Biomek® FX and FX^{P} Laboratory Automation Workstations

Version 3.3 Tutorial

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Beckman Coulter, Inc
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c. Any product claimed to be defective must, if required by the Company, be returned to the factory, transportation charges prepaid, and will be returned to Purchaser with transportation charges collect unless the product is found to be defective, in which case the product must be properly decontaminated of any chemical, biological, or radioactive hazardous material.

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Parts replaced during the warranty period are warranted to the end of the instrument warranty.

Note: Performance characteristics and specifications are only warranted when Beckman Coulter replacement parts are used.

Safety Information

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle. Please pay special attention to the specific safety information associated with these symbols.

WARNING: If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the equipment may be impaired.

Warning and Caution Definitions

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle.

The exclamation point symbol is an international symbol which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing is attempted.

WARNING: A WARNING calls attention to a condition or possible situation that could cause injury to the operator.

CAUTION: A CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator's work.

When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a Beckman Coulter Service Engineer for any servicing of equipment requiring the removal of covers or panels.

High Voltage

This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) wall outlets. Use only multiplug power strips provided by the manufacturer.

Disposal of Electronic Equipment

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- the device was put on the European Market after August 13, 2005.
- the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Chemical and Biological Safety

Normal operation of the Biomek FX may involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility's waste disposal procedures.
- Operate the Biomek FX in accordance with the instructions outlined in this manual, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

Note: Observe all warnings and cautions listed for any external devices attached or used during operation of the Biomek FX. Refer to applicable external device user's manuals for operating procedures of that device.

Moving Parts

To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the Biomek FX.
- Keep the Biomek FX work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user's manual for the Biomek FX. Prior to cleaning equipment that has been exposed to hazardous material:

- Appropriate Chemical and Biological Safety personnel should be contacted.
- The Chemical and Biological Safety information contained in this user's manual should be reviewed.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

Important

It is your responsibility to decontaminate components of the Biomek FX before requesting service by a Beckman Coulter Service Engineer or returning parts to Beckman Coulter for repair. Beckman Coulter will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

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1 Multichannel Pod—Getting Started With Biomek® Software

1.1 Introducing Biomek Software

Note: Unless otherwise noted, all information in this tutorial refers to both the Biomek® FX and FX^P instruments.

Welcome to Biomek Software and the Biomek FX Laboratory Automation Workstation.

Biomek Software controls the Multichannel Pod on your Biomek FX Laboratory Automation Workstation and is designed to:

- do a substantial amount of method building work for you.
- allow you to take as much direct and precise control over the method-building process as you want.

The flexibility that results from this combination gives the Biomek FX Laboratory Automation Workstation its power.

1.1.1 Using this Tutorial

Tip

For effective learning, print this tutorial before use, leaving your computer screen free for viewing Biomek Software.

This tutorial is designed to help you become comfortable using Biomek Software (version 3.3) with your Multichannel Pod on the Biomek FX. The chapters in this tutorial can be completed consecutively or, depending on the learning required, may be completed in any order. This format will allow advanced users to complete only the chapters that include the topics they need to learn. Generally, the topics in subsequent chapters increase in complexity. Chapters for the Span-8 Pod on the Biomek FX are also available.

The chapters in this tutorial for the Multichannel Pod can be used with the following configurations:

- Single Multichannel Pod.
- Dual Multichannel Pods.
- Hybrid system with a Multichannel Pod and a Span-8 Pod.

In addition to the step-by-step instructions in this tutorial, you will also see boxes containing useful information in the following forms:

BIOMEK CONCEPT

These boxes contain information to help you understand important features and capabilities of Biomek Software or the Biomek FX Laboratory Automation Workstation. While the step-by-step instructions may be completed without reading the information in these boxes, the information will enhance your knowledge and give you a fuller picture of what Biomek Software and your instrument can do.

Tip

The information in these boxes offer suggestions on how to use your instrument and software to enhance the activities you want to do in your laboratory.

1.1.2 What You'll Learn in Getting Started with Biomek® Software

In this chapter, you will learn:

- How to launch Biomek Software and what the method-building process looks like
- How to set up the deck for a liquid transfer
- How to build a liquid-transfer method
- How to run a method
- How to save and check in a method

1.1.3 Launching Biomek Software

From the Start menu, select **All Programs>Beckman Coulter>Biomek Software**.

BIOMEK CONCEPT Accounts & Permissions

Beckman Coulter Accounts & Permissions is an integrated set of features built into Biomek Software that assists users in complying with 21 CFR Part 11 requirements for closed systems. Permissions provide the ability to control user access to specific program operations. Refer to the *Biomek Software User's Manual,* Chapter 2, *Using Accounts & Permissions*.

If Beckman Coulter Accounts & Permissions is enabled on your system, you must have an account established and log in using that account name and password in order to fully complete this tutorial. For more information, contact your system administrator.

1.1.3.1 Viewing the Main Editor

The main editor (Figure 1-1) is your starting point for building liquid-handling methods for the Biomek FX Laboratory Automation Workstation. You will choose method steps from a step palette and place them into the Method View in a linear fashion. The configuration for each of these steps appears in the Configuration View.

CAUTION: Do not change the pod settings in Hardware Setup after the system has been installed; changing the pod settings will necessitate another service call.

If using an instrument with a Multichannel Pod and a Span-8 Pod, the Multichannel Pod should be set as the default pod when completing this tutorial. To do this:

Select the default pod by choosing it from the pod icon on the toolbar (Figure 1-1).

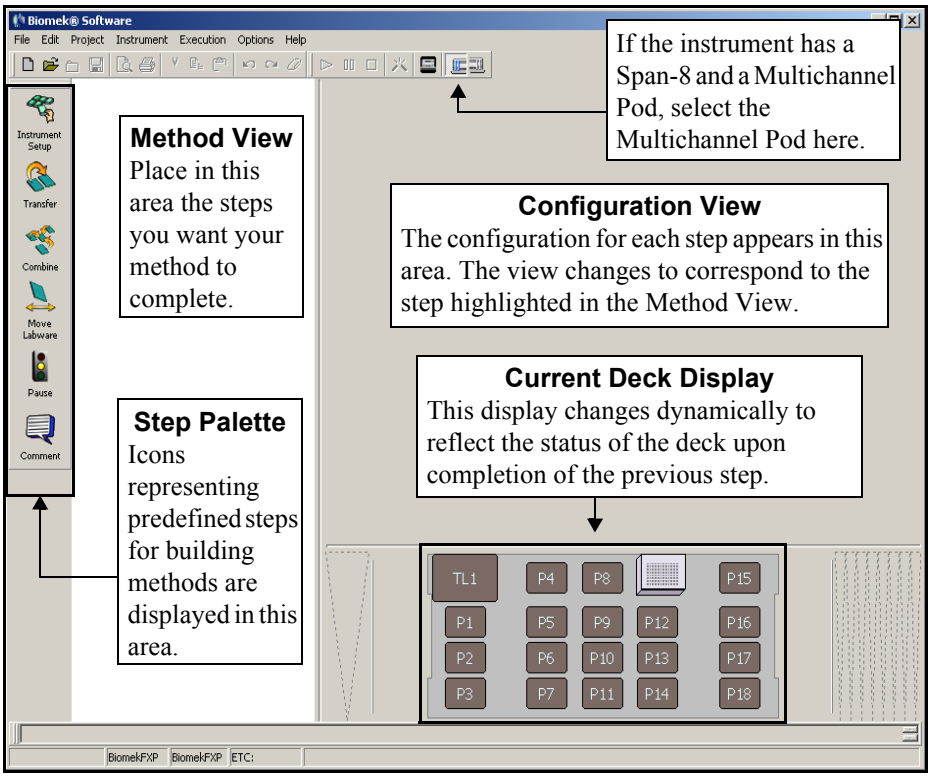

Figure 1-1. Biomek main editor

1.2 Beginning a Method

To begin a method, you have the option of creating a new method or opening an existing method you've completed, named, and saved. In this tutorial, you'll create a new method.

But before you create a new method, get into the habit of ensuring you are using the correct project file.

1.2.1 Introducing Project Files

BIOMEK CONCEPT
Project File

A project file stores information about liquid types; labware and tip types; well patterns; and pipetting templates and techniques as revisions that are used by a method file to configure the actions of the instrument. Project files store a history of all changes, additions, and deletions of items from the project file. Methods are associated with projects and contain all of the items required to perform the method.

While project files may be created, revised, deleted, saved, imported, and exported, in this tutorial you will use the project file on your system that was created or imported when your instrument and Biomek Software were installed.

View Figure 1-2 to learn where project file information is accessed or viewed from the main editor.

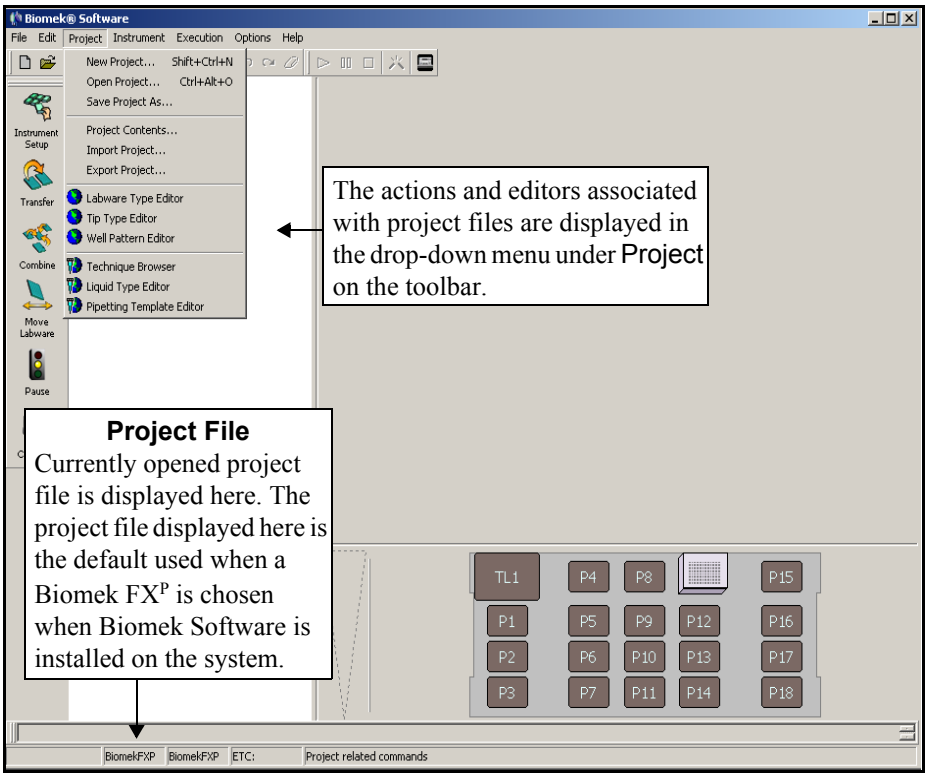

Figure 1-2. Project file

BIOMEK CONCEPT
Method

A method is a series of steps that control the operation of the Biomek FX instrument. The step palettes in the main editor present a group of icons representing the steps available for a method. To build a method, you simply select the step icon you want, and drag it into the method-building space (Method View) in the main editor. Place and configure each step to perform the operations as desired.

1.2.2 Creating a New Method

To create a new method:

Go up to the toolbar and select **New Method** (Figure 1-3).

This creates the beginning for your new method. It's a good idea to expand the Biomek editor to fill the entire screen.

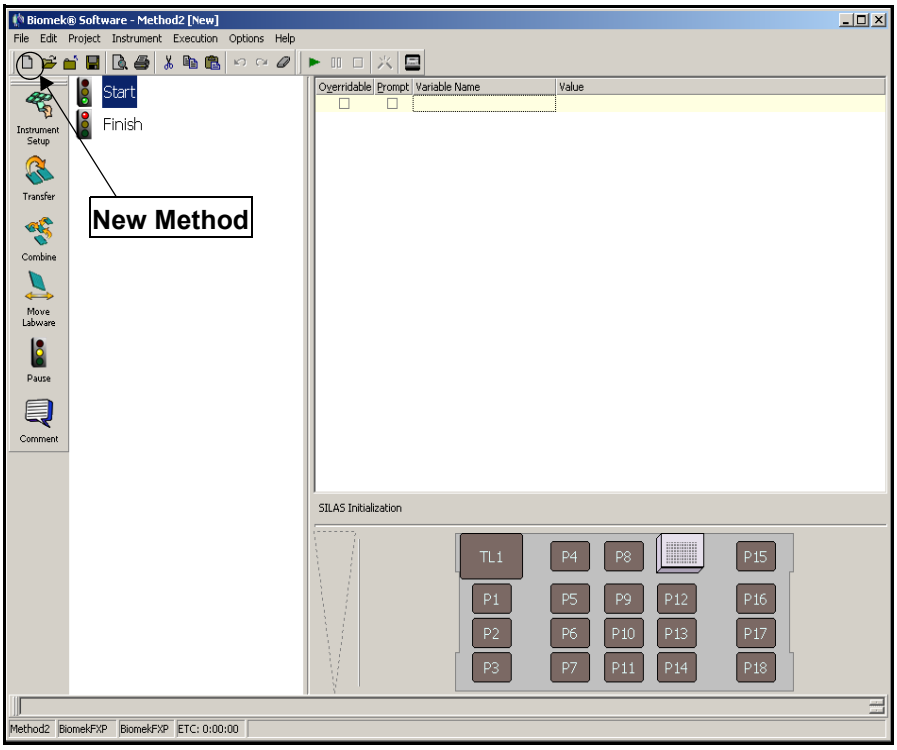

Figure 1-3. Main editor when a new method is created.

1.2.3 Understanding the Start and Finish Steps

As you can see (Figure 1-3), the method view of the main editor now contains the Start and Finish steps that appear automatically when you create a method. These two steps are always there and indicate the beginning and end of your method. You'll insert all the rest of the steps you want the Biomek FX instrument to complete between Start and Finish.

When the Start step is highlighted in the method view, you are presented with the opportunity to create some variables in the configuration view. Ignore this configuration for our first chapter in this tutorial.

If you want to know more in-depth information on the Start configuration right now, refer to the *Biomek Software User's Manual,* Section 13.2.1, *Configuring the Start Step*.

You'll learn more about using the Finish step in Section 1.4.4, *Determining the Estimated Time for Completion (ETC) of the Method*.

1.3 Setting Up the Deck

BIOMEK CONCEPT
Deck Editor

The Deck Editor is used to define and change the deck configurations stored in the current instrument file. A deck is a software representation of the Biomek instrument deck and can be stored and used for multiple methods; however, the software deck must always match the physical deck of the instrument used in the method. Refer to the *Biomek Software User's Manual,* Chapter 6, *Preparing and Managing the Deck*.

Setting up the deck includes:

- ensuring the current deck used in Biomek Software via the Deck Editor matches the physical deck of the instrument.
- configuring the Instrument Setup step to tell the software what labware and what deck position each labware piece occupies on the deck.

1.3.1 Ensuring the Deck in Biomek Software is Correct

To avoid hardware crashes, it's important that the deck in Biomek Software matches the physical deck of your instrument. If you wish to run these tutorial methods on hardware rather than in simulation and your deck varies from what is shown, you may have to modify the methods to work with your hardware (Figure 1-4).

Note: Automated Labware Positioners (ALPs) are removable and interchangeable platform structures installed on the deck to allow automated assays to be performed.

The steps in this tutorial assume the current deck in the software includes a 96-Channel Tip Wash ALP on the back row, the second column from the right since you will use this ALP later. You can also create a deck in the Deck Editor for use only when completing this tutorial.

To do this:

1. View the current software deck Figure 1-4.
2. Ensure a 96-Channel Tip Wash ALP is placed on the position in the back row, the second column from the right (Figure 1-4). See the sidebar tip to add this ALP.

Tip

To add a 96-Channel Tip Wash ALP to your deck, choose **Instrument> Deck Editor**. The current deck appears. Then from All, highlight **WashStation96** and drag it to the proper position. You may have to delete an ALP from the deck before you add the WashStation96. Then choose **Renumber** to ensure your deck matches the deck on Figure 1-4.

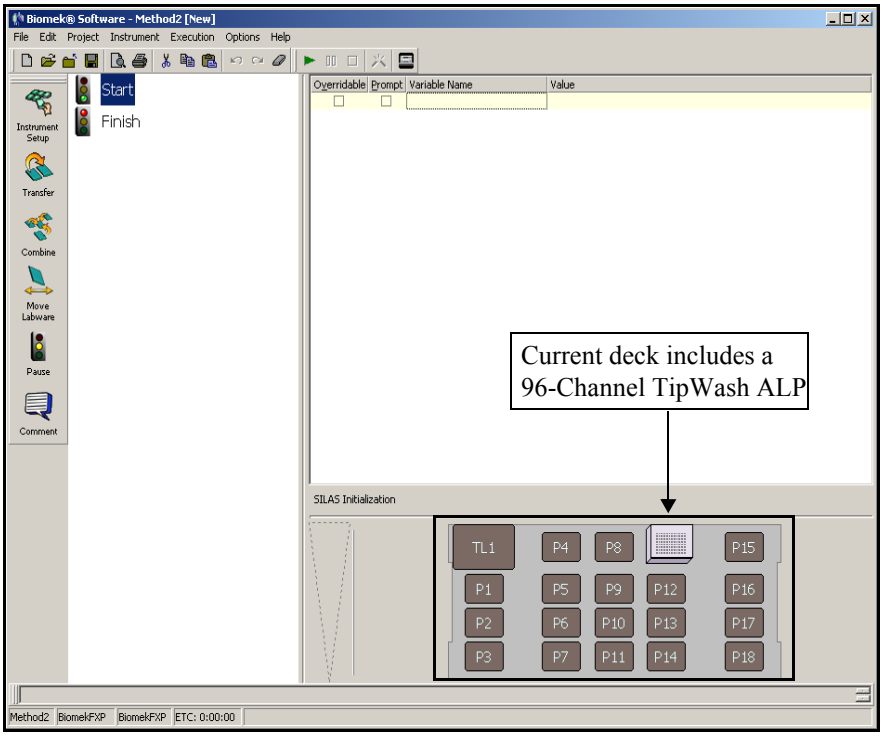

Figure 1-4. Current deck includes a 96-Channel Tip Wash ALP

Tip

If the Instrument Setup step, or any step, is inserted into the wrong location in the Method View, you can drag and drop it to the proper location.

1.3.2 Configuring the Instrument Setup Step

The next activity of this tutorial is to configure the Instrument Setup step for your liquid-transfer procedure. You will place on the deck:

- Tips
- Source reservoir
- Destination microplate

To insert the Instrument Setup step:

1. Choose (highlight) **Start** in the Method View
2. Hover the cursor over the **Instrument Setup** icon in the step palette. As you hover, look at the Method View and you'll see a black bar appear just below Start. This black bar indicates the insertion point where your next step will appear. In this case, it's where the Instrument Setup step will be inserted.
3. Click the **Instrument Setup** icon to insert the step. The Instrument Setup configuration appears (Figure 1-5).
4. If you created a deck just for use with this tutorial, select the deck you created in **Deck**.

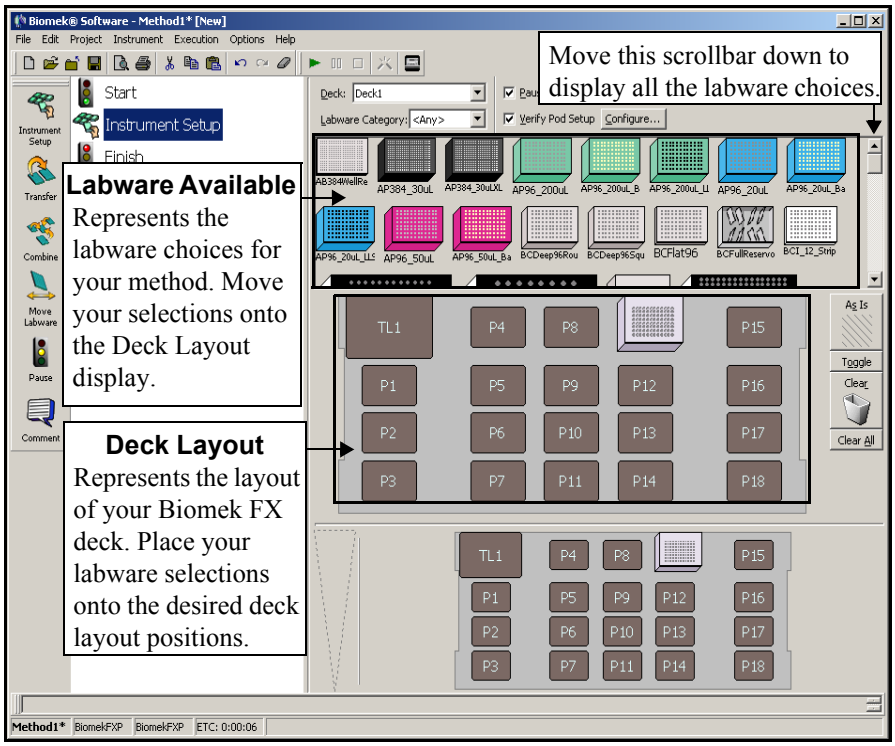

Figure 1-5. Instrument Setup step configuration

Using the Instrument Setup step you just inserted, you'll learn how to select and place:

- AP96 200 µL tips onto TL1 (Tip Loader 1) deck position
- Reservoir onto P4 (Position 4) deck position
- BCFlat 96 microplate onto P5 deck position

To select and place your labware:

1. Click the **AP96_200 µL** tips icon, then click on **TL1** deck position in the Deck Layout. Notice that when you hover the cursor over the tip box on the Deck Layout, a tool tip identifies the deck position and labware. This procedure applies to all the labware you place on the deck.
2. Using the above procedure, place a **Reservoir** onto the **P4** deck position.
3. After you have positioned the reservoir on the deck, double-click it or right-click and select **Properties**. This opens Labware Properties (Figure 1-6). Each piece of labware added to the Deck Layout is configured using Labware Properties. The information provided in Labware Properties is used when a pipetting technique is selected or when tips are loaded and unloaded.

Note: A technique instructs the Biomek instrument how to perform pipetting operations, such as an aspirate, dispense, and mix.

Tip

Using Labware Properties, tips may be configured to be loaded back to the tip box or to the trash.

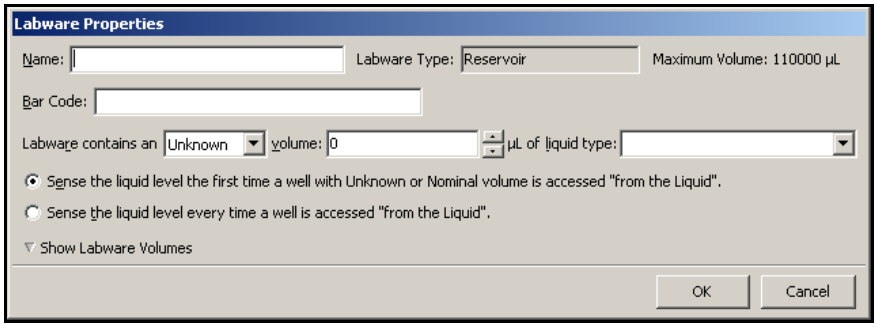

Figure 1-6. Labware Properties for Reservoir

Tip

It's helpful to name your labware on the deck. You can assign a name that identifies the contents of the labware, or a descriptive name that fits the work being done in your laboratory. This can reduce confusion considerably. The name can then be used in other steps and appears in log files.

4. In Labware Properties, you can give the reservoir a name. You'll name this one "Rsvr," but in general you can assign labware any name you want. Type **Rsvr** in the Name field. After configuration is complete, the name will appear over the reservoir in the Current Deck display (Figure 1-7).
5. In Labware contains an, select **Known**.
6. In the Volume field, type **100000**. This means you know you have 100,000 microliters of liquid in the source reservoir.
7. Choose **Water** from the Liquid Type drop-down menu, or type **Water** into this field.
8. Leave Bar Code blank for this tutorial, but it can be used to identify a specific plate in certain methods.
9. Leave the default Sense the liquid level the first time a well with Unknown or Nominal volume is accessed "from the Liquid" selected. You'll learn more about liquid level sensing, only available with a Span-8 Pod, later in the Span-8 tutorial.
10. Choose **OK**.
11. Place a **BCFlat96** microplate onto the deck in position **P5**.
12. Double-click on the **P5** microplate, or right-click and select **Properties**.
13. Type **Dest** in the Name field to represent the destination.
14. In Labware contains an, select **Known**.

15. In the Volume field, leave this value at **0**.
16. Do not specify a Liquid Type for this destination plate since it is presently empty.
17. Choose **OK**.

That's it. Your deck is now set up for transferring liquid, and the main editor should look like Figure 1-7.

Tip

You can set the properties (name, volume, and liquid type) as you've just done in these steps, then drag the labware back up, and drop it into the Labware Available display once you've selected the Custom labware category. This labware will retain the properties you set and be available to use in other methods when you access Instrument Setup.

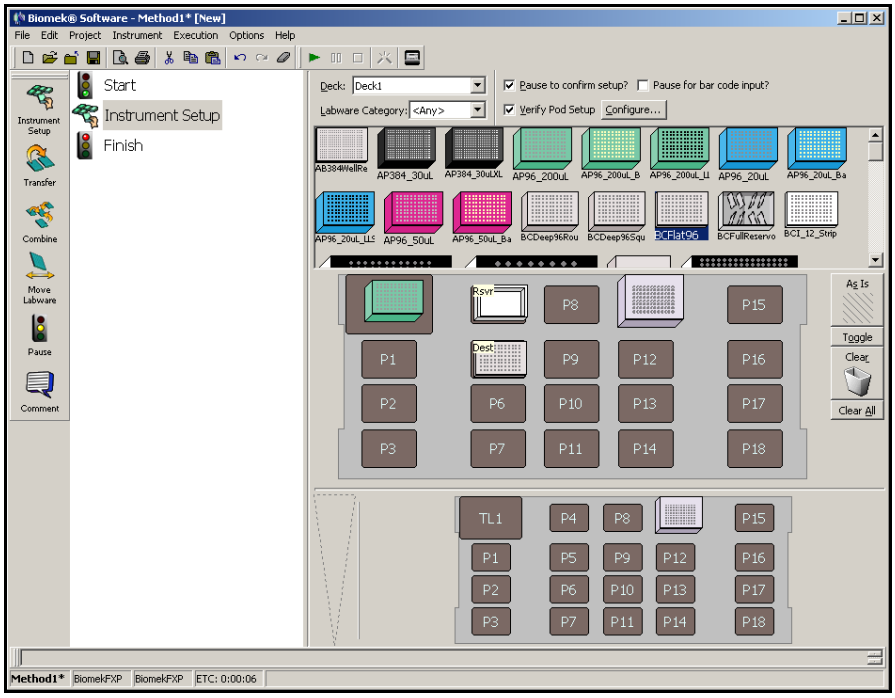

Figure 1-7. Instrument Setup step completed

1.4 Transferring Liquid

BIOMEK CONCEPT
Transfer Step

The Transfer step for the Multichannel Pod transfers liquid from one source to one or more destinations. The Transfer step will by default complete the following: load tips, aspirate liquid, dispense liquid, and unload tips. This concept eliminates the need to insert four separate steps, although occasionally a method may require these steps be performed individually. These individual steps will be covered in Chapter 3 of this tutorial.

Now you are ready to insert and configure your procedure to transfer liquid. Biomek Software provides a Transfer step on the Basic Step palette that makes it easy to accomplish this task.

Configuring the Transfer step includes configuring:

- Tip handling.
- Source labware
- Destination labware

1.4.1 Configuring Tip Handling

To set up a liquid transfer, insert the Transfer step into the Method View in the main editor, and configure the Tip Handling by completing the following:

1. Highlight the **Instrument Setup** step.
2. Choose the Transfer icon from the step palette, and insert it into the method by dragging and dropping it after the Instrument Setup step. The Transfer step configuration appears (Figure 1-8). Notice the Current Deck display at the bottom of the editor is now populated to illustrate your deck setup since it changes dynamically to match the state of the deck at the start of the current step.

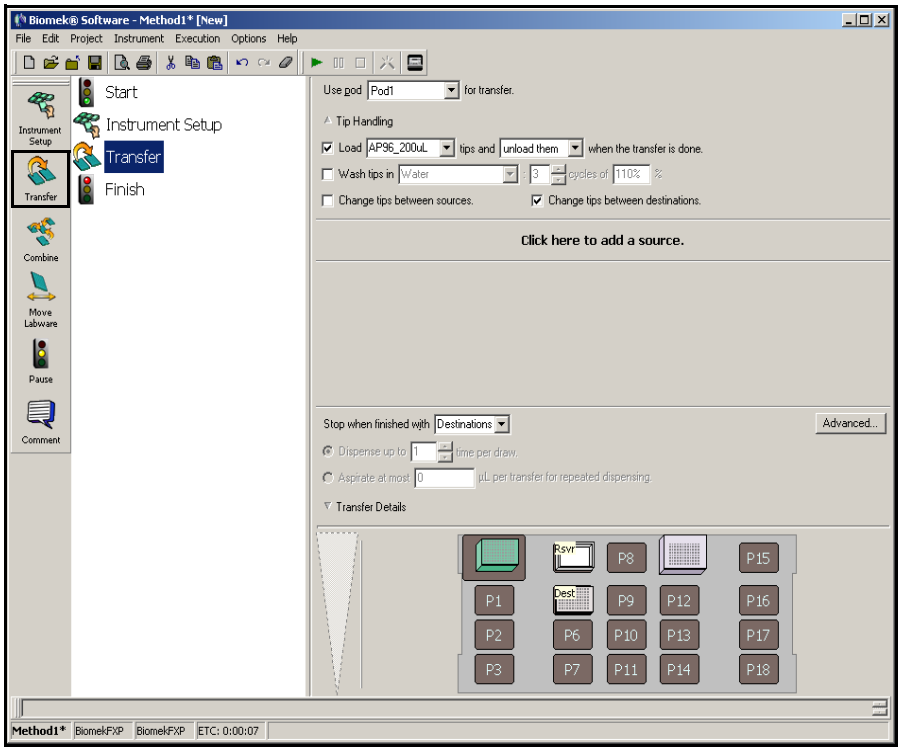

Figure 1-8. Transfer step inserted

3. In Tip Handling, make sure **Load** is checked.
4. Make sure the type of tips displayed is **AP96_200µL**, the type of tips you configured in Instrument Setup.

5. Make sure **unload them** is selected in the next field.
6. Check **Change tips between sources**.
7. Uncheck **Change tips between destinations**.
8. Your tips are configured for your liquid transfer, so click the **up arrow** next to Tip Handling (Figure 1-8). This collapses the Tip Handling configuration to allow more room for labware configuration. A simple text description of the way tips will be handled is displayed in place of the expanded Tip Handling configuration.
9. You will not be changing the Transfer Details section at this time, so click the **down arrow** next to Transfer Details to collapse the Transfer Details configuration to a summary. This gives you more room for the Source and Destination configurations. The editor now looks like Figure 1-9.

Tip

To make sure your tips are going to behave the way you want during method execution, simply collapse the Tip Handling configuration at any time during the process. The sentence displayed describes what you have configured and how the tips will be handled. If the description is not how you want the tips handled, expand the configuration and change it.

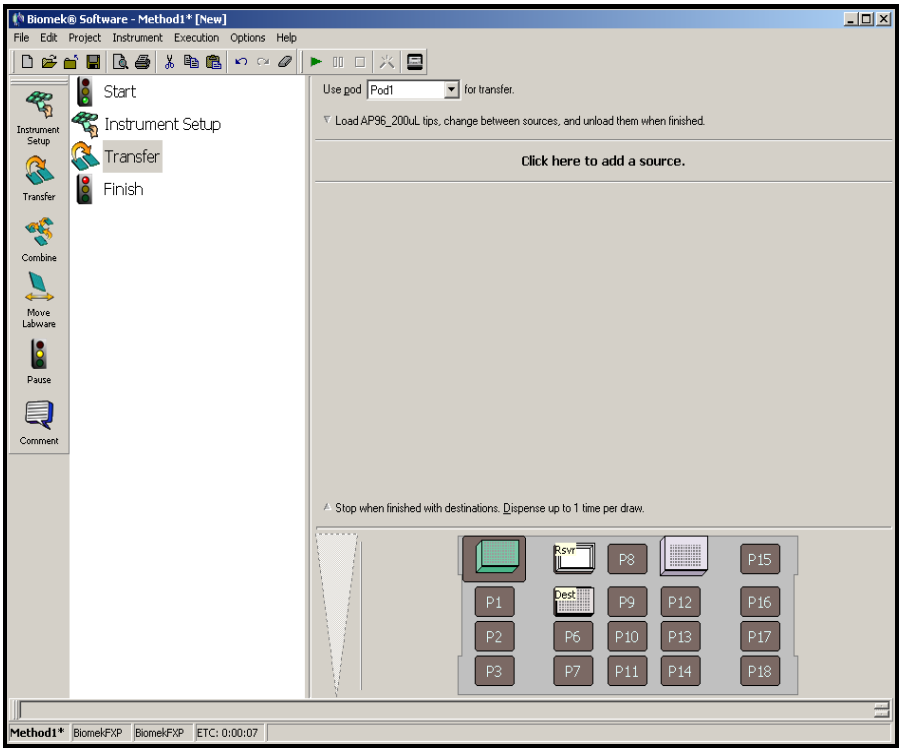

Figure 1-9. Tip Handling configured and collapsed

1.4.2 Configuring Source Labware

Now you will configure the source labware. Here you will specify from which labware liquid will be aspirated and the height to which the tip descends into the labware before aspirating.

To configure the reservoir named Rsvr as the source labware:

1. Click on **Click here to add a source**.
2. Click on **Rsvr** labware on the P4 position in the Current Deck display. As you can see, the information you supplied during Instrument Setup is displayed in the source labware configuration.
3. Right-click on the large tip illustration next to the reservoir graphic in the configuration and choose **Measure from Bottom**.
4. To adjust and set the aspirate height to which the tip descends into the reservoir, place the **mouse cursor over the tip illustration**. When the cursor turns into a hand, hold the left mouse button down to move the hand up and down until the depth is as close to **1.00 mm from bottom** as you can get. Then adjust the height precisely to 1.00 mm using the Tip described in the margin. There is a slight break in the bottom of the source reservoir graphic with the large tip that indicates that the reservoir is wider than the graphic can display.

Tip

After you click on the tip, you can adjust the height more precisely by using the up or down arrow keys on your keyboard to change the height by 0.10 mm or you can use the Page Up and Page Down keys to change the height by 1.0 mm with each press of the key. You can also right-click on the graphic, then select **Custom Height** from the menu that appears.

The source labware is complete, and the editor now looks like Figure 1-10.

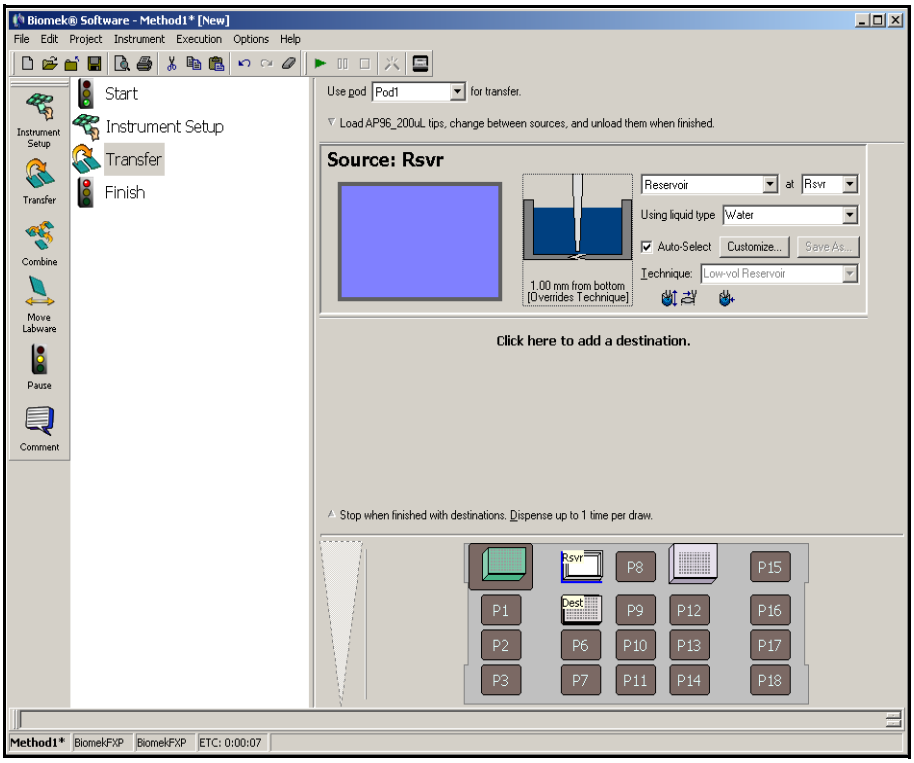

Figure 1-10. Configured source labware

1.4.3 Configuring Destination Labware

Here you will configure where you want the water from the source reservoir to be dispensed. In this case, you want to dispense water into the BCFlat 96 microplate on deck position P5.

Tip

If you accidentally open too many destination configurations, just right-click on the title in the configuration. Click **Delete** from the popup menu and the entire configuration goes away.

To do this:

1. Click the **Dest** microplate in the Current Deck display. This one operation accomplishes the same tasks as steps 1 and 2 of Section 1.4.2, *Configuring Source Labware*. Notice that the source labware configuration fields are now replaced with a brief sentence summary of the setup. When the source configuration is collapsed, it can be opened by clicking anywhere in the collapsed configuration area.
2. The Volume field is highlighted in the destination configuration, which allows you to designate the amount of liquid to be dispensed. For this tutorial, you're transferring 100 µL; so type **100** into the Volume field. This means you will be dispensing 100 µL into each of the 96 wells; so in this case, you're dispensing a total of 9600 µL into the 96-well microplate.
3. Right-click on the large tip illustration and choose **Measure from Bottom**.
4. Set the dispense height in the large tip illustration to **1.00 mm from bottom**, using the same technique as you used for setting the aspirate height.

The destination labware is now configured and the editor looks like Figure 1-11

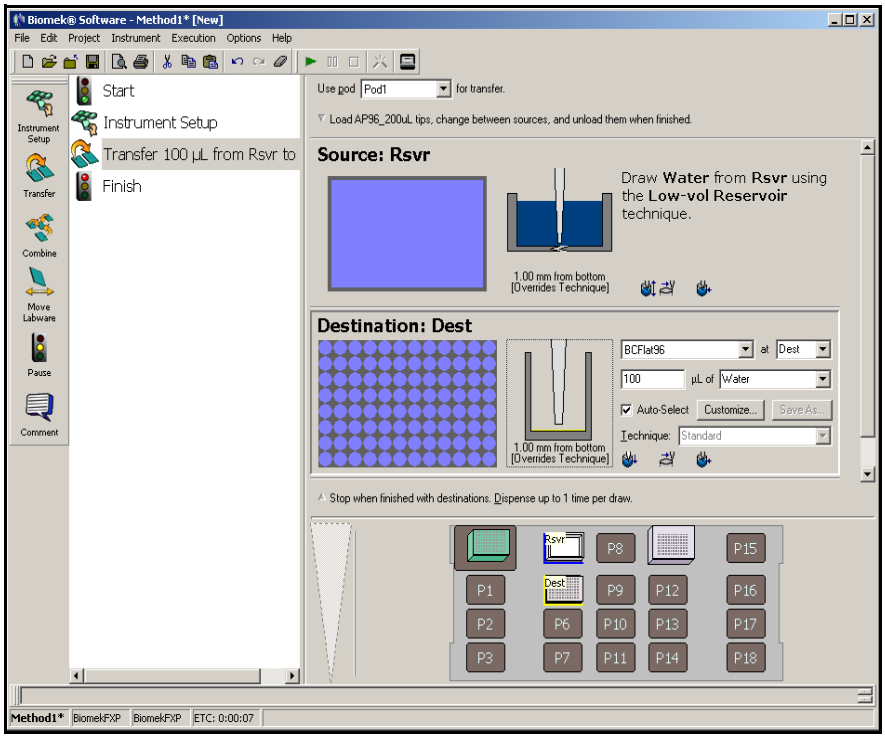

Figure 1-11. Configured destination labware

1.4.4 Determining the Estimated Time for Completion (ETC) of the Method

Your liquid transfer is set up, so let's see how long it will take to run the entire method by using the Finish step.

To do this:

1. Click on the **Finish** step in the Method View.
2. Check the status bar at the bottom of the editor for a display of the ETC. For this method, the ETC is approximately 51 seconds (Figure 1-12). It's all right if your ETC varies slightly.

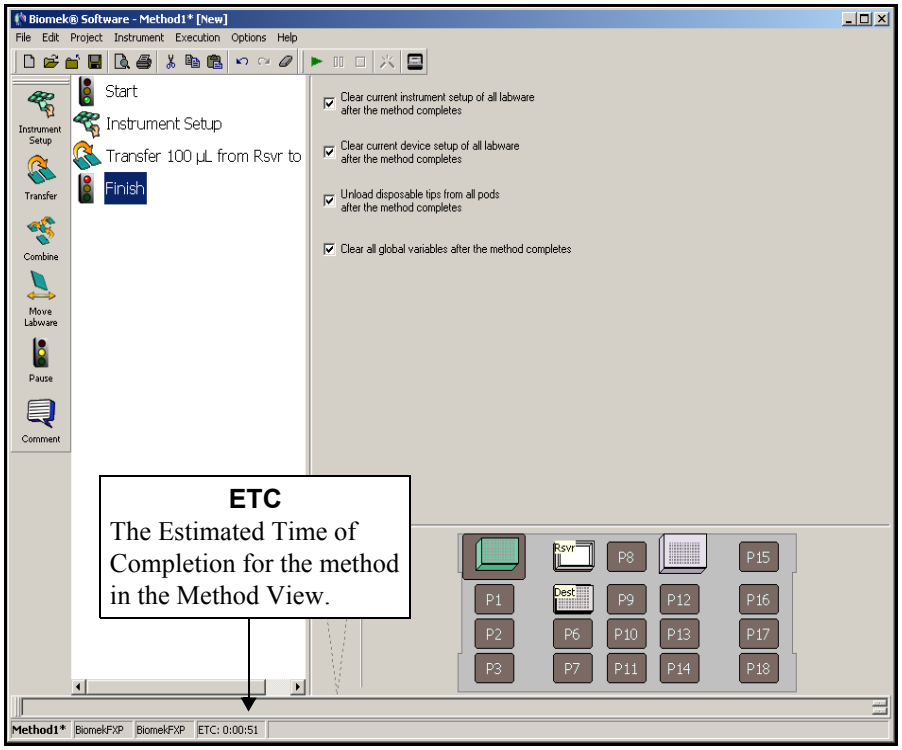

Figure 1-12. Finish step displaying the ETC

Congratulations! You've just built a liquid transfer method using Biomek Software that:

- Prepared the main editor for a new method.
- Set up the deck and the configured the labware you want to use using an Instrument Setup step.
- Added and configured a liquid transfer using a Transfer step.

1.5 Running the Method

Now that you've built a method, let's run it.

1.5.1 Validating the Method and Confirming the Deck Setup

When you select **Run**, the method will be validated internally to check for errors. After this validation is complete, a deck confirmation prompt will appear over the main editor (Figure 1-13). This prompt displays the deck setup as interpreted by the software.

If you wish, you can also view the method in the Biomek Simulator. Refer to Section 1.5.2, *Viewing the Method in the Biomek Simulator*.

To confirm the deck setup:

1. Click on the green arrow button on the toolbar or from the Execution menu, choose **Run**. A deck confirmation appears (Figure 1-13).
2. Visually confirm the physical deck and pod setup matches the deck confirmation, included labware placement and tip state on the pod, before continuing with the method.

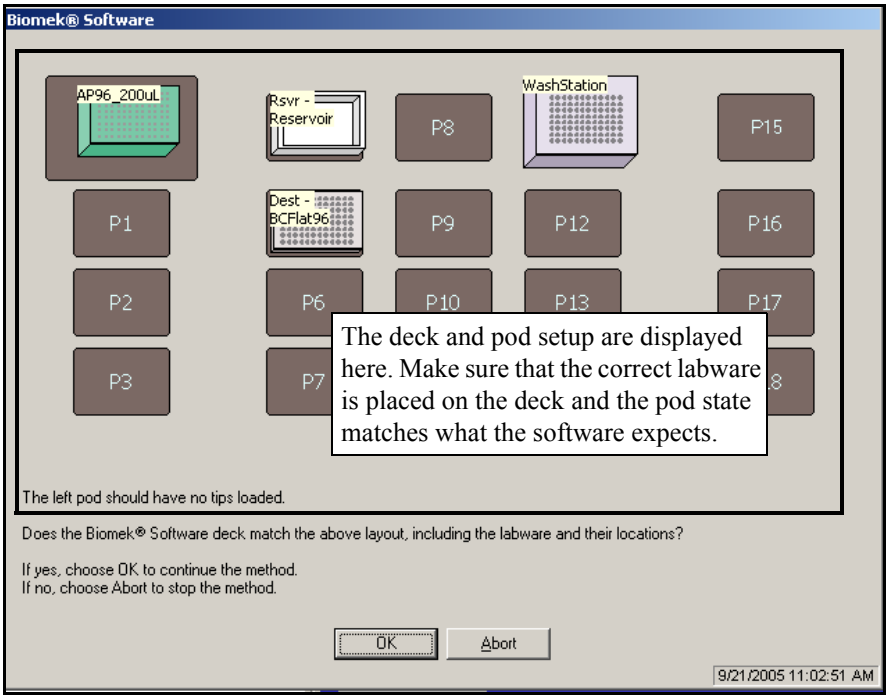

Figure 1-13. Deck confirmation prompt

3. If the physical deck does not match the deck shown, move or place labware on the deck so that it does match. Alternatively, you may choose **Abort** and adjust the Instrument Setup step to match your physical deck setup.
4. When the physical deck setup matches the deck shown, choose **OK**. The method runs as soon as you choose OK. You can visually follow the run in the Method View; steps are highlighted as the step is executed.

1.5.2 Viewing the Method in the Biomek Simulator

CAUTION: Make sure the proper port is selected in Hardware Setup. Simulate is used only when running methods on the Biomek Simulator. To run methods on the instrument, choose the com port to which the instrument is connected.

When a method is run in simulation, the Biomek Simulator appears, showing an animated 3-D model of the instrument performing the method. Setting the simulation mode is configured in Hardware Setup (Figure 1-15).

BIOMEK CONCEPT
Hardware Setup

Hardware Setup is used to configure Biomek Software with the appropriate Biomek instrument information, including the Biomek Simulator. While the Beckman Coulter Service Engineer normally installs and configures new devices, it may be necessary to install, configure, and remove other devices using Hardware Setup. Refer to the *Biomek Software User's Manual,* Section 5.2, *Accessing Hardware Setup*.

If you wish to view the method in simulation:

1. From the toolbar, choose **Instrument>Hardware Setup**. Hardware Setup appears (Figure 1-14).

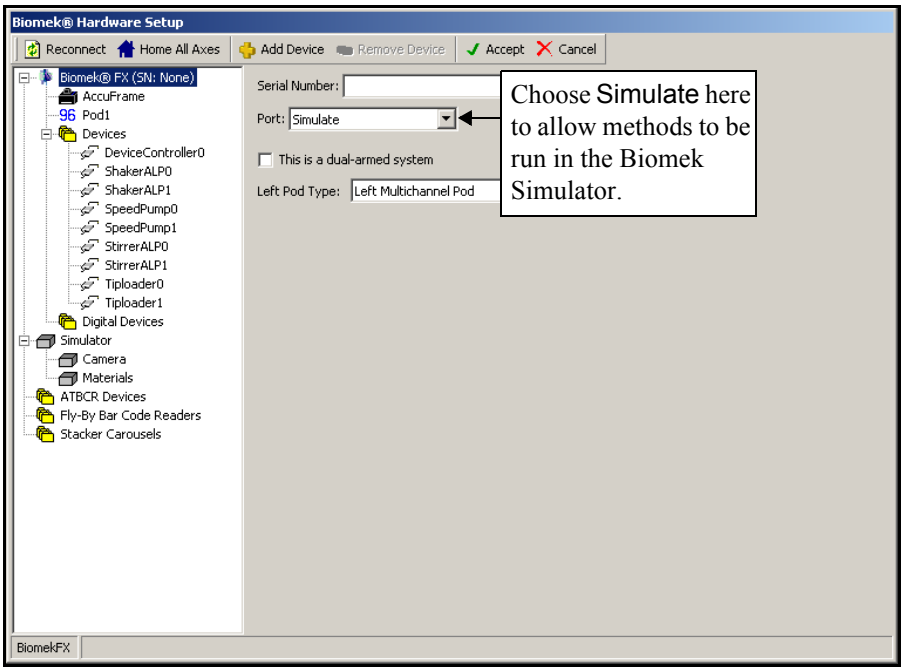

Figure 1-14. Hardware Setup

2. From Port, choose **Simulate**.
3. Choose **Accept**. Now, when a method is run, an animated 3-D model of the Biomek instrument is displayed (Figure 1-15). You can now watch a simulation of the Biomek instrument perform the steps in the method.

Tip

The simulator can be a useful tool to test methods to ensure that they are performing as expected without using up valuable reagents or tips, and can also save time not only in set up, but also by running at an accelerated speed. Refer to the *Biomek Software User's Manual,* Section 5.4, *Configuring the Biomek Simulator*, for more information on the simulator.

If you wish to run the method on hardware, you must go back to Hardware Setup and change the Port from Simulate to the Com port your Biomek instrument is connected to on your PC.

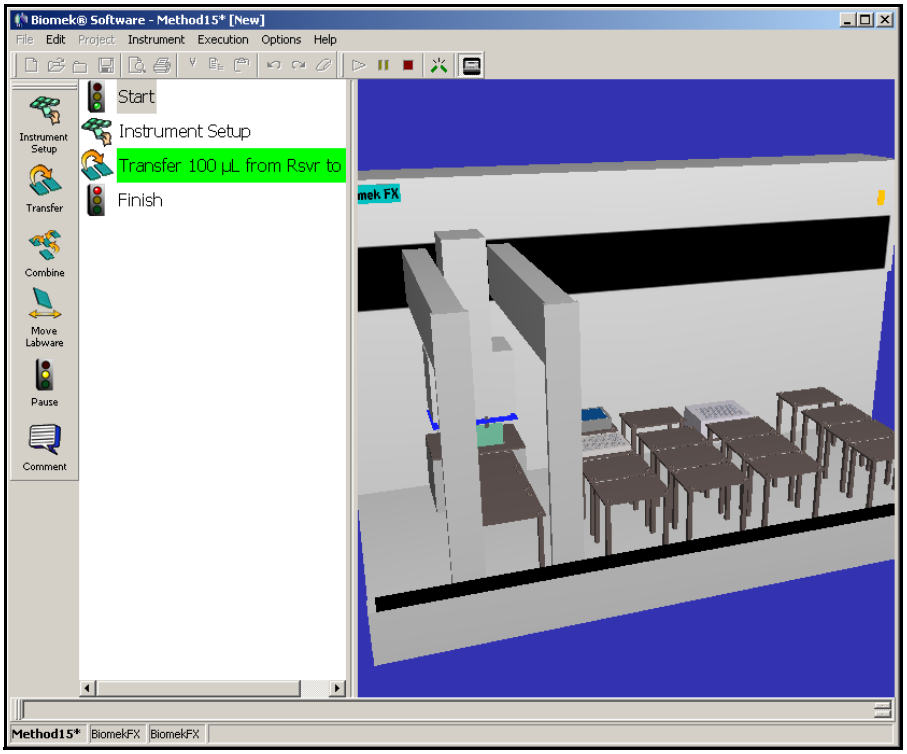

Figure 1-15. Running a method in simulation

1.6 Saving the Method

BIOMEK CONCEPT
Saving Methods

Methods may be saved at any time during their development. Saving a method automatically checks in the method, creating a record of the revision that preserves the method configuration at the time it was saved. Revisions may be accessed from the revision history at a later time. If any project items, such as labware definitions or techniques, change after the method is saved, when the method is opened next, the latest definitions are used. Refer to the *Biomek Software User's Manual,* Section 13.9, *Saving a Method* and Section 13.12, *Viewing Method History* for more information.

You will save the method you've just created.

To save your method:

1. Choose the **Save Method** icon on the toolbar.
2. In Method Name, type the file name under which your method will be saved. For this chapter, type **Getting Started Tutorial** (Figure 1-16).

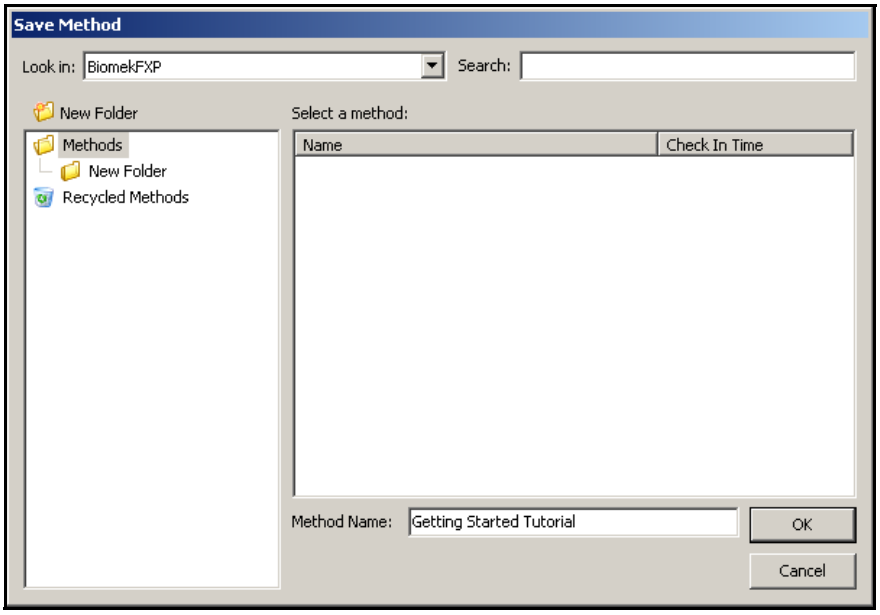

Figure 1-16. Save Method

3. Choose **OK**. Now notice how the method name in the Biomek main editor has changed to Getting Started Tutorial [Revision 1] (Figure 1-17).

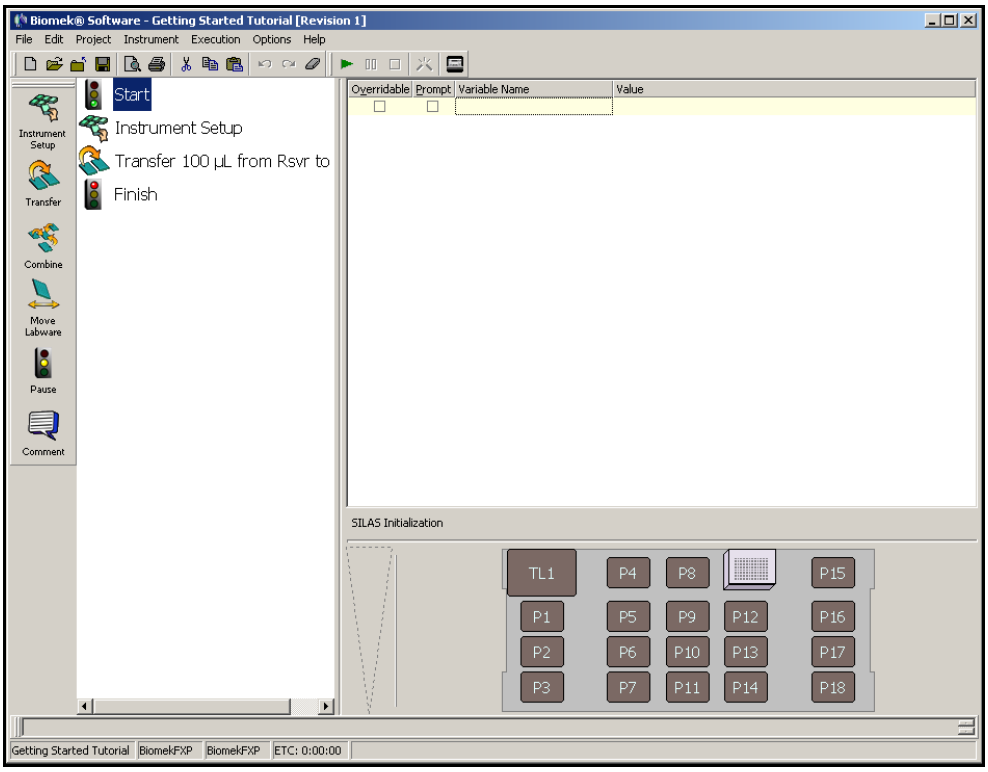

Figure 1-17. Method name has changed

Now go to Chapter 2 in this tutorial to learn how to use more steps in a method.

2 Multichannel Pod—Using More Steps in a Method

2.1 Introduction to Using More Steps in a Method

In the first chapter of this tutorial (refer to Chapter 1, *Multichannel Pod—Getting Started With Biomek® Software*), you learned how to:

- Launch Biomek Software.
- Set up the deck for a liquid transfer.
- Build a liquid-transfer method.
- Save, run, and check in a method.

If you already know how to complete these tasks in Chapter 1, *Multichannel Pod—Getting Started With Biomek® Software*, you can just start with this chapter or subsequent chapters.

2.1.1 What You'll Learn in Using More Steps in a Method

This chapter will help you develop the skills to create basic transfer methods for tasks such as reaction setup and reformatting from one density to another; for example, transferring liquid from 96 to 384-well plates. You will also learn how to pause the system to add more labware to the deck and handle errors. Using Single Step to perform single operations to improve method development will also be described.

More specifically, the step-by-step instructions in this chapter will teach you how to:

- Transfer liquid from two sources to a single destination.
- Mix contents in labware.
- Move labware on the deck using the gripper tool on the Multichannel Pod.
- Respond to errors.
- Add more labware to the deck once a method has started to run.
- Use the Multichannel Pod with a 96-channel head with 384-well plates.
- Group steps logically in the Method View.
- Perform single operations with the Biomek FX.

2.1.2 Setting Up Your Deck for Using More Steps in a Method

Using what you learned in Chapter 1, launch Biomek Software and configure an Instrument Setup with the following:

- Place **AP96 20µL** tip boxes on TL1 and P1.
- Place **BCFlat96** plates on P5 and P6 and name them **Source1** and **Source2**. Give these source plates a **Known** volume of **150** µL of **Water**.
- Place a **CostarFlat384Square** on P9 and name it **Dest**. Give this destination plate a **Known** volume of **0** µL.

Your deck should look like Figure 2-1. Now go to the next activity to learn how to create a method using other steps in your methods.

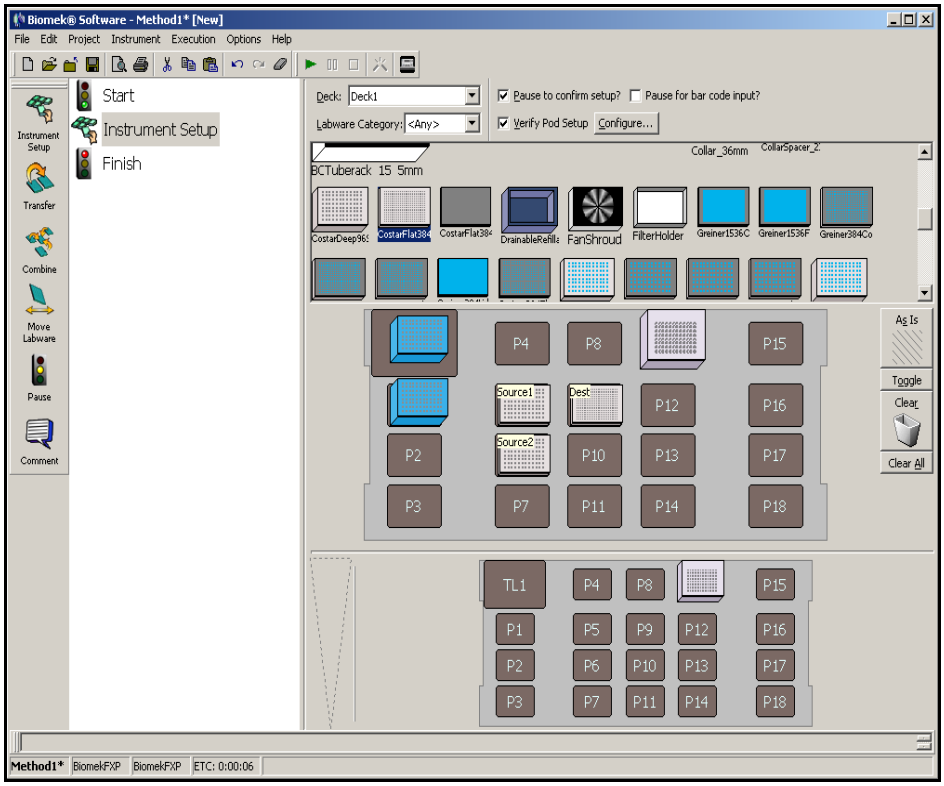

Figure 2-1. Completed Instrument Setup step

2.2 Transferring Liquid from Multiple Sources to a Single Destination

To transfer liquid from one or more sources to a single destination, a Combine step is used. It is similar to a Transfer step which uses a single source and one or more destinations.

Like the Transfer step, the Combine step will by default complete the following:

- load tips
- aspirate liquid
- dispense liquid
- unload tips

For this activity, you will use the default tip handling, configure the two sources, configure the destination, and configure transfer details. The configured Combine step will transfer the sources from two 96-well source plates to a 384-well destination plate.

2.2.1 Configuring Tip Handling

To set up the Combine step, you will insert the Combine step in the Method View and use the default settings for Tip Handling.

1. Ensure you deck is configured according to the instructions in Section 2.1.2, *Setting Up Your Deck for Using More Steps in a Method*.
2. Add a **Combine** step after the Instrument Setup step.
3. In Tip Handling, make sure **Load** is checked.
4. Make sure the type of tip displayed is **AP96_20µL tips**, the type you configured in Instrument Setup.
5. Make sure **unload them** is selected in the next field.
6. Check **Change tips between sources** and make sure **Change tips between destinations** is not checked.

7. Collapse **Tip Handling**. Your main editor should look like Figure 2-2.

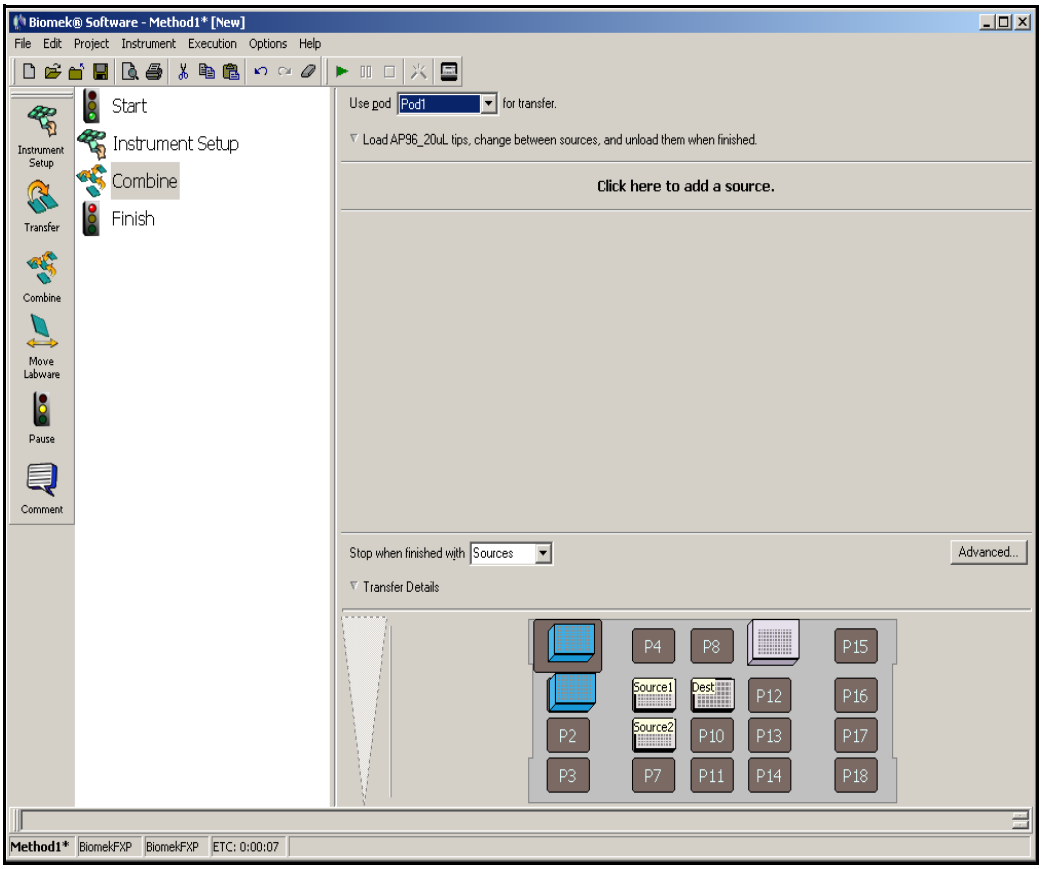

Figure 2-2. Combine step inserted and Tip Handling collapsed

2.2.2 Configuring Source Labware

To configure the two 96-well plates from which you're going to aspirate:

1. Click on **Click here to add a source** (Figure 2-2)
2. Click on **Source1** sitting on P5.
3. In the Volume field, designate the amount of liquid to be aspirated. For this method, you're aspirating 40 µL, so type **40** into the Volume field.
4. Click on **Dest** in the Current Deck Display to add a destination. You will configure the destination in the next section, but must choose it here to activate another source option.
5. Click on the next **Click here to add a source**.
6. Click on **Source2** sitting on P6 and type **20** into the Volume field.

Tip

The well volume display shows how much liquid is in the well. If the liquid is not visible in the display, you may be accidentally trying to aspirate from an empty destination rather than a source.

The source plates are now configured, and the editor should now look like Figure 2-3.

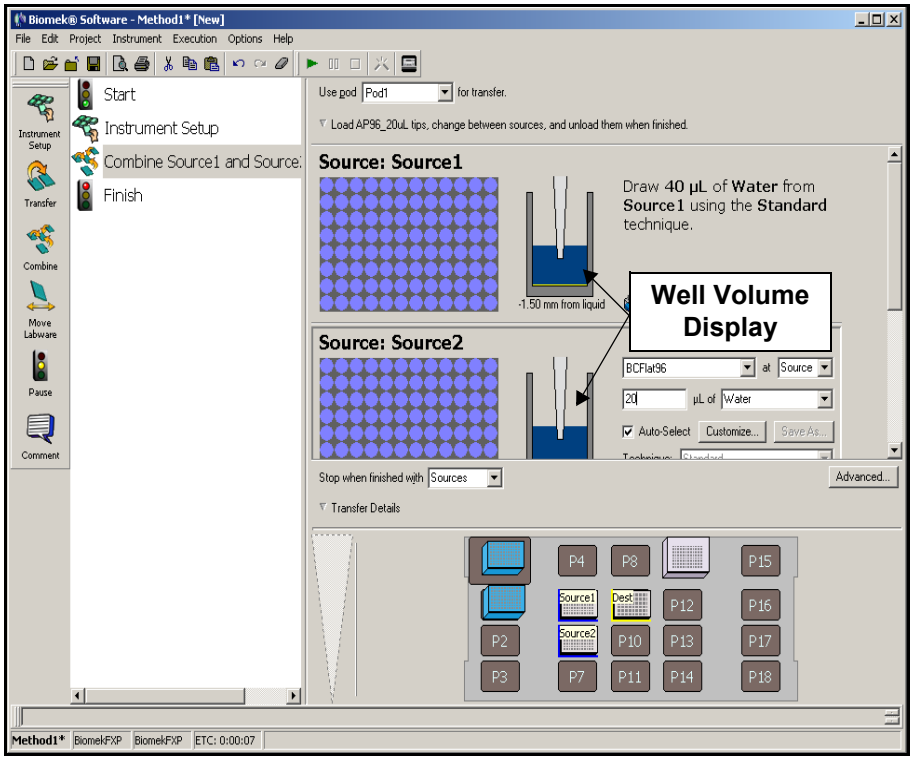

Figure 2-3. Source labware for Combine step configured.

2.2.3 Configuring Destination Labware

The next task is to designate where you want to dispense the aspirated liquid. For this method, you want to dispense into Dest on deck position P9. To accomplish this:

1. Click anywhere in the **Destination:Dest** configuration.
2. If Transfer Details is not opened, click on the arrow next to it.

3. From Stop when finished with, choose **Destinations** (Figure 2-4).

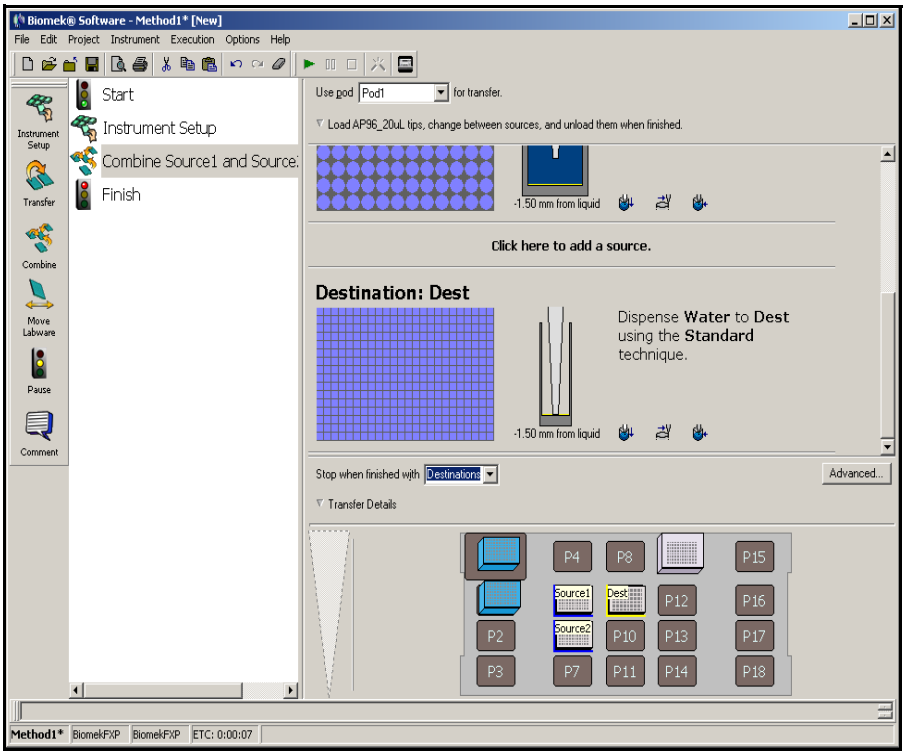

Figure 2-4. Stop when finished with Destinations chosen

That's it. You've just configured a Combine step to aspirate liquid from two sources in order to dispense it to a single destination.

The Combine step uses the Source and Destination configurations and the stop condition to configure a series of operations. When the method is executed, the Combine step will transfer liquid from each of the source plates twice so that the each well of the desination plate receives exactly one source. Later in this chapter, you will transfer to specific quadrants of a 384-well microplate using a 96-channel head.

Go to the next activity to learn how to mix the liquid in the destination plate after dispensing.

2.3 Mixing Contents in Labware

BIOMEK CONCEPT
Techniques

Techniques are sets of predefined and stored values, including aspirate and dispense height, tip touch and other properties that affect pipetting. Based upon these stored sets of values and properties, the appropriate pipetting technique is selected automatically. If you want control over this otherwise automatic function, you can choose Customize for each source and destination in a liquid transfer. This customizing option is also available via the Technique Editor. Refer to the *Biomek Software User's Manual,* Chapter 10, *Understanding and Creating Techniques*.

In the Transfer and Combine steps, you can alter liquid-handling functions that extend beyond simple aspirating and dispensing. For example, you can turn off the tip touch feature, activate the pre-wet function, or configure mixing operations. These modifications are accomplished through customizing the technique and are used to control the pipetting process.

Let's suppose you wish to mix the contents of the destination plate once liquid from the two sources has been dispensed.

To complete this task:

1. Click the **Dest** labware in the Deck Display, or click in the gray area around the destination configuration. This expands the destination labware configuration.
2. In the Destination configuration fields, select **Customize**. The Technique Editor opens to the Dispense tab (Figure 2-5).

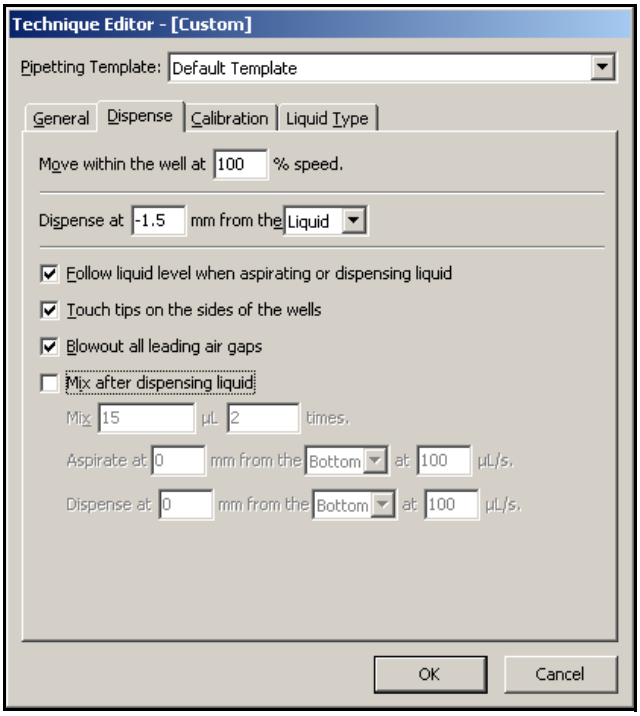

Figure 2-5. Dispense tab of the Technique Editor

2.3.1 Configuring "Mix after dispensing liquid"

Now, you will configure the options to mix the contents in the destination after dispensing.

To mix after dispensing:

1. From the Dispense tab (Figure 2-5), check **Mix after dispensing liquid**. The fields for this option are enabled. We'll allow some of the defaults to remain except for the amount and number of times to mix.
2. In Mix, enter **15**. This specifies the amount of microliters that will be aspirated and dispensed during mixing.

3. In time, enter **2.** (After configuration, time changes to times.) This specifies the number of times you want to mix the liquid after dispensing. You're finished configuring the mix after dispensing process. The Technique Editor should now look like Figure 2-6.

Tip

Pipetting from the Bottom can sometimes cause wells to overflow, or it can contaminate the tips. Aspirating from the liquid would be a good choice in these cases. This is not a concern for this tutorial, so you are leaving the default in the mix operation for this technique.

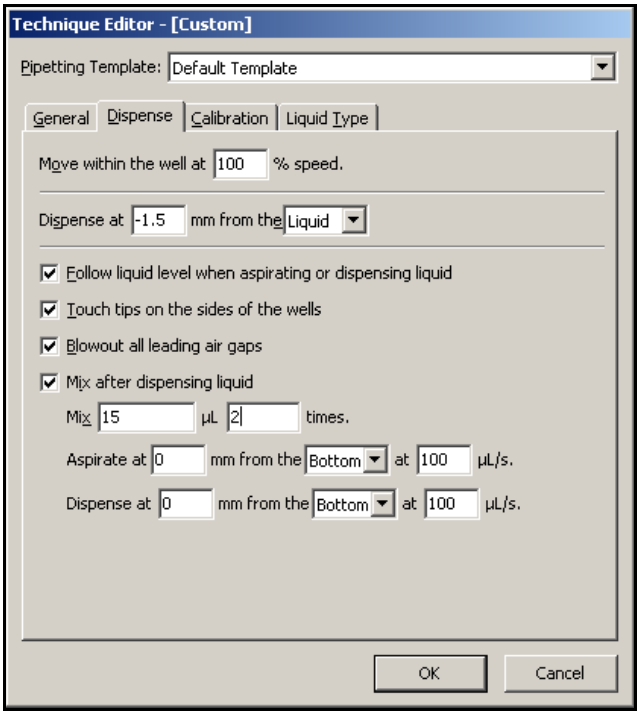

Figure 2-6. Configured mix in a Custom technique

4. Choose **OK** to close the Technique Editor.

After you configure the mix operation and return to the Combine step configuration, you see an icon indicating that the technique now includes mixing. This is one of several icons that indicate the different procedures in the pipetting technique. You will also notice that the Auto-Select option is turned off when the technique has been customized.

2.4 Moving Labware Around the Deck

On the step palette, you can see a Move Labware step icon. When you insert and configure this step in your method, the instrument behaves as follows:

- The pod moves over the selected labware and moves down.
- The gripper tool fingers on the pod extend, then squeeze and grip the labware.
- The pod moves up and carries the labware to the designated position.
- The pod moves down, and gripper tool fingers release the labware at the new position.

2.4.1 Moving Labware Using the Gripper Tool

For this part of the tutorial, let's suppose that the contents of the destination plate have settled, and you need to shake up the contents. Let's suppose further that you have a shaking ALP installed somewhere on the deck.

To move labware on the deck:

1. Highlight the **Combine** step in the Method View.
2. Insert the **Move Labware** step from the step palette after the Combine step. The Move Labware configuration appears (Figure 2-7).

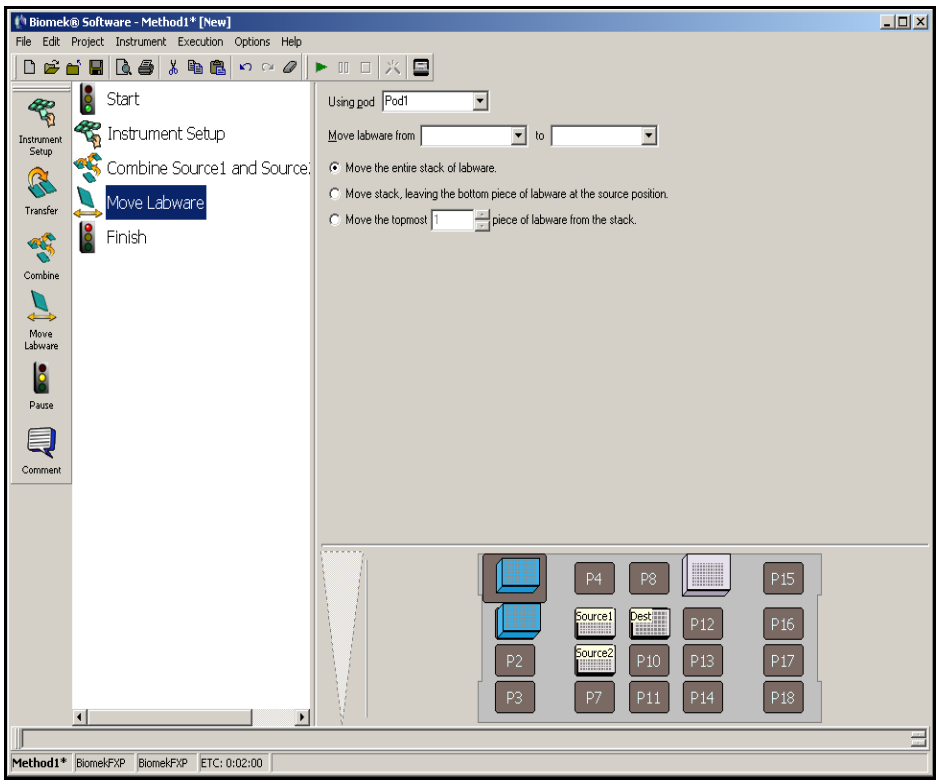

Figure 2-7. Move Labware step configuration

3. Choose **P9** into the Move labware from field.
4. Choose **P6** into the to field.

5. Keep Move the entire stack of labware selected.
6. Now, click on the **Finish** step in the Method View following the Move Labware step to validate the method.

OOPS!!! All the red you see indicates an error (Figure 2-8). But that's all right—just keep going in this tutorial to recover.

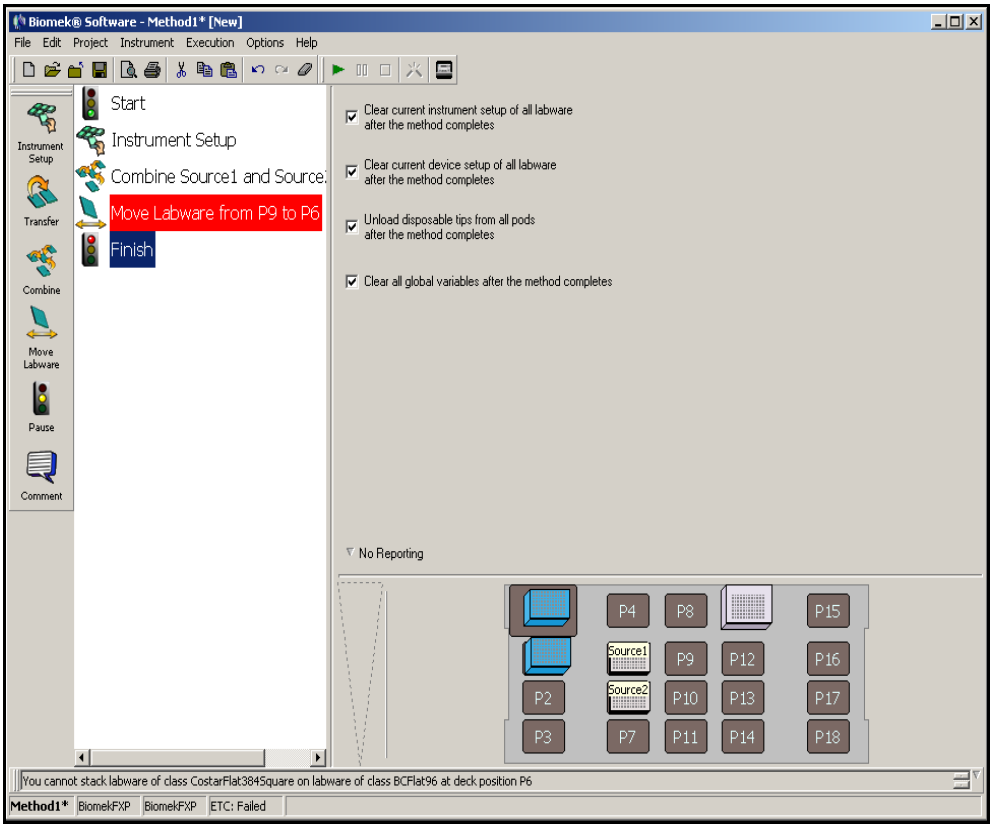

Figure 2-8. Main editor with error displayed

2.5 Responding to an Error Message

For this activity, you'll learn how to locate and respond to the error message you just received.

2.5.1 Locating the Error

Biomek Software alerts you to errors in several ways, including:

- The step with the error appears in red in the Method View when any step following the error is highlighted.
- A tool tip describes the error when you hover the cursor over the step causing the error.
- An associated red error message is displayed in the status bar at the bottom of the editor.
- The error is displayed in an error bar just above the status bar.

There are other errors that are displayed in error message boxes. These errors state the problem and display appropriate recovery options. Refer to the *Biomek Software User's Manual,* Chapter 26, *Handling and Preventing Errors*.

2.5.2 Correcting the Error

In this tutorial, the error message is You cannot stack labware of class CostarFlat384Square on labware of class BCFlat96 at deck position P6. This means you've configured the software to stack labware on top of labware that is not allowed by the software. You'll learn more about stacking labware on top of other labware in Chapter 3. So for now, we'll correct the error.

To correct the stacking error:

1. Click on the **Move Labware** step in the Method View.
2. In the to field, change the position to **P14**.
3. Click the **Finish** step again in the Method View.

There, the error has been corrected.

Biomek Software continually validates the steps as you progress through building your methods. When you highlight any step, the steps above that point in the method will be validated. If an error is encountered, the step causing the error will be highlighted in red.

2.6 Adding Labware During a Method Run

Suppose that you want to add another reagent to the destination plate, but you don't want to place the second reagent on the deck until after the first Transfer is complete (perhaps it can only be exposed to light or open air for a very short time). You can do this by adding a second Instrument Setup step to your method to indicate to the software that there is more labware now on the deck.

But before you add a second Instrument Setup step, you will:

- Move the pod to a new location by configuring a Move Pod step to make sure the pod is moved to a part of the deck where it won't prevent you from physically adding more labware.
- Pause the instrument by configuring a Pause step to give you enough time to physically add more labware.

2.6.1 Moving the Pod to a New Location

The Move Pod step on the Intermediate step palette repositions the pod away from the positions on the deck you want to reach manually. Since you are going to be adding more labware to the deck to prepare for another liquid transfer, you will need to move the pod away from the deck locations affected before pausing the system and adding more labware.

However, since the Move Pod step is on the Intermediate step palette, you will need to display that step palette before you can add this step to your method.

2.6.1.1 Displaying the Intermediate Step Palette

BIOMEK CONCEPT
Step Palettes

Steps are grouped on step palettes based upon the complexity of the operations they control and the depth of knowledge required to configure them, athough some steps are grouped on step palettes according to the specific pod or instrument they control.

To add the Intermediate step palette to the main editor:

1. Right-click anywhere in the space below the Basic step palette.
2. Choose **Intermediate**. Your main editor now looks like Figure 2-9.

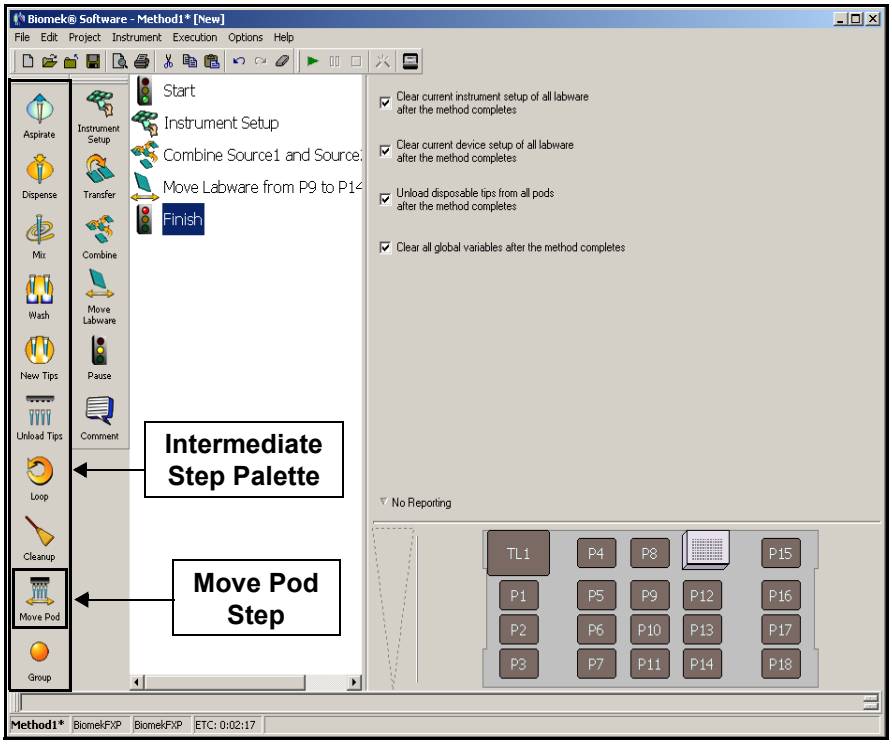

Figure 2-9. Intermediate step palette displayed

2.6.1.2 Configuring the Move Pod Step

To configure the Move Pod step:

1. Insert the **Move Pod** step in the Method View after the Move Labware step (Figure 2-9).
2. From the Location drop-down menu, choose **WS1**. This instructs the pod to move and stop over the 96-Channel Wash ALP (WS1) (Figure 2-10).

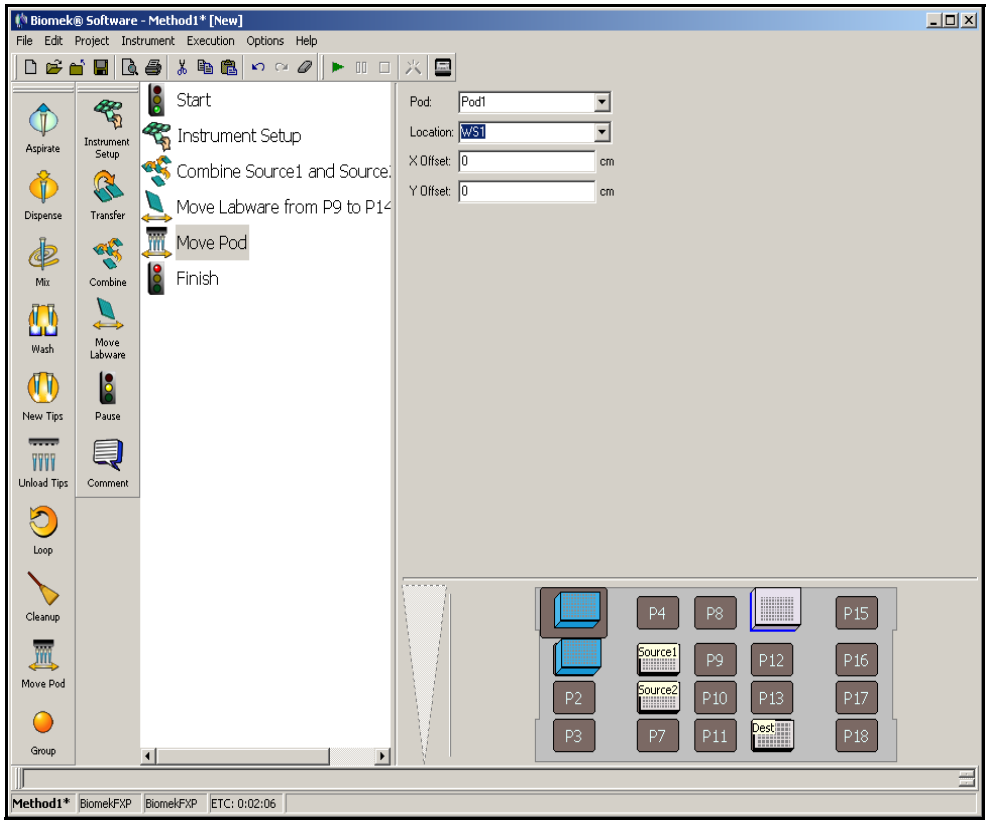

Figure 2-10. Configured Move Pod step

3. Click **Finish** to validate the method.

2.6.2 Pausing the Instrument

The instrument may be paused during a method run for either a specified amount of time or for an indefinite period of time by adding and configuring a Pause step. Depending on the purpose of the pause, you configure the step in one of the following ways:

- If you want to incubate a piece of labware in a specific position for a specific amount of time, you configure the desired time in seconds that you want that position to be idle and unavailable for interaction with the instrument.
- If you want to move labware manually during a method (either move it around on the deck, or remove it from the deck to take it to a device, such as a reader), you configure the step to pause the instrument for an indefinite period of time.

When the Pause step occurs during a run, the pod remains in the position of the last operation.

2.6.2.1 Configuring the Pause Step

You will configure the Pause step for indefinite amount of time to add more labware to the deck manually. The software will display a message on top of the main editor until you complete your manual operations, at which time it will resume the method run.

To configure the Pause step for an indefinite amount of time:

1. Insert a **Pause** step into the Method View below the Move Pod step.
2. Choose **Pause the whole system and display this message:**
3. Type in the message: **Remove the two used tip boxes at TL1 and P1 and place a new AP96 20µL on TL1. Place a reservoir containing a Known volume of 100000 µL water at P7.** The main editor should now look like Figure 2-11.

Tip

If you physically move the pod during a Pause step, then when you choose OK on the prompt to unpause the system, the pod returns to the correct place to continue the method.

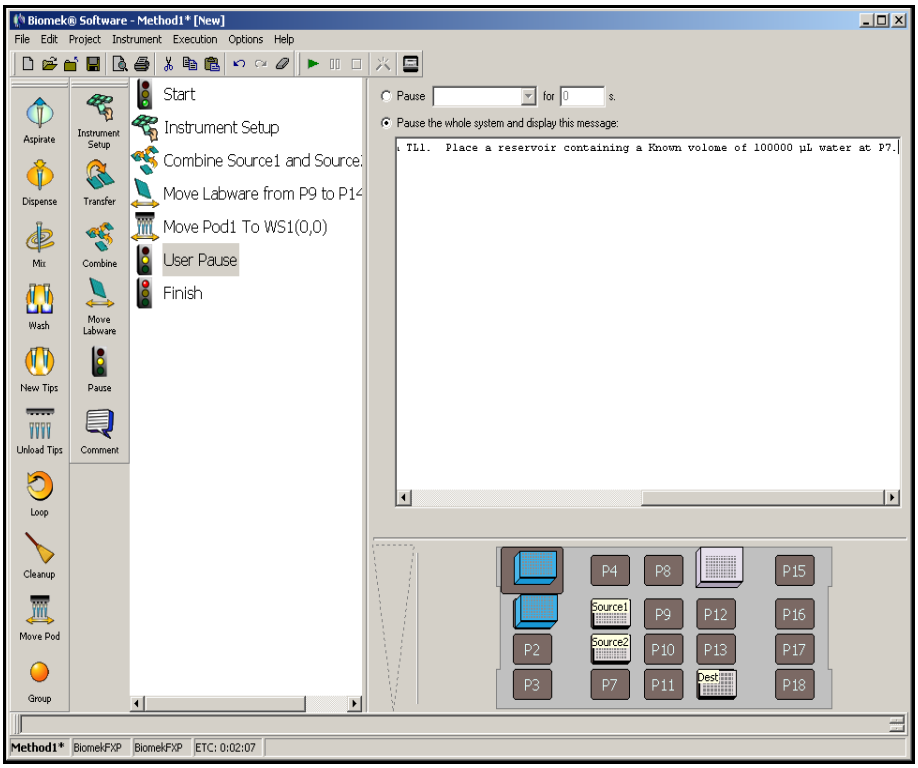

Figure 2-11. Pause configuration with message inserted

4. Click **Finish** to validate the method.

When the method is run, you will see a prompt similar to Figure 2-12 that will remain displayed until you choose **OK**.

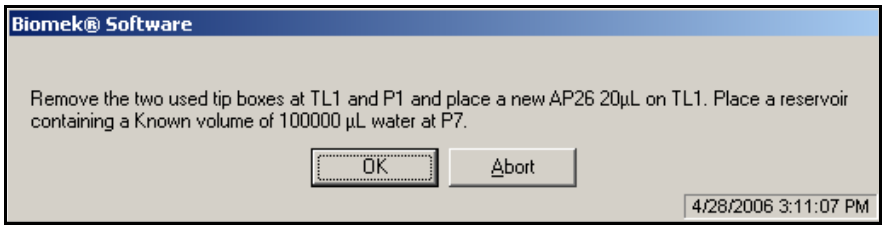

Figure 2-12. Pause prompt displaying the configured message

2.7 Adding a Second Instrument Setup Step

Now that you have prepared to add more labware to the deck during a method, you are ready to configure a second Instrument Setup step. You will insert a second Instrument Setup step after the Pause step. The second Instrument Setup step not only indicates the current state of the deck, but it also allows you to add more labware.

To configure the second Instrument Setup step:

1. Insert an **Instrument Setup** step into the Method View below the User Pause step. This opens a second Instrument Setup step configuration.
2. Choose **Toggle** under the As Is square. This lets the software know that all deck positions are to remain as they are. The main editor should now look like Figure 2-13.

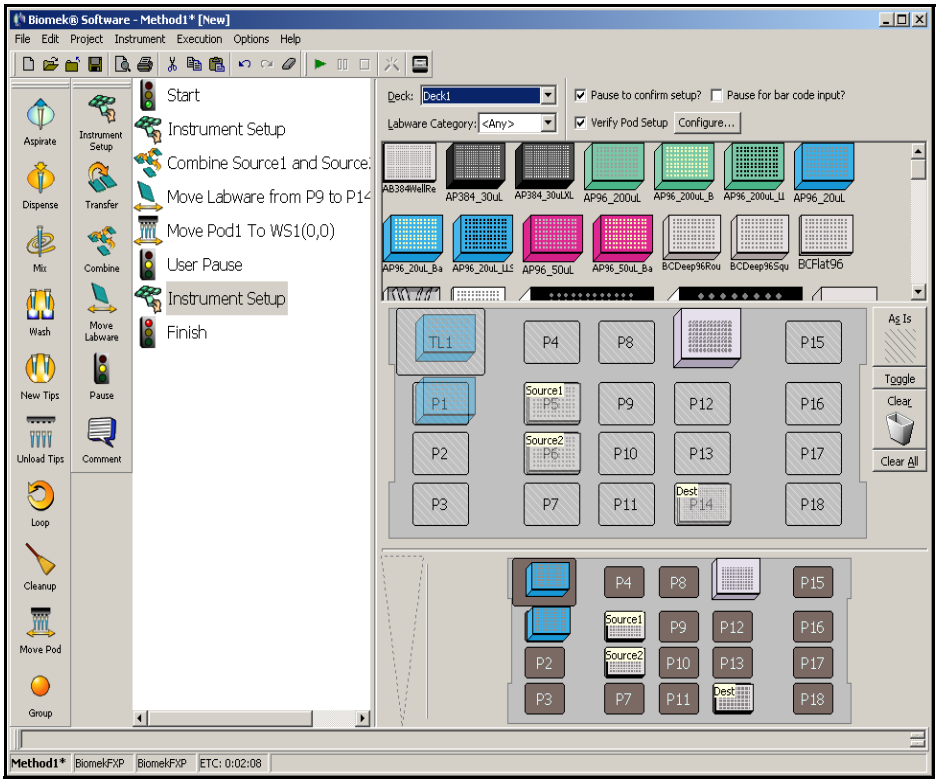

Figure 2-13. Adding an Instrument Setup step and toggling all deck positions As Is

3. Select **Clear** and then click on **TL1** and **P1**. This removes the used tip boxes. Now the main editor should look like Figure 2-14.

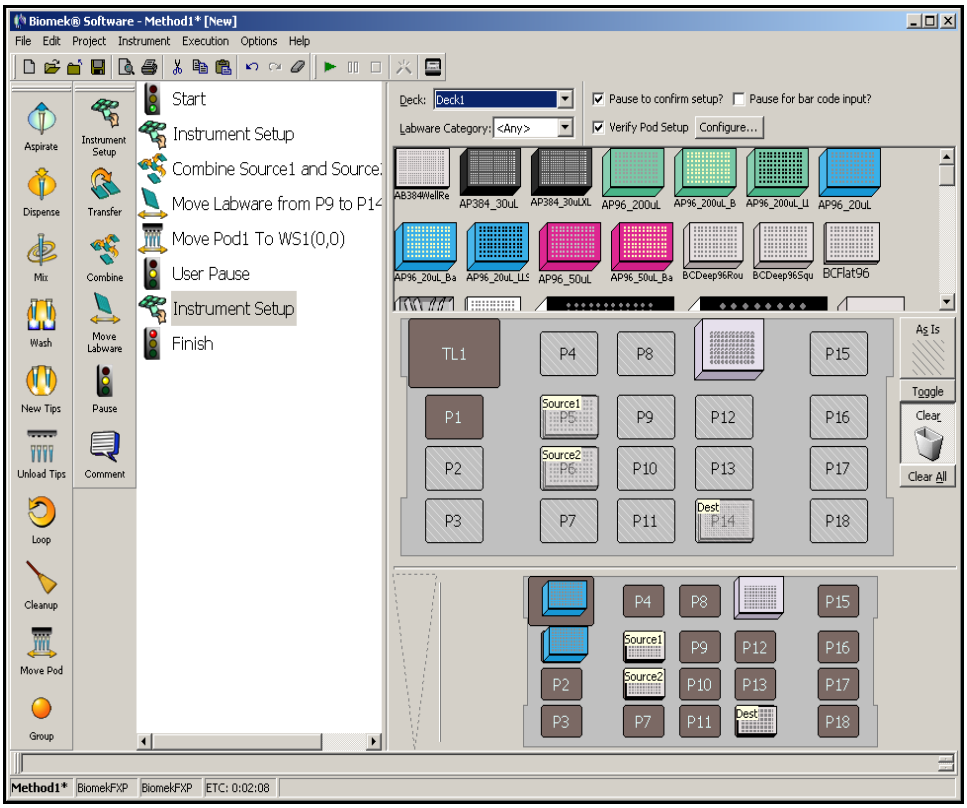

Figure 2-14. Using Clear to remove used tip boxes

2.7.1 Adding Labware to the Deck

Now you will add the labware to prepare for next liquid-handing process which is a Transfer step using the 96-Channel Multichannel Head with 384-well labware. For this next liquid-handling process, you will need to add another tip box and an additional source reservoir. This new labware was what you configured to display when the instrument paused for manual placement.

1. Place a **AP96 20µL** tip box on TL1.
2. Place a **Reservoir** on P7 and configure it to have a **Known** volume of **100000** of **Water**.

Now you are ready to configure your next liquid-handling procedure using the 96-Channel Multichannel Pod with 384-well plates.

Tip

Even though the software finds tips automatically, you have to ensure there are enough tips on deck to do the job. If you don't, you will get an error message.

2.8 Transferring to 384-Well Plates Using a 96-Channel Pod

BIOMEK CONCEPT
Quadrants

Quadrants allow a 96-channel pod to access 96 wells in a 384-well plate, or a 384-channel head to access 384 wells of a1536-well microplate, simultaneously. The 384-well and 1536-well microplates are divided into four sets of equally spaced wells or quadrants. Using quadrants, it would take four passes of the Multichannel Pod to access all wells of a microplate.

Biomek Software gives you the ability to transfer liquid between 96-well and 384-well plates using a 96-channel pod or 384-well and 1536-well plates using a 384-channel pod. You can select quadrants in microplates as sources or destinations.

An illustration of the quadrants is displayed in Figure 2-15.

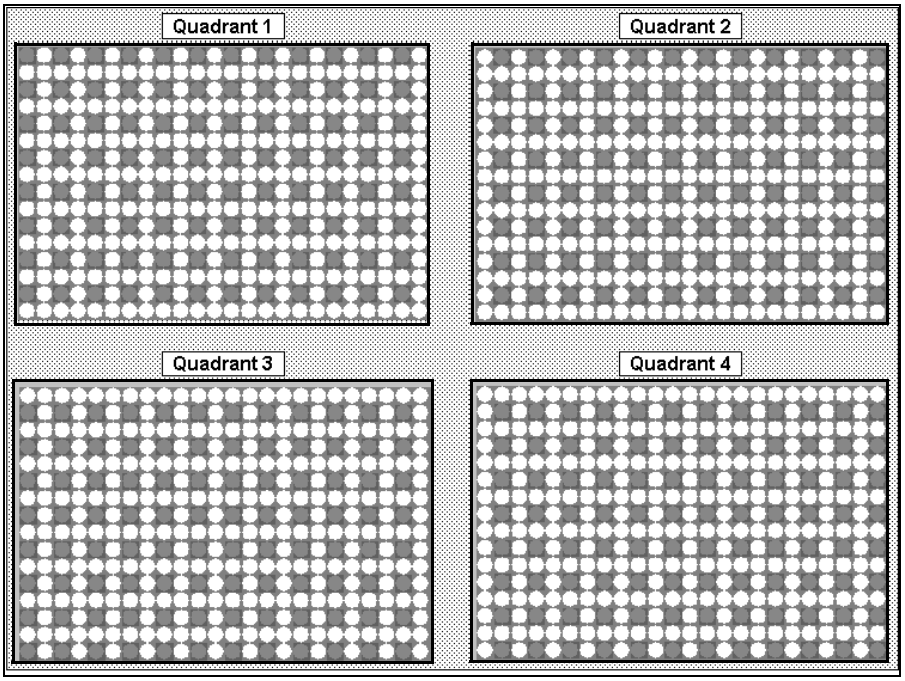

Figure 2-15. Illustration of the quadrants in a 384-well microplate

2.8.1 Configuring Quadrants in a Transfer Step

To learn how to use and configure quadrants, you will need to add and configure a new Transfer step to the method after the second Instrument Setup step.

1. Insert a new **Transfer** step after the second Instrument Setup step in the Method View. The Transfer step configuration appears.
2. In Tip Handling, make sure that **Change tips between sources** and **Change tips between destinations** are deselected. This will mean that only one box of tips is used to complete this transfer.
3. Select the **Reservoir** on P7 as the source labware.
4. Select **Dest** on P14 as the destination labware and specify a volume of **50** µL.
5. Double-click on the graphic of **Dest** to zoom in. This is where you will select Quadrant 1 and Quadrant 4.
6. Click the first well of the first column and first row. Quadrant 1 is selected.

7. Hold down **Ctrl** or **Shift** and click on any well that is in Quadrant 4. The main editor should look like Figure 2-16.

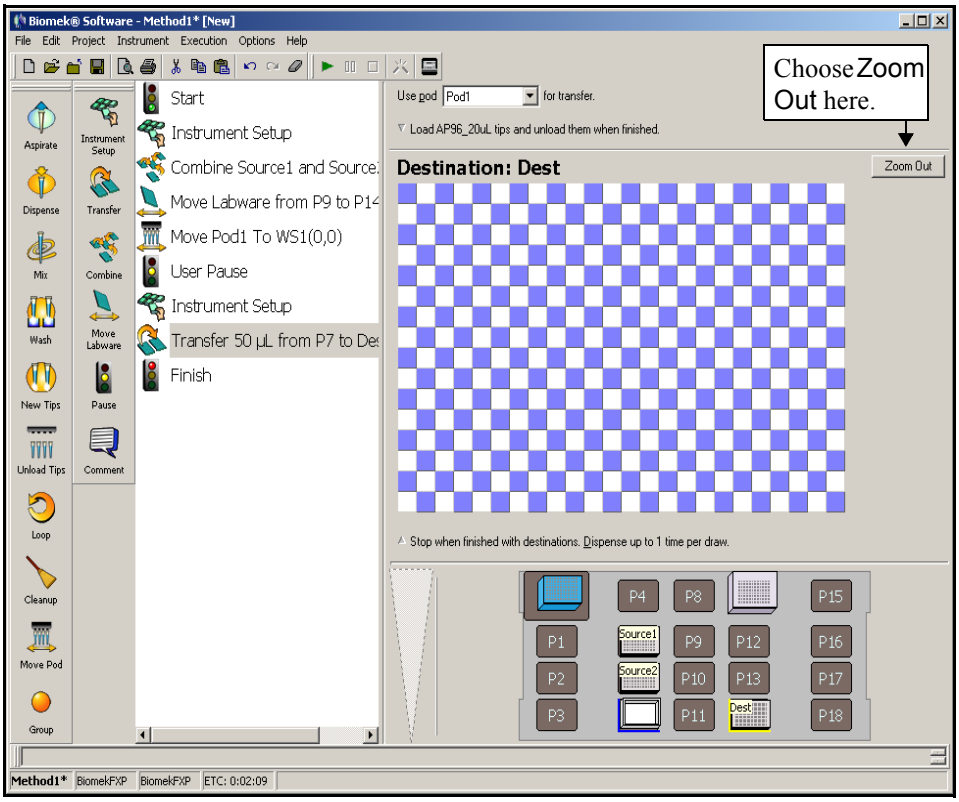

Figure 2-16. Quadrants 1 and 4 chosen in destination labware

8. Choose **Zoom Out**. The graphic returns to the zoom out display.
9. Highlight the **Finish** step to validate the method.
10. Run the method to see how the Multichannel Pod transfers liquid using quadrants.

As you can see, all the steps in the Method View make your method look complex. Go to the next section to learn what you can do about that.

2.9 Using a Group Step

BIOMEK CONCEPT
Group Step

The Group step allows you to "nest" a series of connected steps together, and give the group a logical name that appears in your Method View. Then, when you open your method, the Group step appears collapsed with the connected steps hidden. This makes the method appear shorter, and it allows you to see more of the method without scrolling the Method View up or down. You simply double-click on the Group step in the Method View to expand it and expose the nested steps.

To prevent your method from appearing too complex, you can group steps together logically under one heading by inserting the Group step into the Method View. This group of steps is hidden in the Method View under the name that you've given it during configuration of the Group step.

2.9.1 Configuring the Group Step

For this activity, you will group under one heading the Move Pod, Pause, and Instrument Setup steps you already have in your method. These steps are all associated with the preparation for the liquid transfer using quadrants.

To group these steps:

1. Insert the **Group** step from the Intermediate Step Palette in the Method View after the Move Labware step.
2. Double-click on the **Group** step to reveal the next End Group step. Scroll the Method View to the left if necessary.
3. In Group Label of the step configuration, enter **Pause to add labware** (Figure 2-17). After the Group step is configured and another step later in the method is highlighted, it will be named in the Method View whatever it has been labeled in Group Label. In this example, the Group step will be named Pause to add labware in the Method View (Figure 2-18).

Tip

If you want to use a configured Group step in other methods, drag and drop the labeled Group step from the Method View to any step palette on the main editor. It will then always be available on that step palette for use in other methods. This works for any configured step that will be used in other methods.

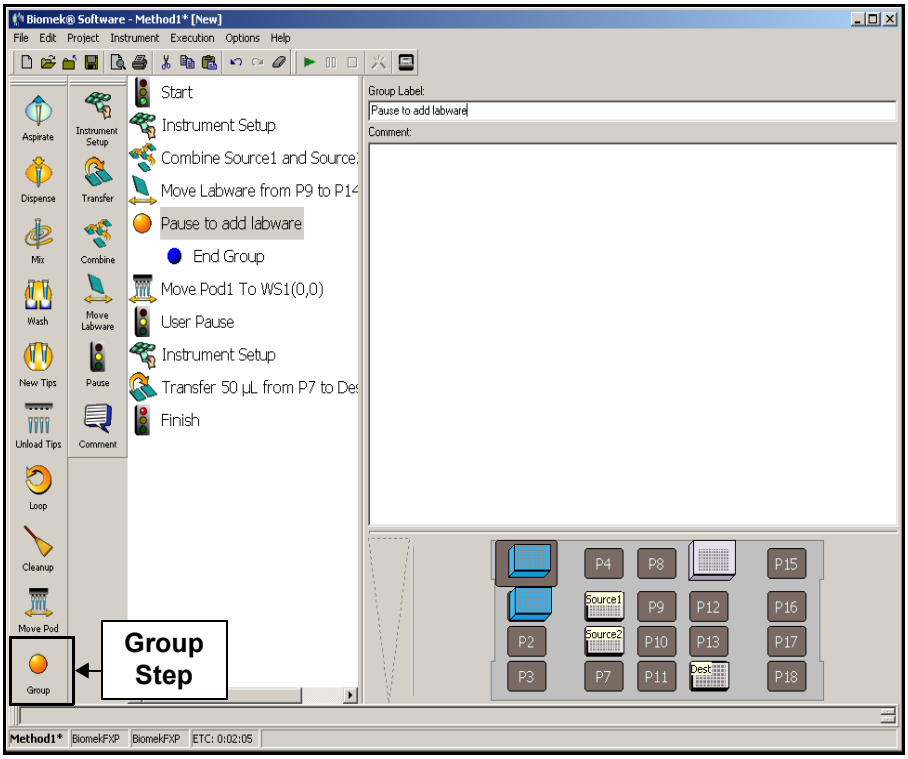

Figure 2-17. Configured Group step

4. Highlight the **Move Pod** step, then drag and drop it into the Group step above End Group.

Tip

Like the Group step, the Comment (Figure 2-18) step does not initiate any actions on the instrument. It is used to provide descriptive information and notes in the Method View for a method. Refer to the *Biomek Software User's Manual,* Section 16.6, *Comment Step*.

5. Repeat step 4 above for the User Pause, and then the second Instrument Setup step. Make sure the nested steps are in the order shown on Figure 2-18 since it's easy to get the steps out of order when you are dragging and dropping them.

Now the Move Pod, User Pause, and second Instrument Setup steps are nested logically within a Group step (Figure 2-18). You can expand and collapse this step as desired by double-clicking on the Group step.

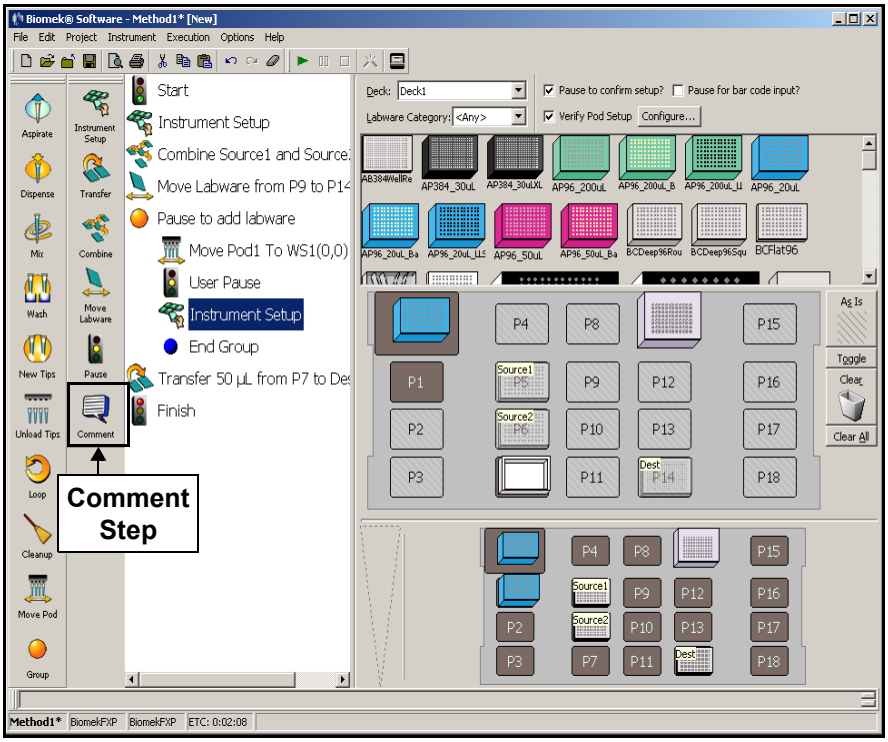

Figure 2-18. Configured Group step with nested steps expanded

Go to the next section to learn how to use Single Step to view each operation of this method.

2.10 Performing Single Operations with the Biomek FX

BIOMEK CONCEPT
Single Step

Single Step pauses the Biomek instrument between each operation in a step, allowing visual verification that the operation is correct. Performing single operations can help when fine tuning a method.

In this activity, you will use Single Step to view each operation of the method you just created.

1. Choose **Execution>Single Step**. Single Step appears (Figure 2-19).

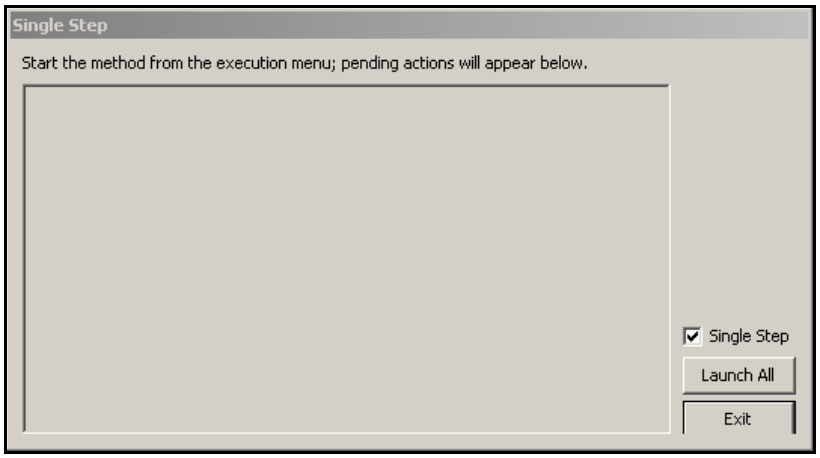

Figure 2-19. Single Step

Tip

Go slowly when using Single Step. It is possible to move too quickly through the method and by-pass the steps that need verification.

2. Choose **Execution>Run**. Single Step with specific operations displayed appears (Figure 2-20). If you have other ALPs configured on your deck, the initialization process for them appears in Single Step.

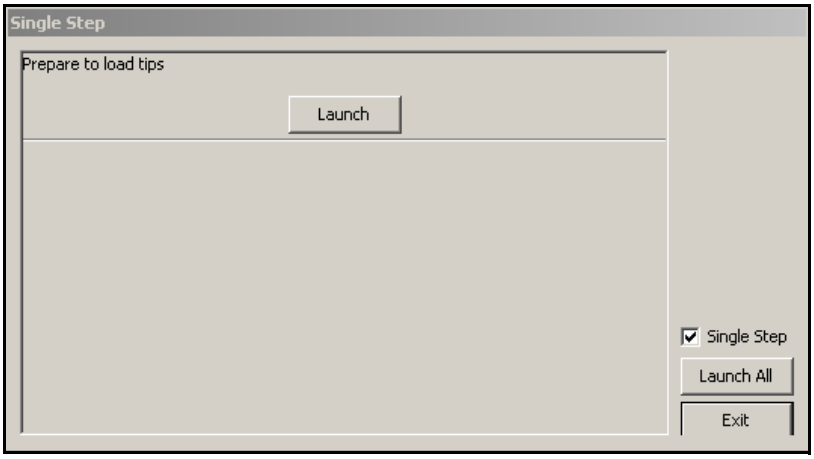

Figure 2-20. Single Step with specific operations displayed

3. Under Prepare to load tips, choose **Launch**. The next operation is launched (Figure 2-21).

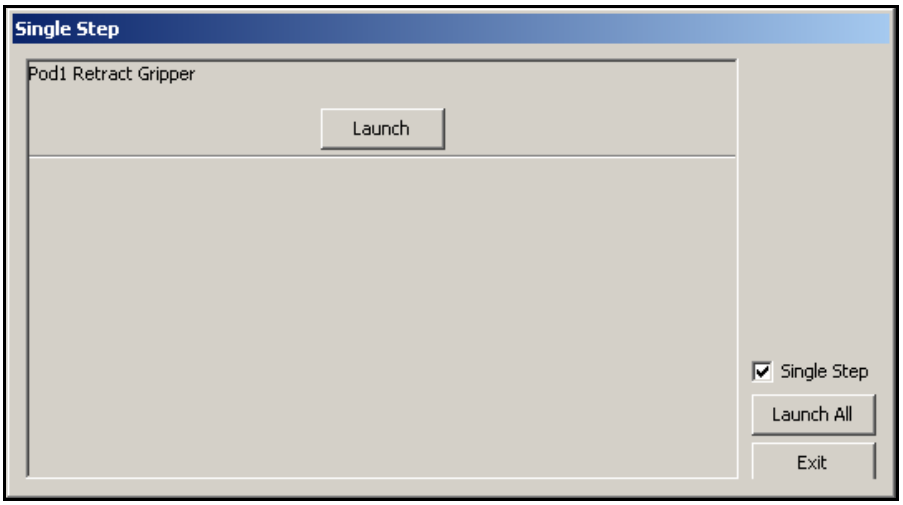

Figure 2-21. Single Step with next operation launched

4. Choose **Launch** again. The Biomek prompt appears. If the physical deck matches the software prompt, choose **OK**. Single Step continues displaying each operation, along with the option to Launch and execute that operation.
5. Continue to view each operation by choosing **Launch** or to stop Single Step, choose **Exit** to allow the method to run without the option to view each operation.
6. If you wish, save this method.

Now move on to the next chapter of this tutorial to learn how to use individual steps to exercise precise control over transferring liquid.

3 Multichannel Pod—Using Individual Steps to Transfer Liquid

3.1 Introduction to Using Individual Steps

In the previous chapters of this tutorial (refer to Chapter 2, *Multichannel Pod—Using More Steps in a Method*), you learned how to:

- Transfer liquid from two sources to a single destination.
- Mix contents in labware.
- Move labware on the deck using the gripper tool on the Multichannel Pod.
- Respond to errors.
- Add more labware to the deck once a method has started to run.
- Use the Multichannel Pod with a 96-channel head with 384-well plates.
- Group steps logically in the Method View.
- Perform single operations with the Biomek FX.

If you already know how to complete these tasks in Chapter 2, *Multichannel Pod—Using More Steps in a Method*, you can start with this chapter or subsequent chapters.

3.1.1 What You'll Learn in Using Individual Steps

This chapter will help you enhance your method-building skills to create more advanced methods using variables to "loop" or repeat tasks. You will also learn how to optimize deck space using stacks and lidded plates and how to conserve tip usage by washing tips.

More specifically, the step-by-step instructions in this chapter will teach you how to:

- Aspirate and dispense liquid independently using individual steps.
- Use a Loop step to repeat actions.
- Load and unload tips independently using individual steps.
- Wash tips at the 96-Channel Tip Wash ALP during a method.
- Handle lids in a method.
- Stack plates in a method.

3.1.2 Setting Up Your Deck for Using Individual Steps

Launch Biomek Software and using an Instrument Setup step, configure the following:

- Place an **AP96_20uL** tip box on TL1.
- Place a **BCFlat96** microplate on P5. Name it **Source1** and configure it to contain a **Known** volume of **300** µL of **Water**.
- Place a **CostarFlat384Square** microplate on P9. Configure it to contain a **Known** volume of **0** µL.

Your deck should look like Figure 3-1. Now go to the next activity to learn how to create a new method using individual transfer steps.

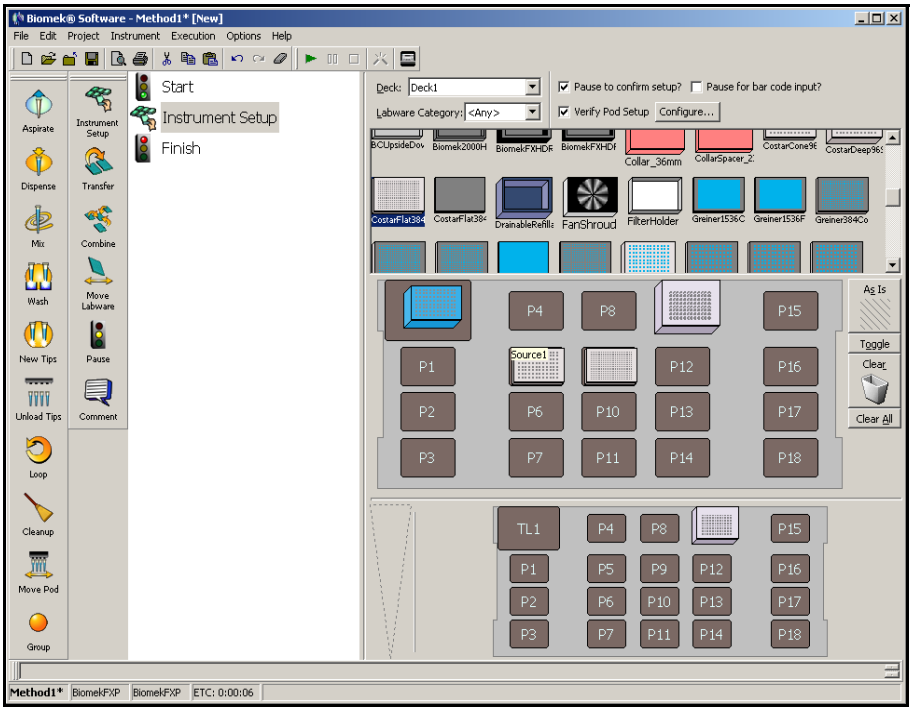

Figure 3-1. Initial Instrument Setup for using individual steps

3.2 Using Individual Steps to Transfer Liquid

In previous chapters, when you wanted to transfer liquid from one plate to another, you used the Transfer and Combine steps to perform all the necessary actions—loading tips, aspirating and dispensing liquid, and unloading tips. Sometimes, however, you want more direct control over these actions; for example, precise control over the order in which samples are transferred or when tips are loaded, unloaded, and washed.

When you need more control over the liquid transfer operation than the Transfer and Combine steps provide, you can configure liquid transfers using individual steps. Unlike the Transfer and Combine steps, these individual steps each perform only one task (e.g., aspirate, dispense, load tips, wash tips, or unload tips).

In this section, you will use the Aspirate and Dispense steps to transfer liquid from the 96-well source plate to the 384-well destination plate.

3.2.1 Aspirating Liquid Using the Aspirate Step

You can use the Aspirate step to aspirate liquid from a microplate or reservoir.

To aspirate liquid from the 96-well plate using the Aspirate step:

1. Ensure you configured the deck according to the instructions in Section 3.1.2, *Setting Up Your Deck for Using Individual Steps*.
2. Drag an **Aspirate** step from the Intermediate step palette to the Method View and drop it after the Instrument Setup step.
3. In the Current Deck Display, click on **Source1** to select it as the labware from which to aspirate.

Tip

Make sure you use an Aspirate step from the Intermediate step palette and not a Span-8 Aspirate step which is used only with the Span-8 pod.

4. In Volume, enter **60** µL.
5. Select **Refresh Tips** (Figure 3-2). This means that at the beginning of the step, before aspirating from the source plate, the pod will load new tips.

Tip

If tips are already loaded, it unloads those tips (along with any liquid in those tips if they are not empty) and loads new tips prior to aspirating. This option should be used only when there are either no tips loaded or the tips are empty.

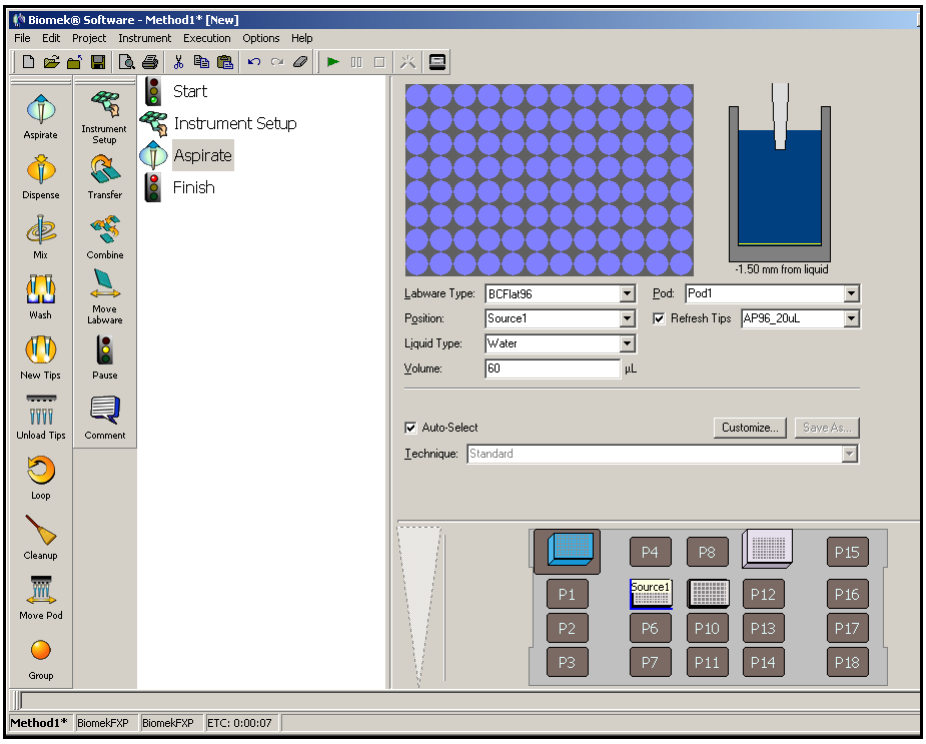

Figure 3-2. Aspirate step configured

3.2.2 Dispensing Liquid Using the Dispense Step

Now that you have aspirated some liquid, you need to dispense it into another piece of labware. In this tutorial, you will dispense the aspirated liquid into one quadrant of a 384-well plate.

To dispense previously aspirated liquid:

1. Drag a **Dispense** step from the Intermediate step palette to the Method View and drop it after the Aspirate step.
2. In the Current Deck Display, click on the 384-well plate on position P9 to select it as the destination.

Tip

Make sure you use a Dispense step from the Intermediate step palette and not a Span-8 Dispense step which is used only with the Span-8 pod.

3. In Volume, enter **60** µL. You will dispense the full amount of liquid you aspirated earlier in the Aspirate step.
4. In the graphic of the 384-well plate, select the second quadrant. Your Dispense step configuration should look like Figure 3-3.

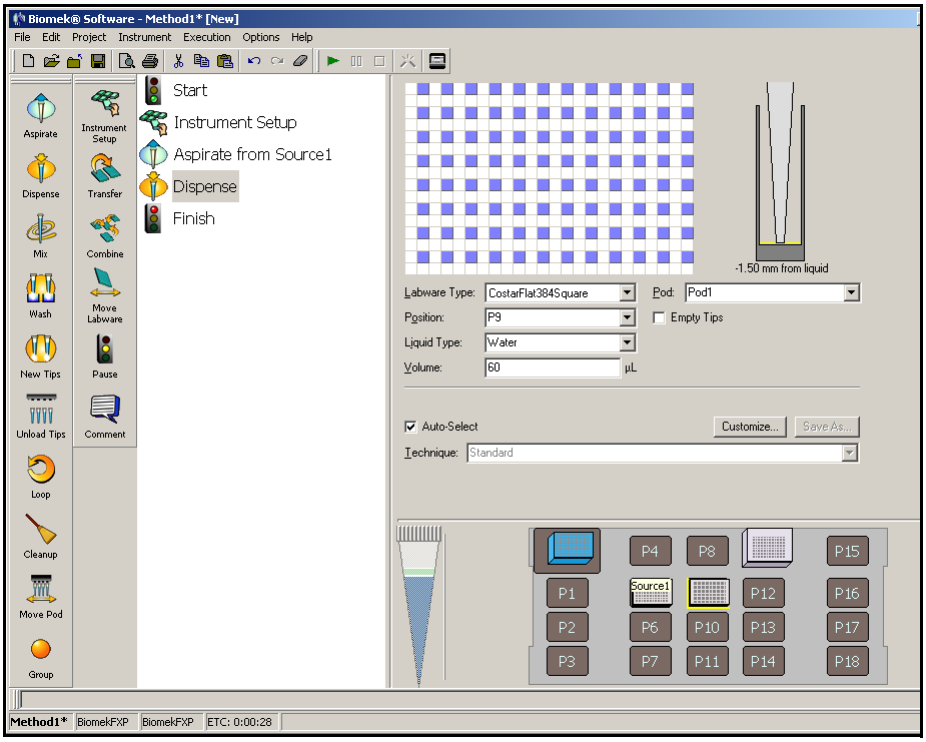

Figure 3-3. Dispense step configured

You have now configured a simple method that aspirates from a 96-well source plate and dispenses into one quadrant of a 384-well destination plate using individual steps. If you like, you can run this method on your Biomek FX instrument or in the simulator (refer to Chapter 1, *Multichannel Pod—Getting Started With Biomek® Software*, for more information on how to do this).

In the next section, you will reconfigure this simple method to use a variable for the volume to aspirate and dispense.

3.3 Using Variables in a Method

BIOMEK CONCEPT
Variables

Using a variable provides several advantages:

- If you want to change a value that is used in several places within a method, you can change it in one place and it is automatically changed everywhere that variable is used.
- The value of a variable can be set at run time and the method is automatically updated appropriately.
- Decisions can be made at run time based on the value of a variable (you will do this in the next chapter).

Variables make it easier to modify a method. When configuring steps, you enter the name of the variable in the desired field; when the method is run, the actual value of the variable is substituted and the action executed.

You will now create a variable for the volume to transfer and use it in the Aspirate and Dispense steps. This includes:

- *Creating a Variable in the Start Step* (Section 3.3.1).
- *Using a Variable in Step Configurations* (Section 3.3.2).
- *Changing the Value of a Variable at Run Time* (Section 3.3.3).

3.3.1 Creating a Variable in the Start Step

The Start step, in addition to being the first step in the method, also can be used to create and name variables that can be used throughout the method. Variables named in a Start step may be used in configuring other steps in the method. Other steps in Biomek Software also allow you to create variables, but those variables are particular to the steps in which they are created. You will work with those variables later in the chapter with the Loop step.

You will now create a variable for the volume to transfer using the Start step.

To create a variable in the Start step:

1. Select the **Start** step in the method view to display its configuration.
2. In Variable Name, enter **Vol**. Don't place a period after Vol.
3. In Value, enter **60**. Your Start configuration should look like Figure 3-4. You have created a variable named *Vol* that has a value of 60.

Tip

Do not use the variable C_Volume. This variable is built into Biomek Software and is associated with tracking volume.

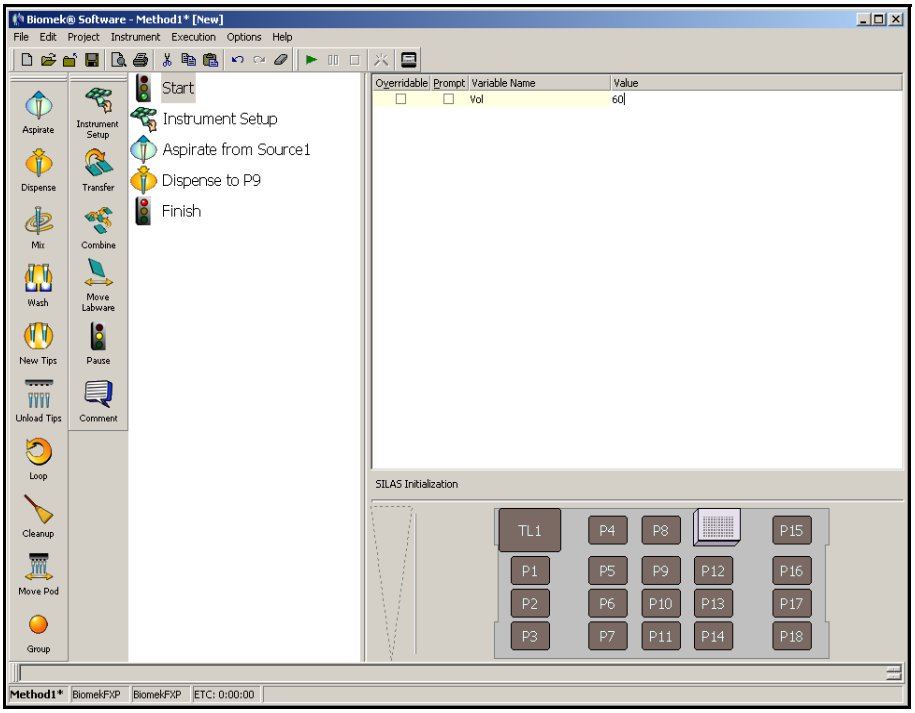

Figure 3-4. Vol variable created in the Start step

3.3.2 Using a Variable in Step Configurations

You will now use the *Vol* variable you created in the Start step to configure the volume to transfer in the Aspirate and Dispense steps.

To use a variable in a step configuration:

1. Select the **Aspirate from Source1** step.
2. In Volume, enter **=Vol** (including the equal sign), as shown in Figure 3-5. Variables are always preceded by an equal sign when entering them into a step configuration field. When the method is run, *Vol* is replaced by the value of the variable; in this case, 60.

Tip

Variable names are not case sensitive, so entering the name VOL, vol, Vol, or VoL are all evaluated the same.

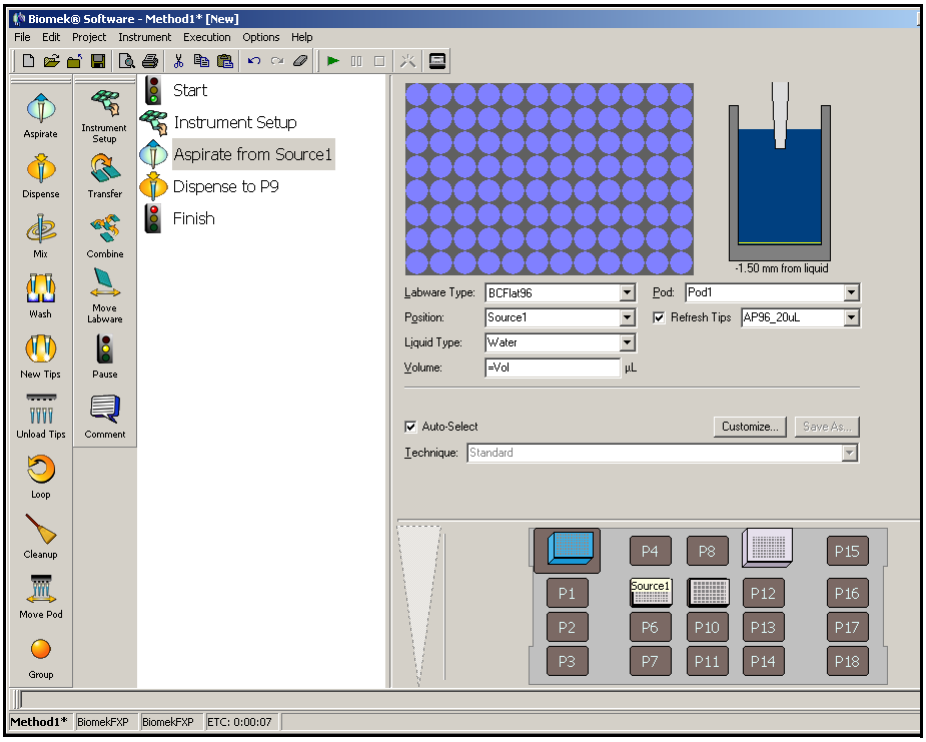

Figure 3-5. Using the variable Vol to configure the Volume

3. Select the **Dispense to P9** step and enter **=Vol** in Volume. The method is now configured to transfer a volume equal to the variable Vol.

To change the volume you want to transfer, you would change the value of the variable *Vol* in the Start step. The Volume specified in both the Aspirate and Dispense steps would then automatically be updated when the value of *Vol* is substituted at run time.

3.3.3 Changing the Value of a Variable at Run Time

Since the value of a variable can be changed throughout the method by changing the value of the variable in the Start step, it makes it easy to quickly reconfigure methods that are similar except for one or two items.

For variables that are created in the Start step, you can configure Biomek Software to prompt you to specify a value for the variable at run time. When configured to do this, a prompt appears for each variable when the method is run. The value that you entered for the variable in the Start step is now the default value for the variable. You can use that value by choosing **OK**, or enter a new value and choose **OK** to change the value of the variable. The method run then uses the specified value for the variable and updates the method accordingly.

To specify the value of a variable at run time:

1. Select the **Start** step.
2. Select the **Prompt** check box for the variable Vol. Your Start configuration should now look like Figure 3-6.

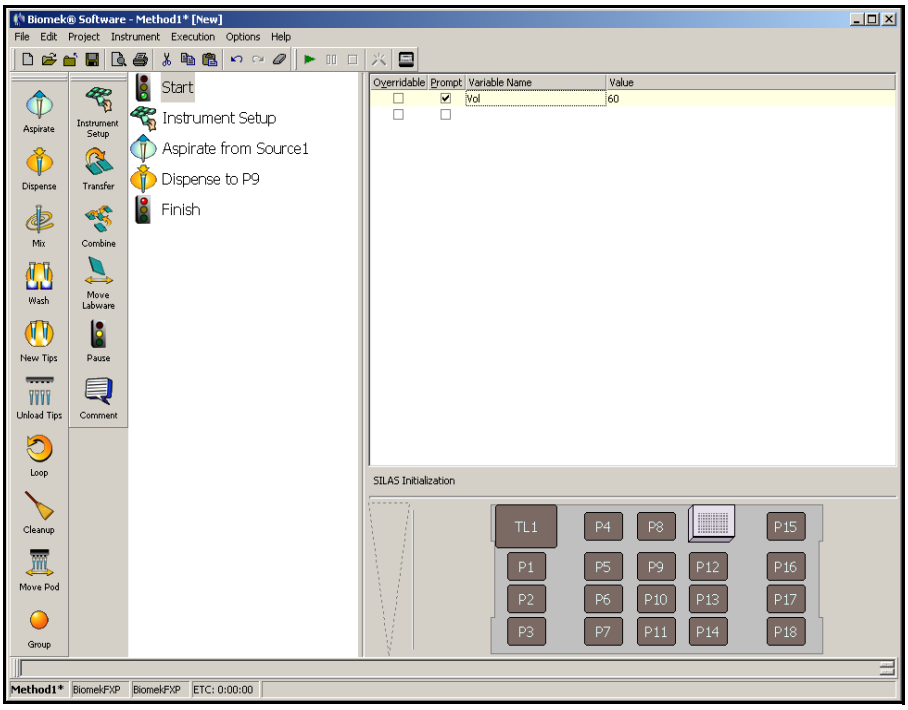

Figure 3-6. Prompting for value of a variable

3. Run the method. A prompt appears allowing you enter a value for the variable (Figure 3-7).

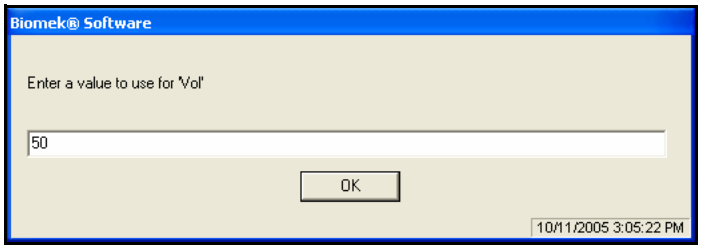

Figure 3-7. Prompt to specify the value of a variable

4. Enter **50** and choose **OK**. If you run the method on your Biomek FX instrument, you should notice that 50 µL was transferred into each well, not 60.

In the next section, you will learn to use variables and a Loop step to perform repeated actions in order to aspirate from four different source plates and dispense into all four quadrants of a 384-well plate.

3.4 Repeating Liquid Transfer Steps Using a Loop

BIOMEK CONCEPT
Loop Step

The Loop step repeats the nested step or steps until its End value is exceeded. For the first cycle of a Loop, its value is the Start value. After completing all steps inside the loop, the value changes by the Increment and the steps are repeated again. This process repeats until the Increment changes the value to be greater than the End value.

The Loop step enables you to repeat one or more steps for multiple cycles. Each cycle or iteration repeats the steps contained inside the Loop. An optional variable may also be created in the Loop step. This variable is assigned a start and end value and increments in regular intervals with each cycle of the Loop.

In this section, you will modify the method to use a Loop step to aspirate from four different 96-well source plates to each of the four quadrants of the 384-well destination plate. In completing this task, you will create a variable in the Loop step and use this variable to reconfigure the Aspirate and Dispense steps as the liquid transfer actions are repeated during method execution.

To do this you will need to:

- add more plates to the deck in the Instrument Setup step (refer to Section 3.4.1, *Modifying the Instrument Setup*).
- use a Loop step to repeat the aspirate and dispense actions (refer to Section 3.4.2, *Repeating Actions Using the Loop Step*).

3.4.1 Modifying the Instrument Setup

In order to transfer liquid from four source plates to the 384-well destination, you need to have four 96-well source plates on the deck. You will now modify your initial Instrument Setup step to include four source plates.

To modify the Instrument Setup step:

1. Select the Instrument Setup step in the Method View to display its configuration.
2. Right-click the **Source1** source plate at position P5 and select **Copy**. When you copy labware like this, all of the labware properties for that piece of labware, including the name and volume information, are also copied.

3. Click on the empty positions **P4**, **P6**, and **P7** to paste copies of Source1 at those positions.
4. Rename the Source1 labware at positions P5, P6, and P7 to **Source2**, **Source3**, and **Source4**, respectively. You should now have four 96-well source plates in positions P4-P7 named Source1 to Source4 (see Figure 3-8).

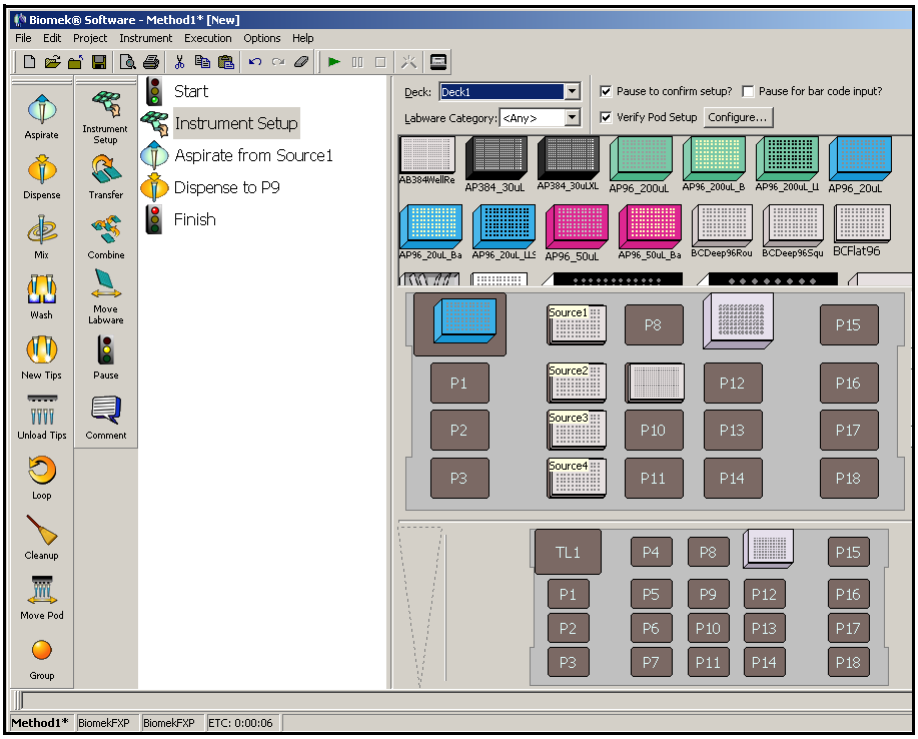

Figure 3-8. Modifying the Instrument Setup step to add more sources

3.4.2 Repeating Actions Using the Loop Step

When you want to repeat actions several times during a method run, you use a Loop step. The Loop step allows you to repeat the actions of one or more steps without inserting and configuring those steps for each time they are to be repeated. Steps to repeat are placed inside the Loop step, or nested.

Biomek Software internally tracks the value of the Loop for each cycle, and Start, End, and Increment values are specified. If desired, a name can be assigned to the Loop value to create a variable. This variable can then be used like any other variable to configure steps, but can only be used with steps contained within the Loop step.

Tip

The Loop step can also be used without using variables, such the variable quad in this example.

To repeat the Aspirate and Dispense steps:

1. Insert a **Loop** step after the Instrument Setup step. The Loop and End Loop icons appear in the Method View, and the Loop step configuration is displayed.
2. In Variable, enter **quad**. This will create a variable named *quad* that can be used to configure steps within the Loop. See the sidebar Tip.
3. In Start, enter **1**. This will be the initial value assigned to the variable *quad* on the first iteration of the loop.
4. In End, enter **4**. This will be the final value for the variable *quad*. When the value of *quad* exceeds the End value, the Loop step ends and the remainder of the method continues.

5. In Increment, enter **1**. The Increment value is how much the value for the variable *quad* changes with each iteration of the Loop. Your Loop step configuration should look like Figure 3-9.

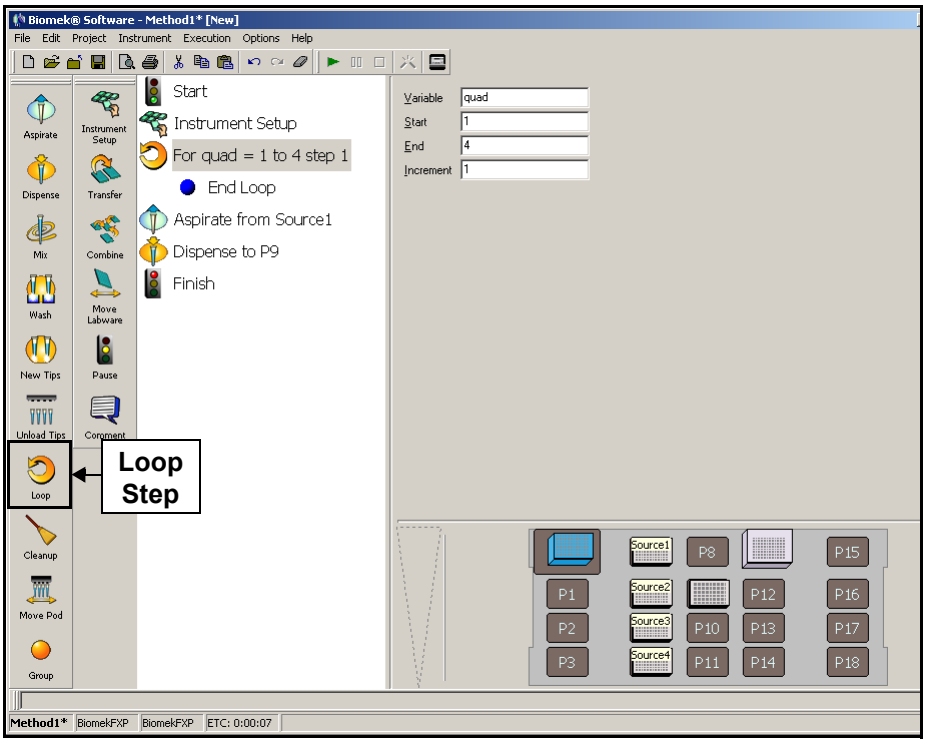

Figure 3-9. Loop step for repeating aspirate and dispense

6. Drag the **Aspirate** and **Dispense** steps between the Loop and End Loop icons.

3.4.3 Using Variables in Expressions

Now that you have configured the Loop step to create the variable *quad* and placed the Aspirate and Dispense steps inside the Loop, you can use the variable *quad* to reconfigure the Aspirate and Dispense steps. You will now use the variable *quad* to configure:

- the source plate from which to aspirate.
- the quadrant on the destination plate to which to dispense.

BIOMEK CONCEPT
Expressions

Expressions combine text, numerical constants, and variables using operators to modify a variable. These operators may perform a number of mathematical operations or combine text strings. Just like with variables, the expression is evaluated and the resulting value is substituted for the expression at run time.

Sometimes, however, the variable by itself is not enough, and you need to use an expression to modify the variable. This is the case to specify the source plate in the Aspirate step. As you recall, the four 96-well source plates are named Source1 Source2, Source3, and Source4. The variable *quad* has values of 1, 2, 3, and 4 for each cycle of the loop.

To specify which plate to aspirate from, you need to append the word "Source" in front of the value for the variable *quad*. This can be accomplished using the operator **&**. This operator combines two strings. In this case, it will combine a constant string "Source" with the value of the variable *quad*. To do this:

1. In the Aspirate step configuration, change the Position to **="Source"&quad** (including the equal sign and with no spaces), as shown below in Figure 3-10. Values in quotes are text strings and are always evaluated as the same value.

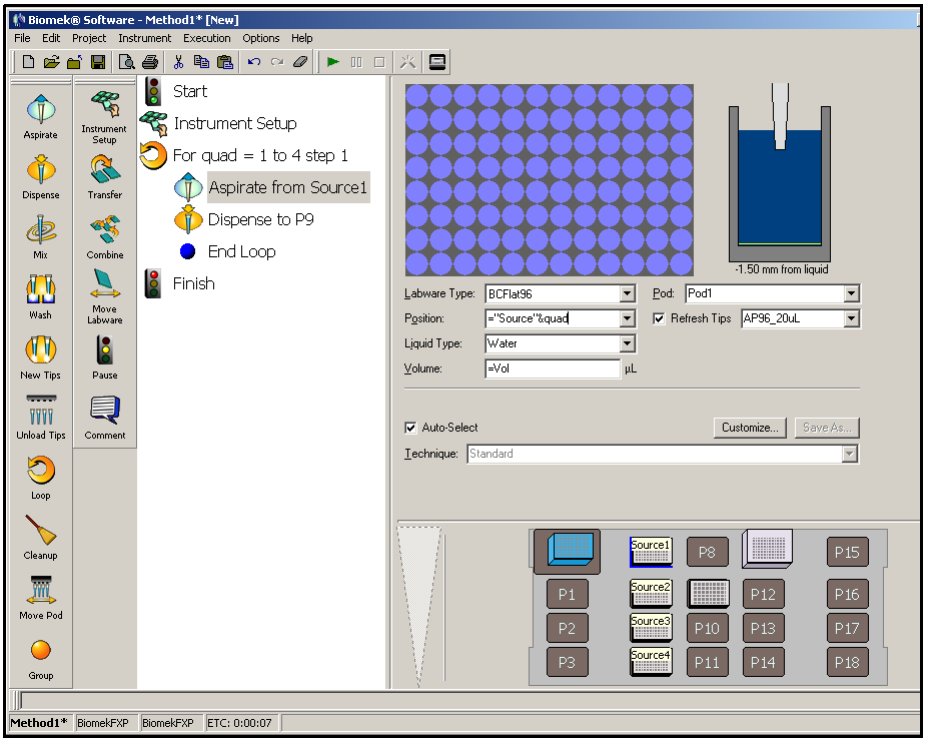

Figure 3-10. Aspirate step using Loop variable quad in an expression

2. In the Dispense step configuration, rather than specifying the wells to transfer to using the graphical representation of the 384-well plate, you will specify the destination wells using the variable quad. To do this, right-click on the graphical representation of the 384-well plate and choose **Specify Selection as Text** in the menu that appears (Figure 3-11). Text Selection is displayed (Figure 3-12).

Figure 3-11. Menu to select Specify Selection as Text

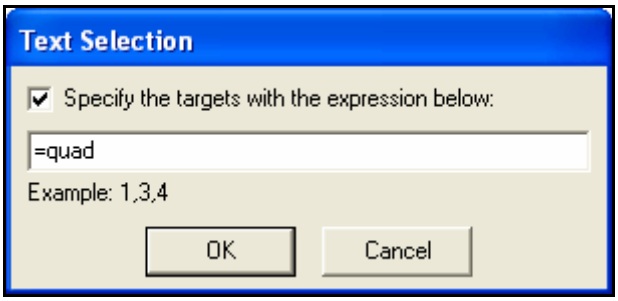

Figure 3-12. Text Selection

3. In Text Selection, enter **=quad**. This will specify the quadrant into which to dispense. Make sure that Specify the targets with the expression below is selected. This means that for the first iteration of the loop, it will dispense into quadrant 1; for the next iteration, it will dispense into quadrant 2; and so on.
4. Choose **OK**.

The Dispense step configuration looks like Figure 3-13. The graphical representation of the labware is grayed out to indicate that the target wells to dispense into are specified by text.

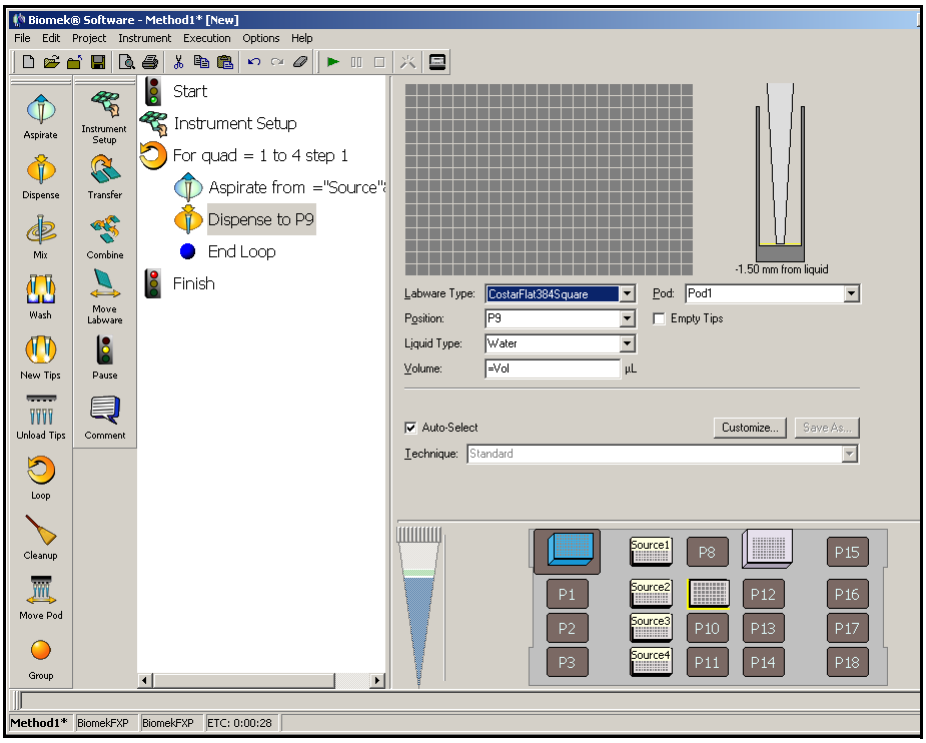

Figure 3-13. Dispense step inside the Loop

3.5 Conserving Tips Using Individual Steps

As the method is currently configured, an error would result because there are not enough tip boxes on the deck. This is because the Aspirate step tries to load tips for each iteration of the Loop, but there is only one tip box on the deck. You will fix this, and conserve tips while doing so, by loading the tips before the Loop and reusing them for each iteration of the Loop. If you have a wash ALP, you will then configure the method to wash the tips after each dispense.

Conserving tips includes:

- *Loading and Unloading Tips Outside the Loop* (Section 3.5.1).
- *Washing Tips Inside the Loop* (Section 3.5.2).

3.5.1 Loading and Unloading Tips Outside the Loop

To load and unload tips outside of the Loop:

1. Insert a **New Tips** step between the Instrument Setup and Loop steps.
2. Select the **Aspirate** step inside the Loop.
3. Deselect the **Refresh Tips** checkbox. This tells Biomek to use whatever tips are already loaded to perform the aspirate instead of loading new tips at the start of the Aspirate step.
4. Insert an **Unload Tips** step after the End Loop icon in the Method View. Your method should now look like Figure 3-14.

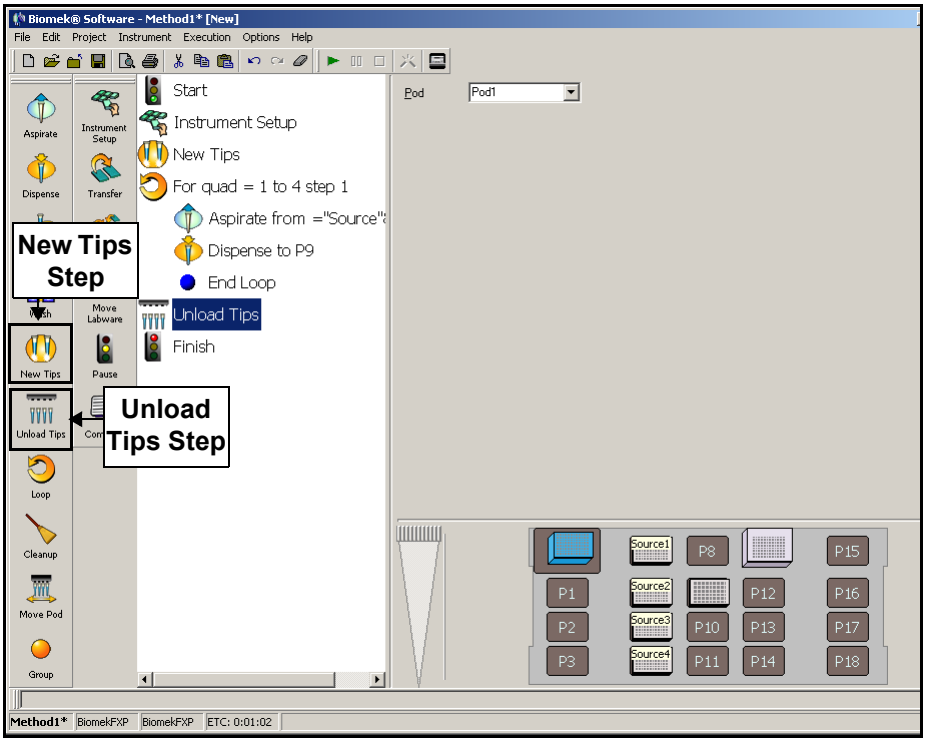

Figure 3-14. Loading and unloading tips outside the Loop

This method loads new tips, uses those same tips to perform four aspirate and dispense operations from the Loop, and unloads the tips after the last iteration of the Loop.

3.5.2 Washing Tips Inside the Loop

BIOMEK CONCEPT
Washing Tips

The Wash step washes tips at the Wash Station by aspirating and dispensing a specified volume of wash fluid a specified number of times.

If you have a wash ALP (called a Wash Station in the software), you will add a Wash step to wash the tips after each dispense.

To wash tips between aspirate actions:

1. Insert a **Wash** step between Dispense and End Loop in the Method View.
2. In Volume, enter **100%**. This means that for each wash cycle, a volume equal to the total volume that was aspirated and dispensed is used to clean the tips.
3. In Wash Cycles, enter **3**. This is the number of times that the Volume of wash fluid is aspirated and dispensed to clean the tips (Figure 3-15).

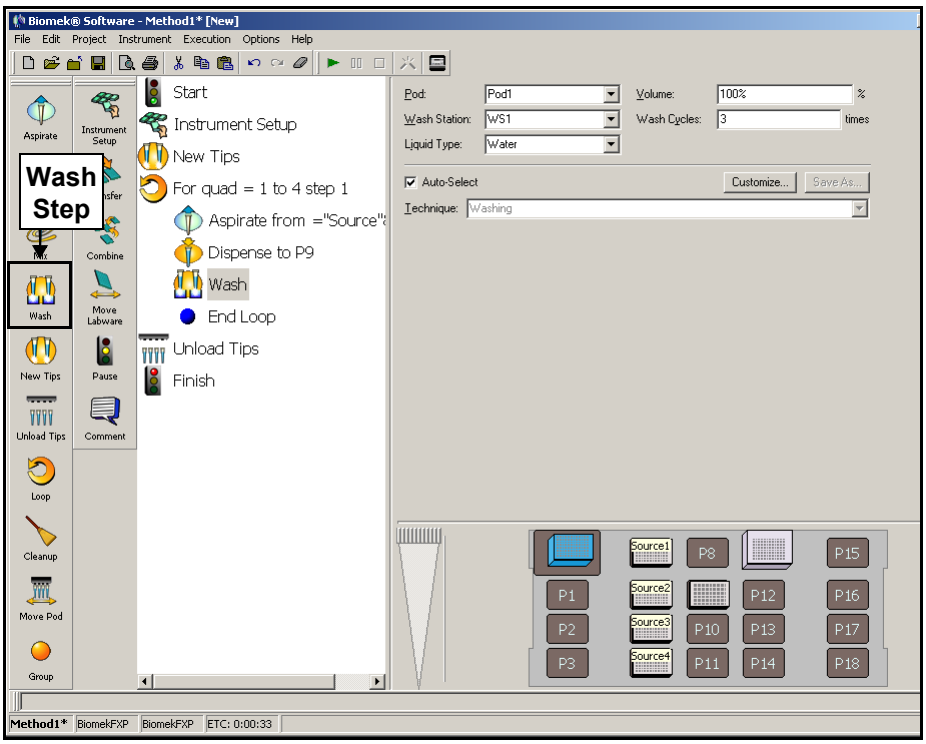

Figure 3-15. Wash tips

3.6 Using Lids in a Method

In chapter 2 you learned about using the Move Labware step to move labware on the deck. Another use of the Move Labware step is to remove lids from lidded microplates or tip boxes. In this section, you will modify the method to use a lidded 384-well plate for the destination.

To use lids in a method:

1. Select the **Instrument Setup** step.
2. In Labware Category, select **Lid** to display only the lids.
3. Drag a **CostarFlat384SquareLid** on top of the CostarFlat384Square plate located on position P9. Your Instrument Setup should now look like Figure 3-16.

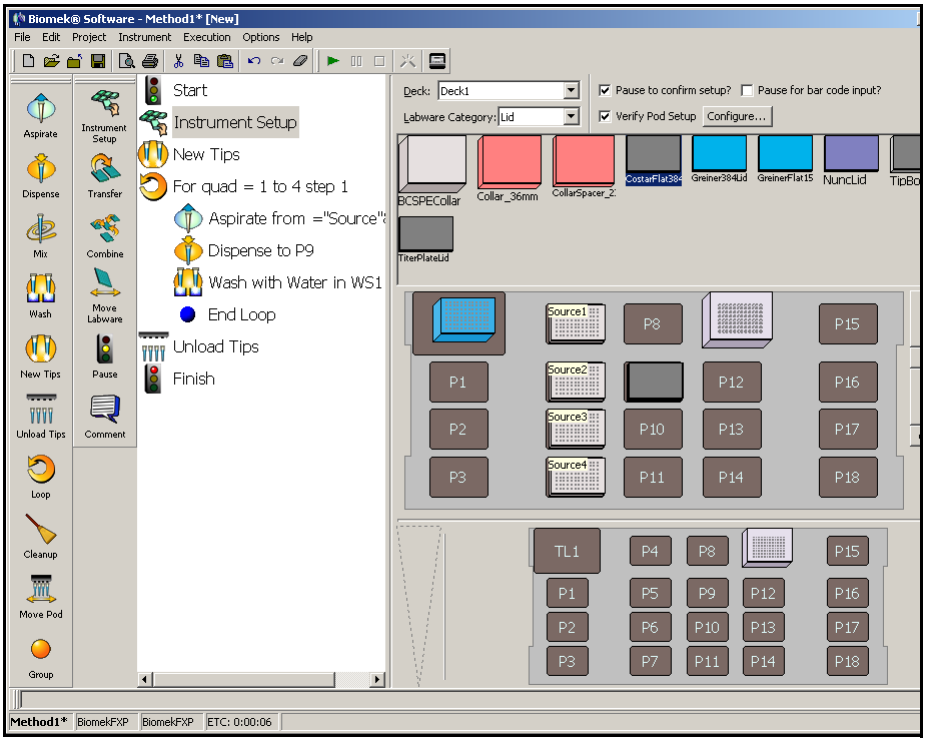

Figure 3-16. Modifying Instrument Setup step to add a lid

4. To remove the lid as part of the method, insert a **Move Labware** step immediately after the Instrument Setup step. It must go before the New Tips step, because the gripper cannot move labware when tips are loaded.

5. Configure the **Move Labware** step to Move labware from **P9** to **P8**.
6. Select the second option to **Move stack, leaving the bottom piece of labware at the source position**. This tells Biomek to pick up only the lid and leave the plate at its current position. Your Move Labware step configuration should look like Figure 3-17.

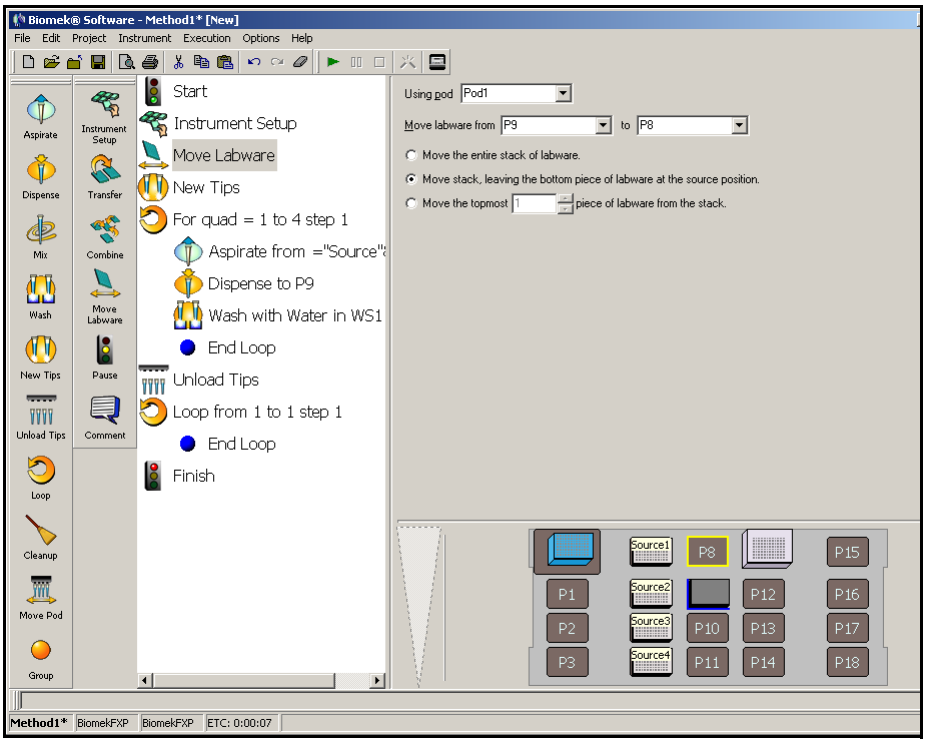

Figure 3-17. Using Move Labware to remove a lid

3.7 Stacking Plates in a Method

BIOMEK CONCEPT
Using Stacks

When stacking or unstacking labware, Biomek Software works from the bottom up. If stacking four microplates on the deck, the top plate is placed on the second plate by the gripper, then the top two plates are placed on the third plate, and finally the top three plates are placed on the bottom plate.

They must be stacked in this way to follow the stacking rules for the Biomek FX gripper. Refer to the *Biomek Software User's Manual,* Section 8.3.6.4.1, *Biomek Stacking Rules*, for more information on using stacks in a method.

The maximum height of stacks of microplates allowed on the Biomek FX instrument is 5.5 cm (2.17 in.). A stack of four standard 96-well plates is approximately 5.2 cm (2.05 in.) tall.

The Biomek FX instrument can also handle stacks of labware, both stacking and unstacking labware stacks in a method using the gripper. In this section, you will use what you have learned previously about the Loop step and the Move Labware step to stack the four source plates.

To do this, you will use another Loop step with a variable that will be used to specify the deck positions from which to pick up labware and an expression to specify to which position to move labware. Once configured, the three cycles of the Loop will move labware as shown in the table below

Table 3-1. Values of variables and expressions used in Move Labware step for cycles

Cycle	Stack value	From ("P"&stack)	To ("P"&(stack-1))
1	7	P7	P6
2	6	P6	P5
3	5	P5	P4
4	4	Value of stack is below End value; breaks out of loop and continues with method.	

To stack the four source plates:

1. Insert a **Loop** step after the Unload Tips step.
2. Configure the Loop step to create a Variable named **stack** with a Start value of **7**, an End value of **5**, and an Increment value of **-1**. Your Loop step should look like Figure 3-18.

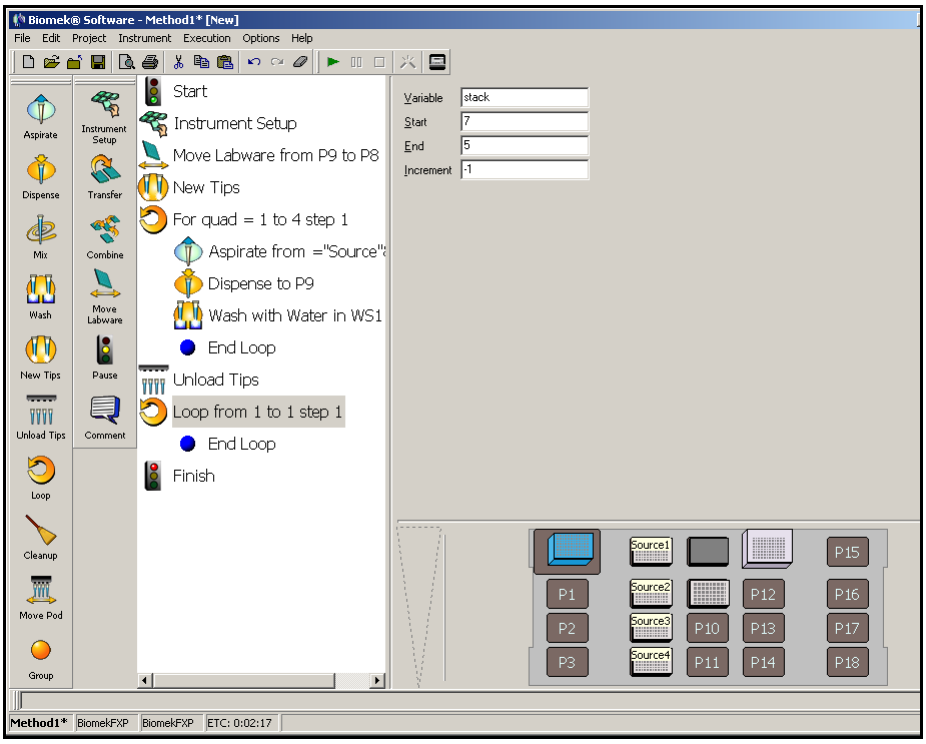

Figure 3-18. Loop to stack plates

Tip

Using the concatenation operator & is a useful way to specify deck positions with a variable, as it appends the value of the variable to the constant character "P".

3. Insert a **Move Labware** step between the Loop and End Loop icons.
4. In Move Labware from, type **="P"&stack.**
5. In to, type **="P"&(stack-1)**. Select the first option, **Move the entire stack of labware**. Your Move Labware configuration should look like Figure 3-19.

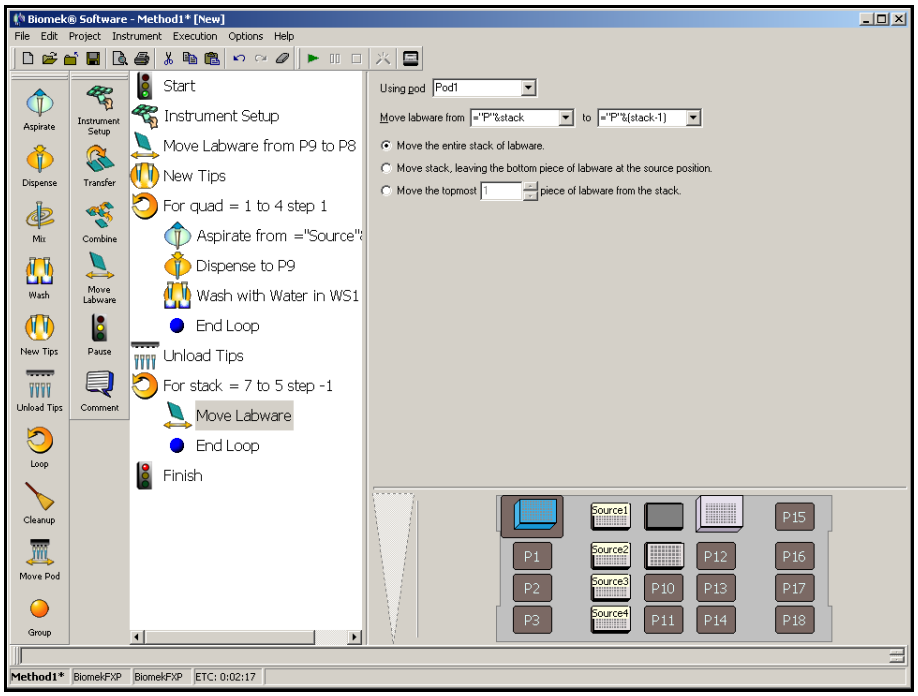

Figure 3-19. Use variables to stack plates

Now move on to the last chapter of this tutorial to learn how to use some advanced features in Biomek Software.

4 Multichannel Pod—Using Worklists and Conditions

4.1 Introduction to Using Worklists and Conditions

To successfully complete the activities in this chapter, you will need to know how to:

- Configure an Instrument Setup step to reflect the physical deck you will set up for the method in this chapter (refer to Section 1.3.2, *Configuring the Instrument Setup Step*).
- Configure Labware Properties for labware you will use in this chapter.
- Configure a Transfer step (refer to Section 1.4, *Transferring Liquid*).
- Configure a Move Labware step.
- Use variables and expressions in Biomek Software (refer to Section 3.3.1, *Creating a Variable in the Start Step*).
- Display step palettes.

4.1.1 What You'll Learn in Using Worklists and Conditions

In this chapter, you will develop the advanced skills to use external data sources, such as a worklist, with a Biomek method. Using a worklist will allow you to create a method using source and destination volumes defined in a text file. You will also learn how to use procedures to run the same set of configured steps several times in a method to eliminate having to configure each step several times and learn how to configure a conditional statement that will allow a step or steps to be executed based on real-time conditions that occur during the method.

More specifically, the step-by-step instructions in this chapter will teach you how to:

- Create a worklist file that defines variables and values.
- Use the worklist file in a Worklist step for executing a sequence of liquid transfers without configuring individual Transfer steps.
- Use an If step to transfer liquid from specific reservoirs using specific tips based on conditional decisions.
- Define a procedure using the Define Procedure step that will run based on the conditional decisions within an If step.
- Run the defined procedure based on the conditional decisions using the Run Procedure step.

4.1.2 Setting Up Your Deck for Using Worklists and Conditions

> **Tip**
>
> Since you will configure six BCFlat96 plates with the same properties, you can place the first one on P9, configure it, and then right-click on it and choose Copy, and then click on P10, P11, P13, P14, and P15. The labware type and the properties are copied to those positions.

Using what you learned in Chapter 1, launch Biomek Software and configure an Instrument Setup as follows:

1. Place a **AP96 200µL** tip box on P1 and name it **TipsA**. Configure Load no more than as **6**. This means that the tips in this tip box will not be used more than 6 times.
2. Place the same tip box type on P2, name it **TipsB**, and configure it the same as P1.
3. Place a **Reservoir** on P5 and name it **A**. Give it a known volume of **100000µL** of **Water**.
4. Place another **Reservoir** on P6 and name it **B**. Give it a **Known** volume of **100000**µL of **Ethanol**.
5. Place **BCFlat96** plates on P9, P10, P11, P13, P14, and P15. Give these plates each a **Known** volume of **100** of **Water** (see sidebar Tip).
6. Place a **TiterPlateLid** on each of the BCFlat96 plates.

Make sure you have the following step palettes on the main editor:

- Basic
- Intermediate
- Advanced
- Specialty

Your deck should look like Figure 4-1. Now go to the next activity to learn how to use worklists and conditions in your methods.

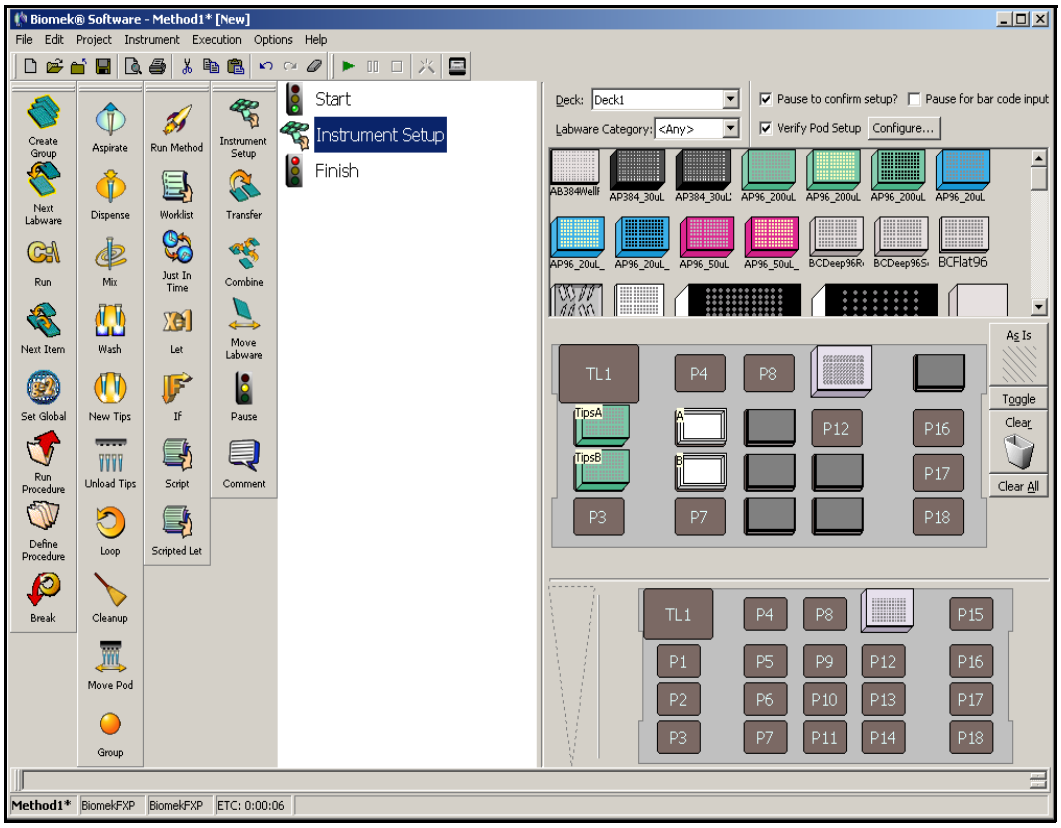

Figure 4-1. Instrument Setup step configured

4.2 Creating a Worklist Text File

A worklist is a text file that contains multiple values for one or more variables. The Worklist step in Biomek Software uses the variables and values defined in the worklist to configure a series of repetitive actions during the method run. This is different from the Loop step that you learned earlier in this tutorial in that a worklist can contain non-incremental values and multiple variables for use during repetitive actions.

Before you can use the Worklist step, you must create the worklist text file and define the variables and associated values. For the method in this chapter, you will create a worklist for a series of transfers using different amounts of liquid for each transfer.

4.2.1 Configuring a Worklist Text File

Tip

If a value such as a plate name or bar code contains a comma, enclose the entire value in double quotes, for example, "Dest1,4".

The first line of a worklist text file defines the variable names. The subsequent lines list the values to be assigned to those variables. The variable and names are separated by commas.

To configure the worklist text file for this tutorial:

1. Using Notepad or Microsoft® Excel, create a text file that looks like Figure 4-2.

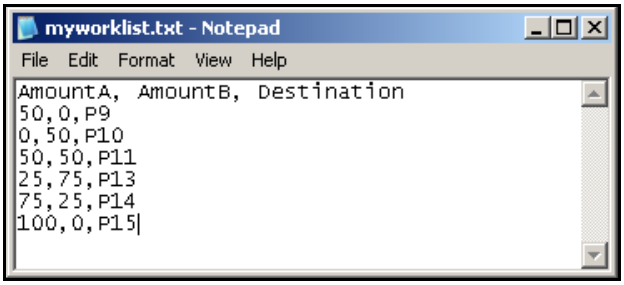

Figure 4-2. Created worklist

Tip

When creating a Worklist text file, white space between values is not important; however, the comma (,) and carriage returns (CRLF) are important to create and use the file properly. Do not enter a carriage return after the last line.

2. Save your text file as **myworklist** in the default My Documents folder.

Now that you have configured the worklist, you will insert and configure a Worklist step to enable the software to use the worklist in the method. Go on to the next section to learn how to do this.

4.3 Configuring a Worklist Step to Use a Worklist

BIOMEK CONCEPT
Worklist Step

The Worklist step offers several advantages:

- Data in a text file is accessible by any Biomek Software method.
- Selecting the text file in the Worklist step configuration copies all of the variable data from the text file to the step without individually entering all the variables.
- A dynamic link exists between the method and the text file. Any updates made to the text file are included in the next method run, although the text file must maintain the original file path to allow the method to find the file.

The Worklist step is located on the Advanced step palette and uses a text file to supply to the method multiple values for one or more variables. Worklist is useful when repetition of the same action is required, but one or more variables needs to change each time the step cycles through the worklist. When a step or group of steps using the variables defined in a text file are placed inside a Worklist step, Worklist automatically performs each step once for each line in the text file.

For this part of the tutorial, we will use a Worklist step to transfer specific amounts of liquid from two different sources to six destination plates. The text file you configured in Section 4.2, *Creating a Worklist Text File*, contains all the needed details.

To configure the Worklist step:

1. Ensure your deck is configured according to the instructions in Section 4.1.2, *Setting Up Your Deck for Using Worklists and Conditions*.
2. Drag and drop a **Worklist** step (Figure 4-3) into the Method View below the Instrument Setup step.
3. From Worklist file in the configuration window, use the Browse button to find and choose **myworklist.txt**, the text file you configured in Section 4.2, *Creating a Worklist Text File*.
4. Make sure **Loop entire worklist** is selected. This ensures that all the values contained in the worklist are used. The Worklist step configuration should look like Figure 4-3.

Tip

You can use Loop from line to execute the loop for only some of the lines in the worklist.

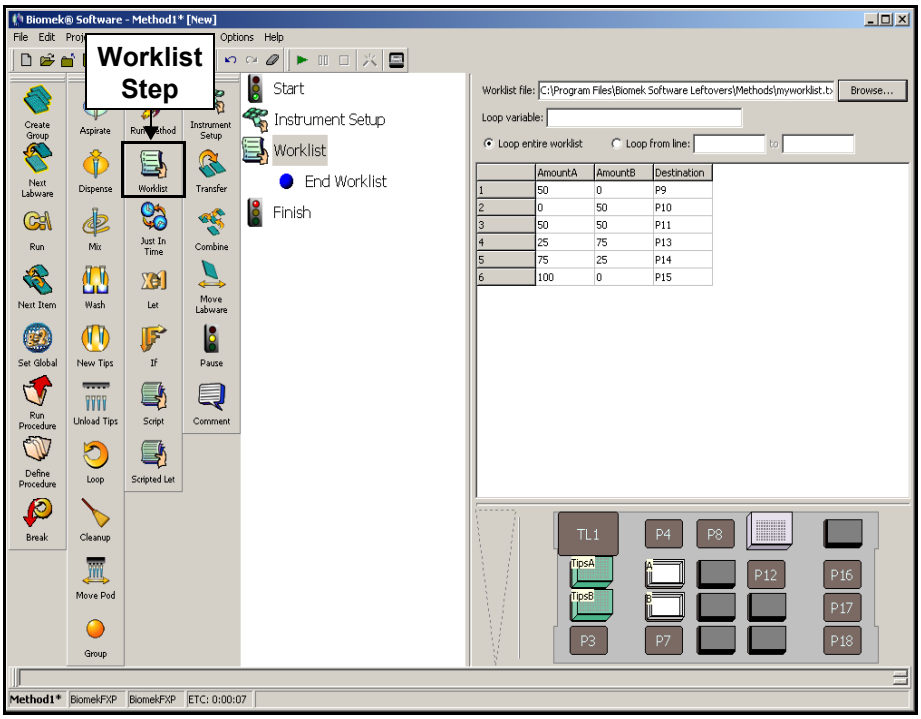

Figure 4-3. Worklist step with text file displayed

Now, you will define a procedure using a Define Procedure step and insert it before the Worklist step. This procedure will be run as the method cycles through the worklist. This procedure will be configured to load tips, transfer liquid, and unload tips.

4.4 Defining and Running Procedures

BIOMEK CONCEPT
Procedures

Procedures offer advantages, such as running the same steps multiple times within a method but configuring them only once. Procedures control the size of the current method in the Method View by listing only the Run Procedure step in the Method View and not all the steps accessed by the procedure.

The Define Procedure step is used to configure and save a series of steps that may be used multiple times in a method without having to reconfigure each individual step within that procedure. The Run Procedure step is inserted into the method and is used to identify the defined procedure to be used in a method. The procedure defined in the Define Procedure step can be run only by inserting a Run Procedure step and choosing the desired procedure in the step configuration.

4.4.1 Defining a Procedure Using the Define Procedure Step

For this part of the tutorial, you will insert and configure a Define Procedure to load specific tips, transfer volumes based on the worklist you configured earlier, and unload tips. The defined procedure will then be run as part of the If step that you will learn about and configure later. You will also create variables in this procedure whose values will be specified in the Run Procedure step. This lets you run the steps in the procedure with different values associated with the defined variables.

1. From the Specialty step palette (Figure 4-4), insert a **Define Procedure** step below the Instrument Setup step.
2. In Procedure, enter **TransferReagent** (Figure 4-4). This becomes the name of your procedure and will appear as Define TransferReagent in the method view.

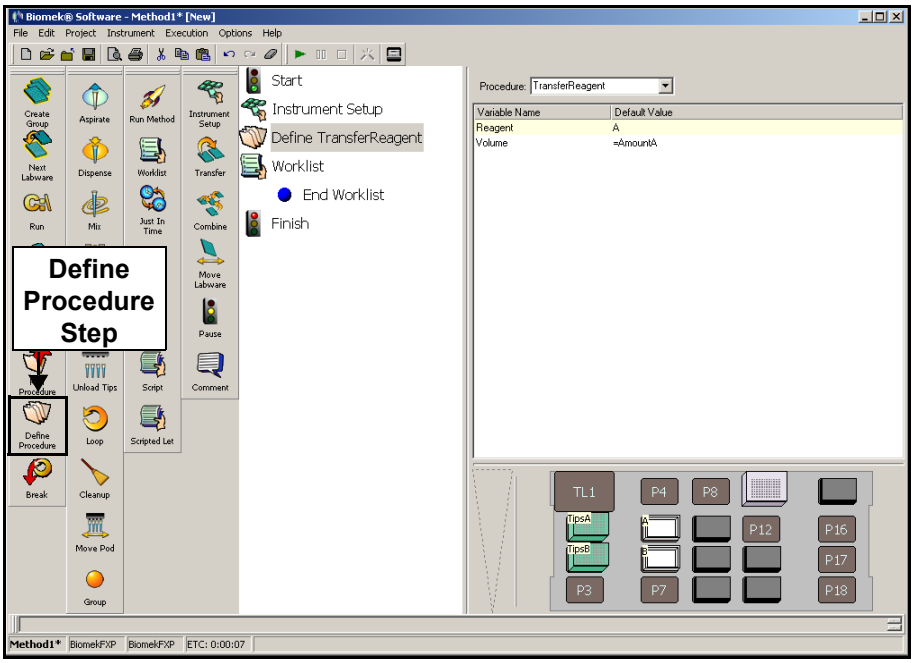

Figure 4-4. Procedure named

3. Under Variable Name, enter **Reagent** and tab over to Default Value and enter **A.**
4. Press Enter on the keyboard, then under Variable Name, enter **Volume** and tab over to Default Value and enter **=AmountA**.
5. Double-click the **Define TransferReagent** step in the Method View to expose End Procedure.

4.4.2 Configuring Steps Inside the Define Procedure Step

To configure the Define Procedure step to remove the lids on the BCFlat 96 microplates, load specific tips, transfer volumes based on the worklist you configured earlier, unload tips and then replace the lids on the BCFlat 96 microplates, the following steps will be configured individually inside the Define Procedure step:

- Move Labware step (refer to Section 4.4.2.1, *Removing Lids Using the Move Labware Step*)
- New Tips step (refer to Section 4.4.2.2, *Configuring Different Tips for Accessing Sources*)
- Transfer step (refer to Section 4.4.2.3, *Transferring Liquid During a Procedure*)
- Unload Tips step (refer to Section 4.4.2.4, *Unloading Tips During a Procedure*)
- Move Labware step (refer to Section 4.4.2.5, *Replacing Lids Using the Move Labware Step*)

The Define Procedure will be run as part of the If step that you will configure later.

4.4.2.1 Removing Lids Using the Move Labware Step

Inserting and configuring a Move Labware step in the Define Procedure step will remove the lids from the destination plates and place the lids in the empty deck position P8 before each liquid transfer.

1. From the Basic step palette, insert a **Move Labware** step into the Define Procedure above End Procedure step. The Define Procedure step now appears as Define TransferReagent step.
2. In Move labware from, enter **=Destination**.
3. In to, choose **P8**.
4. Choose **Move the topmost 1 piece of labware from the stack**. This option allows only the lid (the topmost piece in the stack) to be moved. The main editor should look like Figure 4-5.

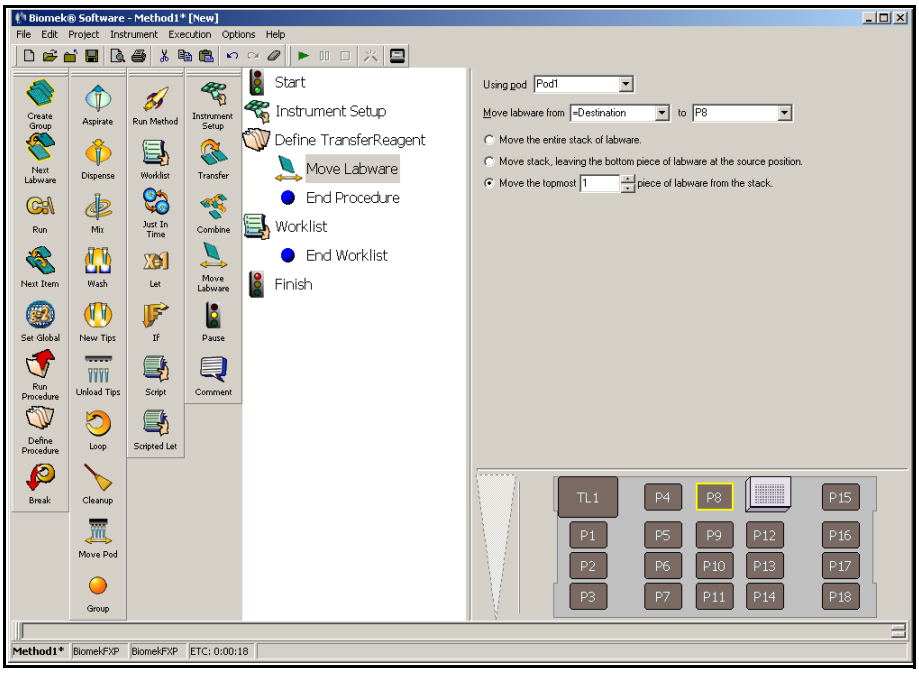

Figure 4-5. Configured Move Labware step

4.4.2.2 Configuring Different Tips for Accessing Sources

Since your reservoirs in this tutorial method contain different liquid types, you will want different tips designated for each reservoir. You will configure the New Tips step as part of your procedure to ensure that the correct tips are loaded to access the correct source reservoir.

1. From the Intermediate step palette, insert a **New Tips** step into the Method View inside the Define Procedure below the Move Labware from =Destination step.
2. In Tips in the configuration view, highlight the field and enter **=“tips”&reagent** (Figure 4-6).

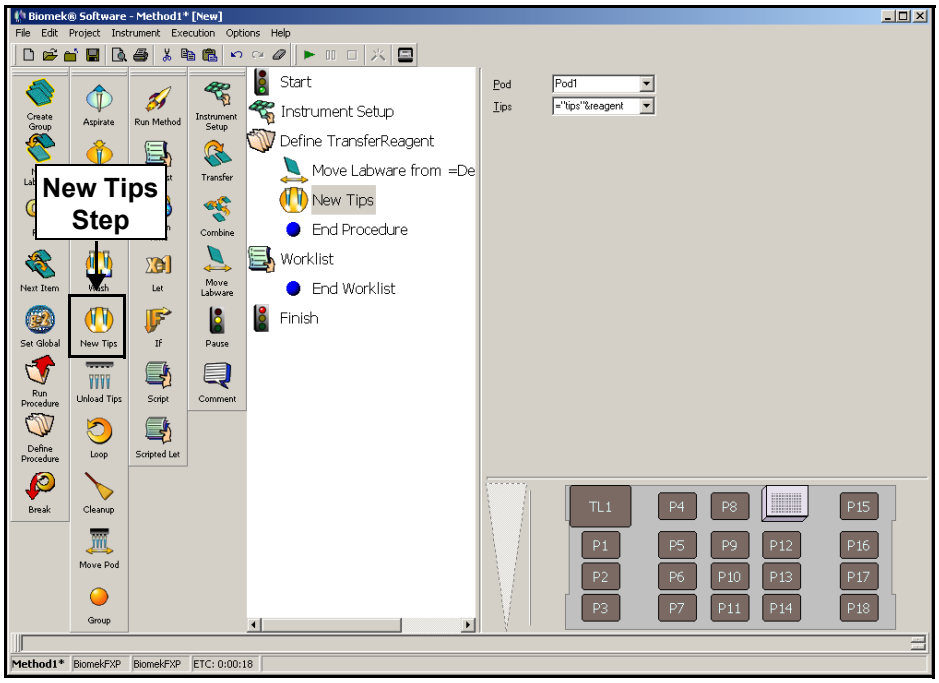

Figure 4-6. New Tips configured

4.4.2.3 Transferring Liquid During a Procedure

To configure the actual liquid transfer that will be executed when the procedure is run, configure the transfer as follows:

1. Insert a **Transfer** step into the Method View below the New Tips step.
2. Uncheck **Load AP96_200µL tips**.
3. Using what you learned in Chapter 1, configure the Source in the Transfer step as **Reservoir** at **=Reagent**. Configuring =Reagent means that the reservoir on the deck that has the same name as the value of the variable Reagent will be used. You’ll configure this variable later in the Define and Run Procedure steps
4. In the Transfer step, configure the Destination as a **BCFlat96** at **=destination**. Configuring =destination means that the destination will be those you configured in the worklist.
5. In the Destination configuration µL field, enter **=Volume**.

4.4.2.4 Unloading Tips During a Procedure

Here you will configure the procedure to unload the tips and put them back in the box after the liquid transfer action. To unload the tips:

1. Insert an **Unload Tips** step below the Transfer step.
2. Click on the **Define TransferReagent** step.Your main editor should look like Figure 4-7, and the variables in the named procedure you just created will be used to specify when new tips are used and which reservoir will be accessed when transferring liquid.

Tip

If you want to reuse a procedure in other methods, drag the configured procedure and drop onto any of the displayed step palettes. A prompt appears to ask if you would like to include the step on that specific palette. You could also create a custom step palette (refer to the *Biomek Software User's Manual,* Section 30.4, *Customizing Step Palettes*). Either way, the procedure may then be used in other methods by dragging and dropping it into the Method View.

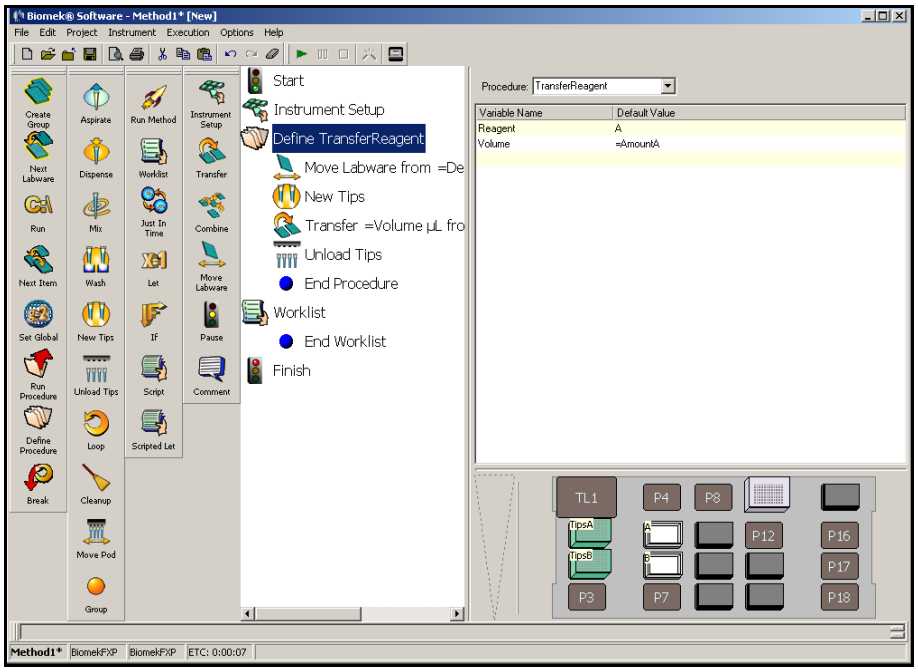

Figure 4-7. Define Procedure step configured

4.4.2.5 Replacing Lids Using the Move Labware Step

Now you'll need to configure a Move Labware step to replace the lid you removed before the liquid transfer.

1. Insert a **Move Labware** step into the Method View above the last End substep.
2. In Move labware from, choose **P8**.
3. In to, enter **=Destination**.
4. Choose **Move the entire stack of labware**.
5. Click on the **Finish** step to validate the method.
6. Double-click the **Define TransferReagent** step to collapse it.

4.5 Configuring the If Step to Use Conditions in a Method

BIOMEK CONCEPT
If Step

The substeps of an If step are:

- Then — if the condition is true, substeps following Then are processed.
- Else — if the condition is false, substeps following Else are processed.
- End — The End substep terminates each If, Then, and Else block of steps.

The If step controls the steps that are executed in a method based on conditional decisions. When If is run, Biomek Software tests the If condition as true or false, then processes the appropriate block of substeps based on the results of the test. (See Biomek Concept sidebar.).

To configure the If steps to use conditions in this tutorial, you will:

- Insert an If step and enter the condition for a transfer from source A.
- Insert a Run Procedure step for the transfer from source A for the Then substep.
- Insert another If step and enter the condition for a transfer from source B.
- Insert a Run Procedure step for the transfer from source B for the Then substep.

After you configure the If steps, you will insert another Move Labware step in the Worklist step to place the lids back on the plates from position P8.

4.5.1 Setting Conditions Using If Steps

For this section of the tutorial, you will configure two If steps that will specify the reagent reservoirs to access and the specific tip boxes for each transfer based on the sources you configured for the Worklist step. You will configure the If steps and insert them into the Worklist step. The transfers will then run the TransfersReagent procedure you configured earlier. The procedure locates the correct tips and transfers the appropriate volume from the correct reservoir.

To configure the If steps:

1. From the Advanced step palette, insert an **If** step into the Method View into the Worklist step.
2. In Condition, enter **AmountA > 0**. The main editor should look like Figure 4-8.

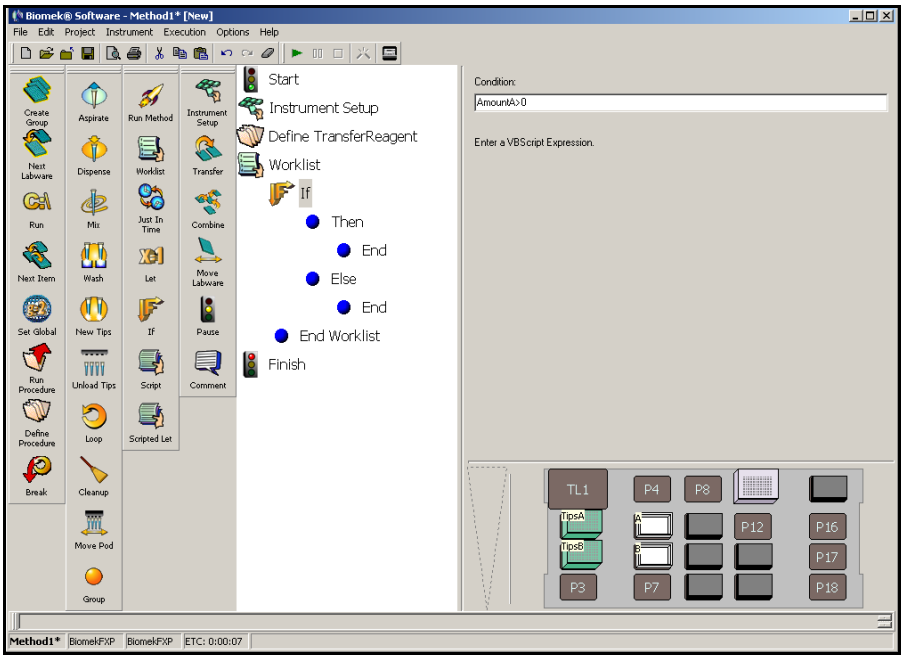

Figure 4-8. Condition entered in If step

3. From the Specialty step palette, insert a **Run Procedure** step into the Method View below the Then substep of the If step.
4. In Procedure, choose **transferreagent**.

For this tutorial, you won't place steps in the Else substep. This means that if the evaluation of the If step is false, no further action occurs. The main editor should now look like Figure 4-9.

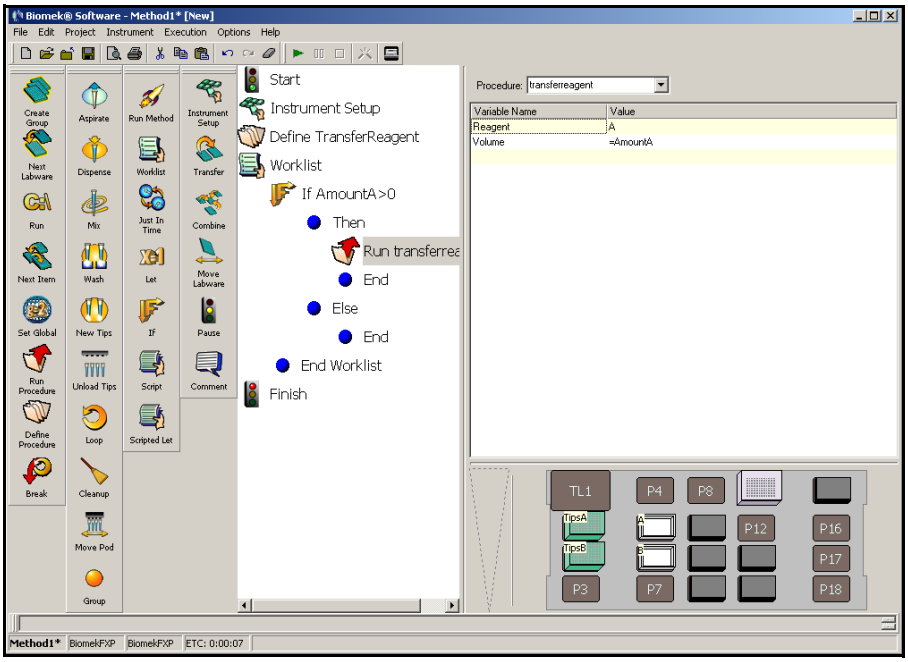

Figure 4-9. transfersreagent procedure inserted as a Then substep

5. Insert another **If** step into the Method View below the last End substep and above End Worklist.
6. In Condition, enter **AmountB > 0**.
7. Insert a **Run Procedure** step into the Method View below the Then substep of the second If step.
8. From Procedure, choose **transferreagent**.
9. Change the Value for Reagent to **B**.

10. Change the Value for Volume to **=AmountB**. You won't use an Else substep here either, so the main editor should look like Figure 4-10.

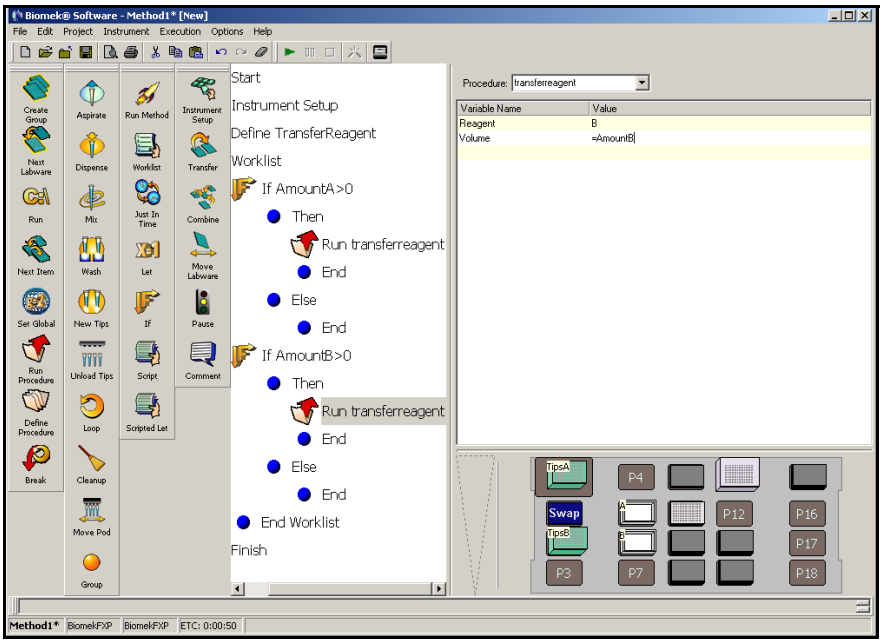

Figure 4-10. Variable name and value changed

Congratulations! You have just created a method using a worklist and If steps with configured conditions. If you would like to see this method run in simulation mode, click the green run button on the toolbar.

As the method runs, you will see how the worklist you created is executed. If liquid from reagent A is transferred, TipsA are used and if liquid from reagent B is transferred, TipsB are used. The amounts transferred and the destination plates used are those you configured in the worklist.

This chapter completes the tutorial for the Biomek FX Multichannel Pod. There are also chapters for the Span-8 Pod, so complete these to learn how to begin using the Span-8 Pod.

5 Span-8 Pod—Getting Started with Biomek® Software

5.1 Introducing Biomek Software

Welcome to Biomek Software and the Biomek FX Laboratory Automation Workstation.

Biomek Software controls the Span-8 Pod on your Biomek FX Laboratory Automation Workstation and is designed to:

- do a substantial amount of method building work for you.
- allow you to take as much direct and precise control over the method-building process as you want.

The flexibility that results from this combination gives the Biomek FX Laboratory Automation Workstation its power.

5.1.1 Using this Tutorial

Tip

For effective learning, print this tutorial before use, leaving your computer screen free for viewing Biomek Software.

This tutorial is designed to help you become comfortable using Biomek Software (version 3.3) with your Span-8 Pod on the Biomek FX. The chapters in this tutorial can be completed consecutively or, depending on the learning required, may be completed in any order. This format will allow advanced users to complete only the chapters that include the topics they need to learn. Generally, the topics in subsequent chapters increase in complexity. Chapters for the Multichannel Pod on the Biomek FX are also available.

The chapters in this tutorial for the Span-8 Pod can be used with the following configurations:

- Single Span-8 Pod.
- Hybrid system with a Multichannel Pod and a Span-8 Pod.

In addition to the step-by-step instructions in this tutorial, you will also see boxes containing useful information in the following forms:

BIOMEK CONCEPT

These boxes contain information to help you understand important features and capabilities of Biomek Software or the Biomek FX Laboratory Automation Workstation. While the step-by-step instructions may be completed without reading the information in these boxes, the information will enhance your knowledge and give you a fuller picture of what Biomek Software and your instrument can do.

Tip

The information in these boxes offer suggestions on how to use your instrument and software to enhance the activities you want to do in your laboratory.

If using fixed tips. . .

If you have fixed tips on your instrument, the instructions in these boxes show you how you can use them rather than the disposable tips which are called out in the tutorial instructions.

5.1.2 What You'll Learn in Getting Started with Biomek® Software

In this chapter, you will learn how to:

- Launch Biomek Software and see what the method-building process looks like.
- Set up the deck for a liquid transfer.
- Build a liquid-transfer method.
- Run a method.
- Save and check in a method.

5.1.3 Launching Biomek Software

From the Start menu, select **All Programs>Beckman Coulter>Biomek Software**.

BIOMEK CONCEPT Accounts & Permissions

Beckman Coulter Accounts & Permissions is an integrated set of features built into Biomek Software that assists users in complying with 21 CFR Part 11 requirements for closed systems. Permissions provide the ability to control user access to specific program operations. Refer to the *Biomek Software User's Manual,* Chapter 2, *Using Accounts & Permissions*.

If Beckman Coulter Accounts & Permissions is enabled on your system, you must have an account established and log in using that account name and password in order to fully complete this tutorial. For more information, contact your system administrator.

5.1.3.1 Viewing the Main Editor

The main editor (Figure 5-1) is your starting point for building liquid-handling methods for the Biomek FX Laboratory Automation Workstation. You will choose method steps from a step palette and place them into the Method View in a linear fashion. The configuration for each of these steps appears in the Configuration View.

CAUTION: Do not change the pod settings in Hardware Setup after the system has been installed; changing the pod settings will necessitate another service call.

If using an instrument with a Multichannel Pod and a Span-8 Pod, the Span-8 Pod should be set as the default pod when completing this tutorial. To do this:

Select the default pod by choosing it from the pod icon on the toolbar (Figure 5-1).

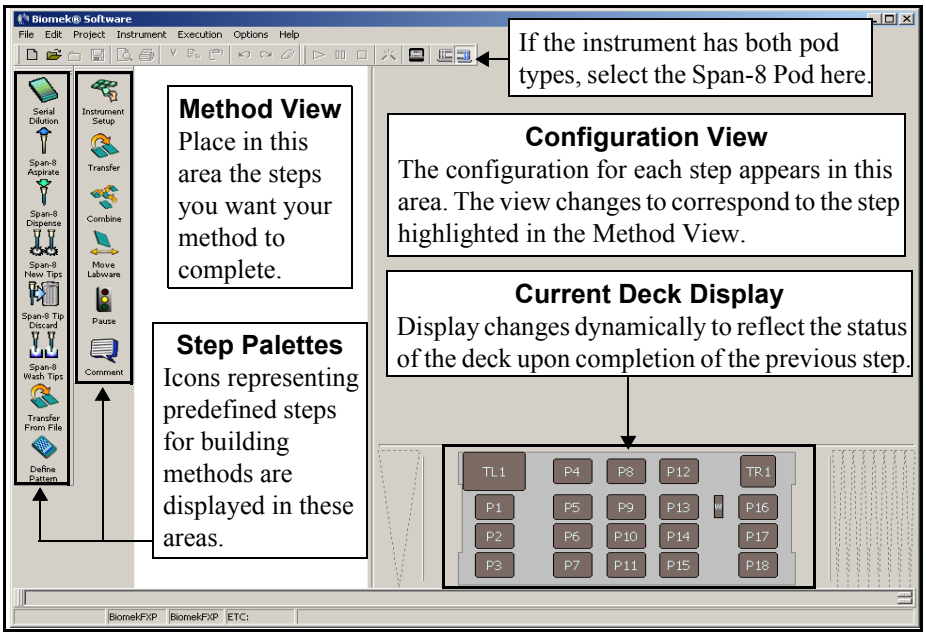

Figure 5-1. Biomek main editor

5.2 Beginning a Method

To begin a method, you have the option of creating a new method or opening an existing method you've completed, named, and saved. In this tutorial, you'll create a new method.

But before you create a new method, get into the habit of ensuring you are using the correct project file.

5.2.1 Introducing Project Files

BIOMEK CONCEPT
Project File

A project file stores information about liquid types; labware and tip types; well patterns; and pipetting templates and techniques as revisions that are used by a method file to configure the actions of the instrument. Project files store a history of all changes, additions, and deletions of items from the project file. Methods are associated with projects and contain all of the items required to perform the method.

While project files may be created, revised, deleted, imported, exported, checked in, or checked out, in this tutorial you will use the project file on your system that was created or imported when your instrument and Biomek Software were installed.

View Figure 5-2 to learn where project file information is accessed or viewed from the main editor.

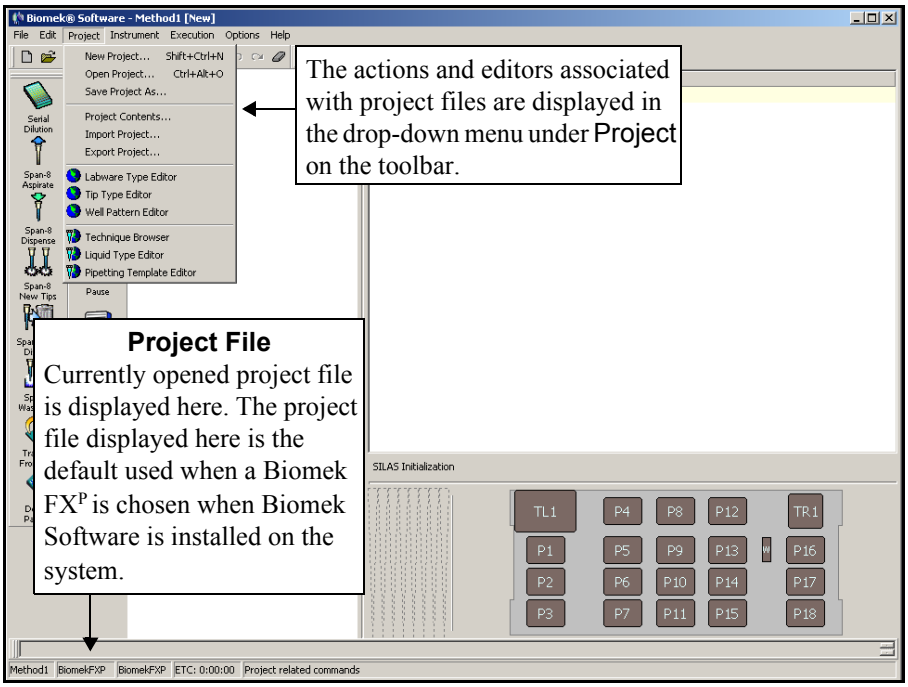

Figure 5-2. Project file

BIOMEK CONCEPT
Method

A method is a series of steps that control the operation of the Biomek FX instrument. The step palettes in the main editor present a group of icons representing the steps available for a method. To build a method, you simply select the step icon you want, and move it into the method-building space (Method View) in the main editor. Place and configure each step to perform the operations as desired.

5.2.2 Creating a New Method

To create a new method:

Go up to the toolbar and select **New Method** (Figure 5-3).

This creates the beginning for your new method. It's a good idea to expand the Biomek editor to fill the entire screen.

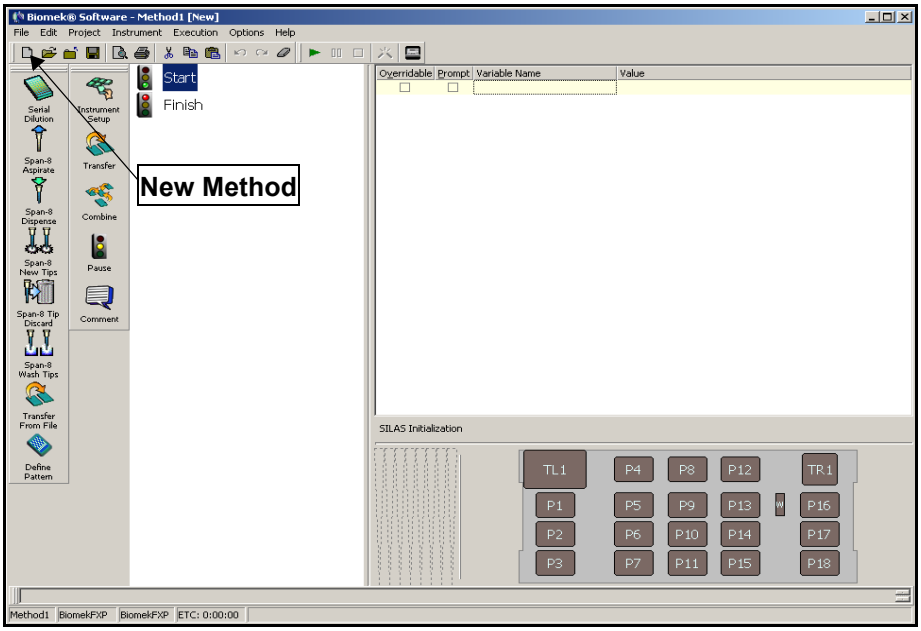

Figure 5-3. Main editor when a new method is created.

5.2.3 Understanding the Start and Finish Steps

As you can see (Figure 5-3), the method view of the main editor now contains the Start and Finish steps that appear automatically when you create a method. These two steps are always there and indicate the beginning and end of your method. You'll insert all the rest of the steps you want the Biomek FX instrument to complete between Start and Finish.

When the Start step is highlighted in the method view, you are presented with the opportunity to create some variables in the configuration view. Ignore this configuration for our first chapter in this tutorial.

If you want to know more in-depth information on the Start configuration right now, refer to the *Biomek Software User's Manual,* Section 13.2.1, *Configuring the Start Step*.

You'll learn more about using the Finish step in Section 5.4.4, *Determining the Estimated Time for Completion (ETC) of the Method*.

5.3 Setting Up the Deck

BIOMEK CONCEPT
Deck Editor

The Deck Editor is used to define and change the deck configurations stored in the current instrument file. A deck is a software visual representation of the Biomek instrument deck and can be stored and used for multiple methods; however, the software deck must always match the physical deck of the instrument used in the method. Refer to the *Biomek Software User's Manual,* Chapter 6, *Preparing and Managing the Deck*.

Setting up the deck includes:

- ensuring the current deck used in Biomek Software via the Deck Editor matches the physical deck of the instrument.
- configuring the Instrument Setup step to tell the software what labware and what deck position each labware piece occupies on the deck.

5.3.1 Ensuring the Deck in Biomek Software is Correct

To avoid hardware crashes, it's important that the deck in Biomek Software matches the physical deck of your instrument. If you wish to run these tutorial methods on hardware rather than in simulation and your deck varies from what is shown, you may have to modify the methods to work with your hardware (Figure 5-4).

Note: Automated Labware Positioners (ALPs) are removable and interchangeable platform structures installed on the deck to allow automated assays to be performed.

The steps in this tutorial assume the current deck in the software includes a Span-8 Wash ALP between the first and second column from the right since you will use this ALP later. You will also need a Span-8 Disposal ALP in the back row, back column position.

If using fixed tips. . .

A Span-8 Disposal ALP is not necessary.

To do this:

1. View the current software deck (Figure 5-4).
2. Ensure a Span-8 Wash ALP is placed between the first and second column from the right and a Span-8 Disposal ALP is placed in the back row, back column position. See the sidebar tip to add these ALPs.

Tip

To add a Span-8 Wash ALP to your deck, choose **Instrument> Deck Editor**. The current deck appears. Then from All, highlight **Span8Wash Left** and drag it to the proper position. To add a Span-8 Disposal ALP, highlight **Span8Trash Right** and drag it to the proper position. You may have to delete other ALPs from the deck before you add these. Then choose **Renumber** to ensure your deck matches the deck on Figure 5-4.

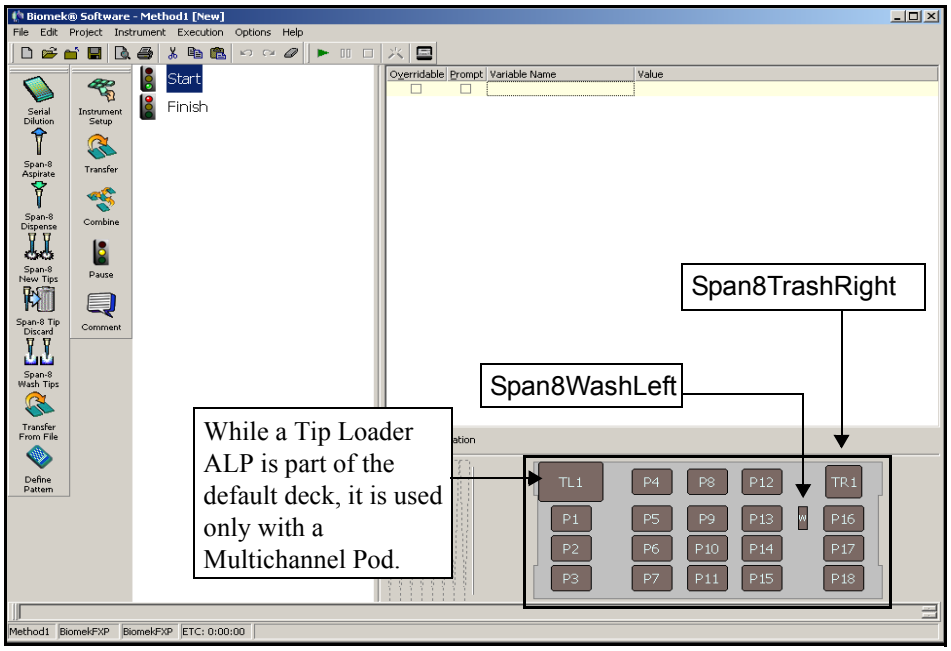

Figure 5-4. Current deck for this tutorial

Tip

If the Instrument Setup step, or any step, is inserted into the wrong location in the Method View, you can drag and drop it to the proper location.

5.3.2 Configuring the Instrument Setup Step

The next activity of this tutorial is to configure the Instrument Setup step for your liquid-transfer procedure. You will place on the deck:

- Tips
- Source reservoir
- Destination microplate

To insert the Instrument Setup step:

1. Choose (highlight) **Start** in the Method View.
2. Hover the cursor over the **Instrument Setup** icon in the step palette. As you hover, look at the Method View and you'll see a black bar appear just below Start. This black bar indicates the insertion point where your next step will appear. In this case, it's where the Instrument Setup step will be inserted.
3. Click the **Instrument Setup** icon to insert the step. The Instrument Setup configuration appears (Figure 5-5).

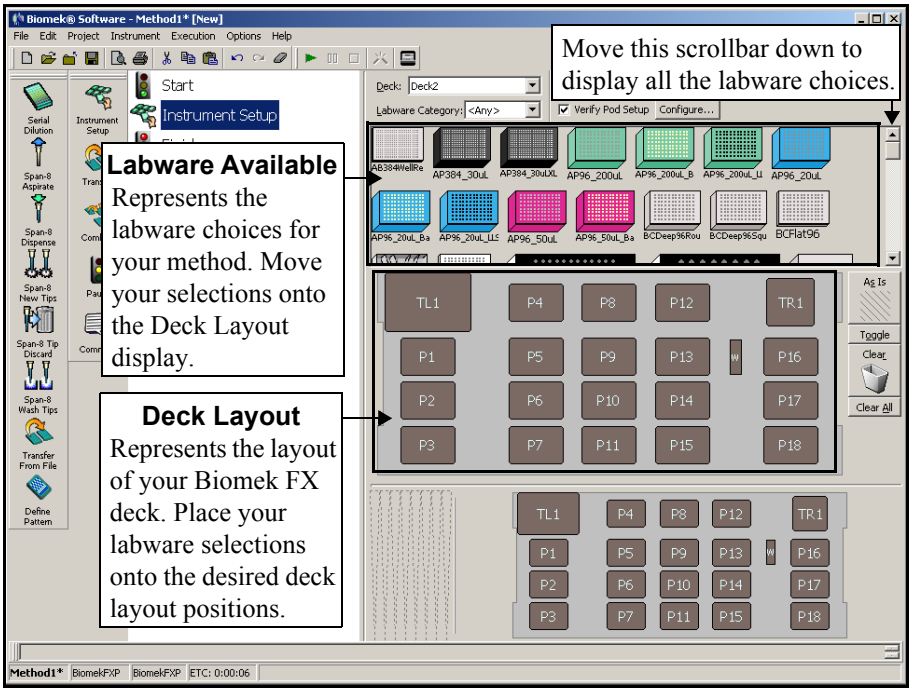

Figure 5-5. Instrument Setup step configuration

Using the Instrument Setup step you just inserted, you'll learn how to select and place:

- Span_8_200µL tips onto P14 deck position
- Reservoir onto P4 deck position
- BCFlat 96 microplate onto P5 deck position

To select and place your labware:

1. Click the **Span_8_200µL** tips icon, then click on **P14** deck position. Notice that when you hover the cursor over the tip box on the Deck Layout, a tool tip identifies the deck position and labware. This technique applies to all the labware you place on the deck.
2. Using the above procedure, place a **Reservoir** onto the **P4** deck position.
3. After you have positioned the reservoir on the deck, double-click it or right-click and select **Properties**. This opens Labware Properties (Figure 5-6). Each piece of labware added to the Deck Layout is configured using Labware Properties. The information provided in Labware Properties is used when a pipetting technique is selected or when tips are loaded and unloaded.

Note: A technique instructs the Biomek instrument in performing pipetting operations, such as an aspirate, dispense, and mix.

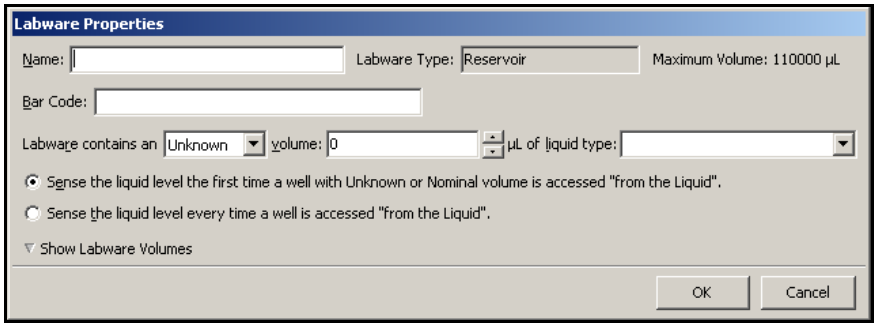

Figure 5-6. Labware Properties for Reservoir

Tip

It's helpful to name your labware on the deck. You can assign a name that identifies the contents of the labware, or a descriptive name that fits the work being done in your laboratory. This can reduce confusion considerably.

4. In Labware Properties, you can give the reservoir a name. You'll name this one "Rsvr," but in general you can assign labware any name you want. Type **Rsvr** in the Name field. After configuration is complete, the name will appear over the reservoir in the Current Deck display (Figure 5-7).
5. In Labware contains an, select **Known**.
6. In the Volume field, type **100000**. This means you know you have 100,000 microliters of liquid in the source reservoir.
7. Choose **Water** from the Liquid Type drop-down menu, or type **Water** into this field.
8. Leave Bar Code blank for this tutorial, but it can be used to identify a specific plate in certain methods.

9. Ignore the two options to Sense the liquid level. Since we have known volumes in the labware, we won't use liquid level sensing in this chapter, but you'll use liquid level sensing in later chapters.
10. Choose **OK**.
11. Place a **BCFlat96** microplate onto the deck in position **P5**.
12. Double-click on the **P5** microplate, or right-click and select **Properties**.
13. Type **Dest** in the Name field.
14. In Labware contains an, select **Known**.
15. In the Volume field, leave this value at **0**.
16. Do not specify a Liquid Type for this destination plate since it is presently empty.
17. Choose **OK**.

That's it. Your deck is now set up for transferring liquid, and the main editor should look like Figure 5-7.

Tip

You can set the properties (name, volume, and liquid type) as you've just done in these steps, then drag the labware back up, and drop it into the Labware Available display once you've selected the Custom labware category. This labware will retain the properties you set and be available to use in other methods when you access Instrument Setup.

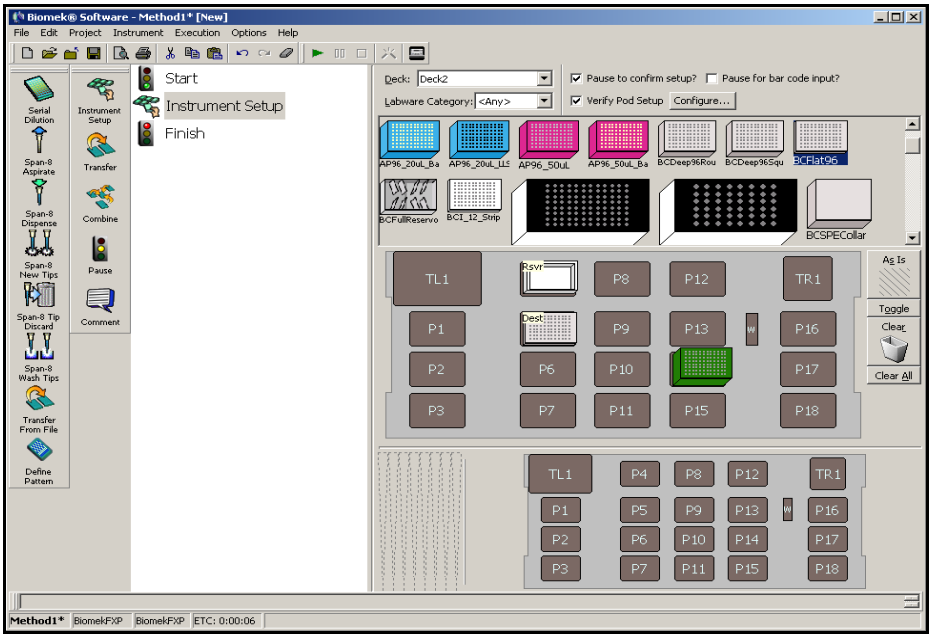

Figure 5-7. Instrument Setup step completed

5.4 Transferring Liquid

Transfer Step

The Transfer step for the Span-8 Pod transfers liquid from one source to one or more destinations. The Transfer step will by default complete the following: load tips, aspirate liquid, dispense liquid, and unload tips. This concept eliminates the need to insert four separate steps, although occasionally a method may require these steps be performed individually. These individual steps will be covered in Chapter 6 of this tutorial.

Now you are ready to insert and configure your procedure to transfer liquid. Biomek Software provides a Transfer step on the Basic step palette that makes it easy to accomplish this task.

Configuring the Transfer step includes configuring:

- Tip handling
- Source labware
- Destination labware

5.4.1 Configuring Tip Handling

To set up a liquid transfer, insert the Transfer step into the Method View in the main editor, and configure the Tip Handling by completing the following:

1. Highlight the **Instrument Setup** step.
2. Choose the Transfer icon from the step palette, and insert it into the method by dragging and dropping it after the Instrument Setup step. The Transfer step configuration appears (Figure 5-8). Notice the Current Deck display at the bottom of the editor is now populated to illustrate your deck setup since it changes dynamically to match the state of the deck at the start of the current step.

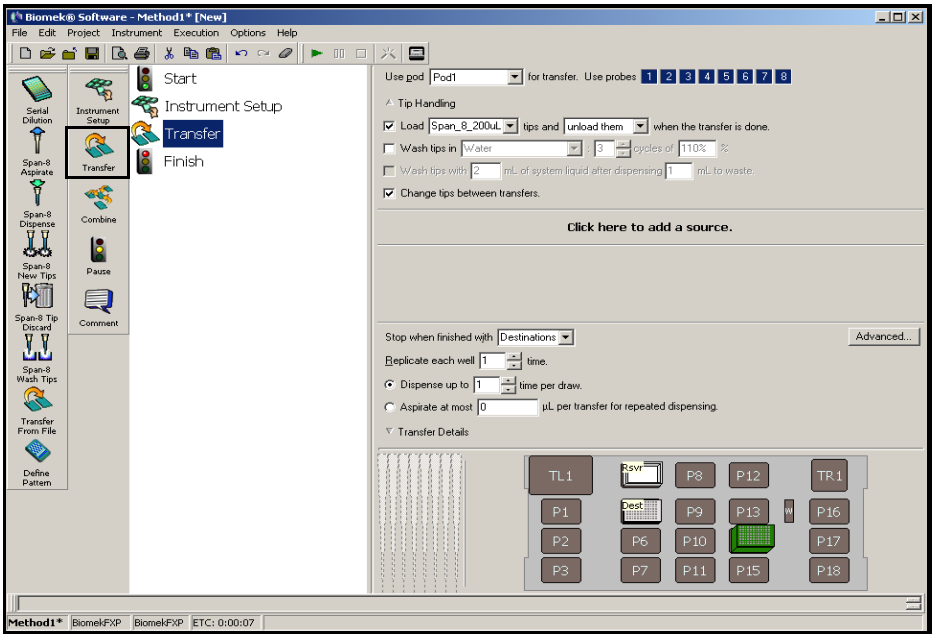

Figure 5-8. Transfer step inserted

3. In Use pod, verify the Span-8 Pod is selected. If you have a hybrid Biomek FX, make sure you choose the correct pod in Use pod; the configuration for the Transfer step should look like Figure 5-8. If you only have a Span-8 Pod on your instrument, the Span-8 Pod Transfer step configuration will be displayed by default.

If using fixed tips. . .

From Use Probes, right-click any probe and selected **Use Fixed Tips**. You won't have to deselect any probes since you'll use all the fixed tips configured on the pod.

4. Make sure the type of tips displayed is **Span_8_200µL**, the type of tips you configured in Instrument Setup.
5. Make sure **unload them** is selected in the next field.
6. Allow the default **Change tips between transfers** to be checked.
7. Your tips are configured for your liquid transfer, so click the **up arrow** next to Tip Handling (Figure 5-8). This collapses the Tip Handling configuration to allow more room for labware configuration. A simple text description of the way tips will be handled is displayed in place of the expanded Tip Handling configuration. The editor now looks like (Figure 5-9).

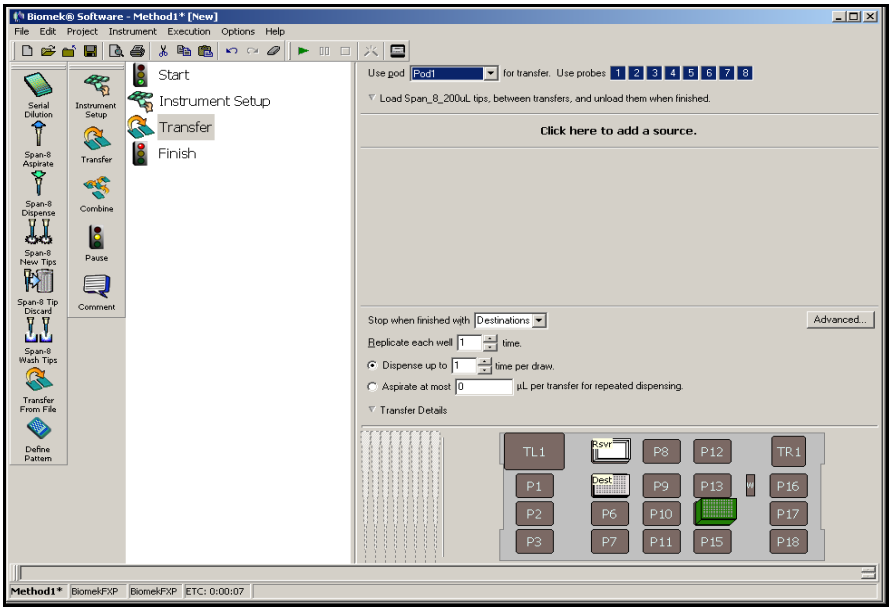

Figure 5-9. Tip Handling configured and collapsed

5.4.2 Configuring Source Labware

Now you will configure the source labware. Here you will specify from which labware liquid will be aspirated and the height to which the tip descends into the labware before aspirating.

To configure the reservoir named Rsvr as the source labware:

1. Click on **Click here to add a source**.
2. Click on **Rsvr** labware on the P4 position in the Current Deck display. As you can see, the information you supplied during Instrument Setup is displayed in the source labware configuration.
3. Right-click on the large tip illustration next to the reservoir graphic in the configuration and choose **Measure from Bottom**.
4. To adjust and set the aspirate height to which the tip descends into the reservoir, place the **mouse cursor over the tip illustration**. When the cursor turns into a hand, hold the left mouse button down to move the hand up and down until the depth is as close to **1.00 mm from bottom** as you can get. Then adjust the height precisely to 1.00 mm using the Tip described in the margin. There is a slight break in the bottom of the source reservoir graphic with the large tip that indicates that the reservoir is wider than the graphic can display.

The source labware is complete, and the editor now looks like Figure 5-10.

Tip

After you click on the tip, you can adjust the height more precisely by using the up or down arrow keys on your keyboard to change the height by 0.10 mm or you can use the Page Up and Page Down keys to change the height by 1.0 mm with each press of the key. You can also right-click on the graphic, then select **Custom Height** from the menu that appears.

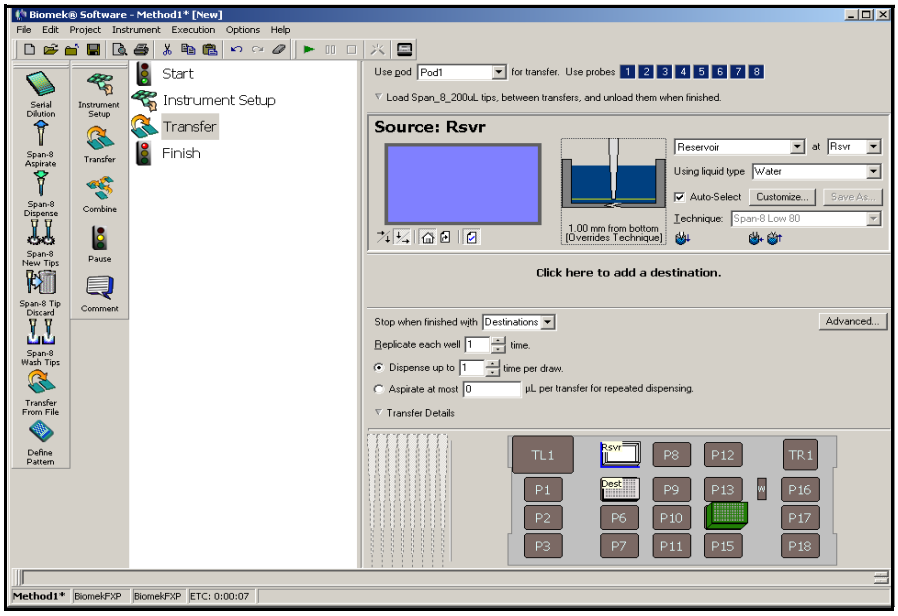

Figure 5-10. Configured source labware

5.4.3 Configuring Destination Labware

Here you will configure where you want the water from the source reservoir to be dispensed. In this case, you want to dispense water into the BCFlat 96 microplate on deck position P5.

Tip

If you accidentally open too many destination configurations, just right-click on the title in the configuration. Click **Delete** from the popup menu and the entire configuration goes away.

To do this:

1. Click the **Dest** microplate in the Current Deck display. This one operation accomplishes the same tasks as steps 1 and 2 of Section 5.4.2, *Configuring Source Labware*. Notice that the source labware configuration fields are now replaced with a brief sentence summary of the setup. If you want to reopen this source configuration for any reason, click anywhere in the collapsed configuration area.
2. Double-click the Destination Labware in the step configuration to zoom in on the labware. All of the wells are selected by default.
3. Since all of the wells are selected by default, select the first well of the first column by clicking on the well. Now the only well that is selected is that first well that you just clicked; all the other wells are deselected. Then, select every other well of the first six columns by holding down Ctrl key and clicking the wells. Your pattern should look like Figure 5-11. You have just configured which wells will be filled with water from the source reservoir Rsvr.

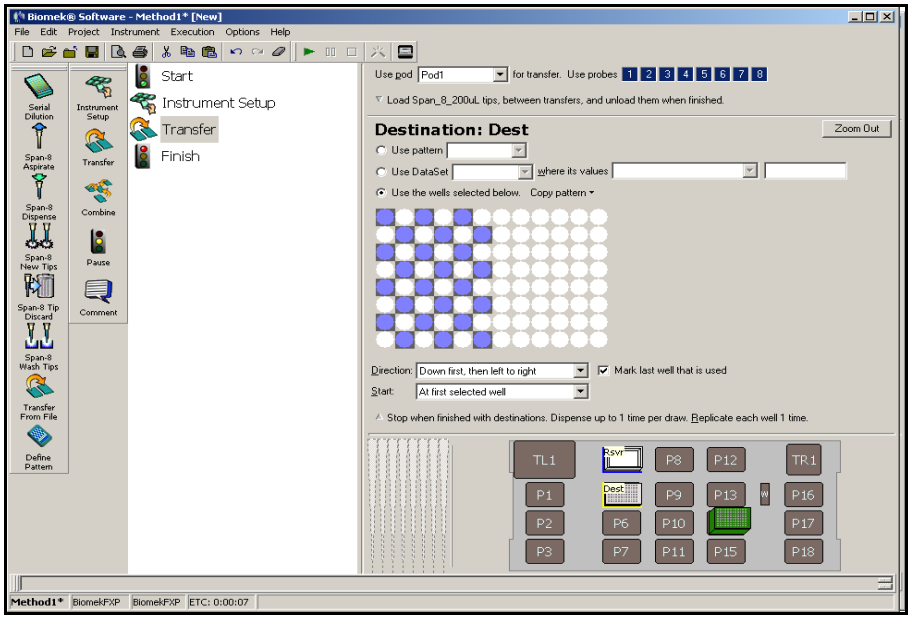

Figure 5-11. Destination labware zoomed in

4. Allow the default selections in Direction, Start, and Mark last well that is used to remain.
5. Choose **Zoom Out**.
6. Select the volume field, which allows you to designate the amount of liquid to be dispensed. For this tutorial, you're transferring 100 µL; so type **100** into the Volume field. This means you will be dispensing 100 µL into each of the wells you selected.

7. Right-click on the large tip illustration and choose **Measure from Bottom**.
8. Set the dispense height in the large tip illustration to **1.00 mm from bottom**, using the same technique as you used for setting the aspirate height.

The destination labware is now configured and the editor looks like Figure 5-12.

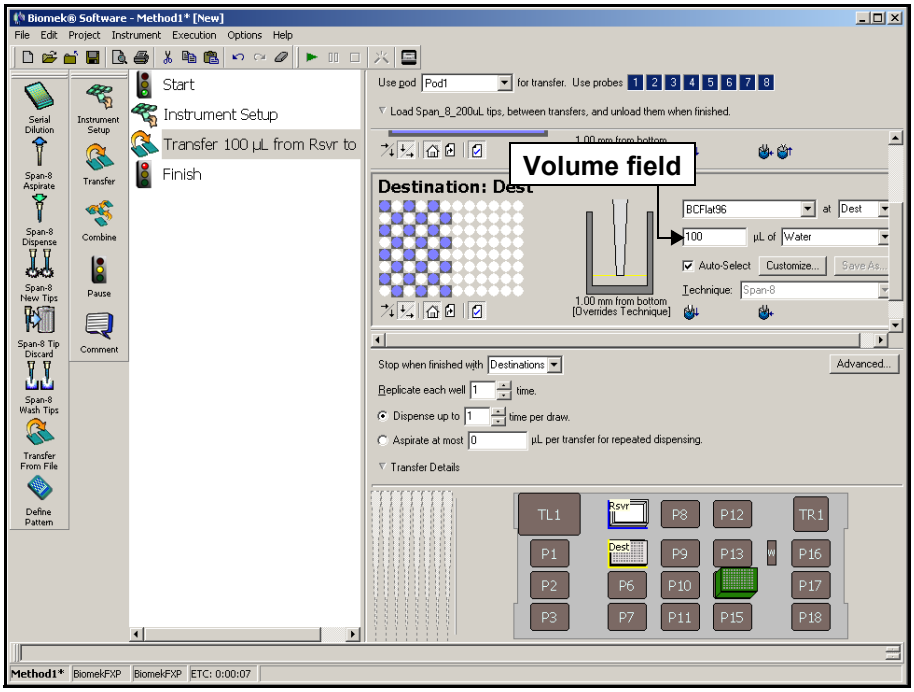

Figure 5-12. Configured destination labware

5.4.4 Determining the Estimated Time for Completion (ETC) of the Method

Your liquid transfer is set up, so let's see how long it will take to run the entire method by using the Finish step.

To do this:

1. Click on the **Finish** step in the Method View.
2. Check the status bar at the bottom of the editor for a display of the ETC. For this method, the ETC is approximately 1:32 (Figure 5-13). It's all right if your ETC varies slightly.

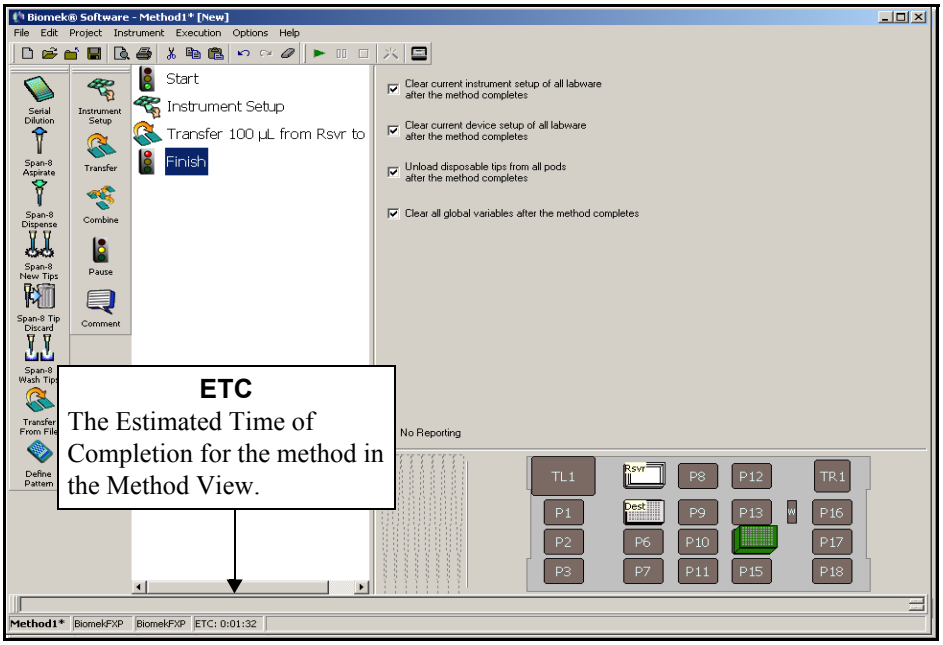

Figure 5-13. Finish step displaying the ETC

Congratulations! You've just built a liquid transfer method using Biomek Software that:

- Prepared the main editor for a new method.
- Set up the deck and the configured the labware you want to use using an Instrument Setup step.
- Added and configured a liquid transfer using a Transfer step.

5.5 Running the Method

Now that you've built a method, let's run it.

5.5.1 Validating the Method and Confirming the Deck Setup

Tip

If the method will be run on hardware, rather than in simulation (refer to Section 5.5.2, *Viewing the Method in the Biomek Simulator*), make sure air is purged from the lines (refer to the *Biomek Software User's Manual,* Section 13.17.6, *Purging Air From Span-8 Pod Before a Method Run (FX and NX-S8 Only)*).

When you select **Run**, the method will be validated internally to check for errors. After this validation is complete, a deck confirmation prompt will appear over the main editor (Figure 5-14). This prompt displays the deck setup as interpreted by the software.

If you wish, you can also view the method in the Biomek Simulator. Refer to Section 5.5.2, *Viewing the Method in the Biomek Simulator*.

To confirm the deck setup:

1. Click on the green arrow button on the toolbar or from the Execution menu, choose **Run**. A deck confirmation appears (Figure 5-14).
2. Visually confirm the physical deck setup matches the deck confirmation.

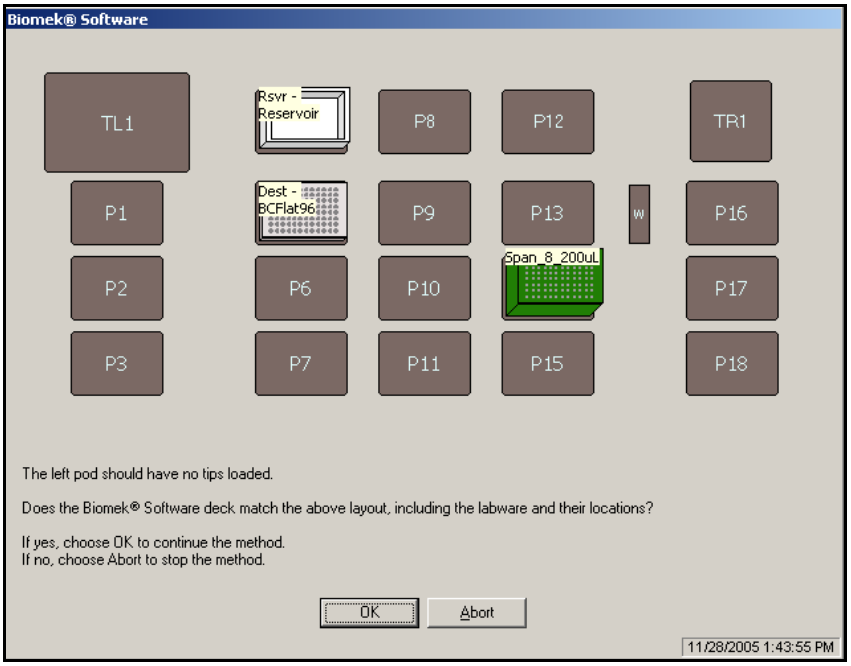

Figure 5-14. Deck confirmation prompt

3. Choose **OK** if the deck confirmation matches the physical deck setup or choose **Abort** and then change the Instrument Setup step to match the physical deck setup. The method runs as soon as you choose OK. You can visually follow the run in the Method View; steps are highlighted as the step is executed.

5.5.2 Viewing the Method in the Biomek Simulator

CAUTION: Make sure the proper port is selected in Hardware Setup. Simulate is used only when running methods on the Biomek Simulator. To run methods on the instrument, choose the com port to which the instrument is connected.

When a method is run in simulation, the Biomek Simulator appears, showing an animated 3-D model of the instrument performing the method. Setting the simulation mode is configured in Hardware Setup (Figure 5-15).

BIOMEK CONCEPT
Hardware Setup

Hardware Setup is used to configure Biomek Software with the appropriate Biomek instrument information, including the Biomek Simulator. While the Beckman Coulter Service Engineer normally installs and configures new devices, it may be necessary to install, configure, and remove other devices using Hardware Setup. Refer to the *Biomek Software User's Manual,* Section 5.2, *Accessing Hardware Setup*.

If you wish to view the method in simulation:

1. From the toolbar, choose **Instrument>Hardware Setup**. Hardware Setup appears (Figure 5-15).

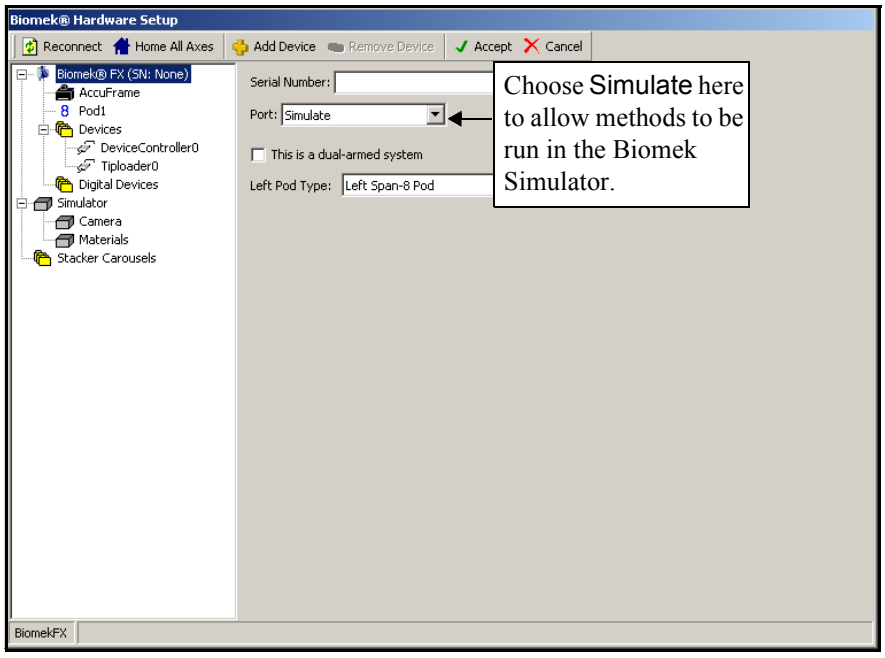

Figure 5-15. Hardware Setup

2. From Port, choose **Simulate**.
3. Choose **Accept**. Now, when a method is run, an animated 3-D model of the Biomek instrument is displayed (Figure 5-16). You can now watch a simulation of the Biomek instrument perform the steps in the method.

Tip

The simulator can be a useful tool to test methods to ensure that they are performing as expected without using up valuable reagents or tips, and can also save time not only in set up, but also by running at an accelerated speed. Refer to the *Biomek Software User's Manual,* Section 5.4, *Configuring the Biomek Simulator*, for more information on the simulator.

If you wish to run the method on hardware, you must go back to Hardware Setup and change the Port from Simulate to the Com port your Biomek instrument is connected to on your PC.

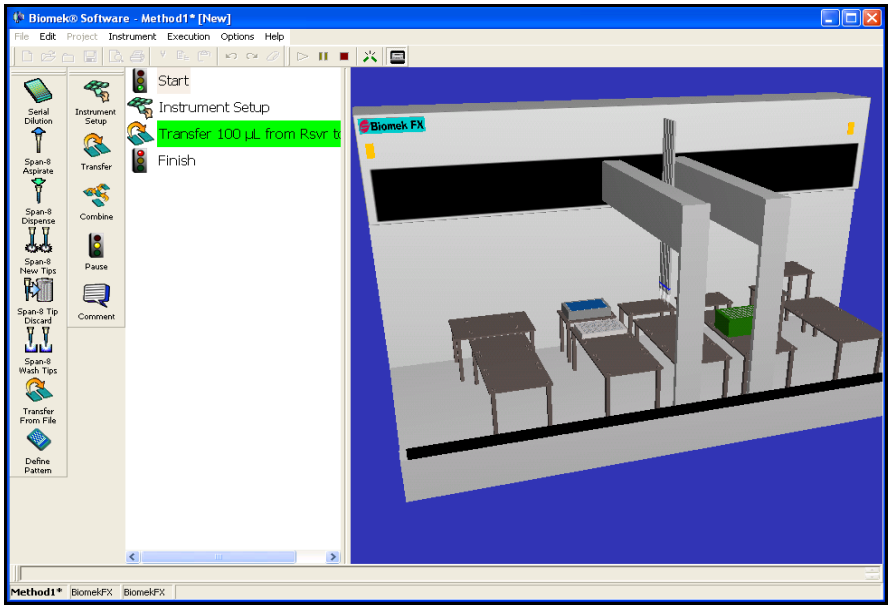

Figure 5-16. Running a method in simulation

5.6 Saving the Method

BIOMEK CONCEPT
Saving Methods

Methods may be saved at any time during their development. Saving a method automatically checks in the method, creating a record of the revision that preserves the method configuration at the time it was saved. Revisions may be accessed from the revision history at a later time. If any project items, such as labware definitions or techniques, change after the method is saved, when the method is opened next, the latest definitions are used. Refer to the *Biomek Software User's Manual,* Section 13.9, *Saving a Method* and Section 13.12, *Viewing Method History* for more information.

You will save the method you've just created.

To save your method:

1. Choose the **Save Method** icon on the toolbar.
2. In Method Name, type the file name under which your method will be saved. For this chapter, type **Getting Started Tutorial** (Figure 5-17).

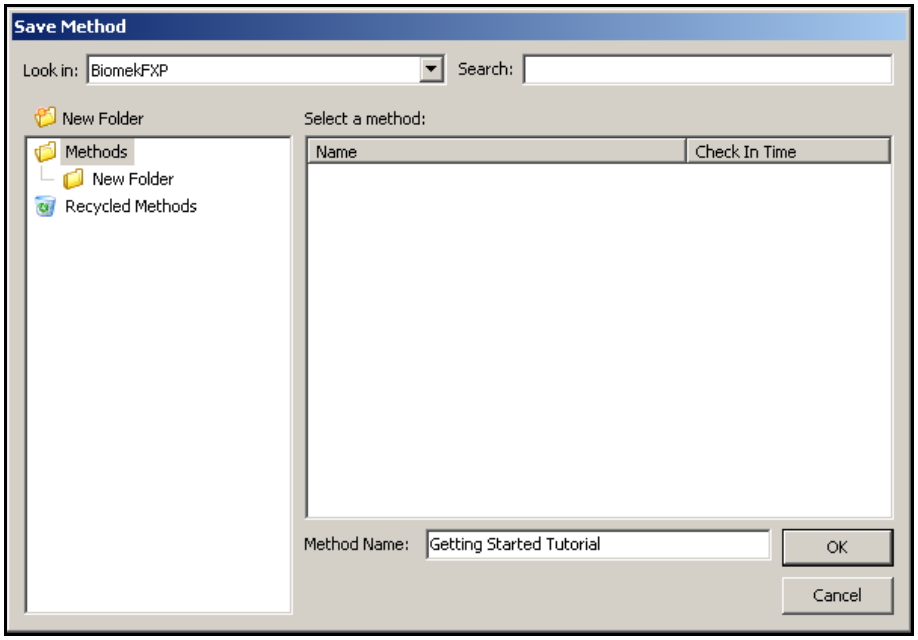

Figure 5-17. Save Method

3. Choose **OK**. Now notice how the method name in the Biomek main editor has changed to Getting Started Tutorial [Revision 1] (Figure 5-18).

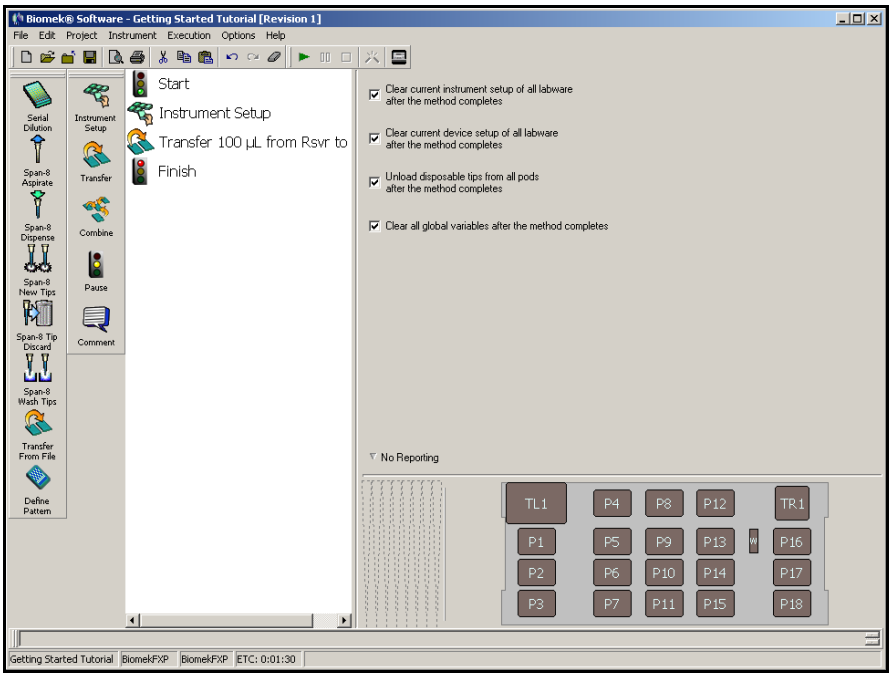

Figure 5-18. Method name has changed

Now go to the next chapter to learn how to use more steps in a method.

6 Span-8 Pod—Using More Steps in a Method

6.1 Introduction to Using More Steps in a Method

In the previous chapter of this tutorial (refer to Chapter 5, *Span-8 Pod—Getting Started with Biomek® Software*), you learned how to:

- Launch Biomek Software.
- Set up the deck for a liquid transfer.
- Build a liquid-transfer method.
- Save, run, and check in a method.

If you already know how to complete these tasks in Chapter 5, *Span-8 Pod—Getting Started with Biomek® Software*, you can just start with this chapter or subsequent chapters.

6.1.1 What You'll Learn in Using More Steps in a Method

This chapter will help you develop the skills to create methods for tasks such as transferring liquid from tubes to plates, liquid level sensing, and serial dilution. You will also learn how to pause the system to add more labware to the deck and handle errors. Using Single Step to perform single operations to improve method development will also be described.

In this chapter, you will learn how to:

- Transfer liquid from two sources to a single destination.
- Mix contents in labware.
- Remove and add labware to the deck once a method has started to run.
- Group steps logically in the Method View.
- Use the automatic serial dilution feature.
- Respond to errors.
- Perform single operations with the Biomek FX.

6.1.2 Setting Up Your Deck for Using More Steps in a Method

Using what you learned in Chapter 5, *Span-8 Pod—Getting Started with Biomek® Software*, launch Biomek Software and configure an Instrument Setup with the following:

- Place an **AP96_200µL_LLS** tip box on P5.
- Place **SmallTuberack_Microfuge** tube racks on P9 and P10 and name them **Tubes1** and **Tubes2**. Give these a **Nominal** volume of **1000** µL of **Serum** and choose **Sense the liquid every time a well is accessed "from the Liquid."** Sensing the liquid level from the Liquid helps pipetting performance since air will not be likely to be aspirated along with the liquid. See the sidebar.
- Place a **BCFlat 96** on P13 and name it **Dest**. Give this microplate a **Known** volume of **0** µL.

Your deck should look like Figure 6-1. Now go to the next activity to learn how to use other steps in your methods.

BIOMEK CONCEPT
Liquid Level Sensing

Liquid level sensing is used to determine the liquid level within a piece of labware using specially designed LLS tips. Refer to the *Biomek® FX and FX-P Laboratory Automation Workstations User's Manual,* Chapter 3.3.1, *Liquid Level Sensing*, for more information.

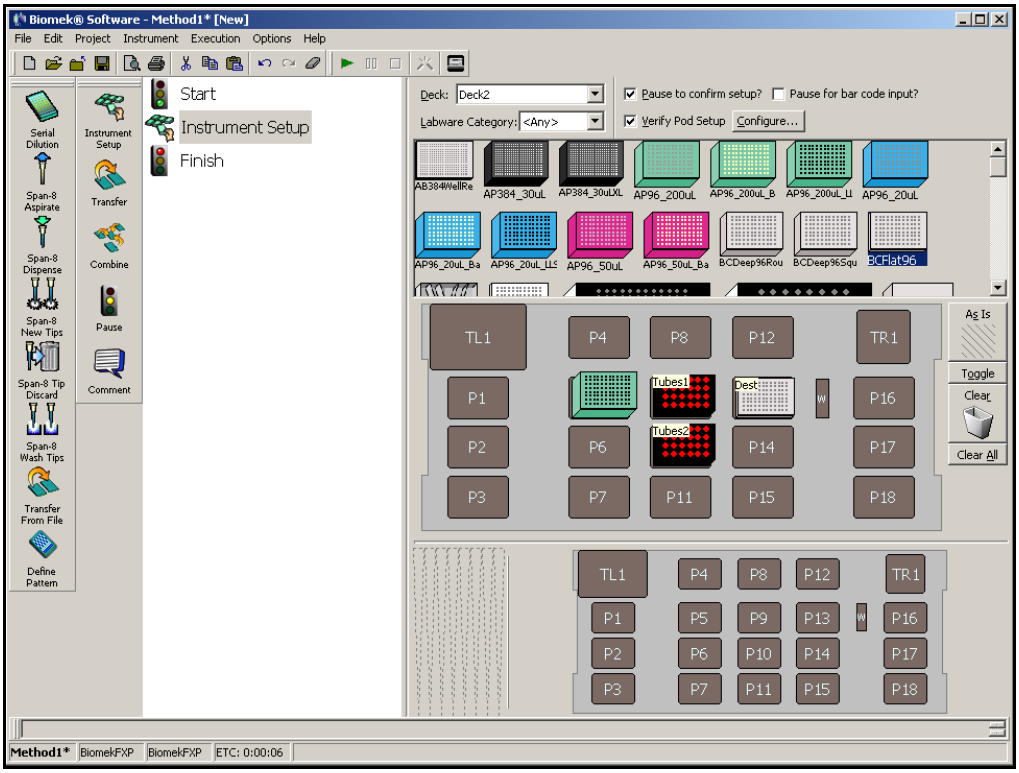

Figure 6-1. Completed Instrument Setup step

6.2 Transferring Liquid from Multiple Sources to a Single Destination

To transfer liquid from one or more sources to a single destination, a Combine step is used. It is similar to a Transfer step which uses a single source and one or more destinations.

If using fixed tips. . .

You won't configure the Combine step to load or unload tips.

Like the Transfer step, the Combine step will by default complete the following:

- load tips
- aspirate liquid
- dispense liquid
- unload tips

For this activity, you will use the default tip handling, configure the two sources, configure the destination, and configure transfer details to perform a tube-to-plate transfer and pool samples into the first column of 96-well plate.

6.2.1 Configuring Tip Handling

To set up the Combine step, you will insert the Combine step in the Method View and configure the step.

To do this:

1. Ensure you deck is configured according to the instructions in Section 6.1.2, *Setting Up Your Deck for Using More Steps in a Method*.
2. Add a **Combine** step after the Instrument Setup step.
3. Make sure the Span-8 Pod and all eight probes are chosen for use. See Figure 6-2.

If using fixed tips. . .

From Use Probes, right-click any probe and select **Use Fixed Tips**.

4. Collapse the **Tip Handling** since you will use the default settings for your liquid transfer. Make sure **Load AP96_200µL_LLS tips, between transfers, and unload them when finished** is displayed. Your main editor should look like Figure 6-2.

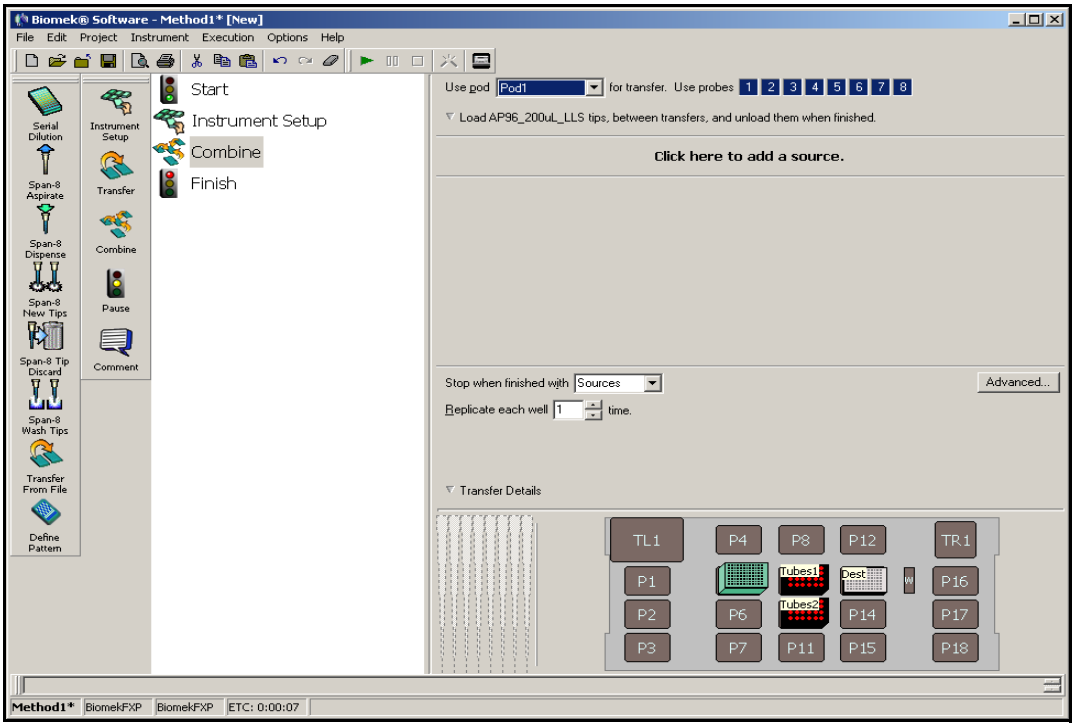

Figure 6-2. Combine step inserted and Tip Handling collapsed

6.2.2 Configuring Source Labware

To configure the two small tube racks from which you're going to aspirate:

1. Click on **Click here to add a source** (Figure 6-2)
2. Click on **Tubes1** sitting on P9.
3. In the Volume field, designate the amount of liquid to be aspirated. For this method, you're aspirating 25 µL, so type **25** into the Volume field.
4. Click on the **Dest** plate in the Current Deck Display to add a destination. You will configure the destination in the next section, but must choose it here to activate another source option.
5. Click on the next **Click here to add a source**.
6. Click on **Tubes2** sitting on P10 and type **25** into the Volume field.

The sources are now configured, and the editor should look now look like Figure 6-3.

Tip

The well volume display shows how much liquid is in the well. If the liquid is not visible in the display, you may be accidentally trying to aspirate from an empty destination rather than a source.

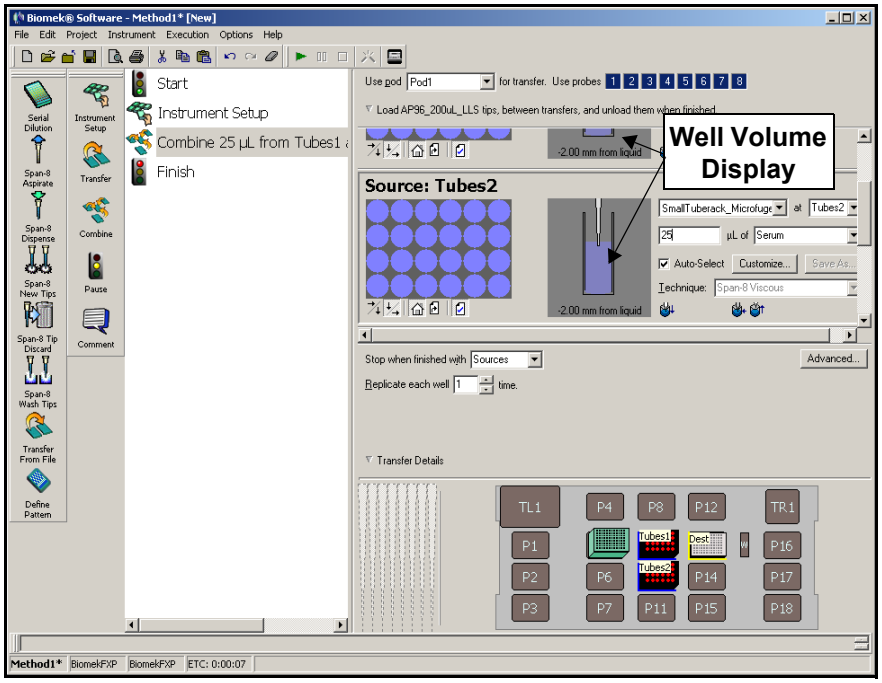

Figure 6-3. Source labware for Combine step configured.

6.2.3 Configuring Destination Labware

The next task is to designate where you want to dispense the aspirated liquid. For this method, you want to dispense into the first column of the 96-well plate on deck position P13. To accomplish this:

1. If necessary, scroll down until you see Destination: Dest. Click anywhere in the **Destination: Dest** configuration.
2. Click on the 96-well plate on P13.
3. Double-click the Destination labware graphic in the step configuration.
4. Select only the wells in the first column.
5. Choose **Zoom Out**.
6. In Stop when finished with, make sure **Sources** is chosen (Figure 6-4).

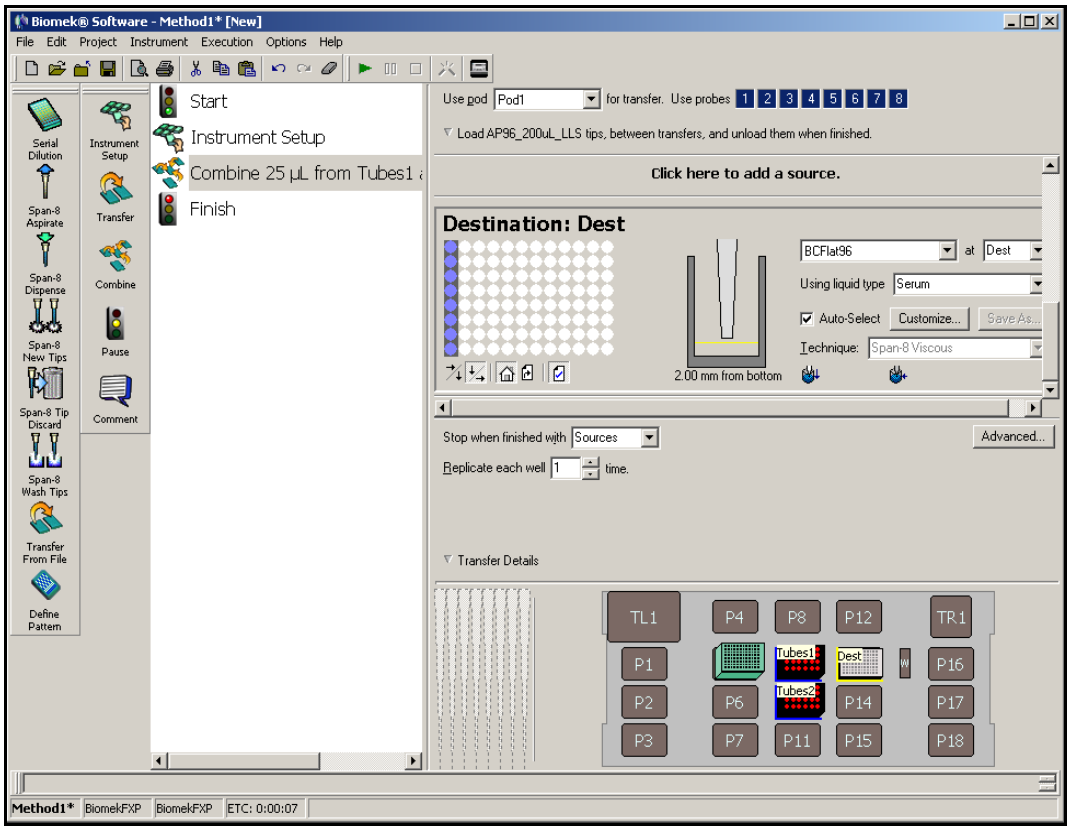

Figure 6-4. Stop when finished with Sources chosen

That's it. You've just configured a Combine step to aspirate liquid from two sources in order to dispense it to a single destination. Go to the next activity to learn how to mix the liquid in the destination plate after dispensing.

6.3 Mixing Contents in Labware

BIOMEK CONCEPT
Techniques

Techniques are sets of predefined and stored values, including aspirate and dispense height, tip touch and other properties that affect pipetting. Based upon these stored sets of values and properties, the appropriate pipetting technique is selected automatically. If you want control over this otherwise automatic function, you can choose Customize for each source and destination in a liquid transfer. This customizing option is also available via the Technique Editor. Refer to the *Biomek Software User's Manual,* Chapter 10, *Understanding and Creating Techniques*.

In the Transfer and Combine steps, you can alter liquid-handling functions that extend beyond simple aspirating and dispensing. For example, you can turn off the tip touch feature, activate the pre-wet function, or configure mixing operations. These modifications are accomplished through customizing the technique and are used to control the pipetting process.

Let's suppose you wish to mix the contents of the destination plate once liquid from the two sources has been dispensed.

To complete this task:

1. Click the **Dest** labware in the Deck Display. This expands the destination labware configuration.
2. In the Destination configuration fields, select **Customize**. The Technique Editor opens to the Dispense tab (Figure 6-5).

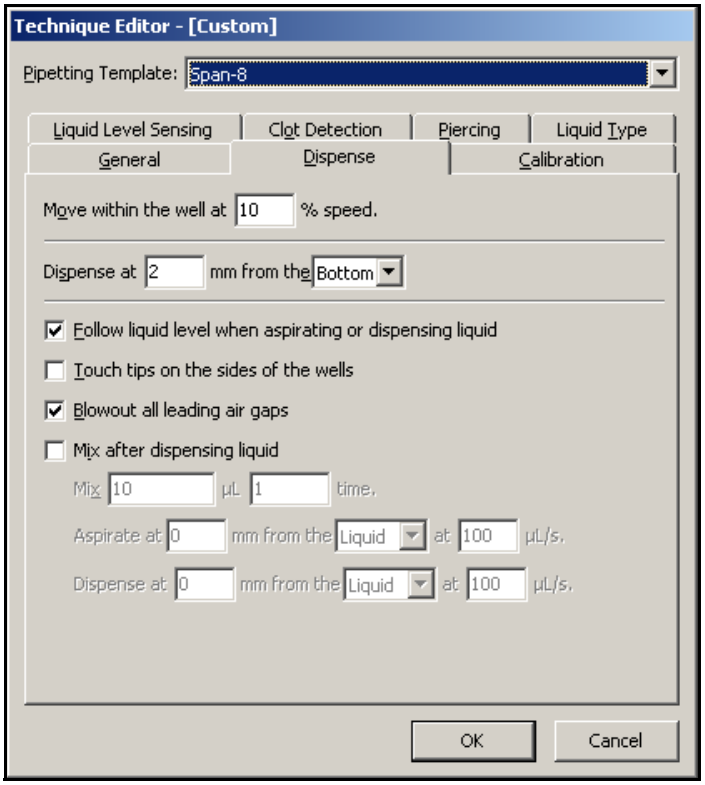

Figure 6-5. Dispense tab of the Technique Editor

6.3.1 Configuring “Mix after dispensing liquid”

Now, you will configure the options to mix the contents in the destination after dispensing.

To mix after dispensing:

1. From the Dispense tab (Figure 6-5), check **Mix after dispensing liquid**. The fields for this option are enabled. We’ll allow some of the defaults to remain except for the amount and number of times to mix.
2. In Mix, enter **25**. This specifies the amount of microliters that will be aspirated and dispensed during mixing.
3. In time, enter **2.** This specifies the number of times you want to mix the liquid after dispensing.

Tip

Pipetting from the Bottom can sometimes cause wells to overflow, or it can contaminate the tips. Aspirating from the liquid would be a good choice in these cases. This is not a concern for this tutorial, so you are leaving the default.

You’re finished configuring the mix after dispensing process. The Technique Editor should now look like Figure 6-6.

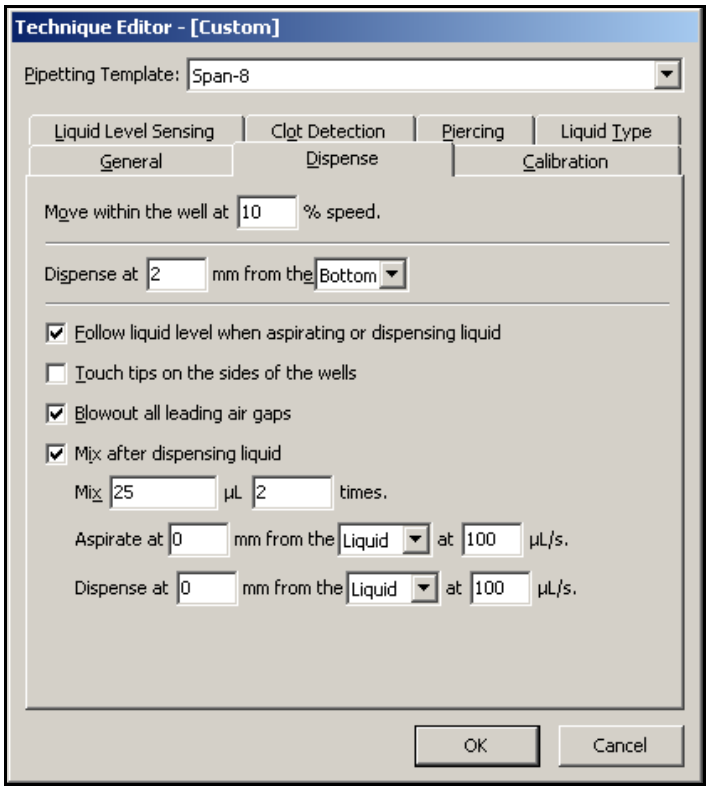

Figure 6-6. Configured mix in a Custom technique

4. Choose **OK**.

After you configure the mix operation and return to the Combine step configuration, you see an icon indicating that the technique now includes mixing. This is one of several icons that indicate different procedures in the pipetting technique. You will also notice that the Auto-Select option is turned off when the technique has been customized.

6.4 Adding Labware During a Method Run

Now you are ready to add labware to the deck for another liquid transfer process. Suppose that you want to add another reagent to the destination plate, but you don't want to place the second reagent on the deck until after the first Transfer is complete (perhaps it can only be exposed to light or open air for a very short time). This means that you will add a second Instrument Setup step to your method to indicate to the software that there is more labware now on the deck.

But before you add a second Instrument Setup step, you will:

- Move the pod to a new location by configuring a Move Pod step to make sure the pod is moved to a part of the deck where it won't prevent you from physically adding more labware.
- Pause the instrument by configuring a Pause step to give you enough time to physically add more labware.

6.4.1 Moving the Pod to a New Location

The Move Pod step on the Intermediate step palette repositions the pod away from the positions on the deck you want to reach manually. Since you are going to be adding more labware to the deck to prepare for another liquid transfer, you will need to move the pod away from the deck locations affected before pausing the system and adding more labware.

However, since the Move Pod step is on the Intermediate step palette, you will need to display that step palette before you can add this step to your method.

6.4.1.1 Displaying the Intermediate Step Palette

BIOMEK CONCEPT
Step Palettes

Steps are grouped on step palettes based upon the complexity of the operations they control and the depth of knowledge required to configure them, athough some steps are grouped on step palettes according to the specific pod or instrument they control.

To add the Intermediate step palette to the main editor:

1. Right-click anywhere in the space below the Basic step palette.
2. Choose **Intermediate**. The Intermediate step palette appears on the main editor (Figure 6-7).

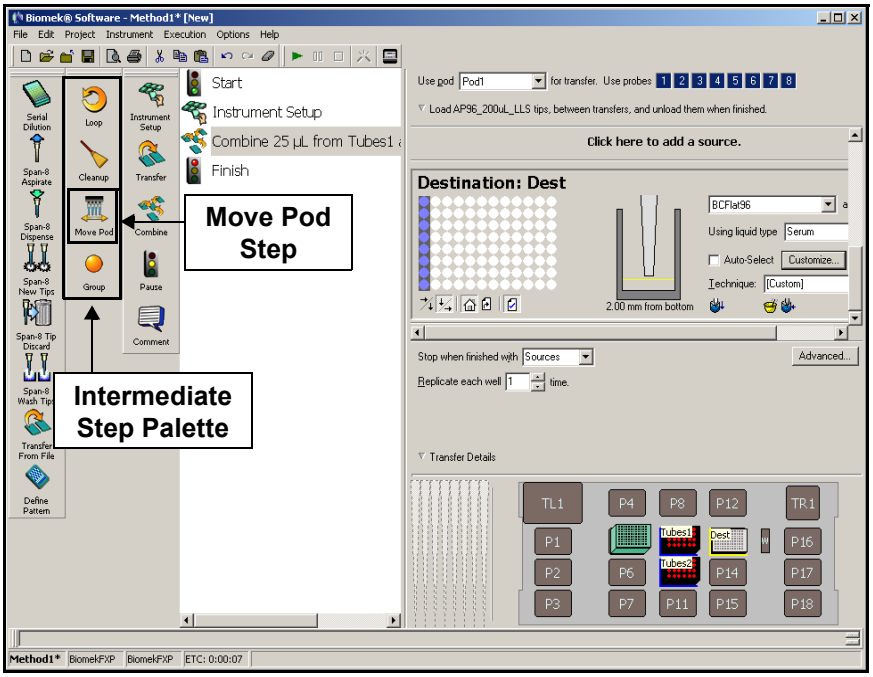

Figure 6-7. Intermediate step palette displayed

6.4.1.2 Configuring the Move Pod Step

To configure the Move Pod step:

1. Insert the **Move Pod** step in the Method View after the Combine step (Figure 6-7).
2. From the Location drop-down menu, choose **P16**. This instructs the pod to move and stop over the P16 position (Figure 6-8).

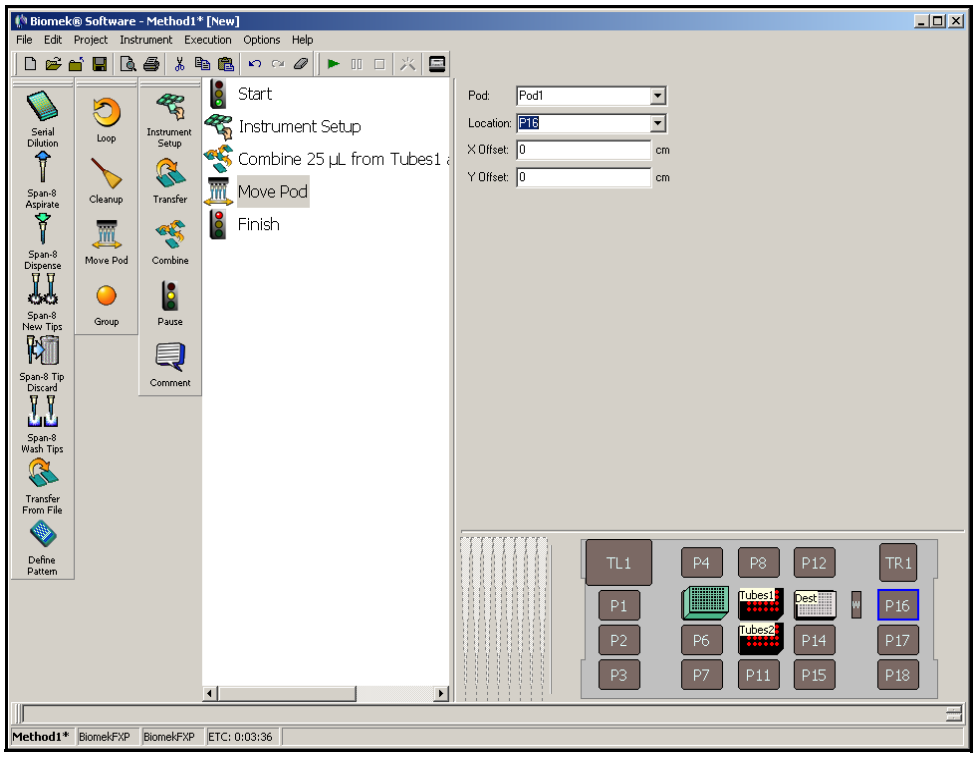

Figure 6-8. Configured Move Pod step

3. Click **Finish** to validate the method.

6.4.2 Pausing the Instrument

The instrument may be paused during a method run for either a specified amount of time or for an indefinite period of time by adding and configuring a Pause step. Depending on the purpose of the pause, you configure the step in one of the following ways:

- If you want to incubate a piece of labware in a specific position for a specific amount of time, you configure the desired time in seconds that you want that position to be idle and unavailable for interaction with the instrument.
- If you want to move labware manually during a method (either move it around on the deck, or remove it from the deck to take it to a device, such as a reader), you configure the step to pause the instrument for an indefinite period of time.

When the Pause step occurs during a run, the pod remains in the position of the last operation.

6.4.2.1 Configuring the Pause Step

You will configure the Pause step for indefinite amount of time to add more labware to the deck manually. The software will display a message on top of the main editor until you complete your manual operations, at which time it will resume the method run.

To configure the Pause step for an indefinite amount of time:

1. Insert a **Pause** step into the Method View below the Move Pod step.
2. Choose **Pause the whole system and display this message:**
3. Replace Paused in the message box by typing in the message: **Remove and store the tube racks at P9 and P10, remove the tip box at P5, and place a diluent reservoir at P14 and two new tip boxes at P5 and P6.**

The main editor should now look like Figure 6-9.

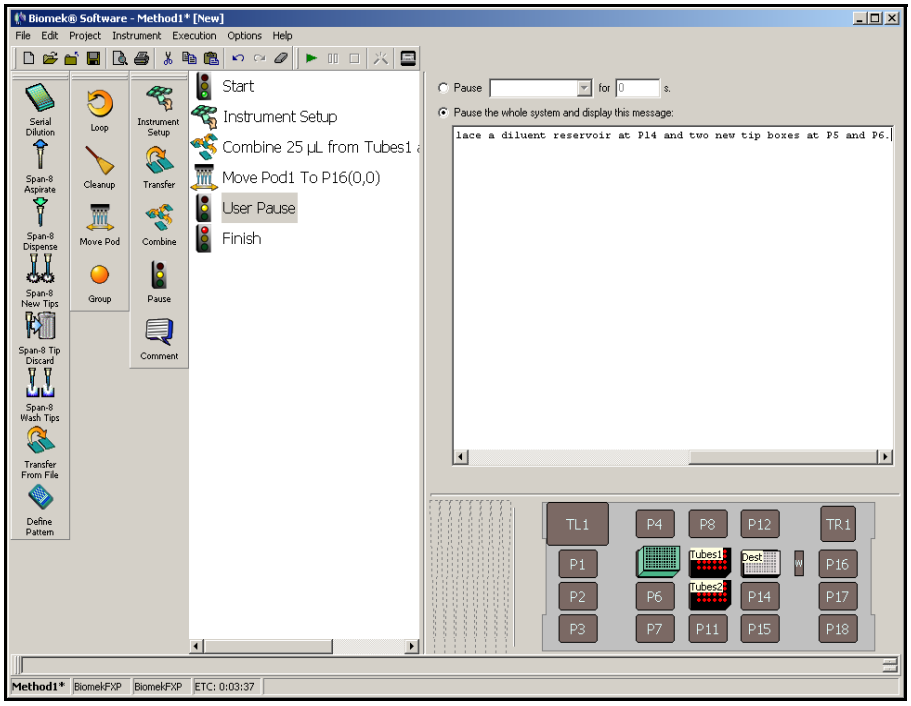

Figure 6-9. Pause configuration with message inserted

When the method is run, you will see a prompt similar to Figure 6-10 that will remain displayed until you choose **OK**.

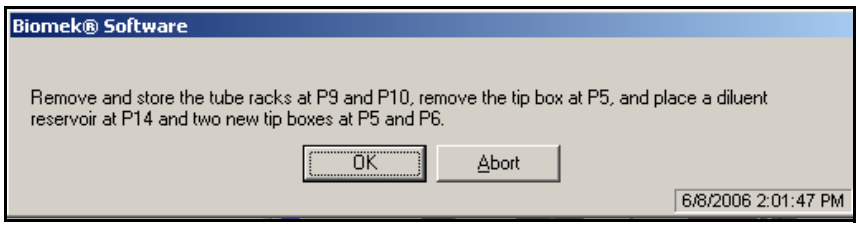

Figure 6-10. Pause prompt displaying the configured message

6.5 Adding a Second Instrument Setup Step

Now that you have prepared to add more labware to the deck during a method, you are ready to configure a second Instrument Setup step. You will insert a second Instrument Setup step after the Pause step. The second Instrument Setup step not only indicates the current state of the deck, but it also allows you to add more labware. But before you will add more labware to the deck, you will remove some labware.

To configure the second Instrument Setup step:

1. Insert an **Instrument Setup** step into the Method View below the User Pause step. This opens a second Instrument Setup step configuration.
2. Choose **Toggle** under the As Is square. This lets the software know that all deck positions are to remain as they are. The main editor should now look like Figure 6-11.

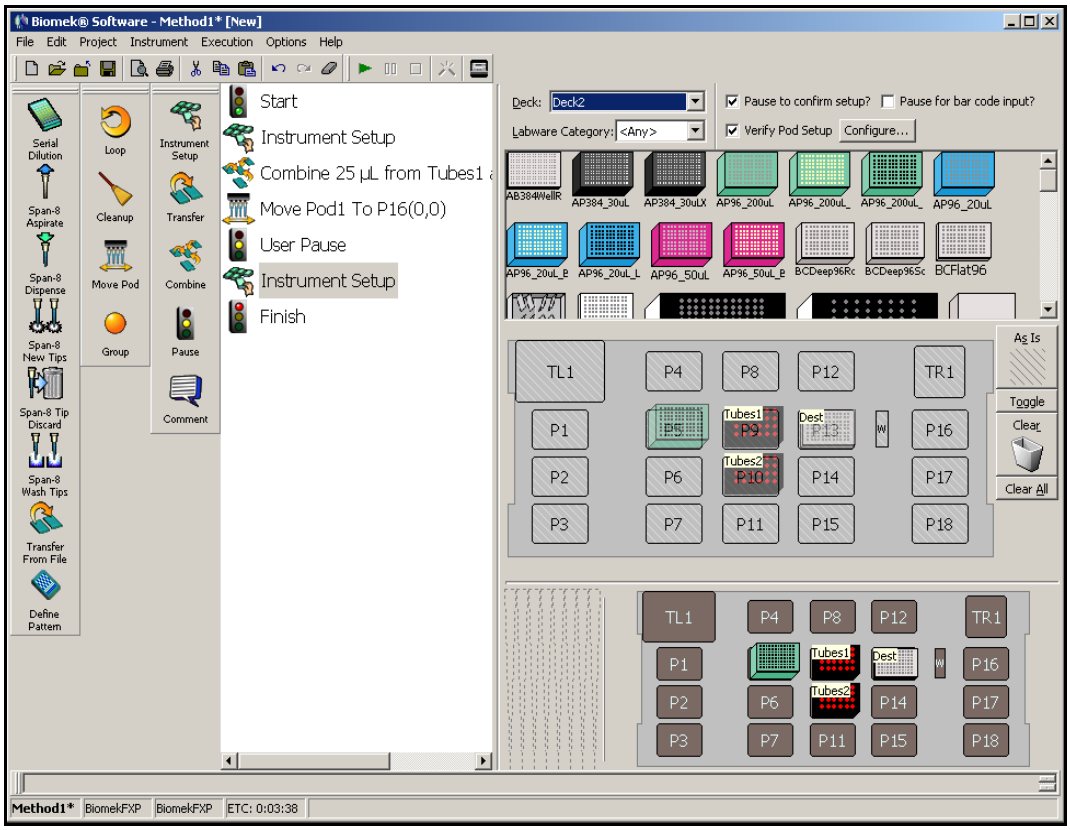

Figure 6-11. Adding an Instrument Setup step and toggling all deck positions As Is

3. Select **Clear** and then click on **P5, P9,** and **P10**. This removes the tube racks and used tip boxes. Now the main editor should look like Figure 6-12.

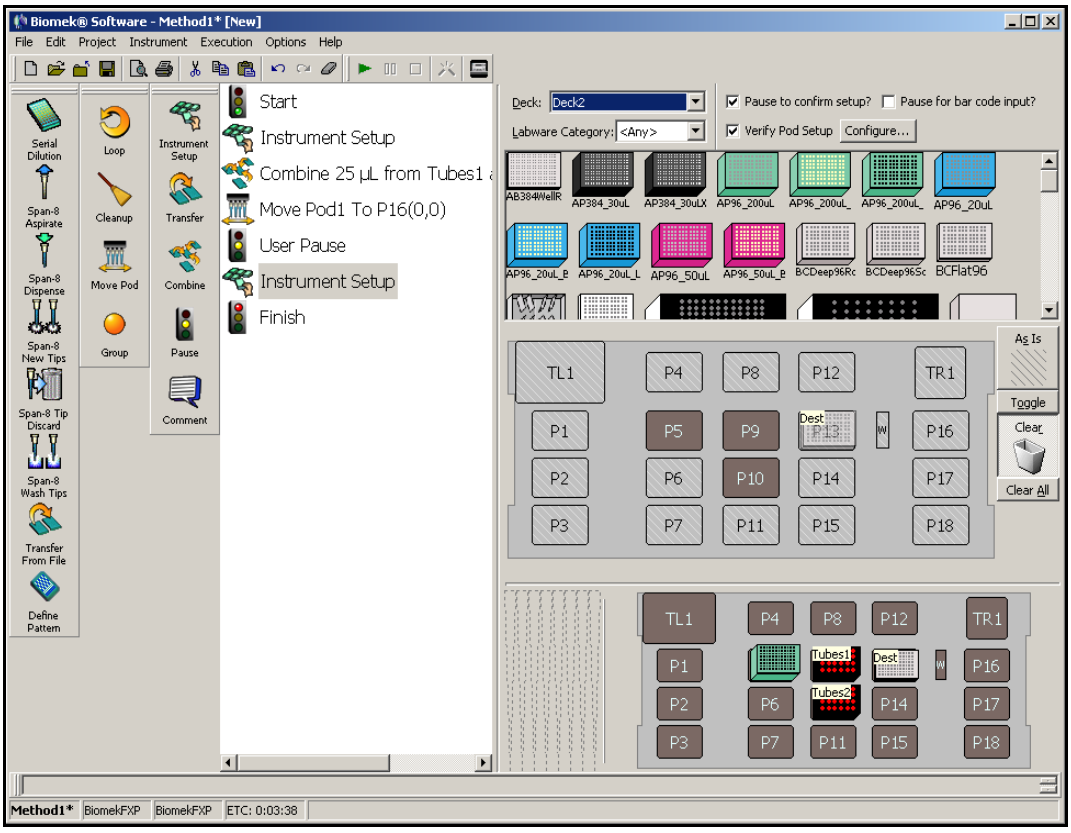

Figure 6-12. Using Clear to remove used tip boxes

6.5.1 Adding Labware to the Deck

Tip

Even though the software finds tips automatically, you have to ensure there are enough tips on deck to do the job. If you don't, you will get an error message.

Now you will add the labware to prepare for next liquid-handing process which is a Serial Dilution. For this next liquid-handling process, you will need to add two more tip boxes and an additional source reservoir. This new labware was what you configured to display when the instrument paused for manual placement.

1. Place a **Reservoir** on P14 and name it **Diluent**. Configure the reservoir to have an **Unknown** volume of **Water**. Make sure Sense the liquid level the first time a well with Unknown or Nominal volume is accessed "from the Liquid" is selected.

If using fixed tips. . .

You won't add tip boxes.

2. Place **Span_8_200µL** tip boxes on positions P5 and P6.

You may think that all the steps in the Method View make your method look complex. Go to the next section to learn what you can do about that.

6.6 Using a Group Step

BIOMEK CONCEPT
Group Step

The Group step allows you to "nest" a series of connected steps together, and give the group a logical name that appears in your Method View. Then, when you open your method, the Group step appears collapsed with the connected steps hidden. This makes the method appear shorter, and it allows you to see more of the method without scrolling the Method View up or down. You simply double-click on the Group step in the Method View to expand it and expose the nested steps.

To prevent your method from appearing too complex, you can group steps together logically under one unique heading by inserting the Group step into the Method View. This group of steps is hidden in the Method View under the name that you've given it during configuration of the Group step.

6.6.1 Configuring the Group Step

For this activity, you will group under one heading the Move Pod, Pause, and Instrument Setup steps you already have in your method. These steps are all associated with the preparation for the serial dilution.

To group these steps:

1. Insert the **Group** step from the Intermediate Step Palette in the Method View before the Move Pod step.
2. Double-click on the **Group** step to reveal the next End Group step. Scroll the Method View to the left if necessary.
3. In Group Label of the step configuration, enter **Pause to remove and add labware** (Figure 6-13).

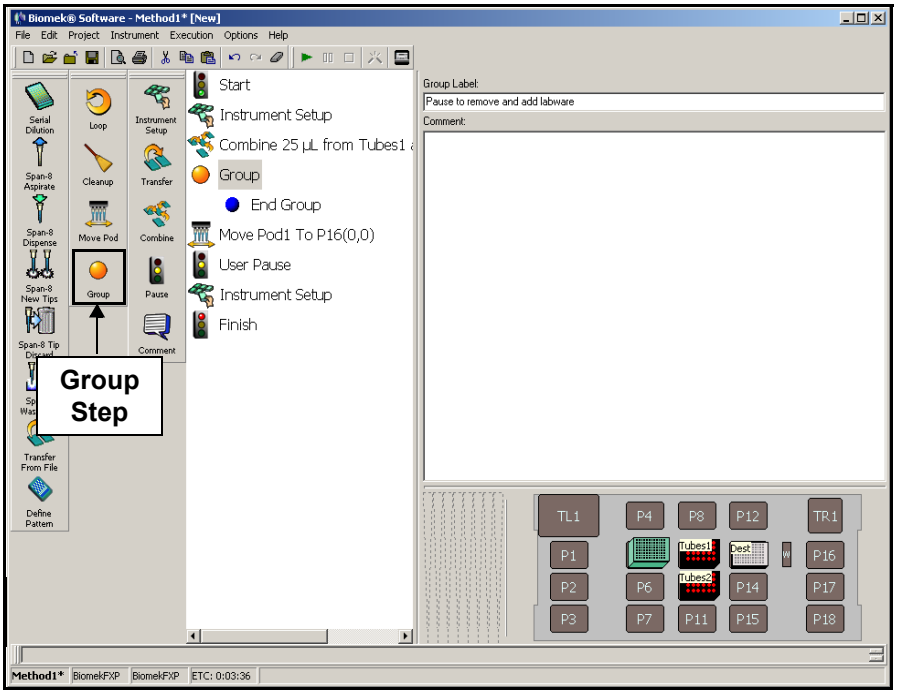

Figure 6-13. Configured Group step

4. Highlight the **Move Pod** step, then drag and drop it into the Group step above End Group.
5. Repeat step 4 above for the User Pause, and then the second Instrument Setup step.

Now the Move Pod, User Pause, and second Instrument Setup steps are nested logically within a Group step (Figure 6-14). You can expand and collapse this step as desired by double-clicking on the Group step title.

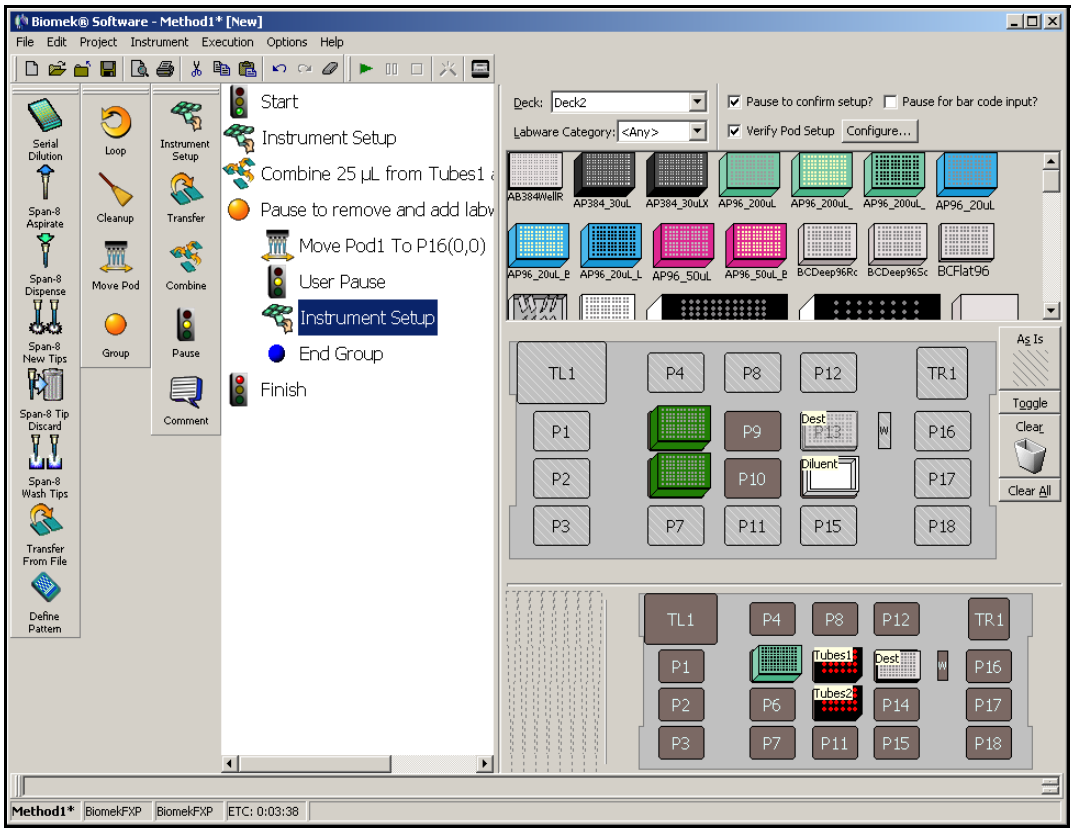

Figure 6-14. Configured Group step with nested steps expanded

Tip

Like the Group step, the Comment step does not initiate any actions on the instrument. It is used to provide descriptive information and notes in the Method View for a method. Refer to the *Biomek Software User's Manual,* Section 16.6, *Comment Step*.

Go to the next section to learn how to perform multiple dilutions of a sample on a single microplate.

6.7 Performing Multiple Dilutions of a Sample

BIOMEK CONCEPT
Serial Dilution Step

The Serial Dilution step completes multiple dilutions by performing the following actions in sequence:

- Transfer a volume of diluent to all selected wells.
- Transfer a volume of sample from the first selected well to the next selected well.
- Mix the solution via the Technique Editor.
- Transfer a volume of solutions to the next selected well.
- Mix the solution via the Technique Editor.
- Repeat transfer and mix operations until all selected wells are used.

The Serial Dilution step is used to perform multiple dilutions of a sample on a single microplate. This step transfers liquid from wells on a microplate to other wells on the same microplate and may also add diluent to those wells.

For this next activity, we will use the sample in the first column in the microplate on P13.

1. Insert a **Serial Dilution** step after the collapsed Group step (Figure 6-15).

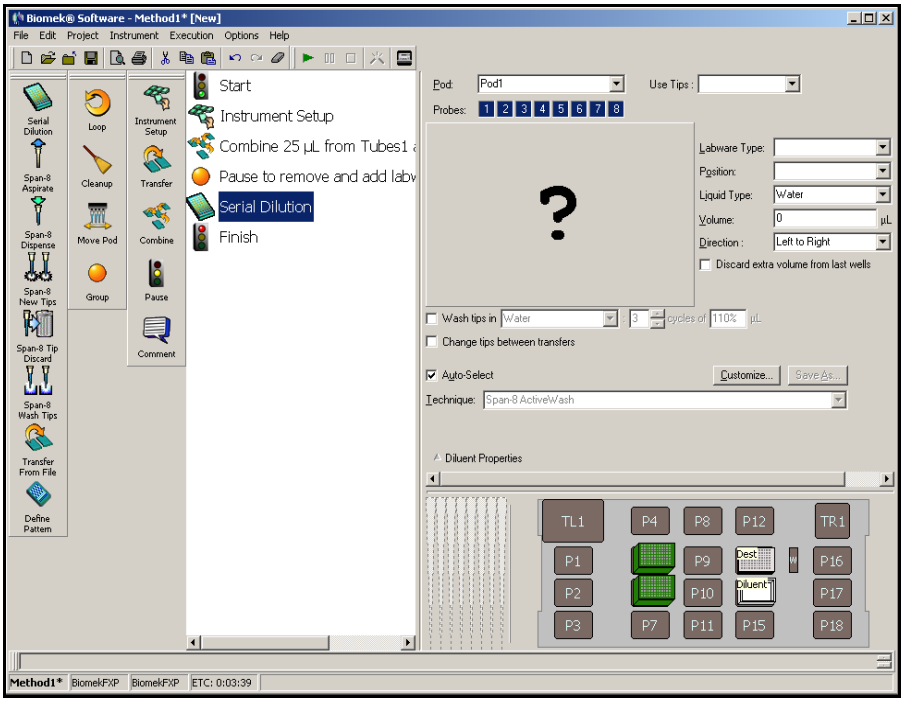

Figure 6-15. Serial Dilution step inserted

2. Make sure the Span-8 Pod and all Probes are used.

If using fixed tips. . .

From Use Probes, right-click any probe and select **Use Fixed Tips**.

3. In Use Tips, choose **Span_8_200µL**.
4. Click on the Dest plate on **P13**.
5. In Volume, enter **50**. This is how much liquid is aspirated from one well to the next.

Tip

With the Serial Dilution step, contiguous wells must be used. In Direction, Left to Right is faster.

6. In Direction, make sure **Left to Right** is selected since you will be diluting across the rows of the plate.
7. Check **Discard extra volume from last wells** and **Change tips between transfers**.
8. Select **Diluent Properties** to expand.
9. Check **Add diluent before transfer**.
10. Select the **Diluent** reservoir at P14. This means the diluent is added to all wells before the sample transfers.
11. Configure the Dilution Ratio to 1:2 by entering **2** in the field. The dilution ratio is a ratio of volume of sample to total solution, so a dilution ration of 1:2 means that for every µL of sample there is 1µL of diluent added, or a 50% dilution.
12. Make sure Change tips between diluent transfers is not checked.
13. Highlight the **Finish** step to validate the method. OOPS!!! All the red indicates an error (Figure 6-16). Go to the next section to learn how to correct this error.

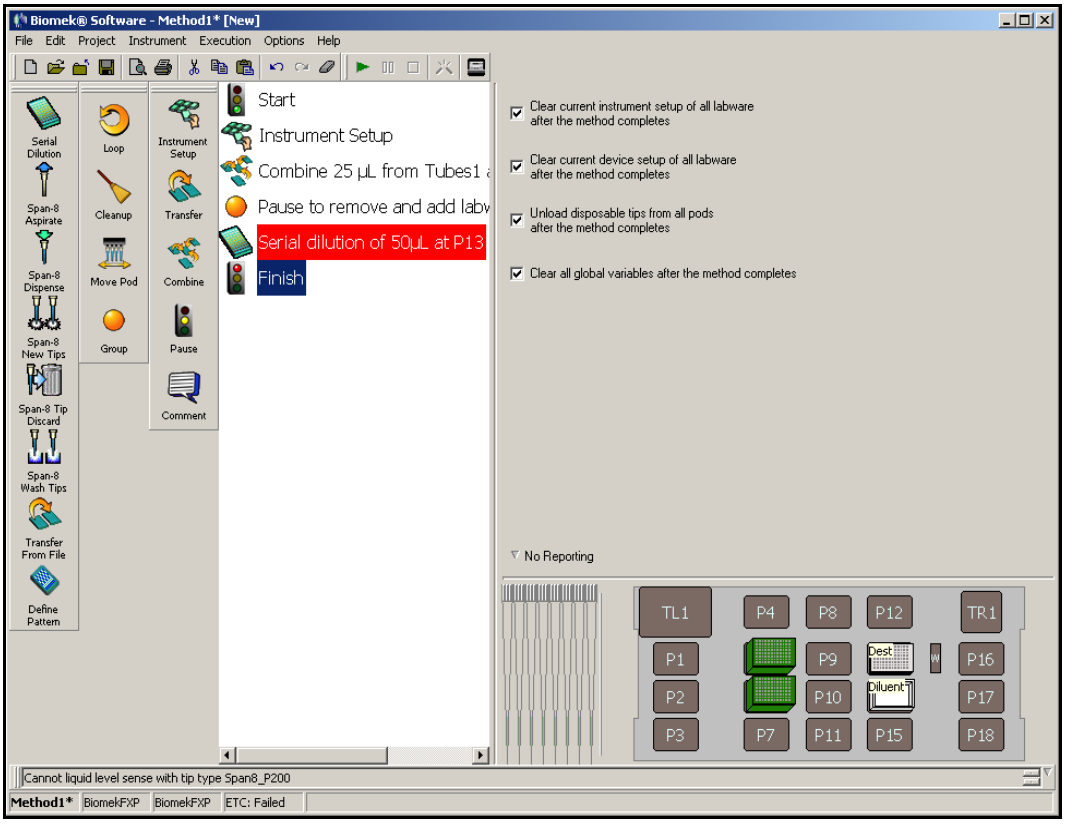

Figure 6-16. Error displayed

If using fixed tips. . .

You won't have an error. But read through the next section to learn about responding to error messages.

6.8 Responding to an Error Message

For this activity, you'll learn how to locate and respond to the error message you just received.

6.8.1 Locating the Error

Biomek Software alerts you to errors in several ways, including:

- The step with the error appears in red in the Method View when any step following the error is highlighted.
- A tool tip describes the error when you hover the cursor over the step causing the error.
- An associated red error message is displayed in the status bar at the bottom of the editor.
- The error is displayed in an error bar just above the status bar.

There are other errors that are displayed in error message boxes. These errors state the problem and display appropriate recovery options. Refer to the *Biomek Software User's Manual,* Chapter 26, *Handling and Preventing Errors*.

6.8.2 Correcting the Error

In this tutorial, the error message is Cannot liquid level sense with tip type Span8_P200. If you recall, you used Span_8_200µL tips in the second Instrument Setup step rather than the AP96_200µL_LLS tips you used in the first Instrument Setup step. Since you have configured Labware Properties for the Diluent reservoir to sense the liquid level and you have not used LLS capable tips, an error is produced.

To correct the error:

1. Expand the **Group** step and highlight the second **Instrument Setup** step.
2. Drag and drop the tip boxes on P5 and P6 to **Clear**.
3. Place **AP96_200µL_LLS** tip boxes on positions P5 and P6.
4. Highlight the **Serial Dilution** step in the Method View.
5. In the Use Tips, change the tip type to **AP96_200µL_LLS**.
6. Click the **Finish** step again in the Method View.

There, the error has been corrected.

Biomek Software continually validates the steps as you progress through building your methods. When you highlight any step, the steps above that point in the method will be validated. If an error is encountered, the step causing the error will be highlighted in red.

Now go to the next section to learn how to use Single Step to view each operation of this method.

6.9 Performing Single Operations with the Biomek FX

BIOMEK CONCEPT
Single Step

Single Step pauses the Biomek instrument between each operation in a step, allowing visual verification that the operation is correct. Performing single operations can help when fine tuning a method.

In this activity, you will use Single Step to view each operation of the method you just created.

1. Choose **Execution>Single Step**. Single Step appears (Figure 6-17).

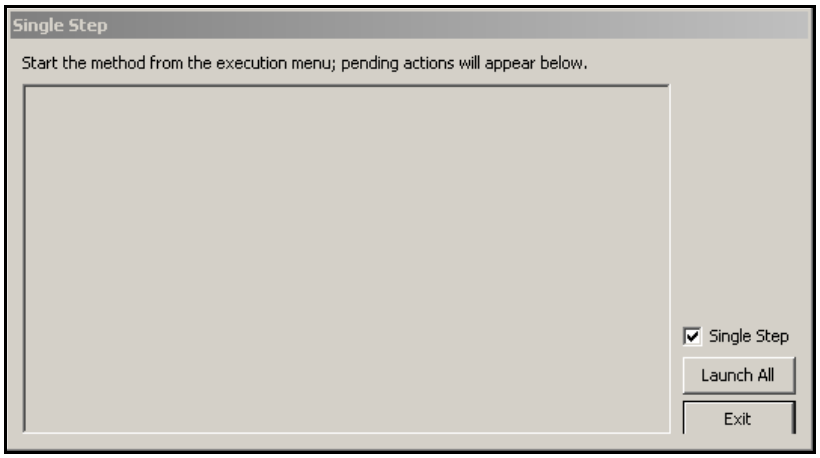

Figure 6-17. Single Step

Tip

Go slowly when using Single Step. It is possible to move too quickly through the method and by-pass the steps that need verification.

2. While Single Step is still open, choose **Execution>Run** from the greyed-out toolbar. Single Step with specific operations displayed appears (Figure 6-18). If you have other ALPs configured on your deck, the initialization process for them appears in Single Step.

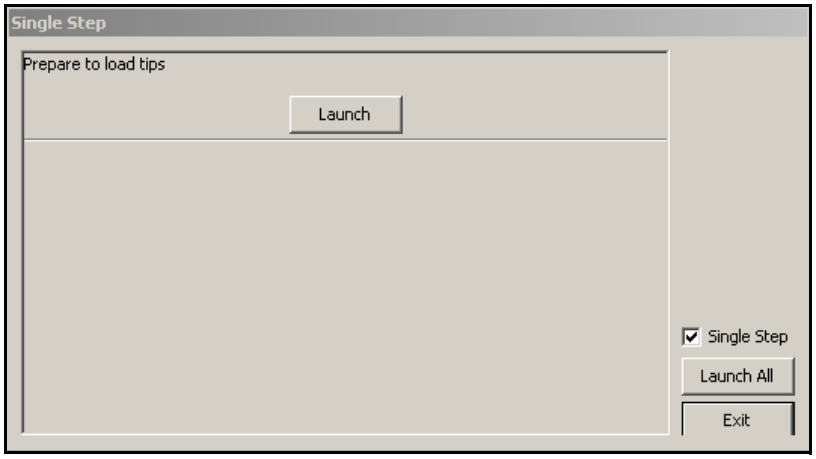

Figure 6-18. Single Step with specific operations displayed

3. Under Prepare to load tips, choose **Launch**. The next operation is launched (Figure 6-19).

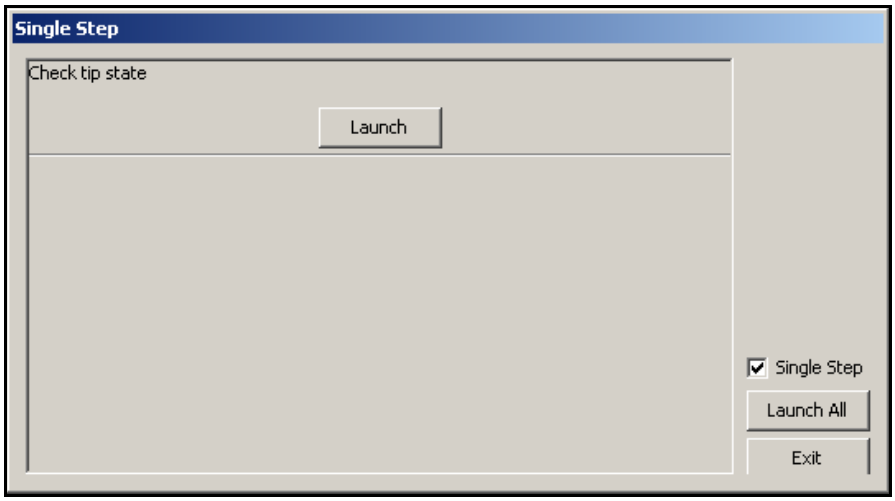

Figure 6-19. Single Step with next operation launched

4. Choose **Launch** again. The Biomek prompt appears. If the physical deck matches the software prompt, choose **OK**. Single Step continues displaying each operation, along with the option to Launch and execute that operation.
5. Continue to view each operation by choosing **Launch** or to stop Single Step, choose **Exit** to allow the method to run without the option to view each operation.

Go to the next chapter to learn how to use individual steps to more precisely control a liquid transfer.

7 Span-8 Pod—Using Individual Steps to Transfer Liquid

7.1 Introduction to Using Individual Steps

In the previous chapters of this tutorial (Chapter 5, *Span-8 Pod—Getting Started with Biomek® Software* and Chapter 6, *Span-8 Pod—Using More Steps in a Method*) you learned how to:

- Launch Biomek Software and build, run, and save a simple transfer method.
- Transfer liquid from two sources to a single destination.
- Mix contents in labware.
- Remove and add more labware to the deck once a method has started to run.
- Group steps logically in the Method View.
- Use the automatic serial dilution feature.
- Perform single operations with the Biomek FX.

If you already know how to complete these tasks, you can start with this chapter or subsequent chapters.

7.1.1 What You'll Learn in Using Individual Steps to Transfer Liquid

This chapter will help you enhance your method-building skills to create more advanced methods using variables, expressions, and "loops" to repeat tasks. You will also learn how to conserve and wash tips and view a log file.

More specifically, the step-by-step instructions in this chapter will teach you how to:

- Aspirate and dispense liquid independently using individual steps.
- Use variables and expressions.
- Use a Loop step to repeat actions.
- Load and unload tips independently using individual steps.
- Wash tips at the Span-8 Tip Wash ALP during a method.
- View a log file associated with a method.

7.1.2 Setting Up Your Deck for Using Individual Steps

Launch Biomek Software, start a new method, and using an Instrument Setup step, configure the following:

1. Place an **AP96_200uL_LLS** tip box on P9.
2. Place a **Reservoir** on P10. Name it **Source** and configure it to contain an **Unknown** volume.
3. Place a **BCFlat96** microplate on P14. Name it **Dest** and configure it to contain an **Unknown** volume.

Your deck should look like Figure 7-1. Now go to the next activity to learn how to use individual steps to transfer liquid in a method.

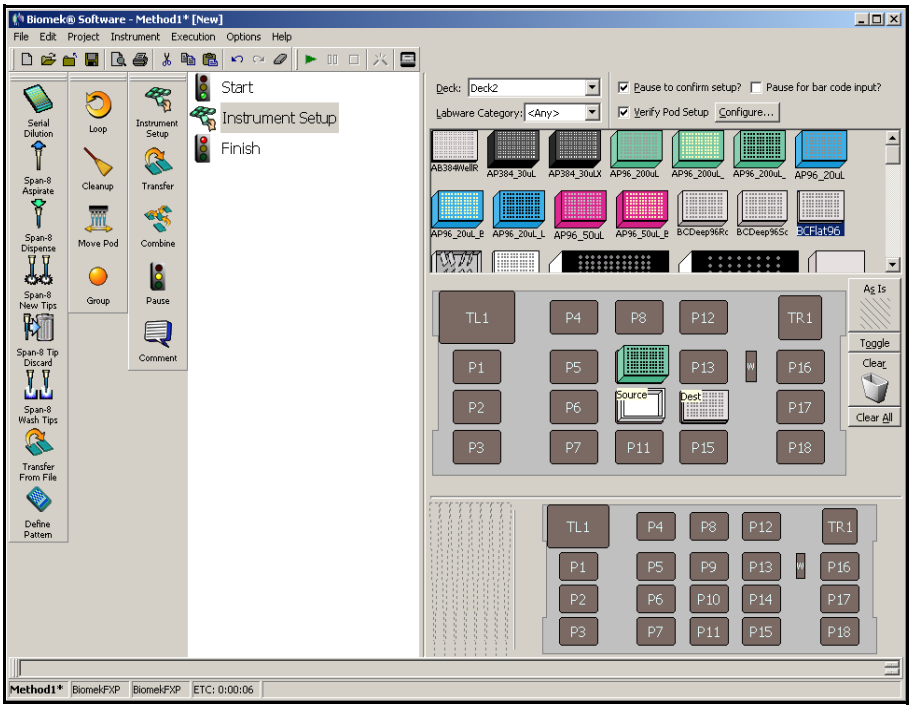

Figure 7-1. Initial Instrument Setup for using individual steps

7.2 Using Individual Steps to Transfer Liquid

In previous chapters, when you wanted to transfer liquid from one plate to another, you used the Transfer and Combine steps to perform all the necessary actions—loading tips, aspirating and dispensing liquid, and unloading tips. Sometimes, however, you want more direct control over these actions; for example, precise control over the order in which samples are transferred or when tips are loaded, unloaded, and washed.

When you need more control over the liquid transfer operation than the Transfer and Combine steps provide, you can configure liquid transfers using individual steps. Unlike the Transfer and Combine steps, these individual steps each perform only one task (e.g., aspirate, dispense, load tips, wash tips, or unload tips).

In this section, you will use the Span-8 Aspirate and Span-8 Dispense steps to transfer liquid from the Reservoir source to the BCFlat96 destination plate.

7.2.1 Aspirating Liquid Using the Span-8 Aspirate Step

You can use the Span-8 Aspirate step to aspirate liquid from a microplate or reservoir.

To aspirate liquid from the reservoir using the Span-8 Aspirate step:

1. Ensure you configured the deck according to the instructions in Section 7.1.2, *Setting Up Your Deck for Using Individual Steps*.
2. Drag a **Span-8 Aspirate** step from the Span-8 step palette to the Method View and drop it after the Instrument Setup step.

3. In the Current Deck Display, click on **Source** to select it as the labware from which to aspirate. Figure 7-2 appears.

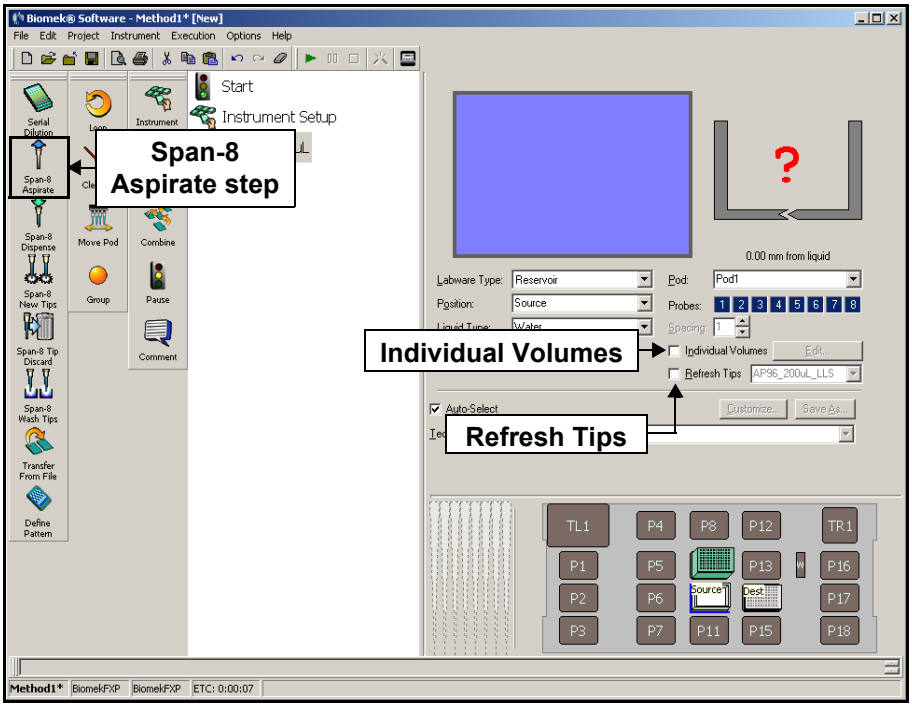

Figure 7-2. Source chosen for Span-8 Aspirate step

4. Check **Individual Volumes** and select **Edit** (Figure 7-2). Individual Volumes appears.

5. In Individual Volumes, alternate the volume aspirated by each probe between **40** and **50** to configure the volumes like Figure 7-3.

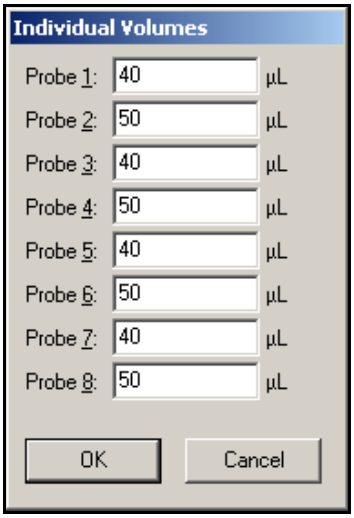

Figure 7-3. Individual Volumes configured for Span-8 Aspirate step

6. After entering the volumes in Individual Volumes, choose **OK**. Note that the Volume on the step configuration is now greyed out.

If using fixed tips. . .

You don't load and unload tips so you won't need to refresh tips. Skip step 7. Go to the next section.

Tip

If tips are already loaded, Refresh Tips unloads those tips (along with any liquid in those tips if they are not empty) and loads new tips prior to aspirating. This option should be used only when there are either no tips loaded or the tips are empty.

7. From the Span-8 Aspirate step configuration, check **Refresh Tips** (Figure 7-2). This will load new tips before the pod aspirates. Make sure AP96_200µL_LLS are chosen. Your Span-8 Aspirate step is configured and the main editor should look like Figure 7-4.

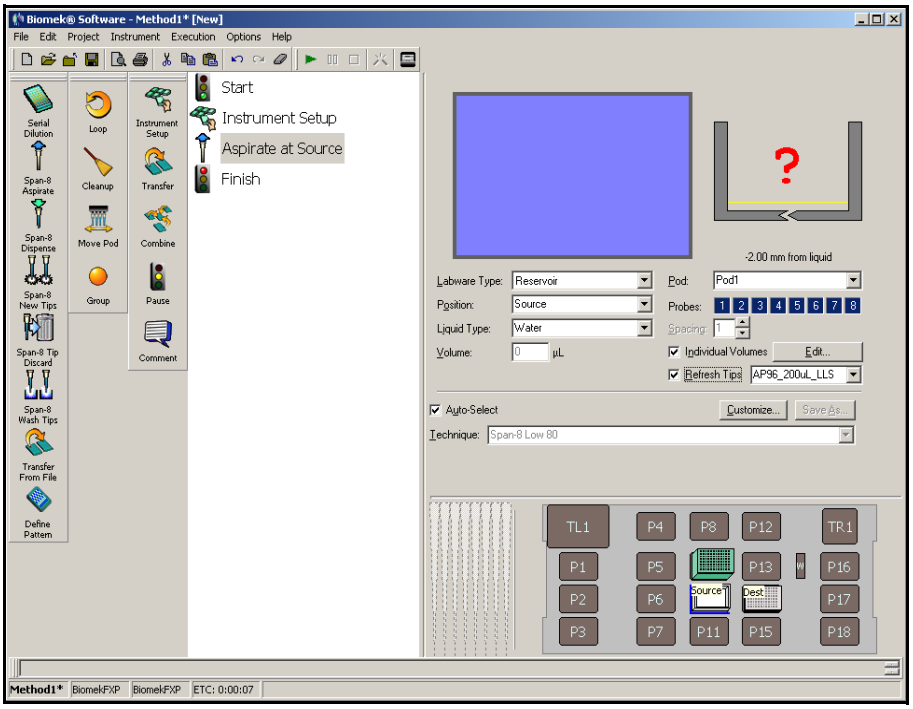

Figure 7-4. Span-8 Aspirate step configured

7.2.2 Dispensing Liquid Using the Span-8 Dispense Step

Now that you have aspirated some liquid, you need to dispense it into another piece of labware. In this tutorial, you will dispense the aspirated liquid into some wells of the Dest plate.

To dispense previously aspirated liquid:

1. Drag a **Span-8 Dispense** step from the Span-8 step palette to the Method View and drop it after the Span-8 Aspirate step.
2. In the Current Deck Display, click on the **Dest** plate on position P14 to select it as the destination.
3. Make sure the first column of the microplate is selected.

4. Check **Individual Volumes** and select **Edit**. Individual Volumes appears. Note that the volumes you configured earlier for the Span-8 Aspirate step are displayed (Figure 7-5).

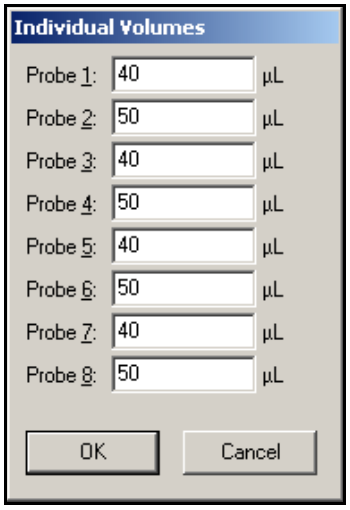

Figure 7-5. Individual Volumes

5. From Individual Volumes, choose **OK**. Note that the Volume on the step configuration is now greyed out.

You have now configured a simple method that aspirates individual volumes for each probe from a reservoir source plate and dispenses into one column of a destination plate using individual steps. If you like, you can run this method on your Biomek FX instrument or in the simulator (refer to Chapter 5, *Span-8 Pod—Getting Started with Biomek® Software*, for more information on how to do this).

In the next section, you will reconfigure this simple method to use a variable for the volumes to aspirate and dispense.

7.3 Using Variables in a Method

BIOMEK CONCEPT
Variables

Using a variable provides several advantages:

- If you want to change a value that is used in several places within a method, you can change it in one place and it is automatically changed everywhere that variable is used.
- The value of a variable can be set at run time and the method is automatically updated appropriately.
- Decisions can be made at run time based on the value of a variable (you will do this in the next chapter).

Variables make it easier to modify a method. When configuring steps, you enter the name of the variable in the desired field; when the method is run, the actual value of the variable is substituted and the action executed.

You will now create a variable for the individual volumes to transfer and use it in the Span-8 Aspirate and Span-8 Dispense steps. This includes:

- *Creating a Variable in the Start Step* (Section 7.3.1).
- *Using a Variable with Expressions in Step Configurations* (Section 7.3.2).
- *Changing the Value of a Variable at Run Time* (Section 7.3.3).

7.3.1 Creating a Variable in the Start Step

The Start step, in addition to being the first step in the method, also can be used to create and name variables that can be used throughout the method. Variables named in a Start step may be used in configuring other steps in the method. Other steps in Biomek Software also allow you to create variables, but those variables are local and are particular to the steps in which they are created. You will work with local variables later in the chapter with the Loop step.

You will now create a variable for the volume to transfer using the Start step.

To create a variable in the Start step:

1. Select the **Start** step in the method view to display its configuration.
2. In Variable Name, enter **Vol**.
3. In Value, enter **50**. Your Start configuration should look like Figure 7-6. You have created a variable named *Vol* that has a value of 50.

Tip

Do not use the variable C__Volume. This variable is built into Biomek Software and is associated with tracking volume.

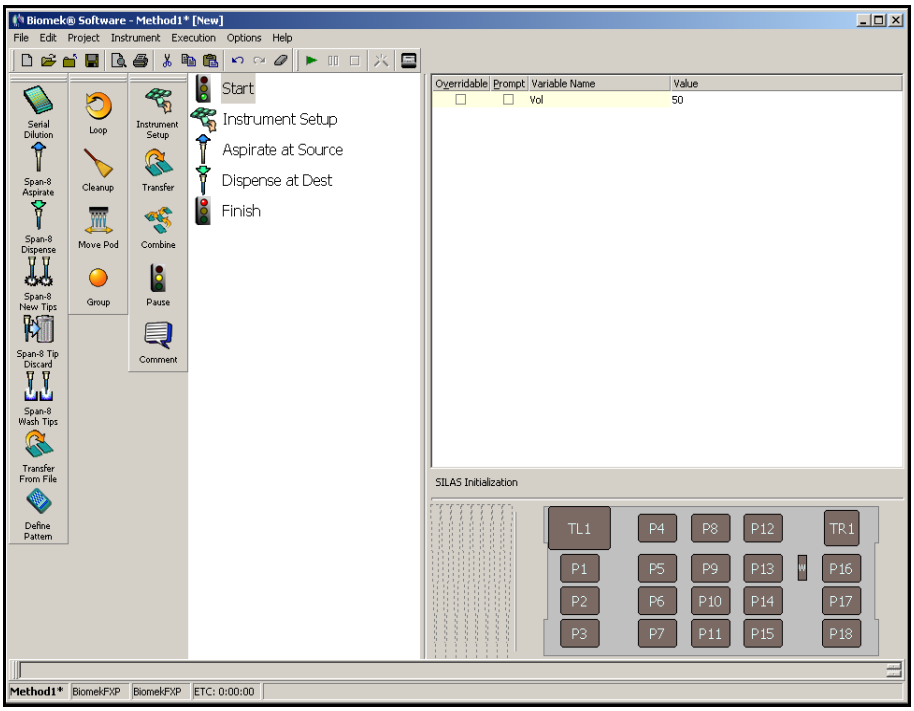

Figure 7-6. Vol variable created in the Start step

7.3.2 Using a Variable with Expressions in Step Configurations

Tip

Variable names are not case sensitive, so entering the name VOL, vol, Vol, or VoL are all evaluated the same.

You will now use the *Vol* variable you created in the Start step with expressions to configure some individual volumes to transfer in the Span-8 Aspirate and Span-8 Dispense steps.

To use a variable in a step configuration:

1. Select the **Aspirate at Source** step in the Method View.
2. Select **Edit** next to Individual Volumes.
3. In Individual Volumes, for Probe 1, enter **=Vol** (including the equal sign), in Probe 2, enter **=Vol+5**, in Probe 3, enter **=Vol+10** as shown in Figure 7-7. Variables and expressions are always preceded by an equal sign when entering them into a step configuration field. When the method is run, *Vol* is replaced by the value of the variable and the expression is evaluated; in this case, 50 µL for Probe 1, 50 plus 5 or 55 µL for Probe 2, and 50 plus 10 or 60 µL for Probe 3.

BIOMEK CONCEPT Expressions

Expressions combine text, numerical constants, and variables using operators to modify a variable. These operators may perform a number of mathematical operations or combine text strings. Just like with variables, the expression is evaluated and the resulting value is substituted for the expression at run time.

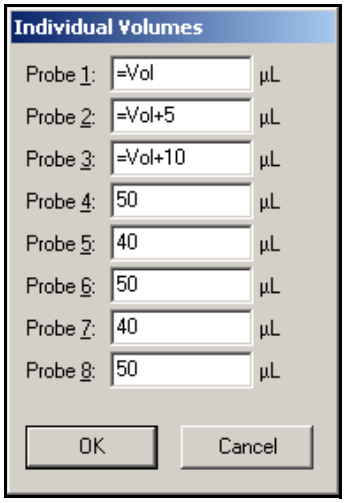

Figure 7-7. Individual Volumes with variables entered

4. Choose **OK**.
5. Select the **Dispense at Dest** step in the Method View and configure Individual Volumes using the same variable and volumes as you did for the Aspirate at Source step. You will need to configure this as it won't automatically display the volumes you configured in the Aspirate at Source step like it did in Section 7.2.2, *Dispensing Liquid Using the Span-8 Dispense Step* since it will only update the first time the volumes are configured for a step.
6. Choose **OK** when Individual Volumes has been correctly configured as shown in Figure 7-7.

To change the volume you want to transfer, you would change the value of the variable *Vol* in the Start step. The volumes for probes specified in Individual Volumes for both the Span-8 Aspirate and Span-8 Dispense steps would then automatically be updated when the value of *Vol* is substituted at run time.

7.3.3 Changing the Value of a Variable at Run Time

Since the value of a variable can be changed throughout the method by changing the value of the variable in the Start step, it makes it easy to quickly reconfigure methods that are similar except for one or two items.

For variables that are created in the Start step, you can configure Biomek Software to prompt you to specify a value for the variable at run time. When configured to do this, a prompt appears for each variable when the method is run. The value that you entered for the variable in the Start step is now the default value for the variable. You can use that value by choosing **OK**, or enter a new value and choose **OK** to change the value of the variable. The method run then uses the specified value for the variable and updates the method accordingly.

To specify the value of a variable at run time:

1. Select the **Start** step.
2. Select the **Prompt** check box for the variable Vol. Your Start configuration should now look like Figure 7-8.

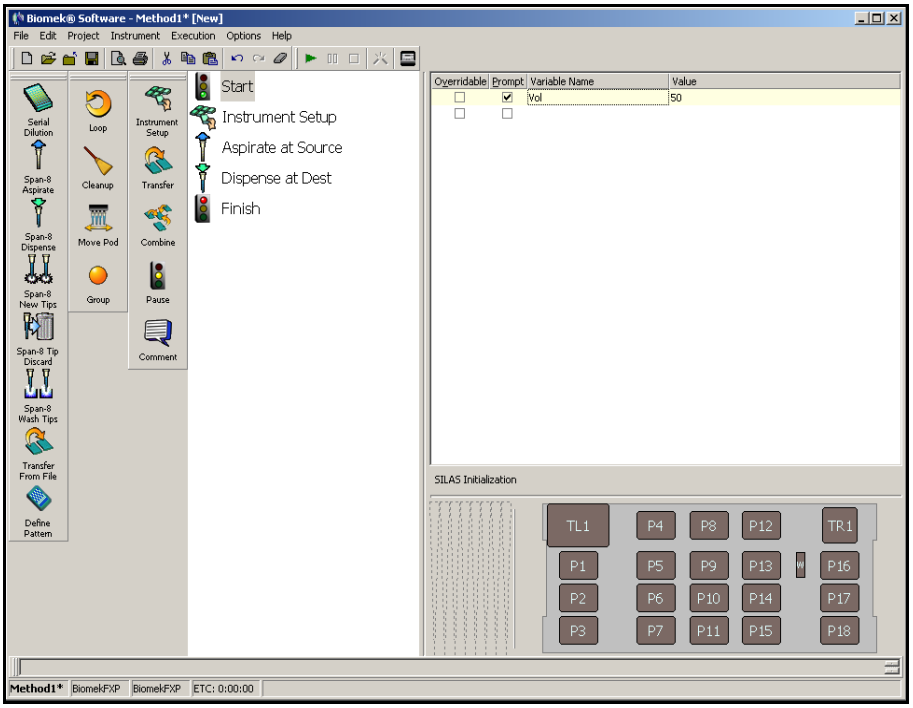

Figure 7-8. Prompting for value of a variable

3. Run the method. A prompt appears allowing you to enter a value for the variable (Figure 7-9).

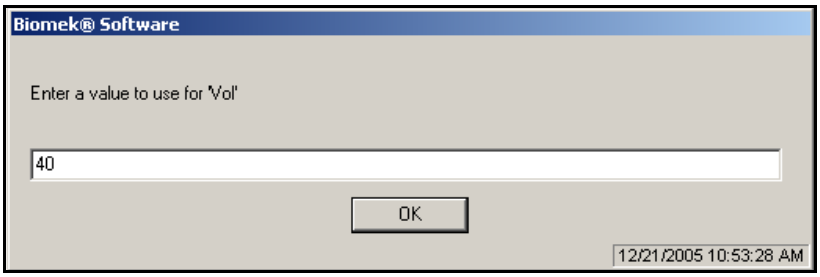

Figure 7-9. Prompt to specify the value of a variable

4. Enter **40** and choose **OK**. If you run the method on your Biomek FX instrument, you should notice that 40µL was transferred for Probe 1, 40 plus 5 or 45 µL for Probe 2, and 40 plus 10 or 50 µL for Probe 3.
5. As in all methods, the deck setup confirmation prompt appears. Respond appropriately.

In the next section, you will learn to use variables and a Loop step to perform repeated actions in order to dispense to the destination plate.

7.4 Repeating Liquid Transfer Steps Using a Loop

BIOMEK CONCEPT
Loop Step

The Loop step repeats the nested step or steps until its End value is exceeded. For the first cycle of a Loop, its value is the Start value. After completing all steps inside the loop, the value changes by the Increment and the steps are repeated again. This process repeats until the Increment changes the value to be greater than the End value.

The Loop step enables you to repeat one or more steps for multiple cycles. Each cycle or iteration repeats the steps contained inside the Loop. An optional variable may also be created in the Loop step. This variable is assigned a start value that is incrementally processed with each cycle of the Loop until it reaches the end value.

In this section, you will modify the method to use a Loop step to aspirate and dispense to all 12 columns of a plate rather than just the first column. In completing this task, you will create a variable in the Loop step and use this variable to reconfigure the Span-8 Aspirate and Span-8 Dispense steps as the liquid transfer actions are repeated during method execution.

7.4.1 Repeating Actions Using the Loop Step

When you want to repeat actions several times during a method run, you use a Loop step. The Loop step allows you to repeat the actions of one or more steps without inserting and configuring those steps for each time they are to be repeated. Steps to repeat are placed, or nested, inside the Loop step.

Biomek Software internally tracks the value of the Loop for each cycle, and Start, End, and Increment values are specified (see sidebar at left). If desired, a name can be assigned to the Loop value to create a variable. This variable can then be used like any other variable to configure steps, but can only be used with steps contained within the Loop step.

To repeat the Span-8 Aspirate and Span-8 Dispense steps:

1. Insert a **Loop** step after the Instrument Setup step. The Loop and End Loop icons appear in the Method View, and the Loop step configuration is displayed.
2. In Variable, enter **column**. This will create a variable named *column* that can be used to configure steps within the Loop.
3. In Start, enter **1**. This will be the initial value assigned to the variable *column* on the first iteration of the loop.
4. In End, enter **12**. This will be the final value for the variable *column*. When the value of *column* exceeds the End value, the Loop step ends and the remainder of the method continues.

5. In Increment, enter **1**. The Increment value is how much the value for the variable *column* changes with each iteration of the Loop. Your Loop step configuration should look like Figure 7-10.

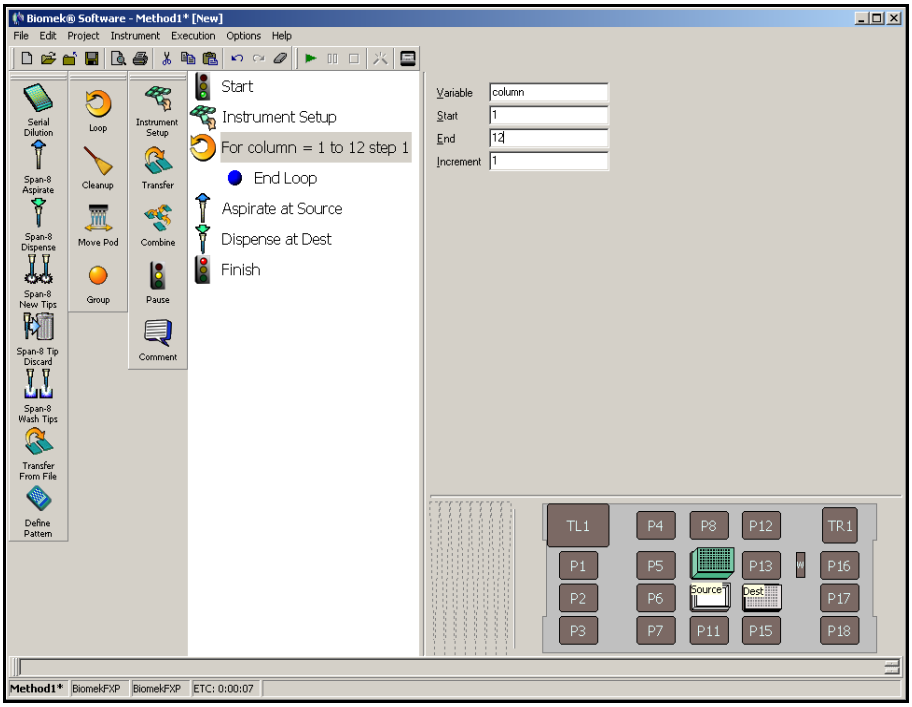

Figure 7-10. Loop step for repeating aspirate and dispense

6. Drag the **Aspirate at Source** and **Dispense at Dest** steps between the Loop and End Loop icons.

7.4.2 Specifying the Column to Dispense to in the Dispense Step

Now that you have configured the Loop step to create the variable *column* and placed the Aspirate and Dispense steps inside the Loop, you can use the variable *column* to reconfigure the Dispense step so all 12 columns on the Dest microplate are dispensed to rather than just the first column you originally configured.

1. Highlight the **Dispense at Dest** step configuration.
2. Right click anywhere in the labware graphic and choose **Specify Selection as Text**. Text Selection appears (Figure 7-11).

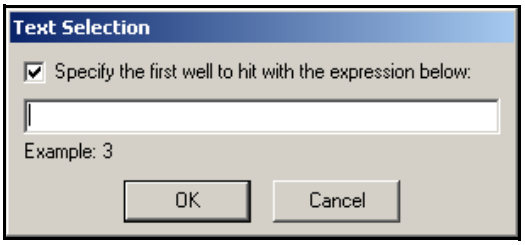

Figure 7-11. Text Selection

3. Enter **=column**. This means that it will dispense using all 8 probes starting with the well number equal to the value of the Loop variable. Since the wells in the first row are numbered 1 through 12, it will aspirate from the column that starts with value of *column*.
4. Choose **OK**.

The Dispense step configuration looks like Figure 7-12. The graphical representation of the labware is grayed out to indicate that the target wells to dispense into are specified by text.

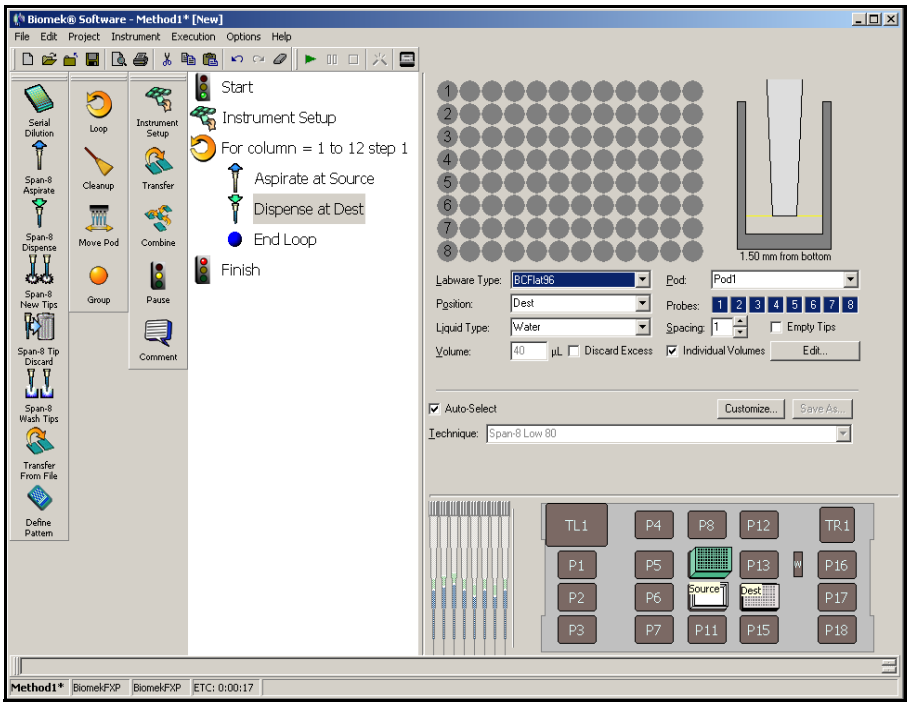

Figure 7-12. Dispense step inside the Loop

7.5 Conserving Tips Using Individual Steps

If using fixed tips. . .

You won't load and unload tips. Go to the next section.

As the method is currently configured, an entire box of tips will be used. This is because the Aspirate step loads tips for each iteration of the Loop. Since the source is a reservoir and cross contamination is not a concern, you could reuse tips for all 12 iterations of the Loop. To accomplish this, you will use a Span-8 New Tips step before the Loop and a Span-8 Tip Discard step after the Loop.

7.5.1 Loading and Unloading Tips Outside the Loop

To load and unload tips outside of the Loop:

1. Insert a **Span-8 New Tips** step between the Instrument Setup and Loop steps.
2. Select the **Aspirate** step inside the Loop.
3. Deselect the **Refresh Tips** checkbox. This tells Biomek to use whatever tips are already loaded to perform the aspirate instead of loading new tips at the start of the Aspirate step.
4. Insert a **Span-8 Tip Discard** step after the End Loop icon in the Method View. Your method should now look like Figure 7-13.

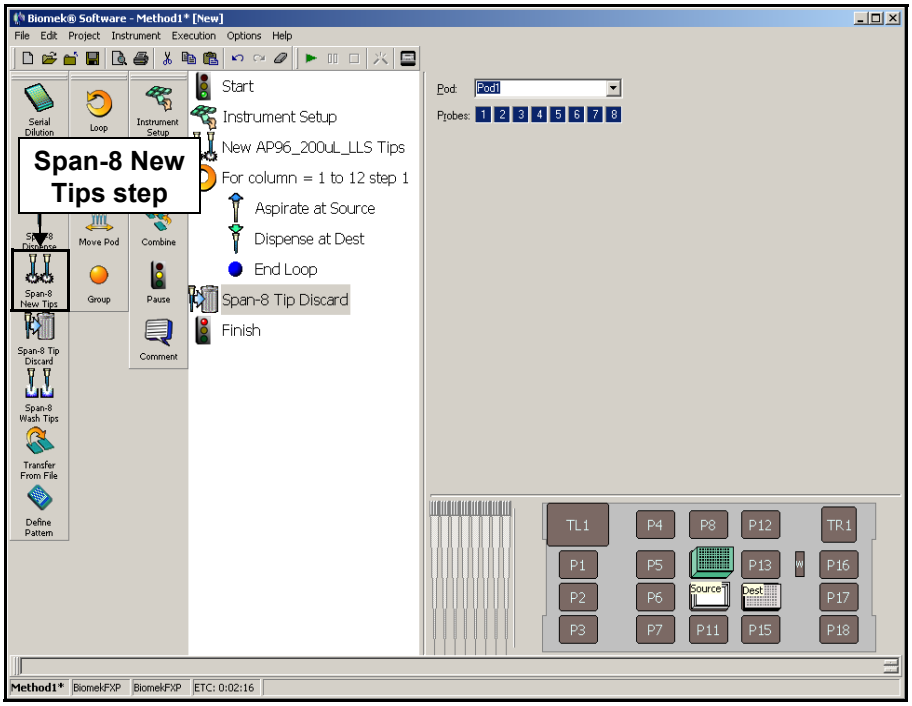

Figure 7-13. Loading and unloading tips outside the Loop

This method loads new tips, uses those same tips to perform all 12 iterations of the Loop, and unloads the tips after the last iteration of the Loop.

7.6 Washing Tip Mandrels

After unloading the tips in the previous section, you can wash the tip mandrels at the Span-8 Wash ALP. If you haven't unloaded the tips, an error will be displayed because disposable tips cannot be washed at the Span-8 Wash ALP.

You probably really wouldn't have a need to wash tip mandrels, but complete this section to learn how to use a Span-8 Wash Tips step. If you are ever using fixed tips, you will want to know how to wash them using this step.

BIOMEK CONCEPT
Washing Tips

The Span-8 Wash Tips step washes tips at the Span8WashALPLeft or Span8WashALPRight by aspirating and dispensing a specified volume of wash fluid a specified number of times.

Now you will add a Span-8 Wash Tips step to wash the tip mandrels.

To wash tip mandrels:

1. Insert a **Span-8 Wash Tips** step after the Span-8 Tip Discard step.

If using fixed tips. . .

Insert a Span-8 Wash Tips step after the Loop step.

2. In Position, choose **W1**.
3. Make sure all eight probes are selected.
4. Select **Passive Wash**.

5. Allow the other default settings to remain. The main editor should look like Figure 7-14.

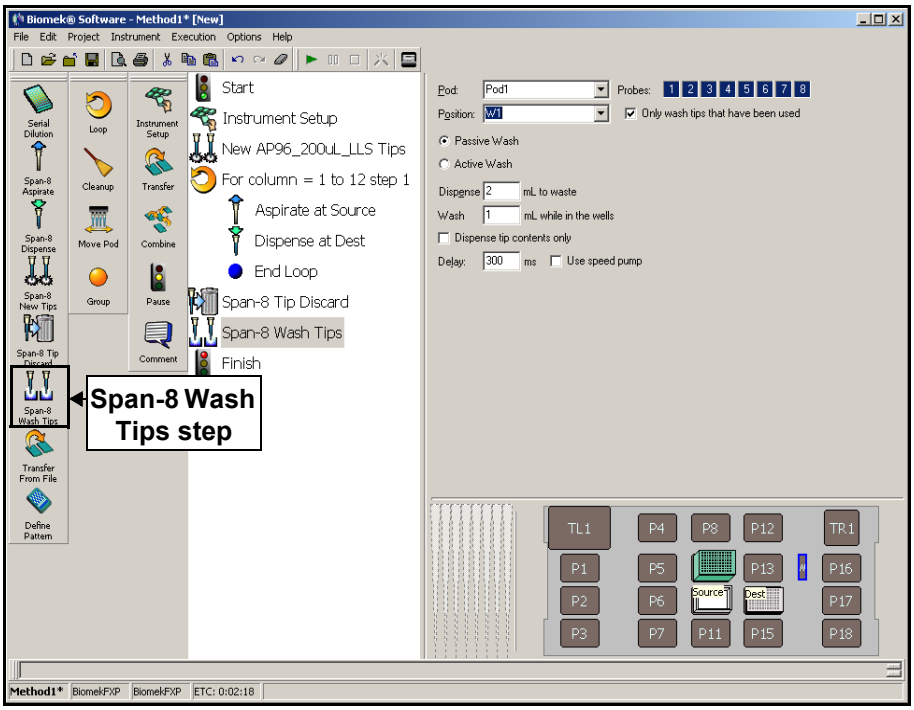

Figure 7-14. Span-8 Wash Tips step configured

6. Highlight **Finish** to validate the method.
7. Choose **Options>Log Configuration>Span8Pipetting**. You will view this log file in the next section (refer to Section 7.7, *Viewing Log Data*).
8. Save and run the method.

Go to the next section to learn how to view the log data associated with the method.

7.7 Viewing Log Data

BIOMEK CONCEPT
Log Files

The following log files are available for the Span-8 Pod:

- Details—captures every operation that occurs during a method run.
- Errors—captures any errors that occur during a method run.
- Span8Pipetting—captures pipetting operations, including location and labware name or type.
- Span8Transfer—captures transfer operations, including location and labware name or type.
- UnifiedPipetting—captures pipetting operations, along with sample IDs for wells.
- UnifiedTransfer—captures transfer operations, along with sample IDs for wells.

Refer to the *Biomek Software User's Manual,* Chapter 27, *Generating and Using Log Data*, for more information.

Logs provide text records of a method run. The contents of the text record, or log file, are based upon the type of log requested. The type of log available is based on the pod type. For example, the logs Errors, Span8Pipetting, and Span8Transfer are by default available for the method you just ran although other logs may be generated. See the side bar.

To view the log data for the method you just ran:

1. Browse to **C:\Documents and Settings\All Users\ Documents\Biomek\Logs** (Figure 7-15).

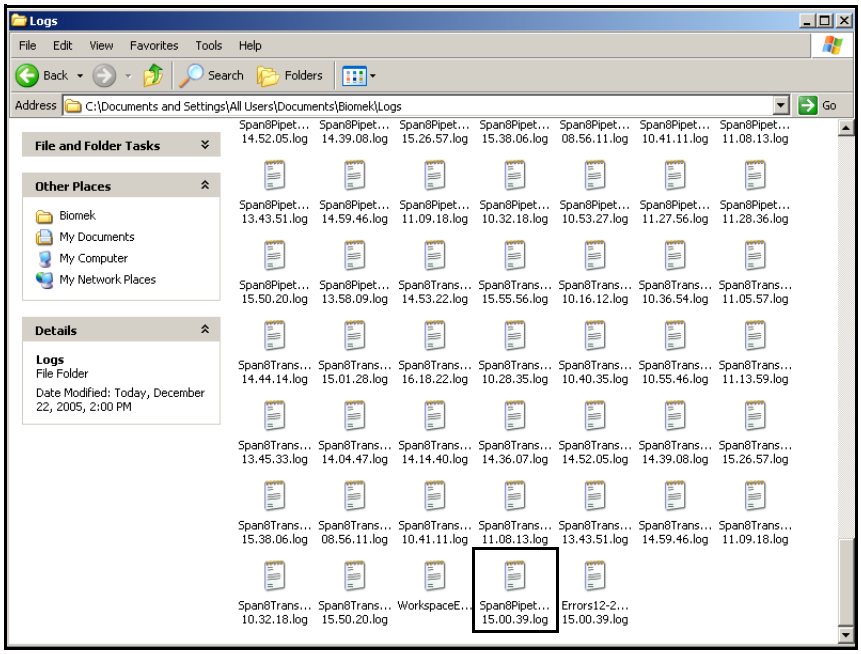

Figure 7-15. Browse to Logs

2. Double-click the latest **Span8Pipetting** log. Figure 7-16 appears.

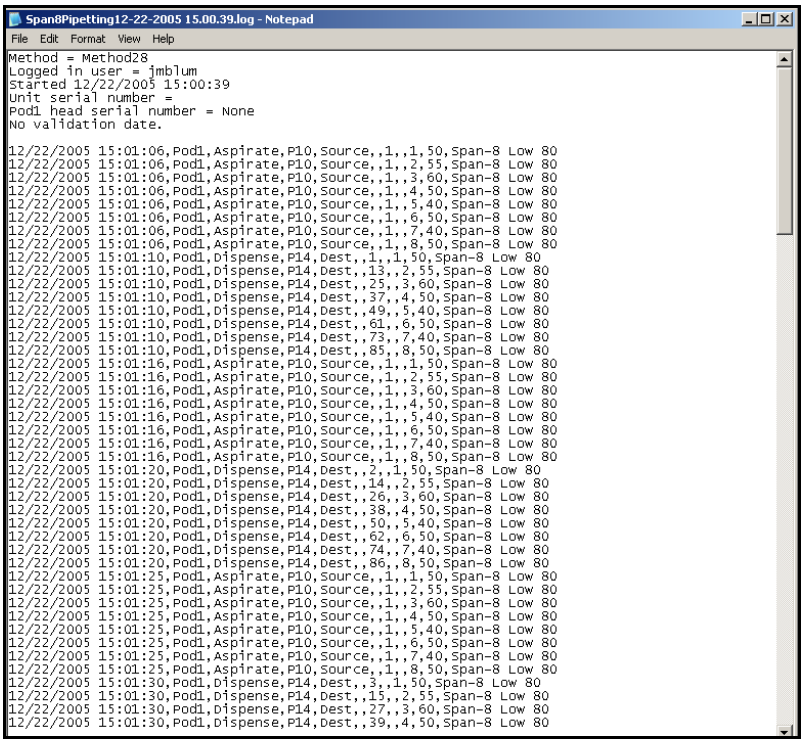

Figure 7-16. Portion of the Span8Pipetting log

3. From Figure 7-16, note the following specifics about the log for the method you just created and ran. The specifics (listed in the order following) are displayed from left to right on each line of the log.
   - Date and time of the method run.
   - Pod which performed the operation (Pod1 or Pod2).
   - Operation (aspirate or dispense).
   - Location where the operation took place.
   - Name assigned to labware in Labware Properties.
   - Well number pipetted to or from.
   - Probe number.
   - Amount of liquid.
   - Technique name.
4. Close the file.

Go to the next chapter to learn how to use worklists and conditions.

8 Span-8 Pod—Using Worklists and Conditions

8.1 Introduction to Using Worklists and Conditions

To successfully complete the activities in this chapter, you will need to know how to:

- Configure an Instrument Setup step to reflect the physical deck you will set up for the method in this chapter (refer to Section 5.3.2, *Configuring the Instrument Setup Step*).
- Configure Labware Properties for labware you will use in this chapter.
- Configure a Transfer step (refer to Section 5.4, *Transferring Liquid*).
- Use variables and expressions in Biomek Software (refer to Section 7.3.1, *Creating a Variable in the Start Step*).
- Display step palettes.

8.1.1 What You'll Learn in Using Worklists and Conditions

In this chapter, you will develop the advanced skills to use external data sources, such as a worklist, with a Biomek method. Using a worklist will allow you to create a method using transfer amounts and destinations defined in a text file. You will also learn how to use procedures to run the same set of configured steps several times in a method to eliminate having to configure each step several times and learn how to configure a conditional statement that will allow a step or steps to be executed based on real-time conditions that occur during the method.

More specifically, the step-by-step instructions in this chapter will teach you how to:

- Create a worklist file that defines variables and values.
- Use the worklist file in a Worklist step for executing a sequence of liquid transfers without configuring individual Transfer steps.
- Use an If step to transfer liquid from specific reservoirs using specific tips based on conditional decisions.
- Define a procedure using the Define Procedure step that will run based on the conditional decisions within an If step.
- Run the defined procedure based on the conditional decisions using the Run Procedure step.

8.1.2 Setting Up Your Deck for Using Worklists and Conditions

Using what you learned earlier, launch Biomek Software, begin a new method, and configure an Instrument Setup step as follows:

1. Place a **AP96_200µL_LLS** tip box on P5 and name it **TipsA**.
2. Place the same tip box type on P6 and name it **TipsB**.
3. Place a **Reservoir** on P9 and name it **ReagentA**. Configure the liquid type as **Ethanol** with an **Unknown** volume.
4. Place another **Reservoir** on P13 and name it **ReagentB**. Configure the liquid type as **Water** with an **Unknown** volume.
5. Place **BCFlat96** plates on P10, P11, P14, and P15. Name them **Plate1**, **Plate2**, **Plate3**, and **Plate4**.

Make sure you have the following step palettes displayed on the main editor:

- Basic
- Intermediate
- Advanced
- Specialty
- Span-8

Your deck should look like Figure 8-1. Now go to the next activity to learn how to use worklists and conditions in your methods.

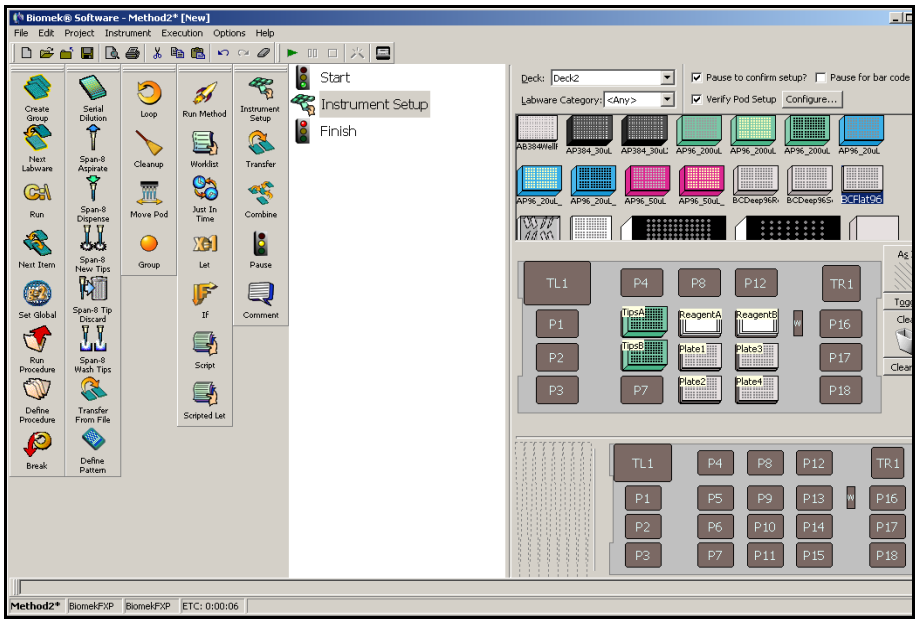

Figure 8-1. Instrument Setup step configured

8.2 Creating a Worklist Text File

A worklist is a text file that contains multiple values for one or more variables. The Worklist step in Biomek Software uses the variables and values defined in the worklist to configure a series of repetitive actions during the method run. This is different from the Loop step that you learned earlier in this tutorial in that a worklist can contain non-incremental values and multiple variables for use during repetitive actions.

Before you can use the Worklist step, you must create the worklist text file and define the variables and associated values. For the method in this chapter, you will create a worklist for a series of transfers using different amounts of liquid for each transfer.

8.2.1 Configuring a Worklist Text File

The first line of a worklist text file defines the variable names. The subsequent lines list the values to be assigned to those variables. The variable and names are separated by commas. If a value such as a plate name or bar code contains a comma, enclose the entire value in double quotes; for example, "Dest1, 4".

Tip

When creating a Worklist text file, white space between values is not important; however, the comma (,) and carriage returns (CRLF) are important to create and use the file properly. Do not enter a carriage return after the last line.

To configure the worklist text file for this tutorial:

1. Using Notepad, create a text file that looks like Figure 8-2.

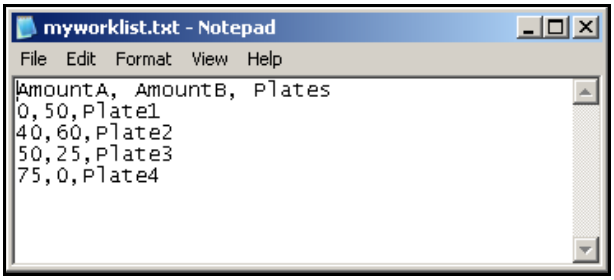

Figure 8-2. Created worklist

2. Save your text file as **myworklist** to the desktop. If you completed the chapters for the Multichannel Pod and want to preserve the worklist you created for that Multichannel chapter, save your text file as **myworklistS-8**.

Now that you have configured the worklist, you will insert and configure a Worklist step to enable the software to use the worklist in the method. Go on to the next section to learn how to do this.

8.3 Configuring a Worklist Step to Use a Worklist

BIOMEK CONCEPT
Worklist Step

The Worklist step offers several advantages:

- Data in a text file is accessible by any Biomek Software method.
- Selecting the text file in the Worklist step configuration copies all of the variable data from the text file to the step without individually entering all the variables.
- A dynamic link exists between the method and the text file. Any updates made to the text file are included in the next method run, although the text file must maintain the original file path to allow the method to find the file.

The Worklist step is located on the Advanced step palette and uses a text file to supply to the method multiple values for one or more variables. Worklist is useful when repetition of the same action is required, but one or more variables needs to change each time the step cycles through the worklist. When a step or group of steps using the variables defined in a text file are placed inside a Worklist step, Worklist automatically performs each step once for each line in the text file.

For this part of the tutorial, we will use a Worklist step to transfer specific amounts of liquid from two different sources to four destination plates. The text file you configured in Section 8.2, *Creating a Worklist Text File*, contains all the needed details.

To configure the Worklist step:

1. Ensure your deck is configured according to the instructions in Section 8.1.2, *Setting Up Your Deck for Using Worklists and Conditions*.
2. Drag and drop a **Worklist** step (Figure 8-3) into the Method View below the Instrument Setup step.
3. From Worklist file in the configuration window, use the Browse button to find and choose **myworklist.txt**, the text file you configured in Section 8.2, *Creating a Worklist Text File*. Choose **myworklistS-8** if you saved the worklist in Section 8.2, *Creating a Worklist Text File* with this name.

4. Make sure **Loop entire worklist** is selected. This ensures that all the values contained in the worklist are used. The Worklist step configuration should look like Figure 8-3.

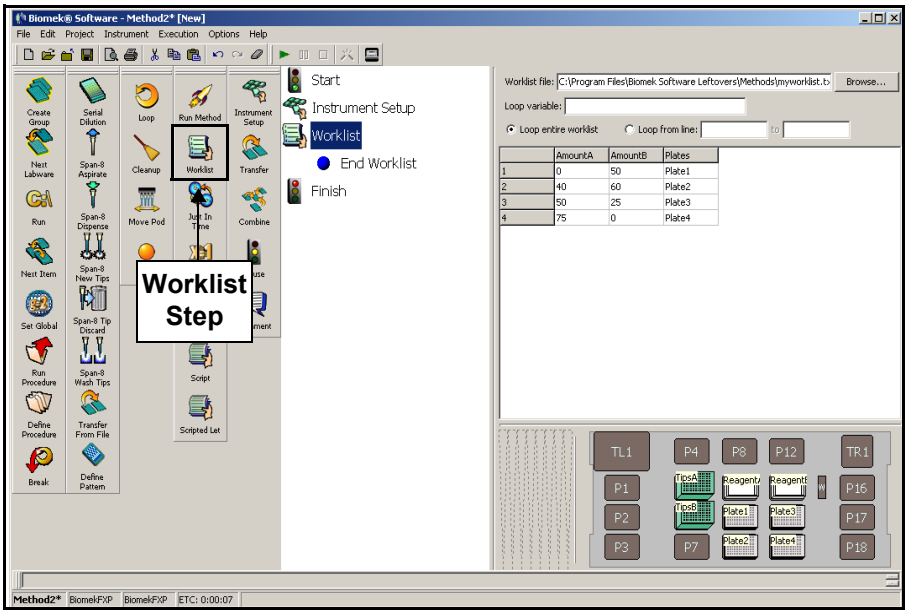

Figure 8-3. Worklist step with text file displayed

Now, you will define a procedure using a Define Procedure step and insert it before the Worklist step. This procedure will be run as the method cycles through the worklist. This procedure will be configured to load tips, transfer liquid, and unload tips.

8.4 Defining and Running Procedures

BIOMEK CONCEPT
Procedures

Procedures offer advantages, such as running the same steps multiple times within a method but configuring them only once. Procedures control the size of the current method in the Method View by listing only the Run Procedure step in the Method View and not all the steps accessed by the step.

The Define Procedure step is used to configure and save a series of steps that may be used multiple times in a method without having to reconfigure each individual step within that procedure. The Run Procedure step is inserted into the method and is used to identify the defined procedure to be used in a method. The procedure defined in the Define Procedure step can be run only by inserting a Run Procedure step and choosing the desired procedure in the step configuration.

8.4.1 Defining a Procedure Using the Define Procedure Step

For this part of the tutorial, you will insert and configure a Define Procedure to load specific tips, transfer volumes based on the worklist you configured earlier, and unload tips. The defined procedure will then be run as part of the If step that you will configure later. You will also create variables in this procedure whose values will be specified in the Run Procedure step. This lets you run the steps in the procedure with different values associated with the defined variables.

1. From the Specialty step palette (Figure 8-4), insert a **Define Procedure** step below the Instrument Setup step.
2. In Procedure, enter **ReagentAddition** (Figure 8-4). This becomes the name of your procedure and will appear as Define ReagentAddition in the method view.

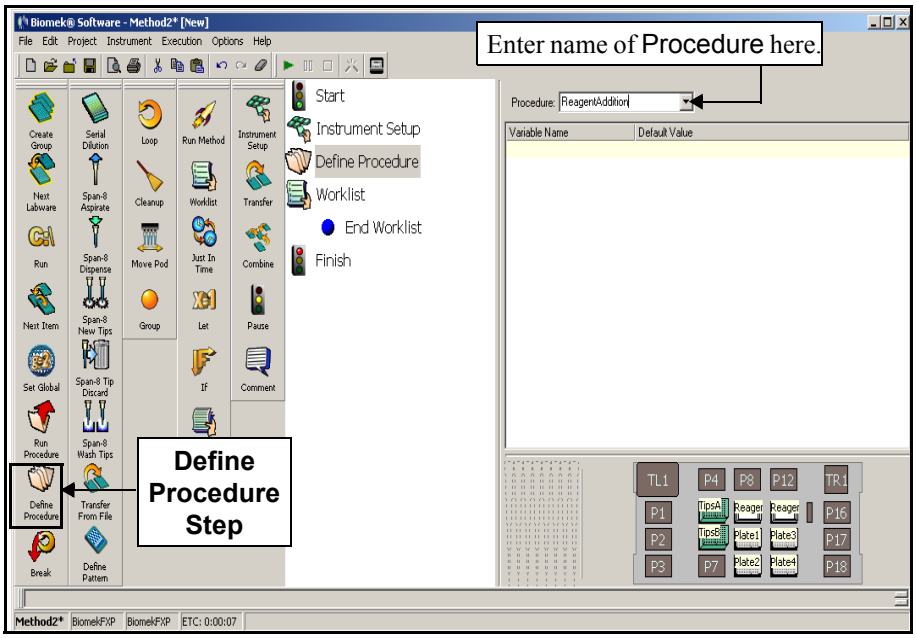

Figure 8-4. Procedure named

3. Under Variable Name, enter **Reagent** and tab over to Default Value and enter **A.** You are entering A since it is part of the default value as part of the expression.
4. Press **Enter** on the keyboard, then under Variable Name, enter **Amount** and tab over to Default Value and enter **=AmountA**.

5. Double-click the **Define Procedure** step in the Method View to expose End Procedure (Figure 8-5). The Define Procedure step now appears as Define ReagentAddition step.

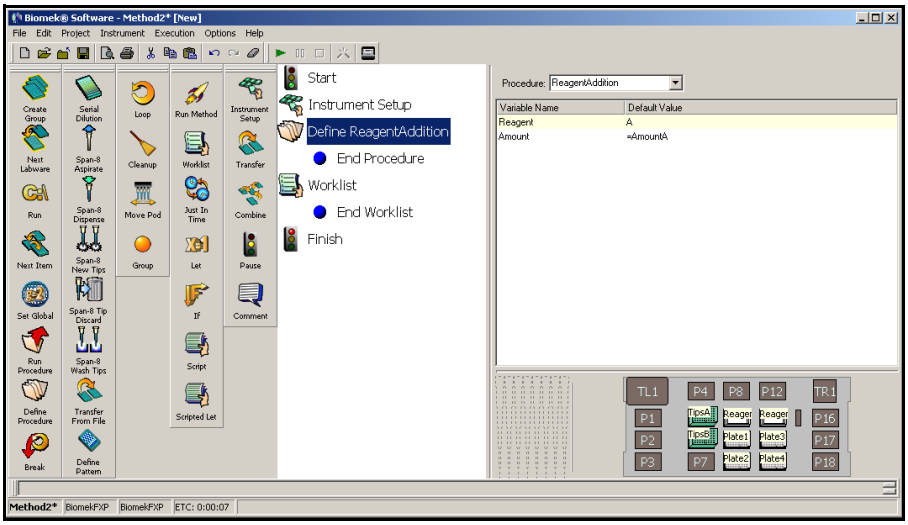

Figure 8-5. Variables entered in Define Procedure step

8.4.2 Configuring Steps Inside the Define Procedure Step

If using fixed tips. . .

You don't load and unload tips so you won't do 8.4.2.1, *Configuring Different Tips for Accessing Sources* or 8.4.2.3, *Unloading Tips During a Procedure*. Just go 8.4.2.2, *Transferring Liquid During a Procedure*.

To configure the Define Procedure step to load tips, transfer volumes based on the worklist you configured earlier, and unload tips, the following steps will be configured individually inside the Define Procedure step:

- Span-8 New Tips step (refer to Section 8.4.2.1, *Configuring Different Tips for Accessing Sources*)
- Transfer step (refer to Section 8.4.2.2, *Transferring Liquid During a Procedure*)
- Span-8 Tip Discard step (refer to Section 8.4.2.3, *Unloading Tips During a Procedure*)

The Define Procedure will be run as part of the If step that you will configure later.

8.4.2.1 Configuring Different Tips for Accessing Sources

Since your reservoirs in this tutorial method contain different liquid types, you will want different tips designated for each reservoir. You will configure the Span-8 New Tips step as part of your procedure to ensure that the correct tips are loaded to access the correct source reservoir.

1. From the Span-8 step palette, insert a **Span-8 New Tips** step into the Method View inside the Define ReagentAddition step.
2. In Tips in the configuration view, highlight the field and enter **=“tips”&reagent** (Figure 8-6). Remember that variable names are not case sensitive.

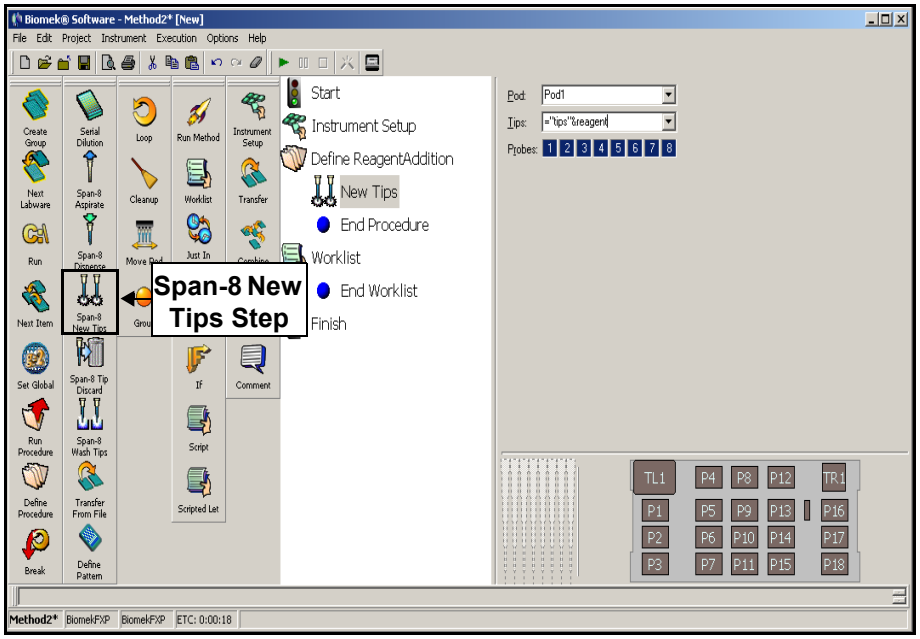

Figure 8-6. Span-8 New Tips configured

8.4.2.2 Transferring Liquid During a Procedure

To configure the actual liquid transfer that will be executed when the procedure is run, configure the transfer as follows:

If using fixed tips. . .

Insert a **Transfer** step below the Define Reagent step and allow the default Tip Handling to remain.

Tip

The deck is inactive when configuring a Transfer step inside the Define Procedure step.

1. Insert a **Transfer** step into the Method View below the Span-8 New Tips step.
2. Deselect **Load AP96_200µL_LLS tips** and **Change tips between transfers**.
3. Using what you learned earlier, configure the Source in the Transfer step as **Reservoir** at **=”Reagent”&Reagent**. Configuring =”Reagent”&Reagent means that the reservoir on the deck that has the same name as the value of the variable Reagent will be used. You’ll configure this variable later in the Define and Run Procedure steps.
4. In the Transfer step, configure the Destination as a **BCFlat96** at **=plates**.
5. In the Destination configuration µL field, enter **=Amount**.

8.4.2.3 Unloading Tips During a Procedure

Here you will configure the procedure to unload the tips after the liquid transfer action. To unload the tips:

1. Insert an **Span-8 Tip Discard** step below the Transfer step.
2. Click on the **Define ReagentAddition** step.Your main editor should look like Figure 8-7, and the variables in the named procedure you just created will be used to specify when new tips are used and which reservoir will be accessed when transferring liquid.

Tip

If you want to reuse a procedure in other methods, drag the configured procedure and drop onto any of the displayed step palettes. A prompt appears to ask if you would like to include the step on that specific palette. You could also create a custom step palette (refer to the *Biomek Software User's Manual,* Section 30.4, *Customizing Step Palettes*). Either way, the procedure may then be used in other methods by dragging and dropping it into the Method View.

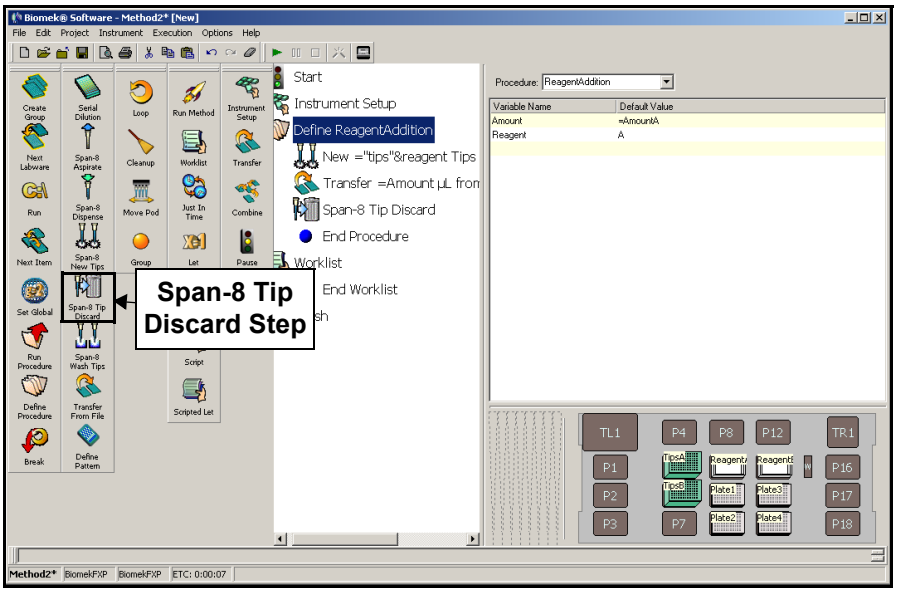

Figure 8-7. Define Procedure step configured

3. Double-click the **Define ReagentAddition** step to collapse it.
4. Click on the **Finish** step to validate the method.

Go on to the next section where you will configure an If step to learn how to use conditions in a method.

8.5 Configuring the If Step to Use Conditions in a Method

BIOMEK CONCEPT
If Step

The substeps of an If step are:

- Then — if the condition is true, substeps following Then are processed.
- Else — if the condition is false, substeps following Else are processed.
- End — The End substep terminates each If, Then, and Else block of steps.

The If step controls the steps that are executed in a method based on conditional decisions. When If is run, Biomek Software tests the If condition as true or false, then processes the appropriate block of substeps based on the results of the test. (See Biomek Concept sidebar.).

To configure the If steps to use conditions in this tutorial, you will:

- Insert an If step and enter the condition for a transfer from source ReagentA.
- Insert a Run Procedure step for the transfer from source ReagentA for the Then substep.
- Insert another If step and enter the condition for a transfer from source ReagentB.
- Insert a Run Procedure step for the transfer from source ReagentB for the Then substep.

8.5.1 Setting Conditions Using If Steps

For this section of the tutorial, you will configure two If steps that will specify the reagent reservoirs to access and the specific tip boxes for each transfer based on the sources you configured for the Worklist step. You will configure the If steps and insert them into the Worklist step. The transfers will then run the ReagentAddition procedure you configured earlier. The procedure locates the correct tips and transfers the appropriate volume from the correct reservoir.

To configure the If steps:

1. From the Advanced step palette, insert an **If** step into the Method View into the Worklist step.
2. In Condition, enter **AmountA > 0**. The main editor should look like Figure 8-8.

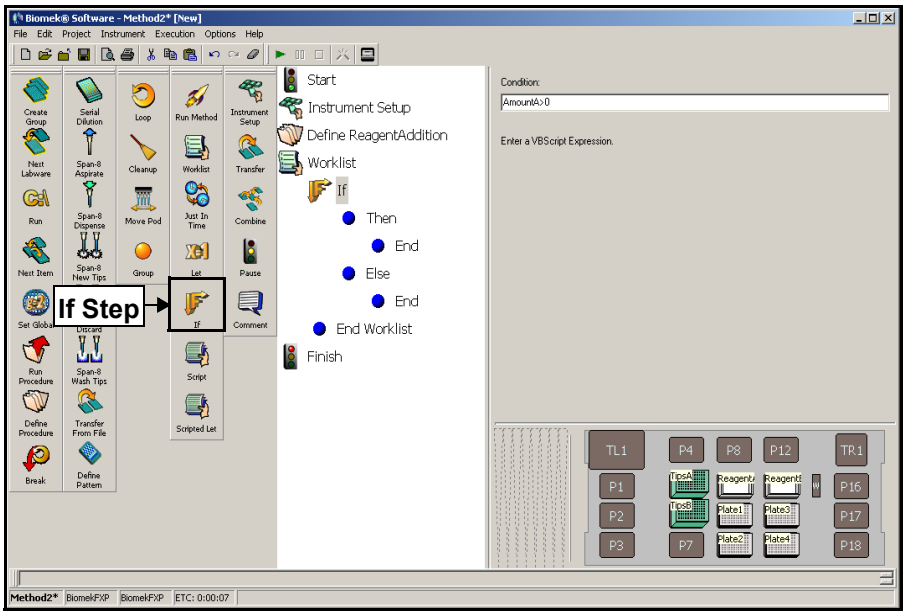

Figure 8-8. Condition entered in If step

3. From the Specialty step palette, insert a **Run Procedure** step into the Method View below the Then substep of the If step.
4. In Procedure, choose **reagentaddition**.

For this tutorial, you won't place steps in the Else substep. This means that if the evaluation of the If step is false, no further action occurs. The main editor should now look like Figure 8-9.

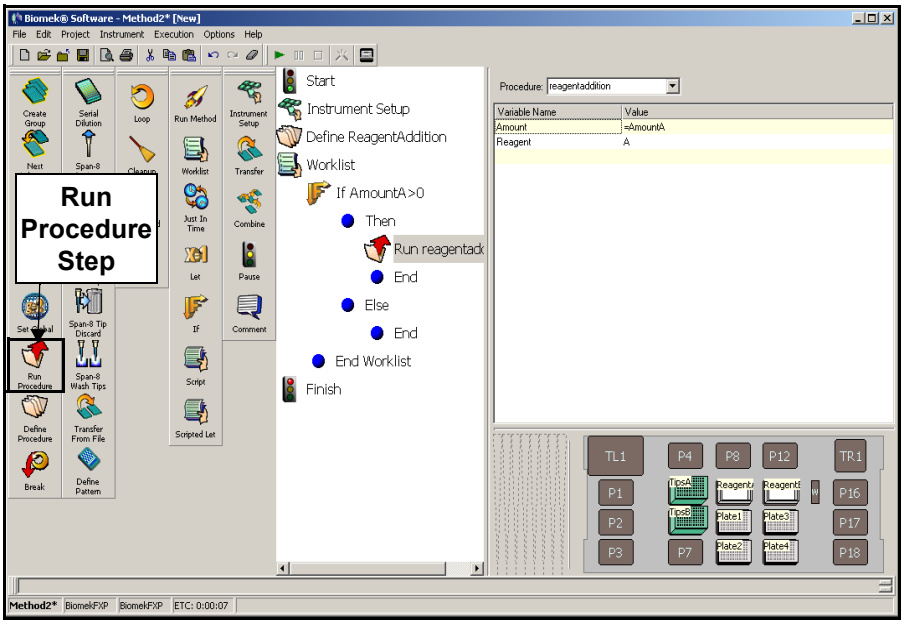

Figure 8-9. ReagentAddition procedure inserted as a Then substep

5. Insert another **If** step into the Method View below the last End substep and above End Worklist.
6. In Condition, enter **AmountB > 0**.
7. Insert a **Run Procedure** step into the Method View below the Then substep of the second If step.
8. From Procedure, choose **reagentaddition**.
9. Change the Value for Amount to **=AmountB**.

10. Change the Value for Reagent to **B**.You won't use an Else substep here either, so the main editor should look like Figure 8-10.

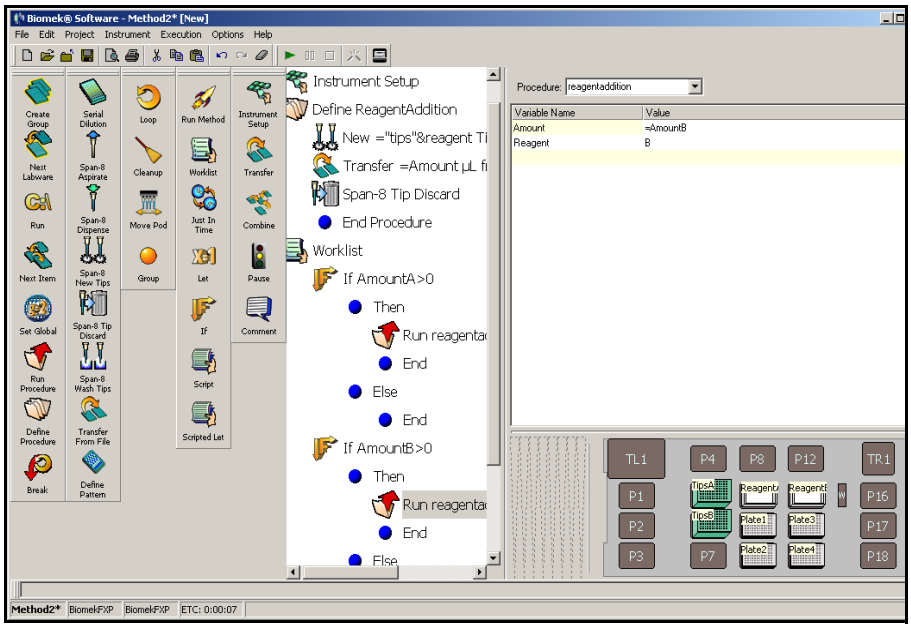

Figure 8-10. Variable name and value changed

Congratulations! You have just created a method using a worklist and If steps with configured conditions. If you would like to see this method run in simulation mode, click the green run button on the toolbar.

9 Span-8 Pod—Using Files to Direct Transfers

9.1 Introduction to Using Transfer from File

To successfully complete the activities in this chapter, you will need to know how to:

- Configure an Instrument Setup step to reflect the physical deck you will set up for the method in this chapter (refer to Section 5.3.2, *Configuring the Instrument Setup Step*).
- Configure Labware Properties for labware you will use in this chapter.
- Configure a Transfer step (refer to Section 5.4, *Transferring Liquid*).
- Use variables and expressions in Biomek Software (refer to Section 7.3.1, *Creating a Variable in the Start Step*).
- Display step palettes.

9.1.1 What You'll Learn in Using Transfer from File

In this chapter, you will use the advanced skills you've developed earlier to configure a Transfer from File step

More specifically, the step-by-step instructions in this chapter will teach you how to:

- Configure a Loop step for hit picking.
- Insert a Define Pattern step inside the Loop step.
- Configure a Transfer from File step using two, supplied .csv files.

9.1.2 Setting Up Your Deck for Using Transfer from File

Using what you learned earlier, launch Biomek Software, create a new method, and configure an Instrument Setup step as follows:

1. Place **AP96_200µL_LLS** tip boxes on P5 and P13.
2. Place **BCDeep96Round** plates on P6, P7, P14, and P15.
3. Name the four plates at P6, P7, P14, and P15 **Samples1**, **Samples2**, **Samples3**, and **Samples4**. Configure these to have an **Unknown** volume of **Serum**.
4. Place a **BCFlat96** on P10 and name it **Dest**. This plate starts out empty, so configure a **Known** volume of **0** µL.
5. Place a **SmallTuberack_Microfuge** at P9 and name it **Primers**. Configure it to have an **Unknown** volume of **Water**.

Make sure you have the following step palettes on the main editor:

- Basic
- Intermediate
- Span-8

Your deck should look like Figure 9-1.

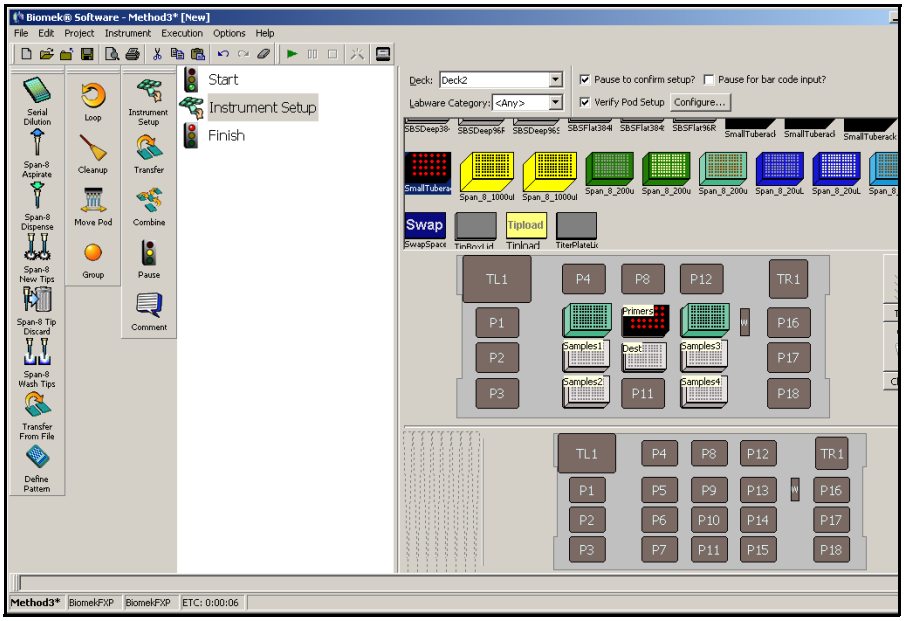

Figure 9-1. Instrument Setup step configured

9.1.3 Copying .CSV Files to the Desktop

Copy the two, supplied .csv files (hits.csv and transferfromfile.csv) to your desktop. These files are located on the CD that included this tutorial.

9.2 Creating a Loop for Hit Picking

Since library compounds are used in drug discovery labs for high throughput screening on large numbers of microplates that produce “hits,” the repeating process of a loop is useful. In this section, you will use the supplied hits.csv file and assume it has identified a number of hits on several microplates that will be processed further in your method. Before you create a loop to further process the hits, you will open the .csv file. Then you will use a Define Pattern step to specify the hits into which liquid will be transferred via a Transfer step.

9.2.1 Viewing the Hits.csv File

The hits.csv file is a simple, two-column file. Each row specifies one well that is a hit which requires further study or processing. The first column specifics on which plate the hit is located, and the second column specifies which well on that plate the hit is located.

To view the .csv file:

1. Double-click hits.csv on your desktop. The hits.csv file opens (Figure 9-2).

A1 = Plate

	A	B	C	D	E	F
1	Plate	Well				
2	samples1	23				
3	samples1	32				
4	samples1	44				
5	samples1	46				
6	samples1	48				
7	samples1	52				
8	samples1	53				
9	samples1	57				
10	samples1	58				
11	samples1	64				
12	samples1	68				
13	samples1	84				
14	samples1	86				
15	samples2	2				
16	samples2	7				
17	samples2	16				
18	samples2	20				
19	samples2	58				
20	samples2	63				
21	samples2	77				
22	samples2	85				
23	samples3	10				
24	samples3	19				
25	samples3	29				
26	samples3	30				
27	samples3	49				
28	samples3	59				
29	samples3	73				
30	samples3	74				
31	samples3	81				
32	samples3	91				
33	samples4	9				
34	samples4	13				
35	samples4	29				
36	samples4	32				
37	samples4	33				
38	samples4	34				
39	samples4	55				
40	samples4	77				
41	samples4	80				

Figure 9-2. Supplied hits.csv file

2. Notice how each row specifies one well that is a hit. Also notice that the first column specifies on which plate the hit is located, and the second column specifies which well on the plate the hit is located.
3. Close the .csv file since it must be closed to use it in the Define Pattern step.

9.2.2 Inserting a Loop Step

To insert the Loop step:

1. Insert a **Loop** step in the Method View after the Instrument Setup step.
2. In Variable, enter **plate**.
3. In Start, enter **1**
4. In End, enter **4**.
5. In Increment, enter **1**.

9.2.3 Inserting a Define Pattern Step

To insert the Define Pattern step:

1. If it is collapsed, double-click the **Loop** step.
2. Insert a **Define Pattern** step inside the Loop step (Figure 9-3).

BIOMEK CONCEPT
Define Pattern Step

The Define Pattern step is used to create patterns and assign names to them for use in other steps in the method, such as a Transfer or Combine step. Well patterns defined using the Define Pattern step are method specific and embedded as part of the method, as compared to well patterns created in the Well Pattern Editor which are project specific and may be used across methods. Refer to the *Biomek Software User's Manual,* Chapter 12, *Creating Well Patterns*, for more information on using the Well Pattern Editor.

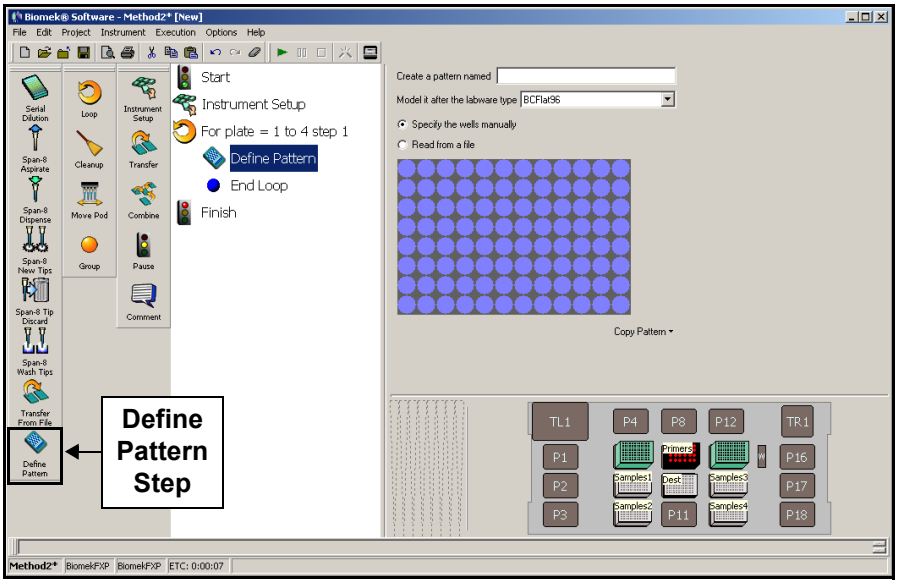

Figure 9-3. Define Pattern inserted inside Loop

3. In Create a pattern named, enter **SamplesToTransfer**.
4. From Model it after the labware type, choose **BCDeep96Round**.

5. Check **Read from file**. The configuration changes (Figure 9-4).

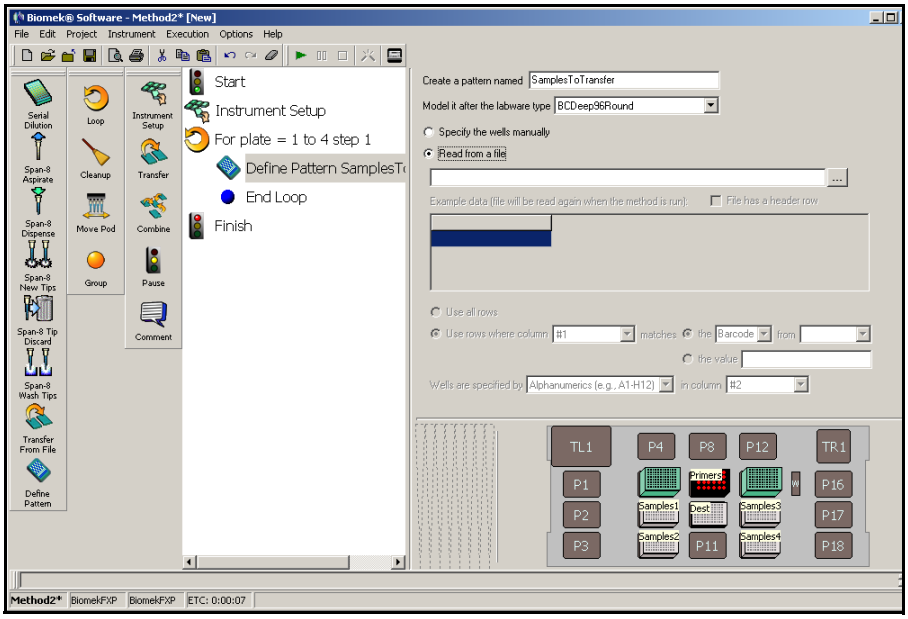

Figure 9-4. Read from File chosen

6. From the Browse button, choose **Hits.csv**. The first few rows display in Example data.
7. Check **File has a header row**.
8. Choose **Use rows where column . . .matches** and select **Plate**.
9. After matches, keep the top option selected and choose **Name.**
10. In from, enter **=”Samples”&plate**. This uses the variable created in the Loop step to specify which sample plate the pattern is for. In this way, each time through the Loop, the pattern will change to match the sample plate that is being processed for that iteration.
11. In Wells are specified by, select **Numerics (e.g., 1-96)**.

12. From in column, select **Well**. The step configuration should look similar to Figure 9-5.

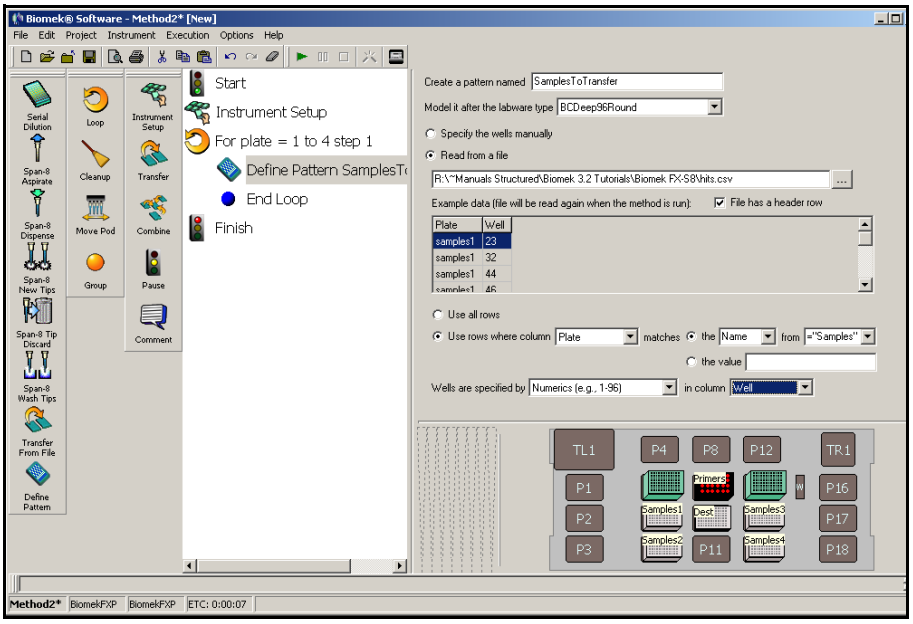

Figure 9-5. Read from file configured

9.2.4 Inserting a Transfer Step

To insert the Transfer step:

1. Insert a **Transfer** step below the Define Pattern step.
2. Allow the default Tip Handling to remain and collapse it.
3. Select **Click here to add a source**.
4. Select **BCDeep96Round** from the labware type drop-down in the Source configuration.
5. In at, enter **=”Samples”&plate**.
6. In Using liquid type, choose **Serum** from the drop-down menu.
7. Double-click the source plate to zoom in on it.

8. Select **Use Pattern** and choose the **SamplesToTransfer** pattern that was created earlier in the Define Pattern step. The main editor should look like Figure 9-6.

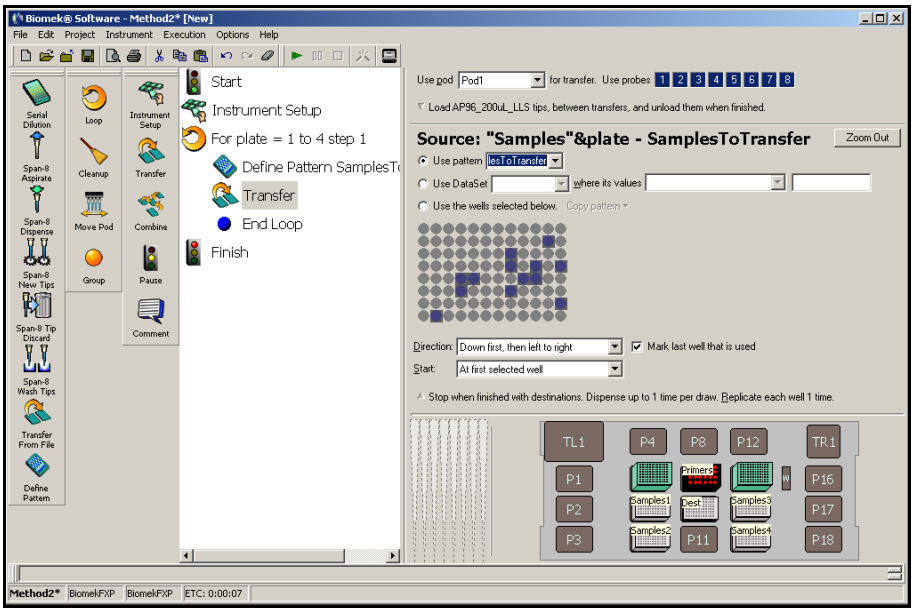

Figure 9-6. SamplesToTransfer pattern chosen

9. Choose **Zoom Out** to return to the main Transfer step configuration.
10. Select **Click here to add a destination**.
11. Click on the **Dest** labware on P10 in the Current Deck Display to select it.
12. Enter a volume of **50** µL. You will be transferring the same volume for each of the hit wells. This is a key difference to using the Transfer From File step you will configure later in this chapter, as Transfer From File allows you to also configure a different volume for each well-to-well transfer.
13. Underneath the destination labware graphic, make sure that **Start after last marked well** and **Mark last well that is used** are selected. These are the last two from the right. If either of these is not selected, then with each iteration of the Loop the Transfer will start with the first selected well (A1).

14. In Transfer Details, select **Stop when finished with Sources**. The main editor should look like Figure 9-7.

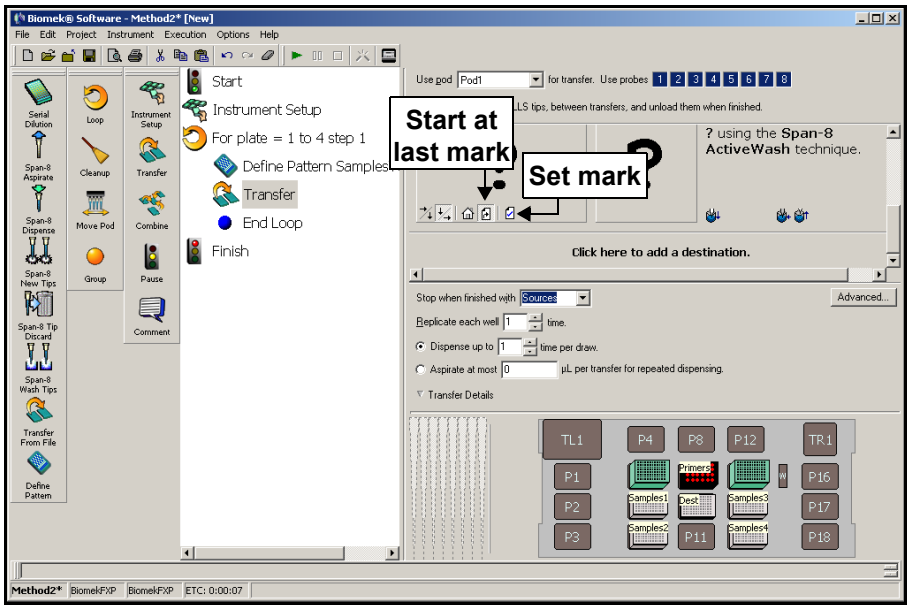

Figure 9-7. Configured Transfer step for inside Loop

9.3 Inserting a Transfer From File Step for Reaction Setup

BIOMEK CONCEPT
Transfer From File Step

The Transfer From File step allows a specified volume to be transferred from a specified source well to a specified destination well by reading data from a comma-delimited text file (.txt or .csv). A comma-delimited text file is a text file that specifies the values of a table by separating each column with a comma and each row with a return.

In this section, you will insert a Transfer From File step to perform a reaction setup. Each of the hit wells that was transferred earlier will need to have two primers added from the Primers tube rack. Which primer is added and the volume of each is different for each sample. The TransferFromFile.csv file that you copied to your desktop at the beginning of the chapter specifies which primer and the volume to transfer for each of the destinations.

First you will view the TransferFromFile.csv file.

9.3.1 Viewing the TransferFromFile.csv File

The TransferFromFile.csv file is a five-column file with each row specifying one well-to-well transfer:

- The first column specifies the source labware.
- The second column specifies which well on the source labware from which to aspirate.
- The third column specifies the destination labware.
- The fourth column specifies the well on the destination labware to which to dispense.
- The fifth column specifies the volume to transfer.

To view the .csv file:

1. Double-click TransferFromFile.csv on your desktop. The TransferFromFile.csv file opens (Figure 9-2).

A1 = Source

	A	B	C	D	E
1	Source	SourceWe	Dest	DestWell	Volume
2	Primers	11	Dest	1	80
3	Primers	5	Dest	1	35
4	Primers	9	Dest	2	80
5	Primers	15	Dest	2	45
6	Primers	5	Dest	3	100
7	Primers	22	Dest	3	100
8	Primers	15	Dest	4	70
9	Primers	14	Dest	4	60
10	Primers	13	Dest	5	35
11	Primers	12	Dest	5	35
12	Primers	20	Dest	6	60
13	Primers	19	Dest	6	65
14	Primers	20	Dest	7	95
15	Primers	11	Dest	7	55
16	Primers	20	Dest	8	90
17	Primers	7	Dest	8	30
18	Primers	11	Dest	9	55
19	Primers	19	Dest	9	90
20	Primers	11	Dest	10	65
21	Primers	23	Dest	10	100
22	Primers	12	Dest	11	40
23	Primers	5	Dest	11	95
24	Primers	23	Dest	12	45
25	Primers	18	Dest	12	65
26	Primers	2	Dest	13	30
27	Primers	15	Dest	13	35
28	Primers	15	Dest	14	70
29	Primers	5	Dest	14	70
30	Primers	9	Dest	15	95
31	Primers	6	Dest	15	80
32	Primers	13	Dest	16	60
33	Primers	14	Dest	16	30
34	Primers	15	Dest	17	85
35	Primers	5	Dest	17	60
36	Primers	1	Dest	18	75
37	Primers	9	Dest	18	60
38	Primers	20	Dest	19	65
39	Primers	14	Dest	19	85
40	Primers	23	Dest	20	60
41	Primers	1	Dest	20	85
42	Primers	14	Dest	21	90
43	Primers	20	Dest	21	35
44	Primers	21	Dest	22	25
45	Primers	16	Dest	22	25
46	Primers	7	Dest	23	70
47	Primers	19	Dest	23	40
48	Primers	19	Dest	24	85
49	Primers	11	Dest	24	30

Figure 9-8. Supplied TransferFromFile.csv file

2. Notice the five columns.
3. Close the .csv file since it must be closed to use it in the TransferFromFile step.

9.3.2 Inserting a Transfer From File Step

1. Insert a **Transfer From File** step after the end of the Loop step (Figure 9-9).

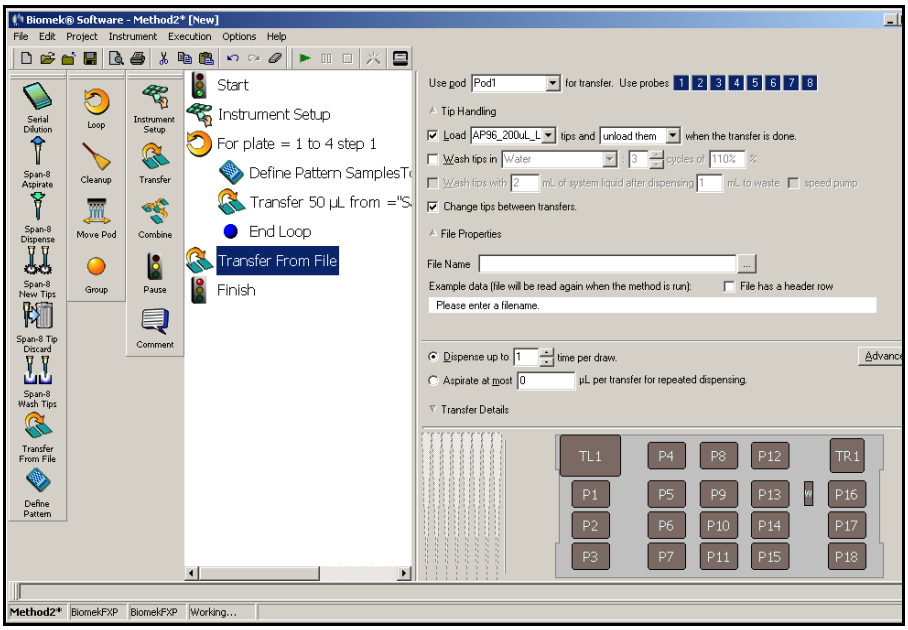

Figure 9-9. Transfer From File inserted

2. Allow the default Tip Handling to remain and collapse it.
3. If it is not displayed, expand the **File Properties** section. This option specifies which file to use and instructs Biomek Software how to use the data contained in the file.
4. From the Browse button, choose **TransferFromFile.csv**. The first few rows display in Example data.
5. Check **File has a header row**.
6. Select all five check boxes beneath Example data.
7. In File specifies source position in column, select **Source**.
8. In File contains source well information in column, select **SourceWell**. This is the only item that is required to be included in the file; everything else can be configured as a regular Transfer step.
9. In File specifies destination position in column, select **Dest**.
10. In File contains destination well information in column, select **DestWell**.
11. In File contains volume information in column, select **Volume**. The final check box instructs the TransferFromFile step to ignore any rows where the Volume is 0. If this is not selected, it will go through the actions of loading tips for the 0 volume transfer without actually transferring any liquid.
12. Collapse **File Properties**. Even though the Source and Destination are specified by the file, the file does not contain any information about the labware and liquid types. This information is needed by the Transfer From File step, so it can select the correct techniques to use for the liquid transfers (refer to Section 9.3.2.1, *Configuring Some Needed Source and Destination Information for the Transfer From File Step*).

9.3.2.1 Configuring Some Needed Source and Destination Information for the Transfer From File Step

1. Click on the Source configuration to open it.
2. In the first drop-down menu, select **SmallTuberack_Microfuge**.
3. In Using liquid type, select **Water**.
4. Click on the Destination configuration to open it.
5. In the first drop-down menu, select **BCFlat96**.
6. In Using liquid type, select **Water**. The main editor should look like Figure 9-10.

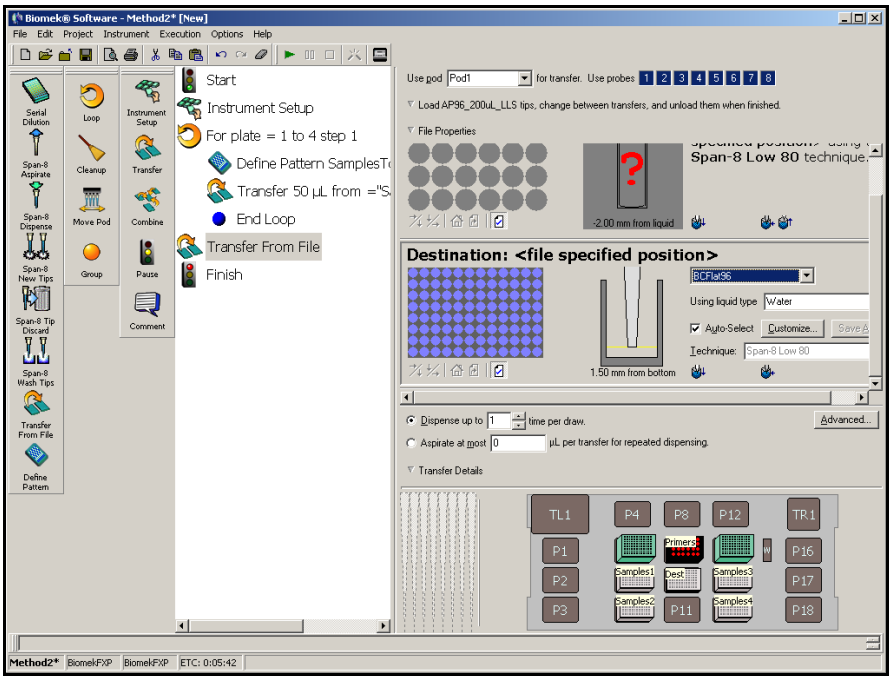

Figure 9-10. Transfer From File configured

You can run the method, but as you can see, it will take about 15 minutes.

Congratulations! You have completed the Span-8 chapters for the Biomek FX.