The blocks on the work surface hold:

- Block 1 96-well rack for 8x12 strips of 1.1 mL tubes for dispensed reagents
- Block 2 96-well rack for microtiter plate used for preparing bead solution
- Block 3 96-well rack on the heating/cooling unit where reactions will take place; used for BeadX tube strips, enzyme tube strips, sample tube strips and product tube strips
- Block 4 96-well rack on heating/cooling unit; used to hold 8-tube strip of ligation mix
- Block 5 96-well rack for piercing tips and filter tips
- Block 6 Reservoir rack for EDTA, ethanol and water reservoirs
- Blocks 7 and 8 Not used

For details on loading each block, see Chapter 3, "Setting Up and Running Protocols."

#### **Software Interface**

The software that provides the interface for setting up and running protocols is already installed. You use the software through a touchscreen panel on the instrument.

For details on starting the system, setting up the work surface and performing a run, see Chapter 3, "Setting Up and Running Protocols."

## **Workflows**

#### **PrepX DNA Library**

- 1. Press the PrepX DNA Library button on the touchscreen to activate the Peltier heating/cooling units.
- 2. Place consumables, reagents, magnetic beads and samples in the racks on the work surface (Figure 1-4).
- 3. Start the protocol run, using the touchscreen interface.
- 4. The samples undergo BeadX processing:
  - a. End repair
  - b. Intermediary bead-based cleanup
  - c. A-Tail
  - d. Intermediary bead-based cleanup
  - e. Adapter ligation
- 5. Final double bead-based cleanup and size selection is performed.
- 6. DNA samples are now ready for amplification and can be placed into the Agilent Bioanalyzer or equivalent DNA analyzer to determine the concentration after being centrifuged briefly.



#### **PrepX DNA Library Processing Schematic**

## **BeadX-High Library**

- 1. Press the PrepX Library BeadX-High button on the touchscreen to activate the Peltier heating/cooling units.
- 2. Place consumables, reagents, magnetic beads and samples in the racks on the work surface (Figure 1-4).
- 3. Start the protocol run, using the touchscreen interface.
- 4. The samples undergo BeadX-High processing:
  - a. End repair
  - b. Intermediary bead-based cleanup
  - c. A-Tail
  - d. Intermediary bead-based cleanup
  - e. Adapter ligation
- 5. Final bead-based cleanup and size selection is performed.
- 6. DNA samples are now ready for amplification and can be placed into the Agilent Bioanalyzer or equivalent DNA analyzer to determine the concentration after being centrifuged briefly.



#### **BeadX-High Library Processing Schematic**

# **Preparing Samples and Reagents**

In this chapter:

Overview 7 Preparing the Library Kit Reagents 7 Preparing 5X AMPure XP Beads 8 Preparing Adapter Mix 8 Preparing 70% v/v EtOH 8 Preparing Ligation Mix 9 Preparing Samples 10

## **Overview**

This chapter describes how to prepare samples and reagents for use in the Apollo 324<sup>™</sup> System.

**IMPORTANT:** We only guarantee the Apollo 324 System to perform using the recommended supplies and materials listed in Appendix B.

## **Preparing the Library Kit Reagents**

The following reagent kits are available:

- PrepX DNA Library Kit Used to prepare genomic DNA and CDNA libraries.
  - Box 1: BeadX reagents and BeadX buffers in Bead Mix strips (blue labeled seal)
  - Box 2: Enzyme strips, enzymes and enzyme buffers
- BeadX-High Library Kit Used to prepare chromatin-immunoprecipitated DNA libraries for sequencing
  - Box 1: BeadX-High reagents and BeadX-High buffers in Bead Mix strips (green labeled seal)
  - Box 2: Enzyme strips, enzymes and enzyme buffer

**NOTE:** The reagent setup is the same for both the PrepX and BeadX-High kits. The only difference is that the kits use different bead solutions.

To prepare either PrepX DNA Library Kit or BeadX-High Library Kit reagents:

- 1. Thaw the reagents from Box 1 and retrieve Box 2 from the refrigerator. Keep all reagents, including ligase enzyme, ligase buffer and annealed adapters on ice.
- 2. Vortex and spin down reagents briefly.

For IntegenX reagents, use an 8-strip adapter for the micro-centrifuge.

3. Visually inspect to be sure that the entire volume is at the bottom of each vial with no air pockets.

## **Preparing 5X AMPure XP Beads**

Preparing the beads can be done in advance.

- 1. Mix the AMPure<sup>®</sup> bead stock. Make sure that all of the settled beads are well homogenized.
- 2. Pipette 10 mL of 1X AMPure into a 15 mL test tube, magnetically pellet the beads and remove 8 mL of supernatant.
- 3. Vortex the remaining 2 mL of 5X bead solution to resuspend the beads.

**IMPORTANT:** Just before starting the protocol, you will aliquot the AMPure beads into an empty 8-well tube strip on the work surface. This step is done last in order to avoid bead settling.

## **Preparing Adapter Mix**

- Resuspend lyophilized adapter oligonucleotides (Adapter 1 and 2) to 100µM each in 10mM Tris-EDTA, pH 8.0.
- 2. Add equal volumes of adapter 1 and adapter 2 to a 1.5 mL microfuge tube to produce a 50μM mixture of both, then vortex and spin down briefly.
- **3.** Aliquot 50 μL of the adapters into PCR tubes, cap the tubes and anneal using the following thermocycling parameters:
  - Step 1: 95 °C for 2:00 minutes
  - Step 2: 0.1 °C/second to 25 °C
  - Step 3: 25 °C for 5:00 minutes
  - Step 4: 4°C hold
- **4.** Pool the annealed adapters into a clean 1.5 mL tube.
- 5. Vortex and spin down briefly.
- Aliquot into single use portions in PCR tubes and cap the tubes. Store frozen at -20 °C.

**NOTE:** Adapters can be prepared in advance and stored at -20  $^{\circ}$ C prior to use, but avoid freeze-thaw cycles.

**7.** Prepare the use-concentration by diluting 10-fold (50μM to 5μM) in 10mM Tris or reagent grade water.

## Preparing 70% v/v EtOH

Combine 28 mL of 100% EtOH and 12 mL of UltraPure water in a sterile 50 mL Falcon tube and mix.

# **Preparing Ligation Mix**

This recipe prepares ligation mix for one run of eight samples. See Table 2-1 for ligation mix component volumes.

- 1. Prepare the ligation buffer and enzyme mix in a sterile 1.5 mL microfuge tube (for one run of eight samples, plus one sample for volume loss to surfaces):
  - a. Thaw, vortex and briefly centrifuge T4 DNA Ligase buffer and T4 DNA Ligase stock tubes.
  - b. Combine 108  $\mu$ L of T4 DNA ligase buffer, 18  $\mu$ L of Annealed Adapter set (5 $\mu$ M) and 9  $\mu$ L of T4 DNA ligase in a 2 mL tube. Keep the mixture on ice.

	1 Sample	8 (+1) Samples
T4 DNA Ligase Buffer	12 µL	108 µL
Annealed Adapter Set (5µM)	2 µL	18 µL
T4 DNA Ligase Enzyme	1 µL	9 µL

Table 2-1 Reagent volumes for ligation mix

- **2.** Pipette 15 μL of the ligation mix into each well of an 8-well tube strip. Temporarily cap the tubes and keep on ice or in the IntegenX 8-well cold block.
- 3. Heat seal the ligation mix with aluminum heat seal:
  - a. Turn on the heat sealer to pre-heat (approximately 10 minutes).

If the temperature can be set, set it to 165  $^{\circ}$  C. If the time can be set, set it between 0.5 to 1 second.

- b. After the plate sealer has pre-heated, retrieve the 8-well cold block from a -20 ° C freezer. Place the strip tube with the ligation mix into the plate holder.
- c. Place the aluminum heat seal on top of the tubes seated in the cold block (correct side up as indicated on the packaging) and place the cold block with the foil seal aligned atop the tubes into the heat sealer deck.
- d. Insert the cold block into the sealer and seal the tubes by applying the heat for between 0.5 to 1 second.

A proper seal is verified by the appearance of embossed circles at the tops of the tubes.

- e. Let the seal cool for 30 seconds and then cut out the 8-well strip tubes with the ligation mix using a utility knife or razor.
- **4.** Return the ligation mix to ice or the cold block until ready to place onto the Apollo 324 work surface.

Visually inspect the tubes to be sure that the entire volume is in the bottom of the tubes, without air pockets.

## **Preparing Samples**

**NOTE:** We recommend using color-coded Axygen<sup>®</sup> strip tubes to ensure correct setup.

- **1.** Pipette 15  $\mu$ L of fragmented DNA sample, 200 ng (13 ng/ $\mu$ L) to 1000 ng (67 ng/ $\mu$ L) concentration, into each well of an Axygen 8-well strip tube.
- 2. To avoid contamination, temporarily cap the sample tubes with 8-strip Axygen caps until they are seated in the work surface.
- 3. Place the sample on ice until ready to load onto the Apollo 324 work surface.

**IMPORTANT:** The instrument is calibrated for Axygen tubes only. Using other types might result in run failure.

# **Setting Up and Running Protocols**

In this chapter:

Overview 11 Work Surface Positions 12 Before Beginning a Protocol Run 12 Using the Interface 13 Setting Up a Run 17 Running a Protocol 26

## **Overview**

This chapter describes how to set up and run protocols. The work surface setup window in the touchscreen interface is mapped to the work surface blocks on the instrument. The window provides guidance to verify how you place reagents, samples and consumables, and then run the protocols.

There are two types of runs, each requiring a slightly different setup.

- Eight samples The default setup of eight samples fills the available blocks on the work surface. For details, see "Setting Up a Run" on page 17.
- Fewer than eight samples Tubes must be loaded specifically in the middle of the block to avoid side-to-side torque. For details, see "Setting Up Runs for Fewer Than Eight Samples.".

For details on preparing the samples and reagents before loading them in the instrument, see Chapter 2, "Preparing Samples and Reagents."

**IMPORTANT:** You can run PrepX DNA Library and BeadX-High Library kits simultaneously. For details on placing the Bead Mix strips, see "Loading Samples and Reagents."

## **Work Surface Positions**

The Apollo 324<sup>™</sup> work surface contains blocks for the reagents and consumables for the protocol.

- Block 1 96 (eight each in 12-tube strips) 1.1 mL tubes for reagents dispensed by the pipette head
- Block 2 96-well microtiter plate for preparing solutions and dispensing waste products
- Block 3 BeadX<sup>™</sup> tube strips, AMPure 5X bead strip, enzyme tube strips, sample tube strip and empty tube strips (for product and ligation reaction)
- Block 4 8-tube strip for ligation mix
- Block 5 Piercing tips and dispensing pipette filter tips for the pipette head
- Block 6 Reservoirs for buffers and reagents (EDTA, ethanol and water)
- Blocks 7 and 8 Empty, not used

#### Figure 3-1 Apollo 324 work surface positions



## **Before Beginning a Protocol Run**

Make sure that the following is done before beginning any run:

- Apollo 324 System is powered on
- · Work surface is clean, clear of materials and ready for loading
- · Reagents are prepared for the protocol; use fresh, prepared reagents
- Sample has been fragmented and adapter/ligation mixture has been prepared.
- All racks are inserted in the correct block locations, with the consumables and reagents specified
- Waste tip box has been emptied



An accumulation of discarded pipettes and tips in the waste tip box can cause a run to fail. Be sure to pull out the internal drawer and check that the waste tip box has been emptied.

**NOTE:** For details on preparing samples and reagents for use in the Apollo 324 system, see Chapter 2, Preparing Samples and Reagents.

**NOTE:** For instructions on placing consumables, reagents and sample for the run, see "Setting Up a Run."

# **Using the Interface**

The Apollo 324 System is controlled by software that has a touchscreen interface for setting up and running protocols. Use the stylus on the touchscreen of the instrument to make your selections.

## Starting the System

**1.** Power on the instrument.

The software start-up window is displayed for a few seconds, and then the IntegenX splash screen appears. When you power on the instrument, the temperature in the heating/cooling units (Blocks 3 and 4) adjusts to  $18 \degree$ C.



After the splash screen disappears, the initial Start window is displayed, showing the available protocols. You can choose to set up and run a protocol or run automated maintenance scripts.





After the software is launched, the pipette head of the Apollo 324 System will initialize.

**IMPORTANT:** Pressing in the center of the window or pressing and holding any of the buttons will initiate calibration of the touchscreen. For details, see "Calibrating the Touchscreen" in Chapter 4, "Maintenance Procedures."

 To initiate a protocol, in the Start window, press the PrepX DNA Library or the PrepX Library BeadX-High button.

The work surface setup window is displayed.

On the instrument work surface, the left-hand heating/cooling unit (Block 3) remains at 18 °C, while the right-hand unit (Block 4) cools to 4 °C. While it is cooling, the **COOLING** button is displayed; when it reaches 4 °C, the **RUN** button appears.



3. While the unit is cooling, pre-load consumables, samples and reagents on the work surface.

**NOTE:** You can place the consumables (tubes, plates, reservoirs and tips) on the work surface before starting the software and loading the samples and reagents. For details, see "Placing Consumables on the Work Surface."

For instructions on loading reagents and samples, see "Loading Samples and Reagents."

For instructions on setting up runs for fewer than eight samples, see "Setting Up Runs for Fewer Than Eight Samples."

### Viewing the Work Surface Setup Window

The work surface setup window depicts the work surface of the instrument. In the setup window, all eight of the work surface blocks are referred to by numbers, starting from the upper left of the window for blocks 1 through 4, and from the lower left for blocks 5 through 8. All eight blocks are active when pressed.



When a block is pressed on the touchscreen, it is magnified so you can easily see how it is set up for the run. The entire setup is still visible in the background, with the active block denoted by a color outline. Instructions describe how the active block should be loaded for the protocol.

After you press a block in the setup window or press **NEXT**, the active block is magnified, and its related instructions are displayed.



### Navigating in the Setup Window

The NEXT and BACK buttons enable you to navigate in the setup window so you can easily view the setup for any block.

- NEXT Magnifies the next sequential block in the setup window.
  - In the setup window with no block selected Magnifies Block 1.
  - In the setup window with an active block selected Magnifies the selected block.
  - From a magnified block window Magnifies the next sequential block.
- **BACK** Allows you to return to the last magnified block or the Start window.
  - If you have returned to the setup window from a magnified block Redisplays the magnified block.
  - If you are in the setup window with no block selected Returns you to the Start window.

#### **Starting Runs**

The **RUN** and **OK** buttons enable you to start a run.

- RUN Allows you to start the run immediately.
  - After reviewing the work surface setup, press **RUN** to start the run.

When you view a magnified block window, the RUN button no longer appears in the background setup window. To make RUN available again, press **OK**.

• **OK** – Allows you continue to set up blocks for the run.

From any magnified block window, you can press **OK** to return to the setup window. A prompt asks if you are ready to perform the run. If you are ready, press **RUN**. If you are not ready, press **NEXT** or **BACK**.

After you have set up several successful runs, and are confident that all blocks on the instrument are correctly loaded for the current protocol, you can quickly review the setup window, close the instrument door and press **RUN** to start the run.

**IMPORTANT:** You must close the instrument door before starting a run.

# Setting Up a Run

This section describes how to load items on the instrument, using the setup window to verify placement. Refer to Figure 3-1 for the actual work surface positions.

There are two types of runs, each requiring a slightly different setup.

- Eight samples The default setup of eight samples fills the available blocks on the work surface. The setup windows only provide guidance for setting up runs for a full eight samples.
- Fewer than eight samples Tubes must be loaded in the middle of the block to avoid side-to-side torque. The setup windows do not give specific instructions for preparing a run for fewer than eight samples. For details, see "Setting Up Runs for Fewer Than Eight Samples."

**NOTE:** The reagent setup is the same for both the PrepX and BeadX-High kits. The only difference is that the kits use different bead solutions.

#### **Placing Consumables on the Work Surface**

Placing the consumables on the work surface can be done in advance.

 Place 96 1.1 mL tubes (eight each in 12-tube strips) in Block 1. Verify that they are properly seated and level.

Apollo 324	🛟 integen X
Block 1 Block 2   Block 1 Block 2   Block 2 Block 2   Block 5 Block 6	Block 1 1.1ml Strip Tubes A B C D E F G H Block 1 12 11 10 9 8 7 6 5 4 3 2 1 1 1 1 1 1 1 1 1 1 1 1 1

2. Place a clean, empty 96-well microtiter plate in Block 2, with the A1 well in the lower left corner.

Apollo 324	🛟 integen X
Block 1 Block 2 Block 2 Block 2 Block 2 Block 2 Block 2 Block 5 Block 6	Block 2   12   10   9   8   7   6   0000000   0000000   4   3   2   1   A B C D E F G H

- 3. Place the piercing tips and dispensing pipette tips into Block 5 as follows:
  - a. Place 24 grey piercing tips into rows 10-12 of Block 5.
  - b. In Block 7, place a full set of dispensing tips (in their plastic carrier tray) on the tip rack and snap the carrier tray securely onto the rack.
  - c. Transfer 72 dispensing tips from Block 7 into rows 1–9 of Block 5. This can be done efficiently using a standard multichannel pipette.



**NOTE:** Do not use the plastic carrier tray in Block 5, as it might cause tips to stick during automatic operation.

4. Place four empty reservoirs in Block 6.



**5.** Verify that the waste tip box is empty.

## **Loading Samples and Reagents**

**IMPORTANT:** You can run PrepX DNA Library and BeadX-High Library kits simultaneously. For details on placing the Bead Mix strips, see step 2.

1. If you have not already done so, press the **PrepX DNA Library** or the **PrepX Library BeadX-High** button in the Start window to initiate the protocol.

The work surface setup window is displayed.

On the instrument work surface, the left-hand heating/cooling unit (Block 3) remains at 18 °C, while the right-hand unit (Block 4) cools to 4 °C. While it is cooling, the **COOLING** button is displayed; when it reaches 4 °C, the **RUN** button appears.

While the unit is cooling, you can place samples and reagents on the work surface.

**IMPORTANT:** Do not load the ligation mix until the Block 4 heating/cooling unit is at 4 °C and the **RUN** button appears (approximately three minutes).



- **2.** Load the empty product tubes, prepared sample and reagents into Block 3:
  - a. Place each of the eight Bead Mix 4-tube strips vertically into rows 9–12 (blue) at the top of the block so that the arrows point up toward the rear of the work surface.
    - PrepX DNA Library Load the BeadX strips (blue labeled seal)
    - BeadX-High Library Load the BeadX-High strips (green labeled seal)
  - b. Place the 8-well strip with the sample (tube caps removed) into row 8 (violet).
  - c. Place the empty 8-well strip for the AMPure beads into row 7 (green).

**NOTE:** Just before starting the protocol, you will aliquot the AMPure beads into these tubes (step 6). This is done later in order to avoid bead settling. For details on preparing the beads, see "Preparing 5X AMPure XP Beads" in Chapter 2.

- d. Place an empty 8-well strip in row 6 (clear).
- e. Place the empty 8-well strip for product in row 5 (red).
- f. Place each of the eight 4-tube enzyme strips vertically into rows 1–4 (yellow) at the bottom of the block so that the arrows point up toward the rear of the work surface.

NOTE: Verify that all tubes are oriented and seated correctly.



Visually inspect the tubes as you place them in the block to ensure that the entire volume is in the bottom of the tubes, without droplets on the side walls, bubbles or void volume. If necessary, centrifuge briefly before placing the tubes.



3. After the RUN button appears, place the heat-sealed 8-well strip with ligation mix into row 1 of Block 4.



- 4. Fill the reagent reservoirs in Block 6:
  - a. Dispense 25 mL of UltraPure water into Reservoir 1.
  - b. Dispense 40 mL of freshly prepared 70% v/v EtOH into Reservoir 3.
  - c. Dispense 15 mL of 0.5M EDTA into Reservoir 4.

**NOTE:** Reservoir 2 remains empty for this configuration, but is included to ensure seating alignment.

itegenX
0.5M EDTA 70% EtOH H2O

5. Blocks 7 and 8 are not used and remain empty.

Apollo 324		integenX
Block 7	Block 8	Block 4
00000000	OOOOOC Bloc OOOOOC and	ks 7 and 8 are empty not used.
Block 5 B		
	Block 7	Block 8
OK BACK	NEXT	

- 6. Load the AMPure beads:
  - a. Remove the empty 8-well strip from row 7 in Block 3.
  - b. Re-vortex the 5X AMPure beads.
  - c. Aliquot 120  $\mu L$  of 5X AMPure beads into each well of the strip.
  - d. Place the strip back in row 7.

**NOTE:** Do not centrifuge the bead strip before placing in row 7.

7. Verify placement of all reagents and consumables and check that all tubes, plates and reservoirs are seated properly.

8. Install the metal retention plates over Block 3 and Block 4 to secure the tubes and keep them stable, aligning their guide pins at the top and bottom.

Rotate the side knobs to lock the retention plates in place.





**9.** Verify that all components are installed according to the setup window. You are now ready to start the run. For instructions, see "Starting a Run."

## Setting Up Runs for Fewer Than Eight Samples

If you are running fewer than eight samples, several of the work surface blocks must be loaded from the center column, to avoid side-to-side torque. Use the procedure in this section to load the blocks, and consult the following table for the columns to load.

**IMPORTANT:** We recommend that you use complete empty strips rather than single tubes.

#### Where to Load Samples and AMPure Beads

For one to seven samples, load the sample and the AMPure beads in the appropriate columns as follows:

Number of Samples	Sample and Beads are Loaded in Columns
1	D
2	D, E
3	C, D, E
4	C, D, E, F
5	B, C, D, E, F
6	B, C, D, E, F, G
7	B, C, D, E, F, G, H

As an example, the following sections describe the procedure for setting up a run for one sample.

#### Placing Consumables on the Work Surface

- 1. Place one 12-tube strip of 1.1 mL tubes into column D of Block 1.
- 2. Place a clean, empty microtiter plate in Block 2, with the A1 well in the lower left corner.
- 3. Place three grey piercing tips in column D, rows 10–12 of Block 5.
- 4. Place nine dispensing pipette tips in column D, rows 1–9 of Block 5.
- 5. Place four empty reservoirs in the positions on Block 6.

#### **Loading Samples and Reagents**

1. If you have not already done so, press **PrepX DNA Library** or the **PrepX Library BeadX-High** button in the Start window.

The work surface setup window is displayed.

On the instrument work surface, the left-hand heating/cooling unit (Block 3) remains at 18  $^{\circ}$ C, while the right-hand unit (Block 4) cools to 4  $^{\circ}$ C. While it is cooling, the **COOLING** button is displayed; when it reaches 4  $^{\circ}$ C, the **RUN** button appears.

While the unit is cooling, you can load the samples and reagents.

**IMPORTANT:** Do not load the ligase mix until the heating/cooling unit is at 4 °C and the **RUN** button appears.

#### 2. In Block 3:

- a. Place one BeadX 4-tube strip in the top of column D (rows 9–12) (blue), with the arrow tab pointing up toward the top of the block. Verify that the orientation of the strip is correct.
  - PrepX DNA Library Load a blue BeadX strip
  - BeadX-High Library Load a green BeadX-High strip
- b. Place the 8-well strip with the sample into row 8 (violet). Use a complete strip rather than a single tube. The sample should be in column D.
- c. Place the empty 8-well strip for the 5X AMPure beads into row 7 (green). Use a complete empty strip rather than a single tube.

**NOTE:** Just before starting the protocol, you will aliquot the AMPure beads into these tubes (step 6). This is done later in order to avoid bead settling. For details on preparing the beads, see "Preparing 5X AMPure XP Beads" in Chapter 2.

- d. Place an empty strip in row 6 (clear).
- e. Place the empty 8-well strip for product in row 5 (red).
- f. Place one 4-tube enzyme strip into rows 1–4 (yellow) of column D, with the arrow tab pointing up toward the rear of the work surface. Verify that orientation of the strip is correct.

**NOTE:** Verify that all tubes are oriented and seated correctly.

- **3.** After the RUN button appears, place the heat-sealed 8-well strip in row 1 of Block 4, with the ligation mix in column D.
- 4. In Block 6, fill the reagent reservoirs as for eight samples.
- 5. Blocks 7 and 8 are not used and remain empty.
- 6. Load the AMPure beads:
  - a. Remove the empty 8-well strip from row 7 in Block 3.
  - b. Re-vortex the 5X AMPure beads.
  - c. Aliquot 120  $\mu$ L of 5X AMPure beads into the column D well of the strip.
  - d. Place the strip back in row 7.
- 7. Verify placement of all reagents and consumables and check that all tubes, plates and reservoirs are seated properly.
- 8. Place the metal retention plates over Block 3 and Block 4 to secure the tubes and keep them stable, aligning their guide pins at the top and bottom.

The plates snap into place with magnets. Tighten the side knobs on the plates to secure them.

9. Verify that all components are installed according to the setup window.

You are now ready to start the run. For instructions, see "Starting a Run."

# **Running a Protocol**

## Starting a Run

When all of the items are on the work surface, close the instrument door.

**IMPORTANT:** You must close the door in order to start the run.

If you are ready to start the protocol, press **RUN**. If you are not ready, press **NEXT**, **BACK** or any block to review the setup and make changes to items on the work surface.



After you press **RUN**, the front door of the instrument locks and the run starts. The status of each step of the run is displayed in a progress bar.

## Monitoring a Run

After a run begins, the progress window shows the status of each step. A countdown timer shows how much time (minutes and seconds) is left for each step.

	Apollo 324		integenX	
(		Eat Time	Status	
	End Repair	00:30		
	A-tailing	01:00		
	Adaptor Ligation	01:00		
	BeadX Sizing	<b>00:</b> 15		
		PrepX DNA Libra	ry	STOP

From this window, you can stop a run completely. The **STOP** button appears only after a run has started.

#### Stopping a Run

There might be situations when you need to stop a run, such as if you need to make an adjustment to an item on the work surface. You can then re-start the protocol.

1. In the progress window, press STOP.

A warning dialog is displayed.

Apollo 324	🛟 integen X
30 Est.Time End Repair 30:00	Status
A-tailing Do you wis	P pressed! sh to stop the run?
Adaptor Ligation Yes	Cancel
BeadX Sizing 00:15	
PrepX DNA Li	brary

2. Press Yes to stop the run.

If you press Cancel, the run will continue.

3. In the Protocol Finished dialog box, press **OK**.

The Start window opens. The instrument door unlocks and you can have access to the work surface. When you are ready, you can start a new run.

#### Errors in a Run

If an error that causes the system to stop functioning, the run stops and an error message is displayed. In the error message window, click **OK** to return to the Start window.

### **Finishing a Protocol**

After the products have been eluted off the beads, the protocol is finished. A message informs you when the protocol is complete.

Apollo 324	🛟 integen X
	PROTOCOL FINISHED
ОК	

- 1. Press **OK** to display the Start window. At this point, the door unlocks.
- 2. Open the door and remove the 8-tube product strip from the instrument.
- 3. Cap the processed samples immediately and store them on ice or at -20 °C.
- 4. Remove and discard used reagents and consumables.



Do NOT attempt to open the instrument door until the "Protocol Finished" message is displayed. Do NOT assume that a protocol is complete until this message is displayed.