ABI™ 3948

Nucleic Acid Synthesis and Purification System

User's Manual

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Contents

1 Introduction to the User's Manual/Instrument 1-1

In This Chapter 1-1
Using This Manual 1-3
Reference Manual and User Bulletins 1-4
Site Preparation and Safety 1-4
Customer Support 1-5
What Is New 1-9
Key Terms Defined 1-10
Basic Instrument Features 1-12
Applied Biosystems Synthesis Support 1-12

2 Initiating a Run 2-1

In This Chapter 2-1
Summary of Oligonucleotide Production Process 2-3
Opening a Synthesizer Window 2-5
Pre-Run Checks and Pre-Run Considerations 2-8
Instrument Preferences Settings 2-11
Auto-Resuming 2-17
Assigning Sequences 2-20
Selecting Chemistry and Order Sequences 2-24
Checking Reagents and Waste/Installing Sample Collector Rack 2-27
Loading OneStep Columns 2-30
Initiating and Monitoring a Run 2-33
Using "Pause After" During a Run 2-35
Post-Run Tasks 2-40
RunFiles 2-42

2 Monitoring a Run 3-1

In This Chapter 3-1
Monitor Chemistry View 3-2
Monitor Instrument View 3-5
Monitor Run View 3-8
Stopping a Run in Progress 3-9

4 Setup Procedures 4-1

In This Chapter 4-1
Pre-Synthesis Tasks 4-3
General Pre-Run Procedures 4-5
Preparing Synthesis Orders 4-8
Working with Phosphoramidite Bottles 4-14
Storing and Handling Reagent Bottles 4-17
Installing Reagent Bottles 4-19
Changing the Argon Tank 4-21
Calibrating Fluid Sensors 4-22
Manual Phosphoramidite Preparation 4-23

5 Maintaining the Instrument. 5-1

In This Chapter 5-1
Scheduling Necessary Maintenance 5-2
Replacing O-Rings, Gaskets, and Seals 5-2
Replacing 2-L and 4-L Inlet Filters 5-3
Manual Control Jaw Leak Testing 5-4
Cleaning the Instrument for Shut Down 5-5

6 Advanced Use of 3948Control 6-1

In This Chapter 6-1
Overview of Cycles, Procedures, and Protocols 6-3
Overview of 3948 Functions 6-4
Valve Functions 6-6
Manual Control 6-7
Sensor-Controlled User Functions 6-7
Support for New Cycles and Procedures 6-10
Creating a New Cycle or Procedure 6-11
Changing an Existing Cycle or Procedure 6-14
Saving and Retrieving Cycles, Procedures, and Functions 6-20

A Restoring B+Tet and Reagent Default Values. A-1

Default Values A-1

B Reinstalling Your Instrument. B-1

In This Appendix B-1
Controller Components B-2
Power PC Setup/Instrument Setup B-3

Installing the Software B-4
3948 Networking B-6
Testing Your Installation/Beginning a Controller Session B-8
Printing B-10
Ending a Controller Session B-10
Scenario of 3948 Use B-11

C Creating and Using Multi-Order Files C-1

In This Chapter C-1
General Information on Multi-Orders C-2
Short Multi-Order Format C-3
Using the Long Multi-Order Format C-4
Generating Multiple Synthesis Orders C-8
Creating Synthesis Order Files Directly C-9

Index Index-1

Introduction to the User's Manual/Instrument 1

In This Chapter

User and Reference Manuals

The *ABI 3948 User's Manual* is one of two manuals in the document set supporting the ABI™ 3948 Nucleic Acid Synthesis and Purification System. The second manual in the set is the *ABI 3948 Reference Manual* (P/N 4303111).

The user's manual is intended to be used for day-to-day operation while the reference manual presents detailed descriptions of the user interface as well as detailed functional information needed to fully understand and utilize the instrument.

Topics Covered

This chapter covers the following topics:

Topic	See page
Using This Manual	1-3
Contents of This manual	1-3
Where to Find System information	1-3
Reference Manual and User Bulletins	1-4
Detailed User Information	1-4
User Bulletins	1-4
Site Preparation and Safety	1-4
Safety Information	1-4
Customer Support	1-5
To Reach Us by E-Mail	1-5
Hours for Telephone Technical Support	1-5
To Reach Us by Telephone or Fax	1-5
Regional Offices	1-6
Fax-on-Demand	1-8
To Reach Us On the Web	1-8
What Is New	1-9
New Features	1-9
Key Terms Defined	1-10
Table of Key Terms	1-10
Instrument Messages	1-10

Topic	See page
Basic Instrument Features	1-12
Automates Oligonucleotide Production	1-12
Phosphoramidite Method of Synthesis	1-12
Pressure-driven Chemical Delivery	1-12
Macintosh 3948Control Software	1-12
Applied Biosystems synthesis support	1-12

Using This Manual

Contents of This manual

The user's manual contains six chapters and a single appendix.

Chapter	Title	Types of Information
1	Introduction to the User's Manual/ Instrument	♦ Purposes of User and Reference manuals ♦ Information about safety and user bulletins ♦ Description of the ABI 3948
2	Setting Up/Initiating a Run/Post Run	♦ Summary of instrument use ♦ How to set up and initiate a run ♦ Checklist of pre-run tasks ♦ Checklist of post-run tasks
3	Monitoring a Run	♦ Basic information needed to evaluate the current run in progress
4	Setup Procedures/ Changing Bottles	♦ Procedures and instructions needed to prepare for a run ♦ Changing phosphoramidite bottles using Autodilution procedures ♦ Changing reagents ♦ Reagent storage and lifetimes
5	Maintaining the Instrument	♦ Schedule of necessary maintenance ♦ Replacement of gaskets and seals ♦ Replacement of 2L and 4L Inlet Filters (PN 200270) ♦ Instrument Shutdown
6	Advanced Use of the Controller	♦ Creating modified cycles and procedures ♦ Saving and retrieving sequences, cycles, procedures, and functions ♦ Setting up instruments on a network
Appen. A	Backup Information	♦ Default Contents of the B+ Tet Calibration View ♦ Default Contents of the Reagent Utilization View
Appen. B	Controller/ Instrument Installation and Start-up	♦ Setting up the Power PC with the instrument ♦ Installing software ♦ Networking ♦ Testing Your Installation
Appen. C	How to Create and Use Multi-order files	♦ Using synthesis Multi-order files

Where to Find System information

For a general understanding of the synthesis and purification system, read the "Basic Instrument Features" on page 1-12 and, in the *ABI 3948 Reference Manual*, the introductory material in Chapters 2, 3, and 5.

Reference Manual and User Bulletins

Detailed User Information

Information of general interest provided in the *ABI 3948 Reference Manual* includes:

- Detailed descriptions of 3948Control windows and commands
- An overview of functions, cycles, and procedures as well as more detailed information on functions
- A complete listing of all chemistry cycles and procedures (provided in Appendix B)
- An instrument plumbing diagram (to be used in conjunction with chemistry listings)

User Bulletins

User Bulletins (UBs) contain technical information that is essential to ABI 3948 instrument operation and related laboratory techniques. UBs are the quickest way to ensure that you have current information. They are produced periodically and mailed to you as they become available.

Please read the UBs before operating your ABI 3948. Current UBs are found under their own tab at the end of the *ABI 3948 Reference Manua*l.

Site Preparation and Safety

Safety Information

For information on the safe operation of the ABI™ 3948 Nucleic Acid Synthesis and Purification System, refer to the *ABI 3948 DNA Synthesizer Site Preparation and Safety Guide* (P/N 903704B).

A few of the sections covered in the *Site Preparation and Safety Guide* are noted below.

Site Preparation

- Preinstallation checklist
- Items shipped with the instrument

Instrument Safety

- Instrument safety user attention words
- Safety alert symbols

Chemical Safety

- Chemical hazard warnings
- MSDSs for chemicals manufactured or distributed by Applied Biosystems
- Waste profiles

Customer Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
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Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	apisupport@sciex.com or api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

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ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844**, then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
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Hong Kong	**852 2756 6928**	**852 2756 6968**
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Singapore	**65 896 2168**	**65 896 2147**
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Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
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Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

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http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
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3	Enter the requested information and your question in the displayed form, then click **Ask Us RIGHT NOW** (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click **Ask Us RIGHT NOW**. You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

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through the Internet for fax or e-mail delivery	a. Access the Applied Biosystems Technical Support Web site at **http://www.appliedbiosystems.com/techsupp** b. Under **Resource Libraries**, click the type of document you want. c. Enter or select the requested information in the displayed form, then click **Search**. d. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click **Deliver Selected Documents Now** (or click the PDF icon for the document to download it immediately). e. Fill in the information form (if you have not previously done so), then click **Deliver Selected Documents Now** to submit your order. **Note** There is a limit of five documents per request for fax delivery but no limit on the number of documents you can order for e-mail delivery.

What Is New

New Features

A number of new features have been added to the ABI 3948 Nucleic Acid Synthesis and Purification System. These include:

- A new Instrument Preferences view (with chemistry as well as software changes to support the new view).

  See the information provided under “Instrument Preferences Settings” on page 2-11.

- Improved Multi-order Synthesis Order processing capability
  - For general information on Multi-order Synthesis Orders as well as text file formats used to creating them, see Appendix C, “Creating and Using Multi-Order Files”
- An auto-resume feature (see the discussion under “Auto-Resuming” on page 2-17)
- Separate flagging of critical messages (critical messages appear in the log on the Monitor Chemistry View
  - For more information on the Monitor Chemistry View, see “Monitor Chemistry View” on page 3-2.
  - For a listing of critical messages, see “Critical Messages” under “System Messages” in Appendix B, Cycles, Procedures, and System Messages.
- Improved jaw leak test with results reported to Microphone (see “Manual Control Jaw Leak Testing” on page 5-4)
- Addition of oligonucleotide names and associated cycle names to the Load view (see page 2-33)
- Addition of “SubStep” and “Loop” count information (outermost loop only) to the Monitor Chemistry view (reflects changes in how instrument performs chemistry - see “Upper Three Panes” on page 3-3)
- Added capability of producing 150-mer sequences
- Addition of column usage information to Bottle Usage View (see “Checking Reagents and Required OneStep Columns” on page 2-27.
- Addition of 100 sensor-based user functions (see “401 SynUpr Wet 401 to 500 UV Dry 500” on page B-21 of the *ABI 3948 Reference Manual*)
- End Row SCP/123 (see “229 End Row SCP/ 123” on page B-8 of the *ABI 3948 Reference Manual*)

Key Terms Defined

Table of Key Terms

The following table lists the key terms needed for operation of the 3948.:

Term	Definition
3948Control Views	
Protocol	Contains the three chemistry cycles needed to produce an oligonucleotide: ♦ Synthesis cycle ♦ Cleavage cycle ♦ Purification cycle When new chemistry cycles are available, you can assign them to a new protocol using the Run Protocol view. For more information on creating a new protocol with your own cycles, see Chapter 6 of this manual.
Begin and End Procedures	These procedures, chosen during Run Setup, are run before and after oligonucleotide production. If you create a new protocol with your own cycles, you may need to create revised versions of these procedures (see Chapter 6 for information on revising these procedures).
Commands	
Abort	Immediately terminates execution of a run or Manual Control action in progress.
Interrupt	Halts the instrument at the first safe step for all active chemistries (synthesis, cleavage, purification, and procedures). There are two ways to initiate an interrupt: ♦ Choose Interrupt from the Synthesizer menu ♦ Press the Interrupt button on the ABI 3948 Front Panel If you use Interrupt to halt the instrument operation, use the Resume command from the Synthesizer menu to restart the instrument
Pause After	Halts the instrument after the designated synthesis, allowing the user to extend the run or do a bottle change if necessary. To initiate a Pause After, choose Pause After from the Synthesizer menu.

Instrument Messages

Instrument Status Messages

The following instrument status messages appear in the upper right corner of all Synthesizer Window views to indicate the current instrument condition:

Term	Indicates
Ready	That the instrument is idle and ready for a run to be initiated
Running	That the instrument is currently running chemistry
Interrupted	That a run in progress has been interrupted
Manual Control	That a manual control action is in progress

Message Waiting/Critical Message Waiting

These messages are presented in the upper left corner of all Synthesizer Window views except the Monitor Chemistry View. They indicate that an important message is currently presented in the log on the bottom of the Monitor Chemistry View. More information on these messages is presented under “System Messages” on page C-38 of the *ABI 3948 Reference Manual*.

Basic Instrument Features

Automatic Oligonucleotide Production

The ABI 3948 Nucleic Acid Synthesis and Purification system completely automates the entire process of oligonucleotide production: synthesis, cleavage, deprotection, purification, quantitation, and sample collection. When used as a system utilizing ABI reagents and columns, this instrument produces high quality synthetic DNA while minimizing synthesis time and cost.

Phosphoramidite Method of Synthesis

The phosphoramidite method of oligonucleotide synthesis is used because of its inherently high coupling efficiency and the stability of the starting materials. The 3´ terminal nucleoside attached to a solid support, which is contained within a disposable column (the OneStep column). Nucleoside bases are added one at a time to the support-bound DNA chain until the sequence is fully synthesized. Solid support synthesis allows excess reagents to be removed by filtration and eliminates the need for purification between base additions.

Pressure-driven Chemical Delivery

Applied Biosystems synthesizers use a pressure driven chemical delivery system to deliver reagents and solvents to a reaction column chamber (OneStep column). Reagent and solvent deliveries also rely on our patented *zero-dead volume* valves which increase reliability, eliminate cross-contamination and reduce cycle costs.

Macintosh 3948Control Software

You can program cycles, functions, and procedures for use in the synthesizer from its Macintosh® 3948Control software. Once you download chemistry protocols, an internal controller/driver within the synthesizer exercises real-time control of the instrument. The ABI 3948 instrument can run preprogrammed protocols or you can create customized cycles. The Macintosh software is also used to fill out the Synthesis Orders used as sequence input for the instrument.

Applied Biosystems Synthesis Support

Applied Biosystems has been perfecting the science of nucleic acid synthesis on automated instruments since 1982. The ABI 3948 is our most advanced instrument to date. Applied Biosystems provides researchers with complete systems, not just instruments. Our DNA synthesis system includes instruments, chemicals, service, and technical support.

All of our chemicals are purified and rigorously analyzed to ensure high yield synthesis. Each phosphoramidite, column reagent, and solvent manufactured at Applied Biosystems is guaranteed. If you are not completely satisfied with the product (and it is used prior to any applicable expiration date and under the correct operating conditions) it will be replaced at no charge.

Applied Biosystems also maintains a large staff of fully trained service engineers strategically located around the world. In addition, technical experts are available to answer any questions about DNA synthesis, analysis, and purification.

Initiating a Run 2

In This Chapter

Topics Covered

This chapter provides all the information needed to set up and initiate a run, and also provides procedures for the operator to perform after the end of the run.

This chapter covers the following topics:

Topic	See page
Summary of Oligonucleotide Production Process	2-3
Reviewing Order Information	2-3
Performing Run Setup	2-3
Initiating the Run	2-4
Removing Tray and Printing Labels	2-4
Opening a Synthesizer Window	2-5
Accessing the Instrument	2-5
Different Passwords	2-7
Pre-run Checks and Pre-Run Considerations	2-8
Importance	2-8
Checks Before Each Run	2-8
Instrument Preferences Settings	2-11
Introduction	2-11
Auto-Resume Feature	2-11
Types of Settings	2-11
Setup Variables	2-12
Setup Choices	2-14
Instrument Dip Switches	2-15
Auto-Resuming	2-16
Theory of Operation	2-16
Ties to Jaw/Block Pressure Testing	2-16
Three Basic Requirements to Enable Auto-Resume	2-17
Assigning Sequences	2-19
Introduction	2-19
Assigning to the Run Setup View	2-19
Assigning Sequences for the Run	2-21
Displaying Sequence Information	2-22

Topic	See page
Removing a Sequence	2-22
Selecting Chemistry and Order Sequences	2-23
Introduction	2-23
Autosorting Procedure	2-23
Manually Sorting Sequences	2-24
Sorting and Processing of Sequences by the System	2-25
Checking Reagents and Waste/Installing Sample Collector Rack	2-26
Introduction	2-26
Checking Reagents and Required OneStep Columns	2-26
Reagent Consumption Information	2-27
When to Renew Reagents	2-28
Loading OneStep Columns	2-29
Introduction	2-29
Loading	2-29
Initiating and Monitoring a Run	2-32
Proper Instrument Preparation	2-32
Starting and Monitoring a Run	2-32
Using Pause After During a Run	2-34
Introduction	2-34
Extending a Run	2-34
Replacing a Reagent Bottle/Removing a Priority Oligonucleotide	2-38
Post-run tasks	2-39
Types of Tasks	2-39
Procedure for post-run	2-39
RunFiles	2-41
Information in the RunFile	2-41
Opening a RunFile	2-41
Printing Labels for Oligonucleotides	2-43

Summary of Oligonucleotide Production Process

Reviewing Order Information

Review incoming synthesis orders or multi-order files to determine they are filled out correctly. Edit the files as necessary. Information on synthesis orders is provided under “About Synthesis Orders” on page 4-38 of the *ABI 3948 Reference Manual* and information on multi-orders is provided in Appendix C, How to Create and Use Multi-order Files.

An order can include the following:

- Customer information, which can include customer name, address, and phone/fax number
- Purchase order number and account number
- Order date
- Comments
- Set Protocol to use and Purify/Crude option

Note You can change the protocol during run setup, but you cannot change the Purify/Crude option.

- Sequence name
- Listing of sequence to produce
- Base composition breakdown

Performing Run Setup

The following table lists the steps in performing run setup.

Step	Action
1	Load the Synthesis Orders into the Sequence Order list using the Open button in the Run Setup View.
2	Transfer the sequences represented by orders from the Sequence order list into the Run Setup table.
3	Select the proper Begin and End procedures.
4	Assign the protocol you want used with each sequence or group of sequences. Any row of three orders must use the same protocol.
5	Autosort orders into the optimal order for processing. This stage is optional. For information, see “Sorting and Processing of Sequences by the System” on page 2-26.
6	Use the Bottle Usage view to determine if sufficient reagents and phosphoramidites are available on the instrument for the next run (see “Checking Reagents and Waste/Installing Sample Collector Rack” on page 2-27). Use the following table to determine the next step.

If quantities in the ABI 3948...	Then...
would run out before urgently needed oligos are produced	change bottles before the run.
would run out after urgently needed oligos are produced	program a Pause After and change bottles during the programmed pause.

Step	Action
7	Load correct OneStep™ columns into the instrument according to the 3´-terminal nucleoside covalently bound to the support (the correct column is indicated in the "Pie chart view," see step 3 on page 31).
8	Check the Instrument Preferences (using the command from the Synthesizer Menu) and make any changes needed for the run. **CAUTION** **To prevent damage to the Sample Collector, it is essential that the rack type chosen is the same as the actual rack type used. To use the red rack (8 x 6 configuration), leave the checkbox blank (default). To use the white rack (4 x 12 configuration), check the box.**
9	Load a Sample collector rack of the type set in Instrument Preferences.
10	Empty waste (primarily sample collector waste bottles, but also empty large aqueous waste and flammable waste bottles, in lower compartment if needed). **Note** More information on emptying waste bottles is provided under "General Pre-Run Procedures" on page 4-5.

Initiating the Run

Press the Start button after loading the columns and monitor the production of oligonucleotides through the Monitor Chemistry View.

Removing Tray and Printing Labels

When the run is completed, take the following steps.

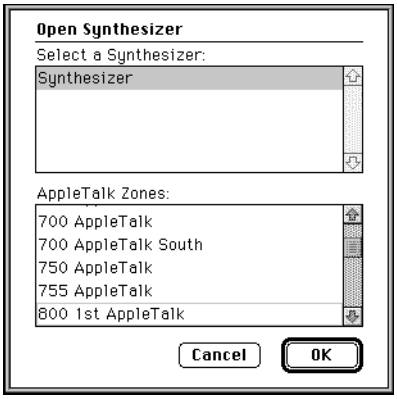
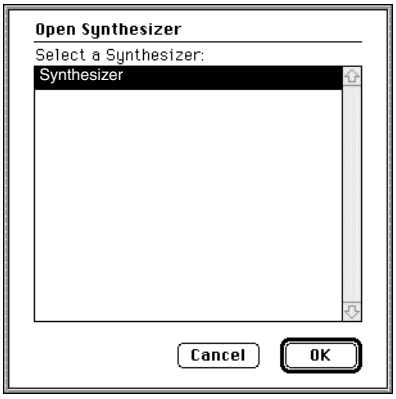
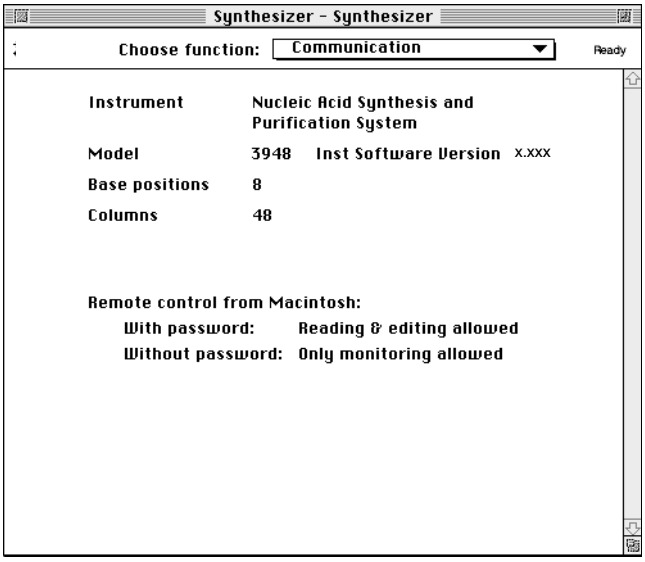
Step	Action
1	Remove the tray containing the completed oligos from the instrument.
2	Open the RunFile generated for the run and use the Print label feature to generate labels for rack or tubes. ♦ For a list of the types of information contained in a RunFile, see "Information in the RunFile" on page 2-42. ♦ For information on how to do label printing, see "Printing Labels for Oligonucleotides" on page 2-44.
3	Affix labels to each tube to identify output oligonucleotides.

Opening a Synthesizer Window

Accessing the Instrument

The following procedure describes how to open a Synthesizer window for the instrument when both the Macintosh® and the instrument are on.

To open a Synthesizer window for the instrument:

Step	Action
1	Double-click the 3948Control application icon to start the application. The splash screen appears briefly while the application is loading, and then one of the Open Synthesizer dialog boxes shown below appears. If the application is already running, then choose Open Synthesizer from the File menu.   **Note** The type of dialog box presented will depend upon the type of connection between the Macintosh and the instrument. If your ABI 3948 instrument is connected to the Macintosh by a network with many AppleTalk zones, a dialog box like that on the left above is presented. If your 3948 is connected directly to the Macintosh (on a single zone LocalTalk network), a dialog box like that on the right above is presented.
2	Select the name of the instrument (*i.e.*, “Synthesizer” as shown in step 1) and click OK. The Synthesizer window appears. 

To open a Synthesizer window for the instrument: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>If the name of the synthesizer you want to use is not visible in the upper pane of the left or right dialog box (step 1), the instrument is either not turned on or is not communicating with the Macintosh.

<table>
<tr><th>If the instrument...</th><th>Then...</th></tr>
<tr><td>is turned off</td><td>turn the instrument on</td></tr>
<tr><td>is turned on and communication is not established (name does not appear in the “Select a Synthesizer” window)</td><td>do one of the following:
♦ Check the cable connections between the instrument and the Macintosh (for systems not on a network), or
♦ Check with your system administrator for a solution (for system components connected by network)</td></tr>
</table>

The window (step 2) will have the name of your instrument assigned to it. After it appears, the Password dialog box will open.</td></tr>
<tr><td>4</td><td>Type in your password and click OK to open access to the database window. If you enter an incorrect password, the message shown in the bottom of the dialog box will appear. If this happens, re-enter your password

Note The instrument will initially not have a password assigned. When no instrument password has been assigned, press Return (or click OK) to gain access to your instrument.</td></tr>
</table>

Different Passwords

There are two types of passwords:

- A Full Access or read and write password gives full access to the instrument (Choose Function pop-up menu (Figure 2-1, left).
- A Monitor Access, or Read Only password, provides access only to the Communication, Monitor Chemistry, Monitor Instrument, Monitor Run, and Power Fail History views (Choose Function pop-up menu (Figure 2-1, right).

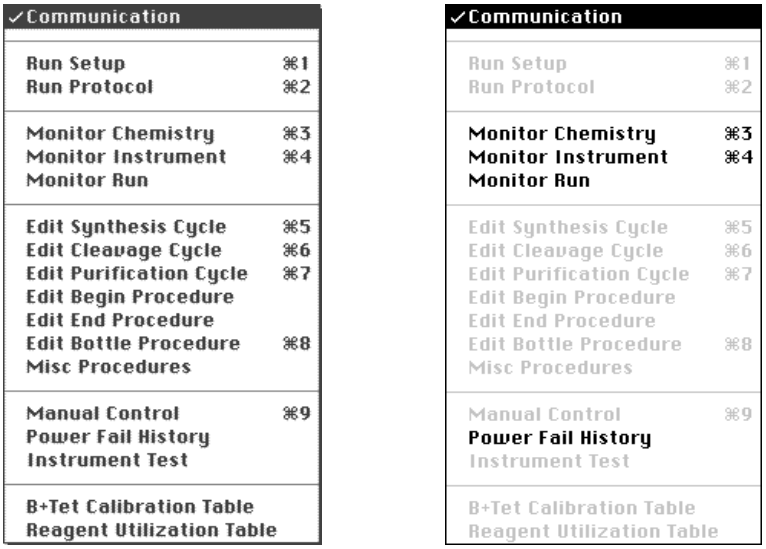


Figure 2-1 Two versions of Choose Function Pop-up menu

Whenever an instrument has been accessed using the Open Synthesizer command (used by default when starting the 3948Control application), you can determine which type of password (if any) was used to access the instrument. By default, the first time the 3948Control application is used to access the instrument, no password is assigned and full access is available as shown in Figure 2-1, left.

For information on setting passwords, see “Change Password Command” on page 4-25 of the *ABI 3948 Reference Manual.*

Pre-Run Checks and Pre-Run Considerations

Importance

It is a good practice to run through the checklist below before starting a run. If you do not know how to perform a step, the list directs you to further information provided in Chapter 4, “Setup Procedures.”

Checks Before Each Run

To prevent common problems and to ensure efficient operation, do the checks below.

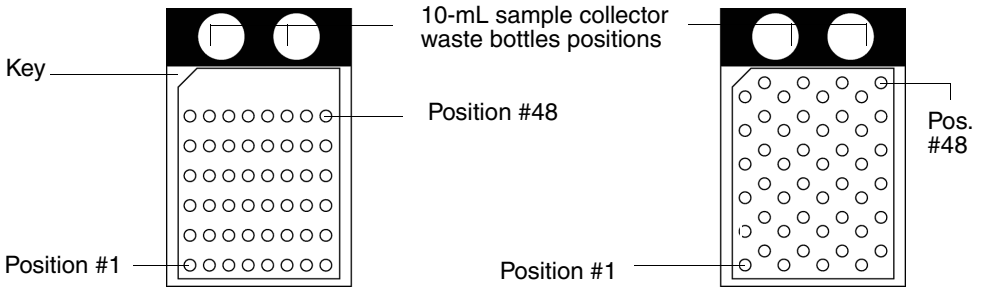
Note Before handling chemicals in response to any step below, be sure to observe the precautions listed under “Precautions to Observe” on page 4-19.

Make these checks before each run:

Check	Action
Synthesis Orders	Have the Synthesis Orders for producing oligonucleotides during the run been generated? If not, refer to “Organizing and Processing Synthesis Order Files” on page 4-11 for instructions on how to produce them.
Argon tank pressure	Check the pressure of the argon tank. The secondary pressure should be between 14 and 15 psi, but 14.5 is best. To get the most precise reading, do not use the low pressure gauge on the tank. Instead, use the system’s input pressure reading. Use ⌘-4 in the 3948Control interface application to present the Monitor Instrument view. Adjust the low pressure valve on the tank as needed to get the desired pressure. Change the tank if it is depleted. Be ready to change the tank when the high pressure gauge drops below 200 psi. With average synthesizer use, an argon tank should last approximately 2 months. To change an argon tank, see “Changing the Argon Tank” on page 4-22.
Bottle seals	Ensure that bottles are installed correctly, with a tight seal, on every position. ♦ All bottle positions must have a bottle attached (empty bottles at unused positions) in order to run the instrument. ♦ Leaking bottles cause an audible clicking sound from the Pressure Regulator Control (PRC) whenever the instrument is idle. **! WARNING ! Ensure that both upper and lower reagent bottle doors are closed during operation.**
Fluid sensors	If necessary, calibrate fluid sensors**.** Sensor calibration is done during instrument setup and is otherwise rarely needed.
Amidites	Check that sufficient phosphoramidites (amidites) are available for the run. If necessary, prepare phosphoramidites using the auto-dilution feature. Procedures for auto-dilution of phosphoramidites are provided in Chapter 4, “Setup Procedures/ Changing Bottles.”

Make these checks before each run: *(continued)*

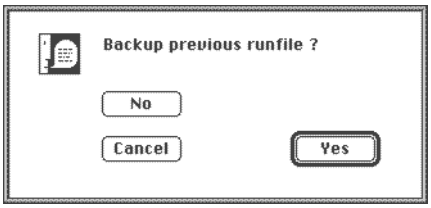
Check	Action
Waste bottles	Check the waste level of the three types of liquid waste. ♦ Flammable liquid waste is collected in a 2 1/2-gallon bottle located in the lower instrument compartment. ♦ Halogenated liquid waste is collected in a 4-L bottle located in the lower instrument compartment. ♦ Sample collector liquid waste (mostly water and acetonitrile), is collected in two 15-mL bottles located to the rear of the sample collector platform (a run of 48 oligonucleotides fills these bottles about 1/2 full). The 3948 generates about 90 mL of flammable waste and about 15 mL of halogenated waste per 20-mer oligo. When a waste bottle is full, it must be emptied and the waste disposed of properly. A waste bottle can be changed prior to a synthesis or when a synthesis is interrupted. For more information on emptying waste containers, see step 5 on page 4-3.
Reagent levels	Check reagent levels of all bottles. This is done using the Bottle Usage command during run setup. Replace bottles with fresh reagents before the beginning of the run or during a pause (set by Pause After) in the run in order to replace particular reagents prior to reagent depletion (see page 27).
Sample collector	Make these two checks for the sample collector: a. Make sure that the sample collector is equipped with either the standard red rack or the optional white rack (rack information is presented in the figure below). Make sure that the rack to be used contains sufficient tubes/vials to collect the samples. If fewer than 48 oligonucleotides will be produced by a run, the tubes/vials in the collector tray must be correctly inserted, as shown below. **CAUTION A sample rack must be inserted key first for the instrument to function and to prevent damage to the instrument.** b. Check Monitor Instrument view to ensure that the type of rack loaded into the sample collector is selected. Use Instrument Preferences to change type.



! WARNING ! Remove all caps when using screw type tubes.

Note The standard red rack (OligoRack™), with the micro-titer format, is intended for delivery to a single customer, and the optional white rack is intended for delivery of individual oligonucleotides to customers.

Make these checks before each run: *(continued)*

Check	Action
RunFile Created?	Was a RunFile generated for the last run? Before loading the turntable, you will be prompted by a dialog box like that shown below to produce a RunFile. If you have not used the Generate RunFile command (⌘ - M keyboard shortcut) since the last run, click Yes to save a RunFile.  **IMPORTANT** Be sure that the information for the last run is documented by a RunFile, because this information is not available after a new run starts. See step 5 on page 41 of the post-run procedure for more information on placing sample tubes in the sample collector carrier.

Instrument Preferences Settings

Introduction

The defaults in the Instrument Preferences window are sufficient for normal instrument operation. Make changes in these settings only after you fully understand the auto-resume feature and how specific parameters on this page affect this feature and instrument operation. For further information, see “Auto-Resuming” on page 2-17 and “Special Functions” in Appendix A (Valves and Functions, Multi-Order Formats) of the *ABI 3948 Reference Manual*.

The Instrument Preferences window enables a number of important chemistry and system changes to be made in a single place. This feature makes the instrument easier to use, facilitates adjustment of the instrument to the specific environmental conditions found in different labs, and supports the Auto-resume feature.

Auto-Resume Feature

The Auto-resume feature gives the instrument the ability to recover from less than catastrophic system problems during unattended instrument operation and continue on to complete a run. The implementation of Auto-resume is intended to strike a balance between throughput and reliable operation. See the discussion of this feature under “Auto-Resuming” on page 2-17.

Deprotection Time Interactions

IMPORTANT As explained below, it is important to understand the relationship between the Deprotect Mins value in Instrument Preferences, the deprotection heater time-out value, and any time entered into the Depro Htr Wait step (Function 169) in the Purification cycle.

Using a Single Deprotection Time

Basically, the time at which the deprotection heater times out is the Deprotect Mins value plus 1 hour, unless a shorter non-zero time value is entered into the MISC field in the Function 169 step of the purification cycle. This means that with a default Deprotect Mins setting of 60 minutes or 1 hour, the deprotection heater will time out in 2 hours with the default MISC field value of zero in Function 169.

Using Multiple Deprotection Times

If different deprotection times need to be used for different cycles, enter the longest time desired in the Deprotect Mins parameter in Instrument Preferences and specify shorter time(s) within the purification cycles (in Function 169). Times entered into the Depro Htr Wait step in Purification cycles only override the Instrument Preferences Deprotect Mins value when they are shorter, i.e., when the deprotection heater has not yet timed out before the purification cycle is run.

Note The user needs to be aware that, with a very long synthesis in progress, it is possible for time-out to occur before the Depro Htr Wait step (Function 169) is ever reached because the long synthesis delays the turntable move and therefore delays the start of the purification cycle containing the Depro Htr Wait step.

Types of Settings

The Instrument Preferences view, accessed by the Instrument Preferences command (Synthesizer menu), allows a number of chemistry and hardware settings to be made in one place:

Parameter Type	Purpose
Setup Variables	Allows the user to change values for key functions in chemistry cycles.
Setup Choices	Allows a number of choices for hardware or operation conditions.
Instrument Dip Switches	Shows the settings of dip switches on the processor (CPU) board. These switches control instrument features and should only be changed by an ABD Service Engineer.

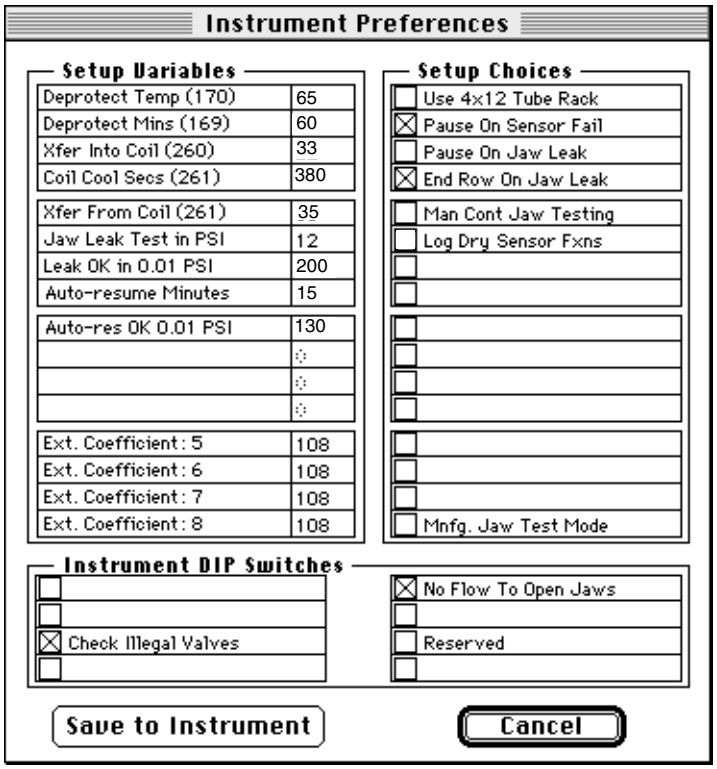


Figure 2-2 Instrument Preferences View

Note The Instrument Preferences choices shown in Figure 2-8 are the default values. If you make changes, you can refer back here to see a list of the default values. Do not check the “Mnfg. Jaw Test Mode” since this function is reserved for manufacturing purposes.

Setup Variables

Rather than having to create custom cycles to implement certain chemistry changes, the values for Setup Variables can be changed on the Instrument Preferences view for use during the next run.

Entries made for Setup Variables, the group of entries on the upper left corner of the view, enable you to implement chemistry changes in one place rather than write custom cycles to implement your changes. The types of changes listed below are possible with these variables.

Deprotect Variables (Deprotect Temp and Deprotect Mins)

These variables allow you, respectively, to set the final deprotect temperature and the minimum time that the final temperature will be held during deprotection.

Deprotect Variables	Default/ Maximum	Description
Deprotect Mins (169)	60/480	The minimum time that the Deprotection coils will remain hot once the final deprotection temperature has been reached.
Deprotect Temp (170)	65	The final temperature at which the Deprotection coils are set for deprotection.

Transfer Variables (Xfer Into Coil/Xfer From Coil/Coil Cool Secs

These variables allow you, respectively:

- To set the temperature at which the deprotection coils are set for the transfer into the coils,
- To Set the time (in seconds) to allow the temperature of the deprotection coils to drop before transfer to purification, and
- To set the time (in seconds) to allow the deprotection coil temperature to drop before a transfer is made from them

Transfer Variables	Default	Description
Xfer Into Coil (260)	33	This variable sets the deprotection coil temperature for the transfer from the cleavage vessels into the deprotection coils.
Xfer From Coil (261)	35	This variable sets the temperature of the coils for the transfer to Purification.
Coil Cool Secs (261)	380	This variable sets the time for the Deprotection coils to cool before the transfer to purification.

Note Values in parentheses above are the numbers of the associated functions for which changes are made.

Jaw Leak Variables (Jaw Leak Test/Leak OK 0.01 PSI)

These variables allow you, respectively, to set the pressure used for the jaw leak test and to set the maximum passing leak rate allowed for the test.

Jaw Leak Variables	Default/ Maximum	Description
Jaw Leak Test	12	This variable sets the pressure in PSI used for this test.
Leak OK 0.01 PSI	2.00/5	This variable sets the maximum passing leak rate (in 1/100th PSI) for Jaw/Block Leak testing.

Note See information provided under "Pause on Jaw Leak Check Box" on page 2-33 for more information.

Auto-Resume Variables (Auto-resume Minutes/Auto-res OK 0.01 PSI)

These variables allow you, respectively, to set the time the system will wait to auto-resume after a delivery failure, and to set the maximum jaw leak rate in 1/100th PSI allowed during testing for auto-resuming to be enabled.

Auto-Resume Variables	Default/ Maximum	Description
Auto-resume Minutes	15	This variable sets the time the system will wait to auto-resume after a delivery failure.
Auto-res OK 0.01 PSI	1.30/1.8	This is the maximum jaw leak in 1/100th PSI allowed during testing for auto-resuming to be enabled.

Note For information on the auto-resume function, see "Auto-Resuming" on page 2-17. More information on this feature is provided in Appendix A.

Extinction Coefficient Variables (Ext. Coefficient 5/6/7/8)

These variables represent 1% of the extinction coefficient values for the contents of Bottles 5–8 and are used to convert ODU (Optical Density Unit) values to pmol/mL.

Extinction Coefficient Variables	Description
♦ Ext. Coefficient 5 ♦ Ext. Coefficient 6 ♦ Ext. Coefficient 7 ♦ Ext. Coefficient 8	These values represent 1% of the extinction coefficient values for the contents of Bottles 5- through 8 and are used to convert ODU values to picomole/mL.

Note Refer to the Chapter 3 of the *ABI 3948 Reference Manual* for more information.

Setup Choices

These checkboxes provide a central place to make the following hardware or operational settings:

Setup Choice Checkboxes	Description
Use 4 x 12 Tube Rack	Used to designate that the white rack will be used (unchecked to use the red rack. A checked box is the setting for using the white rack (4 x 12 configuration). An unchecked (blank) box blank is the setting for using the red rack (8 x 6 configuration).
Pause On Sensor Fail	Used to enable pausing on sensor failure. The instrument will pause on a failed sensor delivery with this box checked. The box is checked by default. The system may auto-resume from this pause if auto-resume is enabled.
Pause On Jaw Leak	Used to enable pausing on a failed leak test. The instrument will pause on a failed leak test with the box checked (unchecked by default). The system will not auto-resume from this pause.
End Row on Jaw Leak	Used to disable a row when a jaw leak occurs. The affected turntable row will drop out of the run on a failed jaw leak test but the run will continue processing other rows (as a default with the box checked).
Man Cont Jaw Testing	Used to enable Jaw/Block testing when jaws are closed manually (Manual Mode). This parameter enables jaw/block testing in Manual Control mode - default is unchecked.
Log Dry Sensor Fxns	Used to enable “dry” sensor readings to be reported to the Microphone log. This parameter enables “dry” sensor flows (flushes and backflushes) report to the Microphone log - normally only “wet” sensor deliveries report. Default is unchecked. **Note** Be aware that enabling this parameter will produce a much larger Microphone file.
Manufacturing Test Mode	For manufacturing use only.

Instrument Dip Switches

These are hand Dip switches on the CPU board whose settings are reflected on this page. They may only be set by taking off a side panel and flipping the switches on the board.

Check Illegal Valves

This parameter is enabled by default. Makes sure no “illegal” value combinations are being attempted by User functions such as delivery of an acid with a base.

No Flow to Open Jaws

When checked, deliveries cannot be made to an open jaw. This box is checked by default.

Reserved

This box is reserved for future instrument releases.

Auto-Resuming

Theory of Operation

Auto-resume is a feature designed to overcome the occasional sensor delivery failure. When an operator is not present to aid recovery, an instrument that has paused due to a sensor delivery failure may automatically restart itself after the period of time specified by the operator. Experience has shown that the delivery will eventually complete as long as the bottle is not empty, so it is useful to have an instrument persist in attempts to complete a run.

The Instrument Preferences view contains the testing pressure in pounds per square inch for jaw/block pressure tests performed within cycles and defines the terms under which the auto-resume feature will be enabled. The term “auto-resume” refers to the 3948 instrument’s ability to wait for a period and then restart itself after being paused by a sensor delivery failure.

Note When a sensor delivery failure occurs involving B+Tet, Auto-resume is disabled.

When an instrument is paused with an auto-resume pending, any indication that an operator is present and aware of instrument status will cause the auto-resume to be cancelled at once. Therefore, operator interventions, such as resuming the run, executing a function in Manual Control, or even “interrupting” the auto-resume pause will cause the auto-resume to be cancelled.

Ties to Jaw/Block Pressure Testing

Auto-resume is closely tied to successful jaw/block pressure testing so that reagents will not be delivered repeatedly when a leak is the source of delivery failure. Basically, two checkboxes on the Instrument Preferences, End Row on Jaw Leak and Pause on Jaw Leak, give two options for instrument operation. Only the End Row on Jaw Leak choice, the default, gives the auto-resume capability. The other option, enabled using the Pause on Jaw Leak checkbox, requires operator intervention (see “Ending Rows” on page 2-19).

When Auto-resume is enabled by checking the End Row On Jaw Leak check box and the jaw test fails, all columns in the module associated with the failed jaw will be deactivated immediately but the instrument will continue with the run. For throughput reasons, this is a useful option for an unattended instrument and is set by default.

Three Basic Requirements to Enable Auto-Resume

For auto-resume to be enabled, three basic requirements must be met.

- The settings in the Instrument Preferences Window must support the auto-resume feature.

  This requirement is met automatically by using the default settings in the Instrument Preferences page (those shown in Figure 2-2) which are intended to implement auto-resuming. Changes can be made in default settings only if they do not change the requirements set in the list below.

- The system must be currently leak tight so that auto-resuming will not result in spillage due to a leak.

  Depending on the results of each individual jaw/block leak test, some active cycles may not be allowed to auto-resume at the same time that others would be.

- The third requirement is that there be no manual intervention while an auto-resume is pending.

  Since auto-resume is an automatic process designed for unattended operation, control is always surrendered to the operator if there is any manual intervention with an auto-resume pending.

To meet these requirements, a number of factors must be in place:

- Pause On Sensor Fail must be checked in the Instrument Preferences view so that sensor delivery pausing is enabled.
- Auto-resume Minutes in the Instrument Preferences Window must be set to some value greater than zero (15 minutes is the default). A value of zero means the instrument will not auto-resume.
- Jaw/block pressure testing must be in effect and the testing pressure must be done at the system maximum 12 psi as specified by the Jaw Leak Test in PSI setup variable in the Instrument Preferences Window. A value of 12 psi is the default for this setup variable.
- Jaw/block pressure testing must be performed with a minimum pressure drop time of 30 seconds. This is the default time provided for by the system but care must be used if this time is modified either within the jaw close step or by setting an alternate default using Jaw Test Times.
- Jaw/block testing must pass the Auto-res OK 0.01 PSI test standard in the Instrument Preferences Window. This allows a maximum pressure drop of only 1.8 psi to pass compared to a maximum allowable drop of 5 psi for the “Leak OK in 0.01 PSI” preference value.

  These setup variable default values are 1.3 psi (Auto-res OK 0.01PSI) and 2.00 psi (Leak OK in 0.01 PSI) entered in 1/100's psi as 130 and 200. It is possible for the instrument to “pass” the jaw/block leak test and run chemistry while not passing a more stringent standard required to enable auto-resume.
- Any intervention by an operator during an auto-resume wait period (Interrupt) will cancel the auto-resume. Such operator interventions include initiating a manual control action, manually resuming the run, or even “interrupting” an instrument that is paused with an auto-resume pending. Also, auto-resume will never go into effect while a procedure or other operation is underway in Manual Control. However, once a run is resumed (by the operator), auto-resuming is re-enabled and will go into effect if there are any future sensor delivery failures within the cycle.

If the above requirements are met, sensor delivery functions will auto-resume when they fail to deliver. The exceptions to this are function number 1, B+TET to Syns, and ramping functions. For information on how these functions work, see the headings with these titles in Appendix A of the *ABI 3948 Reference Manual*.

Ending Rows

If the Pause On Jaw Leak checkbox in the Instrument Preferences Window is checked and the jaw test fails, the run will be paused at that point and will not auto-resume. This may be the preferred option when the instrument is running with an operator in attendance. For information on how to proceed when using this option, see the discussion under 229 End Row SCP/123 in Appendix A (Valves and Functions, Multi-Order Formats) of the *ABI 3948 Reference Manual.*

Note The End Row SCP/123 function should be used only when the system is interrupted because of a jaw leak, not during a Pause After.

If you are using Pause On Jaw Leak, there are several scenarios where End Row SCP/123 could prove useful. If synthesis falters, for example, the row can be terminated while the oligos in cleavage and purification continue on to conclusion. Or a row with a leaky OneStep™ column that appears in the middle of a run can be skipped over while the other oligos, both before and after that row, are run to completion.

When 229 End Row SCP/123 is used to terminate a purification cycle for a given row during a system interrupt, the system is unable to automatically advance the sample collector upon a restart as required. You will be presented with a critical error message announcing that the row was ended and directing you to advance the sample collector by one position.

Moving the sample collector ensures that the oligonucleotides produced are collected in the rows corresponding to their turntable positions.

Assigning Sequences

Introduction

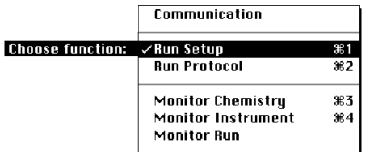
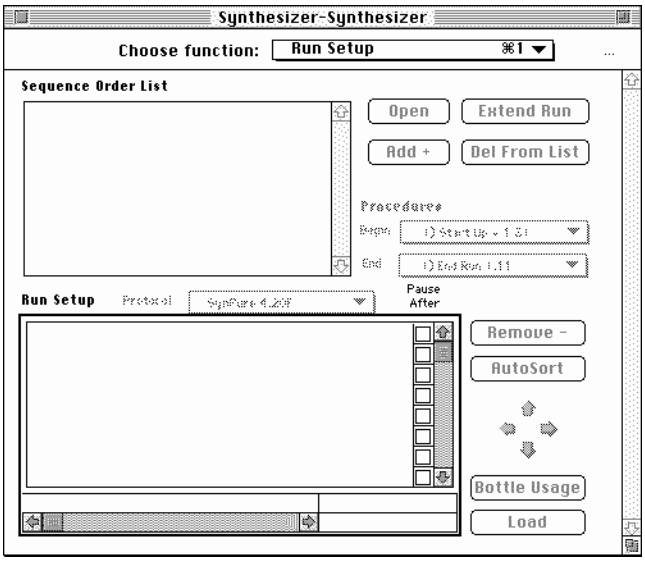
This section describes how to:

- Assign Synthesis Orders for production during a run
- Display information about a Synthesis Order

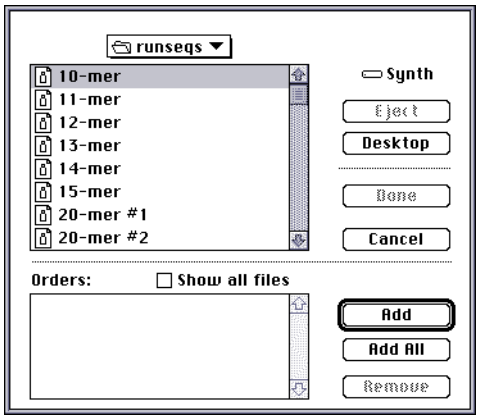
Assigning to the Run Setup View

The following procedure describes how to assign orders to the Sequence Order list for a run.

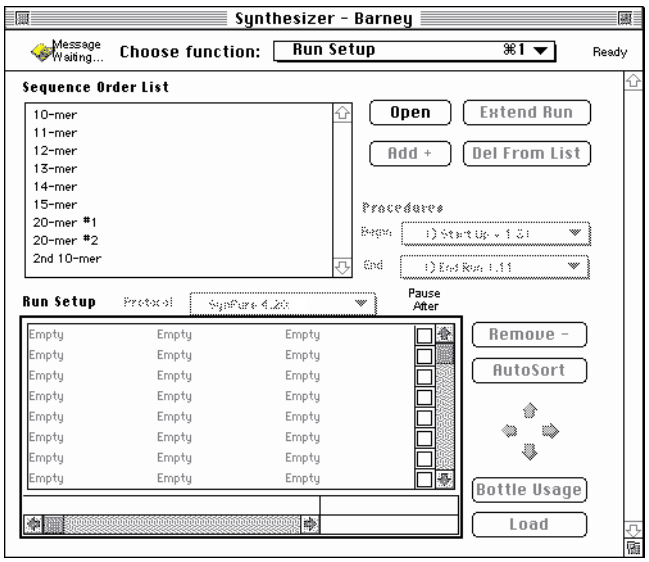
To assign Synthesis Orders:

Step	Action
1	Choose Run Setup from the Choose function pop-up menu.  **Note** Upon initial communication with the instrument, the Macintosh will read the data from the 3948 the first time you choose the popup menu (indicated by the appearance of the Status dialog box). You cannot access the Synthesizer window until all the data is copied from the instrument into the current view. **IMPORTANT** When the Status dialog box is present, it is important to keep 3948Control the active application in the Macintosh Finder. The Synthesizer window appears in the Run Setup view, either immediately or after the application is finished reading all the data from the instrument. 

To assign Synthesis Orders: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Click the Open button (lower figure on previous page) and a Directory dialog box appears.
 
</td></tr>
<tr><td>3</td><td>You can take the following action.
<table>
<tr><th>If you want to...</th><th>Then...</th></tr>
<tr><td>add a single Synthesis Order</td><td>select the Synthesis Order from the top scroll box and click Add.

The order for a sequence moves to the Orders list.</td></tr>
<tr><td>add all the Synthesis Orders</td><td>click Add All.

All the orders move to the Orders list.</td></tr>
</table>
Note Creation of Synthesis Orders is a task that is usually done in advance of starting a run. See “Organizing and Processing Synthesis Order Files” on page 4-11 in Chapter 4 for information on creating Synthesis Orders.</td></tr>
<tr><td>4</td><td>After selecting all the sequences to be synthesized, click Done. The sequences are added to the Sequence Order List in the Run Setup view.
 
</td></tr>
</table>

Assigning Sequences for the Run

To assign sequences for the run:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>You have the following options to select sequences from the Sequence Order List.
<table>
<tr><th>If you want to select...</th><th>Then...</th></tr>
<tr><td>a range of sequences</td><td>select the first sequence in the range (e.g.,10-mer in the figure in step 4 on the previous page), press the Shift key and select the last sequence in the range (e.g. 14-mer, to select sequences in the 10-mer through 14-mer range).</td></tr>
<tr><td>multiple sequences</td><td>select the first sequence (e.g. 10-mer), press the Command (⌘) key and then select the other sequences you want to add to the list (12-mer, 14-mer, etc.).</td></tr>
<tr><td>all the sequences</td><td>select a single sequence and press Command (⌘)-A.</td></tr>
</table></td></tr>
<tr><td>2</td><td>Click Add + to add the sequences you selected from the Sequence Order List to the Run Setup scroll list.

Note See Figure 2-3 on the following page for an example of nine orders added to the Run Setup list.</td></tr>
</table>

Displaying Sequence Information

How to Select the Sequence

You can display information from the original Synthesis Order in the Run Setup scroll box by selecting the sequence in the Run Setup scroll box.

The following information is displayed for an individual sequence:

- Detailed base by base listing between 5´ and 3´ ends
- Purification/crude state of the oligonucleotide to be synthesized (Purify Oligo, Crude DMT-off, or Crude DMT-on)
- Length of the sequence
- Protocol (if selected in Synthesis Order)

Example of the Information Listed

The following is an example of the information listed when you select a sequence from the Run Setup scroll box (Figure 2-3).

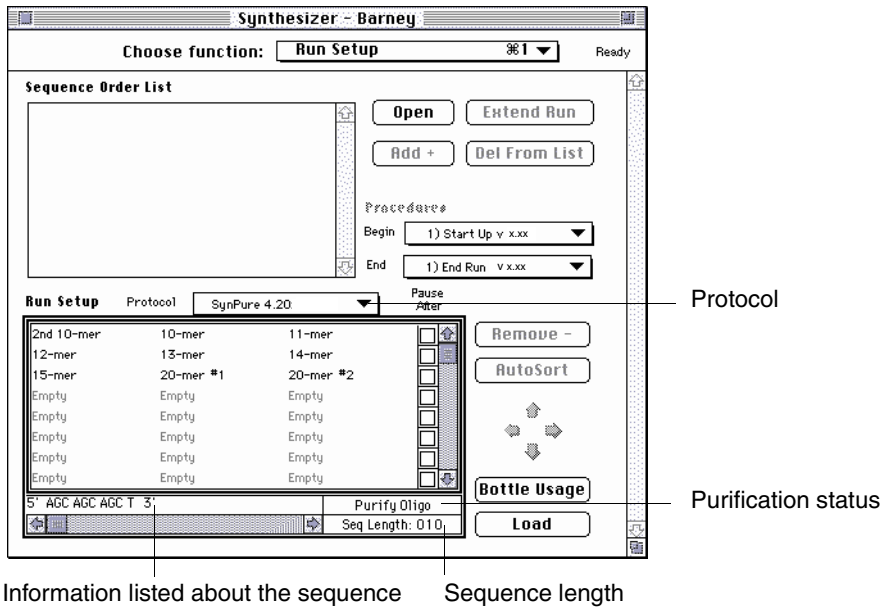


Figure 2-3 Information listed for a selected Synthesis Order

Removing a Sequence

To remove a sequence from the Run Setup scroll box, first select the sequence and then click Remove. The selected sequence returns to the Sequence Order List.

Selecting Chemistry and Order Sequences

Introduction

After you have entered the desired orders/sequences into the Run Setup scroll box, as described under the previous subsection “Assigning Sequences,” do the following to prepare for the run (as described in the procedure below):

- Select Begin and End procedures for the run.
- Assign appropriate protocols to orders.
- Autosort sequences in the correct order for production.

  More information on sorting sequences is provided in “Sorting and Processing of Sequences by the System” on page 2-26.

Note If you want to use any Begin/End procedures or protocols besides the defaults, you will need to develop them before proceeding further (see Chapter 6 for more information).

The procedure below follows the standard process of autosorting sequences using this system feature. For information on manually sorting and assigning protocols, see “Manually Sorting Sequences” on page 2-25.

AutoSort Procedure

Follow this procedure when you are ready to perform the tasks listed above using the AutoSort feature.

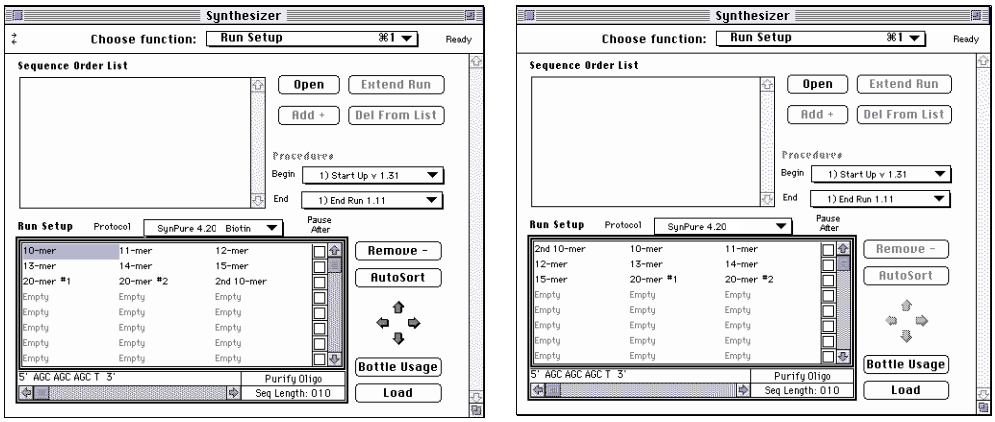
Note In the Run Setup table examples used in the following procedure, seven of the sequences are from 10 to 15 bases in length and two sequences contain 20 bases (sequences are labeled according to the number of bases they contain).

To assign protocols and autosort the sequences:

Step	Action
1	Select the appropriate Begin and End procedures from the pop-up menus for the run.
2	Check the protocol assignments for sequences by selecting each sequence individually to present the name of the assigned protocol in the Protocol name field. If no protocol was assigned, “None” will be displayed in the Protocol name field.
3	To change a protocol assignment for one or more sequences (or assign a Protocol if none was previously assigned), do the following: a. Select the sequence or sequences to receive a particular protocol. b. Choose the appropriate protocol from the Protocol pop-up menu. **IMPORTANT** If you select one or more sequences, either complete rows or any group of two or more sequences, “None” will be displayed in the Protocol name field. The only way to actually see protocol assignments is to individually select sequences. **Note** The same protocol can be assigned to all sequences in the Run Setup scroll box or a different protocol can be assigned to each row in the turntable (every oligonucleotide in a row must have the same protocol). The version number displayed for protocols (v.4.20, etc.) is incremented with each new software release. Once you have assigned protocols to the sequences in the Run Setup scroll box, the AutoSort button becomes available (ungrayed), allowing you to autosort the sequences into the most efficient order for production.

To assign protocols and autosort the sequences: *(continued)*

Step	Action
4	Click the AutoSort button to order sequences for production with the minimum run time (maximum throughput). The figure below shows that autosorting changed the order in which the example sequences are ordered. For more information on autosorting, see “Sorting and Processing of Sequences by the System” on page 2-26. The figure shows the Run Setup table before (left) and after (right) autosorting.



Manually Sorting Sequences

The best throughput on the instrument is attained by autosorting the sequences but you can manually move a set of three sequences to the top of the table to produce them first. Such “priority” oligonucleotides can be removed any time after they are produced when the sample collector is in the open position.

Note The sample collector is closed only when an oligonucleotide is being delivered or the needle is being washed.

The arrow buttons to the right of the Run Setup scroll box allow you to manually assign sequences to a specific position in the desired turntable row. Sequences are moved individually in this way by first selecting a sequence and then using the arrow keys as follows:

- Clicking the Right arrow moves a selected sequence one position to the right.
- Clicking the Left arrow moves a selected sequence one position to the left.
- Clicking the Up arrow moves a selected sequence to the corresponding position in the previous row.
- Clicking the Down arrow moves a selected sequence to the corresponding position in the next row

IMPORTANT Whenever you manually assign sequences, you are making a decision not to autosort them since you are taking the responsibility for ordering the sequences and assigning protocols. Each row of the turntable must be assigned only a single protocol.

Note Be aware that whenever you manually sort sequences, you are likely to obtain less throughput than the instrument would produce using autosorting.

Sorting and Processing of Sequences by the System

Autosorting Orders

When the AutoSort button is used, sequences are ordered according to the following criteria:

- Protocol number in ascending order
- Sequence length
- Starting base in alphabetical order
- Sequence order file name

Order of Sequence Processing

Sequences in the Run Setup table are processed as follows:

- Sequences are processed from the top of the table, with the first row of sequences processed first, the second row next, and so on.
- Each row of three sequences represents a radial row of OneStep™ columns in the turntable which will be processed at the same time with the same protocol. A single protocol can apply to more than one row of OneStep columns but only a single protocol applies within a row.

Note You will not be allowed to continue to load OneStep columns if any row has more than one protocol assigned to the sequences.

Checking Reagents and Waste/Installing Sample Collector Rack

Introduction

Make the following checks before proceeding to load columns for the run:

- Check reagents to ensure that the instrument has sufficient reagents for the run.

  Reagents can be replaced before the run or during a programmed pause (see "How to Prepare for Run Extension" on page 2-25 for information on using the Pause After feature).

- Check waste and empty waste bottles if necessary.

Note The details of checking waste are covered in the Pre-run checklist (see page 2-7).

- Install the proper rack type in the sample collector.

Note The details of checking the rack type are covered in the Pre-run checklist (also see page 2-7).

- Check the Instrument Preferences view to confirm that the rack selected matches the rack installed (see step 8 on page 2-9 for more information).

Checking Reagents and Required OneStep Columns

From the Run Setup View, click the Bottle Usage button. The required volume display appears.

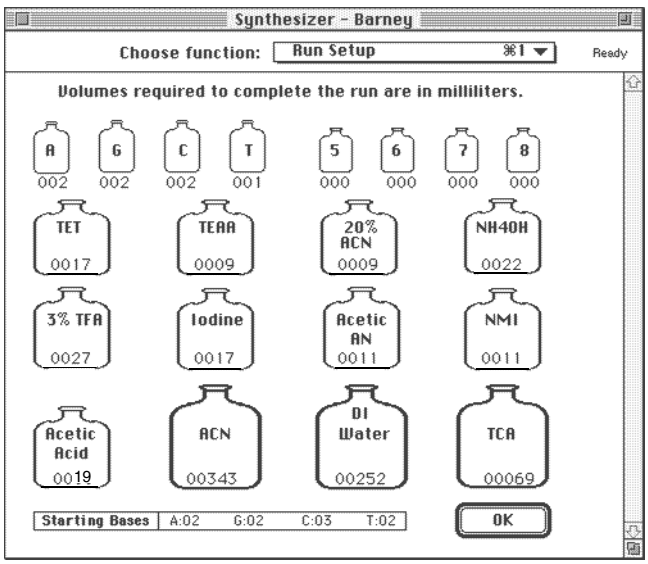


Figure 2-4 Required Reagent Volume display

The volume listed under each bottle is the volume of reagent needed to complete the run. Compare the required volumes listed above with the actual contents of each bottle to determine if new reagent bottles are needed. The capacity of reagent bottles when full is listed in Table 2-1 on the next page.

To determine when to replace reagents, either before the run or during a programmed pause, refer to the decision table in "When to Renew Reagents" on page 2-29.

The information in the "Starting Bases" line at the bottom of the table indicates the number of OneStep columns required for the run.

Note If you examine the display above after you have entered new sequence information for run extension during a Pause After, the volumes will be the volumes required to complete the extended run and the number of additional starting columns required for run extension.

Reagent Consumption Information

The consumption figures presented in the required volumes display are calculated by the 3948Control application using:

- Sequence information
- Data from the Reagent Utilization table

Note The required quantity listed under each reagent bottle is calculated from the corresponding value in the Reagent Utilization table and from the sequence information entered during run setup. Consumption information in the Reagent Utilization table is based upon non-specialty cycles in the current database.

Table 2-1 lists the quantity contained in each type of bottle when they are full. These values are useful in determining whether to change reagent bottles before a run or replace them during a pause.

Note You must first start the run before using the Pause After feature.

Table 2-1 Quantity in reagent bottles when full

Reagent Bottle Position	Description
Phosphoramidite positions A-T	When freshly prepared by autodilution to 0.5 M concentration, the volume of acetonitrile added to these bottles are: ♦ Position A - 44.8 mL ♦ Position G - 46.4 mL ♦ Position C - 47.2 mL ♦ Position T - 52.8 mL
Smaller reagent positions **Note** One reagent in a smaller bottle, 20% Acetic Acid, is located in the lower bottle area.	♦ Bottles in rows two and three represent the reagent bottles contained in the upper compartment ♦ Either 200 or 450 ml types.
Larger reagent positions	♦ Three larger bottles ♦ Either 2 or 4 L in capacity

When to Renew Reagents

Compare the volume indicated as needed in the figure for each bottle with the actual volume present in the indicated bottle to determine if all bottles contain sufficient reagents.

Use the following table to determine your next step.

If there are	And you want to	Then
insufficient reagents present	change one or more reagent bottles before the run	a. Follow the appropriate bottle change procedure in Chapter 4, “Setup Procedures/ Changing Bottles.” b. Proceed with “How to Load OneStep Columns” on page 2-20.
	set a pause to change one or more reagents after estimating how many sequences the instrument can synthesize using reagents currently on the instrument **Note** You can estimate the number of sequences by referring to “Reagent Consumption Information” on page 2-17.	a. Use the Pause After command to set up a pause to occur just after the last row of sequences for which you have reagents. b. Replace the required bottles at the time of an interrupt, see Chapter 4, “Setup Procedures/ Changing Bottles.” c. Proceed with “How to Load OneStep Columns” on page 2-20.

723

Loading OneStep Columns

Introduction

After you have assigned sequences and protocols for a run and ensured that sufficient reagents will be available when needed, continue by loading OneStep columns for the run. Use the procedure below to load OneStep columns into the instrument turntable according to the setup order, as it appears in the Run Setup scroll box.

The information displayed for each position in the figure in step 3 (page 2-31) includes:

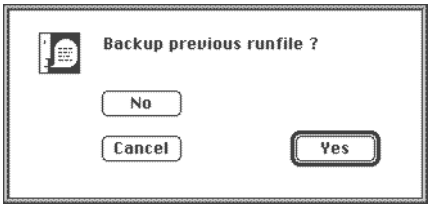
- Individual turntable position (1-48)
- Type of column to be placed in each position by the operator. This is indicated by the letter (A, G, C or T depending upon the 3´-terminal nucleoside covalently bound to the support) and by color with A = green, G = yellow, C = red, and T = blue.
- Protocol name and cycles in protocol
- File name for Synthesis Order/oligonucleotide

Note The bottom position indicator corresponds to the outside of the turntable and the leftmost sequence in each row of the Run Setup table. If only one or two sequences are entered into a row, placeholder columns must be used in the empty positions. No chemistry is performed in the placeholder columns.

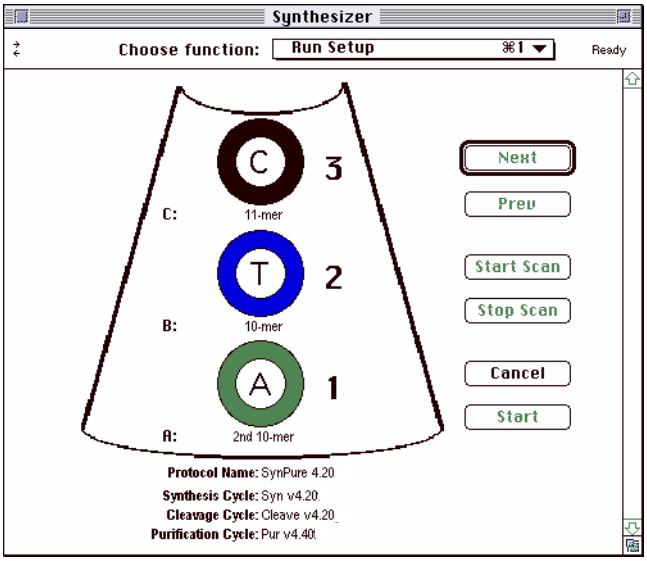
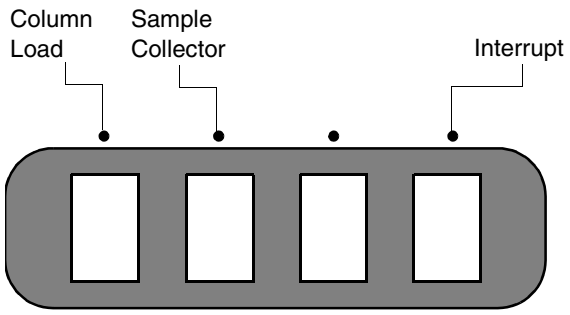
Loading

The following procedure describes how to load OneStep columns using the Load View.

Follow these steps to load OneStep columns:

Step	Action
1	Click the Load button on the Run Setup View. The following dialog box appears. 
2	Click Yes to ensure that the information from your last run is documented. Click No if you have previously obtained the runfile from the last run. The usual practice is to click No.

Follow these steps to load OneStep columns: *(continued)*

Step	Action
3	After you have made a choice in the last step, the Load View appears (clicking Cancel leaves you in the main Run Setup View). The wedge shaped segment represents the area of the turntable currently open for loading.  **Note** Use the Next or Prev buttons to move the turntable.
4	Load the proper OneStep column into each column position in the turntable, following the color and starting base letter designation presented in the view: a. After loading the columns into positions 1 through 3, shown in the above figure, click the Next button to rotate the turntable into its next row (or push the Column Load button on the instrument, identified in the figure below). The display in the Run Setup View changes to reflect the column loading required for the next three positions. b. Continue by loading each successive position as described in step a. c. If you make a mistake in loading, click the Prev button to turn the turntable and display back to the previous loading position. **Note** Inside the front panel door above the turntable is a row of buttons for instrument control, labeled as shown below. The Column Load button has the same function as the Next button on the second Run Setup View. Pressing this button advances the turntable by one position and causes the wedge on the screen to update to the next row. 

Follow these steps to load OneStep columns: *(continued)*

Step	Action
5	This step is optional. After loading is complete, click the Scan button to check for correct loading. The turntable stops at each position long enough for the operator to compare the actual OneStep columns loaded with the position loadings required on the display.
6	When you are satisfied that the proper OneStep columns are loaded, perform the checks listed in the “How to Initiate and Monitor a Run” on page 2-23 before starting the run. **Note** If you are synthesizing only one or two oligonucleotides in a single row on the turntable, you must install placeholder columns (empty One-Step columns) on the unused positions. No chemistry is performed in placeholder columns.

Initiating and Monitoring a Run

Proper Instrument Preparation

You are ready to initiate a run after you have made the checks listed on the pre-run checklist provided on page 2-6. It is good practice to always go through the checklist before initiating a run.

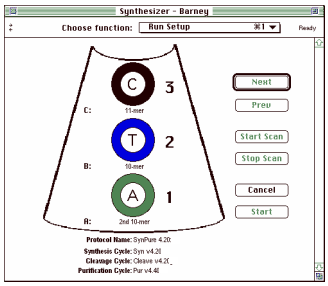
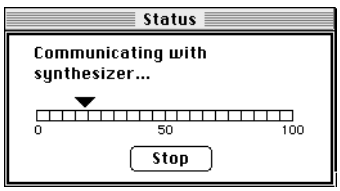
Note This section assumes you have already loaded the 3948 turntable.

You were directed to the Instrument Preferences view, as part of the pre-run checklist, to designate the type of sample collection rack to use for the run. For information on making other settings, refer to “Instrument Preferences Settings” on page 2-31.

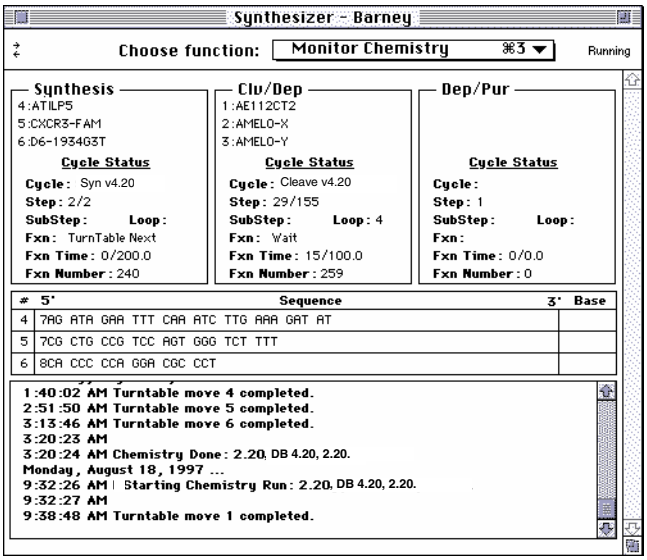
Starting and Monitoring a Run

After going through the pre-run checklist, follow this procedure to start a run.

To initiate and monitor a run:

Step	Action
1	Click Start on the Load View.  The 3948Control application initiates the run by rotating the turntable to the starting position and downloading the following to the instrument: ♦ All Synthesis Orders ♦ Protocols ♦ OneStep column position information While this process is underway, the progress indicator below appears.  **Note** If you click the Stop button, downloading of run information to the instrument stops and the Status box above closes.
2	As soon as loading is complete, the Synthesizer window view changes to the Monitor Chemistry view (which looks like the figure in step 2 on the next page), and the run is initiated. **Note** RunFile information includes all information in the Synthesis Order except run results, which are filled in by the 3948 at the end of the run.

To initiate and monitor a run: *(continued)*

Step	Action
3	Monitor the run using the Monitor Chemistry view.  **Note** The Monitor Chemistry view for your instrument shows activities related to the run on your instrument. The example presented above shows how the view might look with no columns at the purification station yet, three columns at the cleavage station (1, 2, 3), and the following set of columns undergoing synthesis (4, 5, 6). See “Monitor Chemistry View” on page 3-2 for information on using this window to monitor chemistry during a run. If you desire to monitor hardware conditions occurring during synthesis, change to the Monitor Instrument view (see “Monitor Instrument View” on page 3-5).

Using "Pause After" During a Run

Introduction

Once a run is underway, the Pause After feature can be used to enable run extension or to allow changing of a reagent bottle or removal of a priority oligonucleotide before the run is completed. A Pause After can be set any time during a run in progress as long as synthesis chemistry is not yet completed for all loaded turntable positions.

To extend a run, proceed as described below. To change a reagent bottle or remove a priority oligonucleotide, proceed as describe under "Replacing a Reagent Bottle/Removing a Priority Oligonucleotide" on page 2-39.

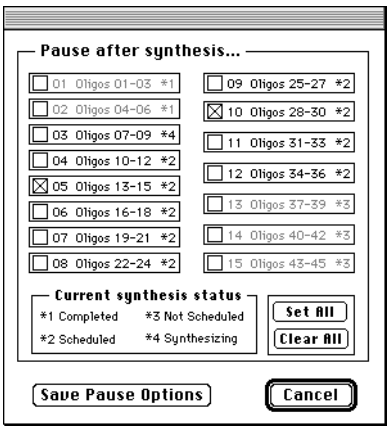
Extending a Run

When preparing for run extension, keep these points in mind:

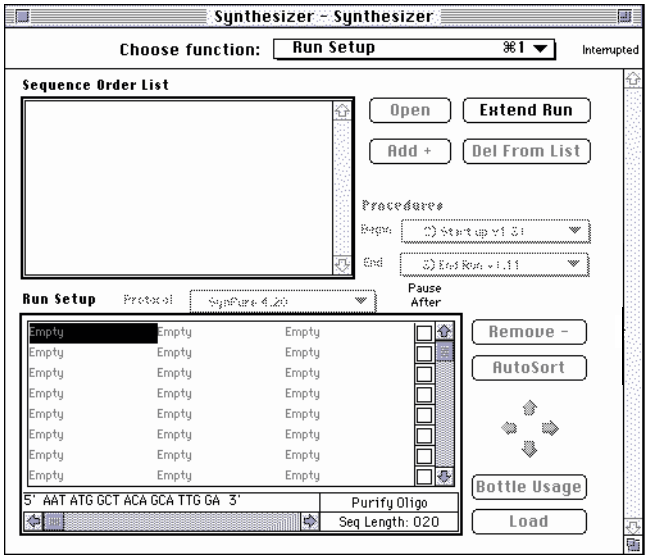
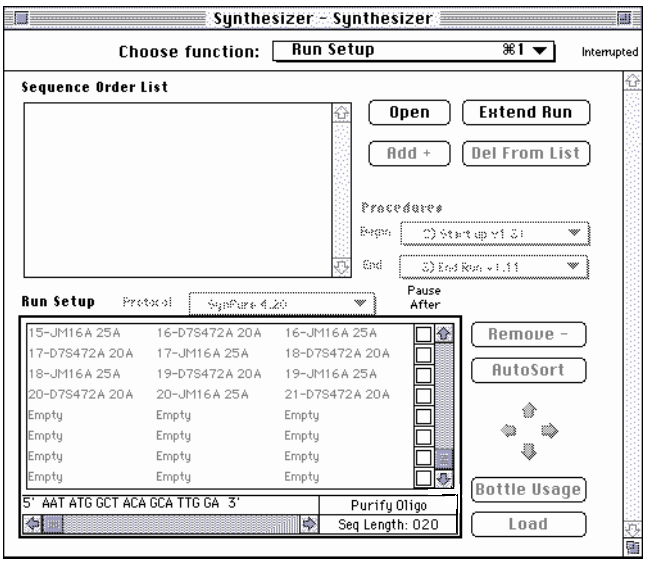
- A run can only be extended when the instrument pauses in response to the Pause After command.
- A pause for run extension occurs after the completion of the synthesis cycle for columns in the synthesis module turntable position.
- Only protocols defined at the start of a run are available at run extension (new protocols cannot be created during a pause).
- To continue an extended run after loading new columns, use the Start button from the Load view. An extended run cannot be started with the Resume command or key.

Note Using the Resume button when extending a run cancels the extension and resumes the current run.

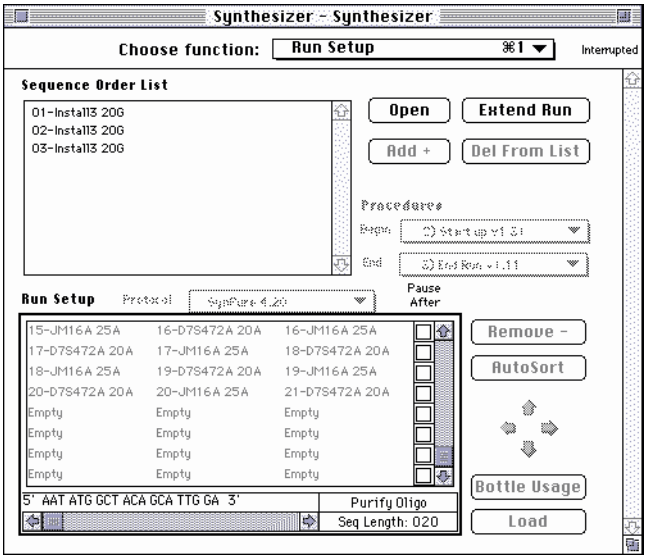
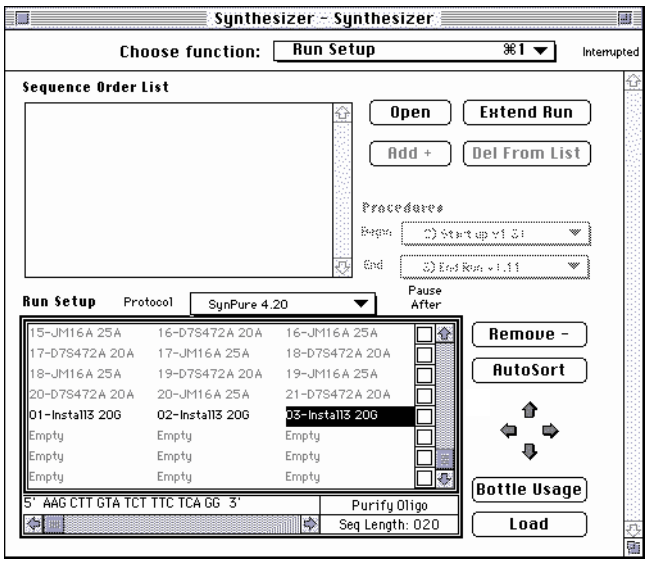
To extend a run:

Step	Action
1	During a run in progress, choose the Pause After command from the Synthesizer menu to present a dialog box like that shown below:  **Note** In this example, the top two dialog boxes are grayed out and marked #1 which indicates that synthesis is complete. A pause can be set after synthesis on any row in bold (marked #2 or "scheduled"). In this example a pause is shown set on rows 5 and 10. At this point, besides clicking another checkbox to set a third pause, the existing checkboxes can be unchecked to deselect the pause set for row 5 or row 10.

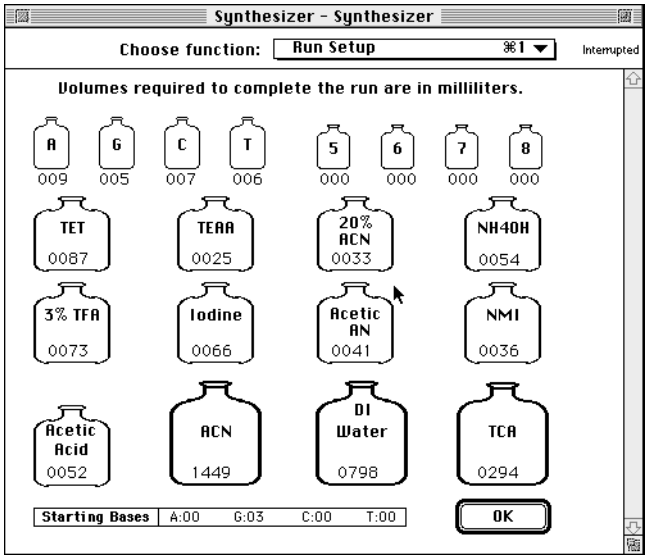
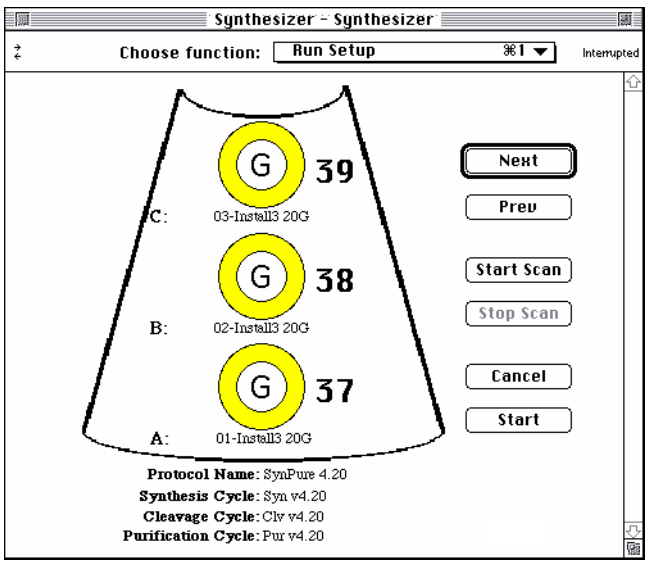
To extend a run: *(continued)*

Step	Action
2	When the instrument pauses in response to the Pause After, choose the Run Setup view of the Synthesizer window. It will look like the figure below:  Three details are important in this figure: ♦ The message “Interrupted” presented at the upper right. ♦ Only the “Extend Run” button is available. ♦ No sequences are shown in the Run Setup scroll box.
3	Click the Extend Run button. This will change the view so that it looks like the figure below:  **Note** The following two changes have been made in the view: 1) the Open button is now available, and 2) the sequences previously loaded into the Run Setup scroll box are grayed out, indicating that no change can be made to them.

To extend a run: *(continued)*

Step	Action
4	Click the Open button and add sequences to the Sequence Order List using the dialog box presented. The view will look like the figure below: 
5	Select the entire group of sequences in the Sequence Order List using Command-A and then click the Add button or individually select each sequence and then click Add. This will move the sequences into the Run Setup scroll box as shown below: 

To extend a run: *(continued)*

Step	Action
6	Click the Bottle Usage button before proceeding to load columns for the new sequences. The Bottle Usage view will appear as shown below:  **Note** The numbers presented for Starting Bases will indicate how many columns need to be added for the run extension and the volumes now show the reagent volumes required to complete the extended run, including both the added sequences and the sequences yet to be produced.
7	Add any reagents needed to complete the extended run and gather the new columns needed for the new sequences. When you are ready to proceed, click OK to return to the prior Run Setup view.
8	Click the Load button. This will present a Load view like that shown below:  **Note** The instrument will automatically move the turntable to the row required for the sequences to be added to the run.

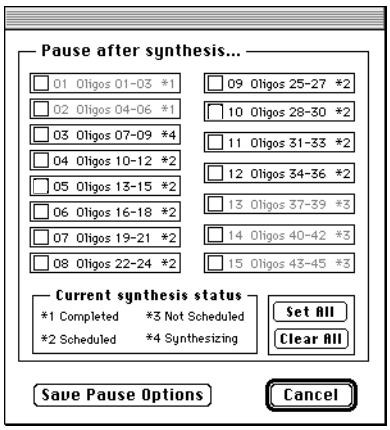
To extend a run: *(continued)*

Step	Action
9	After adding the required columns, click Start on the Load view when you are ready to restart the run.

Replacing a Reagent Bottle/Removing a Priority Oligonucleotide

This procedure is a simplified version of the procedure for run extension. The main difference is that the run is not re-started from the Load view, since no new oligonucleotides are added to the run.

To replace a reagent bottle or remove a priority oligonucleotide, proceed as follows:

Step	Action
1	During a run in progress, choose the Pause After command from the Synthesizer menu to present a dialog box like that shown below: 
2	Click the checkbox for the scheduled row (marked by #2) after which you want the current run to pause. Click “Save Pause Options” to return to the run.
3	When the run pauses in response to the Pause After, change the bottle according to the appropriate procedure listed below or go to step 4 to remove a priority oligonucleotide. ♦ Change phosphoramidite bottles as described under “Working with Phosphoramidite Bottles” on page 4-14. ♦ Change reagent bottles as described under “Installing Reagent Bottles” on page 4-19.
4	If you are removing one or more priority oligonucleotides, use the Sample Collector button on the instrument control panel (see page 31) to extend the Sample Collector tray. Then remove the priority oligonucleotide(s). Push the Sample Collector button a second time to retract the Sample Collector tray.
5	Resume the run using the Resume command or key.

Post-Run Tasks

Types of Tasks

These tasks include preparing sample vials/tubes for customers, emptying the 15-mL waste bottles, disposing of used OneStep columns. The task of placing a new rack in the sample collector carrier, however, could be considered either pre-run or post-run and so is covered in both the Pre-run check list and the procedure for post-run tasks.

Procedure for Post-Run

After each run, complete the following tasks:

- Remove the oligonucleotides produced from the sample collector and label them for the customers.
- Remove used OneStep columns from the turntable and discard.
- Check and empty waste bottles if full.
- Place a new sample rack in the sample collector.
- Verify that the RunFile was created.

CAUTION **Pressing the Sample Collector button while a run is in progress will pause the run.**

Note At the end of the run (and after the production of each oligonucleotide) the instrument automatically extends the carrier to the front of the sample collector compartment to simplify removal of oligonucleotides.

After each run, complete these tasks:

Step	Action
1	At the end of the run or whenever a priority oligonucleotide is to be taken off the instrument, remove the rack containing the oligonucleotide collection tubes from the sample collector. **Note** Keep in mind that you can use either of two types of racks, shown in the figure on page 2-7. The standard red type (OligoRack) delivers oligonucleotides in press top vials (micro-titer format). The second or white type delivers 48 oligonucleotides in screw top tubes. **CAUTION** **Do not remove a rack from the carrier while it is under the delivery needle, as this means that sample will be delivered to vials soon.**
2	Prepare all vials/tubes containing samples for the customer as described below. ♦ For the standard white screw tube rack (OligoRack), cap and label each tube individually for customers. ♦ For the red rack, vials can be sent in the rack after the following steps: – Firmly place the cap strips on each row of tubes. – Cut attachments between caps. – Place top cover (without septum) over rack. – Tape cover in place securely.
3	Remove and dispose of the used OneStep columns from the turntable, using the Column Load button on the instrument (see the lower figure on page 2-21) to rotate the turntable.
4	Empty two 15-mL waste bottles located at the rear of the sample collector platform.

After each run, complete these tasks: *(continued)*

Step	Action
5	Place a new rack in the sample collector carrier for the next run. The rack must be inserted into the carrier with the key end inserted first (toward the back), as shown in the figure on page 9. **Note** New red racks come sealed with 48 vials and a septa sheet. An alternative to using a new rack is to place the number of new vials needed for the next run into an already opened sample rack. In the latter case, be sure to correctly index the vials (vials should be placed from left to right starting at the end opposite to the key). When tubes are inserted into a red rack, only rows 1, 3, 5, 7, 9 and 11 are used. These rows correspond to the openings in the sample tray cover. **IMPORTANT** It is important that the type of rack selected in Instrument Preferences matches the type of rack actually used in the sample collector. See the discussion in step 8 of the procedure for the Pre-Run checklist on page 2-7.
6	Verify that the RunFile for the run was created. **Note** A RunFile is normally automatically generated for the run and placed in the Run folder (stored in the same location used for the 3948Control application). If the RunFile cannot be found, use the Generate RunFile command from the File menu to create a new copy. RunFiles generated by this command are listed in the system as “TRunFile...” by the application.

RunFiles

Information in the RunFile

The 3948Control application generates a RunFile for each run. Run information is normally automatically generated and stored in a file like that shown in step 2 on page 2-43. The information on which a RunFile is based is no longer available from the instrument after the start of the next run.

The following types of information are provided in the RunFile log for each oligonucleotide produced:

- Column position, sequence name and listing for the sequence in each loaded position
- Sample Collector position for labeling the samples
- Begin and End procedure names
- Two measures of the quantity of each oligonucleotide; Optical Density Units and concentration in picomole/microliter
- Name of the Synthesis cycle used
- Name of the Cleavage cycle used
- Name of the Purification cycle used
- Date of completion of Synthesis order (date Synthesis order was generated)

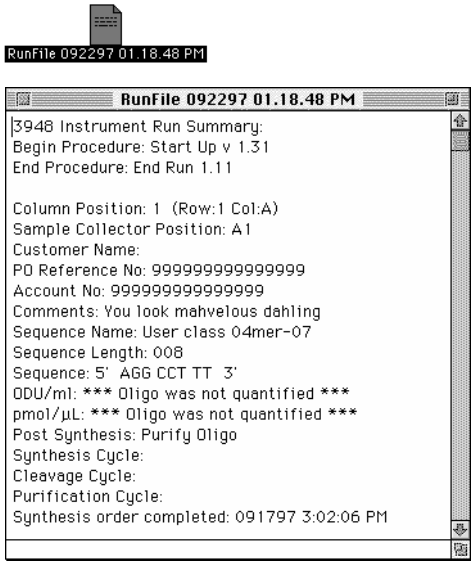
Opening a RunFile

The contents of a RunFile can be accessed by the following procedure.

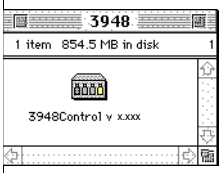
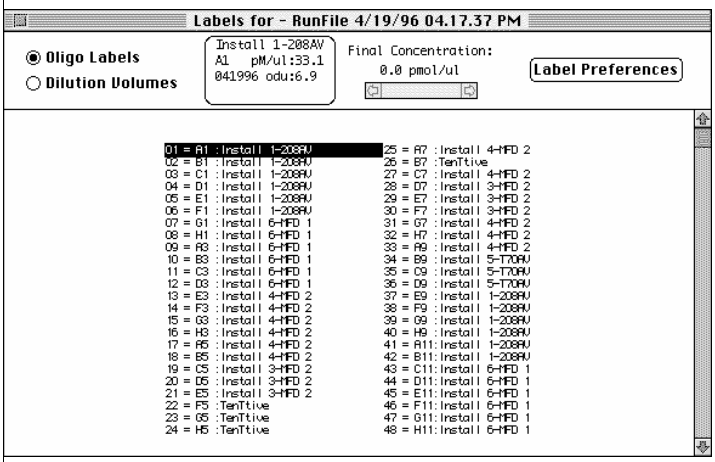
To Open a RunFile:

Step	Action
1	♦ If you just want to look at the file contents or print out the RunFile, open the RunFile as described in step 2. ♦ If you want to open the file to print labels for oligonucleotides, open the RunFile as described in step 3 and then follow the next step.

To Open a RunFile: *(continued)*

Step	Action
2	Open the RunFile in Simple Text by double-clicking on the File icon (the Simple Text application must be present on the Macintosh. The opened RunFile will appear as shown below. 

To Open a RunFile: *(continued)*

Step	Action
3	The RunFile can be opened in two ways in the 3948Control application: ♦ Open the RunFile using the Open command from the 3948Control application, when the application is open. ♦ Alternatively, when the 3948Control application is closed, drag the icon of the RunFile onto application icon (shown below). Either of these actions will open a label print window (also shown below).  

Printing Labels for Oligonucleotides

Once you have opening a RunFile for label printing, proceed as follows.

To print labels from an open RunFile:

Step	Action
1	Put the special label paper into your printer.
2	Click on the scroll bar to display the label area.
3	Choose Print (File menu) to print labels.
4	If your labels do not print correctly (with a print label window like that shown above), use the Page Setup command (File menu) to make the following checks: ♦ Make sure that the print orientation is set to “Portrait.” ♦ Make sure that the scale is set to 100%.
5	If label printing is not centered, click the Label Preferences button, adjust the x and y printer offset values, and print again.

More information on RunFiles is presented under “Sample Labeling Feature” on page 4-33 of the *ABI™ 3948 Reference Manual*.

3 Monitoring a Run

In This Chapter

Topics Covered

The Monitor Chemistry view is used to monitor chemistry in progress on the instrument and displays information as described in this chapter. The Monitor Instrument view displays the values of various parameters during operation and is helpful in troubleshooting instrument operation.

This chapter includes the following topics:

Topic	See page
Monitor Chemistry View	3-2
Introduction	3-2
Parts of the View	3-2
Upper Three Panes	3-2
Middle Pane	3-3
Lower Pane	3-4
Monitor Instrument View	3-5
Introduction	3-5
Parts of the View	3-5
Other Parameters and Instrument Conditions	3-7
Monitor Run View	3-8
Introduction	3-8
Types of Information Provided	3-8
Stopping a Run in progress	3-9
Three Ways to Stop	3-9
Stopping with a Pause After	3-9
Stopping with an Interrupt	3-9
Stopping with the Abort Command	3-10

Monitor Chemistry View

Introduction Use the Monitor Chemistry view, shown in Figure 3-1, to observe the chemistries on all columns as the run progresses. This view displays the names of sequences currently being processed in each of the three processing modules as well as current cycle information for each chemistry module.

Note Cycle names are truncated after 14 characters.

Parts of the View The Monitor Chemistry view has three main parts that include the

- Three upper panes, which report chemistry in progress
- Middle pane, which lists the sequences currently being synthesized
- Lower pane, which presents status and system messages

The details of these portions of the Monitor Chemistry view are called out in Figure 3-1 and explained in the tables for each of the three main parts.

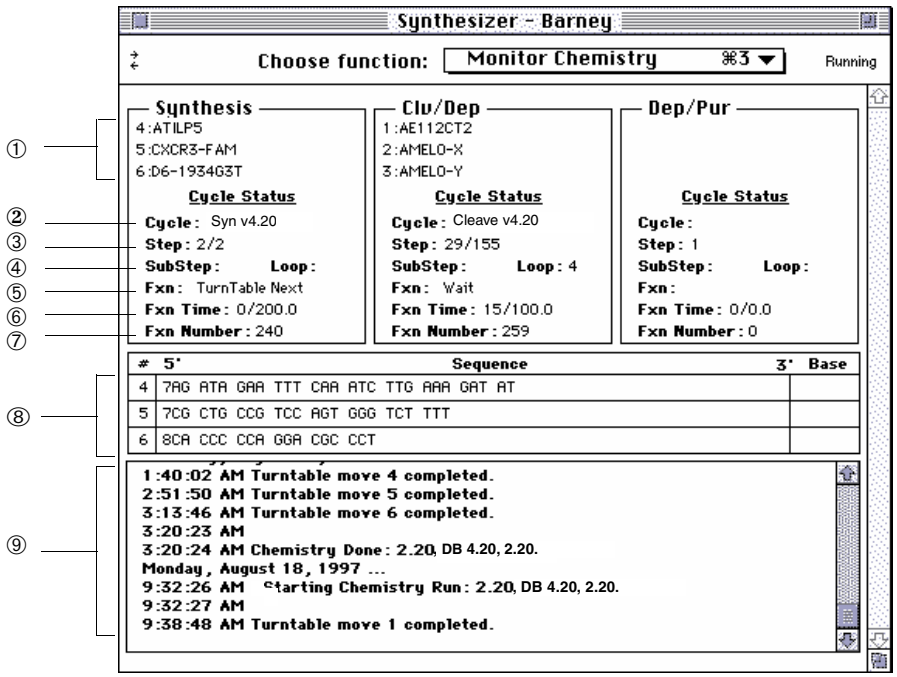


Figure 3-1 Monitor Chemistry view

Upper Three Panes

For each turntable position, the following information is displayed in the upper three panes. The descriptions apply to the fields in all three panes: Each pane reports the chemistry in process in a module (left pane = synthesis, middle pane = cleavage/ deprotection, right pane = deprotection/purification):

Figure 3-1 Bullets	Name of Field	Description
①	Oligonucleotide sequence name and position	These are the turntable positions and names for the three sequences currently being processed in a OneStep column in each module.
②	Cycle Name	Name of the cycle used to process the sequences in each processing module.
③	Step	Step number of cycle currently in progress.
④	SubStep: Loop:	These fields are used to track the progress of a subroutine in progress, SubStep indicates the current subroutine step and Loop indicates the current loop number or repetition number for the subroutine in progress.
⑤	Fxn	Name of function currently in progress.
⑥	Fxn Time	Time that the function has been active. Function time (Fxn Time) is displayed as two numbers. For example, when the Fxn Time is 15/20, 15 represents how many more seconds the function will be active and 20 represents the total time the function should be executed. The first number decrements or counts down from the second number to “0” or completion of execution.
⑦	Fxn number	Number of active function.

Middle Pane

General Explanation

The middle pane, represented in the diagram below, provides information on the base addition in progress (see left upper pane):

⑧

#	5' Sequence 3'	Base
4	6AT CAC AGT CTG ATC TCG AA	13/20
5	7CC TTG GAG ATT CAA ATG TCG TGG TCT TG	13/29
6	5TT TTT TTT TT	11/11

Note If the instrument interrupts during a synthesis, the center pane of the Monitor Chemistry view will become blank instead of showing the progress of sequence synthesis as shown in the figure above.

The sequences currently being synthesized are listed in the middle pane and the bases currently being added are shown highlighted. The counter in the last column indicates that the base being synthesized in position 5, for example, is number 13 out of a total of 29.

DNA Sequence Conventions

Following convention, a DNA sequence is entered 5´ to 3´. Likewise, the sequence listing in the 3948 displays each sequence in a 5´ to 3´ orientation. However, the DNA

sequence is actually synthesized 3´ to 5´, as indicated by the highlighting of the thirteenth base position in two of the sequence listings in the Monitor Chemistry view (the sequence in Position 6 was completed with the 11th base addition). This fact is also indicated by the 13/29 and other numbering to the right of each sequence listing, where the left number increments with each base addition until the value to the right is reached and synthesis is complete for a sequence.

Lower Pane

Status and System Messages

The lower pane presents a series of messages from the instrument listing the times at which major processing events occurred. This listing may also include error and other system messages. The following messages are examples of system status messages. Each message will be prefaced by the time at which an action was taken:

Note Times are instrument times. The instrument clock should be synchronized with the Macintosh clock using the Synchronize clocks command (Synthesizer menu).

⑨ 11:32:17 AM Starting Chemistry Run: 2.20, DB 4.20, 2.20
12:41:55 PM Interrupting system
12:45:05 PM Resuming Chemistry
12:50:10 PM Chemistry Done: 2.20, DB 4.20, 2.20

Note Chemistry versions numbers will change with software releases. A message with only the time displayed and no message is acting as a page break. See “System Messages” on page C-38 in Appendix C of the *ABI 3948 Reference Manual* for a complete list of messages which may appear in the lower pane log.

Using Lower Pane Information

While information in the Monitor Chemistry view is primarily used to keep track of chemistry in progress, the log on the bottom will also inform you of programmed pauses so that you can perform a bottle change procedure or remove a high priority oligonucleotide for a customer. Instructions to the user during procedures (calibration, bottle change, etc.) are presented in the log.

Note When a sensor delivery failure occurs involving B+Tet, Auto resume is disabled.

Monitor Instrument View

Introduction

The Monitor Instrument view, shown below, displays the values of various parameters during operation and is helpful in troubleshooting the instrument. The status for the following items is displayed:

- Valves
- Pressure regulators
- Liquid sensors
- Synthesizer, Cleavage, and Purification jaws
- Other parameters and instrument conditions, including:

  Deprotection coil temperature
  Alarms
  Current turntable and sample collector positions

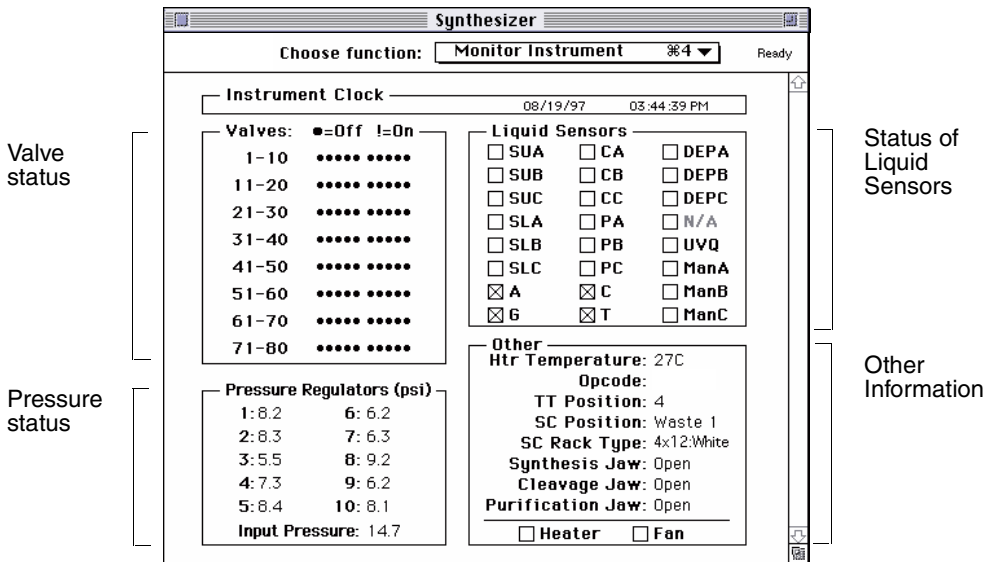
Note The status of chemistry (Ready, Running, Interrupted, Manual Control) is shown on the upper right-hand corner of all views.

Parts of the View

The Monitor Instrument view has four parts:

- A display of valve status
- A display of liquid sensor status
- A display of pressure regulator status
- A display of other information

The main parts of the Monitor Instrument view are called out in the figure and explained in this section.



Note For Rack type, 8x6 Red = standard OligoRack and 4x12 White = optional screw top type rack.

Valve Status

Valve status is represented in groups of 10 valves in ascending order from left to right. Valves that are on are represented by !. Valves that are off are represented by •. For example, when valves 1–10 are represented by ••!!•••••!, you know that valves 3, 4, and 10 are open, and valves 1, 2, 5, 6, 7, 8, and 9 are closed. The valves represented by these numbers are in valve blocks on the plumbing diagram provided in Appendix D (Instrument Plumbing Diagram) of the Reference manual.

Status of Liquid Sensors

The presence of a check in the check box by a sensor label indicates that liquid is detected in the sensor at the current time. When the letters A, B, or C are included as part of a sensor designation, this indicates one of the three column positions in a jaw mechanism.The sensor labels have the following meanings:

- Sensors labeled A, G, C, and T indicate flow from phosphoramidite bottles.
- Sensors labeled “SUA” (Synthesis Upper Position A) through “SUC” (Synthesis Upper Position C) are located on the upper side of the synthesis jaw mechanism.
- Sensors labeled “Man” are located just below the first manifold under the synthesis jaw.
- Sensors labeled “SLA” (Synthesis Lower Position A), “SLB” (Synthesis Lower Position B), and “SLC” (Synthesis Lower Position C) are located closest to the synthesis reagent delivery valve blocks.
- The sensor labeled “UVQ” is for the UV detector.
- Sensors labeled “CA” (Cleavage Column A) through “CC” (Cleavage Column C) are located above the cleavage jaw mechanism.
- Sensors labeled “PA” (Purification Column A) through “PC” (Purification Column C) are located above the purification jaw mechanism.
- Sensors labeled “DEPA” (Deprotection Coil A) through “DEPC” (Deprotection Coil C) are located above the deprotection coils.

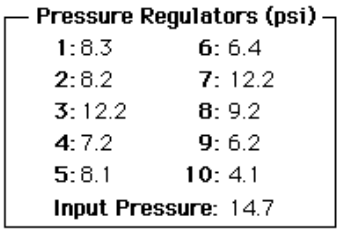
The sensors listed on the Monitor Instrument view can be located by name or number on the instrument plumbing diagram provided in Appendix D of the *3948 Reference Manual*.

Note Position A corresponds to the outer position in a row of columns in the turntable. Position B is the middle position, and Position C is the inner position.

Pressure Regulator/Pressure Status

Pressure regulator readings and Input Pressure are displayed below the valve information. These pressures are accurate within ±0.05 psi. Pressure regulators are always set by functions. Functions that set pressure regulators can be run in the Manual Control view or in Procedures or Cycles.

Note Use the Input Pressure reading to set the input pressure.



Regulators 1, 3, 7, and 10 are block regulators and do not pressurize bottles but are programmed to vary pressures during the Cleavage and Purification cycles. The synthesis block regulator (7) is the only block regulator that does not vary pressure during a cycle. Bottle regulators are kept constant and maintain pressure within ±0.2 psi of the initial pressures.

Other Parameters and Instrument Conditions

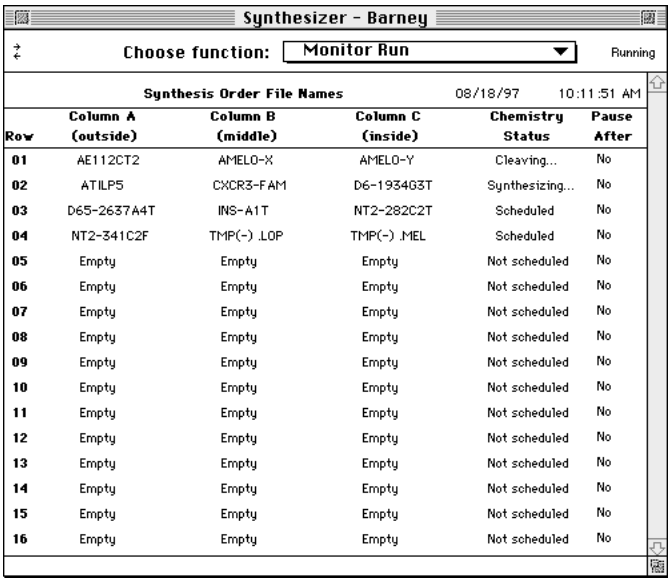
The parameters and conditions listed under the *Other* category on the Monitor Instrument view are as follows

Parameters and Instrument Conditions	Definition
Temp (Temperature) (Start Depro Htr Wait Coil Temp Set Coil Temp)	Current temperature of the deprotection coils - during the Cleavage and Purification cycles, the temperature of the coils is programmed using the functions listed to the left, the MISC data entry for a function is used to designate the temperature. See the Special Functions in Appendix A and Annotated Cycles in Appendix B for more information.
Opcode	Messages presented in this field are only of interest to system developers. No user level information is presented here.
TT Position	This is the current synthesis module turntable position (1-16).
SC Position	This is the current sample collector position (1-48).
SC Rack Type	This is the type of rack in the instrument, either the standard red OligoRack or the optional white type with screw top caps.
Syn Jaw	This is the current status of the Synthesizer jaw mechanism, either Open, Closed, or Moving.
Clv Jaw	This is the current status of the Cleavage jaw mechanism, either Open, Closed, or Moving.
Pur Jaw	This is the current status of the Purification jaw mechanism, either Open, Closed, or Moving.
Heater and fan Check Boxes	If either of these check boxes is checked, the device associated with the check box is turned on.

Monitor Run View

Introduction

The Monitor Run view, shown in Figure 3-4, is another way of observing the progress of an ABI 3948 instrument run. This view is a table of the entire run which provides useful information in tracking the run.



Row	Column A (outside)	Column B (middle)	Column C (inside)	Chemistry Status	Pause After
01	AE112CT2	AMELO-X	AMELO-Y	Cleaving...	No
02	ATILP5	CXCR3-FAM	D6-1934G3T	Synthesizing...	No
03	D65-2637A4T	INS-A1T	NT2-282C2T	Scheduled	No
04	NT2-341C2F	TMP(-) .LOP	TMP(-) .MEL	Scheduled	No
05	Empty	Empty	Empty	Not scheduled	No
06	Empty	Empty	Empty	Not scheduled	No
07	Empty	Empty	Empty	Not scheduled	No
08	Empty	Empty	Empty	Not scheduled	No
09	Empty	Empty	Empty	Not scheduled	No
10	Empty	Empty	Empty	Not scheduled	No
11	Empty	Empty	Empty	Not scheduled	No
12	Empty	Empty	Empty	Not scheduled	No
13	Empty	Empty	Empty	Not scheduled	No
14	Empty	Empty	Empty	Not scheduled	No
15	Empty	Empty	Empty	Not scheduled	No
16	Empty	Empty	Empty	Not scheduled	No

Figure 3-2 Monitor Run view

Types of Information Provided

The Monitor Run view provides the following types of information about the run in the form of a table:

- Row - this column lists the 16 radial rows on the turntable.
- Names of the three Synthesis Orders/sequences on each row of the turntable are listed row by row in three columns.
- Columns of Synthesis Order/sequence names are organized according to turntable position: Column A lists outside side position Synthesis Orders/sequences.
- The fifth column lists the current chemistry status for each row – a particular row will have one of these notations:
  - Scheduled/Not scheduled
  - Synthesizing
  - Cleaving
  - Purifying
  - Completed
- The sixth column, Pause After, will have either “No,” “Yes,” or nothing (blank) after a row to indicate whether a pause has been programmed.

Information in the Monitor Run view is useful in tracking the general course of chemistry and especially useful in preparing for a programmed pause in the run (to change a bottle, remove a high priority oligo, extend the run, etc.).

Stopping a Run in Progress

Three Ways to Stop

A run in progress may be stopped in one of three ways:

- Programming a Pause After
- Using the Interrupt command (or pushing the Interrupt button on the front panel)
- Using the Abort command

Stopping with a Pause After

A run may be programmed to pause after a designated synthesis to extend the run, remove a priority oligonucleotide, or change a reagent bottle. Setting a pause is described in “Using “Pause After” During a Run” on page 2-35.\

Stopping with an Interrupt

A run in progress may be halted with an Interrupt.

To interrupt a run:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>1</td><td colspan="2">Select Interrupt from the Synthesizer pop-up menu or press the Interrupt button on the front of the instrument.

This will interrupt or stop the run at the next safe step. All three chemistry processes (Synthesis, Cleavage, and Purification) must be at safe steps. The new status of Synthesis is indicated by the message “Interrupting Synthesis” in the Lower pane (Monitor Chemistry view). Also, the status message on the upper right-hand corner of views will change from “Running” to “Interrupted.” (“Interrupted” is also displayed for a programmed pause.)</td></tr>
<tr><td rowspan="4">2</td><td colspan="2">Perform the task for which you interrupted the synthesis run. For example, to remove a priority oligonucleotide, do the following:</td></tr>
<tr><td>Step</td><td>Action</td></tr>
<tr><td>a.</td><td>If needed (rack will be out if a Pause After was used), use the Sample Collector button on the 3948Control panel (see the figure on page 2-31) to move the carrier containing the sample rack out of the sample collector.</td></tr>
<tr><td>b.</td><td>Remove the priority oligonucleotide from the sample rack (see the figure on page 2-9 for information on sample positions in the two types of racks).</td></tr>
<tr><td></td><td>c.</td><td>If the button was used in step a, use the Sample Collector button again to move the carrier back into the sample collector.</td></tr>
<tr><td>3</td><td colspan="2">To continue the synthesis run, select Resume from the Synthesizer menu to start chemistry again.</td></tr>
</table>

Stopping with the Abort command

Besides interrupting or pausing, another way to stop a run is using the Abort command (also from the Synthesizer pop-up menu). Using the command will produce the warning shown in Figure 3-2, allowing you to back out if you desire. The status message on all views will state “Ready” for the chemistry state. After using the Abort command, a new run must be started.

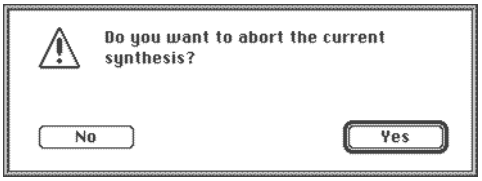


Figure 3-3 Abort Synthesis dialog box

CAUTION **Be aware that there is no way to resume a run once the Abort command has been used (by clicking Yes on the above dialog box).**

4 Setup Procedures

In This Chapter

Topics Covered

This subsection contains the following procedures for tasks which should be done prior to a run: checking argon tank pressure, emptying waste containers, and setting up the sample collector.

The following topics are covered in this chapter:

Topic	See page
Pre-Synthesis Tasks	4-3
Introduction	4-3
List of Tasks	4-3
General Pre-run Procedures	4-5
Checking Argon Tank Pressure	4-5
Empty Waste Containers	4-5
Set up the Sample Collector	4-7
Preparing Synthesis Orders	4-8
Opening	4-8
Assigning a Name	4-8
Types of Information	4-9
Double-checking entry and Saving	4-10
Organizing and Processing Synthesis Order Files	4-10
Organizing Your File System	4-11
Processing Synthesis Orders	4-11
Processing Variations	4-13
Working with Phosphoramidite Bottles	4-14
Introduction	4-14
Removing Bottles	4-14
Placing New Bottles in Position	4-14
Using the Autodilution Procedure	4-15
Storing and Handling Reagent Bottles	4-17
Introduction	4-17
Reagent Storage Conditions	4-17
Important Guidelines	4-17

Topic	See page
Precautions to Observe	4-18
Installing Reagent Bottles	4-19
Introduction	4-19
Installing Upper Position Reagent Bottles	4-19
Installing Reagent Bottles in Lower Positions	4-20
Using UV grade Acetonitrile	4-20
Changing the Argon Tank	4-21
Introduction	4-21
Procedure	4-21
Calibrating Fluid Sensors	4-22
Introduction	4-22
Sensor Calibration Procedure	4-22
Manual Phosphoramidite Preparation	4-23
Things to Consider	4-23
Procedure for Dissolving and Installing	4-24

Pre-Synthesis Tasks

Introduction Before beginning synthesis, you should complete the steps listed below. This list is a simplified version of a pre-synthesis check list ("How to Perform the Pre-run Checklist") provided in Chapter 2 of the manual and is reproduced here as a reminder, since most of these tasks must be done at the synthesizer.

List of Tasks Check the items on this list before starting a run:

To prepare for a run:

Step	Action
1	Check the pressure of the argon tank. Change the tank if the high pressure drops below 200 psi. Checking the system pressure is best done using the Input Pressure value in the Monitor Instrument view. The pressure should read between 14 and 15 psi — the optimum is 14.5 psi.
2	Ensure that bottles are installed correctly, with a tight seal, on every position. Empty bottles must be placed on unused positions.
3	(Optional) Prepare phosphoramidites using the auto-dilution feature (or prepare manually). Procedures for auto dilution of phosphoramidites are provided in this chapter of the manual.
4	(Optional) Calibrate fluid sensors, if necessary. The standard Begin procedure will tell you if sensors need calibrating and pause if they do. Verification of the need for sensor calibration and actual calibration are done using the procedure provided in this chapter of the manual.
5	Check the waste level for the three types of liquid waste. The 3948 generates about 90 mL of flammable waste and about 15 mL of halogenated waste per 20-mer oligo. Flammable waste is collected in a 10-liter bottle in the lower instrument compartment. Halogenated liquid waste is collected in a 4-L bottle located in the lower instrument compartment. The third type of waste, sample collector liquid waste, is collected in two 15 mL bottles located to the rear of the sample collector platform.
6	Check reagent levels of all bottles and make appropriate bottle changes or set interrupts to replace reagents before depletion. The Bottle Usage command, in the Run Setup view, is used to determine whether sufficient reagents are present for the current run. Bottle interrupts are also set in the Run Setup view. Phosphoramidite bottle changes are made using the Autodilute 2g procedure, as described in this chapter (Chapter 4). The current chapter also provides instructions for changing other reagent bottles.
7	Check the run protocol for the correct cycles.
8	Check that the sample collector has a tray containing sufficient tubes to collect the samples. If fewer than 48 oligonucleotides will be produced by a run for a standard red OligoRack, the tubes in the collector rack must be placed in positions corresponding to sample deliveries.

To prepare for a run: *(continued)*

Step	Action
9	Prepare Synthesis Orders needed for the next run. Synthesis Orders are prepared using the 3948Control application (see “Organizing and Processing Synthesis Order Files” on page 4-10). This task is essential but may be done by someone besides the instrument operator.

General Pre-Run Procedures

Checking Argon Tank Pressure

Check the pressure of the argon tank as follows, taking the action indicated:

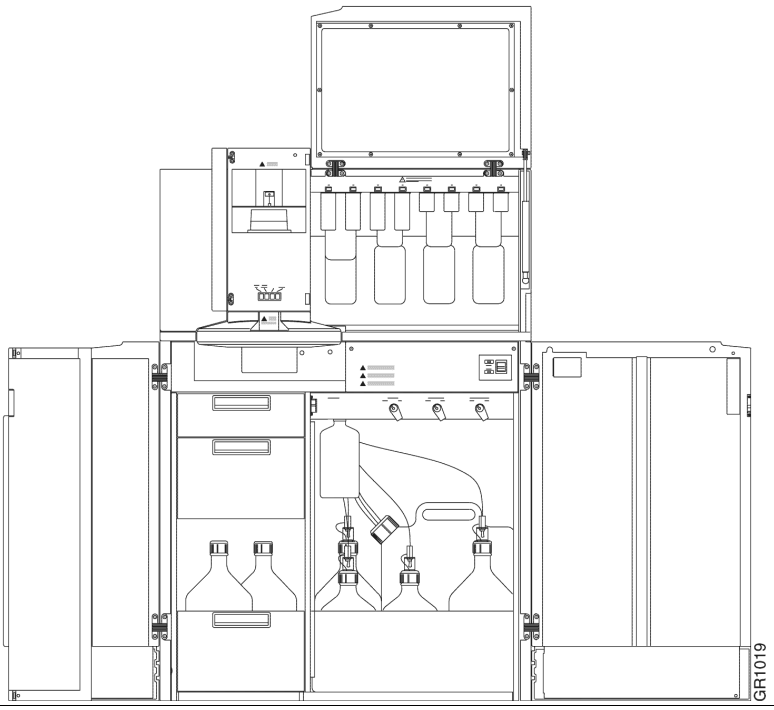
Step	Action
1	Check the high pressure gauge. If the pressure is below 200 psi, the tank is depleted and should be replaced (see “Change the Argon tank as follows:” on page 4-12 of this chapter for the procedure). If the pressure is above 200 psi, proceed with step 2.
2	Check the low pressure and adjust as necessary. This is best done using the Input Pressure reading provided by the system in the Monitor Instrument view. The pressure should read between 14 and 15 psi, 14.5 is optimum. If the pressure is outside this range, adjust the pressure as needed referring to the Input Pressure reading.

Note A size 1A tank of 2500 psi argon should last approximately 2 months.

Empty Waste Containers

Waste containers must be emptied to prevent a full waste bottle, or waste allowed to condense in waste or vent lines from causing back pressure which interferes with reagent flows. The levels of the three types of waste containers (flammable, halogenated, and sample collector) are checked when a synthesis is not in progress.

To check the levels of the three types of waste containers:

1	Open the door of the large lower reagent compartment. This compartment contains two of the three types of waste containers: 1) the 2 1/2 gallon container for aqueous and flammable waste, and 2) the 4-L container for halogenated waste. 

To check the levels of the three types of waste containers: *(continued)*

2	Check the levels of the two waste bottles before each run and determine if they should be emptied: ♦ the aqueous and flammable waste container should be emptied if is much more than 1/3 full (the instrument generates 85 mL flammable waste per 20-mer synthesis and purification or about 4 L per run of 48 oligonucleotides). ♦ the halogenated waste container should be emptied if it is more than 2/3rds full (the instrument generates 15 mL of halogenated waste per 20-mer synthesis and purification of about 3/4 L per run). CAUTION **A full waste bottle can cause back pressure that will interfere with reagent flows. Emptying the waste bottle(s) before they become full prevents the forcing of waste into the waste or vent line.**
3	Before emptying a waste bottle, locate an extra cap for the next step.
4	Unscrew the cap assembly and immediately recap the bottle to prevent the release of vapors.
5	Place the liquid from each waste container into the proper sealed container. Sealed containers for the aqueous and flammable wastes should be labeled "WASTE FLAMMABLE." Sealed containers for the halogenated waste should be labeled "WASTE HALOGENATED." Dispose of the waste following applicable government regulations. ! WARNING ! **CHEMICAL WASTE HAZARD. Synthesizer waste can cause injury, illness, or death, and must be disposed of carefully. Avoid inhalation and skin contact. Refer to the Waste Profile and MSDSs in the ABI 3948 Site Prep and Safety Guide for details. All waste must be disposed of as a regulated hazardous waste in accordance with applicable federal, state, and local government regulations. Wear appropriate eyewear, clothing, and gloves when handling waste for disposal.**
6	After disposal, securely screw the cap assembly (removed in step 4) on to the emptied waste bottle.
7	Check the routing of the waste and vent lines. Route all waste and vent lines so that waste cannot form a "liquid plug." Waste that is allowed to condense in waste or vent lines can cause back pressure that will interfere with reagent flows. Waste condensed in a line should be considered a clog. **IMPORTANT** Waste bottles are the low pressure side of the delivery system and must always be kept vented to atmosphere. Be sure each vent line is properly routed to a fume hood. If a vent line is blocked, back pressure will be generated and will inhibit the deliveries of reagents and solvents. See the ventilation drawing in the ABI 3948 Site Prep and Safety Guide to verify proper ventilation requirements.
8	Empty the two 10-mL bottles for sample collector waste which are located behind the sample collector platform.

Set up the Sample Collector

The Sample Collector can use either an OligoRack™ or a screw top type rack.

To set up the Sample Collector, proceed as follows:

Step	Action
1	Press the Sample Collector button (see Figure 2-17 on page 2-17) to extend the sample collector tray.
2	Either use a new OligoRack™ or screw top rack, with a full complement of 48 vials/tubes, or prepare a rack with sufficient vials/tubes to collect the number of oligonucleotides to be produced by the next run. **Note** New OligoRacks and screw top tube racks come with a complete set of 48 vials/tubes and may be opened if a lesser number is desired. If an OligoRack has been previously opened, place tape on the lid to keep it in place.
3	Place the OligoRack™ or screw top tube rack on the sample collector tray. The key on a rack must be oriented away from you as you insert the rack.
4	Press the Sample Collector button again to retract the sample collector tray. The Begin procedure will home the fraction collector. An alternative way to home the fraction collector is to use manual control. **CAUTION Check Instrument Preferences to ensure that the type of rack loaded into the sample collector is selected (see step 7 of the procedure on page 2-9 for more information).**

Preparing Synthesis Orders

Opening

A Synthesis Order, like that shown in Figure 4-1, can be obtained whenever the 3948Control application is running by choosing the New Synthesis Order command from the File menu. (It also appears if you click Cancel in the Open Synthesizer dialog box when you first load the Control application.)

This section will only describe how to make entries in the basic Synthesis Order created upon loading or upon use of the New Synthesis Order command. For a general overview of Synthesis Orders, see “Organizing and Processing Synthesis Order Files” on page 4-10. For information on how to create Multiple Order files, see Appendix C, Creating and Using Multi-Order Files.

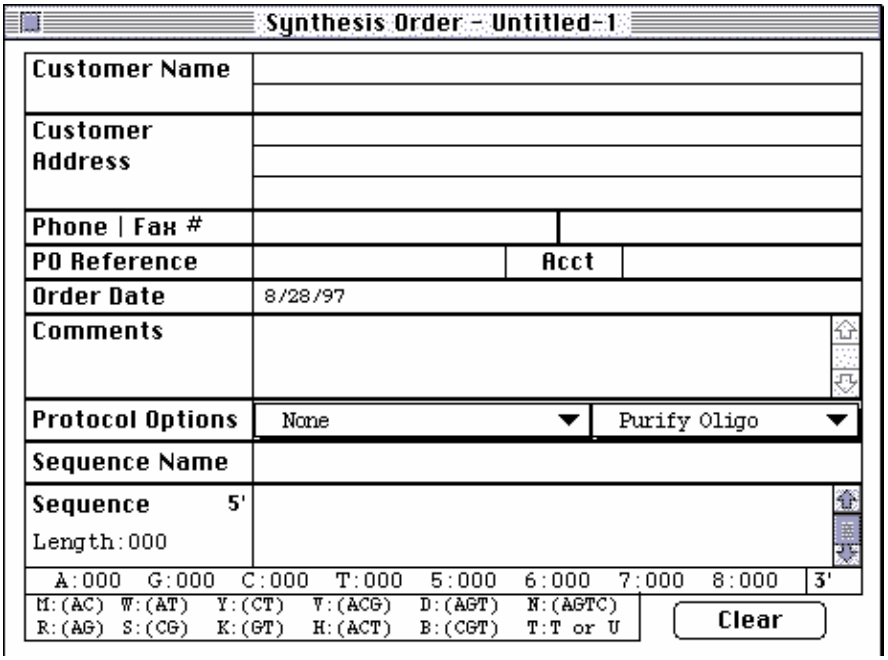


Figure 4-1 Initial Synthesis Order

Assigning a Name

The name you assign to a Synthesis Order is important because the name uniquely identifies a sequence. Sequences input as sequence orders are ordered in the instrument by length as well by the protocol assigned to the sequence input by the Synthesis Order.

Types of Information

The only default information on a Synthesis Order is the “Entry Date” provided by the Macintosh. The following information can be entered by the user on a Synthesis Order:

Table 4-1 Description of Synthesis Order Fields

Field Name	Description
Customer Name	A 48 character per line field is provided for the customer’s name or name and identification number.
Customer Address	A 48 character per line field is provided for entry of the customer’s address.
Phone/Fax #	Two 24 character fields are provided – one for a customer’s phone number – a second for a facsimile number.
PO Reference/ Acct	Two 16 character fields – one for entering the purchase order number – a second for an account number.
Order Date	The current date is entered into this field by the Macintosh when the Synthesis Order is created.
Comments	A large character field is provided for your comments. Everything that an operator will need to complete the order should be entered into this field. Since the user may not know the exact name and spelling of protocols to be used, sufficient information should be entered in this field to allow the operator to correctly assign the protocol. **Note** The most important information should be entered first since only a portion of the comments field will make it into the Run file.
Protocol Options	Two pop-up menus are presented: ♦ The first allows you to assign any of the 16 protocols, which may be created in a Synthesizer database, for production of an oligonucleotide. ♦ The second pop-up menu allows you to specify how to process the sequence after synthesis. Besides the default (1)*Purify Oligo*, you can specify that the sequence be either (2) *Crude - DMT off*, or (3) *Crude - DMT on*. The first choice specifies that the oligonucleotide be purified, the second and third choices bypass purification with the second choice specifying that a crude oligonucleotide be detritylated and the third choice specifying leaving trityl on.

Table 4-1 Description of Synthesis Order Fields *(continued)*

Field Name	Description
Sequence Name	A 31 character field is provided for the sequence name. **Note** The name in this field is not used to identify sequences during processing. The name of the Order is used instead for this purpose.
Sequence	This entry field enters nucleotides in codon groups Entry can be made by typing in a sequence or by importing a sequence using the "Import Sequence" command (File menu). Besides the four bases, valid entries include 5, 6, 7, and 8 (bottle positions, and single character IUB ambiguity codes (IBU codes are listed at the bottom of Figure 4-1 on page 4-8); entry of ambiguity characters by parenthesis is not allowed. CAUTION **Be careful not to enter characters other than A, G, C, or T as the last character because such an entry is unacceptable. The last position in a sequence, which corresponds to the 3' end of the sequence, cannot be ambiguous.** As you enter a sequence, the composition of the sequence is indicated by the number of bases of each type at the bottom of the Synthesis Order form.

Double-checking Entries and Saving

After completing the Synthesis Order form, do the following:

Step	Action
1	Go over all your entries on the form and check them out, referring to the information listed in the table in the previous section above.
2	Select the sequence and choose the Read Selection command (Edit menu). A synthesized Macintosh voice will read the sequence to you and you can double-check it by comparing the spoken sequence to a sequence listing.
3	When you are through checking out the form, save it to a file keeping the following in mind: ♦ Remember to use a name that uniquely identifies the sequence and also provides length information. ♦ A hard copy of the order should be printed for a record or to accompany the Synthesis Order file in placing the order for production of the oligonucleotide.

Note When a Synthesis Order is opened by the operator in setting up a run, the instructions provided in Chapter 2 apply for entering the order into the instrument setup for a run and sending it to an instrument.

Organizing and Processing Synthesis Order Files

To give you some idea of how you might set up your file system to support the ABI 3948 and process Synthesis Orders, the file structure shown in Figure 6-9 and the procedure presented in "Processing Synthesis Orders" are offered together as an example. You can adopt this organization and process or modify them as you desire to meet your needs.

Organizing Your File System

As you'll notice upon examining Figure 6-9, the key elements in the example file system are the "To Do" and the "Done" folders. The To Do folder contains unprocessed Synthesis Orders and the Done folder contains completed Orders. The Synthesis Order file names are assigned when saving the form and should contain both a name and number component so that orders can be uniquely identified both by customer name and order of processing.

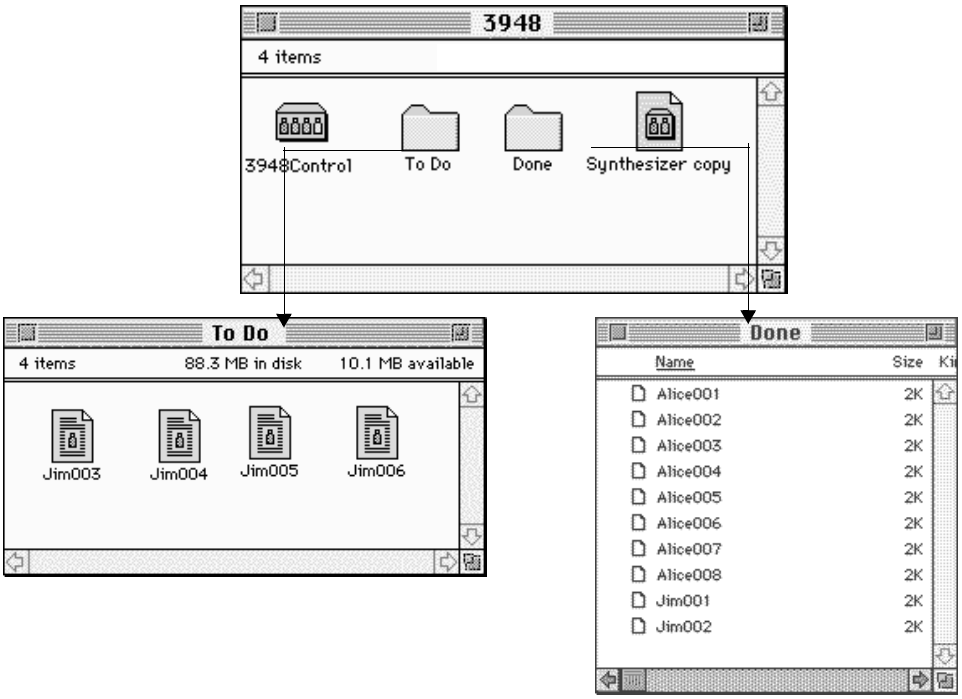


Figure 4-2 Example file organization

Note Synthesis Orders in a "To Do" folder are easily accessed from the Run Setup view for processing. After processing, the Synthesis Orders are placed into the same folder used for the Macintosh 3948Control application and must be dragged to a Done folder if they are to be archived there.

Processing Synthesis Orders

Since each sequence and the instructions needed to synthesize it are contained in a Synthesis Order, using the ABI 3948 instrument can be thought of as processing Synthesis Orders. The procedure below (or a similar set of steps) should be followed for each sequence to be synthesized:

Note Before proceeding, make a backup copy of the Synthesizer Window. This is done upon first establishing communication with a synthesizer and backs up the contents of the

synthesizer, producing an icon for the file like that entitled “Synth Copy” in Figure 6-9. The contents of this file can be used later to restore your synthesizer to its original configuration.

Process Synthesis Orders as follows:

Step	Action
1	Start the 3948Control application and prepare the Synthesis Orders: Create and fill out a Synthesis Order for each sequence to be synthesized (when no previous order exists), saving each order using a name to uniquely identify both the requestor and the particular order number. While filling out each Synthesis Order, check to see that the proper information is entered for the order. Check by asking the following questions: Is the identity of the customer clearly identified? Is the name of the customer clearly stated by the Order name so that the customer can be identified without re-opening the file? Is the sequence entered correctly, using the proper conventions? See discussion under “Sequence” on page 4-10 (Table 4-1). Is the protocol appropriate for the oligonucleotide ordered? Save the Synthesis Orders to the “To Do” folder.
2	As part of preparing for a Model 3948 run, import Synthesis Orders to the synthesizer: a. Directly open a Synthesizer window. b. Import Synthesis Orders and prepare for processing as described under “Assigning Sequences” on page 2-20. c. Use the Save command (File menu), with an active Synthesizer window. d. Save the Synthesis Orders (still in the To Do folder).
3	Upon completion of a run: a. Give or send the oligonucleotide and a printout of the Synthesis Order to each customer. b. Drag order file to the Done folder. c. If customer requests a repeat order, ask for the unique name assigned to the Synthesis Order, look up the file in the Done folder, and repeat steps 1 through 3.

Processing Variations

The previous procedure described processing Synthesis Orders one-by-one using the single order Synthesis Order. Since the 3948Control application now has the capability of generating multiple single order Synthesis Orders from a single Multiple Synthesis Order file, the process of producing Synthesis Orders might differ as described below:

Process Synthesis Order Variations:

Step	Action
1	Receive a Multiple Synthesis Order text file from a customer. **Note** Multiple Synthesis Order files may contain information needed to generate up to 999 single order Synthesis Order files in either the short or long format. See "New Synthesis Order/Creating Multiple Order Files" in Chapter 4 of the Reference Manual for more information.
2	Generate single order Synthesis Order files from the Multiple Order file. All that is required to generate single order Synthesis files is to open the file from the 3948Control application and then click the Make Order Files button.
3	Check for errors.
4	Produce oligonucleotides specified in the single order Synthesis Order files as described in the previous procedure.
5	Give or send the oligonucleotides and a printout of each Synthesis Order to the customer
6	Throw out Multiple Order Synthesis Order file when synthesis is completed rather than save it (if it is known that the contained Synthesis Orders are "one offs" or if repeat orders are never done). Since the customer is providing the Multiple Order files, it is really the customer's responsibility to archive the files needed to reproduce a needed sequence. Since Synthesis Order files can also be read by database programs, such as Excel, FileMaker Pro, etc., it is very easy for a customer to maintain a custom database of orders rather than the raw files. Applied Biosystems *can not offer technical support for such customer developed databases*.

Working with Phosphoramidite Bottles

Introduction

The phosphoramidites are bottled as powders and sealed under argon. In this state, they are stable for at least one year from the date of shipment. The ABI 3948 accommodates installation of phosphoramidites in powder form. Powdered phosphoramidites are dissolved in acetonitrile on the instrument using the Auto Dilution feature as described under “Using the Autodilution Procedure” on page 4-15.

Note When you use an Autodilution procedure, a bottle must be present at each bottle position (1–8 and TET) since all phosphoramidite bottles are pressurized simultaneously by a common pressure source.

Removing Bottles

Remove old bottles as described below:

Step	Action
1	Remove a bottle by firmly pulling it straight down while pressing the black button above its receptacle. If the bottle seems to stick, carefully move it side to side while pulling it off.
2	Wipe the delivery line with a lint-free tissue.

Placing New Bottles in Position

Sufficient phosphoramidites must be present on the instrument to complete a run.

Replace phosphoramidite bottles as described below and then auto-dilute using the procedure on the next page:

Step	Action
1	Firmly push each bottle up around its receptacle while pressing the black button. As necessary, maneuver the bottle into place by carefully moving it side to side while pushing.
2	When the bottle is correctly engaged, release the button and it will return to its *out* position. If the button remains in, the bottle is not seated properly and must be repositioned.

Using the Autodilution Procedure

The standard autodilution procedures are intended for use in preparing powdered A, G, C, and T phosphoramidites for use on the instrument. The procedures autodilute either all four powdered phosphoramidites at one time or each powdered phosphoramidite individually, depending on the procedure chosen.

Note Leave the old phosphoramidite bottles on the instrument until prompted to replace them with new bottles.

Autodilute powdered phosphoramidites as follows:

Step	Action
1	Select the Edit Bottle Procedure view from the Choose function pop-up menu.
2	Choose the appropriate autodilution procedure from the Edit Bottle Procedure pop-up menu. These are the five choices (version numbers are represented by x's below since they are subject to change): ♦ AutodiluteACGT vx.xx ♦ Autodilute-A v1.xx ♦ Autodilute-G vx.xx ♦ Autodilute-C vx.xx ♦ Autodilute-T vx.xx The following volumes of acetonitrile are added to the phosphoramidite bottles at listed positions to achieve 0.05 M concentration: ♦ Position A - 44.8 mL ♦ Position G - 46.4 mL ♦ Position C - 47.2 mL ♦ Position T - 52.8 mL **Note** There are no autodilute procedures for positions five through eight. These positions are intended for manually diluted specialty reagents.
3	Click Execute to start the procedure.
4	When reagents have been rinsed and flushed into the old phosphoramidite bottles, the following message is presented in the lower pane of the Monitor Chemistry view: Place new 2g amidite on synthesizer.
5	Remove the old phosphoramidite bottles from the positions on which new amidites are to be installed. This will be position A, G, C, T or all of them, depending on the autodilute procedure chosen.
6	Place the powdered phosphoramidites properly on the instrument. Put A in position A, G in position G, etc., so that each phosphoramidite is correctly positioned. Save the rubber septum for each bottle for possible use. **Note** Make sure that the bottles are correctly positioned and installed so that they seal tightly on every position.
7	Place an empty bottle at unused positions (Positions 5, 6, 7 and 8). This is necessary during both autodilution and normal operation since all phosphoramidite bottle positions are pressurized simultaneously with a single valve.

Autodilute powdered phosphoramidites as follows: *(continued)*

Step	Action
8	Select the Resume command (from the Synthesizer menu) to continue the autodilute procedure. Refer to the lower pane of the Monitor Chemistry view to determine when autodilution is complete. **Note** The autodilute procedure will fill the bottles to about the 2/3 level with acetonitrile. The dilution rate is different for each phosphoramidite, levels in the four types of bottles will not be even. The Autodilute procedure takes about 45 minutes.

Storing and Handling Reagent Bottles

Introduction

Proper storage and handling of chemicals is an important aspect of laboratory management. This subsection describes the appropriate storage conditions for the chemicals used with your Applied Biosystems 3948 instrument and the precautions to observe while handling them.

Reagent Storage Conditions

The table below lists the lifetimes of reagents on the instrument, recommended storage temperatures, and the shelf lives you may expect:

Reagent/Phosphor	Lifetime on Instrument	Storage Temperature	Shelf Life
N-methylimidazole/Tetrahydrofuran	6 wk	Room temperature	1 yr
Acetic Anhydride/ Pyridine/tetrahydrofuran	6 wk	Room temperature	1 yr
Trichloroacetic acid/DCM	6 wk	Room temperature	1 yr
Tetrazole/acetonitrile	2 wk	Room temperature	1 yr
Acetonitrile/water	1 yr		1 yr
Iodine/water	6 wk	4 °C	6 months
Ammonia	1 wk	4 °C	1 month after opening
Anhydrous acetonitrile (4 L)	6 wk	Room temperature	1 yr
Triethylammoniumacetate	6 wk	4 °C	1 yr
Trifluoroacetic acid/Water	6 wk	Room temperature	1 yr
20% Acetic Acid in H_2O	3 months	Room temperature	1 yr

Please follow the storage recommendations given above for each reagent. Improper storage of chemicals used on Applied Biosystems instruments can impair optimum instrument performance and can compromise reagent bottle integrity when pressurized under normal instrument operation.

Note Cooler temperatures may cause crystal formation in the Tetrazole solution. Gentle warming and agitation will dissolve these crystals.

Important Guidelines

The guidelines listed below should be followed while storing, handling, and using chemicals on the instrument to ensure optimum instrument performance and safe usage:

- Change a reagent bottle either before beginning a run or after an active run has been paused at the appropriate point.
- Store all chemicals away from direct sunlight. Chemicals exposed to direct sunlight may have shorter shelf lives and may detrimentally affect instrument performance and final product quality.
- Avoid direct sunlight on the instrument during operation of your Applied Biosystems instrument. Elevated reagent temperature from direct sunlight will increase reagent vapor pressures, which can alter reagent flow, and in extreme cases may result in the reagent bottle fracturing due to gas pressurization under normal operating conditions.

Precautions to Observe

Follow the precautions listed below while handling and using chemicals.

- Do not inhale vapors.
- Work in a well ventilated area.
- Always use eye protection and wear gloves and a lab coat.
- Do not leave any chemicals uncapped.
- If any chemical is ingested, immediately consult a physician.
- If there is any physical contact with the skin or eyes, wash immediately with ample water for 15 minutes and consult a physician.
- Refer to the MSDSs that are included in the Site Preparation and Safety Manual (PN 903704) for further instructions about storing and handling each reagent.

Observe the precautions presented below as “Note,” “IMPORTANT,” and “WARNING” while handling and using chemicals or performing tasks on the 3948. This type of information is presented elsewhere in the 3948 User’s and Reference manuals under the captions “Note,” “Important,” “Caution,” and “WARNING.”

Note The phosphoramidites, tetrazole, and acetonitrile are atmosphere-sensitive. Upon opening one of these bottles, quickly place it on the instrument to prevent water contamination.

IMPORTANT The disposable bottle seal forms an airtight seal between each cap assembly and bottle. It is designed for single use and should be replaced with each bottle change. Bottle seals are supplied at no charge when you order the chemical reagent kits. To order inserts separately, use the following part numbers (P/N): P/N 400501 for 450 mL bottles, P/N 400790 for 200 mL bottles.

CAUTION To prevent damage to the Sample Collector, it is essential that the rack type chosen is the same as the actual rack type used. To use the red rack (8X6 configuration), leave the checkbox blank (default). To use the white rack (4X12 configuration), check the box.

! WARNING ! CHEMICAL HAZARD. To prevent bottle explosion and severe physical injury, bottles subjected to reuse must be replaced every six weeks. Because certain chemicals reduce the integrity of glass bottles, repeated use beyond this recommended time length may result in the bottle fracturing when it is pressurized under normal operating conditions.

Installing Reagent Bottles

Introduction

Reagent bottles are located in two compartments on the instrument. The 200- and 450-mL bottles are located in the upper right compartment with the phosphoramidite bottles. Each bottle position has its position labeled on the bulkhead and bottles screw into threaded caps mounted at each position. The 4- and 2-L bottles are placed in the large lower compartment, below the phosphoramidite and smaller reagent bottles.

Note One exception: 20% Acetic acid (250/450 mL) is placed in the lower compartment.

Installing upper Position Reagent Bottles

The reagents contained in the upper compartment, in 200/450 mL sizes, include:

- Iodine (450)
- Ammonia (200)
- Acetonitrile/water (200)
- Triethylammonium acetate (200)
- Tetrazole (450)
- N-methylimidazole (450)
- Acetic anhydride 450)
- Trifluoroacetic acid/water (450)

For information on changing phosphoramidites, see“Working with Phosphoramidite Bottles” on page 4-14. We recommend using bottle change procedures for the phosphoramidites, bottles 5–8 (procedure back flushes the delivery lines with acetonitrile and argon).

Be sure to vent bottles without change procedures prior to removal from the instrument.

Ammonia and tetrazole each have a bottle change procedure in the Edit Bottle Procedure view:

- The ammonia procedure vents the bottle, back flushes the line, and then prompts you to replace the bottle.
- The tetrazole procedure back flushes the delivery line with acetonitrile and then argon before prompting you to replace the bottle.

After bottles are replaced, the procedures are continued by choosing Resume from the Synthesizer menu.

! WARNING ! Wear gloves when changing bottles to avoid direct contact with chemical reagents.

To replace a bottle (using a change procedure/replacing other bottles):

Step	Action
1	Remove a bottle as follows: ♦ Slowly unscrew the cap, turning counterclockwise. ♦ Remove the disposable bottle seal. ♦ Recap the bottle to minimize residual vapor release. **IMPORTANT** The disposable bottle seal forms an airtight seal between each cap assembly and bottle. It is designed for single use and should be replaced with each bottle change. Bottle seals are supplied at no charge when you order the chemical reagent kits. To order inserts separately, use the following part numbers (P/N): P/N 400501 for 450-mL bottles, P/N 400790 for 200-mL bottles.
2	Install a new bottle as follows: ♦ Open the new bottle. ♦ Place a new bottle seal inside the bottle neck. ♦ Screw the bottle snugly into its threaded cap on the instrument by turning it clockwise. **Note** The upper compartment receptacles have a ratchet cap assembly. A built-in torque-limiting feature reduces the possibility of overtightening. Do not continue to turn the bottle when clicking starts as this can cause the cap assembly to leak.

Installing Reagent Bottles in Lower Positions

The reagents contained in the lower compartment, in 4-L or 2- L sizes, include acetonitrile, trichloroacetic acid, and deionized water. Acetonitrile is contained in the 4-L bottle (2.5-L in some countries).

To replace a bottle in the lower compartment, proceed as follows:

Step	Action
1	In the lower bottle compartment, locate the 3-way relief valve for the bottle to be replaced. Each valve is labeled with the name of the reagent to which the valve is connected.
2	Turn the valve handle 90° to the right to release the pressure on the bottle. After turning, the valve indicator will point to “Vent” on the panel.
3	Remove the cap assembly and then screw it on a fresh bottle.

Using UV grade Acetonitrile

Use HPLC or UV grade acetonitrile with a specification of less than 100 ppm of water. A higher water content will lead to a significant decrease in synthesis efficiency (coupling).

IMPORTANT Although our specifications for Acetonitrile water content is less than 100 ppm, we strongly recommend using Acetonitrile with the lowest water content available (less than 10 ppm is desirable and available in most countries). The higher the water content in the Acetonitrile used, the lower the oligonucleotide yield and purity and the shorter the useful lifetime of the phosphoramidites on the instrument.

Changing the Argon Tank

Introduction

Monitor the level before each run. Change the tank when the pressure falls below 200 psi. You can replace an empty argon tank before beginning a synthesis or when a synthesis has been interrupted.

! WARNING ! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets, chains, or clamps.

Procedure

Change the argon tank as follows:

Step	Action
1	Close the tank valve.
2	In the Manual Control view, activate Function 65, “SynLowBlk flush,” for 90 seconds. The two tank regulator gauges should fall to zero.
3	Disconnect the regulator from the tank and clean the threads on the fittings.
4	Cap the empty tank, remove the cap from the full tank, and attach it to the regulator. For maximum gas lifetime, wrap the threads with teflon tape before installing.
5	When the regulator is tightly attached to the tank, turn the regulator knob counterclockwise.
6	Open the tank valve.
7	Turn the regulator knob clockwise until the gauge reads approximately 14.5 psi (not greater than 15 psi). **Note** If the inlet pressure exceeds 15 psi, the pressure relief valve may open. If this occurs, a hissing sound will be heard at the rear of the instrument adjacent to the inlet gas line.
8	Activate Function 65 in Manual Control and adjust the regulator to 14.5 psi with the function active.

Note A size 1A tank of 2500 psi argon should last about two months.

Calibrating Fluid Sensors

Introduction

When the instrument has been reset or a new software database has been downloaded to the instrument, the liquid sensors require calibration so that they can determine the difference between a liquid and gas reading. The sensors are calibrated using the procedure below and then the Sensor Verification procedure is performed to verify calibration.

Note Liquid sensors do not need to be re-calibrated when the instrument is turned off or when there is a power failure.

Sensor Calibration Procedure

The procedure below is used whenever it is necessary to calibrate sensors:

To calibrate sensors, proceed as follows:

Step	Action
1	Check the following, adjusting pressures and/or replacing bottles if needed: ♦ Make sure the inlet pressures is at its proper value (see “Check/Set the pressure of the argon tank” on page 4-2 for the procedure). ♦ Make sure that the acetonitrile and water bottles contain at least 300 mL each (see “Installing reagent bottles in lower positions” on page 4-11 for information on replacing bottles). ♦ Make sure that the phosphoramidite bottles (A, G, C, T) contain at least 5 mL each (see “Changing Phosphoramidite Bottles” on page 4-6 for information on replacing bottles).
2	In the Misc Procedure view, select “Sensor Calibration” from the Procedure pop-up menu.
3	Click the Execute button. The turntable will move to the first “load” position (column positions 1-3 visible) and you will be prompted, in the lower pane of the Monitor Chemistry view, to place used columns in these positions. New columns can be used if used columns are not available, although the columns should not be subsequently used for synthesis.
4	After you have loaded the indicated positions (1-3), select “Resume” (from the Synthesizer menu) to move the turntable to the next load position.
5	Load columns in the second load position (column positions 4-6), select Resume, and load columns in the third column position (column positions 7-9 visible).
6	After you have loaded the third set of columns, click Resume to enable the sensor calibration procedure to continue.
7	When the instrument is finished with calibration, select the Instrument Test View to see the sensor calibration values. The liquid calibration number should be about twice the liquid calibration number. If a calibration error is suspected, the calibration procedure can be run again.
8	Remove and discard the columns from the calibration positions.

Manual Phosphoramidite Preparation

Things to Consider

Since phosphoramidites are extremely sensitive to acid, oxygen, and water, you must take special care when dissolving them. The guidelines in the following table will help avoid contamination, prevent degradation, and ensure high coupling yields.

Type of Factor	Guidelines
Proper Use of Acetonitrile	♦ Use anhydrous acetonitrile with less than 100 ppm water to dissolve the phosphoramidites. **Note** *Do not* use HPLC-grade acetonitrile. Its higher water content will decrease coupling efficiency. ♦ After opening acetonitrile, keep it blanketed with argon to avoid contamination with air. ♦ When transferring acetonitrile to a phosphoramidite bottle, use a clean, dry, glass syringe with a needle. Follow these precautions: – Store the syringe in a 100–120 °C oven to prevent atmospheric moisture contamination. – Keep a syringe dedicated to acetonitrile transfer. – Use acetonitrile to rinse the syringe. *Do not* use water. – *Do not* contaminate the acetonitrile bottle with traces of phosphoramidites (i.e., do not allow the syringe needle to contact the phosphoramidites).
Dilution with Proper Amount of Acetonitrile	When preparing phosphoramidites, add the correct amount of acetonitrile to each phosphoramidite as shown in the table below.

Volumes of Acetonitrile Added to Phosphoramidites

ß-Cyanoethyl Phosphoramidites	Weight of Phosphoramidite (grams)	Volume of Acetonitrile (mL)	Part Number
dA^{Bz}	2.0	46.6	401159
dG^{ibu}	2.0	48.5	401161
dG^{dmf}	2.0	48.5	401165
dC^{Bz}	2.0	48.0	401160
T	2.0	53.7	401162
Dye Phosphoramidites	(milligrams)	—	—
6-FAM	85	1.0	401527
HEX	105	1.0	401526
TET	100	1.0	401533
Other	—	—	—
Biotin	85	1.0	401395
Biotin	250	3.0	401396
Phosphalink	70	1.0	401717
CE Aminolink TFA	250	3.3	

Procedure for Dissolving and Installing

Follow this procedure to dissolve phosphoramidites:.

To dissolve phosphoramidites:

Step	Action
1	Prepare the phosphoramidite bottle as follows: a. Pull back the aluminum tab in the direction of the arrow. *Do not* yet remove it, simply expose the septum. b. Place a needle (any gauge) without a syringe into the rubber septum. This vents the pressure in the bottle when the anhydrous acetonitrile is added. Venting also prevents accidental splashing when the phosphoramidite bottle is opened and placed on the instrument.
2	Unscrew the cap from the anhydrous acetonitrile bottle and quickly replace it with a clean rubber septum. The acetonitrile is bottled under argon. Since argon is heavier than air, argon should still blanket the acetonitrile after the septum transfer.
3	Remove the syringe/needle from the oven and allow it to cool to room temperature.
4	Pierce the septum of the acetonitrile bottle with the needle and remove the correct amount of acetonitrile.
5	Pierce the septum of the phosphoramidite bottle a few millimeters with the needle/syringe and slowly add the acetonitrile. Make sure the needle does not touch the phosphoramidite powder or solution.
6	When finished adding the acetonitrile, remove both the venting needle and the needle/syringe and gently swirl the bottle to dissolve the phosphoramidite.
7	Once it is dissolved, use the appropriate Chg (Change) base procedure from the Edit Bottle Procedure menu to replace the existing phosphoramidite bottle on the instrument. See “Working with Phosphoramidite Bottles” on page 4-14 for instructions on removing and replacing phosphoramidite bottles. **Note** A separate Chg base procedure is provided for each phosphoramidite position (A, G, C, T, 5, 6, 7, and 8). You will be prompted during the procedure when to remove the old bottle and put on the new bottle you have just prepared. The procedure will blanket the contents of the new bottle with argon to protect the contents.

Maintaining the Instrument 5

In This Chapter

Topics Covered

This chapter provides the information needed for preventive maintenance of the instrument.

The contents of the chapter are listed below:

Topic	See page
Scheduling Necessary Maintenance	5-2
Introduction	5-2
Required Maintenance Items	5-2
Replacing O-Rings, Gaskets, and Seals	5-2
Replacing O-rings	5-2
Replacing EPR and Kalrez Gaskets	5-3
Replacing Disposable Bottle Seals	5-3
Replacing 2-L and 4-L Inlet Filters	5-3
General	5-3
Procedure	5-3
Manual Control Jaw Leak Testing	5-4
Special Considerations	5-4
Jaw/Block Pressure Test Procedure	5-4
Cleaning the Instrument for Shut Down	5-5
Cleaning	5-5
Shutting Down and Restoring the Instrument	5-5

Scheduling Necessary Maintenance

Introduction

Your ABI 3948 Nucleic Acid Synthesis and Purification system is designed to require very few regular maintenance procedures. Following the necessary maintenance schedule will help prevent service calls and downtime.

Required Maintenance Items

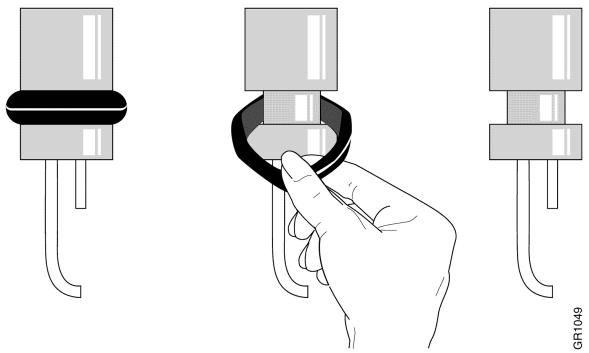
The maintenance schedule required for the ABI 3948 is listed in the table below:

Instrument Part	Replacement Schedule
O-rings	Change annually; examine monthly
Gaskets	Change biannually; examine monthly
Argon tank	Change when pressure falls below 200 psi; monitor before each run
Waste bottle	Empty as needed
Sample collector needle	Adjust as required **! WARNING ! Do not over tighten set screw.**

Replacing O-Rings, Gaskets, and Seals

Replacing O-rings

Follow this procedure to replace a phosphoramidite O-ring (P/N 221014):

Step	Action
1	Remove the O-ring by gripping it with a hemostat and pulling it away from its groove. Be careful not to mar the white Teflon™ insert that holds the O-ring. 
2	Check that the Teflon is free of particulates before inserting the O-ring. Push the new O-ring into the groove with your fingers.
3	If, after a period of use, a white precipitate appears on a gasket or O-ring, clean it with a cotton-tipped swab moistened with acetonitrile.

Replacing EPR and Kalrez Gaskets

EPR and Kalrez™ replacement gaskets for 2L and 4L bottle caps have the part numbers listed in the following table:

Part Number	Description
004297	Kalrez (TCA bottle-2L)
004498	EPR (acetonitrile bottle-4L and DI H_2O bottles-2L)

- To replace the gasket, simply pull the old gasket out and put the new gasket in its place. Make sure the new gasket lies flat in the cap assembly.

Note Kalrez gaskets are also used in the jaw assemblies to seal on the synthesis/purification columns. These gaskets are not user accessible.

Replacing Disposable Bottle Seals

Ratcheting bottle caps are designed to prevent the bottle breakage that results from over-tightening. These caps are used with disposable seals.

Disposable bottle seals have the part numbers listed in the following table:

Part Number	Bottle Volume
400790	200 mL (10 per package)
400501	450 mL (10 per package)

To use a disposable bottle seal, insert the seal in the mouth of the bottle. Use a disposable seal only once; discard it when the bottle is changed.

Replacing 2-L and 4-L Inlet Filters

General

Inlet filters (P/N 200270) fit all 2-L and 4-L bottle cap assemblies (except the TCA bottle) and should be ordered if the cap assembly lacks one. The filters should be changed annually.

Procedure

- To change filters, simply unscrew the filter from the delivery line of the bottle cap assembly and screw on the new filter.

CAUTION The filters are not compatible with the TCA reagent; do not use one on the TCA inlet line.

Manual Control Jaw Leak Testing

Special Considerations

The Jaw Close functions (Functions 232, 234, and 236) may be used to simply close the jaws or they may be used to invoke a pressure test of the jaw seal and associated valve blocks when the jaw closes. These functions only close jaws when used without either a Time or a Misc (Miscellaneous) field entry.

Using both Time and Misc entries for these jaw functions enables leak testing. The pressure drop test time is defined in the Time field and the maximum allowable pressure drop is declared in the Miscellaneous field in 1/100's of a psi, except as described in step 2 of the procedure below.

Function 325 is used to define the default values in whole seconds for the pressure drop time (Time field) and setting time (Misc field) for any jaw/block pressure test. Unless this function is executed with new values in these fields before executing a pressure test, the default of 30 seconds will be used for both values.

Jaw/Block Pressure Test Procedure

The Jaw/Block pressure test is performed from the Manual Control view as follows, using the Jaw Close functions:

Step	Action
1	Go to the Manual Control view and select the desired Jaw Close Function (Function 232, 234, 236, or 325).
2	Choose one of the following ways to set the maximum allowable pressure drop value for the test: ♦ If the Misc field is to be used to specify the maximum allowable pressure drop, proceed with step 2. ♦ If the value for the Leak OK in 0.01 PSI parameter from the Instrument Preferences Window is to be used, check the Man Cont Jaw Testing check box in the Instrument Preferences Window (unchecked by default). Once this check box is checked, no Misc entry is required in the Manual Control View. Proceed with step 3. **Note** If the Instrument Preferences parameter and manual control Misc field both have zero values, no jaw/block pressure testing will be performed.
3	Enter the desired pressure drop (non-zero) pass/fail value in the Misc field.
4	Enter the desired time for the pressure drop test time in the Time field.
5	If values other than 30 seconds are desired for settling time and pressure drop time for any test, do the following with Function 325 before executing a jaw/block pressure test function: ♦ Select Function 325 in the Manual Control view. ♦ Enter the new value desired for settling time in whole seconds in the Misc field. ♦ Enter the new value desired for pressure drop time in whole seconds in the Time field. ♦ Execute Function 325 by clicking the Start button.
6	Execute the jaw/block pressure test by clicking the Start button.

Cleaning the Instrument for Shut Down

Cleaning

It is necessary to remove all reagents and run clean up procedures if you plan to not operate the instrument for more than 2 weeks. We recommend that you discard phosphoramidites rather than store them for reuse. This is because phosphoramidites that are frozen and thawed will show some loss of activity regardless of technique.

IMPORTANT You must properly shut down the instrument if you plan to leave the instrument idle for more than 2 weeks. Failure to properly shut down the instrument could cause clogs that interfere with reagent deliveries.

Shutting Down and Restoring the Instrument

To shutdown and restore the instrument:

Step	Action
1	Execute the Clean Cols (Misc Procedures View) procedure first. This procedure will prompt you to place columns on the Turntable and Resume each time. It is recommended to use empty cartridges for this procedure
2	Execute the Clean Lines (Misc Procedures View) procedure. This procedure will prompt you to remove all reagents, except Acetonitrile. Place empty bottles in all positions (except Acetonitrile). You then need to Resume the procedure. The procedures in steps 1 and 2 deliver acetonitrile through all reagent delivery lines and column positions, depositing it in the empty bottles. Subsequent Argon flushes will blow all lines dry. **Note** You should discard phosphoramidite bottles after removal.
3	The instrument can now be left indefinitely in its present state (with empty bottles in all positions except Acetonitrile). **IMPORTANT** It is important to have a bottle at each position.
4	When you are ready to restore the instrument to operation, do the following: a. Place new reagent bottles at empty positions. b. Replace the Acetonitrile with a new, unopened bottle. c. Select the AutodiluteAGCT procedure (Edit Bottle Procedure menu) and Execute. d. Place new powdered amidites in positions A, G, C, and T when prompted by the procedure and Resume the function.

Advanced Use of 3948Control

6

In This Chapter

Topics Covered

This section provides you with practical information useful in using the 3948Control application with the instrument.

This chapter covers the following topics:

Topic	See Page
Overview of Cycles, Procedures, and Protocols	6-3
Standard Protocols Cover Most Use	6-3
Subcomponents of a Protocol	6-3
Contents of the Standard Protocols	6-3
Overview of 3948 Functions	6-4
System and User Functions	6-4
Function Names	6-4
Time and Miscellaneous Field Entries	6-4
Sensors	6-5
Valve Functions	6-6
Types of Valve Functions	6-6
Uses of Valve Functions	6-6
Manual Control	6-7
Introduction	6-7
Creating Your Own Functions	6-7
Creating Your Own Procedures	6-7
Sensor-Controlled User Functions	6-7
Description	6-7
Components of Sensor User Function Names	6-7
Locations of Sensors for Sensor-Controlled User Functions	6-7
Use of Parallel with Serial Sensor User Functions	6-8
Sensor-Controlled User Function Example	6-8
Support for New Cycles and Procedures	6-10
Introduction	6-10
Guidelines for Modifying Procedures and Cycles	6-10

Topic	See Page
Creating a New Cycle or Procedure	11
Choosing the Type of Cycle or Procedure	11
Procedure	12
Changing an Existing Cycle or Procedure	14
Types of Changes	15
Procedure for Changing Values	16
Procedure for Inserting New Functions	19
Procedure for Deleting Unnecessary Steps	20
Saving and Retrieving Cycles, Procedures, and Functions	20
Backing Up the Synthesizer Setup	20
Restoring a Synthesizer Setup	20
Other uses for the Save command	20
Exporting and Importing Cycles and Procedures	20

Overview of Cycles, Procedures, and Protocols

Standard Protocols Cover Most Use

If you intend to use the standard protocols provided with the instrument, you will have very little to do except import the Synthesis Orders containing sequences you want to produce. The three standard protocols which come with the instrument provide the capability for production of purified oligonucleotides for standard, dye, and Biotin primers.

Subcomponents of a Protocol

Each standard protocol comes with its own set of cycles. These include a Synthesis cycle, a Cleavage cycle, and a Purification cycle. Deprotection is initiated by the Cleavage cycle and concluded by the Purification cycle. The process of producing a new protocol involves creating a new set of these cycles and assigning them to the new protocol using the Run Protocol view.

Note The Synthesis and Cleavage cycles are identical in the three protocols provided. The three protocols differ only in using different Purification cycles.

Contents of the Standard Protocols

The procedures and cycles provided for use by the three standard protocols include those listed below. The process of creating new procedures or cycles involves choosing the most appropriate existing procedure or cycle and then copying and editing it to produce the new procedure or cycle. (The version numbers in the Protocol and Cycle names below are represented by x's because they change with software upgrades.)

SynPure x.xxx Protocol

- Syn vx.xxx
- Cleave vx.xxx
- Pur vx.xxx

SynPure x.xx x Dye Protocol

- Syn vx.xxx
- Cleave vx.xxx
- Pur vx.xxx Dye

Synpure x.xx Biotin Protocol

- Syn vx.xxx
- Cleave vx.xxx
- Pur vx.xxx Biotin

Note If you need or want more information about the chemistry on these procedures and cycles, refer to the following portions of the Reference manual:.

- Chapter 3 for chemistry information
- Appendix A for a discussion of the functions constituting a cycle
- Appendix B for standard cycle and procedure information
- Appendix C for a complete listing of functions

Overview of 3948 Functions

System and User Functions

The permanent non-programmable functions available on the instrument are considered “System” functions to distinquish them from another function category provided on the instrument, “User” functions. More information on User functions is provided below and later on in this chapter.

System Functions

There are three fundamental types of functions on the 3948.

- *Valve functions* that deliver reagents and gas throughout the system.
- *Non-valve hardware functions* that control parts of the 3948 such as the turntable, pressure regulators, or sample collector.
- *Logical or cycle directive functions* such as Begin Loop, Sel Pur Cols, or If Cyc Greater. Logical functions help to define the behavior of other functions or affect the order of progression through the steps in a cycle.

Each of the three types of functions above are covered separately in Appendix A of the Reference manual.

User Functions

Two types of User functions are provided on the instrument, general purpose user functions and special user functions.

- General purpose user functions contain no preset values and each valve to be used must be specified by the user.
- Special user functions perform sensor-controlled deliveries. Information on using these types of User functions is provided under “Sensor-Controlled User Functions” on page 6-7.

Function Names

Functions have abbreviated names which describe the action they perform. For example, Function 44, ACN to SynCol A, delivers acetonitrile to Column A. The third class of functions, Special Logical (Cycle Directive) functions, are functions whose behavior cannot be anticipated by relating the function name to the instrument's reagents or mechanical components (see “Overview of Special Functions” on page B-2 of the *ABI 3948 Reference Manual* for more information).

Time and Miscellaneous Field Entries

In all cases where time values are used, the following rules apply.

- The Time field contains a time in seconds and tenths of a second.
- The Misc (Miscellaneous) field is used to declare any other type of step parameter such as a pressure or temperature setting.

Sometimes a function's behavior is modified simply by having a non-zero instead of a zero in the Misc field. Although any non-zero value will work, ABI cycles use 999 with these functions to distinguish between “non-zero” and the specific non-zero values required by other functions.

Sensors

How Functions use Sensors

Valve functions may be sensor controlled or not and work as described below.

- All serial and parallel functions work with liquid/gas sensors while block functions do not.
- Sensor functions execute for the time specified in the step unless their sensor is activated (i.e., the step field SNS equals YES).

  In this case, execution stops either when the sensor is triggered or when time runs out, whichever occurs first (see also 'Retries' later).
- Parallel functions turn off the delivery to each column or coil separately as each corresponding sensor triggers.
- With Flush and Back Flush functions, sensors are used to detect dryness. All other sensor functions execute until the sensor becomes wet.

Locations of Sensors

A majority of the sensors are located on the flow paths leading through the jaws that clamp on the OneStep™ columns in the turntable. Most of these sensors are located above the columns and detect the delivery of reagents through the columns. The sensors above the columns are also used to verify that columns have been flushed dry with gas or that the cleavage or purification vessels have been drained (back flushed) until empty.

Use of Extra Sensors

To minimize the consumption of expensive amidites, extra sensors are placed between the reagent block and the columns in the synthesis system. These sensors allow the delivery of short slugs of reagent that are then pushed into position to saturate the columns. The upper synthesis sensors control this final push so that it is stopped at the right time to leave these short slugs resting over the columns.

Other sensors include one that detects the arrival of a sample at the UV cell, three sensors that detect the arrival of samples into the deprotection coils, and four others used to verify deliveries out of the primary amidite bottles (A, G, C, and T—bottles 1 through 4).

Retries

Any function executing with sensor(s) active will retry a delivery or a dry up to six times if the sensor does not report success initially (except Base+Tet). Each retry executes for as long as the time specified unless the sensor triggers during the retry and ends the function.

Retries work as follows:

- Delivery functions will wait fifteen seconds between retries to ensure bottle repressurization between attempts.
- Drying functions (back flushes/drains and flushes) do not wait between retries.
- Parallel functions will retry only those columns that have not already been successful and may retry each column a different number of times.

Any function that retries more than four times will issue a message to serve notice that trouble may be developing.

Note Ramping functions will retry only four times instead of the usual six and do not report retries, only failures.

Valve Functions

Types of Valve Functions

There are three basic types of valve functions:

- Block functions

  Block functions do not deliver to columns or coils.

- Serial functions

  Serial functions deliver to a specific column or coil and will only execute if their designated column is active.

- Parallel functions

  Parallel functions will, within a given area such as cleavage, deliver to all active columns or coils at the same time. In an instance where only a single column is active, a parallel function will behave like the corresponding serial function dedicated to that one column.

Note Associated parallel and serial functions are always clustered together on the function list in the order: Parallel Function, Serial A, Serial B, Serial C.

Uses of Valve Functions

Valve functions do the following:

- A valve function opens or closes a valve or set of valves simultaneously to perform a specific delivery or task for a specified time.

  For example, Reagents can flow through the column and then to either the waste bottle, deprotection coils, or the UV detector and the collection vial.

- Valve functions direct the reagent deliveries in the Synthesis, Cleavage/ Deprotection, and Purification cycles that are necessary to produce oligonucleotides on the 3948.

- Valve functions also constitute the following procedures:
  - Bottle change
  - Auto-dilution
  - Begin and end
  - Miscellaneous
  - flow test

The above procedures as well as the three types of chemistry cycles are documented in Appendix B of the *ABI 3948 Reference Manual.*

Manual Control

Introduction

Valve functions are used automatically by the system to perform chemistry or other tasks when you run a procedure or a chemistry protocol. If you desire, you can manually activate valve as well as other types of functions using the Manual Control View whenever the instrument is not active.

Creating Your Own Functions

Besides activating functions which are already defined, the 3948 provides you with the capability of creating your own functions. As described under “Exercising Manual Control of a Function” on page 6-4 of the *ABI 3948 Reference Manua*l, you can create more than 100 user-defined functions in the Manual Control View and combine them with the standard set of functions to customize procedures.

Creating Your Own Procedures

Customized procedures may also be executed from within the Edit view used to create them (see *Execute Button* on page 6-14 of the Reference manual). During synthesis, the Monitor Chemistry menu displays each function as it is activated.

Sensor-Controlled User Functions

Description

The last 100 functions on the 3948 function list (SynUpr Wet 401 to 500 UV Dry 500) are user functions that can perform sensor-controlled deliveries. Like standard user functions, the valve list for these functions can be defined by the operator. These functions offer the additional advantage of having sensors pre-assigned to them.

Sensors for sensor-controlled user functions are engaged in the usual fashion by setting the SNS field in the step to YES. The particular sensor or set of sensors tied to a sensor user function is specified in the default function name. For example, “SynUpr Wet 401” (Fxn 401) has sensor 401 (which corresponds to sensor 1) pre-assigned to it. As with standard user functions, the function name may be edited as well as the valve list.

Components of Sensor User Function Names

There are three components to the default names provided for sensor user functions:

- The first part of the default name identifies the sensor or sensors associated with the function.
- The second component spells out whether the function is wet or dry, that is, whether the function's sensor(s) trip upon detecting wetness (liquid delivery) or dryness (gas delivery, flush or back flush).
- The final component of the default function name lists the function number, in keeping with the convention for standard user functions.

Locations of Sensors for Sensor-Controlled User Functions

Sensors for this type of user function are located as follows:

- SynUpr, SynMan, and SynLow refer respectively to:
  - the upper synthesis sensors numbered 1 to 3
  - the synthesis manifold sensors numbered 22- to 24
  - the lower synthesis sensors numbered 4 to 6

- Sensors 7 to 9 are the Clv sensors
- Sensors 10 to 12 are the Coil sensors
- Sensors 14 to 16 are the Pur sensors
- Sensor 21 is the UV sensor

See the Plumbing Diagram in Appendix D of the *ABI 3948 Reference Manual* to verify which sensors are associated with which columns. In some instances, the column alphabetic order and sensor numeric order are reversed.)

Use of Parallel with Serial Sensor User Functions

Parallel user functions are used with their associated serial user functions as follows:

- Parallel sensor user functions are always grouped on the function list together with their associated serial functions. So SynUpr Wet 401 is followed by SynUprWet A 402, SynUprWet B 403, and SynUprWet C 404.

  This grouping of sensor functions into sets is crucial to the operation of parallel user functions. **Parallel sensor-controlled user functions do not use their own valve lists to execute, they use the valve lists of their associated serial functions instead**.

- Parallel functions turn on only the valves for active columns by merging together the valve list for each active column's associated serial function and excluding the valve lists of functions associated with inactive columns.

  When one column finishes its delivery before the others, its single set of valves is deactivated. Any other active column’s valves will remain on until its sensors trigger.

- Programming a valve list for a parallel function is done by entering the appropriate valve list into each associated serial function.

  It is desirable to enter the complete, three-column valve set into the parallel function's valve list so that the entire valve list may then be viewed under one function in Manual Control.

Sensor-Controlled User Function Example

Background

As explained above (in boldface), sensor-controlled parallel user functions execute the valve list settings made in the associated serial functions. This means that sensor-controlled user functions are always used in groups of four functions, the parallel function for the group and the three serial functions associated with it.

For example, a user might want to deliver Tetrazole (TET) until it tripped the upper sensors (the sensors above the synthesis columns) instead of the manifold sensors as the standard TET to SynCols (Fxn 48) parallel function would do. This can be done using these four functions:

- SynUpr Wet 401
- SynUpr Wet A 402
- SynUpr Wet B 403
- SynUpr Wet 404

Procedure

Using these functions requires the following three steps:

Step	Action
1	Put the desired valve lists in the associated serial functions, referring to the entries in the next table.
2	Call the parallel function (SynUpr Wet 401) in a cycle and make the following entries: a. Turn the sensors on (YES in the SNS field). b. Set a trip time (value in seconds in the TIME field) greater than required for tripping sensors (perhaps 26 seconds).
3	Run the cycle in which you called the parallel function to execute it.

The following valve list entries are required for the functions in this example:

Function	Valves
SynUpr Wet A 402	Valve 3 (Tetrazole)
	Valve 15 (Column A)
SynUpr Wet B 403	Valve 3 (Tetrazole)
	Valve 14 (Column B)
SynUpr Wet C 404	Valve 3 (Tetrazole)
	Valve 13 (Column C)

Note The value set for trip time can be any value greater than actually needed for the sensor(s) to trip. The actual trip values will be reported to the Microphone file.

Support for New Cycles and Procedures

Introduction

Although the standard chemistry provided with the ABI™ 3948 Nucleic Acid Synthesis and Purification System fulfils the synthesis needs of most users, the instrument supports users in developing new chemistry. New chemistry can be developed through making copies of existing chemistry cycles and procedures in the seven editable views and then editing them:

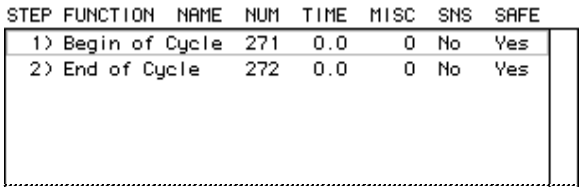
- Edit Synthesis Cycle
- Edit Cleavage Cycle
- Edit Purification Cycle
- Edit Begin Procedure
- Edit End Procedure
- Edit Bottle Procedure
- Miscellaneous Procedures

Since the user interfaces for all seven Edit views are identical, the procedures below can be used to create a new version for any of the seven kinds of cycles or procedures.The most likely changes you will make to a cycle are (1) insert one or more new steps, (2) delete one or more steps, and (3) change the time for an existing procedure step.

Guidelines for Modifying Procedures and Cycles

The guidelines listed below apply when modifying existing procedures and cycles for use in a new protocol:

- Copy the desired cycle or procedure into an empty position and then edit it to develop a new procedure or cycle. A cycle in an empty position looks like the figure below.



STEP	FUNCTION NAME	NUM	TIME	MISC	SNS	SAFE
1)	Begin of Cycle	271	0.0	0	No	Yes
2)	End of Cycle	272	0.0	0	No	Yes

Note The default cycles or procedures in your instrument are write protected and cannot be edited unless first copied into an editable position.

- Take care when creating or editing Cleavage and Purification cycles that the timing for shared use of the deprotection coils is set carefully so that conflicts are not created between cleavage and purification chemistries.

Note Before creating altered cycles, be sure that you understand the current chemistry by following ABI cycles as examples. Do this by reading the annotated cycle information and special function information provided in Appendix B of the Reference manual.

Creating a New Cycle or Procedure

Choosing the Type of Cycle or Procedure

Information on the types of cycles or procedures which may be copied from is listed in the table below. The procedure for modifying is listed in the next subsection.

Most often, a new cycle or procedure will be a variation of an existing cycle or procedure. The fastest way to produce this type of modified cycle/procedure is to start with a copy of the desired type of cycle/procedure and then make changes. To begin developing a new cycle or procedure, choose and name an empty location and then choose a cycle/procedure to copy from. Empty cycles or procedures will have the names listed in the following table in the various Edit views:

Table 6-1 Cycles and Procedures to Copy From

Type of Edit Cycle or Procedure View	Description
Edit Synthesis Cycle	*Syn Cycle 02* through *Syn Cycle 20* (19 empty cycles are available in a new instrument); the default synthesis cycle is used in all three protocols (unlabeled as well as dye and biotin oligonucleotide).
Edit Cleavage Cycle	*Clv Cycle 0*2 through *Clv Cycle 10* (9 empty cycles are available in a new instrument); the default cleavage cycle is used in all three protocols.
Edit Purification Cycle	*Pur Cycle 04* through *Pur Cycle 10* (7 empty cycles are available in a new instrument). Three Purification cycles are provided: ♦ Pur vx.xxx is provided for purification of unlabeled oligonucleotides. ♦ Pur vx.xxx Dye is provided for purification of dye-labeled oligonucleotides. ♦ Pur vx.xxx Biotin is provided for purification of Biotin oligonucleotides.
Edit Begin Procedure	*Beg Proc 02* through *Beg Proc 10* (9 empty procedures are available in a new instrument); “Start Up vx.xx” is the standard Begin procedure used in all protocols).
Edit End Procedure	*End Proc 0*3 through *End Proc 10* (8 empty procedures are available in a new instrument). ♦ The standard End procedure is used to clean up after the termination of a normal run, where the cycles have already “tidied up” after themselves. ♦ The “Clean” procedures are used to clean out the instrument when a run has been aborted in an unknown state and any or all major areas of the instrument could require cleaning.

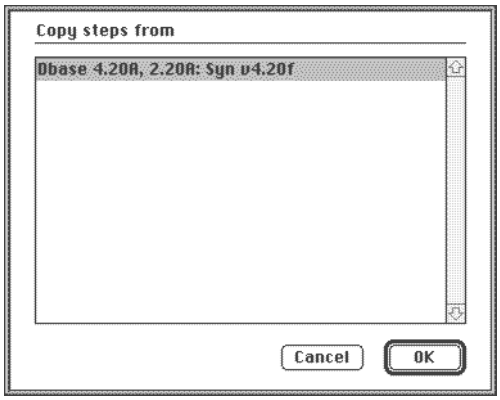
Table 6-1 Cycles and Procedures to Copy From *(continued)*

Type of Edit Cycle or Procedure View	Description
Edit Bottle Procedure	*Bottle Proc 16* through *Bottle Proc 20* (5 empty procedures are available in a new instrument); ♦ AutodiluteAGCT is provided for autodilution of all four phosphoramidite positions at once and four other procedures are provided for autodilution at individual positions. ♦ Additional procedures are provided for changing tetrazole and ammonia bottles as well as the monomers at positions 5 through 8.
Misc (miscellaneous) Procedure	*Miscellaneous 1*2 - 20 (10 empty miscellaneous procedures are provided in a new instrument). Procedures are provided for calibrating sensors and verifying sensor calibration, setting system pressures, and a number of clean/wash procedures as well as test procedures.

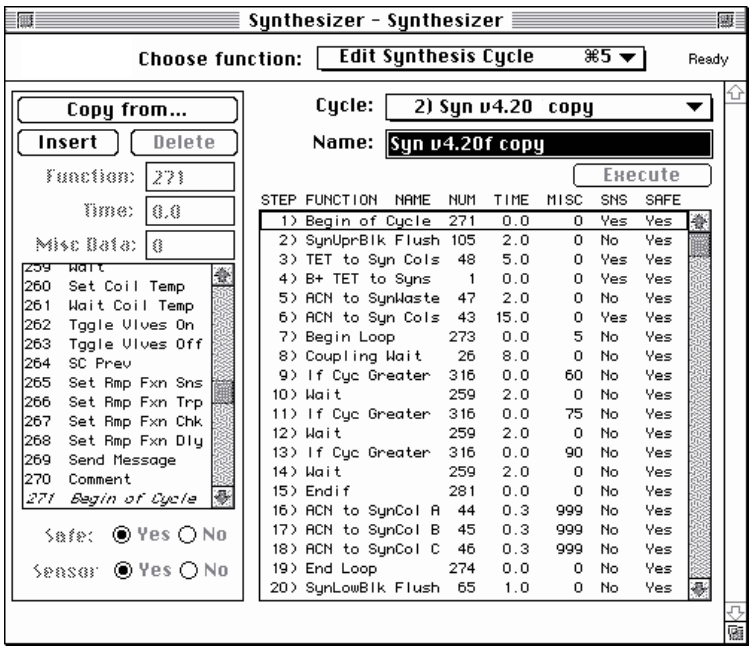
Procedure

This procedure is used whenever you want to create a new cycle or procedure.

To create a new cycle or procedure:

1	Choose an empty cycle or procedure from the Cycle pop-up menu in the appropriate view. Possible choices are listed in Table 6-1 on page 6-11.
2	Click the “Copy from” button to present a dialog box like that shown in the figure below.  The dialog box shown above will show any non-empty cycle (only a single Synthesis cycle is shown for new instruments). **Note** If you have more than one Synthesizer or database window open, cycles/procedures from all these sources will be available for copying. An alternate way of choosing a new cycle is to use the Import Cycle command from the File menu. This will let you select a cycle you have stored outside of the application.

To create a new cycle or procedure: *(continued)*

3	Select the cycle/procedure you desire and click OK. This will change the Edit view so it appears as shown in Figure 6-3, containing the particular cycle/procedure you have chosen. The new cycle will initially have “copy” appended to the original name. 
4	Change the name of the cycle by selecting the name of the copied or imported cycle and then typing in the new name. (“New cycle/procedure” was used as a name in the Figure 6-4 example). **Note** The name of the new cycle will be appended with “copy” only when the Copy from button is used.

Changing an Existing Cycle or Procedure

Types of Changes

The three major changes are:

- Changing values for an existing function

  Changes to existing or new functions include changing time, MISC, and SNS (sensor) values or changing safe step status.

- Adding a new function and then setting values
- Deleting an existing function

Three procedures are provided to support making the kinds of changes listed above. The last step in each procedure is to choose the Save command to save your changes to the instrument.

Note Another way to send changes to the instrument from an active database is to use the Send to Synthesizer command.

You will see the new cycle or procedure you created the next time you pull down that type of Cycle or procedure menu in the applicable Edit view of your Synthesizer database. The revisions you make in existing cycles or procedures can be seen upon opening them.

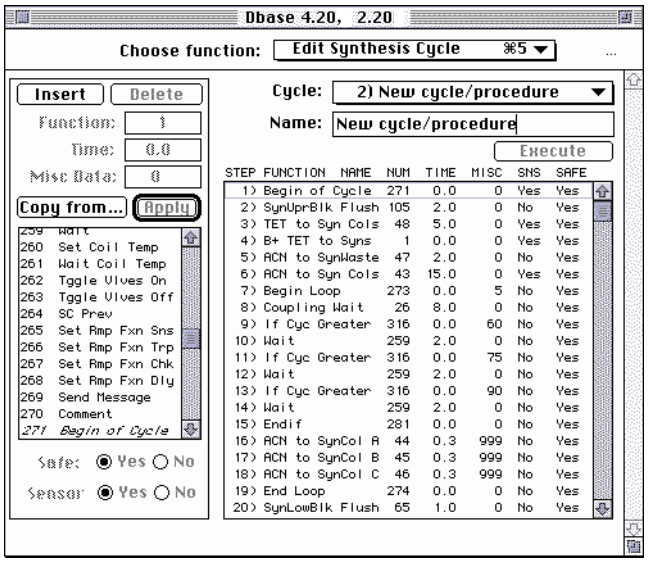
Note If you create a new cycle or procedure in an inactive Synthesizer database, one not currently associated with an instrument, the Save command only saves the changes to the database file in your Macintosh. Your changes will not be available in the synthesizer until you use the Send Copy to Synthesizer command. The use of the Send Copy to Synthesizer command replaces the database currently resident in the instrument with the new database.

Procedure for Changing Values

Use this procedure whenever you want to change an existing cycle or procedure.

Note This procedure may only be used with copies of the original cycles or procedures since the originals are locked.

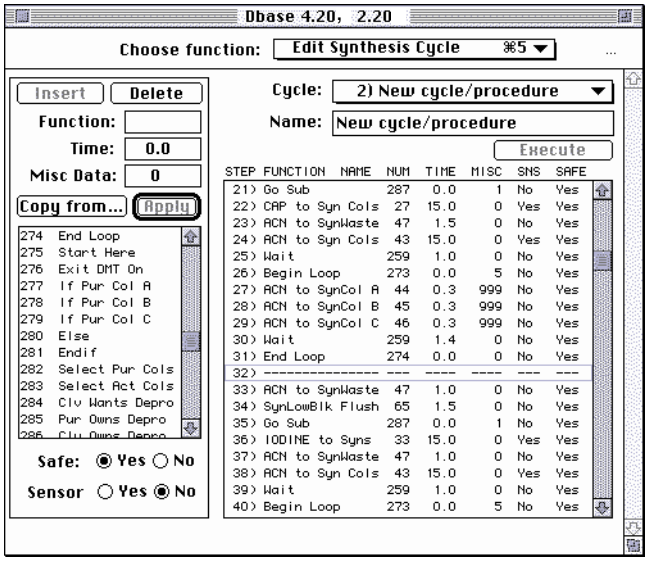
To change a value or values for an existing function:

Step	Action
1	Determine the type of change you want to make to the cycle.
2	Select the desired step in which to change a value. This will highlight the step in the Edit view as shown in the figure below.  **Note** Function, Time, and Misc Data values can not be entered for Begin and End steps and these steps can not be deleted. Although Misc Data and Sensor entries may not apply to some steps, all of the controls which are used to modify a cycle (Insert, Delete, Function, Time, Misc Data, Safe, and Sensor) are open for entry as soon as a step is selected.
3	Click the Function/Time/Misc Data (whichever applies) entry field to select it for entry, placing the insertion point where you want to add a digit. You may also double-click on the field or drag through the existing value to select the entire entry. (See steps 3–5 of the next procedure for more information on the Function, Time, and Misc parameter fields.) **Note** Double-click on the field or select an existing value, either the entire field or one or more digits.
4	Type in the new value.
5	For Safe and Sensor parameter changes, click either Yes or No buttons. (See step 6 of the next procedure for more information).
6	When you have completed your changes, choose Save from the File menu to save your changes to the instrument.

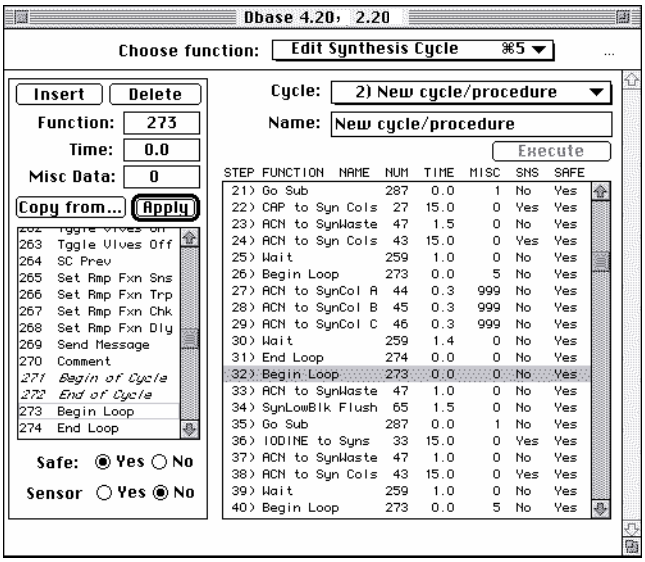
Procedure for Inserting New Functions

Note This procedure may only be used with copies of the original cycles or procedures since the originals are locked.

To insert a new function into an existing cycle or procedure:

Step	Action
1	Determine where you want to insert a step and then select the step above this position. This may require scrolling to bring the desired step into view.
2	Click the “Insert” button. **Note** Notice that an empty step appears to indicate where the additional step will be placed, as shown below. 

To insert a new function into an existing cycle or procedure: *(continued)*

Step	Action
3	Select the function you desire to enter and then click the Apply button to make the insertion. If you know the number, you can type it into the “Function” field. If you don’t know the number, select the function from the Function list to the left of the view. You may have to scroll the function list to see the one of interest. As soon as you click Apply, the function will appear as shown below. 
4	Enter the time for the function (Time column), if applicable.

To insert a new function into an existing cycle or procedure: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>For functions which require MISC column entries, make the appropriate entries in the Misc Data field in the upper left corner of the Edit view.

Note The five functions listed in the following table are examples for the use of the MISC field. The numbers in parenthesis are function numbers):

<table>
<tr><th>Example Functions</th><th>MISC Column Entries</th></tr>
<tr><td>Depro Heater Wait (#169)</td><td>The Misc value for this function is the number of minutes the deprotection heater waits.</td></tr>
<tr><td>Begin Loop (273)</td><td>For this function the value is the number of times the loop will be executed. All the steps found between the Begin Loop and the End Loop will be executed this number of times.</td></tr>
<tr><td>Set Coil Temp (260)
Wait Coil Temp (261)
Start Depro Htr (170)</td><td>For these functions the value represents temperature in degrees centigrade.</td></tr>
<tr><td>Set Pres Reg 1 (301)</td><td>For this function the value represents the pressure in psi.</td></tr>
<tr><td>Pres Reg On (312)</td><td>This function is used immediately following a Set Pres Reg function to turn the pressure regulator on.

The MISC value designates which pressure regulator to turn on; i.e entering “1” for MISC for this function turns on Pres Reg 1 at the pressure entered for the immediately preceding Set Pres Reg 1 function.</td></tr>
</table>

Note Many other functions need MISC entries too — see “Special Functions” in Appendix A of the ABI 3948 Reference Manual for a full discussion of these functions. Some functions will behave differently if the MISC field is non-zero instead of zero but the actual value is not important.</td></tr>
<tr><td>6</td><td>If desired, change the Sensor designation for the step. The default designation of any new step is “No,” which designates that a liquid sensor not be used for the step.

If the step you add requires the use of a liquid sensor, click “Yes” after Sensor on the left side of the view. This will change the designation on the cycle line from “No” to “Yes,” enabling the use of a liquid sensor.

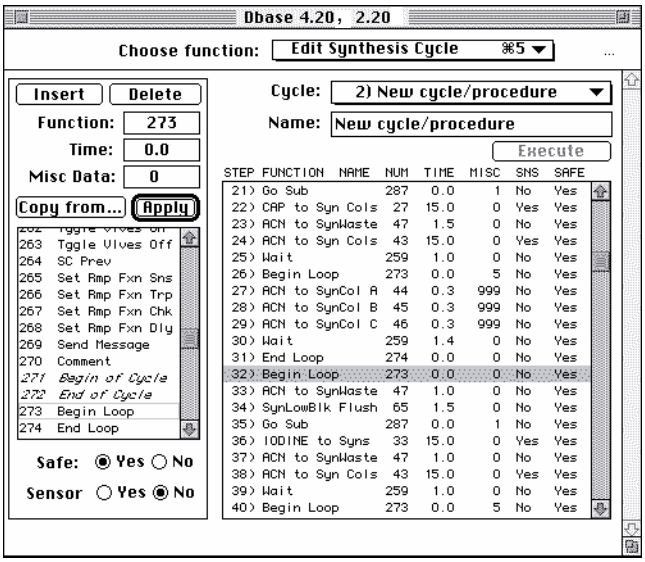
IMPORTANT In order for a “Yes” sensor designation to have any meaning for a function, an actual liquid sensor must be associated with a liquid pathway activated by a valve during execution of the function. Liquid sensor information is presented during a run in the Monitor Instrument view. If you desire to have “dry” sensor flows (such as occur during flushes and backflushes) logged in the Microphone file, enable the “Log Dry Sensor Fxns” parameter in the Instrument Preferences view.

Note “Log Dry Sensor Fxns” is normally disabled because enabling generates a large volume of such messages.</td></tr>
<tr><td>7</td><td>When you have completed your addition of new functions, choose Save from the File menu to save your changes to the instrument.</td></tr>
</table>

Procedure for Deleting Unnecessary Steps

Note This procedure may only be used with copies of the original cycles or procedures since the originals are locked.

To delete unnecessary steps:

Step	Action
1	Scroll the cycle listing down to the area of interest.
2	Select the cycle step you wish to delete or the first in a series you want to delete, as shown in Figure 6-7. If you want to delete a number of consecutive steps, hold down the shift key and drag downward to select a group of steps for deletion. **Note** Figure 6-7 shows the same step selected as just added. This would be the case if you changed your mind after adding a step.
3	Click Delete to delete the selected step or steps. 
4	Repeat steps 2 and 3 as many times as needed to add or delete steps to complete editing of the cycle.
5	When you have completed your deletions, choose Save from the File menu to save your changes to the instrument.

Saving and Retrieving Cycles, Procedures, and Functions

Backing Up the Synthesizer Setup

Use the “Save a Copy in” command to back up your instrument, since this command saves all the cycles, procedures, and functions stored in your 3948 Synthesis and Purification system to a Macintosh file in a single operation. Such a back up of the instrument should be done as part of initial instrument setup and then done periodically. Since the process is time consuming, it is best done at the end of the day or between instrument runs.

Backing up the contents of your instrument, as you would your hard disk, is recommended on a regular basis. One such backup copy should be kept and backup should be done on a periodic basis, such as once a week or once a month.

Restoring a Synthesizer Setup

At a later time, if desired, the “Send Copy to Synthesizer” command is used to restore information to the synthesizer by overwriting any new cycles, procedures, or functions entered since saving the original Synthesizer window. When a new copy is downloaded, the liquid sensors should be recalibrated.

Other uses for the Save command

Besides being used for backup, the Save command is useful if you create a lot of custom cycles and procedures, or if you have any custom functions. The command is particularly useful with custom functions since this is the only way to save such functions.

Exporting and Importing Cycles and Procedures

Although the Export commands can be used to save a single cycle or procedure and then read them back into a Synthesizer window, most users will not have to use this feature because of the many storage locations in the instrument. The synthesizer itself has room for up to:

- 20 Synthesis cycles,
- 10 Cleavage/deprotection and Purification cycles,
- 10 Begin procedures
- 10 End procedures
- 20 Bottle change procedures
- 20 Miscellaneous procedures

The Export and Import commands are useful, of course, if you find the need for storing and using more custom cycles or procedures than allowed by the synthesizer.They are also useful in sending or receiving a single cycle or procedure from a colleague using a disk, or over a network in large laboratory settings.

Synthesis Orders can be input in the form of text files with special formats. These formats are described in Appendix C, How to Create and Use Multi-Order Files.

Restoring B+Tet and Reagent Default Values

A

Default Values

Procedure for Restoration

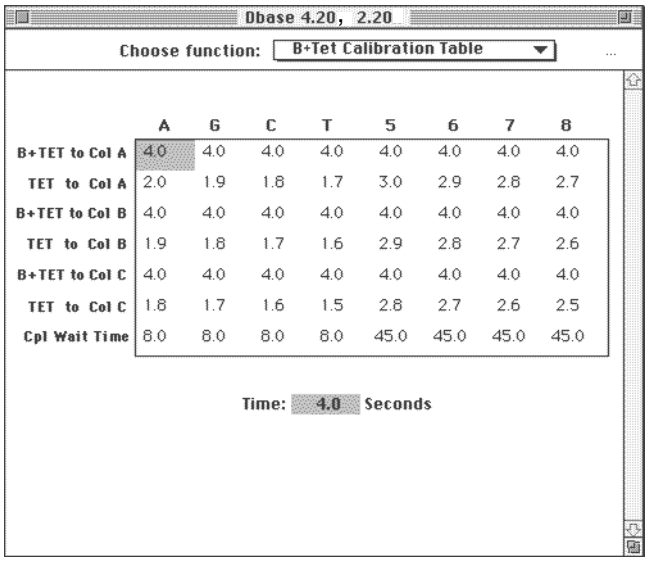
This appendix is provided to show you how to restore the default contents of the B+Tet Calibration and Reagent Utilization views should you inadvertently change or lose the contents of these views. These views can be restored in one of two ways:

- Open the off-line copy of the database and then download the database using the Send to Synthesizer command.
- Open the off-line copy of the database and then use the opened database as a reference while you manually type in appropriate values.

Note Manual entry is made by selecting a field in the table for entry and then entering the value in seconds into the Time entry field at the bottom.

B+Tet Calibration View

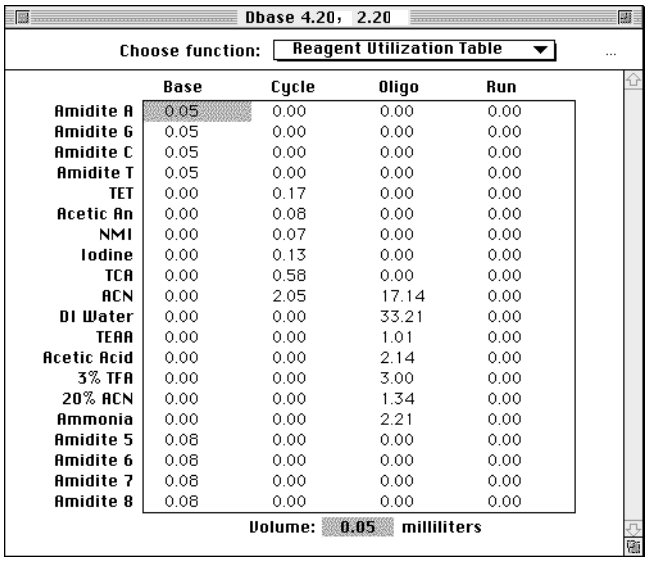
This figure contains the default values of the B+Tet Calibration view.



Reagent Utilization View

This figure contains the default values of the Reagent Utilization Table.

Note Manual entry is made by selecting a field in the table for entry and then entering the volume in milliliters into the Volume entry field at the bottom.



	Base	Cycle	Oligo	Run
Amidite A	0.05	0.00	0.00	0.00
Amidite G	0.05	0.00	0.00	0.00
Amidite C	0.05	0.00	0.00	0.00
Amidite T	0.05	0.00	0.00	0.00
TET	0.00	0.17	0.00	0.00
Acetic An	0.00	0.08	0.00	0.00
NMI	0.00	0.07	0.00	0.00
Iodine	0.00	0.13	0.00	0.00
TCA	0.00	0.58	0.00	0.00
ACN	0.00	2.05	17.14	0.00
DI Water	0.00	0.00	33.21	0.00
TEAA	0.00	0.00	1.01	0.00
Acetic Acid	0.00	0.00	2.14	0.00
3% TFA	0.00	0.00	3.00	0.00
20% ACN	0.00	0.00	1.34	0.00
Ammonia	0.00	0.00	2.21	0.00
Amidite 5	0.08	0.00	0.00	0.00
Amidite 6	0.08	0.00	0.00	0.00
Amidite 7	0.08	0.00	0.00	0.00
Amidite 8	0.08	0.00	0.00	0.00

Volume: 0.05 milliliters

Reinstalling Your Instrument

B

In This Appendix

Purpose Although your ABI™ 3948 instrument is initially service installed, information is provided in this appendix to allow you to set up the instrument again or re-establish communication between the Macintosh and the instrument should you lose it. Instructions include procedures for testing installation as well as service and troubleshooting information to aid you if you have problems later. In addition, this appendix provides you with a summary of operation once you become familiar with the instrument.

Topics Covered This appendix contains the following topics:

Topic	See page
Controller Components	B-2
Macintosh Requirements	B-2
Power PC Setup/Instrument Setup	B-3
Introduction	B-3
Set Up the Macintosh Power PC	B-3
Configuring the Macintosh	B-3
Connecting the Two Communication Cables	B-3
Installing the Software	B-4
Introduction	B-4
Installing Microphone LT Software	B-4
Installing the System Software	B-5
3948 Networking	B-6
Things to Consider 7	B-6
Testing Your Installation/Beginning a Controller Session	B-8
Initiating Communication	B-8
Printing	B-10
Printing Capabilities	B-10
Ending a Controller Session	B-10
Quitting	B-10
Scenario of 3948 Use	B-11
Procedure	B-11

Controller Components

The parts listed below are those required for the ABI™ 3948 Macintosh controller. If any parts applicable to your synthesizer system are missing or damaged, contact your local Applied Biosystems service representative before continuing.

Part	Part Number[a]
Cable, Macintosh to 392/4	201001
Cable, Macintosh PhoneNet	254248
3948 Software, v2.20 (includes two diskettes)	604530
Microphone LT application	201427

a. Table list contains only major kit parts.

Macintosh Requirements

The ABI 3948 is provided with a Macintosh Power Macintosh (4400/200) but can be used with a Macintosh PowerPC machine with a hard disk of at least 75 MB, at least 16 MB of memory, and System 7.6.1 or later software.

Power PC Setup/Instrument Setup

Introduction

The Macintosh provided with the 3948 is placed in close proximity to the instrument because it provides most of the user interface for the system, including loading information for OneStep™ columns.

Two cable connections are required between the Macintosh, functioning as a system controller, and the 3948 instrument. One cable enables the 3948 Control software on the Macintosh computer to communicate with the instrument through the AppleTalk port. The second cable enables the software to communicate with the Microphone application through the serial port.

Set Up the Macintosh Power PC

Connect and power up the computer and monitor as described in the manuals that accompany the Macintosh.

Configuring the Macintosh

Configure the Macintosh as described below.

To configure:

Step	Action
1	Select settings for the 7.6.1 operating system as follows: a. Pull down the Apple menu and choose Control Panels. b. From the Control Panels menu, choose Extensions Manager. c. At the top, under the Sets pull-down menu, find 7.6.1 and select.
2	Pull down the Apple menu, choose Memory and then do the following: a. Set the Cache size to Operating system default. b. Set the RAM Disk to OFF. c. Set Virtual Memory to the ON position.
3	Reboot the Macintosh to activate all new settings.

Connecting the Two Communication Cables

This procedure enables serial and AppleTalk communication:

Step	Action
1	Connect the serial communication cable (“Macintosh to 394,” P/N 201001) from the Macintosh modem port phone icon) to the HOST serial port (at the right rear when facing the rear) of the ABI 3948.
2	Connect the AppleTalk communication cable (P/N 254248, quantity 2) from the Macintosh printer port (printer icon) to the AppleTalk port (at the right rear of the ABI 3948). Make sure that the spare ports on the 8-pin mini DIN connectors are properly terminated.

Installing the Software

Introduction

These installation instructions assume you will be completely loading software from floppy disk when software has not been loaded before. Instructions are provided for installing both System Software and the Microphone application.

Installing Microphone Software

Microphone is a computer-to-computer communications program. Each ABI 3948 ships with a purchased copy of Microphone LT. The ABI 3948 uses Microphone for installation of image and database software and for diagnostic purposes during operation.

Install the Microphone software as follows:

Step	Action
1	Choose “Communications” from the Settings menu. Make the following assignments and click OK: MethodMicrophone Standard Modem DriverStandard Baud rate19,200 Data bits8 ParityNone Stop bitsAuto Flow ControlHardware Connector port
2	Choose “Terminal” from the Settings menu. Make the following assignments and then click OK. Delete Keydelete Cursorflashing underline Terminal typeVT102/ANSI Rows24 Columns80 Font size9 point Capture on CR“X” Strip 8thBitnot checked
3	Choose “Text Transfer” from the Settings menu. Make the following assignments and click OK: Wait for echonone End outgoing lineCR Save text asMicrosoft Word Flow control“X” While Sending “X” While Reading”
4	Choose “Save Settings.” Name the Settings, “Microphone” or Microphone ABI 3948”.

Install the Microphone software as follows: *(continued)*

Step	Action
5	Do the following: a. To activate Microphone with the correct settings, simply double-click on the file the new settings were saved as. b. To make it easy to access Microphone, make an alias of the Microphone Settings document and drag it into the Macintosh Apple Menu Items folder. Putting a space in front of the Microphone alias file name will place the alias at the top of the Apple menu, making it easy to launch. c. The Microphone settings file alias may also be placed in the Startup Items folder if you want to launch Microphone on startup.

Installing the System Software

Install system software as follows:

Step	Action
1	On the Macintosh hard drive, create a folder titled “ABI 3948.”
2	Locate the ABI 3948 System Software Disk (P/N 604530, Version 2.20). It should be located in the top drawer of the instrument.
3	Drag the software (3948 Control v2.20, 3948 Image v2.20, and 3948 Database v4.20) from the ABI 3948 System Software disk to the ABI 3948 folder on the hard drive. **Note** It may be convenient to make an alias of the ABI 3948 Control software document and drag it into the Macintosh Apple Menu Items folder. Again, putting a space before the alias file name will put the alias at the top of the menu.
4	Open the instrument software by double-clicking the 3948 Control v2.20 document.

Note Running other applications (except Microphone) while the 3948 Control program is active is not recommended.

3948 Networking

Things to Consider

Information is provided on the following in this section:

- Communications Based Upon LocalTalk
- Isolating the Instrument in a LocalTalk Zone
- Status Information and Remote Viewing

Communications Based Upon LocalTalk

Communications with the 3948 are based on LocalTalk (AppleTalk). This means that the instrument may be connected directly to a network and that the link between the 3948 and the Macintosh 3948Control software is a network link. However, various network topologies can be problematic, as are certain devices that may be placed on a network.

Two key principles should be followed to avoid networking problems with the 3948:

- The instrument should as isolated as is practical from general network traffic and
- The instrument should only communicate directly with its controlling Macintosh—all other networking activities should be managed through this Macintosh.

Isolating the Instrument in a LocalTalk Zone

To isolate the 3948 from general network traffic, place it in the same LocalTalk zone as its controlling Macintosh and avoid including too many other devices in the same zone. This LocalTalk zone should be connected to any larger network by way of a hardware bridge or router. If the larger network is based on EtherNet, the device linking the 3948 zone into the network must be able to convert between AppleTalk and EtherNet protocols.

The 3948's networking capabilities are derived from the OligoNet™ communications package developed for the Applied Biosystems 392 and 394 DNA Synthesizers. Experience has shown that at least four 3948s (with their controlling Macintoshes) may reside within a single LocalTalk zone but that placing more than sixteen 394s within one zone can be problematic. Before placing a 3948 on a network with other 3948s, the instrument should be turned off. When the instrument is then turned on it will “see” the other 3948s on the network and establish a separate unique network name for itself.

In keeping with a philosophy of isolating the 3948 from excessive network traffic, sequence orders for the instrument should be collected separately by a computer outside of the 3948's LocalTalk zone and then forwarded to the controlling Macintosh for downloading to the instrument at run start.

Status Information and Remote Viewing

The 3948 sends out status information whenever it receives a demand for such information. A controlling or viewing Macintosh will require this information at least once every two seconds. Therefore, having multiple computers connected to a single instrument can place an undesirable communications burden on an instrument that is busy performing chemistry, particularly if the network is busy and messages must be re-sent frequently.

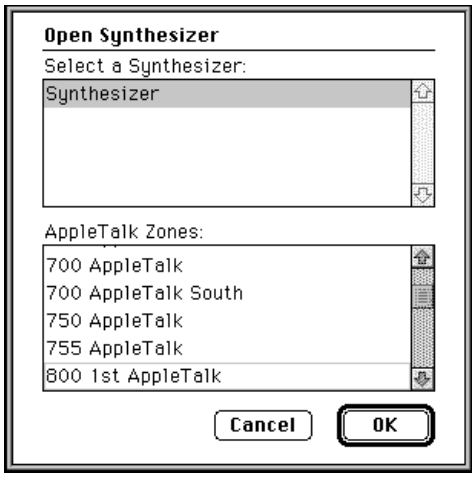
To avoid overloading the 3948 with status request messages, remote viewing of the instrument's activities are best handled by reflecting the controlling Mac's instrument view to the remote site rather than by having the remote viewer demand status directly

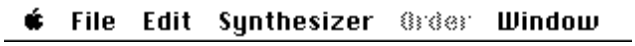
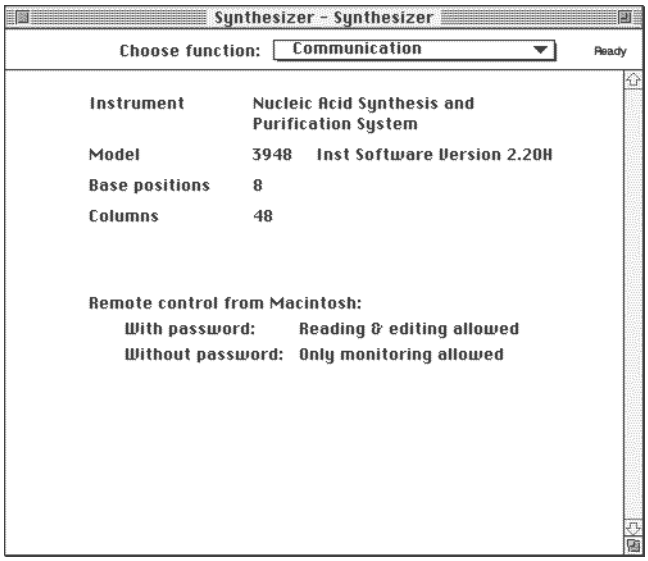
from the 3948. This applies both to viewing the instrument from off site by modem and to accessing the 3948 from another station on the network. Third-party software packages such as Timbuktu™ may be utilized for this purpose.

Testing Your Installation/Beginning a Controller Session

Initiating Communication

The first step in checking out communication between a Macintosh computer used as a controller and the ABI 3948 on the network is to start one or more synthesizers as described below.

Step	Action
1	Turn the 3948 power switch on. This switch is located on the right front of the instrument. Wait until the sample collector and the turntable stop moving before proceeding to step 2.
2	Start the Macintosh and load the 3948 Controller program by double-clicking the icon. This will briefly present a splash screen on the Macintosh monitor and then the Open Synthesizer dialog box appears.  Whenever the 3948 Controller is launched, the splash screen is presented briefly before the above dialog box opens. The Open Synthesizer dialog box contains names of synthesizers available on the network. If there are no names listed and you know your instrument is operational, see your system administrator. You can change the name of the instrument using the Change Name command (this command is chosen from the Synthesizer menu). **Note** This figure shows multiple AppleTalk zones. If only a single zone existed, the second scroll box would not exist.
3	Select the name of the synthesizer to be accessed. If more than one AppleTalk zone exists, this will require selecting the proper zone before selecting the synthesizer. **Note** The synthesizer name will already be selected if only a single instrument is present in the active AppleTalk zone.

Step	Action
4	Click OK to open communication with the synthesizer name selected, the default name assigned to the synthesizer. (If the Use Sounds command in the Edit menu is checked, some beeping will be heard.) The menu bar changes (as shown below) and the Communications View window appears. Until you open a Synthesizer window, all of the Synthesizer commands on the menu bar are grayed out.  

The presentation of the Communications View above for your synthesizer indicates that communications is established. This view shows:

- The model number of your instrument,
- The version of software,
- The number of base positions and the number of columns with which it is equipped, and
- The level of access permission set at the synthesizer.

By using the various views of this window, you can monitor your synthesizer as well as prepare all the operating information such as sequences, cycles, functions, and procedures for use on the instrument. Chapter 6 of the *ABI 3948 Reference Manual* provides you with a detailed description of how to use this window. More general information on using the 3948Control program is provided in the User's Guide in Chapter 2, "Setting Up/Initiating a Run/Post Run."

If for any reason you have failed to establish communication between the 3948 Control program and your instrument, you may need to contact your system administrator.

Printing

Printing Capabilities

The 3948Control provides you with the capability to print out the contents of three types of windows:

- Synthesis Orders (discussed in Appendix C, Creating and Using Multi-Order Files)
- Synthesizer windows (see Chapter 5 of the *ABI 3948 Reference Manual*, Communication View and Operational Views)
- Run files on any Macintosh-compatible printer connected into the network in which the Macintosh computer participates

Note When the 3948 Controller is used to open a Run File, a second type of file (a Multi-Order file) is created for use in printing labels. This feature is described in two places, “Printing Labels for Oligonucleotides” on page 2-44 and “Sample Labeling Feature” on page 4-33 of the *ABI 3948 Reference Manual*.

You can print the following Synthesizer window views:

- Edit Sequence view
- Edit Cycle views (Synthesis, Cleavage, Purification)
- Edit views for the Begin, End, Bottle, Shutdown procedures
- Edit Function view

Ending a Controller Session

Quitting

Use this procedure to end a 3948Control session:

Step	Action
1	End a session with the 3948Control application by choosing Quit from the File menu. An open and unmodified Synthesizer window will close as the application quits.
2	When you have made changes to an open window, either a Synthesizer window or a Synthesis Order, you will be prompted to save your changes. Follow the prompts to save or discard your changes.

Scenario of 3948 Use

Procedure This procedure outlines the main activities needed for use of the 3948Control software with your instrument. More detailed procedures are presented in Chapter 2, “Initiating a Run” and Chapter 3, “Monitoring a Run”.

Step	Action
1	Start the 3948 Controller session.
2	If desired sequence is not already stored in the synthesizer, prepare sequence information in one of two ways: Prepare/Open a separate Synthesis Order for each sequence to be synthesized containing the protocol needed for synthesis. Write/identify Synthesis Orders needed for the next run. Choose the Protocol to be used. Choose the Purify option to be used.
3	On the Run Set Up view, assign the Synthesis Orders, the protocols, Begin and End procedures, and set up the run order to be used for a synthesis. *Optional*: write/import custom cycles, begin and end procedures needed for synthesis.
4	Set up instrument with reagents, collection tubes in sample collector, etc.
5	Load the required OneStep columns
6	Initiate syntheses by clicking Start on the Run Setup view. The 3948 Controller program initiates the process of synthesis by rotating the turntable to the correct position and then downloading the required sequence, protocol, and OneStep™ column position information to the Synthesizer. As soon as downloading is complete, the Synthesizer window view changes to the Monitor Chemistry view and the run is initiated.
7	Monitor progress of synthesis using Monitor Chemistry view on the Macintosh.

Creating and Using Multi-Order Files

C

In This Appendix

Topics Covered This appendix contains the following topics:

General Information on Multi-Orders	C-2
Introduction	C-2
Programs used to Create Multi-Order Files	C-2
Two Multi-Order Formats	C-2
Short Multi-Order Format	C-3
Introduction	C-3
Short Format Syntax	C-3
Using the Long Multi-Order Format	C-4
Introduction	C-4
Rules	C-4
Character Limits	C-5
Examples	C-5
Generating Multiple Synthesis Orders	C-8
Procedure	C-8
Creating Synthesis Order Files Directly	C-9
Differences Between Short and Long Text Files	C-9

General Information on Multi-Orders

Introduction

A Multi-order File is a text file that contains the order information for more than one oligo. The purpose of the Multi-order file is to facilitate the inclusion of the ABI 3948 instrument into the oligo order processing stream. Orders placed into a Multi-order file may come from any source, including a database or spreadsheet being used to track oligo order entry and billing. Oligo order information may be entered into a Multi-order file in any of these ways:

- Manually
- Copied and pasted from electronic order sources (e-mail, web page, etc.)
- Transferred from an order database using the database's programming capabilities

In a well-automated system, order information might be entered only once. All oligo information could be entered by the customer with the order tracking and billing information to be added on receipt of the order. The order flow would then follow this path:

Oligo Order Entry/Billing System -> Multi-order File -> Synthesizer Orders -> 3948 -> RunFile -> Oligo Order Entry/Billing System

Programs used to Create Multi-Order Files

A Multi-order File is a simple ASCII text file, like a 3948 RunFile. It may be created using SimpleText or a more full-featured word processing program such as Word, Ami Pro or WordPerfect. If such a word processor is used, the file must be saved as “text only” so as not to include any proprietary formatting information that might otherwise be embedded with the text.

Two Multi-Order Formats

There are two formats for Multi-order files, a Short format and a Long format. Which format you use depends upon how much order entry information you want to have transferred to the RunFile or for labeling purposes. The choice of format may also depend on how readily the order system may be programmed to automate the use of one format or another. If a great deal of programming capability is present, it is possible to skip the Multi-order file entirely and to generate Synthesis Order files directly from an oligo order database. This option is discussed at the end of this section.

Short Multi-Order Format

Introduction

Short format Multi-order text files may be given any Macintosh file name. The first word in the file, must be “SYNTHMOSFORMAT” (SYNTHesis Multiple Order Short FORMAT) and it must appear in uppercase letters. The oligo name must always precede the sequence but there is no need for spacing between orders (spacing is only used in the example to make it easier to read).

The Short format Multi-order file is the simplest file to create automatically using database programming tools. The key disadvantage to using this format is that no customer information can be passed through the Sequence Order Files to the RunFile.

The only information that is used with the Short format is the name of an oligo and its associated sequence. All oligo orders created from a Short format file will be set to the same Purify Option as selected by the radio buttons in the Make Order file. The example in the next section shows both the simplicity and the limitations of the Short Multi-order format.

Short Format Syntax

The format of the Short format Multi-order text file is listed below.

Note The name SYNTHMOSFORMAT is not the title of the document or file header but an essential name which must appear before any other text to enable correct syntax checking by the 3948Control application. The bold typeface is used only to emphasize this name and is not required in an actual file.

SYNTHMOSFORMAT

User class 33mer-7
TGACCATTAGATCAAGCTTG

User class 33mer-8
A GCT TGT ATC TTT CTC

User class 33mer-9
TGACCATTAGATCAAG

User class 33mer-10
TGT ATC TTT CTC AGG

User class 33mer-11
TGA CCA TTA GAT CA

User class 33mer-12
TGA CCA TTA GAT CAA GCT

Using the Long Multi-Order Format

Introduction The advantages that come with using the Long Multi-order format include being able to include customer information in the oligo orders that will appear in the RunFile as well as having the ability to specify multiple trityl/purification options within one file. It will be harder to program the automatic creation of a Long format file by an oligo order database than a Short format file.

When the Long Multi-order format is used, information entered into any field of one oligo order is retained and included in subsequent orders. This helps to minimize the amount of manual entry required when a single customer orders several oligos at one time. The only exceptions to this are the SEQ_NAME and SEQ_TEXT fields. These two fields must be entered separately for every oligonucleotide in the file.

Each time a SEQ_TEXT field is encountered by the Multi-order file parser, a new Sequence Order file is generated and the information in the SEQ_NAME and SEQ_TEXT fields is cleared. Unless new information is entered, all other fields will appear unchanged when the next Sequence Order file is created.

Rules The following rules must be followed carefully to avoid introducing errors into the order files:

- A Multi-order file document may be given any Macintosh file name.
- The tag “SYNTHMOLFORMAT” must appear at the top of the document (SYNTHesis Multiple Order Long FORMAT).
- The tags must be in upper case. Tags need not be in BOLD type. Any mix of upper- or lowercase characters may be used in the entry fields.
- Any tags which are used in the oligo order *must* be followed with a tab and then the pertinent information. If an information field is left empty after a tag, an error will occur and it is possible that incorrect Sequence Order Files will be produced.
- A sequence entered after the SEQ_TEXT tags is not required to have the bases grouped into threes (codons). Besides A, G, C, T, 5, 6, 7, and 8, only the single-character redundancy codes listed in the 3948 Sequence Order window may be used.
- The only fields required for the Multi-order long format to work are the SEQ_NAME and SEQ_TEXT. All other information can appear in any order, but it must appear before the SEQ_NAME and SEQ_TEXT.
- Each SEQ_NAME in the file must be distinct from the others.
- The SEQ_TEXT field must be the last field for the oligo order.
- A field entry may not exceed the maximum character limit of the field. The character limits for each field are as shown below.

Character Limits

The character limits listed below apply to each of the fields in this type of Multi-order text file.

SYNTHESIZER	none This tag was used in OligoNet™ orders but is not needed in any 3948 order. If it is used, the only correct entry is the word “none” as shown here.
ENTRY_DATE	32 characters
CUSTOMER	48 characters
CUSTOMER1	48 characters
ADDRESS1	48 characters
ADDRESS2	48 characters
ADDRESS3	48 characters
PHONE	24 characters
FAX	24 characters
POREF	16 characters
ACCNT	16 characters
COMMENTS	255 characters Note: only 49 characters of the COMMENTS field will appear in the RunFile.
PURIFYOPTION	Purifyoligo, Crudedmton, or Crudedmtoff Enter one choice. Capitalization is optional.
SEQ_NAME	30 characters
SEQ_TEXT	150 bases

Examples

Example 1 illustrates the use of a Long Multi-order file to specify several orders for a single customer. As shown by the fourth oligo ordered, the Purify option may be changed as needed by including the tag specifying the change somewhere in the order. If the Purify option is not set in the initial order, it defaults to the value selected by the radio buttons in the Multi Order file window.

Example 1

Note The name SYNTHMOLFORMAT is not the title of the document or file header but an essential name which must appear before any other text to enable correct syntax checking by the 3948Control application. The bold typeface is used in the example only to indicate the essential first line and the names of the fields.

SYNTHMOLFORMAT	
CUSTOMER	John Smith
CUSTOMER1	University of Foster City
SYNTHESIZER	None
ADDRESS1	123 Delmonico Ct.
ADDRESS2	Foster City, CA 95008
ADDRESS3	USA
PHONE	650-xxx-xxxx
FAX	650-xxx-xxxx
POREF	111111
ACCNT	222222
ENTRY_DATE	7/12/97
COMMENTS	Priority 1 oligo
SEQ_NAME	Oligo-10
SEQ_TEXT	ACT TCG GCG ATC

SEQ_NAME Oligo-11
SEQ_TEXT ACT TCG GCG ATC

SEQ_NAME Oligo-12
SEQ_TEXT ACT TCG GCG ATC

PURIFYOPTION Crudedmton
SEQ_NAME Oligo-13
SEQ_TEXT ACT TCG GCG ATC

The Multi-order format can be used for several customers as in the example that follows involving customers A, B, and C. Follow the same rules in example 1. When a new customer's oligo orders are entered, also update the customer information as shown in Example 2.

Example 2

Note The name SYNTHMOLFORMAT is not the file header but an essential name which must appear before any other text to enable correct syntax checking by the 3948Control application. The bold typeface is used in the example only to indicate the essential first line and the names of the fields.

SYNTHMOLFORMAT
CUSTOMER Customer A
CUSTOMER1 University of Foster City
SYNTHESIZER None
ADDRESS1 123 Delmonico Ct.
ADDRESS2 Foster City, CA 95008
ADDRESS3 USA
PHONE 650-xxx-xxxx
FAX 650-xxx-xxxx
POREF 111111
ACCNT 222222
ENTRY_DATE 7/12/97
COMMENTS Priority 1 oligo
PURIFYOPTION Crudedmton
SEQ_NAME Oligo-10
SEQ_TEXT ACT TCG GCG ATC

SEQ_NAME Oligo-11
SEQ_TEXT ACT TCG GCG ATC

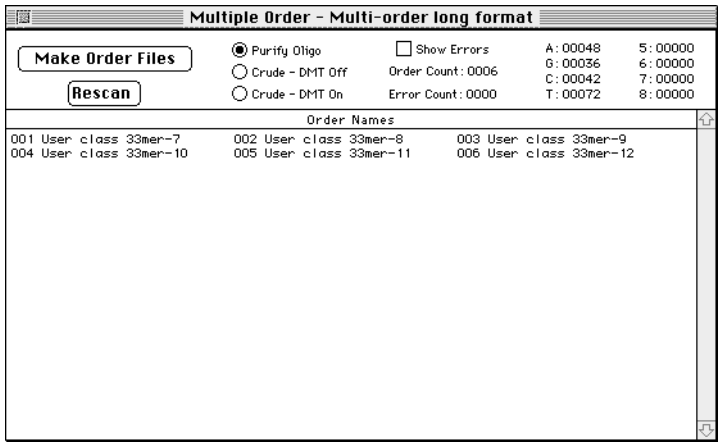
SEQ_NAME Oligo-12
SEQ_TEXT ACT TCG GCG ATC

CUSTOMER Customer B
CUSTOMER1 University of Some City
SYNTHESIZER None
ADDRESS1 4536 Twenty Second Ave.
ADDRESS2 Some City, CA 95XXX
ADDRESS3 USA
PHONE 650-xxx-xxxx
FAX 650-xxx-xxxx
POREF 123123

ACCNT	234234
ENTRY_DATE	7/12/97
COMMENTS	Priority 2 oligo
PURIFYOPTION	Purifyoligo
SEQ_NAME	Oligo-13
SEQ_TEXT	ACT TCG GCG ATC
SEQ_NAME	Oligo-14
SEQ_TEXT	ACT TCG GCG ATC
SEQ_NAME	Oligo-15
SEQ_TEXT	ACT TCG GCG ATC
CUSTOMER	Customer C
CUSTOMER1	University of Foster City
SYNTHESIZER	None
ADDRESS1	123 Delmonico Ct.
ADDRESS2	Foster City, CA 95008
ADDRESS3	USA
PHONE	650-xxx-xxxx
FAX	650-xxx-xxxx
POREF	5678
ACCNT	98765
ENTRY_DATE	7/12/97
COMMENTS	Priority 2 oligo
PURIFYOPTION	Crudedmtoff
SEQ_NAME	Oligo-16
SEQ_TEXT	ACT TCG GCG ATC
SEQ_NAME	Oligo-17
SEQ_TEXT	ACT TCG GCG ATC
SEQ_NAME	Oligo-18
SEQ_TEXT	ACT TCG GCG ATC

Generating Multiple Synthesis Orders

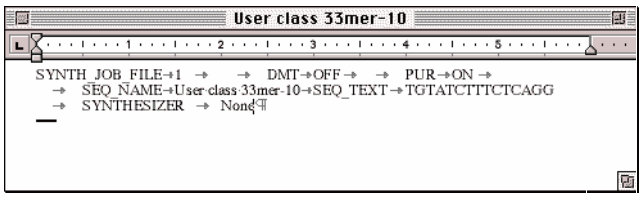
Procedure Once a Multi-order text file is created, in either format, save and close the file and then proceed as follows to create multiple Synthesis Orders:

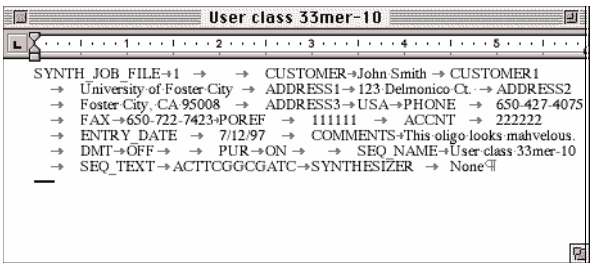
Step	Action
1	Launch the 3948 Control application.
2	Select Open from the File menu and open the Multi-order file you created. The Multiple Order window will appear as shown below:  **Note** There may be a zero error count showing but not all errors will have been checked for at this point.
3	Always click the Show Errors checkbox and then click Rescan to verify there are no hidden errors. (If there are any errors, re-edit your Multi-order file to correct them and repeat this process.) **Note** Errors can be produced by entering too many characters into fields for a Synthesis Order. Address error messages stating that a field is too long by editing the source text file and then producing another Multiple Order Long Format file. Be careful to make an entry in each required field because this will cause the next and all following fields to have incorrect entries.
4	Click Make Order Files. Following a process similar to that for RunFldrs and RunFiles, the Synthesis Order Files created will be put in a date- and time-stamped folder where the 3948Control application resides. The folder will be named “OrdrFldr[date&time]”.

Creating Synthesis Order Files Directly

Differences Between Short and Long Text Files

Like a Multi-Order File, a Sequence Order File is a simple ASCII text file. Two examples of Sequence Order Files created from Multi-Order Files are shown here, the first from a Short Format file and the second from a Long Format file:





Both of the above examples demonstrate that a Sequence Order File is a simple text file with identifying tags preceding fields that contain order information. Every tag and field is separated from its neighbors by a <tab>.

The Short format example shows the minimum amount of information that must be included in any Sequence Order File and the order in which the information must be presented for the 3948 to correctly produce the desired oligo. The Long format example shows the use and positioning order of every possible tag that might be included in a Sequence Order File. Beyond the minimum required set of tags, only those tags that are desired need to be included in a Sequence Order File. The tags that are chosen for use must appear in the order shown.

The process of automating the direct creation Sequence Order Files will be about as difficult as implementing the automatic creation of Long format Multi-Order files. Some difficulty is added by the need to handle the possibility of duplicated order file names.

The advantage of not using the Multi-Order File is that order files may consistently be created in the same order folder so that the 3948 operator always knows exactly where to locate the day's orders and there is no need to do housekeeping on a proliferation of 3948-generated “OrdrFldrs”.

Index

Numerics

3948
  new features 1-9
3948 functions
  overview 6-4
  three fundamental types 6-4
3948 Networking B-6

A

ABI 3948
  Method of control 1-12
Abort
  defined 1-10
  definition 1-10
Abort command
  one way to stop a run 3-10
Acetonitrile
  volumes added to phosphoramidites 4-23
acetonitrile bottle
  installing 4-20
Amidites
  pre-run check 2-8
application support
  Applied Biosystems 1-12
argon
  pressure 4-3
Argon tank pressure
  pre-run check 2-8
Assigning sequences
  for the Run 2-22
  procedure for assignment to the Run Setup view 2-20
Auto-Resume feature
  general 2-11
Auto-Resume Variables
  Auto-res OK 0.01 PSI 2-14
  Auto-resume Minutes 2-14
Auto-resuming
  theory of operation 2-17
  ties to jaw/block pressure testing 2-17
AutoSort
  procedure 2-24
Autosorting sequences
  rules for ordering 2-26

B

B+Tet and reagent default values
  restoring A-1
B+Tet Calibration view
  default values A-1
Backing up the instrument 6-20
Begin and End Procedures
  defined 1-10
Begin and End procedures
  definition 1-10
bottle consumption indication 2-28
bottle installation
  check for proper seal 2-8
  lower positions 4-20
  upper position 4-19
Bottle seals
  pre-run check 2-8
bottle seals
  replace with each bottle change 5-3

C

calibration of fluid sensors/verification of calibration 4-22
Changing an existing cycle or procedure
  procedure for changing values 6-15
  procedure for deleting unnecessary steps 6-19
  procedure for inserting new functions 6-16
Check Illegal Valves parameter 2-15
Checks before each run 2-8
chemical delivery system
  pressure driven 1-12
Chemistry monitor
  middle pane 3-3
  upper three panes 3-3
clogs
  can be caused by improperly shutting down instrument 5-5
Communication cables
  connecting B-3
Communications
  based upon LocalTalk B-6
Communications view, information displayed B-9
controller
  also fills out Synthesis Orders 1-12
  real time control 1-12
Controller components
  list of B-2
Controller session
  Beginning a B-8
  ending B-10
Creating a new cycle or procedure
  first step is to choose existing type 6-11
  procedure 6-12
Critical Message Waiting 1-10
Customer support
  information 1-5
cycle editing
  likely types of changes 6-15
Cycles and Procedures
  guidelines for modifying 6-10
Cycles, procedures, and functions
  saving and retrieving 6-20
Cycles, procedures, and protocols
  overview 6-3

D

Deprotect Mins (Inst. Pref.)
  relationship with deprotection heater time-out and Depro Htr Wait step 2-11
Deprotect Mins variable 2-13
Deprotect Temp variable 2-13
Deprotect Variables
  Deprotect Temp and Deprotect Mins 2-13
deprotection
  using a single deprotection time 2-11
  using multiple deprotection times 2-11

E

Edit Cycle or Procedure views
  description of views 6-11
Empty 6-11
enabling sensors
  procedure during cycle editing 6-18
End Row on Jaw Leak parameter 2-15
Ending a Controller Session B-10
ending rows
  information on using End Row SCP/123 function 2-19
  using Pause On Jaw Leak checkbox 2-19
EPR and Kalrez gaskets
  replacing 5-3

Exporting and importing
cycles and procedures 6-20
Extending a Run
procedure 2-35
Extinction Coefficient Variables
Ext. Coefficient 5/6/7/8 2-14

F

fluid sensors
calibrate and verify before run if necessary 2-8
Function names
generally describe actions they perform 6-4
Functions
creating your own 6-7
Time and Miscellaneous field entries 6-4
Functions with MISC column entries
examples 6-18

G

gasket replacement procedure 5-2
General 4-5

I

initiating a run
list of tasks 2-1
Initiating Communication
procedure B-8
initiating run
procedure 2-4
Inlet Filters
2-L and 4-L 5-3
installing reagent bottles
precautions to observe 4-17
Installing software B-4
instrument
new features 1-9
instrument control
real time 1-12
instrument control buttons
identified 2-31
Instrument Dip Switches
description 2-15
Instrument Dip Switches (Inst. Pref.)
purpose 2-12
Instrument features
overview 1-11
Instrument functions
overview 6-4
Instrument maintenance
scheduling 5-2
Instrument messages
list of status messages 1-9
Instrument Preferences
deprotection time interactions 2-11
general information 2-11
illustration of default view values 2-12
types of settings 2-12
Instrument setup B-3
Instrument use
procedure B-11
Interrupt
definition 1-10
one way to stop a run 3-9
Isolating the instrument
in a LocalTalk zone B-6

J

Jaw Close functions
may be used to close jaws or invoke pressure testing 5-4
Jaw leak testing
using jaw close functions 5-4
Jaw Leak Variables
Jaw Leak Test 2-14
Leak OK 0.01 PSI 2-14
Jaw/Block pressure test
procedure for testing 5-4

K

Key terms
definition 1-10
key terms
table of 1-10

L

Liquid Sensors status
in Monitor Instrument view 3-6
locations of reagent bottles 4-19
Log Dry Sensor Fxns parameter 2-15
Long Multi-Order format
character limits C-5
Example 1 C-5
Example 2 C-6
rules C-4
lower position bottles
sizes 4-20

M

Macintosh
configuring B-3
Macintosh Power PC
setting up the B-3
Macintosh requirements
for system B-2
maintenance
schedule of 5-2
Maintenance items
needed parts and replacement schedule 5-2
Man Cont Jaw Testing
parameter 2-15
manual
list of chapter contents 1-3
Manual Control
jaw leak testing 5-4
one way to execute functions 6-7
manual set
how to use 1-1
Manufacturing Test Mode
parameter 2-15
Message Waiting 1-11
Method of synthesis
Phosphoramidite 1-12
Microphone installation B-4
Mixed Bases
special considerations 2-10
modifying cycles and procedures
guidelines 6-10
Monitor Chemistry view
description of parts of view 3-2
purpose of 3-2
using information 3-4
Monitor Instrument view
detailed description 3-5, 3-6
other parameters and instrument conditions 3-7
parts of the view 3-5
types of information presented 3-5
Monitor Run view
types of information provided 3-8
Monitor Synthesis view
detailed description 3-2
monitoring chemistry 3-4
Multi-Order files
general information C-2
programs used to create C-2
short format C-3
two formats C-2
Multiple Synthesis Orders
generating C-8

N

Networking
things to consider B-6
New Cycles and Procedures 6-10
types of 6-10
types which may be created 6-10
New Synthesis Order command 4-8
Next or Prev buttons
used to move turntable during loading 2-31
No Flow to Open Jaws
parameter 2-15

O

oligonucleotide production
description of process 1-12
OneStep Columns
checking before a run 2-27

OneStep columns
procedure for loading 2-30
Ordering sequences
for a run 2-24
O-rings, gaskets, and seals
replacement procedure 5-2

P

passwords
types of 2-7
Pause After
defined 1-10
definition 1-10
one way to stop a run 3-9
use during a run 2-35
Pause On Jaw Leak checkbox, use of 2-19
Pause On Jaw Leak parameter 2-15
Pause On Sensor Fail parameter 2-15
phosphoramidite
method of synthesis 1-12
phosphoramidite bottles
levels of freshly autodiluted bottles 2-28
preparing 4-14
removing 4-14
Phosphoramidite preparation
things to consider 4-23
Phosphoramidites
procedure for manually dissolving and installing 4-24
phosphoramidites 2-8
preparing and installing 4-14
Post-Run
procedure 2-40
Post-Run tasks
types of tasks 2-40
Power PC setup B-3
Pre-Run
checks and considerations 2-8
pre-run check 2-8
Pre-Run procedures 4-5
pre-run procedures, list of
set up sample collector 4-7
Pres Reg On function
use of MISC value 6-18
pressure regulator information 3-6
Pressure Regulator/Pressure Status
in Monitor Instrument view 3-6
Pre-Synthesis
list of tasks 4-3
Printing
capabilities B-10
printing B-10
Printing labels
for oligonucleotides 2-44
Procedures
Creating your own 6-7
Protocol
defined 1-10
definition 1-10
protocol
subcontents of 6-3

R

rack
illustration of two types 2-9
reaction column chamber
OneStep column 1-12
reagent bottles
quantities when full 2-28
Reagent consumption
basis of volume calculation 2-28
Reagent levels
pre-run check 2-9
Reagent Utilization view
default values A-2
Reagents
checking for sufficient reagents 2-27
when to renew 2-29
reagents
locations on the instrument 4-19
Reference manual
Part number 1-1
types of information 1-4
Reinstalling the instrument B-1
Removing a priority oligonucleotide
during a run 2-39
removing tray and printing labels
procedure 2-4
Replacing
2-L and 4-L Inlet Filters 5-3
EPR and Kalrez gaskets 5-3
gaskets 5-2
O-rings 5-2
seals 5-2
Replacing a Reagent Bottle
during a run 2-39
Reserved parameter 2-16
Restoring the instrument 6-20
Run extension
procedure 2-35
Run Setup
procedure for performing 2-3
Run Setup Order table
rules for autosorting 2-26
Run Setup scroll box
removing a sequence 2-23
RunFile
accessing the contents 2-42
information in the file 2-42
prompt to generate 2-10

S

sample collector
home before a run 4-3
pre-run check for sufficient tubes 2-9, 4-3
Scenario of instrument use B-11
Seal replacement procedure 5-2
Selecting chemistry
for a run 2-24
sending cycles to instrument 6-14
sensor calibration procedure 4-22
Sensor-Controlled functions
components of function names 6-7
description 6-7
locations of sensors 6-7
use of parallel with serial functions 6-7
Sensors
how functions use 6-5
locations 6-5
retries 6-5
use of extra sensors 6-5
Sequence information
types of information displayed 2-23
Sequence processing
order of 2-26
service information B-11
Setup Choices (Inst. Pref.) 2-15
purpose 2-12
Setup Variables (Inst. Pref.)
description of variables 2-13
purpose 2-12
Short Multi-Order format syntax C-3
Shutting Down and Restoring the Instrument
procedure 5-5
Site preparation and safety 1-4
Software installation B-4
Sorting sequences
manually 2-25
procedure for autosorting 2-24
Special Logical functions
behavior goes beyond names 6-4
Standard protocols
contents of 6-3
three 6-3
status and system messages 3-4
Status information
viewing remotely B-6
Stopping a Run
three ways to stop 3-9
Synthesis Order files
creating directly C-9
Synthesis Orders
organizing and processing 4-10
pre-run check 2-8
reviewing information 2-3
Synthesis run
procedure for starting and monitoring 2-33

Synthesis support 1-12
Synthesizer Window
opening 2-5
System and User functions 6-4
System functions
three fundamental types 6-4
System information
where to find 1-3
System Software
installing B-5

T

Technical support
E-Mail 1-5
regional offices 1-6
telephone support hours 1-5
Telephone technical support
fax-on-demand 1-8
numbers for various products 1-5
testing installation B-8
Think-Jet printer B-10
Transfer Variables
Coil Cool Secs 2-13
Xfer From Coil 2-13
Xfer Into Coil 2-13
turntable position display
information provided for
loading 2-30
types of runs
pre-programmed or custom 1-12

U

upper position reagents
list and sizes 4-19
Use 4 x 12 Tube Rack
parameter 2-15
User bulletins
contents and purpose 1-4
User functions
sensor-controlled 6-7
two types 6-4
UV grade Acetonitrile
strongly recommended 4-20

V

Valve functions
types of 6-6
uses of 6-6
Valve Status
in Monitor Instrument view 3-6

W

waste
checking level of 2-9, 4-3
disposal of 2-9, 4-3
web site address 1-8

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