, and appropriate performance. Beckman Coulter has established part numbers for the devices and for accessory integration kits, and, in most respects, treats them as OEM devices. Beckman Coulter assumes ownership for support of these devices after delivery, either directly or through contractual arrangements with the device manufacturers. SAMI software releases include drivers for Core Partner Stations, so these devices may be easily added to your Core System at any time. Customers may be assured of the highest quality support for devices in this category.

CORE COMPLIANT DEVICES

This new category was established to allow Beckman Coulter to provide the latest technology to customers more rapidly. Core Compliant Devices have demonstrat-

ed technical compatibility with Beckman Coulter Core Systems. These devices will not be sold through, or by, Beckman Coulter. Most devices in this category will achieve partner status after a thorough evaluation of reliability, service, and support issues. Beckman Coulter will provide the SILAS™ module, robot motions, and mounting hardware, but support of the devices themselves will be the responsibility of the device suppliers.

CLASS 1 CUSTOM DEVICES

This category was created to allow Beckman Coulter to provide some custom integrations more rapidly and at a substantially reduced cost! Class 1 Custom Device status is applicable when the device appears to be “automation friendly” with robot accessibility and standard communication protocols. A disclaimer provided on Class 1 Custom Device quotations addresses cases where we find that integration is not possible or is simply too complex or costly to be a reasonable option for our customers. Liquid Handlers are strictly excluded from this class, but most other types of devices, such as detectors, incubators, dispensers, and washers, are open for consideration. Beckman Coulter will provide the SILAS module, robot motions, and mounting hardware, but all support of the devices themselves will remain the responsibility of the device suppliers.

CLASS 2 CUSTOM DEVICES

This category will be used to integrate devices that require extensive engineering. Liquid handlers and transportation devices will be the most common examples of Class 2 Custom Devices. Pricing will be based on the findings of a thorough engineering feasibility assessment. Beckman Coulter will provide the SILAS™ module, robot motions, and mounting hardware, but all support

of the devices themselves will remain the responsibility of the device suppliers.

SO WHAT'S THE BIG DEAL?

Beckman Coulter's Continuum is a process to accelerate the adoption of new technologies onto the Core System platform. We can respond faster to most custom integration requests, and at a greatly reduced cost to you. You can rest assured that

we will be continuing to upgrade the status of successful new devices, improving our support as we go along. And you can have confidence that through the adoption of developing technologies, your Core System is a sound investment for today and tomorrow.

For more information, please contact your Beckman Coulter sales consultant.



Multimek™ Tip Wash Station Substitution Reminder

MARK SPECTOR, PH.D.
BECKMAN COULTER, INC.

As a reminder, in March of 1999, Beckman Coulter, Inc., substituted a Teflon anodized aluminum tip wash manifold for the original CCS version in the Multimek™ Tip Wash Station (P/N 148102). The CCS version was made of a black Delrin material. The reasons for the engineering change were to improve material compatibility with common solvents (*i.e.*, DMSO) and improved waste flow.

The manifold stands about 0.265" higher and the inner diameter of each well is about 0.025" narrower than the CCS version. The thickness of the wall of each well is about 0.010" more in the new version. The reasons for the narrower dimension are the machining tolerances required for working with aluminum.

A minor configuration modification in the system setup may be required. The default height for the tip wash station in the Multimek Pro

software is 24.0 mm. The height of the wash station can be adjusted by modifying the resource.ini file in the pipette directory. The new height required is 30.0 mm. The field service engineer, or Multimek user, needs to save this change in the resource.ini file and the wash/mix node will recognize the height change.



Higher Throughput Launched on Core Systems with SAMI® 3.0

JAN ALLEN PURSLEY
BECKMAN COULTER, INC.

Want to improve the throughput on your Beckman Coulter Core System? Think you might need a faster transportation device? Or a quicker liquid handler? Or 38,400 wells per plate? Not really! While all these might help increase throughput, there's something much simpler that you can do: upgrade to SAMI® 3.0!


3.0 is the third release of the popular NT-based SAMI software. At first glance, SAMI 3.0 looks no different from SAMI NT. In fact, the main benefit to SAMI 3.0 is completely

invisible on the user interface: *throughput*. SAMI 3.0 provides up to 20% higher throughput, simply by directing the robot to move more efficiently and with forethought. Using SAMI NT, ORCA® was instructed to go to a safe position before running up and down the rail. SAMI 3.0, along with some creative training from your Beckman Coulter service engineer, can now instruct ORCA to move directly from station to station. SAMI can also tell ORCA to pick up labware in a position that's appropriate for where the labware is to be placed next. This forethought eliminates many time-consuming re-grips.

from one or more methods, during the course of a run! Figure 1 represents the See-It! view of a method that has long incubations. Long incubations typically implies that most of the Core System hardware has nothing to do, and could be performing other work for you. In Figure 2, the anticipated plate reads have been added, and the work load has been dynamically rescheduled to optimize hardware utilization. So with a little forethought, you can get more out of your Core System!

SAMI 3.0 includes many new features and enhancements that are a direct result of listening to SAMI users. Most requests start with, "It would be nice if..." and in SAMI 3.0, we have addressed nearly 20 of these requests, such as:

- run start delay
- simplified resource level settings
- user control of labware grouping and scheduling sequence
- method title, author, and description fields
- improved data formatting
- advanced lidding control
- method parameters altered in response to a bar code

SAMI 3.0 is available on all new core systems, and as an upgrade to existing systems. Your SAMI NT methods may be run on SAMI 3.0 without editing. Contact your Beckman Coulter Automated Solutions sales representative and request Technical Information Bulletin T-1875A for more details. 

SAMI 3.0 delivers other new features that let you plan ahead and save time. For example, you may need to perform a task today that involves long incubation steps. Later that day, you know you may need to automate another task, such as read some plates, but you aren't sure how many. SAMI 3.0 has a new feature that allows you to dynamically reschedule additional families,

Tips & Tricks

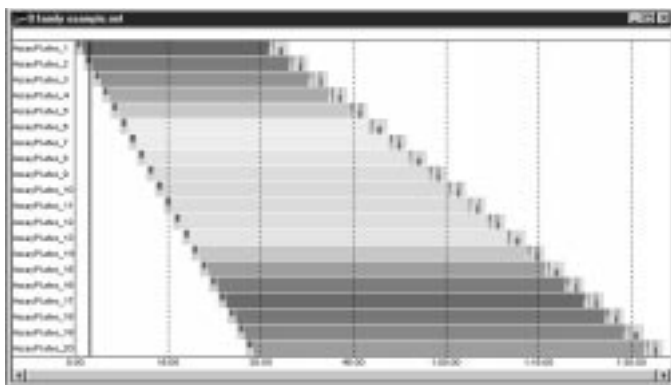


Figure 1. See-It! graph of run in progress.

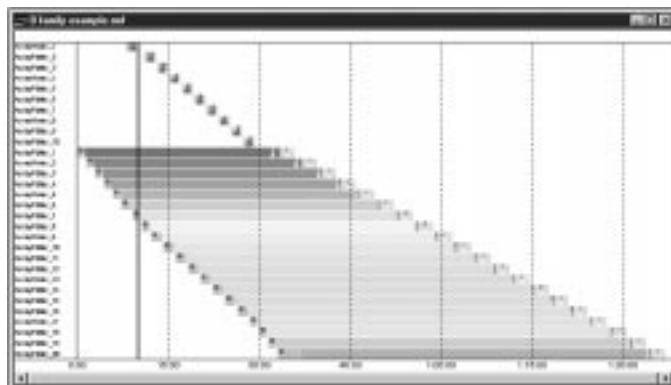


Figure 2. See-It! graph of schedule after the addition of plate read families.



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B1999-3638-CB-8

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