390218-AA
January 2002

CEQ™ 8000

Genetic Analysis System

DNA Sequencing Customer Training Guide

Beckman Coulter, Inc.
4300 North Harbor Boulevard, Fullerton, CA 92835

Copyright, Licenses and Trademarks

I Table of Contents

II Overview

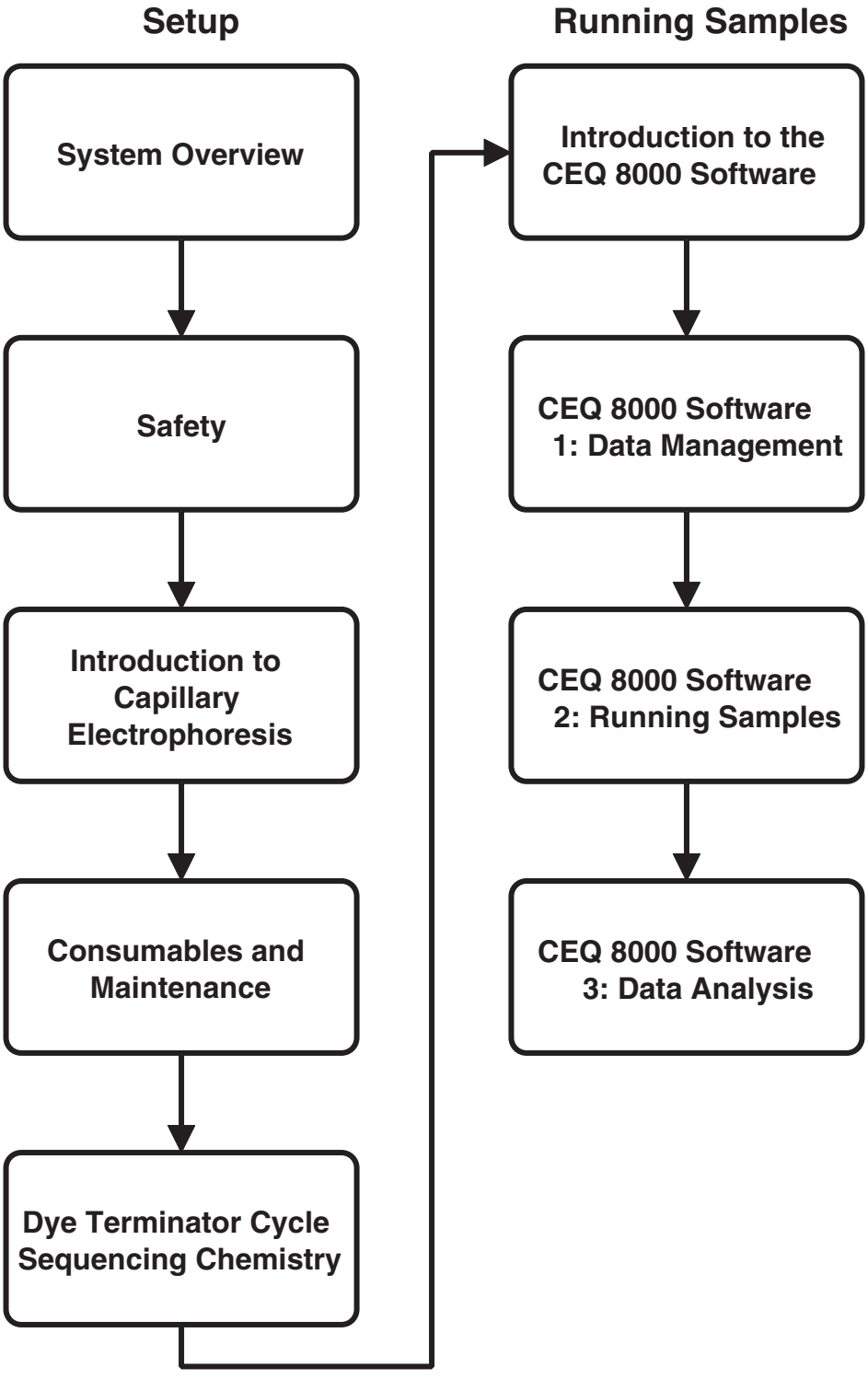
TRAINING COURSE OVERVIEW

Welcome to the application portion of the Beckman Coulter, Inc. training program for the CEQ™ 8000 Genetic Analysis System. During this training, our Application Specialist will guide you through the basic operation of the system, review safety and maintenance, and review the Dye Terminator Cycle Sequencing (DTCS) chemistry.

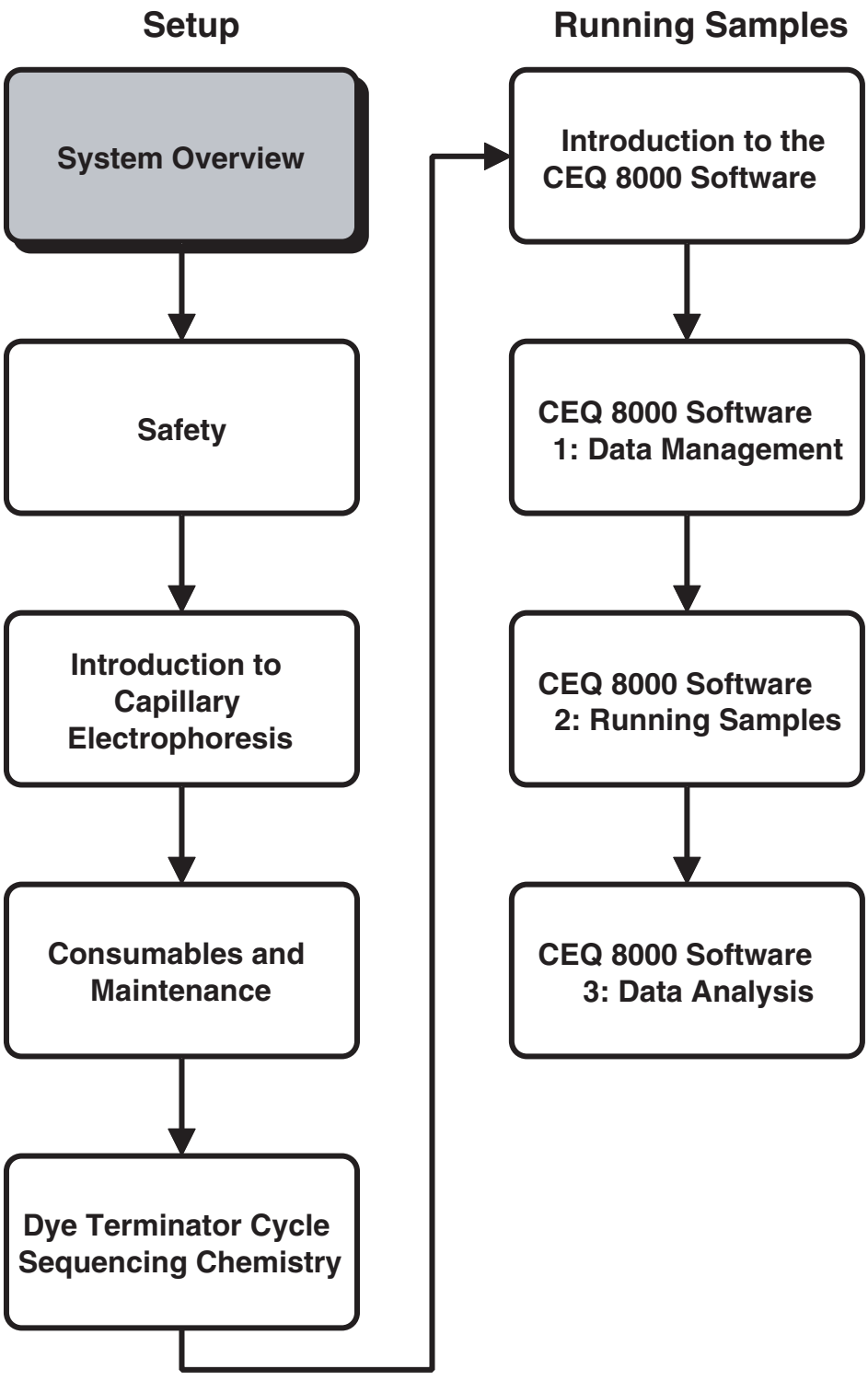
The purpose of the Application Training is to introduce you, the operator, to the system and ensure that you can adequately perform basic functions.

The following prerequisites have been defined and sent to you earlier to ensure a successful Application Training:

1. The operator needs to be available without interruption for the entire training session.
2. No more than two operators will be trained as part of the installation.
3. The operator must have reagents ready for sample preparation prior to the trainer's arrival.
4. Operators must possess a working knowledge of the following molecular biology techniques:
   - DNA template purification and preparation
   - PCR amplification
   - Dye Terminator Cycle Sequencing
   - Ethanol Precipitation
5. Operators must possess a fundamental knowledge of computers and the Windows NT operating system or user interface.
6. This training is organized in ten sections, each containing several concise learning modules. Most modules include a “skill check” to ensure mastery of the fundamental elements of the CEQ 8000 software, hardware and chemistry. Your trainer will guide you through each module. A map of these modules is shown on page II-ii and a highlighted map precedes each module to indicate the learning progress. Please take a moment to familiarize yourself with the training map before we begin.


Setup
System Overview
Safety
Introduction to Capillary Electrophoresis
Consumables and Maintenance
Dye Terminator Cycle Sequencing Chemistry
Running Samples
Introduction to the CEQ 8000 Software
CEQ 8000 Software 1: Data Management
CEQ 8000 Software 2: Running Samples
CEQ 8000 Software 3: Data Analysis

1 System Overview



OVERVIEW

This section describes the main hardware components of the CEQ 8000 Genetic Analysis System. You will learn operational considerations for each module and the terms commonly used when discussing the software and hardware.

Hardware Component Descriptions

Figure 1 shows the user accessible hardware components of the CEQ 8000 System.

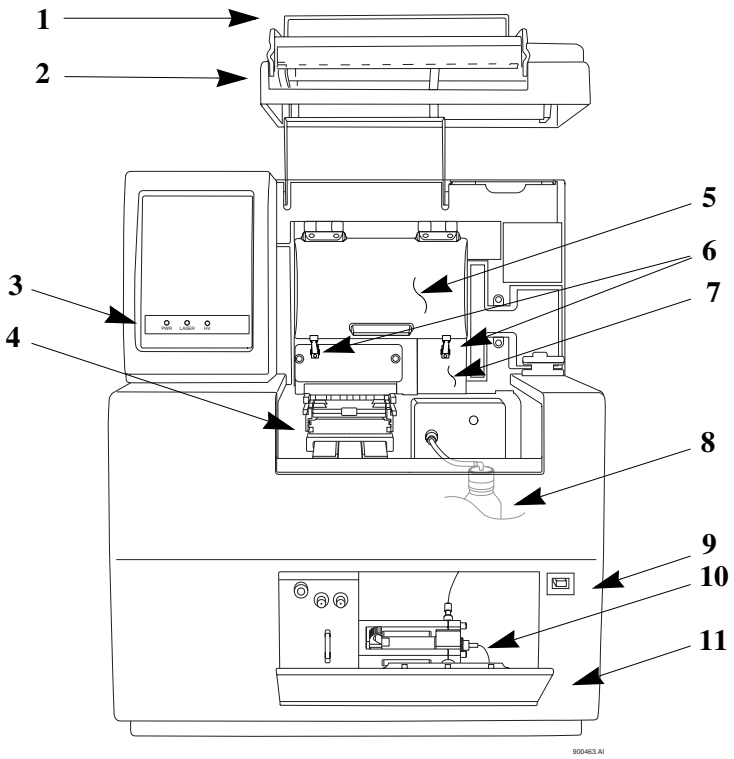


Figure 1: User Accessible Hardware Components

1	Sample Access Cover (extended)	7	Manifold Access Cover
2	Capillary Access Cover (extended)	8	Gel Waste Bottle
3	Status Indicators	9	Power Switch
4	Plate Holders & Sample Transport	10	Gel Pump
5	Capillary Temperature Control Cover	11	Gel Pump/Gel Cartridge Access Cover
6	Rubber Latches		

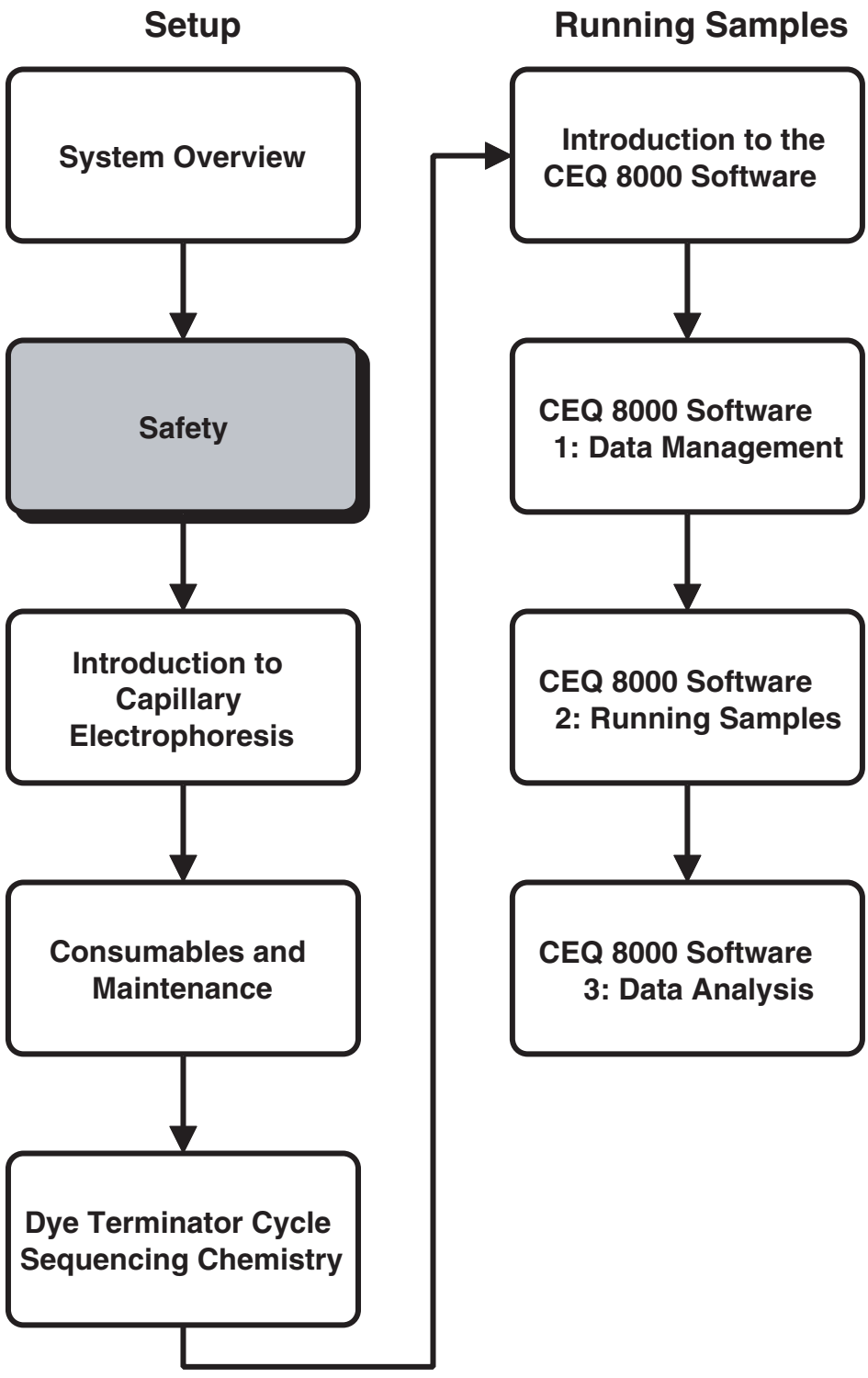
SUMMARY

Upon completion of this section, the user will:

- Describe the purpose of the CEQ 8000 System.
- List and describe the user accessible hardware components.

Now that we have a “big picture” look at the Instrument, we are ready to learn the details required for successful operation. First, however, we should consider personal and equipment safety. We will discuss this in the next section.

2 Safety


Setup
Running Samples
System Overview
Safety
Introduction to Capillary Electrophoresis
Consumables and Maintenance
Dye Terminator Cycle Sequencing Chemistry
Introduction to the CEQ 8000 Software
CEQ 8000 Software 1: Data Management
CEQ 8000 Software 2: Running Samples
CEQ 8000 Software 3: Data Analysis

OVERVIEW

This section provides safety instructions for the CEQ 8000 hardware and accessories. You will review:

- Instrument safety features
- Chemical and biological safety
- Electrical safety
- Moving parts

CEQ 8000 SAFETY

All safety instructions should be read and understood before installation, operation or maintenance is attempted.

Safety Features

Review the location and action of the following safety features:

- ON/OFF Switch
- Safety Interlocks
- Fuse Replacement and Voltage Selection

Safety Notices

Review meaning and placement:

- International Safety Symbol
- High Voltage Symbol

Chemical and Biological Safety

Normal operation of the system involves the use of many reagents which may be toxic, flammable or biologically harmful.

- Observe all cautionary information printed on the original solution containers prior to use.
- Operate the system in an appropriate enclosure and take all necessary precautions when using pathologic or toxic materials to prevent the generation of aerosols.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near the powered-up instrument.
- Wear appropriate lab attire (safety glasses, gloves, lab coat, and breathing apparatus) when working with hazardous materials.

Electrical Safety

- Always disconnect power to the system before performing maintenance operations
- Refer servicing that requires removal of covers to qualified personnel.

Moving Parts

To avoid injury due to moving parts or damage to the autosampler, you must observe the following:

- Keep loose clothing and hair away from the tray area.
- NEVER attempt to exchange vial segments while the tray is moving.
- NEVER attempt to physically restrict movement of tray assembly.

Electrostatic Discharge

- Ground yourself before working with system
- Carpeting
- Contact Beckman Coulter, Inc. Field Service if you have questions

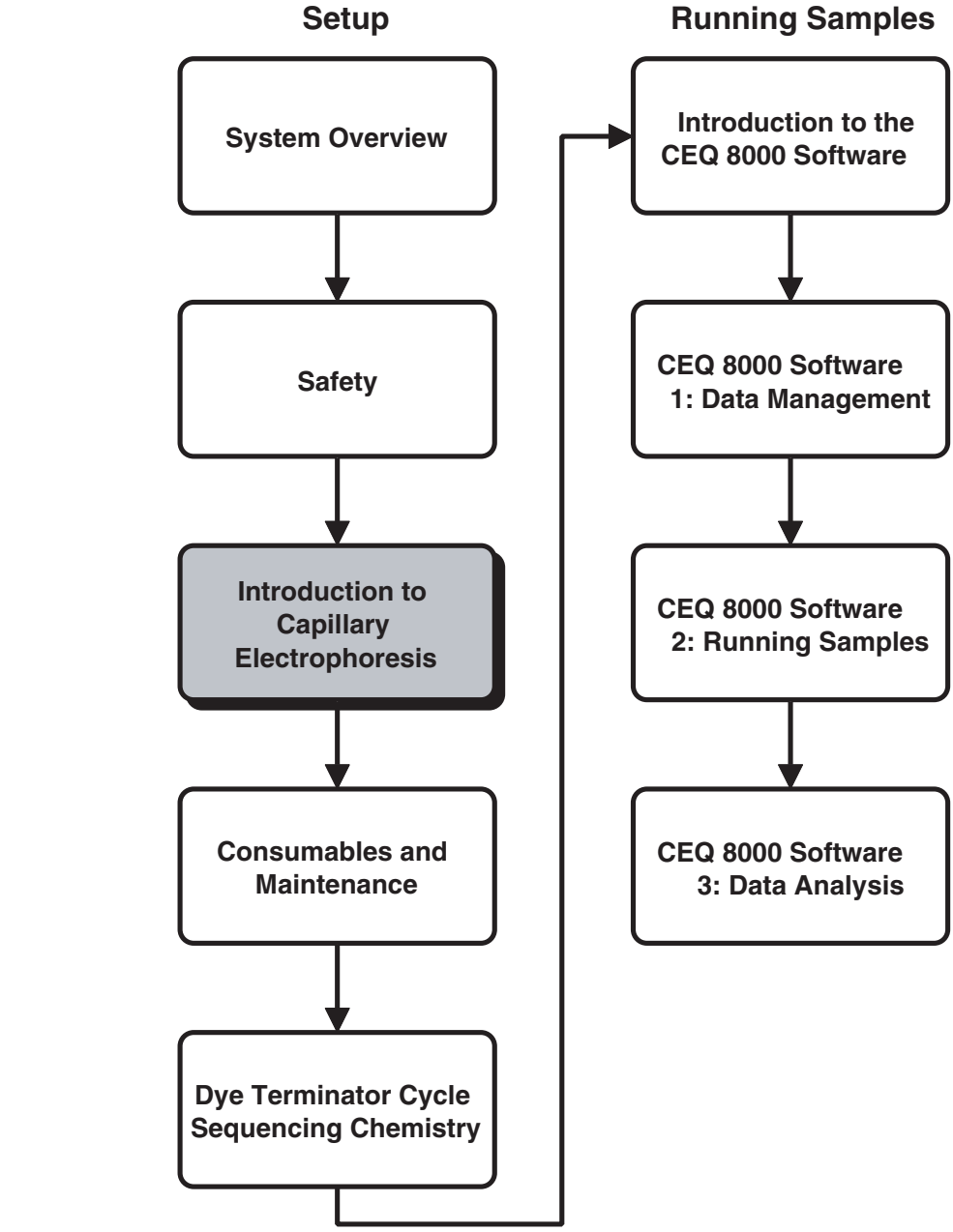
SUMMARY

Upon completion of this section, the user will:

- List and describe the relevant safety hazards, features, precautions and procedures.

This completes the safety portion of the CEQ 8000 Instrument training. For more detailed information regarding safety, refer to the appropriate sections in the individual module manuals, and the CEQ 8000 User's Guide. If the product is used in a manner other than specified in these manuals, the safety and performance of the equipment could be impaired.

3 Introduction To Capillary Electrophoresis



OVERVIEW

This section describes the basic concept of capillary electrophoresis – the principle behind the separation mechanism for the CEQ 8000. While it is similar to slab gel electrophoresis, when performed in a capillary there are a few more considerations critical to obtaining good results.

In theory, capillary electrophoresis occurs when an Electric Field (E) is applied to an electrolyte solution within a capillary, causing ions to migrate.

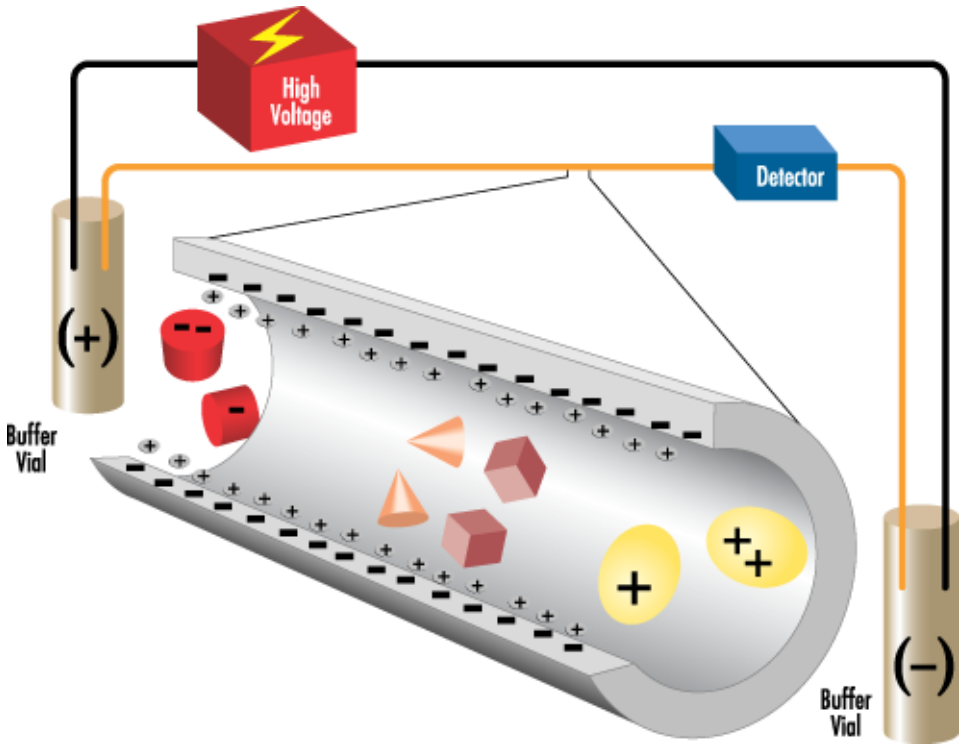


Figure 2: Capillary electrophoresis

The applied field is the driving force that causes the ions to move. This movement is referred to as electrophoretic migration. Having a negative net charge, DNA fragments are likewise affected by the applied field, moving toward the anode (+) in the example above. DNA fragments are separated as they migrate by a size exclusion sieving effect. These dye-labelled fragments will be detected by fluorescence and in turn be rendered into a sequence. Polyacrylamide gels are commonly used as the electrolyte solution to provide the sieving medium for the separations.

Note ***A natural electrostatic pumping effect, called electroosmosis or electroosmotic flow (EOF), may also be a factor with fused silica capillaries and can negatively affect the separation. For DNA sequencing, this is effectively suppressed by coating the capillaries internally with an inert polymer.***

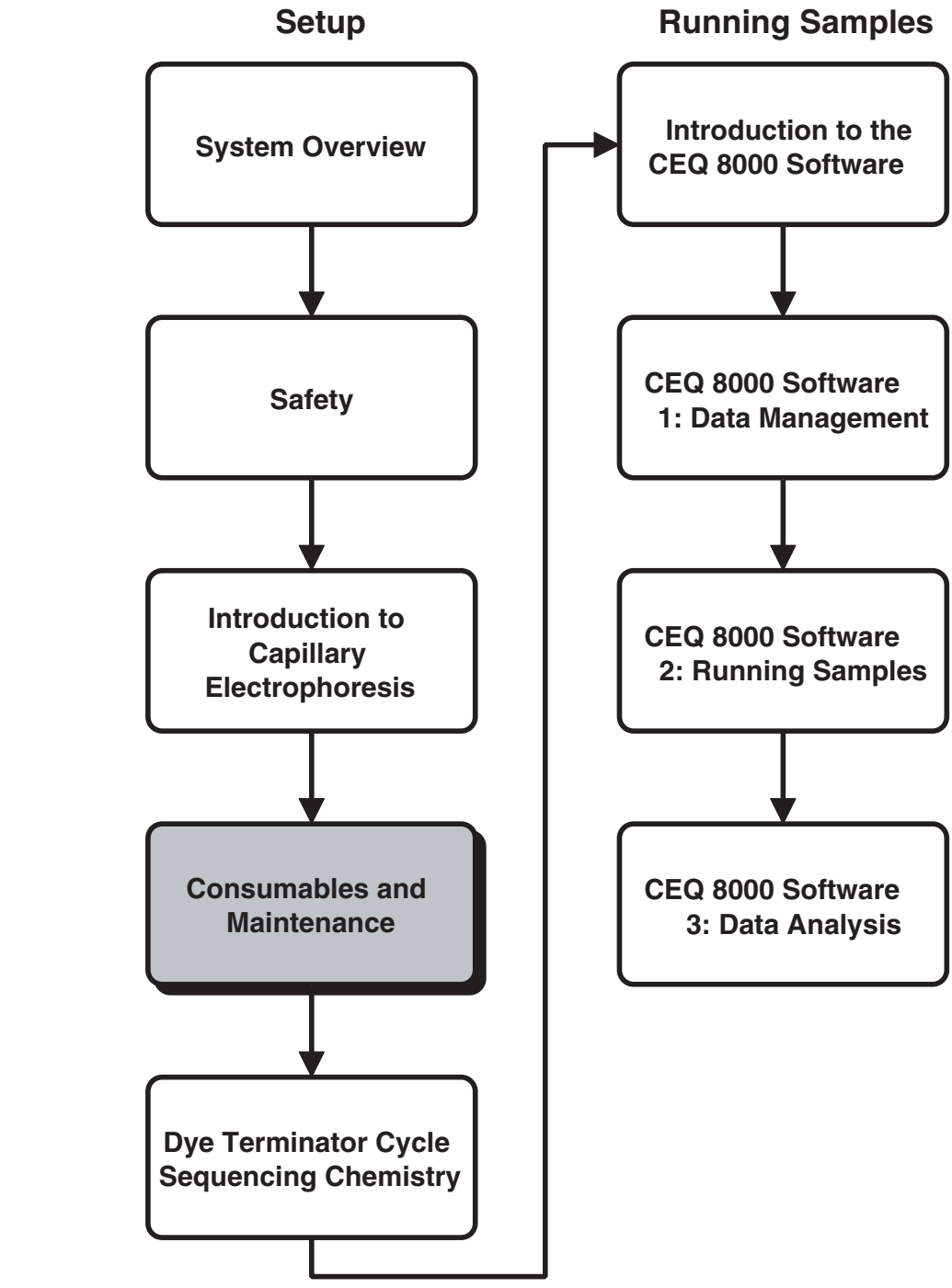
Critical Factors:

- Excess salt/impurities in sample matrix
- Sample loading amount
- Capillary temperature (affects gel resolution)
- Voltage

SUMMARY

Principles of capillary electrophoresis are important because they define the operating basis of the CEQ 8000. Now lets review Consumables and Maintenance.

4 Consumables and Maintenance



OVERVIEW

This section provides routine maintenance and biological waste disposal procedures. It also provides a list of the consumable materials used in the system.

Maintenance Overview

In order to ensure consistent operations, several important hardware and chemistry kit components must be identified and maintenance procedures for these components must be addressed. The full CEQ system is comprised of three necessary components required for successful operation:

- The CEQ 8000 instrument
- Central Processing Unit (CPU)
- Monitor Interface

Figure 3: CEQ 8000 DNA Analysis System

The CEQ 8000 instrument consists of several sub components.

Routine Maintenance

Use Figure 4, User Accessible Hardware Components, to locate hardware components referenced in this chapter.

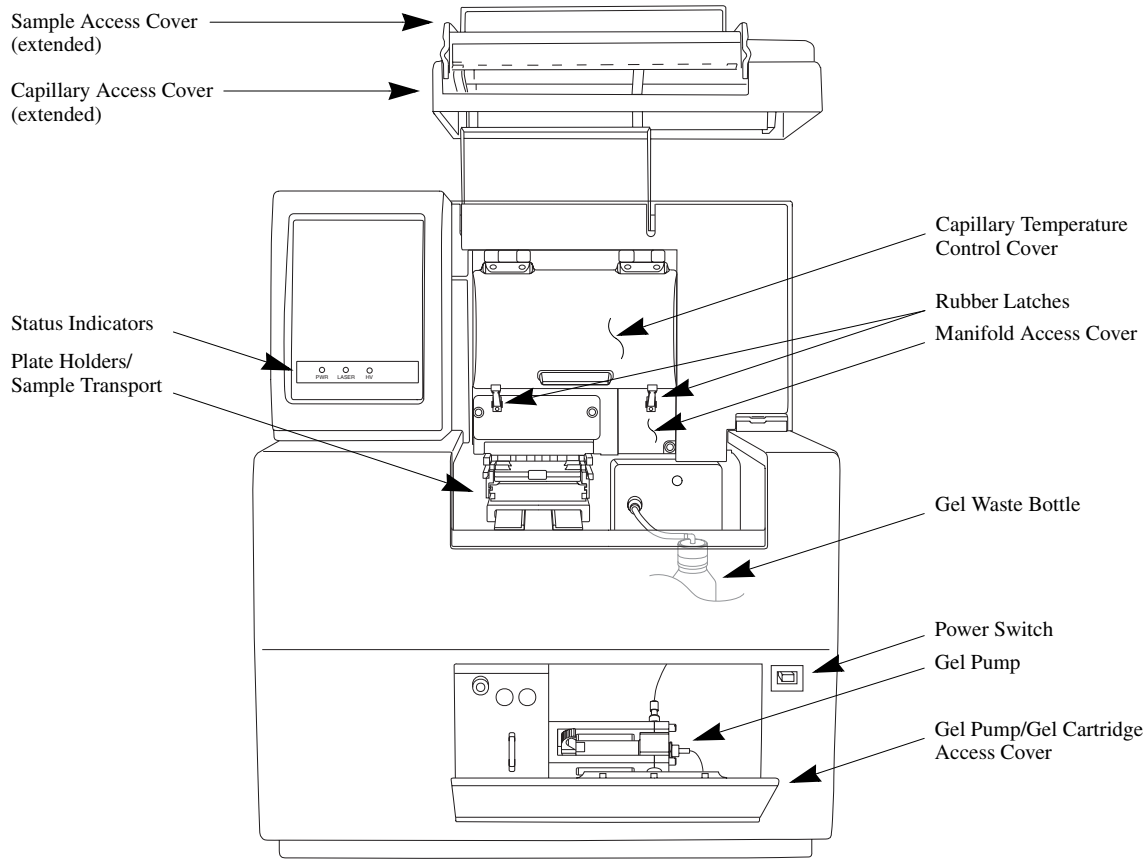


Figure 4: User Accessible Hardware Components

Capillary Array

The capillary array is extremely fragile and must be handled with care. When handling the capillary array, always hold the tab on the electrode block. Handling the array on or near the capillaries may result in breakage. The array fitting is also fragile and must be handled with care. When handling the array fitting, always hold the tab that extends perpendicular to the detection window. **DO NOT** handle the array fitting on or near the detection window. This may result in breakage or excess oil deposits. When installing the capillary array, install the electrode block portion and then insert the array fitting into the manifold. Place the plenum in its proper orientation and secure it by tightening the screws located at the bottom most portion. When an array is not on the instrument, the manifold plug should be used in it's place to prevent drying of the gel system.

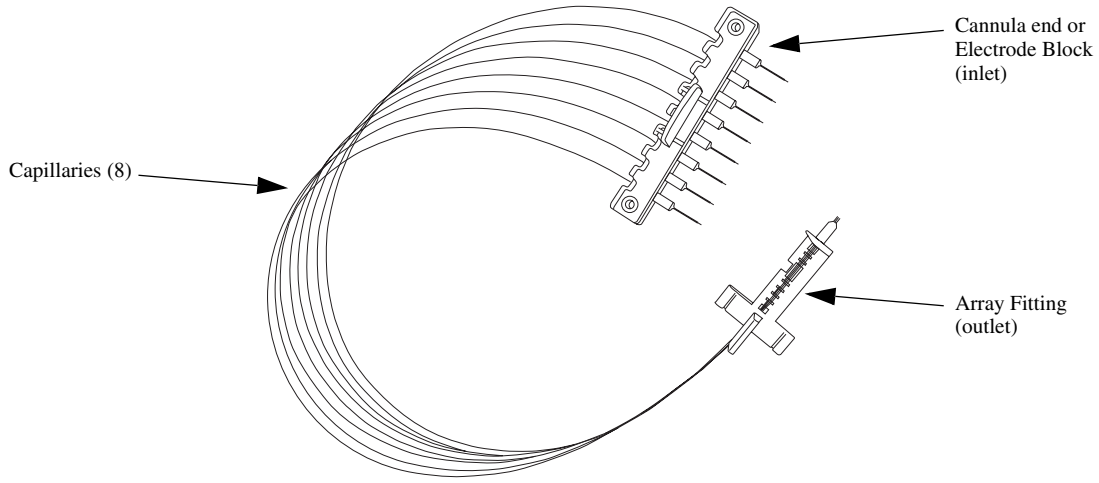


Figure 5: Capillary Array

Install Capillary Array

Use this option to install the capillary array. Verify or change the information in the **Install Capillary Array** dialog box and then install the array.

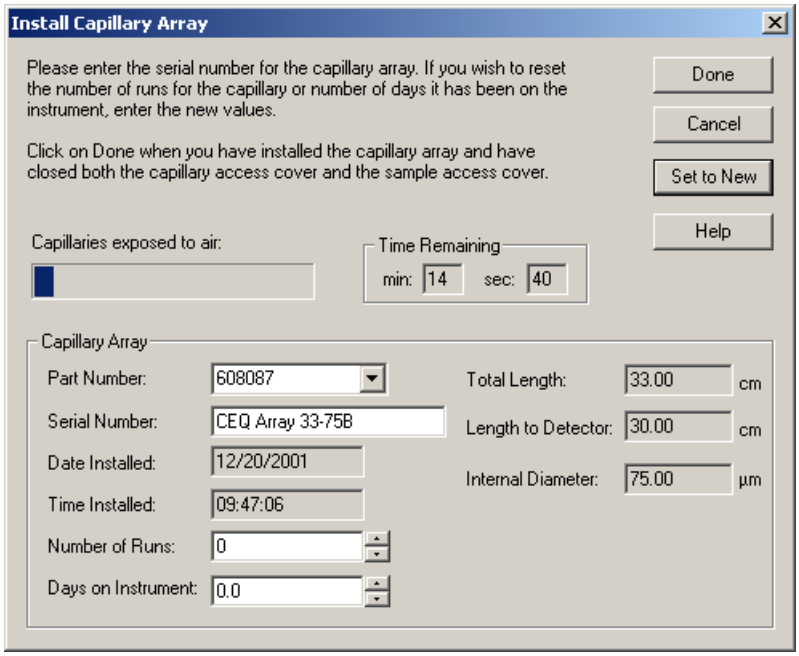


Figure 6: Install Capillary Array dialog box

Capillaries exposed to air — While in the load position, the capillaries are exposed to air. The system will start counting down from fifteen minutes. If you do not load a plate after fifteen minutes has elapsed with the capillaries exposed to air, the capillaries may become damaged.

Time Remaining — The system will start counting down from fifteen minutes after exposing the capillaries to air.

Part Number — Select the part number of the capillary array from the **Part Number** drop-down menu. The 53 cm capillary array (P/N 608015) and the 33 cm capillary array (P/N 608087) are already entered in the system.
If you are installing a new type of capillary array that has never been installed in the CEQ System, click on the **New** icon. Enter the capillary array information in the fields of **New Capillary Array** dialog box, then click on **OK**. This new part number will be available in the drop-down menu for selection in subsequent capillary array installations.

Serial Number — Type the new serial number in the **Serial Number** text box. (The serial number is used to track the capillary array along with the samples that have used the array.)

Date Installed — The date the capillary array was installed.

Time Installed	The time the capillary array was installed.
Number of Runs	If the capillary array has resided on the instrument before, enter the number of runs imparted on the capillary array using the spin control. If the array is new, specify "0."
Days on Instrument	If the capillary array has resided on the instrument before, use the spin control to enter the days the capillary array has been on the instrument. If the array is new, specify "0."

Release Capillary Array

Use this option to remove the current capillary array. If you intend to install a new capillary array, select the **Replace capillary array** radio button. If not, select the **Install manifold plug** radio button. If you are installing a plug, do so and then select **OK**. If you wish to only clean the capillaries, select clean capillaries. See "***Routine Maintenance***", "***Cleaning the Capillary Array***" in the ***User's Guide***.

Remove Capillary Array

Use this dialog box (Figure 7) to specify that you have removed the capillary array.

The manifold plug prevents the gel in the system from drying out.

For instructions on cleaning the capillary windows, refer to your CEQ 8000 User's Guide.

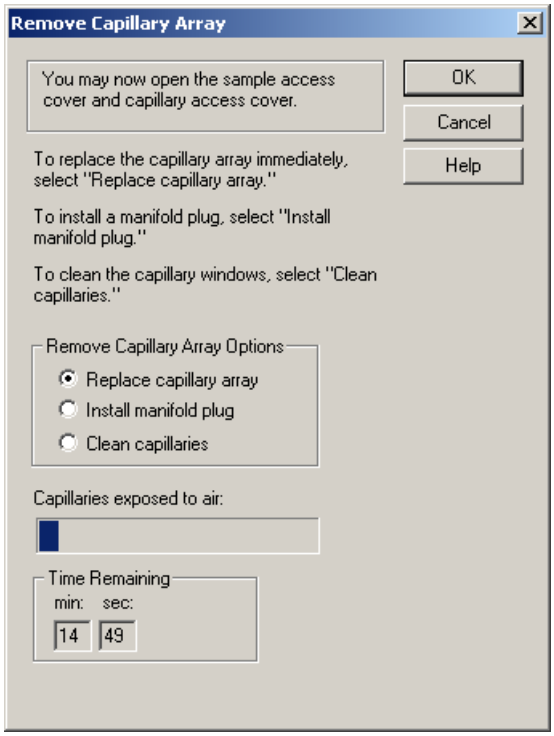


Figure 7: Remove Capillary Array dialog box

Replace capillary array	Select **Replace capillary array** if you are going to immediately replace the capillary array. The **Install Capillary Array** dialog box is displayed.
Install manifold plug	Select **Install manifold plug** if you are not going to replace the array, installing the manifold plug instead. The **Install Manifold Plug** dialog box is displayed.
Clean capillaries	Select **Clean capillaries** if you are going to clean the capillary windows. (This will inform the system to leave the install date instead of changing the install date to the current date automatically.)

Install Manifold Plug

Use this item if you do not plan to install a capillary array immediately. A gel manifold plug must be installed to preserve the gel or capillaries. The **Install Manifold Plug** dialog box (Figure 8) will be displayed. Click on **Done** after you have installed the manifold plug.

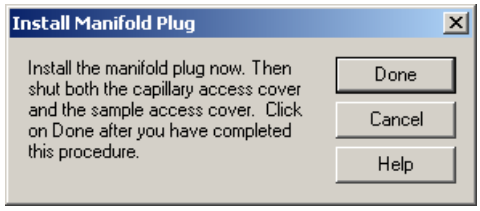


Figure 8: Install Manifold Plug dialog box

Manifold Plug Interface

When removing the manifold plug, acrylamide deposits may accumulate around the manifold plug interface. Gel accumulation may prevent complete contact between the interface and array fitting, resulting in possible pressure leakage. Prior to installing the array fitting, check to see if gel deposits around the interface are present. If gel deposits are present, use a damp cotton swab or kim wipe to gently remove the deposits. Use a dry cotton swab or kim wipe to dry the interface area.

Cleaning the Capillary Array

CAUTION **The capillary array windows must be kept free of any contaminants. Otherwise, high backgrounds and/or drifting baselines will occur. All background counts should be below 6000 RFUs.**

Water used during this procedure must be fresh, distilled, deionized water (18 Mohm/cm water).

1. Select **Replenish | Release Capillary Array** from the Run menu.
2. Open the Sample Access Cover (Figure 4) and lift to the vertical locking position.
3. Open the Capillary Access Cover and lift to the vertical locking position.
4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position.

5. Loosen the Manifold Access Cover captive screw (Figure 9), remove the cover and set it aside.

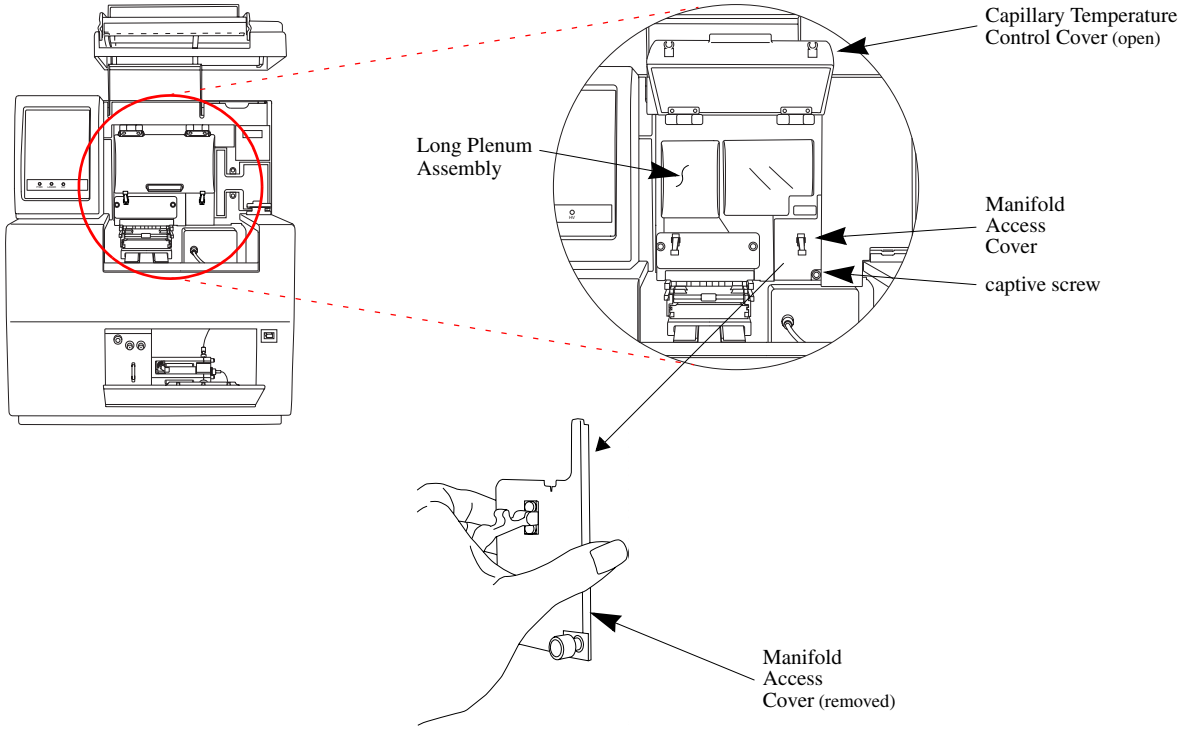


Figure 9: Manifold Access Cover

6. Lift the Eject Lever (Figure 10) to release the Array Fitting.
7. Grasp the Array Fitting tab (Figure 10) and then:
   a. Pull the fitting approximately one inch out of the manifold.
   b. Touch the tip of the fitting to the bottom of the Optics Base Plate.
   c. Hold and wait five seconds for the gel strand to dry.
   d. Pull the fitting away from the instrument.
   e. Use a tissue to wipe gel strands off of the instrument.
8. With the array fitting in hand, blow dust and debris off of the windows with compressed gas (Texwipe Microduster III, P/N: TX2511).
9. Using a water-moistened swab (Texwipe Swab, P/N: TX754B), gently wipe the Detection Window by stroking in one direction *only*.

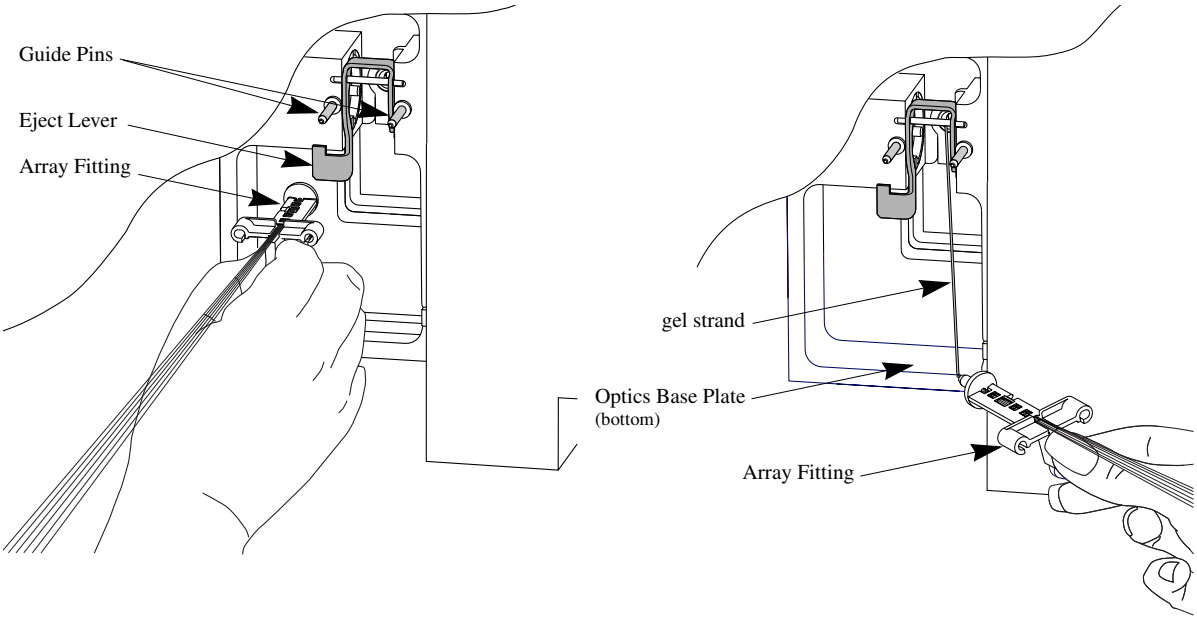


Figure 10: Cleaning the Detection Window

10. With a new water-moistened swab, repeat wiping on the other side of the window.
11. With a dry swab, gently wipe the windows to remove excess water, again repeating on the backside with a new, dry swab.
12. Blow compressed gas on the windows to remove all excess water.

CAUTION **Care must be taken not to invert the compressed gas bottle, otherwise propellant will contaminate the capillary windows.**

13. If dried gel or other debris remains on the window rinse the optical window of the capillary array with deionized water and gently wipe the windows with a dry swab to remove the excess water.
14. While holding the Array Fitting tab (of the clean capillary array), align the Array Fitting with the manifold opening and guide pins. Push the fitting into the manifold until it is completely seated against the bases of the Guide Pins. See Figure 12.
15. Replace the Manifold Access Cover and tighten the captive screw.
16. Lower the Capillary Temperature Control Cover and secure the two rubber latches.
17. Lower the Capillary Access Cover and Sample Access Cover to their locking positions.From the **Remove Capillary** dialog box (Figure 11), select the **Clean Capillaries** radio button and click **OK**.

18. From the **Remove Capillary** dialog box (Figure 11), select the **Clean Capillaries** radio button and click **OK**.

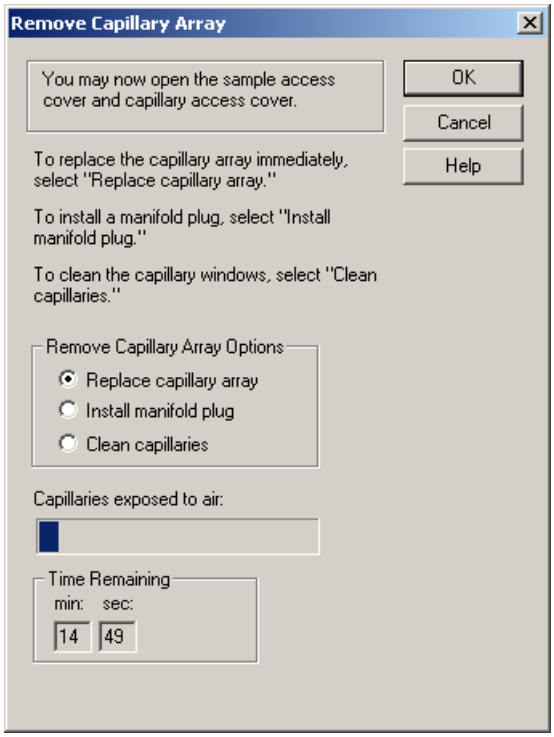


Figure 11: Remove Capillary Dialog

19. From the **Install Capillary Array** dialog box (Figure 12), click **Done** to finish process.

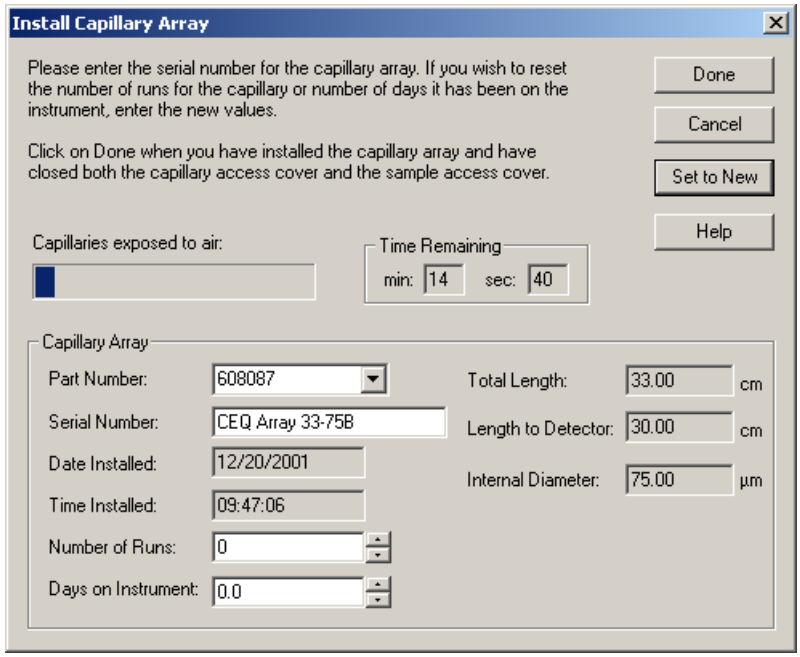


Figure 12: Install Capillary Array Dialog

CAUTION **After cleaning the Detection Windows, perform the Optical Alignment procedure and monitor the baseline. If background levels are above 6000 RFU counts, repeat the cleaning process.**

Wetting Tray

The wetting tray is where the capillaries rest when installed on the CEQ but not in use. The wetting tray should be partially filled with deionized water, approximately ¾ full. When the capillary array is on the instrument, but idle, the wetting tray should be filled once a week. When the instrument is in use, it is recommended that the wetting tray be changed after each run.

Figure 13: Wetting Tray

Cleaning the Wetting Tray

1. Rinse the Wetting Tray with deionized water and dispose of the rinse in the liquid waste container.

Filling the Wetting Tray

CAUTION **No more than one 96-well plate should be processed without replenishing the Wetting Tray.**

Periodically check the liquid level in the wetting tray. Liquid level should NEVER be allowed to rise into the eight cannula recesses of the wetting tray lid, nor drop below the fill level indicator line (9mL minimum). The top surface of the wetting tray lid must remain clean and dry under any and all circumstances.

Replacing the Wetting Tray

Removing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the menu.
2. Open the Sample Access Cover (Figure 4) and lift to the vertical locking position.
3. Remove the Sample Plate and set it aside.
4. Rotate the Wetting Tray Retainers outwards to release the Wetting Tray.
5. Lift the Wetting Tray vertically.

Filling with D.I. Water

1. Remove the lid of the Wetting Station and fill with deionized water to the indicator line.
2. Close the lid.

Installing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the menu.
2. Open the Sample Access Cover (Figure 4) and lift to the vertical locking position.
3. Insert the Wetting Tray into the receptacle between the Sample and Buffer plates (Figure 14) and then gently press it down into the well.
4. Rotate the Wetting Tray Retainers inwards to lock the Wetting Tray in place.

5. Install the Sample Plate.

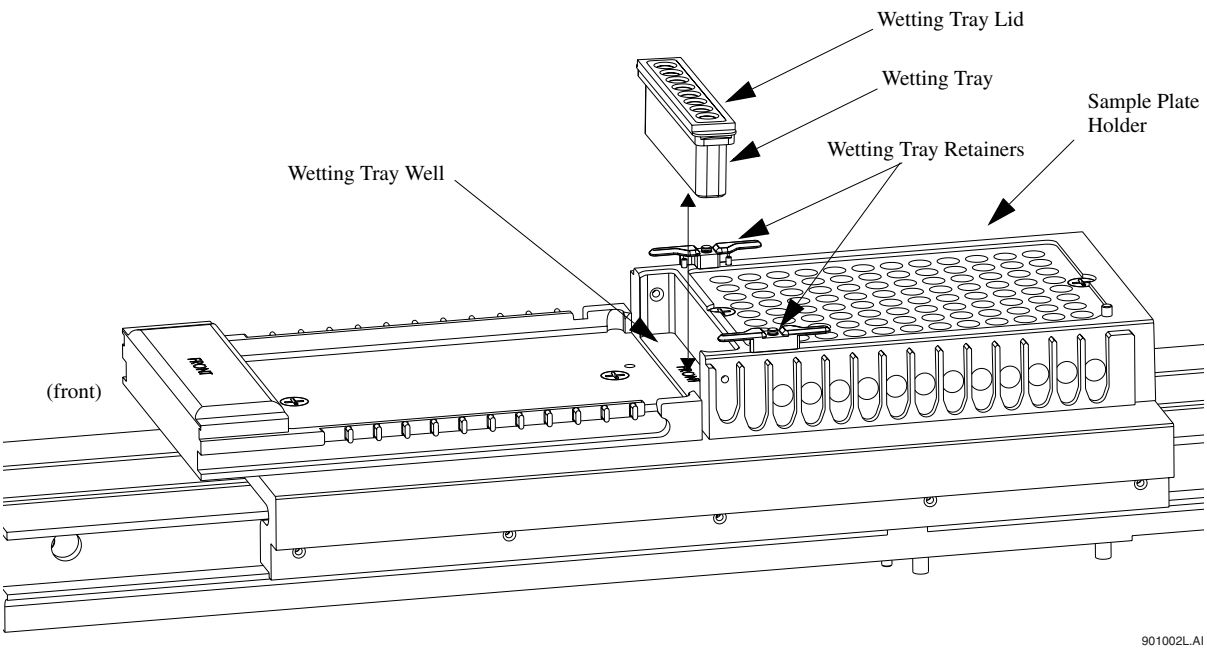


Figure 14: Replacing the Wetting Tray

6. Close the Sample Access Cover and then click the **Done** button of the **Replace Wetting Tray** dialog box (Figure 15).

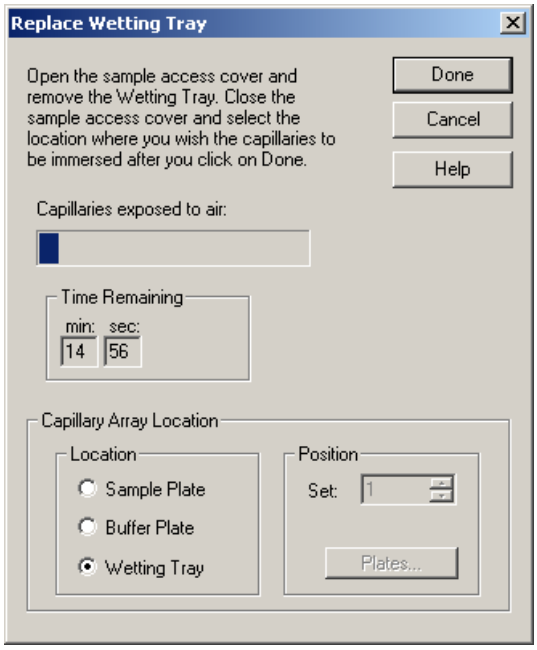


Figure 15: Replace Wetting Tray Dialog

Gel Cartridge

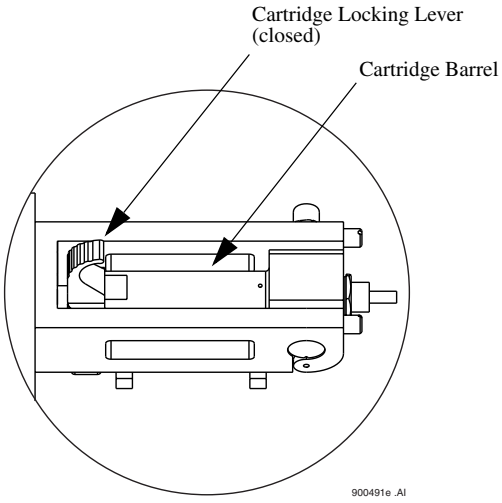


Figure 16: Gel Cartridge

When not in use, insert a gel plug (preferably a used gel cartridge) to prevent drying of the gel system.

Install Gel Cartridge

This menu item is used to install a gel cartridge.

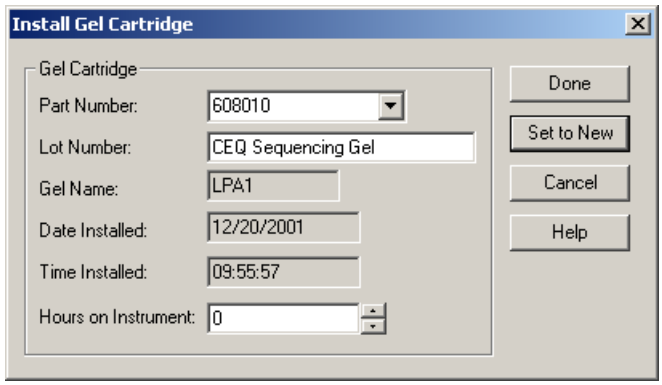


Figure 17: Install Gel Cartridge Dialog

Part Number	Select the part number of the gel cartridge from the drop-down menu.
Lot Number	Type the number in the **Lot Number** text box.
Date Installed	The date the gel cartridge was installed.
Time Installed	The time the gel cartridge was installed.

Hours on instrument	If the gel cartridge has resided on the instrument before, enter the hours it has been on the instrument. If the gel cartridge is new, enter "0." Use the spin control to revert or advance the number of hours. You may also enter hours manually.
Set to New	If the gel cartridge is a new cartridge, enter the new lot number or click on the **Set to New** button.

Release Gel Cartridge

Use this dialog box (Figure 18) to remove the gel cartridge or, if you are not going to install a working gel cartridge, to insert a clean empty cartridge or gel pump plug into the cartridge chamber. The clean empty cartridge or gel pump plug prevents the gel in the system from drying.

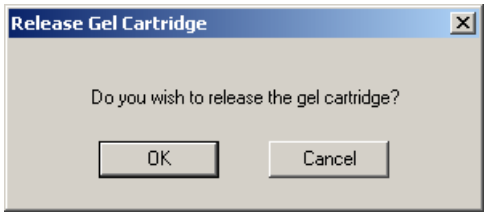


Figure 18: Release Gel Cartridge Dialog

If **OK** is selected, the following dialog box is displayed as the instrument lifts the plunger.

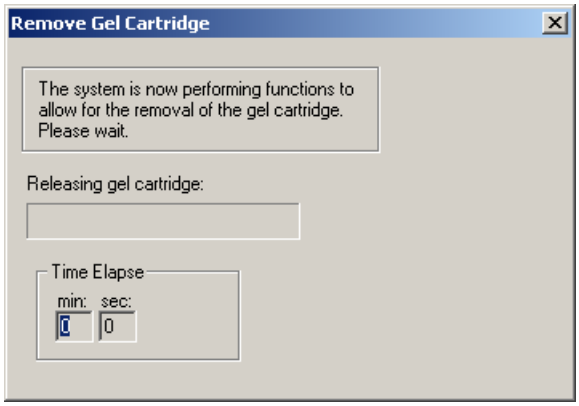


Figure 19: Remove Gel Cartridge Warning Dialog

Once the gel cartridge can be accessed, the **Remove Gel Cartridge** dialog box is displayed.

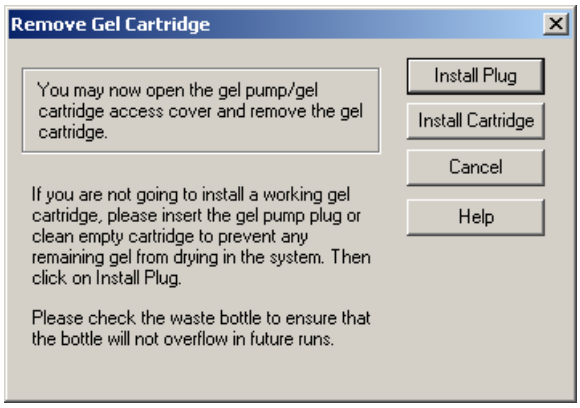


Figure 20: Remove Gel Cartridge Dialog

Install Gel Cartridge Dialog

Use this dialog box to install a gel cartridge.

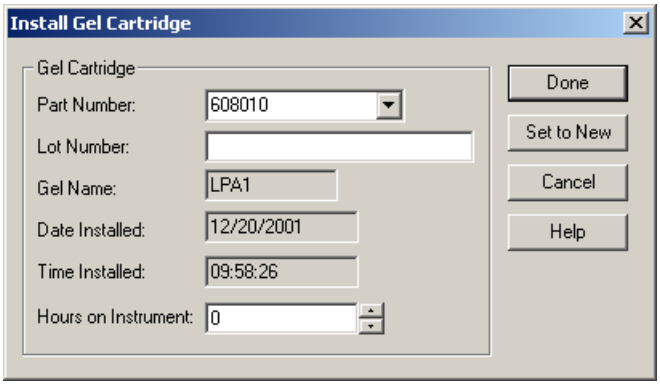


Figure 21: Install Gel Cartridge Dialog

Part Number	Select the part number of the gel cartridge from the **Part Number** drop-down menu.
Lot Number	If the gel cartridge is a new cartridge, enter the new part number and lot number or click on the **Set to New** button.

Date Installed and Time Installed	The system will automatically update the date and time of the gel cartridge installation.
Hours on Instrument	If the cartridge is new, click on **Set to New** and the hours on the instrument text box will revert to "0." If you are installing the previous gel cartridge, do not change the lot number or the hours on the instrument, as they will be correct. If you are installing a gel cartridge that is not new, or the previous cartridge, enter its lot number, and adjust the hours it has been on the instrument based on its prior instrument life.

The lot number is an alphanumeric text box for your own identification purposes.

When removing a gel cartridge, always note the lot number and the hours on the instrument if you are planning on using it for more than one session.

WARNING **If the gel cartridge has been on the instrument for more than 72 hours, it is likely that the gel will produce undesirable results.**

Waste Bottle

Figure 22: Waste bottle

The waste bottle accumulates all of the gel that is used during a manifold purge. Periodically check the volume on waste. When the waste bottle becomes more than half full, remove it and place a new waste bottle in its place.

Replacing the Gel Waste Bottle

This procedure assumes that a used (more than half full) waste bottle is being replaced with an empty waste bottle.

It is recommended to replace gel waste while carrying out sample plate loading or unloading operations to prevent activating the Sample Access Cover alarm.

1. Remove the cap from the new waste bottle.
2. Open the Sample Access Cover (Figure 4) and lift to the vertical locking position.
3. Remove the cap from the used waste bottle and pull the bottle out of the instrument.
4. Place the cap from the new bottle over the used waste bottle and secure.
5. Thread the new bottle onto the cap attached to the instrument and set the bottle into position.
6. Close the Sample Access Cover.
7. Dispose of the full waste bottle according to procedures.

Consumables

Below are the consumables and storage conditions required for DNA sequencing on the CEQ 8000.

Consumables List

Table 1 provides a list of the required consumable items for the Sequence Analysis system.

Table 1: Consumable Items Required for Sequence Analysis

Item	P/N	QTY	Description	on Instrument Life	Shelf Life
DTCS Kit (DNA Sequencing RXN Kit)	608000	1	Dye Terminator Cycle Sequencing Kit for 96 reactions. Includes: • DNA polymerase • CEQ Dye Terminators (ddUTP, ddGTP, ddCTP, ddATP) • dNTP Mix Solution • Sequencing Reaction Buffer • pUC18 Control Template • -47 Sequencing Primer • Glycogen • Mineral Oil • SLS	N/A	1 year @ –20°C (frost-free freezer)
			or		
DTCS Quick Start Kit	608120	1	DTCS Quick Start Kit includes: • DTCS Quick Start Master Mix • pUC18 Control Template • -47 Sequencing primer • Glycogen • Mineral Oil • SLS	N/A	1 year @ –20°C (frost-free freezer)

Item	P/N	QTY	Description	on Instrument Life	Shelf Life
CEQ Separation Gel I	608010	1	11.5mL of gel in CEQ 8000 compatible container. Sufficient for 12 runs (96 samples).	3 Days	8 months @ 4°–6°C
CEQ Separation Buffer	608012	1	Each container has a screw top and pour tip. The container has enough buffer (30mL) to fill a CEQ System 96-well, flat bottom Buffer Plate. (Each well being ¾ full.) 4-Pack	fresh, each run	1 year @ 4°–6°C
DNA Separation Capillary Array 33–75B	608087	1	Eight capillaries, 75µm i.d., 33 cm long, 200 o.d. complete with electrode block and detector array fitting. Ready for installation into CEQ 8000.	4 weeks	8 months @ 4°–6°C

Materials that are provided by Beckman-Coulter:

Sample Microtiter Plates (P/N 608801)

96 Well Plates for sequencing buffer (P/N 373660)

Materials required but not provided by Beckman-Coulter, Inc.:

sterile dH_2O	Molecular Biology Grade
95% (v/v) ethanol/dH_2O,	store at -20°C
70% (v/v) ethanol/dH_2O	store at -20°C
3M Sodium Acetate, pH 5.2	Sigma, Cat # 430771 store at room temperature
100mM Na_2-EDTA, pH 8.0	store at room temperature
Sterile 0.5mL microcentrifuge tubes	
0.2mL thin wall thermal cycling tubes or plates	
Thermal cycler with heated lid	
Texwipe Swabs	P/N: TX754B (VWR)
Texwipe Microduster III	P/N: TX2511B (VWR)
Sterile H_2O	store at room temperature

SKILL CHECK

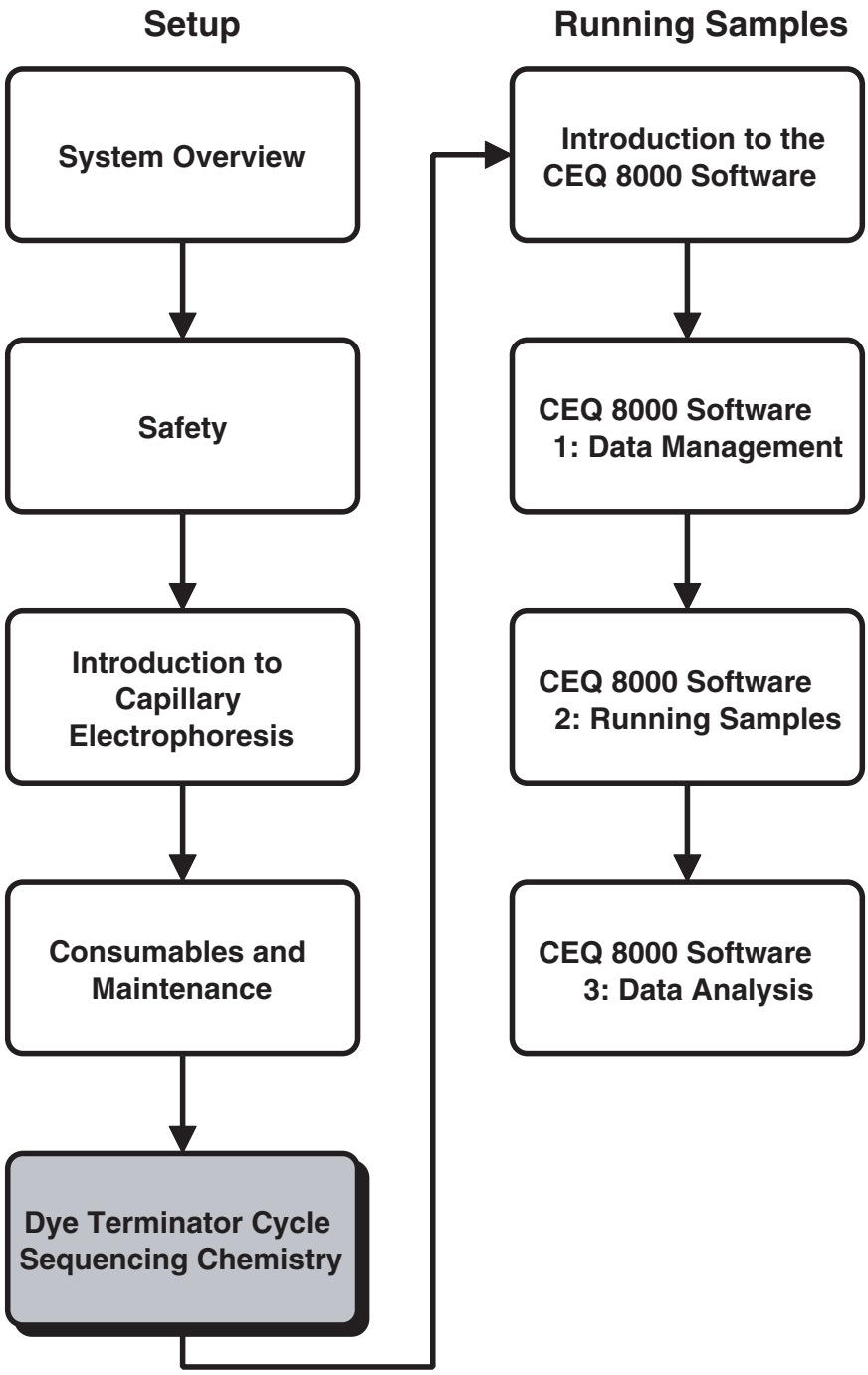
After reviewing the CEQ 8000 Maintenance Overview and Consumables Installation portion of the training, you should have a working knowledge of the following:

- Storage condition of consumables required but not supplied by Beckman Coulter, Inc.
- Storage condition of consumables provided by Beckman Coulter, Inc.
- The proper installation and removal of the capillary array, gel cartridge and waste bottle.

SUMMARY

Understanding the proper methods for storing consumables will ensure consistent results. Before storing consumables, take time to designate which ones will be stored under what conditions. Failure to consider these storage requirements may result in unacceptable sequencing results and possible system damages.

5 Dye Terminator Cycle Sequencing Chemistry



OVERVIEW

This section considers DNA sample preparation using DTCS Kit P/N (608000). We will discuss:

- Different DNA template purification methods and satisfactory commercial minipreps
- The importance of the quantitation and verification of DNA
- Specific primer design considerations
- Setting up your sequencing reaction
  - Making a master mix
  - Determining the correct amount of DNA and primer per reaction
  - Setting up the reaction with all components
- Thermal cycling conditions and possible modifications
- Post-Reaction Clean-up
  - Working knowledge of the different clean-up methods
- Resuspension of your product
- Skill Check

This protocol represents the DTCS kit instructions. Please contact your Beckman Coulter, Inc. representative or* www.Beckman.com *for the latest version of DTCS Kit Instruction.

Dye Terminator Cycle Sequencing Chemistry

The reaction mixture of the dye terminator cycle sequencing chemistry includes your DNA template, polymerase, dNTP mix, primer, individual dye conjugated ddNTP's, reaction buffer and water. The Thermal Cycling Method includes three main steps: Denaturation, Annealing, and Extension.

SAMPLE PREPARATION

Template Preparation

Template preparation is probably the most critical factor in obtaining good sequence data from the CEQ 8000. The kits listed below have been used successfully for a number of different templates. We strongly recommend the use of agarose gels to check the quality of DNA before starting the sequencing reaction.

Purification of DNA Template:

- The QIAGEN Qiawell and Qiaprep DNA isolation kits are recommended (dsDNA and ssDNA)

Other Purification kits:

- Promega Wizard
- Boehringer Mannheim - High Pure Plasmid Isolation Kit
- BioRad Plasmid Miniprep Kit
- 5'–3' Perfectprep Plasmid DNA Kit
- Viogenic Mini - M Plasmid DNA Miniprep System
- Bio 101 RPM kit
- Standard Alkaline Lysis Preps (**NO PHENOL**!)

CsCl Preps have been found to cause low signal and current instability problems.

For PCR products:

- Qiagen QIAquick PCR purification kit is recommended
- PCR products must be homogeneous

Resuspension of Purified Products:

- The purified DNA template should be resuspended in sterile deionized H_2O or 10mM Tris-HCl pH 8.5

Notes:

- Do not resuspend in TE or any reagent with EDTA

Template Quantitation:

For Commercial Minipreps:

- Readings should be taken at wavelengths of 260nm and 280nm. Pure preparations of DNA should have an OD260/OD280 ratio of 1.8 to 2.0.
- Make sure there is NO RNA in the DNA preparation.

For Manual Minipreps:

- Quantitation should be performed by gel electrophoresis and run with a known standard.

For PCR products:

- PCR products should be quantitated and verified by gel electrophoresis and run with a known standard.

Primer Consideration

Primer design is critical to good sequencing. The conditions for thermal cycling listed in the DTCS protocol work well for the -47 primer and other universal primers. Some primers may require change in the cycling conditions for optimal performance. Since other primers (e.g. SP6, T3, T7, -21 primer) are shorter and may have a less than optimal T_m, sequencing protocols using these primers may require a lower annealing temperature in the cycle sequencing (thermal cycling) protocol.

When designing your primers, there are several conditions that should be considered:

- Have a size of 20-24 bases
- GC content should be approximately 50%
- T_m should be greater than 50°C with an optimum of 60°C
- Avoid primer dimerization (for primer design, we recommend OLIGO 6.0 software)
- Purifications by HPLC or OCP columns is highly recommended
- Working primer stock of 3–5µM is satisfactory for the DTCS chemistry

Notes:

Setting Up Your Sequencing Reaction

DTCS Master Mix

It is recommended to make a master premix with the components of the DTCS sequencing kit. This master mix may be aliquoted and frozen. One kit can provide for 100 sequencing reactions.

Figure 23: CEQ DTCS Kit

Prepare sequencing reactions in a 0.2mL thin wall PCR tubes, tube strips or 96-well PCR plate.

All reactants should be kept on ice while preparing the sequencing reactions and should be vortexed and spun down prior to use.

Notes:

Making Premix:

Component	Vol. needed for 100 reactions
10X sequencing buffer:	200.0µL
dNTP mix:	100.0µL
ddUTP Dye Terminator:	200.0µL
ddGTP Dye Terminator:	200.0µL
ddCTP Dye Terminator:	200.0µL
ddATP Dye Terminator:	200.0µL
DNA polymerase:	100.0µL
TOTAL amount:	1200.0µL

Mix the reaction components thoroughly. Consolidate the liquid to the bottom of the tube by briefly centrifuging. Aliquot 100µL in to microcentrifuge tubes and store at –20°C. **Use 12µL of the mix per reaction**. A 100 µL aliquot is enough for eight sequence runs.

Notes:

DNA Template Amount

Find the amount of DNA template needed for your sequencing reaction by using the following two tables.

DNA Template	0.5–6.0µL

The DNA should be in water or 10mM Tris-HCl pH 8.5 EDTA should not be present, as it can inhibit the sequencing reaction.

The table below gives suitable template quantities for sequencing reactions according to DNA type.

dsDNA	50–100fmol
ssDNA	25–50fmol
Purified PCR product	25–100fmol

Notes:

Shown below is a table correlating length and amounts for dsDNA.

Table 2: Estimating the dsDNA concentration**

Size (kilobase pairs)	ng for 25 fmol	ng for 50 fmol	ng for 100 fmol
0.2	3.3	6.5	13
0.3	4.9	9.8	20
0.4	6.5	13	26
0.5	8.1	16	33
1.0	16	33	65
2.0	33	65	130
3.0	50	100	195
4.0	65	130	260
5.0	80	165	325
6.0	100	195	390
8.0	130	260	520
10.0	165	325	650
12.0	195	390	780
14.0	230	455	910
16.0	260	520	1040
18.0	295	585	1170
20.0	325	650	1300
48.5	790	1500†	1500†

** For ssDNA, the values (ng) should be divided by 2.
† Do not use more than 1.5µg of template DNA.

Notes:

Primer Amount

Determine the amount of primer needed in your sequencing reaction.

- The optimum ratio for dsDNA is greater than or equal to 40:1 primer:template.
- Higher ratios may improve the quality of the sequencing results in some cases.

Custom Primer	(3–5µM stock) 2.0µL
-47 sequencing primer (in kit)	(1.6pMol/µL) 2.0µL

H_2O (non-DEPC treated)

The total reaction volume should be adjusted to 20µL with water

H_2O	xxµL

PreHeat Treatment

For certain plasmid DNA templates, the following preheat treatment improves both signal strength and current stability. Preheating the sample prior to adding the primer and sequencing reactants causes nicking of the DNA. This helps produce a more efficient cycle sequencing reaction.

To preheat:

1. Combine water and template to a volume of 6.0µL
2. Heat the template at 96°C for one minute and then cool to room temperature.

If the RFU signal profile declines steeply when using this treatment, change the heating conditions to 86°C for 5 minutes. If the current is low or unstable following this treatment, increase the treatment to 96° for 3 minutes.

Notes:

Preparing your sequencing reaction using the DTCS Standard Kit:

dH_2O (to adjust total volume to 20µL)	XµL
DNA template†	0.5–6.0µL
Primer	2.0µL
Master Mix	12.0µL
Total	20.0µL

† Use 0.5 µL for pUC18 control template

Preparing your sequencing reaction using DTCS Quick Start Kit

dH_2O (to adjust total volume to 20µL	0–9.5µL
DNA template† (see Template Preparation)	0.5–10.0µL
Customer supplied or M13(-47) Sequencing Primer (1.6pmol/µL or 1.6µM))	2.0µL
DTCS Quick Start Master Mix	8.0µL
Total	20.0µL

† Use 0.5 µL for pUC18 control template

Mix reaction components thoroughly. Consolidate the liquid in the bottom of the tube or well by briefly centrifuging before thermal cycling.

- Always run a pUC control sample (included in DTCS kit) with your samples.
- After combining in 0.2mL tube or thermal cycling plate, cover, and mix gently by vortexing.
- Spin down.
- Place in thermal cycler.

Thermal Cycling Conditions

The following cycling parameters were optimized for the control template and primer, but should work in most other cases:

96°C	20 sec.
50°C	20 sec.
60°C	4 min.

For 30 cycles followed by holding at 4°C

- The cycling conditions can be modified based on the primer or template.
  - The annealing temperature should be dictated be the primer's T_m and logically cannot exceed the extension temperature.
  - A two step cycle, which combines the annealing and extension steps, has been shown to work for certain primer-template combinations. The two step cycling procedure is only applicable when the T_m for the primer is at least 5°C higher than the extension temperature.
  - The 4 minute extension time can be reduced for very clean plasmid templates or short PCR products that yield sufficiently high signal on the CEQ 8000. We suggest that the extension time not be reduced lower than one minute.
  - The number of cycles is optimized to give the best results for time spent cycling versus amount of signal generated. Increasing the cycles with increased primer may help in certain cases where low signal is a problem.
- Use a thermal cycler with a heated lid. Removing sample from an oil overlay can give rise to sample losses.
- Thermal cycling conditions have been shown to work well for MJ Research PTC 200 and 100 models and for PE 2400, 9600 and 9700 models. The use of other models may involve the modification of conditions.

Notes:

Post Reaction Cleanup

We recommend Ethanol Precipitation for post-sequencing reaction clean-up. Other methods can be used but should result in lower signal yields.

- Sephadex G50 in the Millipore Multiscreen plate
- Edge Biosystems AGTC filtration block
- Princeton Separations CentriSep 96 filter plates

The following protocol is for single tube Ethanol Precipitation:

1. Prepare a labeled sterile 0.5mL microcentrifuge tube for each sample.
2. To each labeled tube, add 4µL Stop Solution (equal volumes of 3M NaOAc pH 5.2 + 100mM EDTA pH 8.0 prepared daily from stock solutions previously listed) and 1µL of 20mg/mL glycogen (supplied with the kit).

A stop solution "premix" which includes the glycogen may alternatively be made just prior to starting the sample cleanup as follows (amounts below are per row of 8 samples):

- 20µL 3M NaOAc pH 5.2
- 20µL 100mM EDTA pH 8.0
- 10µL Glycogen

Pipette 5µL of this stop solution premix into each sample tube before transferring your sequencing reactions into them.

Make sure that the stop solution is prepared immediately before use and kept at room temperature. If the stop solution is chilled or is left at room temperature for too long the EDTA will precipitate. The glycogen should be stored at –20°C with the kit.

The salt and glycogen facilitate precipitation of the DNA from the sequencing reactions. Precipitation will be inefficient without them and will lead to low signal and potential color imbalance (low A signal may be observed) when run on the CEQ.

3. Transfer the sequencing reactions to the appropriately labeled tube and mix thoroughly. All components must be mixed for efficient precipitation.

Notes:

4. Add 60µL cold 95% (v/v) ethanol/water from –20°C freezer and mix thoroughly. Immediately centrifuge at 14,000rpm at 4°C for 15 minutes. Carefully remove the supernatant with a micropipette (the pellet should be visible).

   For multiple samples, always add the cold ethanol/water immediately before centrifugation.

   Thorough mixing is very important at this stage, otherwise, precipitation will be less than optimal and could lead to low signal from the CEQ. Do not leave the samples for extended periods of time before centrifuging because this will precipitate some of the salt and dye terminators that the ethanol precipitation is designed to eliminate. Leaving samples at –20°C following addition of the ethanol may also have a detrimental effect. Ideally, the samples should be centrifuged immediately following addition of the ethanol.
5. Rinse the pellet two times with 200µL 70% (v/v) ethanol/water from –20°C freezer. For each rinse, add the cold ethanol (***do NOT mix***) and centrifuge immediately at 14,000rpm at 4°C for a minimum of 2 minutes. After centrifugation, carefully remove all of the supernatant with a micropipette.

   The purpose of the rinses is to remove the residual salts that remain after removing the supernatant from the precipitation step. Since salts are preferentially loaded by electrokinetic injection on the CEQ, fewer sequencing fragments will be loaded. As the salts are removed, the pellet may become invisible. Do not disturb the pellet when adding the 70% ethanol, this will cause the pellet to dislodge and could lead to the entire sample being lost during the processing of the sample. Simply allowing the 70% ethanol to cover the pellet is sufficient to reduce the salt remaining in the sample.

The following protocol is for Ethanol Plate Precipitation in a CEQ sample plate:

1. Spin down the CEQ sample plate after thermal cycling at maximum speed for 30 seconds to ensure that any condensation is at the bottom of the well.
2. To each well add 4ul Stop Solution (equal volumes of 3M NaOAc pH 5.2 + 100mM EDTA pH 8.0 prepared daily from stock solutions previously listed) and 1ul of 20ug/µL glycogen (supplied with kit).

Notes:

A stop solution "premix" which includes the glycogen may alternatively be made just prior to starting the sample cleanup as follows (amounts below are per row of 8 samples):

- 20µL 3M NaOAc pH 5.2
- 20µL 100mM EDTA pH 8.0
- 10µL Glycogen

Pipette 5µL of this stop solution premix into each well before transferring your sequencing reactions into them.

Make sure the stop solution is prepared immediately before use and kept at room temperature. If the stop solution is chilled or is left at room temperature for too long the EDTA will precipitate. The glycogen should be stored at –20°C with the kit.

The salt and glycogen facilitate precipitation of the DNA from the sequencing reactions. Precipitation will be inefficient without them and will lead to low signal and potential color imbalance (low A signal may be observed) when run on the CEQ.

3. Add 60µl cold 95% (v/v) ethanol/water from –20°C freezer and cover plate with Seal and Sample Aluminum Foil Lids (Beckman Coulter, Inc. P/N 538619). Invert the plate 5–10 times vigorously or vortex plate.
4. Immediately centrifuge the plate according to the following chart:

Centrifuge	Rotor	G	RPM	Time
Beckman Allegra 25R	S5700	5,957	5,700	2 minutes
Beckman Allegra 25R	Microplate Carrier	3,007	4,100	10 minutes
Beckman GS-15R	S2096	1,107	3,000	30 minutes

For multiple samples, always add the cold ethanol/water immediately before centrifugation.

Notes:

Thorough mixing is very important at this stage, otherwise, precipitation will be less than optimal and could lead to low signal on the CEQ. Do not leave the samples for extended periods of time before centrifuging because this will precipitate some of the salt and dye terminators that the ethanol precipitation is designed to eliminate. Leaving samples at –20°C following the addition of the ethanol may also have a detrimental effect. Ideally, the samples should be centrifuged immediately following addition of the ethanol.

5. After centrifugation remove the plate and place 3–4 folds of paper towels on the centrifuge plate holder. Carefully remove the foil lid and gently invert the plate to remove the supernatant. Do not turn the plate right side up before spinning as this may disrupt the DNA pellets.
6. Place the inverted plate onto the paper towel lined plate holder and spin at 300 rpm for 20 seconds.
7. Rinse the DNA pellet with 200µl 70% (v/v) ethanol/water from –20°C freezer. **DO NOT** mix or vortex the plate.

The purpose of the rinses is to remove the residual salts that remain after removing the supernatant from the precipitation step. Since salts are preferentially loaded by electrokinetic injection on the CEQ, fewer sequencing fragments will be loaded. As the salts are removed, the pellet may become invisible. Do not disturb the pellets when adding the 70% ethanol, this will cause the pellets to dislodge and could lead to the entire sample being lost during the processing of the sample. Simply allowing the 70% ethanol to cover the pellets is sufficient to reduce the salt remaining in the sample.

8. Immediately centrifuge the plate according to the following chart:

Centrifuge	Rotor	G	RPM	Time
Beckman Allegra 25R	S5700	5,957	5,700	2 minutes
Beckman Allegra 25R	Microplate Carrier	3,007	4,100	3 minutes
Beckman GS-15R	S2096	1,107	3,000	5 minutes

9. After centrifugation gently invert the plate to remove the supernatant. Place the inverted plate onto the paper towel lined plate holder and spin at 300 rpm for 20 seconds.

Notes:

10. Repeat the rinse (steps 7–9).
11. Vacuum dry the samples for 10 minutes. If you are not using a speed vac with a plate rotor to dry the DNA pellets, be careful to apply and release the vacuum slowly.

Resuspension of Samples

- Resuspend your samples by adding 40µL of sample loading solution (SLS), wait 10 minutes and then gently vortex and spin.
- Add one drop of mineral oil to the top of every sample.

Notes:

SKILL CHECK

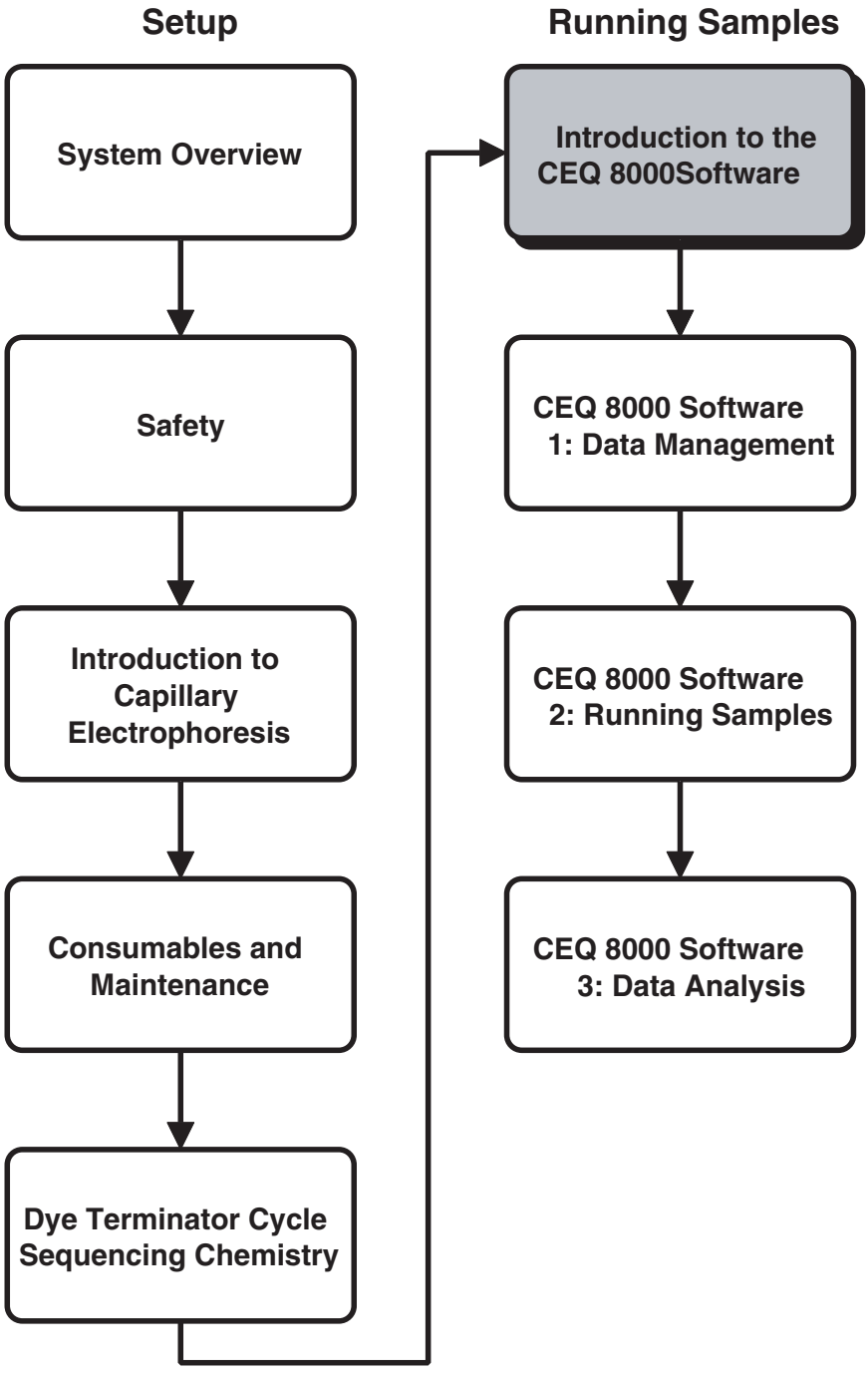
After completing the Sample Setup portion of the training, you should have a working knowledge of the following:

- Different DNA template purification methods and satisfactory commercial minipreps
- The importance of the quantitation and verification of DNA
- Specific primer design considerations
- Setting up your sequencing reaction
  - Making a master mix
  - Determining the correct amount of DNA and primer per reaction
  - Setting up the reaction with all components
- Thermal cycling conditions and possible modifications
- Post-Reaction Cleanup
  - Working knowledge of the different cleanup methods
- Resuspension of your product

SUMMARY

The key to sequencing success on the CEQ 8000 is ultimately DNA template preparation, purification and primer design. Be sure to follow the current protocols included in the DTCS Kit.

6 Introduction to the CEQ 8000 Software



OVERVIEW

We will discuss:

- CEQ software organization
- Work flow through the software modules
- General purpose of each module

Software Module Descriptions

The software subsystem provides the interface for direct or preprogrammed control of the system. The software also provides for data capture and analysis. Software terminology and the main modules of the program are discussed below.

Main Menu

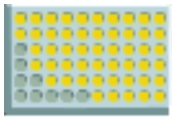
The CEQ 8000 Main Menu provides access to all six modules. Each of the icons and their associated modules are described in Table 3.

Figure 24: Main Menu

If the Shortcut Bar (Figure 25) is displayed, single mouse click on the appropriate module.

Figure 25: Shortcut Bar

Table 3: Software Module Descriptions

Module	Description
	***Sample Setup Module*** Use this module to create, save and modify sample plates. Sample plates are used to assign methods to control sample sets and determine the sequence of methods that will be used to produce data.
	***Run Module*** Use this module to run sample plates and control individual functions of the instrument.
	***Sequence Analysis Module*** Use the Sequence Analysis module to view, analyze, compare, manipulate and print base sequence data produced by sample runs.
	***CEQuence Investigator Module*** This icon accesses the CEQuence Investigator module. This module is used to compare sequence(s) to create a consensus that will then be compared against a reference.
	***Fragment Analysis Module*** Use the Fragment Analysis module to view, analyze, compare, manipulate and print fragment data produced by sample runs.

Module	Description
	***Data Manager Module*** Use this module to modify and print database items.
	***Exit*** When selected, the Exit icon closes any active modules and shuts down the CEQ 8000 System software.

Sample Setup Module

The Sample Setup module is used to create, save, modify and print sample plates.

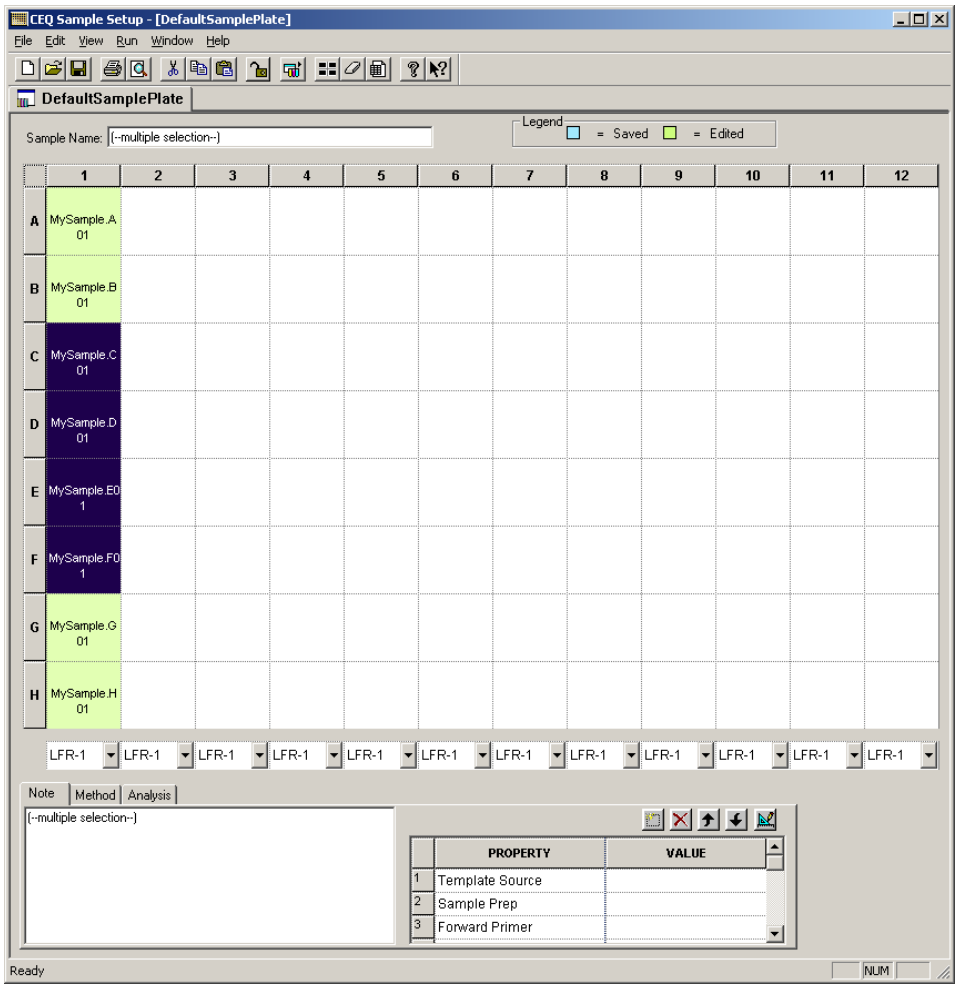


Figure 26: Sample Setup Main Window

Notes:

Run Module

The Run module window allows you to execute preprogrammed sample plates and control individual functions of the software.

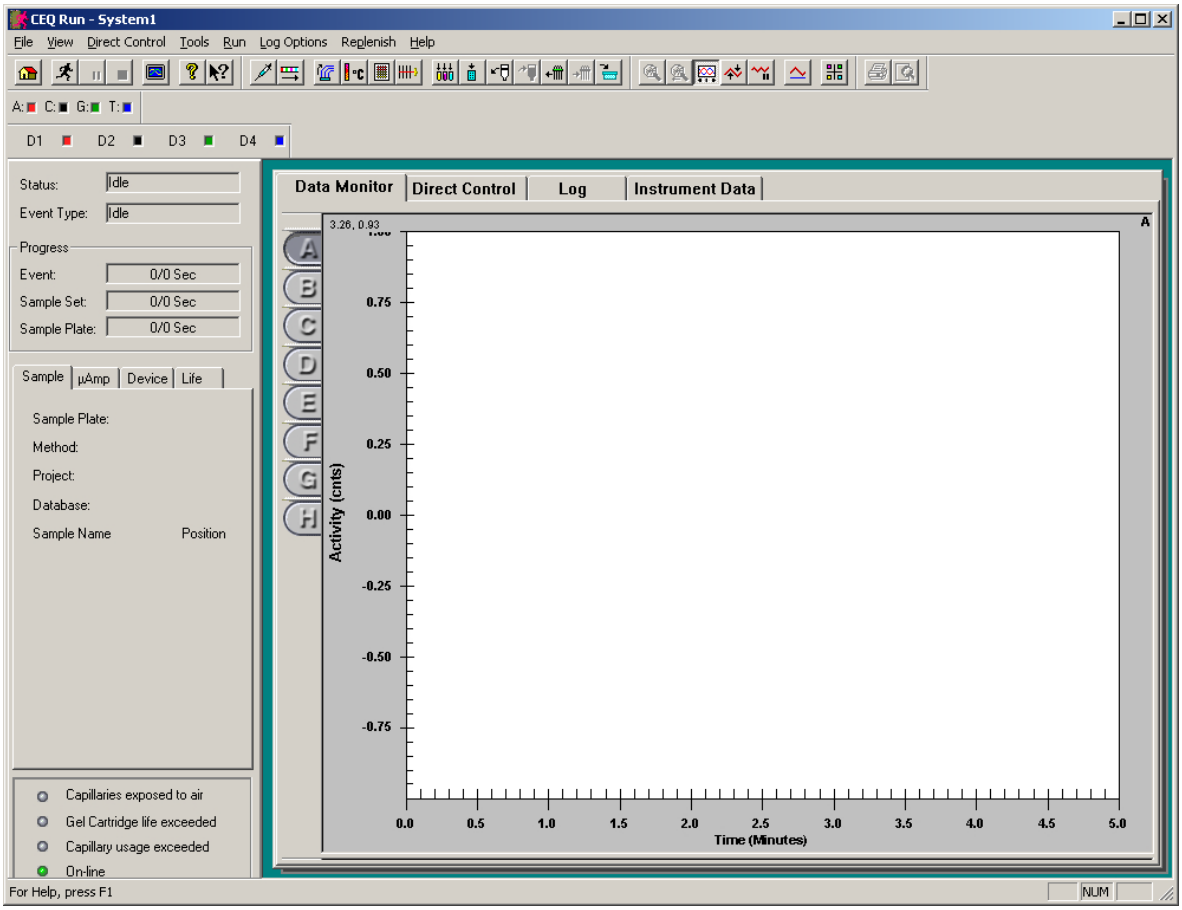


Figure 27: Run Module Main Window

Notes:

Sequence Analysis Module

This session describes how to use the Sequence Analysis module to view, analyze, compare, manipulate and print data of the following types:

- Raw Data*
- Current Data*
- Voltage Data*
- Analyzed Data
- Base Sequences
- Optical Scan Data*
- Baseline Data*
- Quality Parameters*

* These types of data cannot be manipulated.

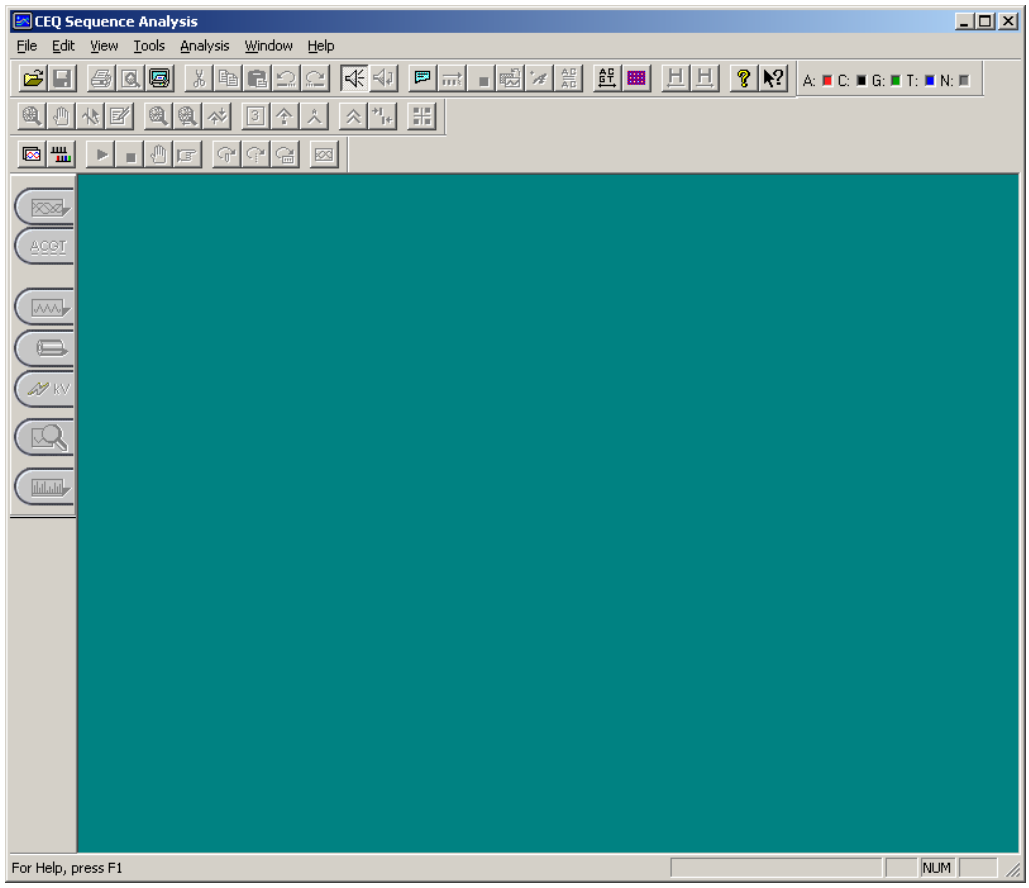


Figure 28: Sequence Analysis Module Main Window

Notes:

Data Manager Module

The Data Manager module is used to create, save and modify databases containing:

- Fragment Analysis Parameters
- Fragment Results
- Locus Tags
- Methods
- Optical Scan Data
- Sample Data
- Sample Plates
- Sample Plate Results
- Sequence Analysis Parameters
- Sequence Results
- SNP Locus Tags
- Standards

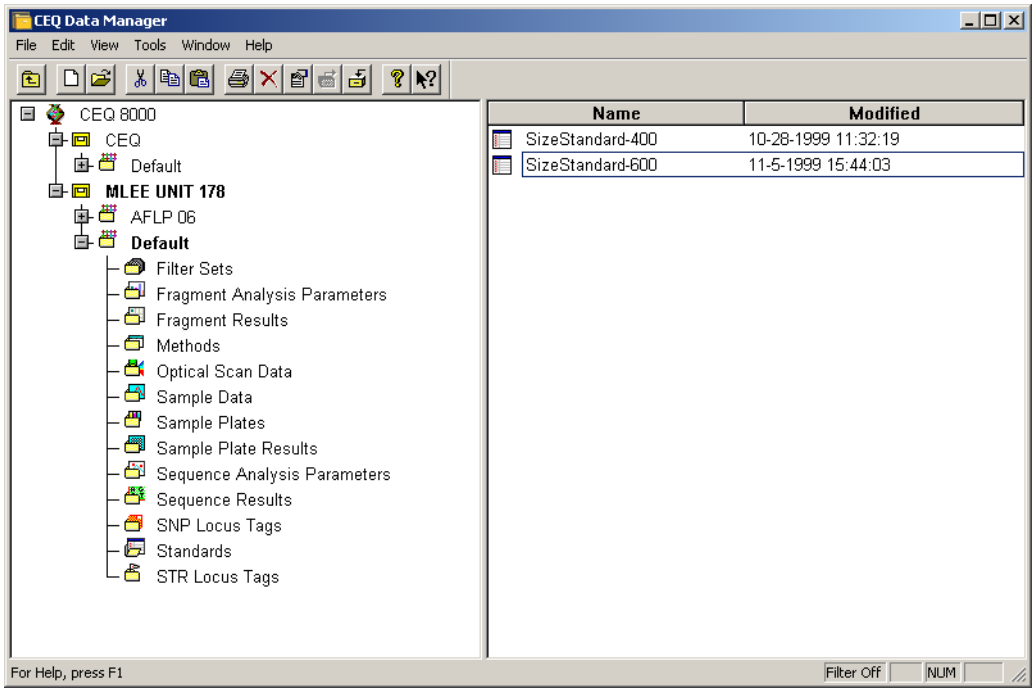


Figure 29: Data Manager Module Main Window

Notes:

Sequence Investigator Module

CEQuence Investigator is a new software module for comparing sequences to known reference sequences. For each comparison, one or two new sequence results, in either orientation, are used to form a consensus for the comparison. The software automatically determines and matches the orientation of the sequences, forms the consensus, aligns the consensus with the reference sequence, and provides a detailed report of the differences.

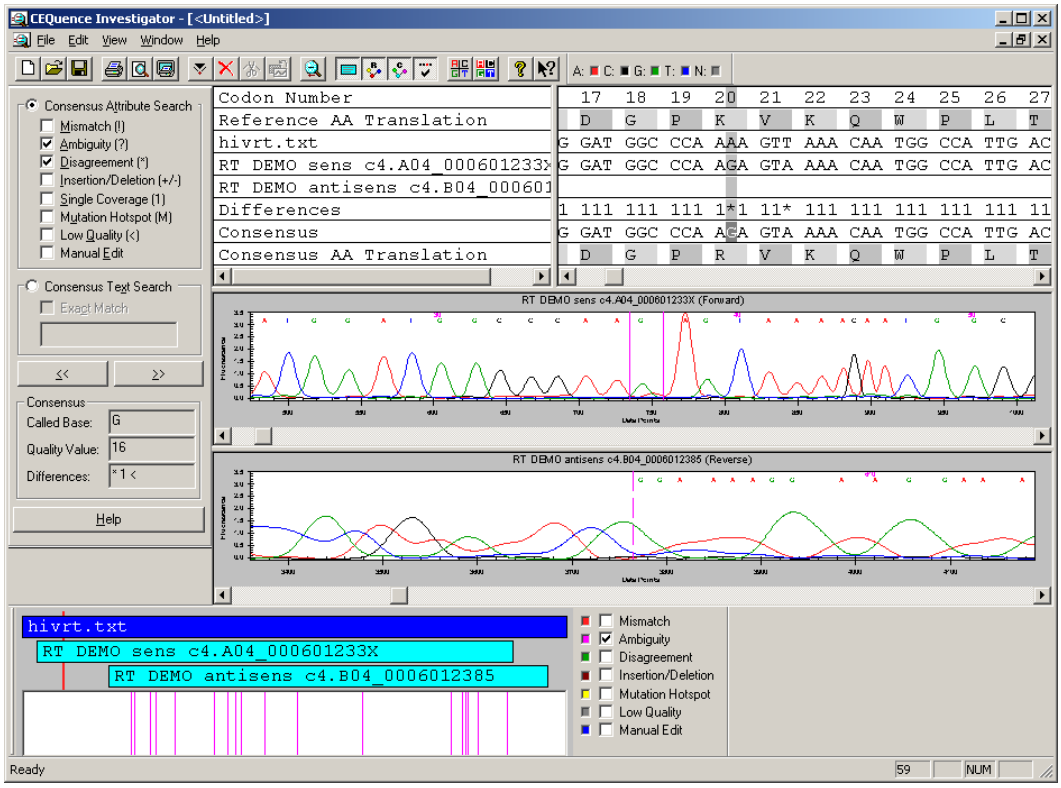


Figure 30: Sequence Investigator Module Main Window

Notes:

SKILL CHECK

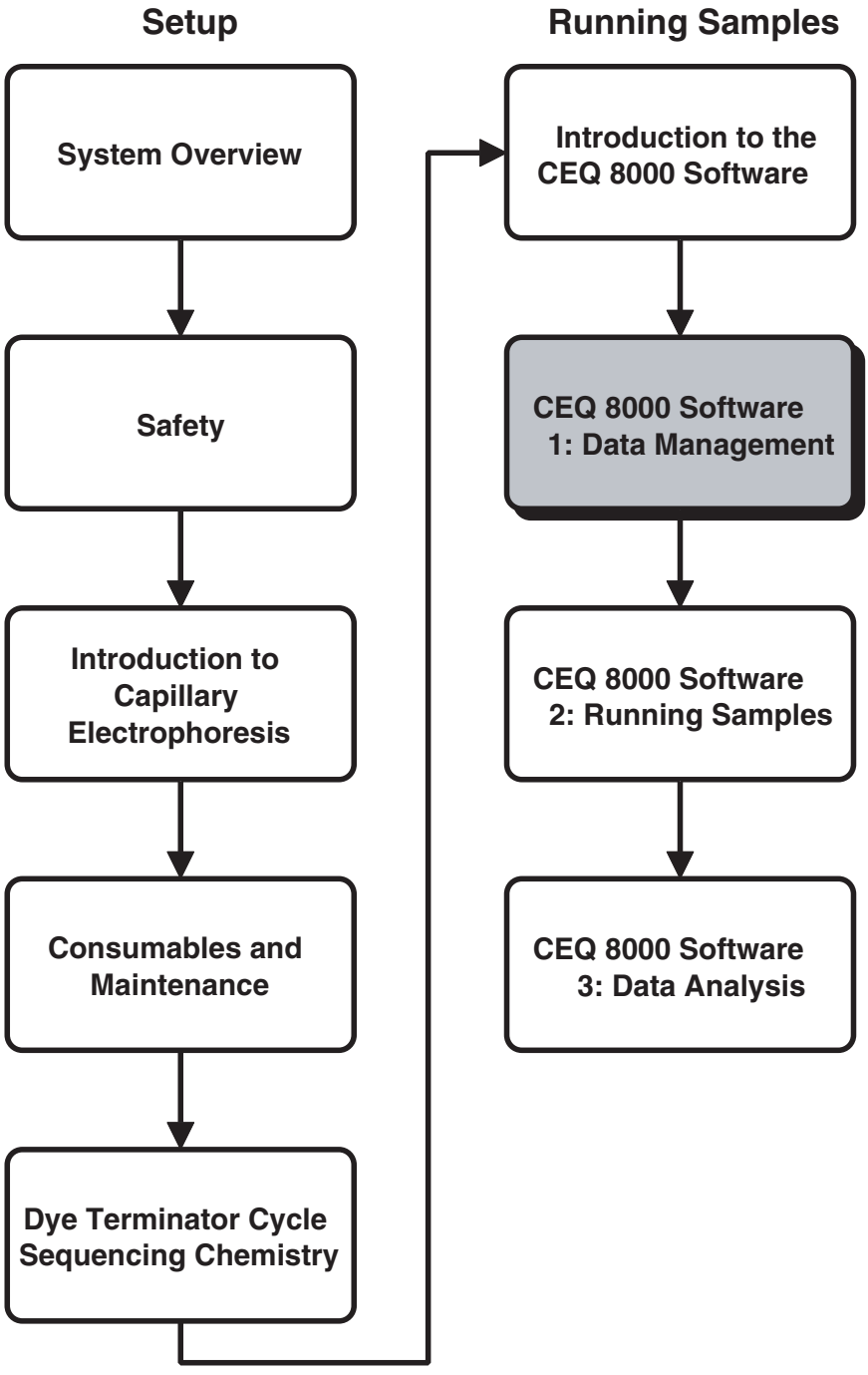
After completing section 6 Introduction to the CEQ 8000 Software training, you should have a working knowledge of the following skills:

- Know the function of each of the software icons
- Be able to move through the various CEQ 8000 windows
- Understand the general work flow for sequencing through the software

SUMMARY

After completing section 6 Introduction to the CEQ 8000 Software training, you should now be able to locate and move through the various windows of the CEQ Software.

7 Software1: Data Management



OVERVIEW

In this section we will explain the procedure for creating and changing databases and project folders. We will also go through how to export database information.

We will discuss:

- Creating a database
- Creating a project
- Archiving a database

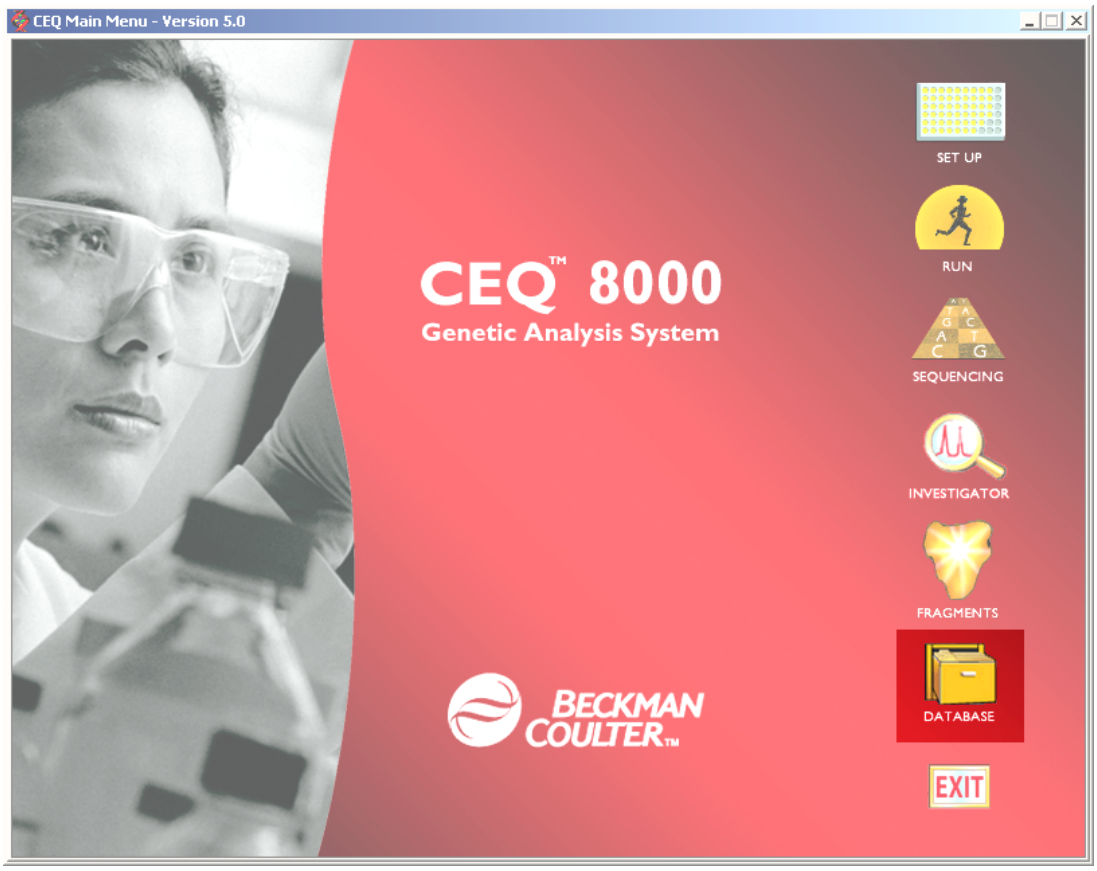


Figure 31: Main Menu, Database Manager Icon

Understanding data management will allow the user to create main databases and project files. Data archiving is also possible through data management.

Note ***If you have any databases that are greater than 500MB it is recommended that you create a new database or archive the data onto CD-ROM***

Creating Main Databases

To create a main database, select the Data Manager icon. Inside the data management program, select **File** and **New Database**. A dialog box will appear and you will be prompted to select a name for the new main database. Each database name contains a .MDB suffix. Within the dialog box, you may choose Set as Working Database. When this box is checked, you will notice that your new main database is highlighted. Please note that you may create a new database during a sequencing run. Though this is possible, you will not be permitted to set your new database as a working database until the sequencing process has ended. To move from database to database, highlight the database you want to set as working database, right click and select **Set as Working Database**.

Deleting the CEQ.MDB file will inactivate the CEQ 8000 software.

Creating New Project Files

To create a new project file, select the data manager icon. Inside the data management program, select **File** and **New** (new project). New project will appear on the screen. Rename the new project by highlighting and right clicking it.

Archiving Data

If you have any databases that are greater than 500MB, you may want to archive the data on CD-ROM. To do so, perform the following procedure.

1. Insert a blank CD-R (recordable) disc into the CD writer.
2. Select the Adaptec Easy CD Creator/Easy CD Creator Deluxe program.
3. The Adaptec Easy CD Creator Wizard is displayed. Click on **Cancel**.
4. Make sure that the *Data CD Layout* tab is on top. Select the CD icon in the lower left-hand area.
5. Select the **Edit** menu, then the Rename menu option to label your CD disc.
6. Select the appropriate directory from the upper left-hand area file explorer and drag and drop data from the upper right-hand area into the lower left-hand CD disc icon to prepare the disc layout.
7. Select the **File | Create CD** or click on the **Create CD** toolbar button 

to start recording.
8. Click on the **Create CD** radio button on the CD Creation Setup dialog box and click **OK**.
9. After the recording has completed, the disc will be ejected automatically.

SKILL CHECK

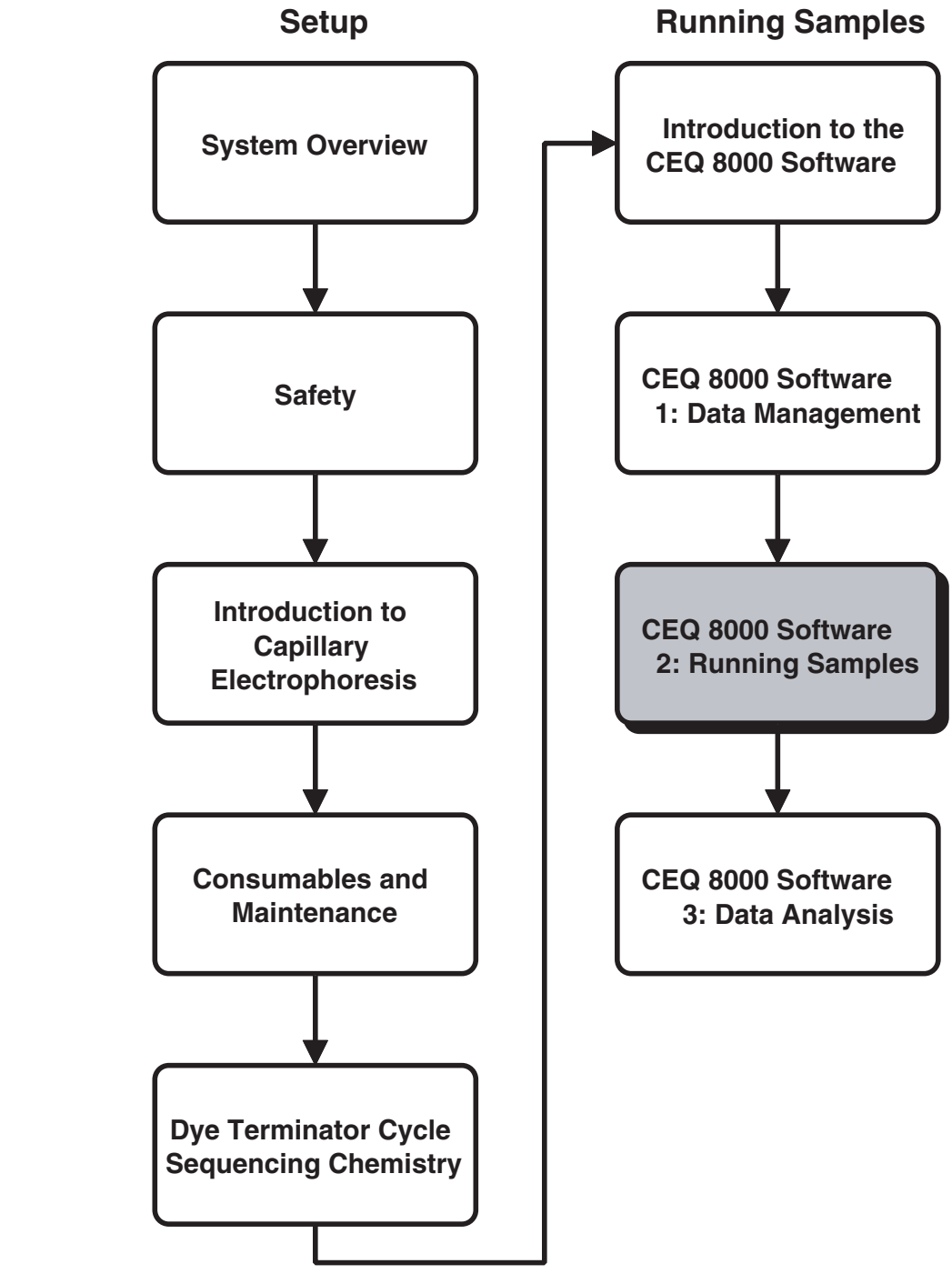
After reviewing the Data Management portion of the training, you should have working knowledge of the following:

- The procedure for creating main databases
- The proper procedure for creating new projects
- Procedure for archiving database information

SUMMARY

Understanding the proper procedure for creating a main database, project files and archiving must be gained before beginning the software portion of the training.

8 Software 2: Running Samples



OVERVIEW

In this section we will explain how to set up a sample sheet and instruct the CEQ to run the samples.

We will discuss:

- Setting up a sample sheet
- Choosing and editing a method
- Understanding and editing the analysis parameters
- Saving the plate
- Replenishing the consumables
- Running the samples

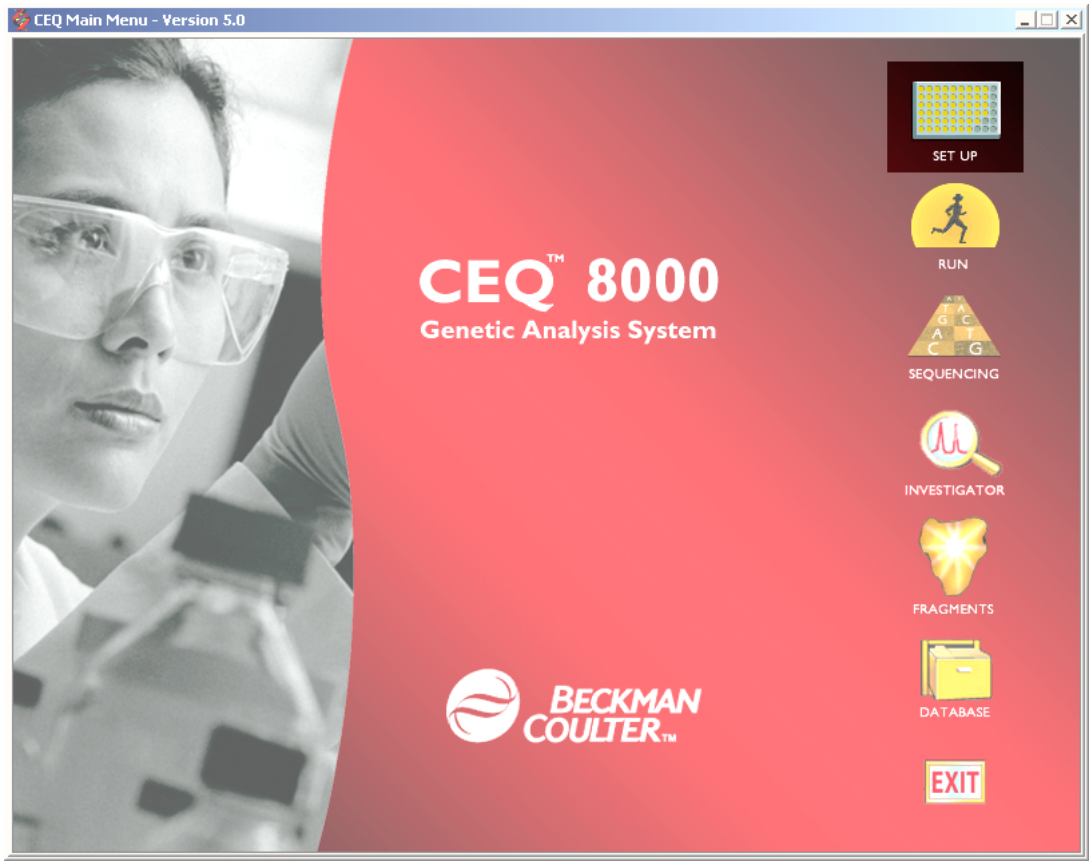


Figure 32: Main Menu, Sample Setup Icon

CREATING A NEW SAMPLE PLATE

Open the **Sample Setup** module and follow the steps outlined below.

To create a new sample plate, perform the following steps.

1. Select **File | New** from the menu.
2. Select (highlight) the cell or cells where the sample(s) will reside.
3. Enter the name of the sample(s) in the **Sample Name** text box and then press the **Enter** key.
    a. Assign a method to each sample set. To edit the method or create a new method, see “Creating or Editing a Method” on page 8-5, and then return here.
    b. To automatically analyze data after a sample set run, click on the **Analysis** tab and then on the **Automatic Analysis** check box in the **Analysis** window. The sample data will be analyzed with the parameter set selected from the drop-down menu. see “Assigning Parameter Sets” on page 8-7, and then return here.
    c. To automatically print a report after a sample set run, see “Specifying Sample Plate Print Options” on page 8-8, and then return here.
    d. To automatically export data after a sample set run, see “Exporting Data” on page 9-16, and then return here.
4. Select **File | Save As** from the menu.
5. In the **Save As** dialog box:
    a. Select a **Project Name** from the drop-down menu**.**
    b. Enter a name for this plate in the name field.
    c. Select **OK** to save the plate.

NAMING SAMPLES

To name a single sample:

1. Select the cell where the sample resides in the plate.
2. Enter the name of the sample in the **Sample Name** text box and press the **Enter** key.

To name contiguous samples:

1. Select the cells where the samples reside in the plate by clicking and dragging the mouse cursor.
2. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.

To name all wells in the plate:

1. Click the **Select All**  icon.
2. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.

To name multiple samples that are not within the same sample set:

1. Select the first cell.
2. Hold down the Control key while selecting the additional samples.
3. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.

ASSIGNING METHODS

The method is the program of events the system uses to collect the data. The method controls the hardware, i.e. the temperatures, voltages and times, which work together to gather optimal data. The system comes with several methods that are optimized for the different software applications. The *Frag* and *SNP-1* methods are used to collect data for fragment analysis, while the *LFR* methods are used to collect sequence data.

LFR-1 is the standard Long fast read sequencing method for the CEQ 8000. LFRa, b and c are alternative methods that can be used, depending on individual application and the desired amount of sequence data. LFRa typically yields longer read lengths but also requires additional separation time. LFRb can yield approximately 700 bases in a shortened separation time compared to the standard LFR-1 method. LFRc reduces the separation time for applications that require only several hundred bases of sequence data. The separation method parameters can be viewed by selecting the Method tab after assigning one of the LFR methods to a sample set.

To assign a method to one sample set:

At the bottom of the selected sample set, select a method from the drop-down menu.

To apply a method to multiple sample sets:

1. Highlight the desired sample sets.
2. Select **Edit | Auto-Fill Method Name** from the menu.
3. In the **Choose Method** dialog box, select a method from the drop-down menu.
4. In the **Auto Fill** area of the dialog box, select **Selected sample sets only** and then click **OK**.

To apply a method to all sample sets:

1. Select **Edit | Auto-Fill Method Name** from the menu.
2. In the **Choose Method** dialog box, select a method from the drop-down menu.
3. In the **Auto Fill** area of the dialog box, select **All sample sets** and then click **OK**.

CREATING OR EDITING A METHOD

If you wish to edit the method or create a new one, it is recommended that you used a voltage between 4.0 and 5.8 kV and a temperature between 35 and 55°C.

If the longest fragments migrate past the detector prior to the end of data collection, the analysis may fail as the system attempts to analyze insignificant baseline data. If this is the case, try checking the **PCR Product** check box so the system will attempt to find the end of significant data. If the analysis still fails, enter an **Analysis Stop** time to force the analysis to only use data prior to the stop time. This will prevent the system from analyzing insignificant data.

To create and/or edit a method, perform the following steps.

1. Select **Edit | Method** from the Sample Setup module menu.
2. Highlight the desired method in the **Choose Method to Edit** dialog box and click **OK**.
3. In the **Method - Capillary Temperature** dialog box:
   a. Enter a temperature between 35 and 60° C.
   b. Select the **Wait for Cap Temp** option (if desired).
   c. Click **OK** to exit the **Method** dialog box or continue with step 4 to make additional changes to the method.
4. Select **Denature** from the **Event** list and then:
   a. Enter a duration 0 and 180 seconds (if 0 is selected, there will be no denaturation).
   b. Click **OK** to exit the **Method** dialog box or continue with step 5 to make additional changes to the method.
5. Select **Pause** from the **Event** list and then:
   a. Enter a time duration between 0 and 10 minutes.
   b. Select **OK** to exit the **Method** dialog box or continue to step 6 to make additional changes to the method.

6. Select **Inject** from the **Event** list and then:

   a. Enter an injection voltage between 0.1 and 12.0 kV.
   b. Enter a time duration in seconds.
   c. Click **OK** to exit the **Method** dialog box or continue with step 7 to make additional changes to the method.

7. Select **Separate** from the **Event** list, and then:

   a. Enter the separation voltage between 0.1 and 12.0 kV.
   b. Enter a time duration in minutes.
   c. Click **Advanced** to specify the two separation phases for the method. Click **OK** to return to the **Method** dialog box.
   d. Click **OK** to exit the **Method** dialog box or continue with step 8 to make additional changes to the method.

8. In the **Save As** dialog box:

   a. Select a **Project Name** from the drop-down menu.
   b. Enter a name in the name field.
   c. Click **OK**.

ASSIGNING PARAMETER SETS

Analysis parameter sets define the conditions specific to an experiment used in data processing. *Sequence Analysis Parameter Sets* define the start and end times for the analysis of raw data, the threshold below which bases will be called as 'N's, and the information necessary to detect the start of data to be analyzed: the delay between the detected start of data and the start of data analysis, the signal to noise ratio, the minimum duration, and the threshold above which data will be considered peaks.

For automatic sequence analysis, select a Sequence Analysis Parameter Set and click the Automatic Analysis check box. The selected parameter set can also be edited from this window.

Sequence Analysis Parameter Sets can only be created or edited in the Sequence Analysis module.

To assign an analysis parameter set to one sample (well):

1. Highlight the desired sample.
2. Select the parameter set from the drop-down menu in the *Analysis* tab.

Do not mix Sequencing and Fragment Analysis Parameter Sets within the same sample set (column).

To assign an analysis parameter set to one sample set:

1. Highlight the desired sample set.
2. Select the desired parameter set from the drop-down menu in the *Analysis* tab.

To apply an analysis parameter set to multiple sample sets:

1. Highlight the desired sample sets.
2. Select the desired parameter set from the drop-down menu in the *Analysis* tab.

Analysis Tab

You can view the sequence analysis parameter set used to analyze the open results. The *Analysis* tab lists the Analysis Parameter Set name and the defined values for that parameter set, as shown below.

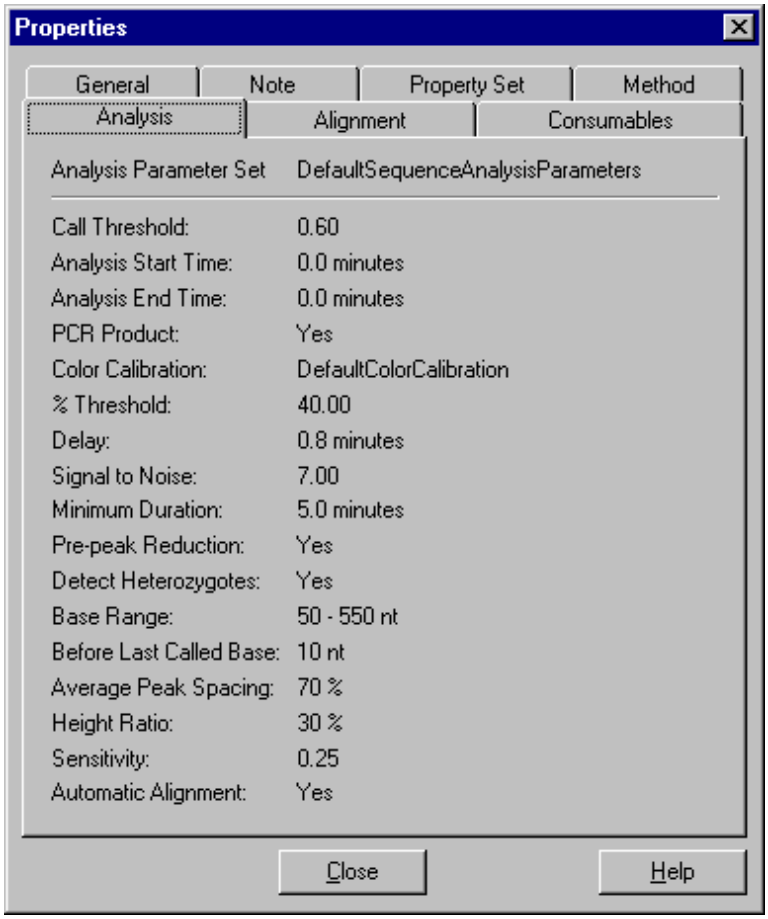


Figure 33: Analysis Tab, Properties Dialog

SPECIFYING SAMPLE PLATE PRINT OPTIONS

To define the print options and report format of the currently open sample plate, perform the following steps.

1. With a cell highlighted, select the **Analysis** tab at the bottom of the window.
2. Select the **Print Report** check box to print a report immediately after completion of the run.
3. Select the **Edit Print Format For Plate** button.
4. In the **Report Format** dialog box, verify or change the **Printer**, **Page Layout** and **Copies** options.
5. In the **Sample Elements** area of the **Report Format** dialog box, select each element to be printed.

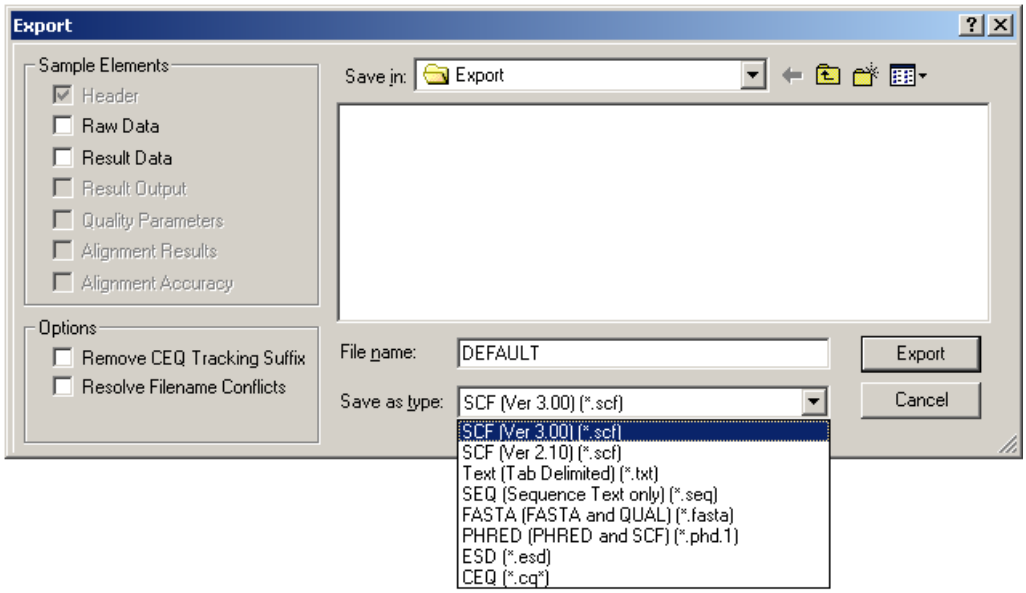
6. If desired, select the **Options** button and verify or change the **Raw Data**, **Sequence Analysis Result Data, Base Sequence Grouping** and **Current Trace Options** selections in the **Print Options** dialog box and then click **OK**.
7. If desired, select the **Colors** button and verify or change the trace colors of the **Raw Data** and/or **Analyzed Data** and then click **OK**.
8. Select **OK** from the **Report Format** dialog box to save the changes and close the dialog box.

SPECIFYING SAMPLE PLATE EXPORT OPTIONS

To specify the type of file and the included elements for export, perform the following steps.

1. With a cell highlighted, select the **Analysis** tab at the bottom of the window.
2. Select the **Export Data** check box.
3. Select the **Edit Export Options For Plate** button.

Figure 34: Sample Plate Export Dialog



4. Select the file type. Some sample elements are grayed-out, or automatically checked when the different file types are selected. If the element is grayed-out and unchecked, the option is not available for that file type. If the element is grayed-out and checked, that item is automatically exported. These options differ for sequence data and fragment data.
5. In the **Sample Elements** area of the **Export** dialog box, select the type(s) of data to be exported (**Header, Raw Data**, **Result Data, Result Output,** and/or **Quality Parameters**, **Alignment Results** and/or **Alignment Accuracy**).

6. In the **Options** area of the **Export** dialog box, select the desired file naming options.
7. Locate the target folder and then click **OK**.

RUNNING A SAMPLE PLATE

To run a sample plate, open the *Run Module* from the *Main Menu* or the *Shortcut Bar*.

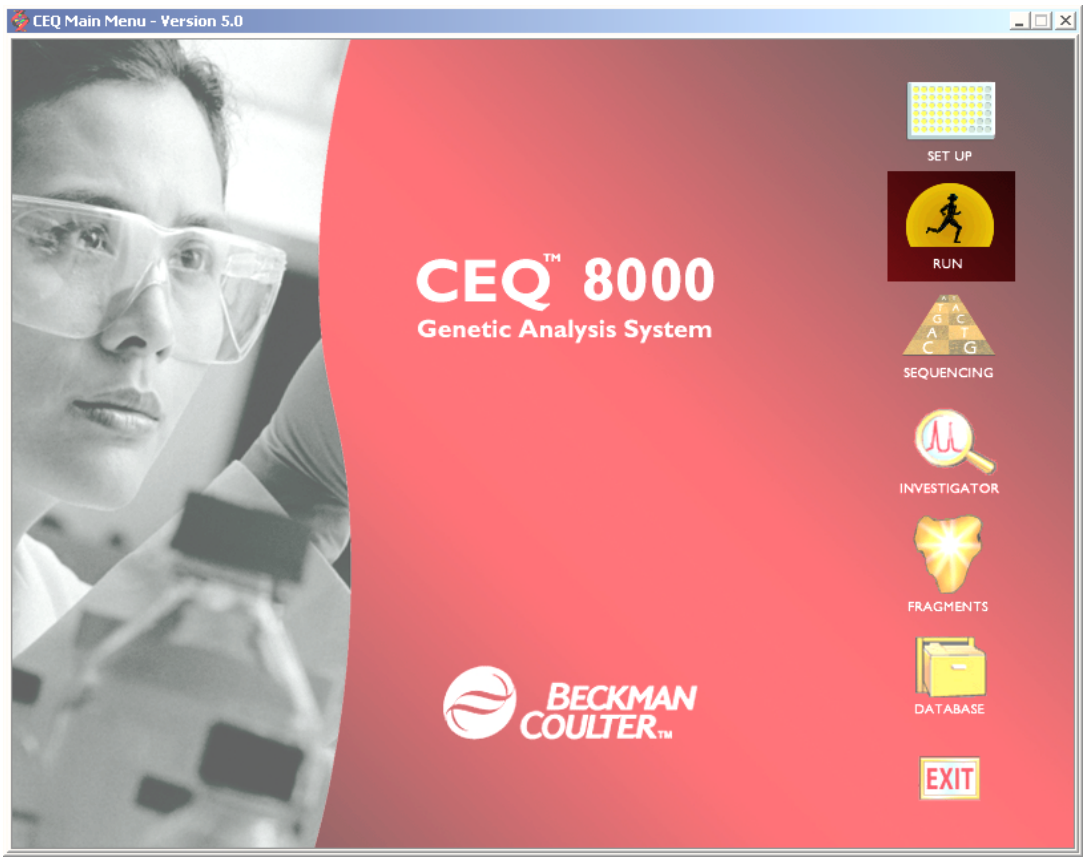


Figure 35: Main Menu, Run Icon

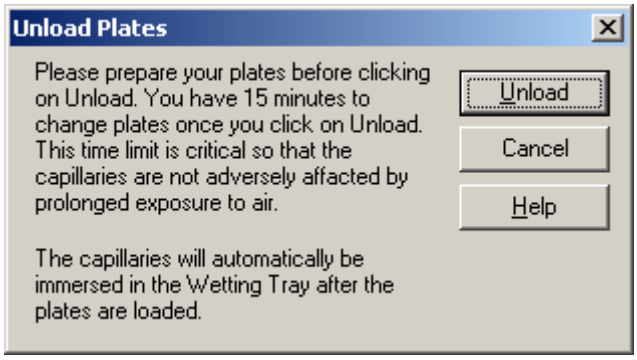
Before running your sample plate, you must ensure that the following items have been performed:

- Capillary Array has been installed
- Gel Cartridge has been installed and/or replaced
- Wetting Tray has been filled

Loading the Sample Plate

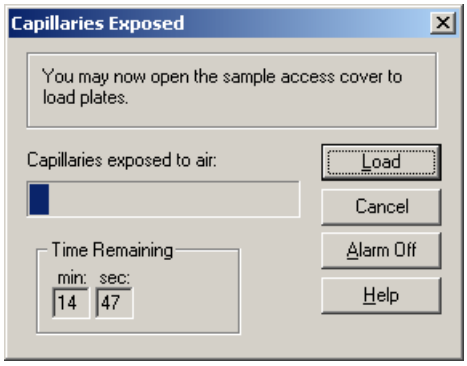
1. Select **Direct Control | Unload Plates** from the menu.

Figure 36: Unload Plates Dialog



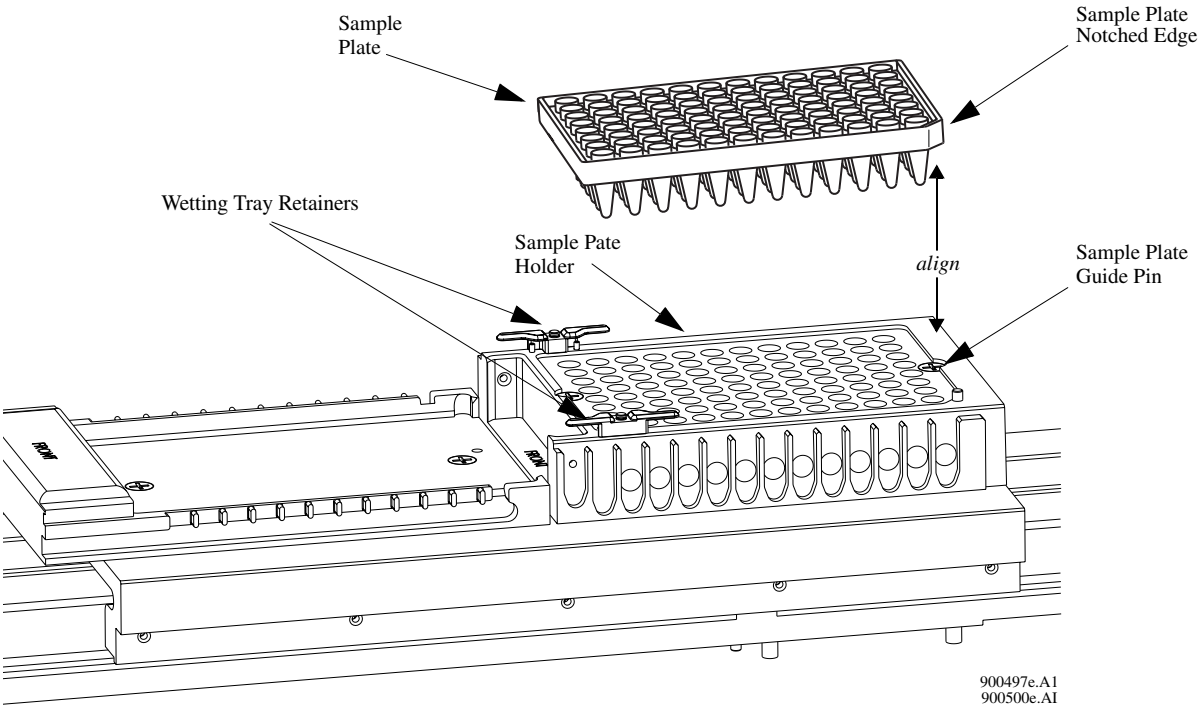
2. Click **Unload**. The Capillaries Exposed dialog (Figure 37) will appear.

Figure 37: Capillaries Exposed Dialog



3. Open the Sample Access Cover and lift to the vertical locking position.
4. Make sure the Wetting Station is installed.
5. Align the Sample Plate Guide Pin with the notched corner of the sample plate and gently lower the plate into position (Figure 38).

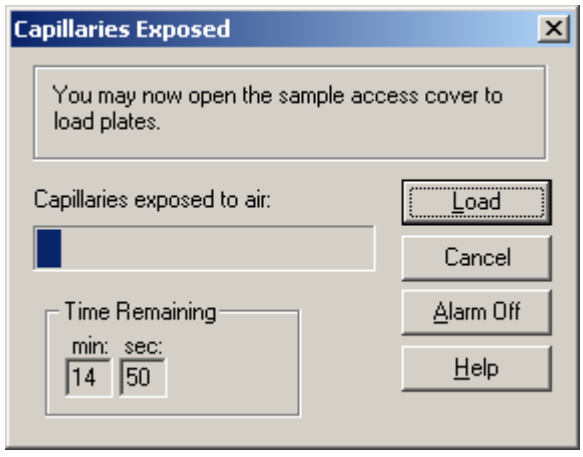
Figure 38: Loading the Sample Plate



6. When finished positioning the plate, close the Sample Access Cover and then click the **Load** button of the **Capillaries Exposed** dialog box (Figure 39).

CAUTION **The separation gel within the capillaries will dry out if the capillaries are left exposed to the air for more than 15 minutes.**

Figure 39: Capillaries Exposed Dialog



Running a Sample Plate

To Run a sample plate that has been created and saved, perform the following steps:

1. Click on **RUN** on the toolbar. Then **Start Sample Plate**.

Run Module
Run Menu

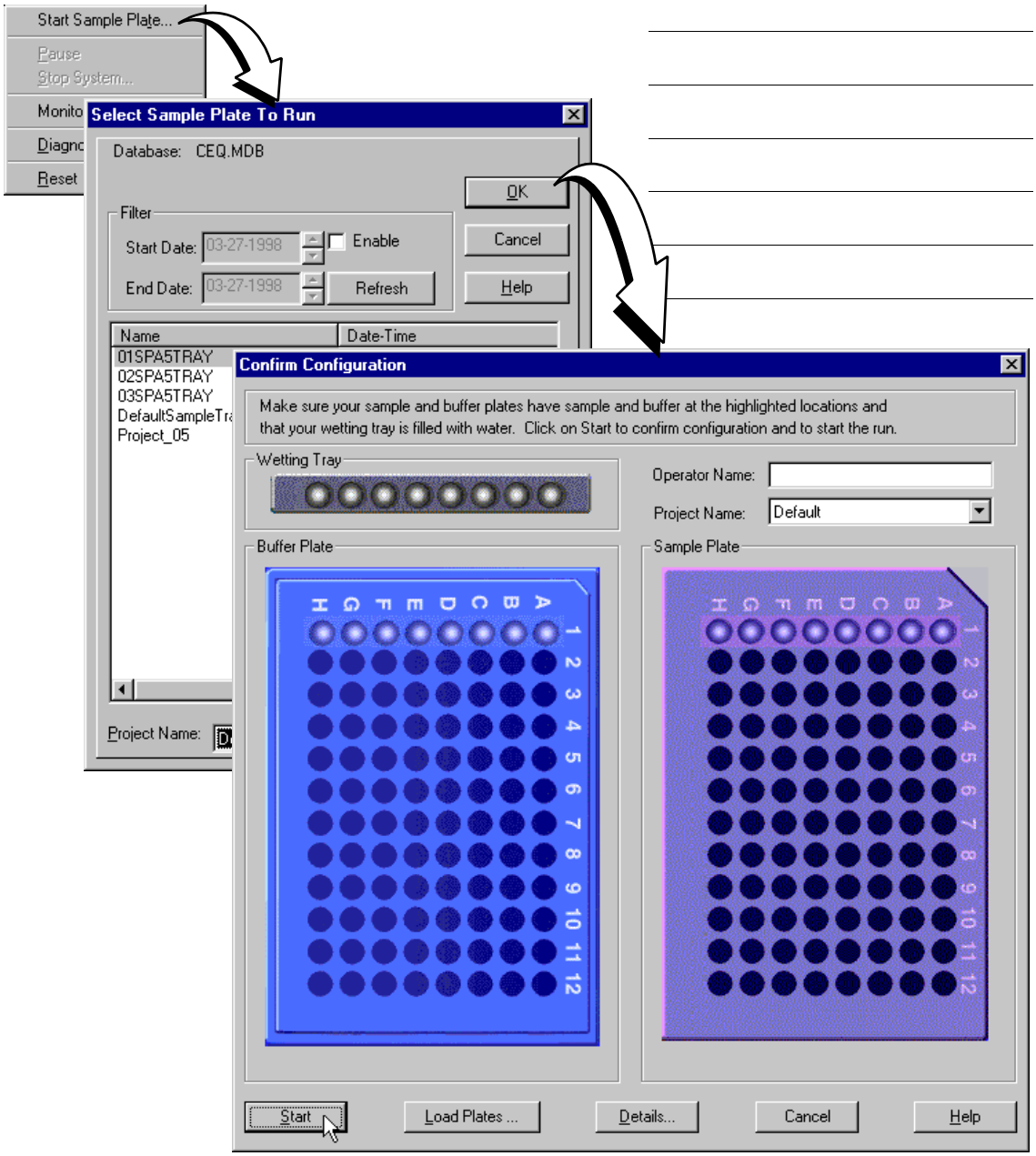


Figure 40: Running a Sample Plate

2. Choose the desired plate to run.

NOTES:

3. **CLICK** on **LOAD PLATES** and then load your sample plate and buffer plate in the proper location in the CEQ.
4. Make sure your plates have been loaded, wetting tray is filled, and all other components have been replenished. In the Confirm Configuration dialog box, verify the sample and buffer set locations and click **Start**.

Status Monitor

The Status Monitor displays the state of the current run. Figure 41 shows the status monitor and Table 4 describes the areas.

Figure 41: Run Module, Status Monitor Displays

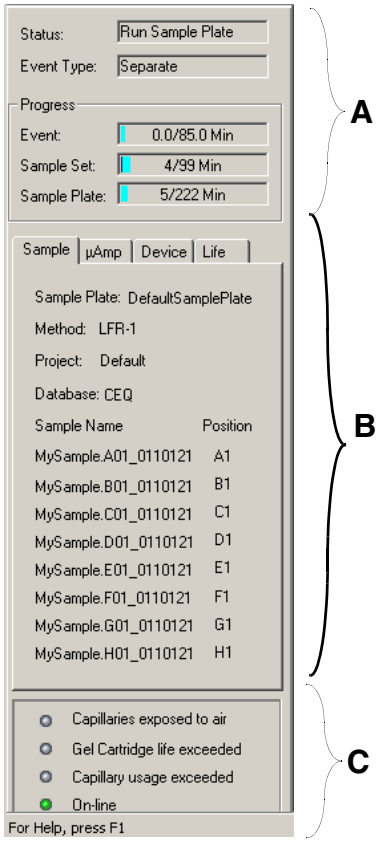
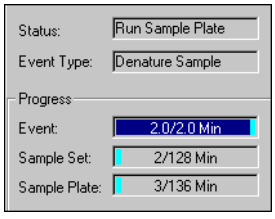
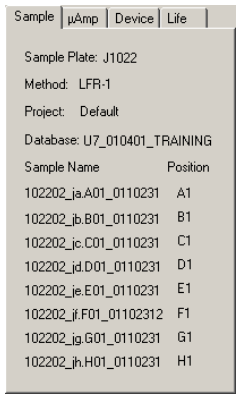
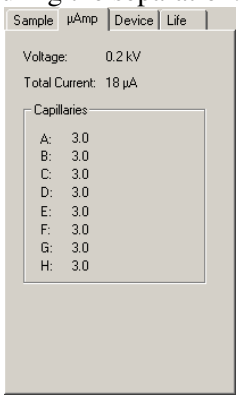
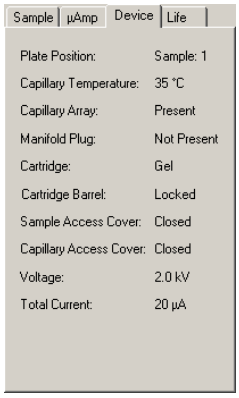
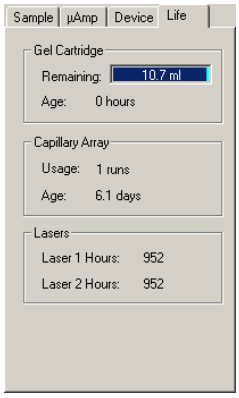
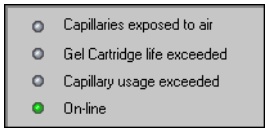


Table 4: Run Module, Status Monitor Displays

Item	Function
A	***Progress Indicator*** - This area of the Status Monitor shows the Status, Event Type and Progress of a running sample plate or process. Note ***Times displayed are approximations.*** 

Item	Function
B	***Information Tabs*** - Toggles between the tabs shown below. • ***Sample*** Lists the sample plate, sample names and the method being used.  • ***µAmp*** Shows the approximate overall microamperes (µA) and voltage used during the separation.  • ***Device*** Displays the condition or value of devices or parameters.  • ***Life*** Displays the remaining gel, capillary usage and laser hours. 
C	***Indicators*** - Provides indicators that pertain to capillary usage, gel cartridge life and on-line status. When the life of the gel or capillary array is exceeded, the associated indicator turns red. When the system is on-line, the indicator is green. 

❑ View Current, Voltage while sample is running

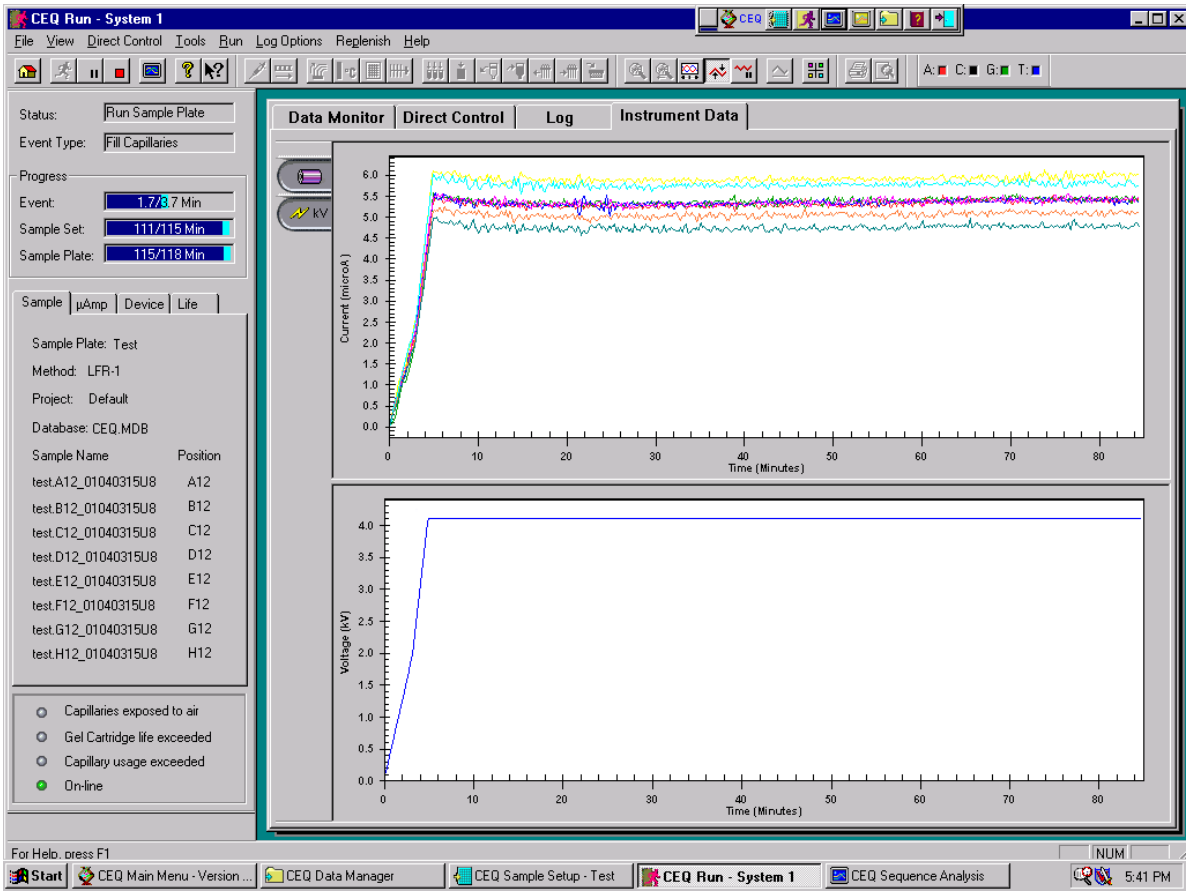


Figure 42: Viewing the Instrument Data Window - Current and Voltage

❑ Monitor baseline

You must perform the optical alignment procedures prior to

monitoring the baseline.

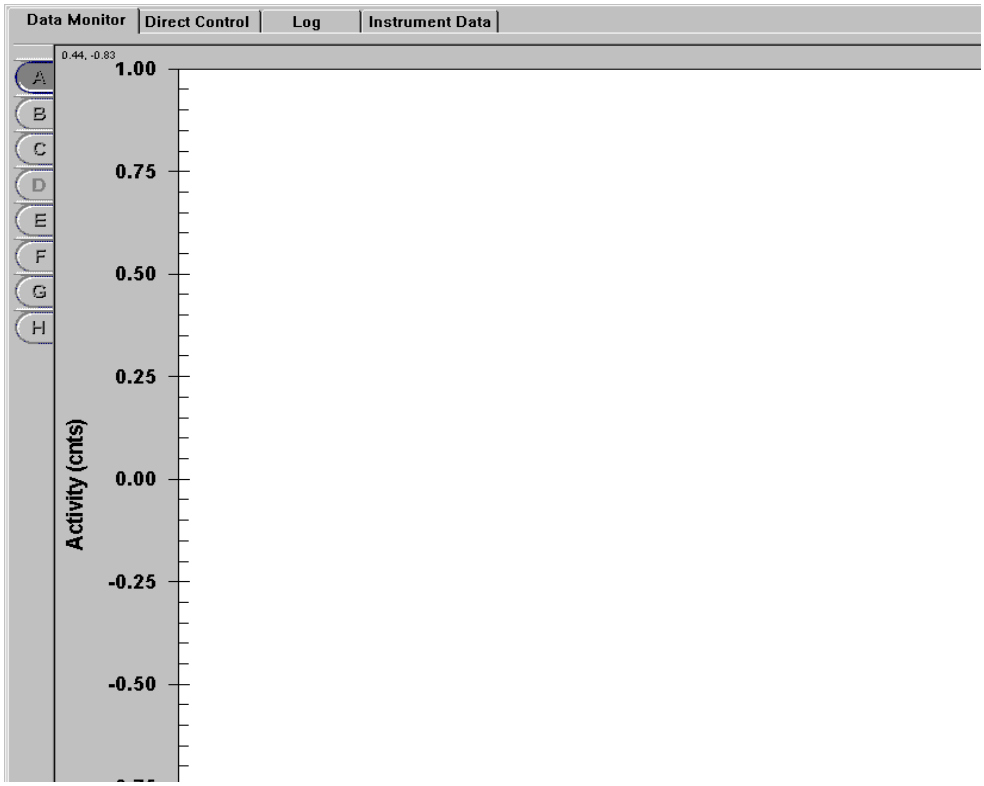


Figure 43: Data Monitor Window

Consumables Tab

You can view the information for all of the consumable products used to collect raw data associated with the currently open sequence result. The capillary array information, the gel information and the buffer information are shown here, as defined in the Run module.

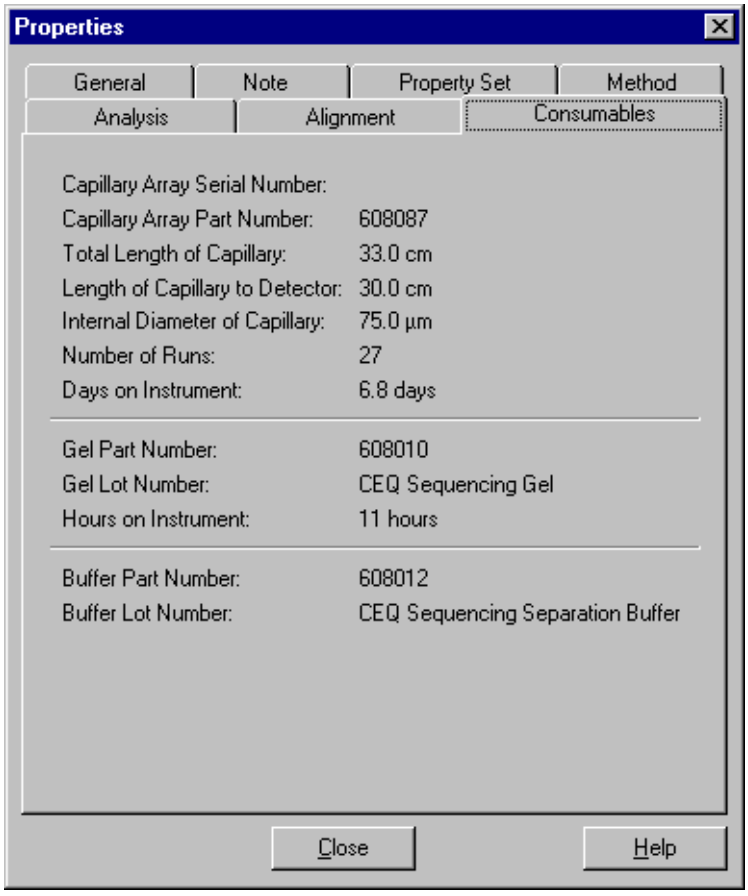


Figure 44: Consumables Tab, Properties Dialog

You can view the gel cartridge volume by clicking the* Life *tab on the Status Monitor.

SKILL CHECK

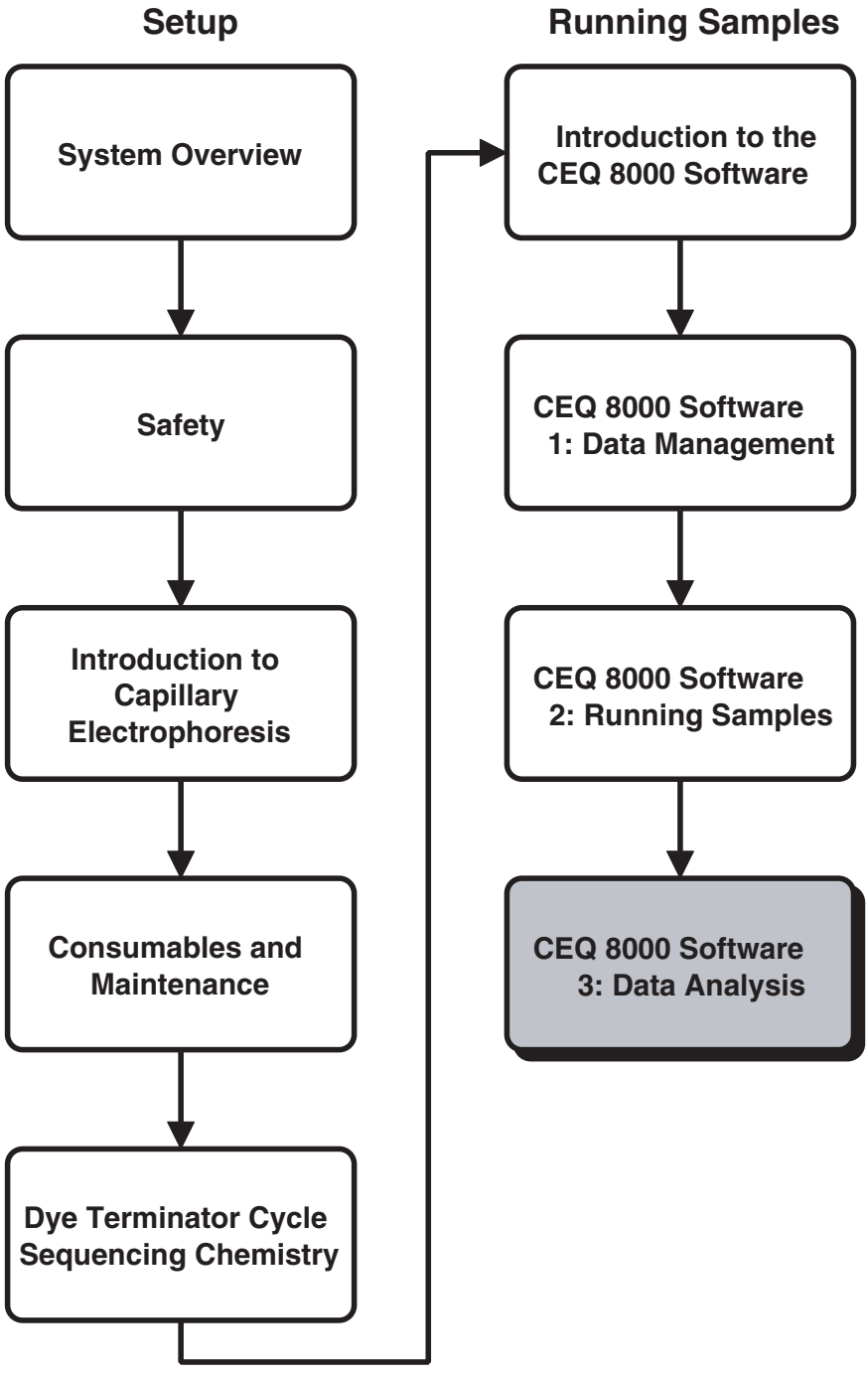
After completing this section you should have a working knowledge of the following:

- Setting up a sample plate
  - Naming your samples
  - Choosing a method
  - Naming and saving your plate
  - Print options
- Editing the methods
- Assigning analysis parameters
- Editing and understanding the different analysis parameters
- Running your sample plate
  - Loading your plates
  - Choosing the correct plate to run
- Understanding the current and voltage
- Monitor Baseline
- Understanding the different tabs in the Run window
- Viewing data as the instrument is running

SUMMARY

After completing this section you should be able to set up your sample sheet and run your sample plate. The most important aspect of the Sample Setup window is properly naming, assigning a specific method, and choosing the proper analysis parameters for your sample plate. In the Run window, you should now have a working knowledge of the different tabs and run options and should be able to load and run your plate. The most important and vital aspect of the Run Window is being able to view the different fields (e.g. the voltage, current, etc.) and knowing what the status and what the condition of your instrument is.

9 Software 3: Data Analysis



OVERVIEW

In this section we will explain how to open data files and view them in various formats including raw data, analyzed data, and base sequence.

We will discuss:

- Selecting files to open
- Viewing files together
- Reanalyzing data
- Exporting Data

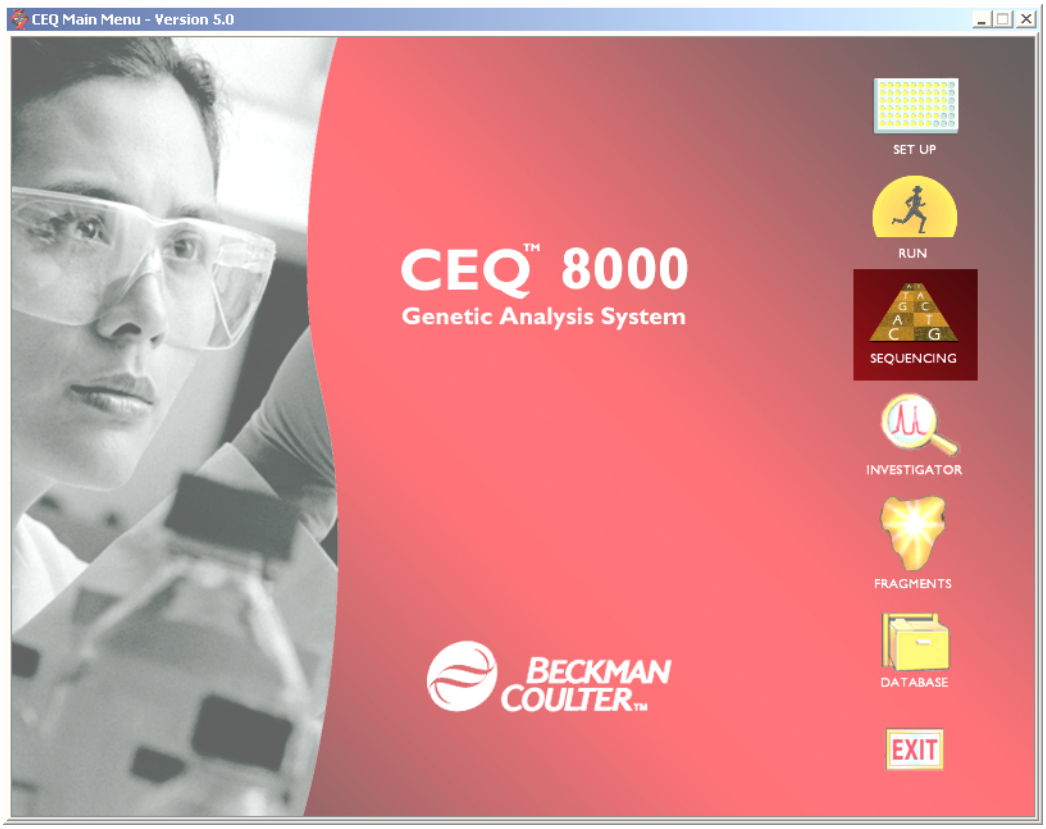


Figure 45: Software 3: Data Analysis

Understanding data analysis will allow the user to analyze sample sequence data and reanalyze existing data. Data archiving is also possible through both data management and Data Analysis.

Analyze Sample Sequence Data

To analyze raw data, select the Analysis icon. Once inside the Analysis program, select **File | Open | Sample Plate Results**. A sample plate with all of the sequences that were run will appear in a virtual, 96-well format. Select the samples you desire by clicking the corresponding well.

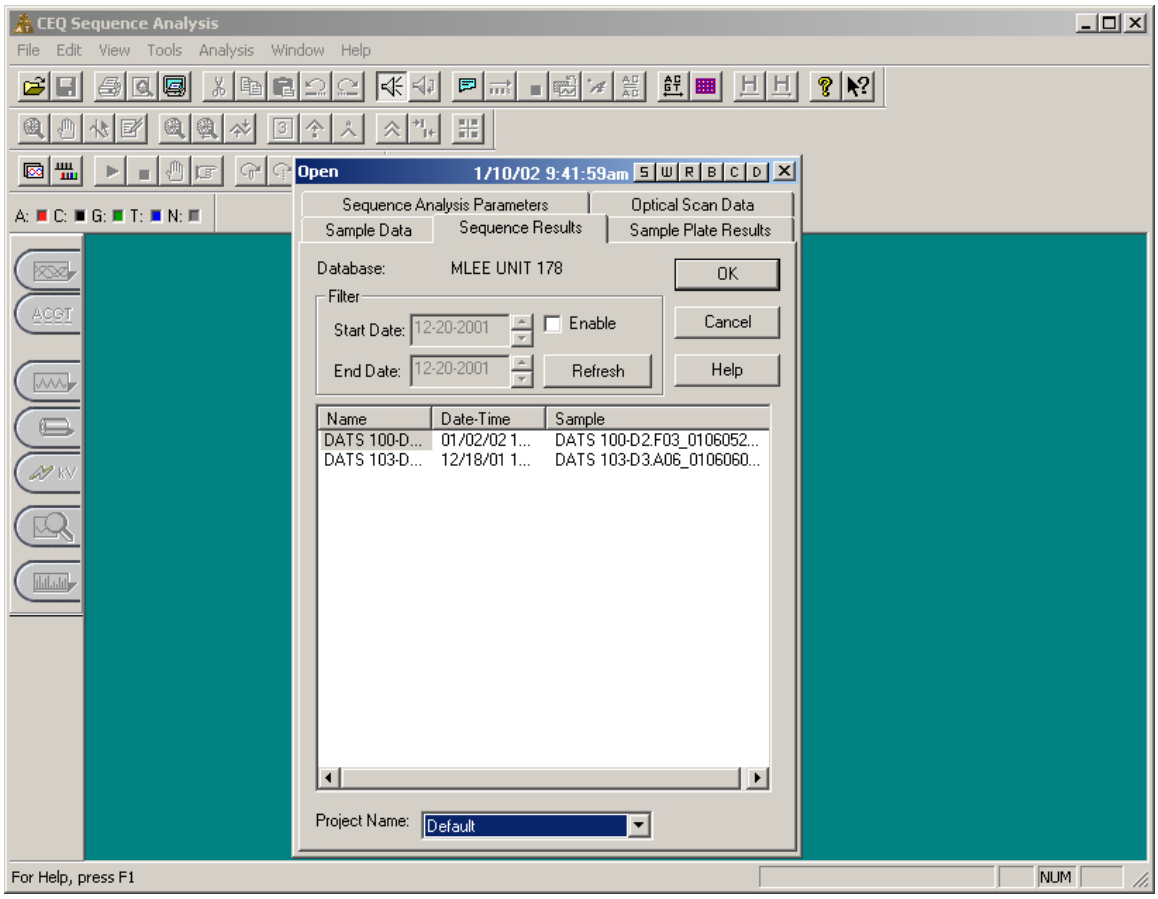


Figure 46: Sample Data Results dialog box

Select **Open** and the samples will appear window by window. To view all of your selections, select **Window | Tile Vertically**. Each window will tile so that they may be viewed at the same time.

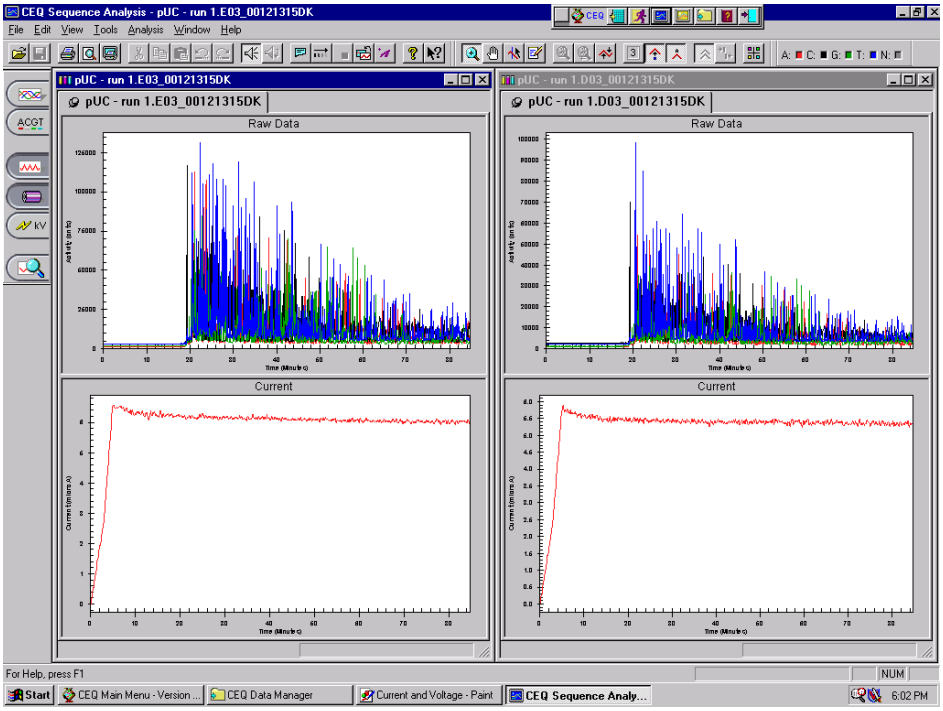


Figure 47: Results Screen – Vertical Tile View

You will find that you are able to move between analyzed and raw data. You will also be able to view the base calls in ASCII text format along with current and voltage profiles. To reanalyze samples:

1. Launch the Sequence Analysis application and open a sample data file.
2. Select **Analysis | Analyze** or click on the **Analyze** icon on the Standard toolbar.
3. Click **Edit** in the Working Parameters dialog box.
4. The Sequence Analysis Parameters Editor dialog box will open, as shown in Figure 48.

To reanalyze after you have altered one or more of these settings, select **OK** and the software will reanalyze the sample(s) based on the new analysis parameters that you have made. To save the new analysis parameters, select **Save As**, assign a new name and click **OK**.

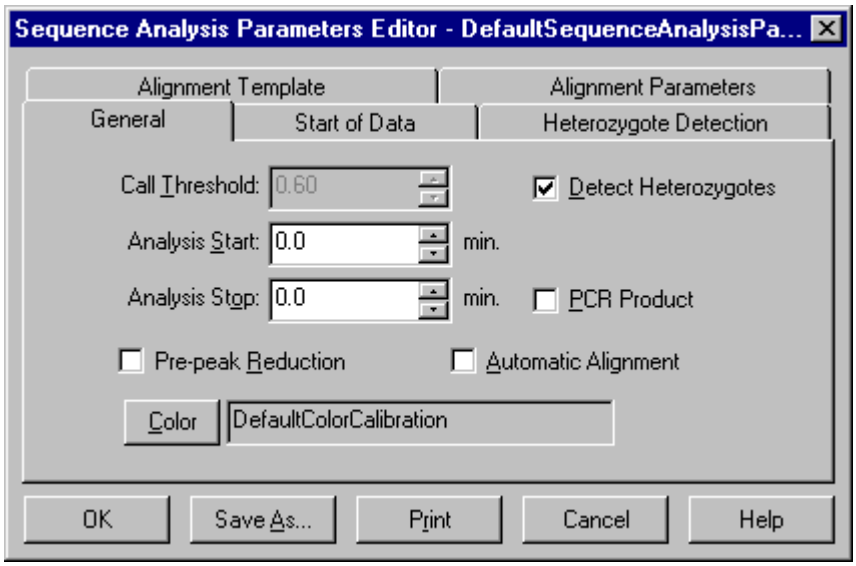


Figure 48: Default Analysis Parameters

General Tab

The *General* tab is displayed by default.

Call Threshold

This value is the threshold for the probability of correctness, below which a called base will be changed to an "N." The range is 0.00 to 1.00 and the default is 0.60.

If Detect Heterozygotes is checked, this field is disabled.

Detect Heterozygotes

When this item is checked, the analysis will include heterozygote detection based on the remaining parameters specified in the *Heterozygote Detection* tab.

When the Detect Heterozygotes check box is checked, the Call Threshold is disabled (grayed) but the current Call Threshold value remains in the text box. The system will use a value of zero for the Call Threshold and will not call "Ns" when attempting to detect heterozygotes.

Analysis Start

Enter the time in minutes from the start of raw data to the time when data analysis is to begin. The range is 0.0 to 180.0 and the default is 0.0.

Analysis Stop

Enter the time in minutes from the start of the raw data to where data analysis will end.

If you enter an Analysis Stop time and check PCR Product (used to determine the end of analyzable data), the system will use the earlier of the two stop times to conclude the analysis.

PCR Product

This check box indicates that the sample is a PCR product and signals the system to end analysis when a sudden drop of signal lasts for more than two minutes. This prevents the analysis of baseline data.

Prepeak Reduction

This check box is used to reduce the inclusion of "prepeaks," which are artifacts that may appear one base prior to the significant peak or near the end of a series of the same base.

Color Calibration

The values in the color matrix show the cross talk between the filters and the emissions for A, C, G, and T. The base calling algorithm is designed to remove signal due to cross talk. Click on Final Values to view the computed color calibration after the run. Click on **Initial Values** to view the color calibration values prior to the run. If the color calibration successfully removed peaks due to cross talk, you might want to save it to apply to data with residual cross-talk signal. If you are going to reanalyze data using a known good color calibration, do not specify that the color calibration is to be recomputed.

Check the **Compute Color Matrix** check box to recompute the color calibration next time you analyze data. (This option is not available if accessed from the Parameters Used to Compute Sequence menu option under the View menu.)

Check the **Use Final Values as Initial Values** check box to use the final values to recompute the color calibration next time you analyze data. Otherwise the system will use the initial values to recompute the color calibration. (This option is not available if accessed from the Parameters Used to Compute Sequence menu option under the View menu.)

Start of Data Tab

Select the **Start of Data** tab to define the parameters that the system will use to define the start of analyzable data when you do not enter an Analysis Start time on the *General* tab.

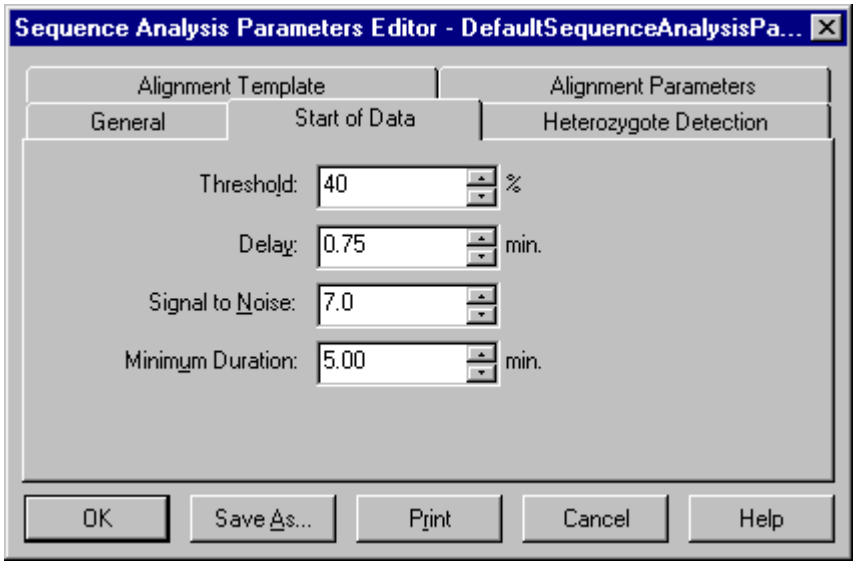


Figure 49: Start of Data Tab

Threshold

The percent threshold is the percent of the maximum of the energy profile of all the data above which the signal must rise before start of data is detected. The data must stay above the threshold for the amount of the minimum duration without gaps lasting greater than 21 seconds. The range is 0 to 100 and the default is 40.

Delay

The start of data Delay is the delay, in minutes, between the detected start of data and the data that will be analyzed. The range is 0.00 to 180.00 and the default is 0.75 minutes.

Signal to Noise

This is the signal to noise ratio for significant data. The range is 3.00 to 1000.00 and the default is 7.00.

Minimum Duration

The minimum duration is the duration, in minutes that the signal to noise threshold specified above has to be exceeded with no gaps greater than 21 seconds. The range is 2.00 to 50.00 minutes and the default is 5.00.

If a value outside of an expected range is entered, a message box will be displayed requesting that a valid value be entered.

Heterozygote Detection Tab

Select the **Heterozygote Detection** tab as shown in Figure 50 to define the parameters the system will use to detect heterozygotes. These parameters will only be used if the Detect Heterozygote option is selected in the *General* tab.

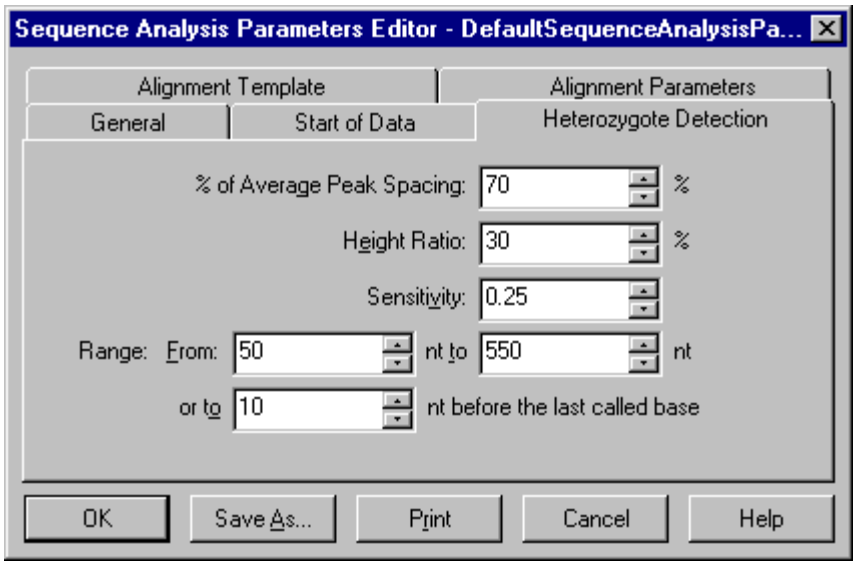


Figure 50: Heterozygote Detection Tab

% of Average Peak Spacing

Use this parameter to determine the range around each peak that the system will search for a possible heterozygous peak. The system will determine the local peak to peak spacing using an average of the spacing for a 10 base range around the base of interest. The system will then find the specified percent (e.g., 70%) of the local peak to peak spacing and look in that range from the center of each peak for a possible heterozygous peak. For instance, if you enter a value of 70%, the system will look 35% of the average peak spacing to the right and 35% to the left for additional peaks. The range is 0 to 100% and the default is 70%.

Height Ratio

Use this parameter to define the minimum percent of the primary peak's height that a second peak falling within the defined range must be to be considered a heterozygous peak. The range is 0 to 100% and the default is 30%.

Heterozygote Display Color

The heterozygous bases will be bold and red, by default. You can change the color of the heterozygotes in the base sequence pane by selecting **Tools | Heterozygotes Display Color**. Select a new color and click **OK**. The color of the ambiguity codes will be changed in the base sequence text.

You mat search for heterozygotes by using the Heterozygote Backward Search  ***and the Heterozygote Forward Search***  ***icons in the Standard toolbar.***

Sensitivity

Use this parameter to define the sensitivity of the system when looking at the sharpness of the peak. A high value leads to a high degree of sensitivity and will detect "peaks" that are not very well formed, i.e., "blips" in the baseline. A lower value will lead to a low degree of sensitivity and will not detect "peaks" unless they are well-define and are shaped more like a DNA fragment peak. The range is 0 to 1.0 and the default is 0.25.

Range

This value defines the nucleotide range in the called sequence in which the system will look for heterozygous peaks. The system will not look for heterozygous peaks outside this nucleotide range. The range max is dependent upon the length of the analyzed sequence for the current sample.

Range From (Minimum)

If **Range From** is zero, detection will start at the first called base. The range is 0 to 2000 and the default is 50.

Range to (Maximum)

If **Range to** is zero, the algorithm will use the **Range End** value.

If both **Range From** and **Range to** are zero, detection will continue to the end of the called base sequence.

If **Range End** is zero, the system will use the **Range to** value.

If both **Range From** and **Range End** have a non-zero value, the algorithm will choose the *earlier* of the two values.

Range End ("or to")

This item specifies how many bases, before the last called base, to stop looking for heterozygotes.

If the value in **Range End** is zero, the algorithm will use the **Range to** value.

If both **Range End** and **Range to** are zero, detection will continue to the end of the called base sequence.

If **Range to** is zero, the system will use the **Range End** value.

If both **Range to** and **Range End** have a non-zero value, the algorithm will choose the earlier of the two values.

If a value is outside of an expected range, a message box will be displayed requesting that a valid value be entered.

The system will use the IUB ambiguity codes in Table 5 to represent heterozygous bases.

Table 5: IUB Ambiguity Codes

Code	Definition	Mnemonic
A	Adenine	A
C	Cytosine	C
G	Guanine	G
T	Thymine	T
R	AG	puRine
Y	CT	pYrimidine
K	GT	Keto
M	AC	aMino
S	GC	Strong 3 H bonds
W	AT	Weak 2 H bonds
B	CGT	Not A
D	AGT	Not C
H	ACT	Not G
V	ACG	Not T
N	AGCT	aNything

The system will represent the heterozygous bases in the Analyzed Data and in the Base Sequence Data.

If a value is outside of an expected range, a message box will be displayed requesting you to select a valid value.

You may specify heterozygote detection when you analyze data in the Sequence Analysis module. Using this feature will determine the possibilities for bases at any location and enter an IUB ambiguity code, depending on the possible bases that exist at that location. This is useful for determining base identities for diploid individuals having two variants of a gene or polymorphic locus.

Alignment Template Tab

When the **Alignment Template** tab is clicked, the dialog box in Figure 51 is shown.

If the Perform Alignment is checked on the *General* tab, the options on this tab along with the information on the *Alignment Parameters* tab will be used for the alignment operation.

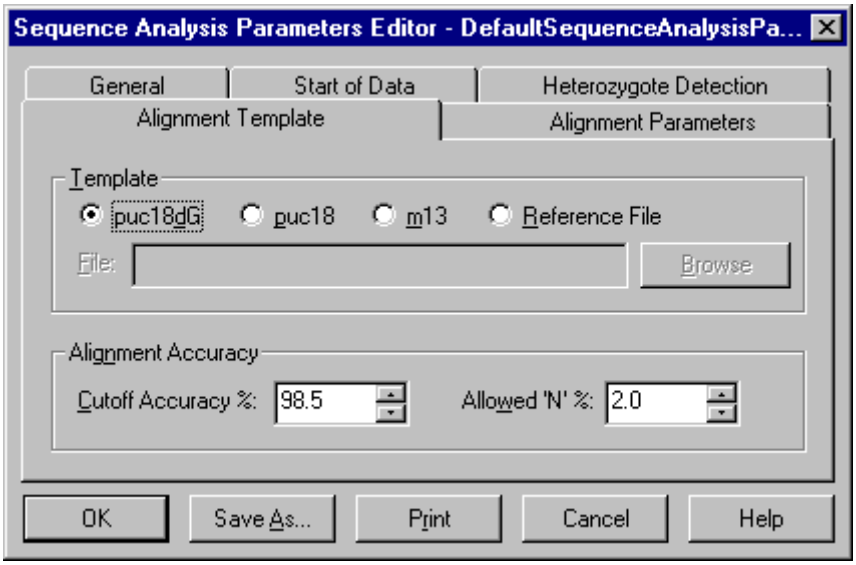


Figure 51: Alignment Template Tab

Template

You may choose from three templates that are stored as part of the software, or you may choose a reference file from a disk.

If the Reference File radio button is not selected, then the edit box and the browse button below it are disabled.

If the Reference File radio button is selected, then the edit box below it and the browse button are enabled, allowing you to enter the path to the desired reference file or to browse for a file.

Browse

The Browse button will allow to search for Text files (*.txt) and Sequence files (*.seq).

Cutoff Accuracy

Enter the desired Cutoff Accuracy. The alignment will detect the base number in the called sequence at which the accuracy falls below this Cutoff Accuracy value. The range is 0.0 to 100.0% and the default is 98.5%.

Allowed N's

Enter the percentage of Allowed N's that will be allowed to occur before they are counted as errors when determining the alignment accuracy. The range is 0.0 to 100.0% and the default is 2.0%.

If a value is outside of an expected range, a message box will be displayed requesting you to select a valid value.

Alignment Parameters Tab

When the **Alignment Parameters** tab is clicked, the dialog box in Figure 52 is shown.

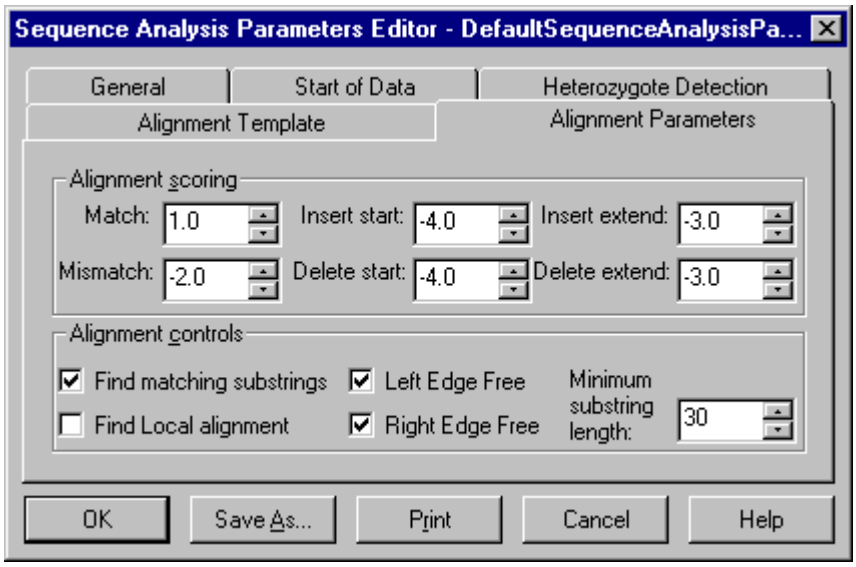


Figure 52: Alignment Parameters Tab

The information on this tab is used to define the parameters used to perform alignments with the active sequence. The system uses a scoring matrix to perform the alignment. The alignment that produces the highest total score is the one that will be displayed. In general, Matches should be given higher scores than non-matches. The definitions of the scoring values are described below. Clicking on the **Alignment Parameters** tab will provide the following options.

The **Match** text box is used for the value of a match between a base position of the sequence and the alignment sequence. The range is –1000.0 to 1000.0. A value of "1.0" is the default value.

The **MisMatch** text box is used for the value of a mismatch between a base position of the sequence and the alignment sequence. The range is –1000.0 to 1000.0. A value of "–2.0" is the default value.

The **Insert start** text box is used for the value of an insertion of a base in the sequence that does not exist in the alignment sequence. The range is –1000.0 to 1000.0. A value of "–4.0" is the default value.

The **Insert extend** text box is used for the value of a consecutive insertion of a base in the sequence that does not exist in the alignment sequence. The range is –1000.0 to 1000.0. A value of "–3.0" is the default value.

The **Delete start** text box is used for the value of a deletion of a base in the sequence that does not correspond to the alignment sequence. The range is –1000.0 to 1000.0. A value of "–4.0" is the default value.

The **Delete extend** text box is used for the value of a consecutive deletion of a base in the sequence that does not exist in the alignment sequence. The range is –1000.0 to 1000.0. A value of "–3.0" is the default value.

Check **Find matching substrings** to optimize the alignment to find matching substrings first, then running the alignment algorithm only on the bases remaining outside the substrings, reducing execution time and storage space.

Check the **Find Local alignment** to use only substrings with the highest score, and ignore the rest, as opposed to using every character from both strings to compute the similarity score.

Check **Left Edge Free** and/or **Right Edge Free** to specify that overhanging bases on the template or sequence will not be counted in the alignment score.

Enter the number of bases needed to constitute a substring in the **Minimum substring length** text box to specify a minimum length for detected substrings. The range is 5 to 2000. A value of "30" is the default value.

If a value is outside of an expected range, a message box will be displayed requesting you to select a valid value.

Sequence Result Properties

The sequence result properties are used to view Method, Analysis, Alignment and Consumable information, in addition to the General, Notes and Property set information previously available in this dialog box.

You will be able to view the Properties dialog box by selecting **File | Properties** when a sequence result is open.

If no alignment has been performed on the open sequence result, the Alignment tab will not be shown.

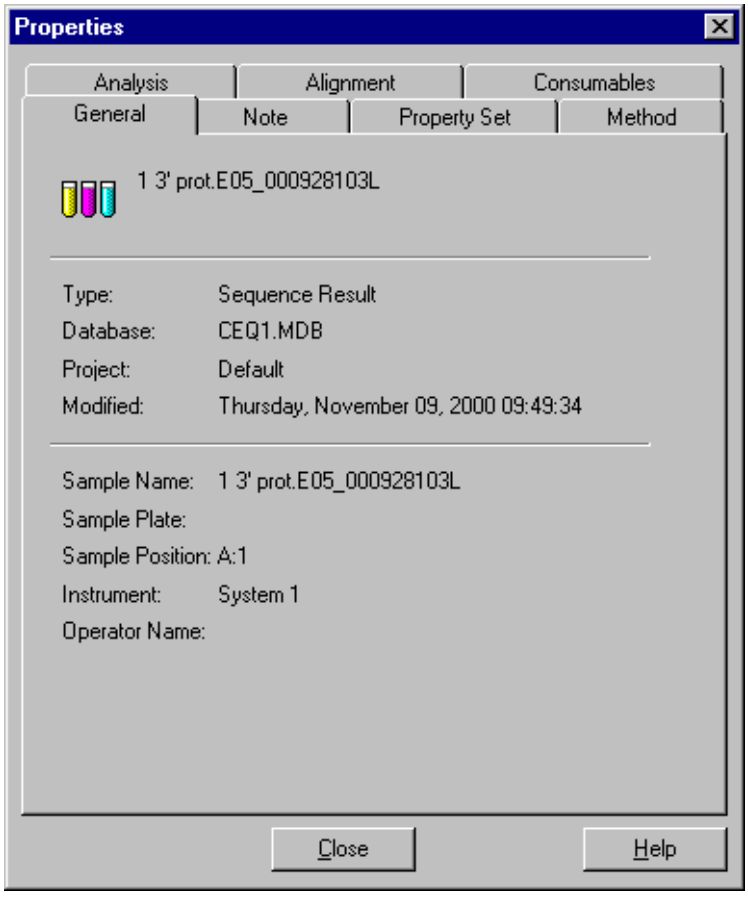


Figure 53: General Tab, Properties Dialog

Exporting Data

To archive sample sequence results, select **File | Export**. The selected samples will then be exported to where ever you have designated it to go. For your convenience, create a CEQ Files folder in the C drive of your computer. When exporting, select that folder and you will be able to readily enter it when you are ready to save on a CD. To write your data on a CD, save your sample results in the CEQ Files folder first. Once this is accomplished, select start from the bottom right corner of your screen. From the Start icon, go to the Programs icon and choose Adaptec Easy CD Creator. Once in this program, you will be asked what type of CD you are creating. Select Data CD. From there select CEQ Files in the pull down menu box and double click it. Highlight all of the information you desire and select the Add icon. Select Finish and you will be prompted through the completion of the writing process.

Sample Data or Result Data

To export sample data or result data from the Sequence or Fragment Analysis modules, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the appropriate tab (**Sample Data** or **Sequence Results**), highlight the desired name and then select **OK**.
3. With the data displayed, select **File | Export** from the menu.
4. From the **Export** dialog box, locate the target folder and then click **Export**.

Sample Plate Results

To export sample plate results from the Sequence or Fragment Analysis modules, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sample Plate Results** tab, highlight the desired name and then select **OK**.
3. Select **View | Toolbars** from the menu. Select the **Sample Plate** check box, then click **Close**.
4. From the **Sample Plate** dialog box, select the sample(s) to export and then click **Export**.
5. From the **Export** dialog box, locate the target folder and then click **Export**.

Sequence Result Export Enhancements

Header information is exported with a sequence result in text format (*.txt) and has been expanded to include the information found in the Properties dialog box. You may export all of the property information when exporting a sequence result in text format.

You can also open the exported file in any text editor and/or in MS Excel.

1. Launch the Sequence Analysis application and open a sequence result.
2. Select **File | Export** to open the Export dialog box.
3. Select a target folder and file name.
4. Select Text (Tab Delimited) (*.txt) in the Save as type text box.
5. Make sure Header is selected in the Sample Elements section, as shown in Figure 54.

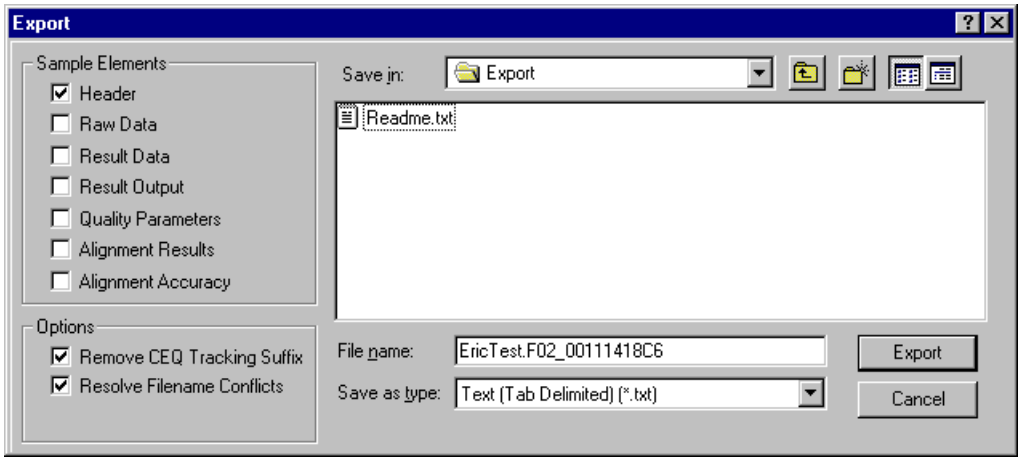


Figure 54: Sequence Analysis Export Dialog

6. Click **Export**.
7. When you view the exported file, the header will include the properties information associated with the sequence result.

Sequence Result Report Enhancements

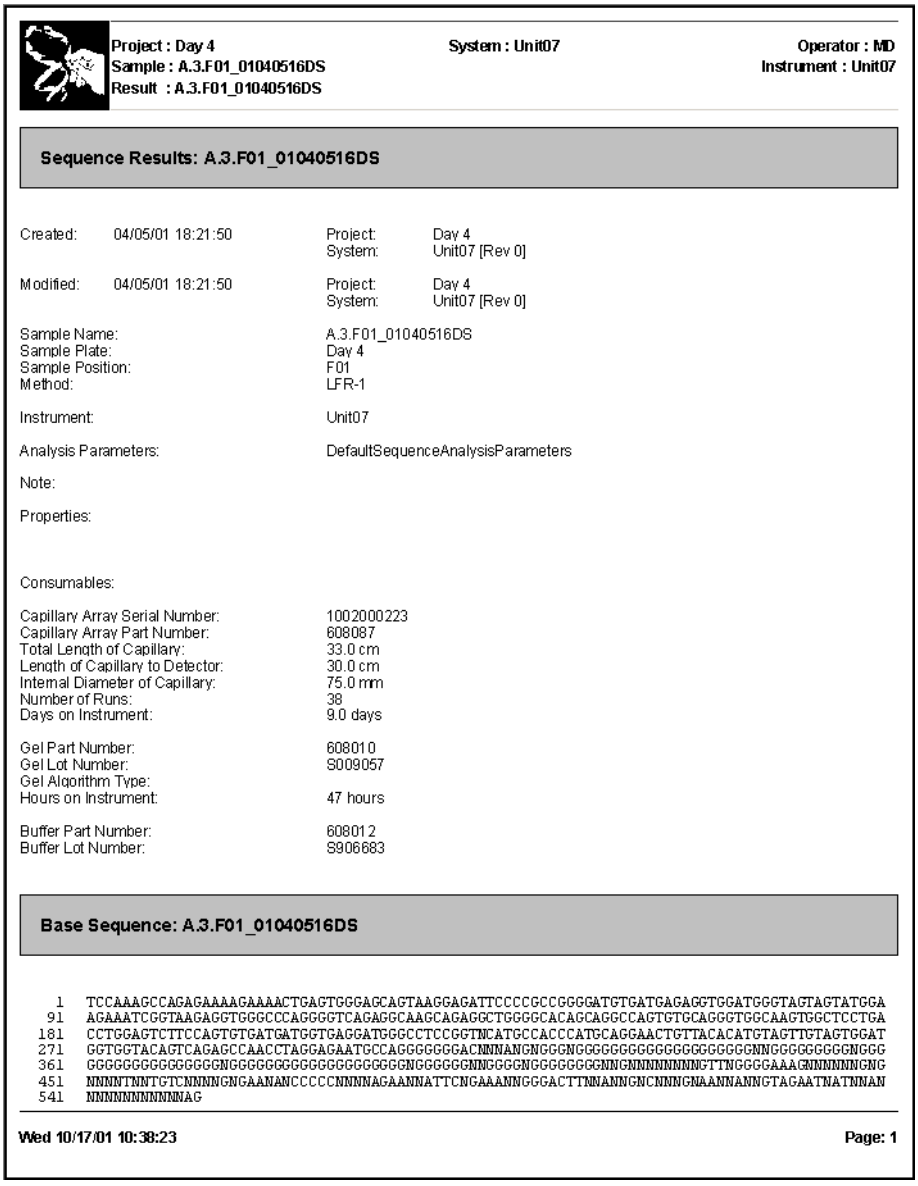
The sequence result report has been modified to include additional information when certain Sample Elements are selected in the Report Format dialog box. The following table describes what each sample element includes.

Header	The header contains summary information for the sample such as sample name, position, the method under which it was run, the sample plate name, the analysis parameter set name, and capillary serial number. In addition, the sample note, property set and consumables information associated with the sample will be printed.  ***This is the same information found on the General, Note, Property Set and Consumables tabs in the Properties dialog box.***
Raw Data	The raw data is the unanalyzed data from the sample run.
Result Data	The result data is data that has undergone final processing to produce result data. If the data has been analyzed using a sequence analysis parameter set, the data with each peak assigned a base is printed.
Result Output	The result output is the final output in text form. If the data has been analyzed using a sequence analysis parameter set, the called sequence will be printed in text form. This section of the report corresponds to the Base Sequence text.
Current	Current is the trace associated with the current output of each capillary or the total current of the sample set run.
Voltage	Voltage is the trace associated with the voltage output of the system during the sample set run.
Analysis Log	The Analysis Log contains all of the steps used to perform the base-calling routine on the raw data.
Run Log	The Run Log contains all of the actions performed and instrument messages for the sample run.
Method Summary	The Method Summary contains the details of the method used to perform the sample run.  ***This is the information found on the Method tab of the Properties dialog box.***

Analysis Parameters	The Analysis Parameters are the parameters used to produce result data and output for a given sample, including heterozygote detection and alignment. If the data has been analyzed using a sequence analysis parameter set, the sequence analysis parameter set will be printed. In addition, the alignment template and the alignment parameters information will be included in this section, when applicable. ***This is the information found on the Analysis and Alignment tabs of the Properties dialog box. No alignment information will be included if an alignment was not performed.*** 
Quality Parameters	Quality parameters are the parameters that indicate that any given base is an A, C, G, or T along with a value to indicate the likelihood that the call is correct.
Alignment Results	The alignment results are the text results of the alignment between a template sequence and a sequence result. The top row of text is the base number, the second line is the template sequence, the third line is the sample sequence and the last line is the consensus.
Alignment Accuracy	The alignment accuracy is the statistical analysis of the accuracy of the sample sequence. The errors in the alignment are grouped into 50-base regions and the number and type of error for each region is displayed.

To define the format and contents of the report, select **File | Report Format**. Select **File | Print Report** to print the report without previewing or formatting prior to printing.

Select **File | Print Preview** to the view the report, shown in Figure 55.



Project : Day 4 System : Unit07 Operator : MD
Sample : A.3.F01_01040516DS Instrument : Unit07
Result : A.3.F01_01040516DS

Sequence Results: A.3.F01_01040516DS

Created:	04/05/01 18:21:50	Project:	Day 4
		System:	Unit07 [Rev 0]
Modified:	04/05/01 18:21:50	Project:	Day 4
		System:	Unit07 [Rev 0]
Sample Name:		A.3.F01_01040516DS	
Sample Plate:		Day 4	
Sample Position:		F01	
Method:		LFR-1	
Instrument:		Unit07	
Analysis Parameters:		DefaultSequenceAnalysisParameters	
Note:			
Properties:			
Consumables:			
Capillary Array Serial Number:		1002000223	
Capillary Array Part Number:		608087	
Total Length of Capillary:		33.0 cm	
Length of Capillary to Detector:		30.0 cm	
Internal Diameter of Capillary:		75.0 mm	
Number of Runs:		38	
Days on Instrument:		9.0 days	
Gel Part Number:		608010	
Gel Lot Number:		S009057	
Gel Algorithm Type:			
Hours on Instrument:		47 hours	
Buffer Part Number:		608012	
Buffer Lot Number:		S906683	

Base Sequence: A.3.F01_01040516DS

```
   1  TCCAAAGCCAGAGAAAAGAAAACTGAGTGGGAGCAGTAAGGAGATTCCCCGCCGGGGATGTGATGAGAGGTGGATGGGTAGTAGTATGGA
  91  AGAAATCGGTAAGAGGTGGGCCCAGGGGTCAGAGGCAAGCAGAGGCTGGGGCACAGCAGGCCAGTGTGCAGGGTGGCAAGTGGCTCCTGA
 181  CCTGGAGTCTTCCAGTGTGATGATGGTGAGGATGGGCCTCCGGTNCATGCCACCCATGCAGGAACTGTTACACATGTAGTTGTAGTGGAT
 271  GGTGGTACAGTCAGAGCCAACCTAGGAGAATGCCAGGGGGGGACNNNANGNGGGNGGGGGGGGGGGGGGGGGGNNGGGGGGGGGNGGG
 361  GGGGGGGGGGGGGGGNGGGGGGGGGGGGGGGGGGNGGGGGNNGGGGNGGGGGGGNNGNNNNNNNNGTTNGGGGAAAGNNNNNNGNG
 451  NNNNTNNTGTCNNNNGNGAANANCCCCCNNNNAGAANNATTCNGAAANNGGGACTTNNANNGNCNNNGNAANNANNGTAGAATNATNNAN
 541  NNNNNNNNNNNAG
```

Wed 10/17/01 10:38:23 Page: 1

Figure 55: Sequence Results Report

SKILL CHECK

After reviewing the Data Analysis portion of the training, you should have working knowledge of the following:

- The procedure for analyzing sequencing data
- Editing and saving sequence analysis parameters
- The correct procedure for reanalyzing analyzed sequencing data
- Procedure for exporting analyzed data information
- Report Format

SUMMARY

Understanding the proper procedure for analyzing sequencing results must be gained in order to receive the full benefit of the CEQ 8000 data analysis portion of the training. Failure to properly analyze data will result in the delivery of unacceptable sequencing results and possible system damages.

10 Materials and Ordering

CONSUMABLE ORDERING INFORMATION

1. Materials that must be ordered through Beckman Coulter, Inc.:
   - CEQ DTCS Dye Terminator Kit (P/N 608000)
   - CEQ DTCS Quick Start Kit (P/N 608120)
   - CEQ Separation Gel (P/N 608010)
   - CEQ Separation Buffer (P/N 608012)
   - CEQ Separation Capillary Array (P/N 608087)
   - Sample Microtiter Plates (P/N 608801)
   - 96 well plates for sequencing buffer (P/N 373660)
2. Material not provided by Beckman Coulter:
   - Sterile Water (Molecular Biology Grade, non-DEPC treated).
   - 100% Absolute Ethanol (stock) for:
     — 95% Ethanol, made from 100%, kept at -20°C
     — 70% Ethanol, made from 100%, kept at -20°C
   - 3M Sodium Acetate, pH 5.2
     — Suggested vendor Sigma, 500mL, cat#S7899
   - 100mM Na2 EDTA, pH 8.0
     — Suggested vendor: Sigma, 100mL (0.5M Na2 EDTA), cat#E7889
   - Sterile 0.5mL microfuge tubes (for sample prep and post-reaction clean-up).
   - 0.2mL thin-wall thermal cycling tubes (or plates) recommended for thermal cycler.
   - Thermal cycler w/ heated lid (Ex. - PE 2400, 9600, 9700 or MJ Research PTC-100, 200).
   - Speedvac or vacuum manifold for drying sequencing products.
   - Refrigerated microcentrifuge for pelleting sequencing products (microcentrifuge in cold room okay)
   - Micropipette set suitable for 0.5µL to 500µL volume range.

11 Advancing Your Skills

We sincerely hope that you are pleased with your new CEQ 8000.

Additional Features

A deeper understanding of the CEQ software and its numerous features can be achieved by exploration, experimentation, and utilization of the supplied documentation. Between the on-line help documents, the supplied manuals, and Beckman Coulter's excellent technical support, there is no question that cannot be answered.

Below is a list of some additional features with brief descriptions. This list is to be used as a road map to help you advance your understanding and familiarity with the CEQ software's many features.

Sample Setup

Property Sets

A list of properties related to your samples can be created and saved as a property set. Property sets are used to annotate samples. The values of each property can be set and assigned to one or many samples.

Methods editing

New methods can be created and saved. Two parameters that are often edited are injection time and the wait for capillary temperature function. Injection time can be changed to increase/decrease the amount of sample injected to optimize raw data signal levels.

Run Module

Optical Alignment

Optical alignment data can be saved for each run and viewed to aid in troubleshooting capillary to capillary signal intensity differences.

Gel Capillary Fill

Performed manually to clean out spent gel or bubbles in the capillary. This is automatically performed before each set of 8 samples.

Manifold Purge

Performed when replacing a gel cartridge or capillary array to remove bubbles from the manifold prior to filling the capillaries with fresh gel.

Sequencing Analysis Module

Preferences (initial views)

Set from the File drop-down menu. Setting the initial view determines which data panes to view upon opening sample results data. For a general overview of sample quality and success the initial view is often set to view raw data and current profile.

Batch Analysis

To reanalyze a batch of samples using new analysis parameters.

Edit Mode

The analysis mode that allows changing, deleting or inserting of bases to the called base sequence data.

Compare View/Align Mode

Compare view is set to allow the comparison of two related sequences. Used in conjunction with align mode to bring the two sequences into alignment with each other (single point alignment only)

Service Alignment

Used with pUC control sequence data only to chow the accuracy and quality of your pUC control included in each sample run.

Quality Parameters

Used to view the call scores and quality values used to perform accurate base-calling. When unzoomed all the way you can get a graph of the quality of your sequence data.

Audio Playback Function

A series of bases can be highlighted and read back to you by the computer when using this function.

Additional Software

HP Direct CD software

This software formats a CD-R disc allowing you to export individual sequence files directly from the CEQ software without having to set up a CD burn from the Adaptec Easy CD creator.

To help you benefit from additional applications features we have provided you with a reference binder and an on-board help menu. Please take some time to familiarize yourself with each of these references. If you would like to purchase additional in-lab training please contact your local service office at 1-800-551-1150.

12 Record of Operator Training

TRAINING SUMMARY

The following pages list the contents of CEQ 8000 operator training. Items initialed by the instructor are relevant to the system trained and were covered. Items marked with an "**X**" are not relevant to the system trained and were not covered.

Instructor Name _____

Operator Name _____

Operator Name _____

Sample Preparation

- ❑ Template DNA preparation and cleanup methods
- ❑ Template quantitation
- ❑ DTCS reaction setup
- ❑ Post DTCS cleanup
- ❑ Proper storage of reagents

Sample Setup Module

- ❑ Enter sample names
- ❑ Edit and assign methods
- ❑ Understand and edit analysis parameters
- ❑ Start a run

Run Module

- ❑ Install and maintain the capillary array
- ❑ Install the gel cartridge
- ❑ Proper storage of above consumables
- ❑ Install and remove the buffer and sample plates
- ❑ Refill the wetting tray
- ❑ Monitor baseline
- ❑ Start a sequencing run
- ❑ View data as a run progresses

Sequence Analysis Module

- ❑ Open sequence results
- ❑ Set analysis parameters
- ❑ Reanalysis of data
- ❑ Export data

Data Manager

- ❑ Set the working database
- ❑ Create a new database
- ❑ Create a new project
- ❑ Export of results
- ❑ When and how to archive a database

Signatures

Operator _____ Date _____

Operator _____ Date _____

The person(s) listed above have received basic instruction from the representative signed below.

Instructor _____ Date _____