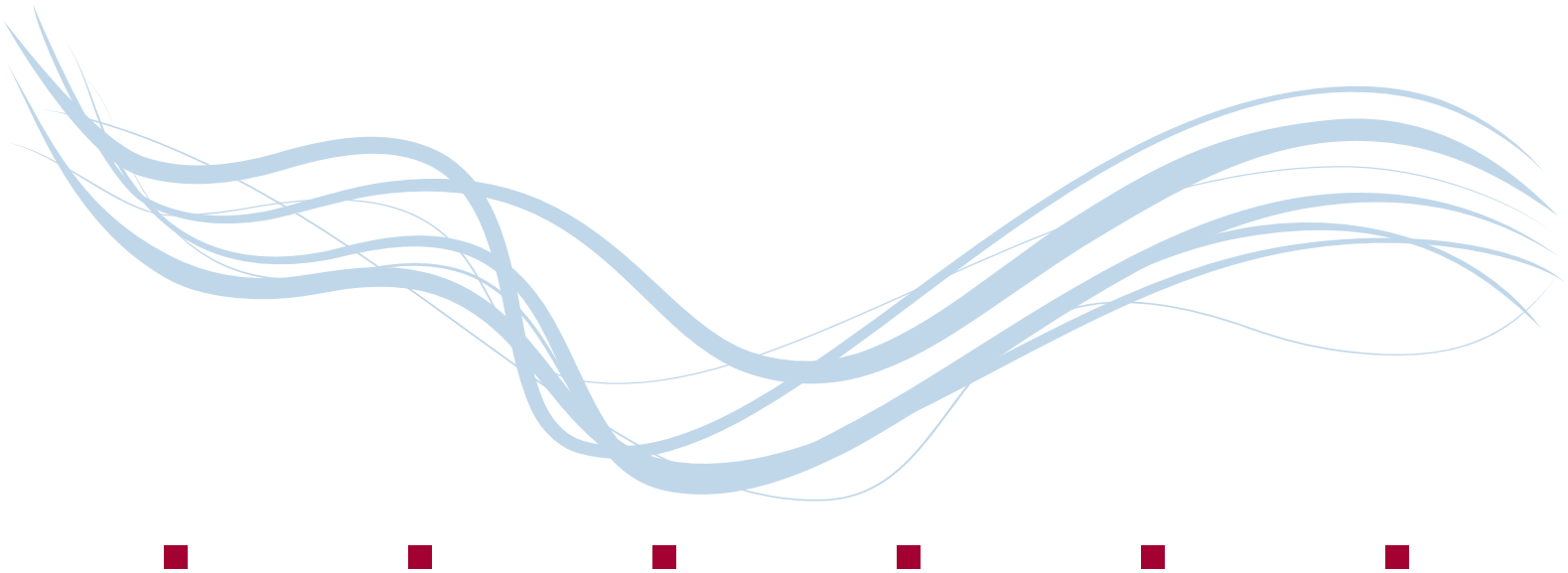
integenX

Apollo 324™ System

PrepX mRNA Library

Protocol



DRAFT – P037497, Rev. A – 9.14.2012

RESEARCH USE ONLY

The Apollo 324 System is for research use only.

WARRANTY

For warranty and liability information, please see IntegenX terms and conditions of sale.

TRADEMARKS

Apollo 324, BeadX and PrepX are trademarks of IntegenX Inc.

Agencourt, AMPure and RNAClean are registered trademarks of Beckman Coulter, Inc.

Covaris is a registered trademark of Covaris, Inc.

LongAmp is a registered trademark of New England BioLabs, Inc.

All other trademarks are the sole property of their respective owners.

IntegenX Inc.
5720 Stoneridge Drive, Suite 300
Pleasanton, CA 94588

925.701.3400

Email: support@integenX.com

www.IntegenX.com

Part Number P037497, Rev. A
9/2012

Revision History

Revision	Date	Description
A	9/2012	Initial release

Contents

Chapter 1

Overview

In this chapter:

This *PrepX mRNA Library Protocol* provides the basic information necessary to use your Apollo 324™ System to prepare cDNA from mRNA samples. It also describes a PCR method for converting the cDNA to libraries.

This document assumes that you know how to use the Apollo 324™ System and the touchscreen interface. For details on using the system, refer to the *Apollo 324 User Guide*.

IMPORTANT: IntegenX recommends that first-time users take advantage of user training offered with the installation of the system. A training video is available at http://integenx.com/324-training-video/.

About the Protocol

The PrepX™ RNA-Seq Library reagent kit for Illumina provides researchers the flexibility to prepare cDNA on the Apollo 324 system, for sequencing on Illumina platforms. The entire process to prepare the cDNA is completed in approximately five hours. PCR is then performed to convert the cDNA to DNA.

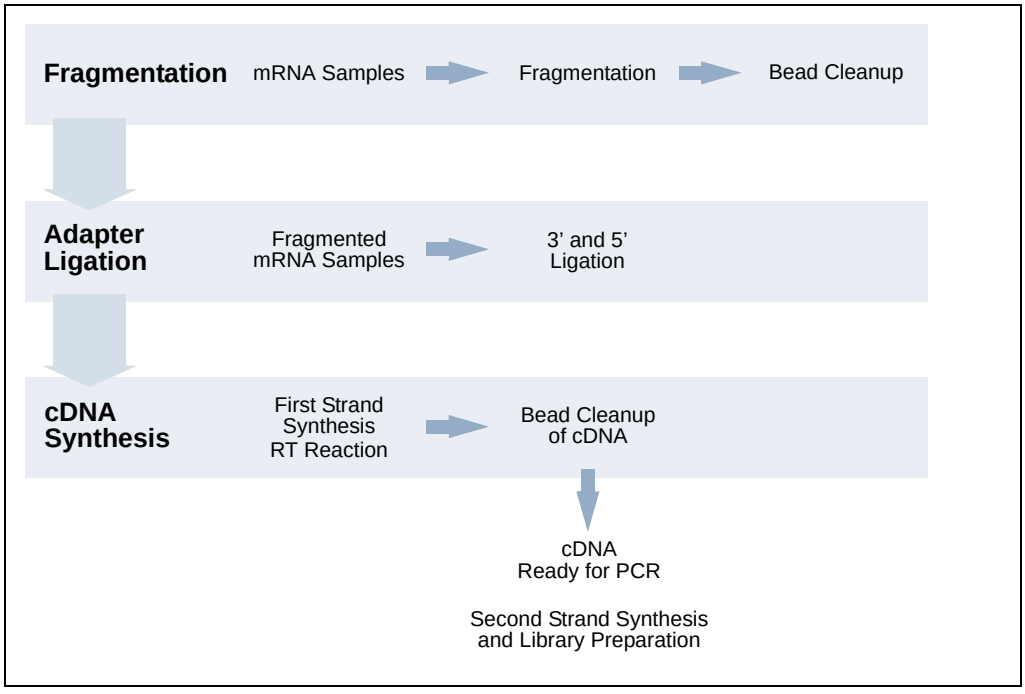
The kit includes the following items, sufficient to process 24 samples:

- PrepX mRNA Enzymes (Box Enz)
  - 24 enzyme strips (four tubes in each strip, blue seal); includes 3’ ligation buffer, 5’ ligation buffer, 3’ ligation enzyme, and 5’ ligation enzyme
  - RNase III Enzyme (one tube, 53 µL)
  - RNase III Buffer (one tube, 53 µL)
  - dNTP (one tube, 106 µL)
  - Murine RNase inhibitors (one tube, 27 µL)
- PrepX mRNA Adapters and Primers (Box AdP)
  - 24 adapter/primer strips (four tubes in each strip, orange seal); includes 3’ SR adapter, 5’ adapter, SR RT primer, and nuclease-free water
  - SR Primer (1 tube, 80 µL)
  - Index 1 Primer (1 tube, 80 µL)
- Molecular biology grade water (1 bottle)

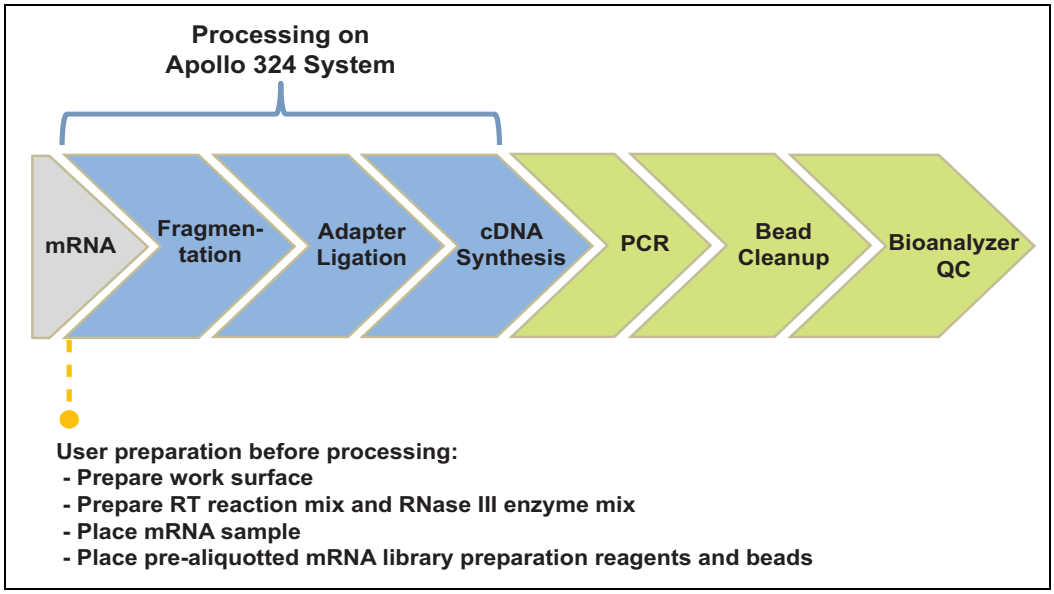
Workflow

1. Press the PrepX mRNA button on the touchscreen to activate the Peltier heating/cooling units.
2. Place consumables, reagents, magnetic beads and mRNA samples in the racks on the work surface.
3. Start the protocol run, using the touchscreen interface.
4. The eight samples undergo processing:
   a. Fragmentation (RNase III) and bead cleanup
   b. 3’ adapter ligation
   c. 5’ adapter ligation
   d. First strand synthesis (RT reaction)
5. Final bead-based purification of single-stranded cDNA
6. The cDNA samples are now ready for second strand synthesis by PCR. The Agilent® Bioanalyzer or equivalent DNA analyzer is recommended to verify library size after PCR and bead cleanup.

Processing Schematic



mRNA Library Preparation Workflow



Chapter 2

Preparing Samples and Reagents

In this chapter:

Overview

This chapter describes how to prepare samples and reagents for the PrepX™ mRNA Library protocol.

IMPORTANT: We only guarantee the Apollo 324™ System to perform using the recommended supplies and materials listed in this document.

Materials for Operation

Reagent Kit

The following reagents are provided in the PrepX RNA-Seq Library Kit. The kit provides sufficient reagents for three 8-sample runs (24 samples).

- PrepX mRNA Enzymes (Box Enz)
  - 24 enzyme strips (four tubes in each strip, blue seal); includes 3’ ligation buffer, 5’ ligation buffer, 3’ ligation enzyme, and 5’ ligation enzyme
  - RNase III Enzyme (one tube, 53 µL)
  - RNase III Buffer (one tube, 53 µL)
  - dNTP (one tube, 106 µL)
  - Murine RNase inhibitor (one tube, 27 µL)
- PrepX mRNA Adapters and Primers (Box AdP)
  - 24 adapter/primer strips (four tubes in each strip, orange seal); includes 3’ SR adapter, 5’ adapter, SR RT primer, and nuclease-free water
  - SR Primer (1 tube, 80 µL)
  - Index 1 Primer (1 tube, 80 µL)
- Molecular biology grade water (1 bottle)

For the list of additional reagents required to run the PrepX mRNA Library protocol, see "Customer-Supplied Reagents and Consumables."

For a complete list of the materials and equipment required for using the Apollo 324 system, see the *Apollo 324 System User Guide*.

Instruments

The following items are required.

Item	Part Number	Supplier
Centrifuge for 0.2 mL 8-tube strips	various	various
Vortex mixer for preparing reagents and samples	various	various
Agilent 2100 Bioanalyzer	G2938C	Agilent®
Agilent High Sensitivity DNA Kit (to perform Bioanalyzer runs for your prepared samples)	5067-4626	Agilent
Thermal cycler	various	various

Customer-Supplied Reagents and Consumables

The following reagents and consumables are recommended.

Customer-Supplied Consumables	Part Number	Supplier
1.1 mL 12-tube strips (Axygen®)	89005-580	VWR®
0.2 mL 8-tube strips (Axygen)	10011-764	VWR
0.2 mL 8-cap strips (Axygen)	10011-786	VWR
15 mL Falcon™ test tube	21008-929	VWR
2 mL screw cap test tube	16466-042	VWR
Piercing tips	300028	IntegenX
Dispensing filter tips	300027	IntegenX
Reservoirs	300031	IntegenX
Micropipette tips (20 µL, 200 µL and 1000 µL)	various	various
Micropipettes (20 µL, 200 µL and 1000 µL)	various	various
Customer-Supplied Reagents for Apollo 324	**Part Number**	**Supplier**
100%, 200 proof EtOH (500 mL)	E7023	Sigma-Aldrich®
Water, Biotech Performance Certified, Bulk	various	various
AMPure® XP Beads (450 mL kit)	A63882	Agencourt®
SuperScript® III Reverse Transcriptase kit	1080-044	Life Technologies™
Customer-Supplied Reagents for PCR	**Part Number**	**Supplier**
LongAmp® Hot Start Taq 2X Master Mix	M0533S (100) or M0533L (500)	New England BioLabs®
100%, 200 proof EtOH (500 mL)	E7023	Sigma-Aldrich®
AMPure XP Beads (450 mL kit)	A63882	Agencourt
Index Primers	Custom	Integrated DNA Technologies, Inc.

The recommended initial purchase quantities and usage per run of the consumables are as follows:

Requirement	Recommended Initial Purchase	Usage per Run	Number of Runs
1.1 mL 12-tube strips (Axygen)	1 box	2	40
0.2 mL 8-tube strips (Axygen)	1 box	7	17
0.2 mL 8-cap strips (Axygen)	1 box	4	31
15 mL Falcon test tube	n/a	2	25
Piercing tips	1 box of 1000	8	125
Dispensing filter tips	1 box of 960	56	17
Reservoirs	1 box of 100	2	50
Micropipette tips (20 µL, 200 µL and 1000 µL)	n/a	varies	varies
Micropipettes (20 µL, 200 µL and 1000 µL)	n/a	varies	varies
Safety glasses, gloves and lab coats as required in your lab	n/a	varies	varies

Decontaminating the Lab Work Space, Instrument, and Pipettes

1. Verify that the waste tip box is empty.
2. Wipe the instrument work surfaces with 70% EtOH sprayed onto a laboratory wipe.
3. Place a large laboratory wipe on the bench surface.
4. Clean the instrument pipettes, as follows:
   a. Remove the metal ejector collar from the pipettes.
   b. Wipe the pipettes and collars with 70% EtOH.
   c. Spray parts with 70% EtOH.
   d. Wipe all parts with laboratory wipes and air dry.

IMPORTANT: We recommend using a set of pipettes dedicated for working with RNA. Always use RNase-free barrier/filtered pipette tips.

Thawing Reagents

1. Thaw at room temperature:
   - 5X FS buffer and 0.1mM DTT from the SuperScript III kit
   - dNTP and RNase III buffer from Box Enz of the PrepX RNA-Seq Library Kit
2. Thaw on ice the frozen reagents from Box Enz and Box AdP.

   Thaw only the reagents that you will be using for the run. Keep all thawed reagents on ice.
3. Once thawed, spin down the reagents briefly prior to use.

   We recommend using a centrifuge with a swinging bucket for the reagent tubes.
4. Visually inspect to be sure that the entire volume is at the bottom of each vial with no air pockets. Gently tap the tubes on surface to level the reagents if they are clinging to the sides of the tubes. Keep on ice and cover until ready to start run.

Preparing the 1X AMPure XP Beads

1. Vortex the 1X AMPure beads. Tap the tubes on benchtop to remove the beads from the tube lid.
2. Aliquot 150 µL of 1X AMPure beads into each tube of the 8-tube strip.

   NOTE: Do not centrifuge the bead strip. Be sure that no air is trapped beneath the bead volume. If there is an air pocket, use a pipette to remove the air.
3. Cap the tubes and set aside at room temperature until ready to load onto the Apollo 324 work surface.

IMPORTANT: Do not place the beads on ice.

Preparing the 100% EtOH Aliquot

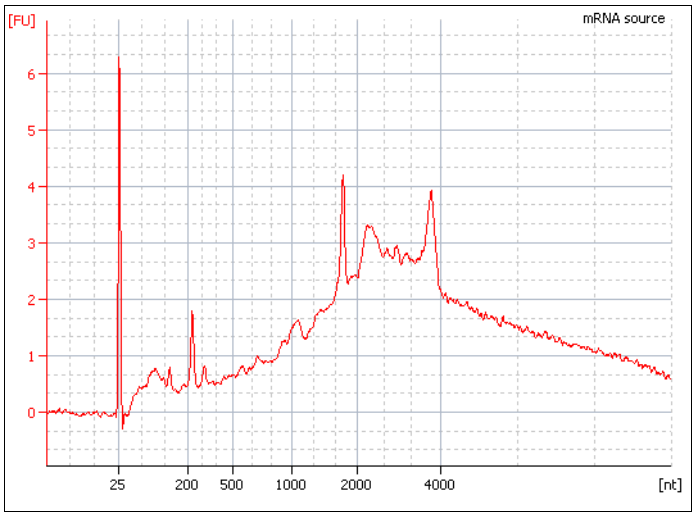
Pipette 15 mL of 100% EtOH into a sterile 15 mL Falcon tube.

Preparing the Molecular Biology Grade Water Aliquot

Pipette 15 mL of molecular biology grade water into a sterile 15 mL Falcon tube.

Confirming Quality of the mRNA Samples

The quality of the mRNA samples must be confirmed by running a Bioanalyzer Pico Chip according to the manufacturer's instructions. Ideally, polyA mRNA with less than 5% rRNA contamination is recommended for mRNA library preparation. An example of a representative polyA mRNA profile is shown in the following figure.



Preparing the mRNA Samples

1. Dilute your mRNA sample to the current working concentration of 0.1–250 ng in a total of 16 uL of nuclease-free water.
2. Pipette 16 µL of the diluted sample into each tube of an Axygen 8-tube strip.

Be very careful when pipetting. Do not allow any air pockets to form under the sample. If there are air pockets, the robot arm will pick up air instead of fluid.

3. Cap the tubes and place 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. We recommend using color-coded Axygen strip tubes to ensure correct setup.

Preparing the Reverse Transcription Reaction Mix

1. Prepare the reverse transcription (RT) reaction mix in a sterile 1.5 mL microfuge tube (for one run of eight samples, plus one sample for volume loss to surfaces) as follows:
   a. Vortex and briefly centrifuge the 5X FS buffer, 0.1mM DTT and dNTP tubes.
   b. Briefly centrifuge the Murine RNase Inhibitor.
   c. Briefly centrifuge the SuperScript III.
   d. For each sample, combine the reagents, using the volumes shown in the following table.

   Table 2-1 Reagent volumes for RT reaction mix

Component	Volume for 1 sample (µL)	Volume for 8 (+1) samples (µL)
5X FS Buffer	16	144
0.1mM DTT	8	72
dNTP	4	36
Murine RNase Inhibitor	1	9
Superscript III	2	18
Total	**31**	**279**

   e. Keep the mixture on ice. Pipette mix and centrifuge briefly.
2. Pipette 31 µL of the RT reaction mix into each tube of an 8-tube strip.
3. Temporarily cap the tubes and keep on ice or in the IntegenX 8-well cold block.

Preparing the RNase III Enzyme Reaction Mix

1. Prepare the RNase III enzyme reaction mix in a sterile 1.5 mL microfuge tube (for one run of eight samples, plus one sample for volume loss to surfaces) as follows:
   a. Vortex and briefly centrifuge the RNase III enzyme tube.
   b. Vortex and briefly centrifuge the RNase III buffer tube.
   c. For each sample, combine the reagents, using the volumes shown in the following table.

   Table 2-2 Reagent volumes for RNase III enzyme reaction mix

Component	Volume for 1 sample (µL)	Volume for 8 (+1) samples (µL)
RNase III Buffer	2	18
RNase III Enzyme	2	18
Total	**4**	**36**

   d. Keep the mixture on ice. Pipette mix and centrifuge briefly.
2. Pipette 4 µL of the RNase III enzyme reaction mix into each tube of an 8-tube strip.
3. Temporarily cap the tubes and keep on ice or in the IntegenX 8-well cold block.

Setting Up and Running the Protocol

In this chapter:

Overview

This chapter describes how to set up and run the protocol, providing guidance for placing the reagents, samples and consumables on the work surface, and then running the protocol. The work surface setup window in the touchscreen interface is mapped to the work surface blocks on the instrument. The window provides guidance for placing reagents, samples and consumables, and then running the protocol.

The default setup for eight samples fills the available blocks on the work surface.

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

For details on placing:

- Consumables, see “Placing Consumables on the Work Surface.”
- Samples and reagents, see “Loading Samples and Reagents.”

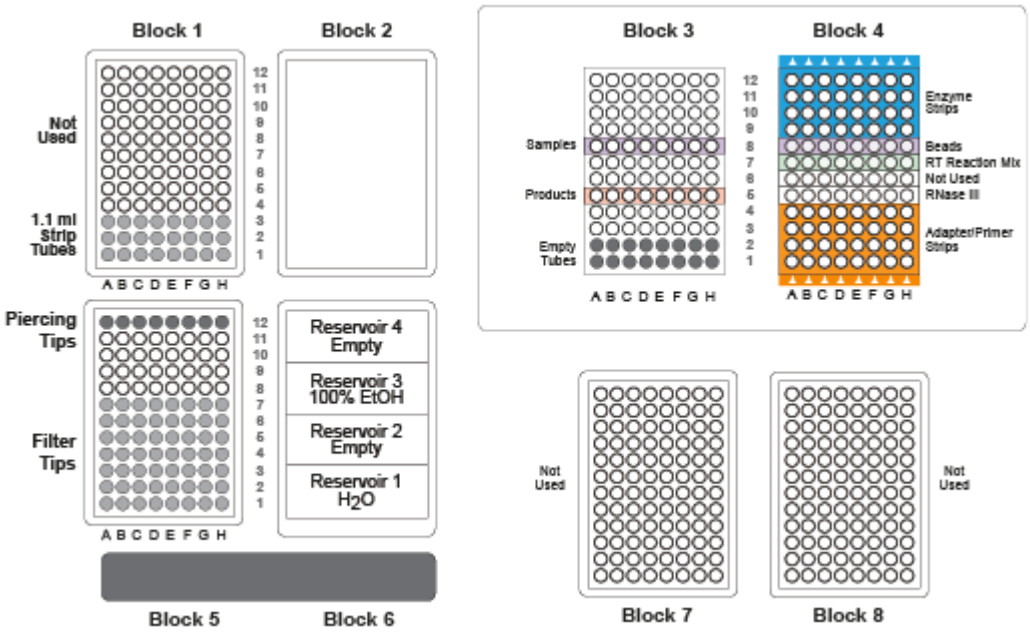
An accumulation of discarded pipettes and tips in the waste tip box can cause a run to fail. Be sure to open the waste tip box access door and check that the box has been emptied.

Setting Up a Run

This section describes how to load items on the instrument, using the setup window to verify placement.

Work Surface Layout

The following illustration shows the layout of the work surface and placement of the consumables, reagents and samples for the protocol run.



Setup Window

The setup window in the touchscreen interface provides guidance for setting up runs. In the setup window, the NEXT and BACK buttons enable you to navigate so you can easily view the setup for any block.

- **NEXT** magnifies the next sequential block.
- **BACK** returns to the last magnified block or the Start window.

For details on using the touchscreen interface, refer to the *Apollo 324 User Guide*.

Launching the Software

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 °C and 4 °C, respectively.

   After the splash screen disappears, the initial Start window is displayed.

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

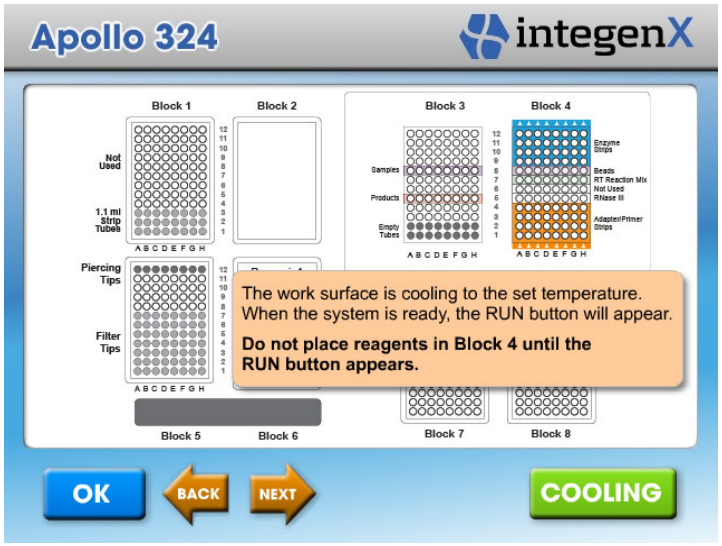
IMPORTANT: Pressing in the center of the window or pressing and holding any of the buttons for several seconds will initiate calibration of the touchscreen. If you accidentally do so, refer to the *Apollo 324 System User Guide* for details on calibrating the touchscreen.

2. To select the protocol, in the Start window, press **PrepX mRNA Library**.

   The work surface setup window is displayed.

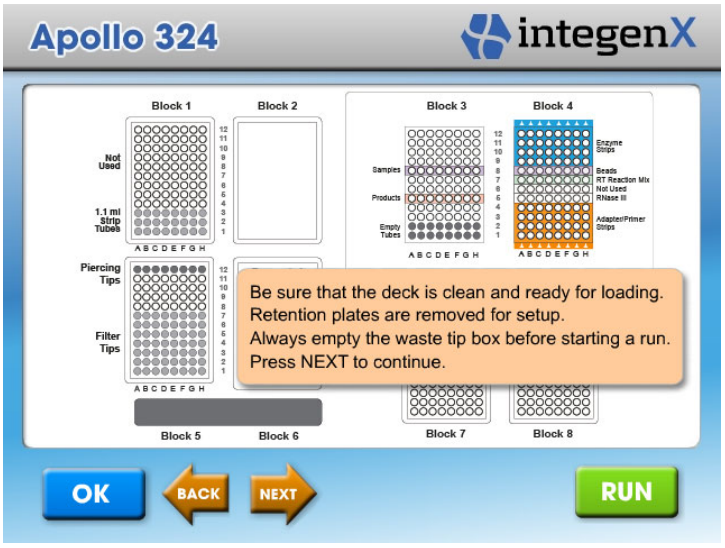
   On the instrument work surface, the left-hand heating/cooling unit (Block 3) remains at 18 °C, while the right-hand unit (Block 4) cools to 4 °C.

   While Block 4 is cooling, the **COOLING** button is displayed.



During cooling of Block 4

   When Block 4 reaches 4 °C, the **RUN** button appears.



When Block 4 reaches 4 °C

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

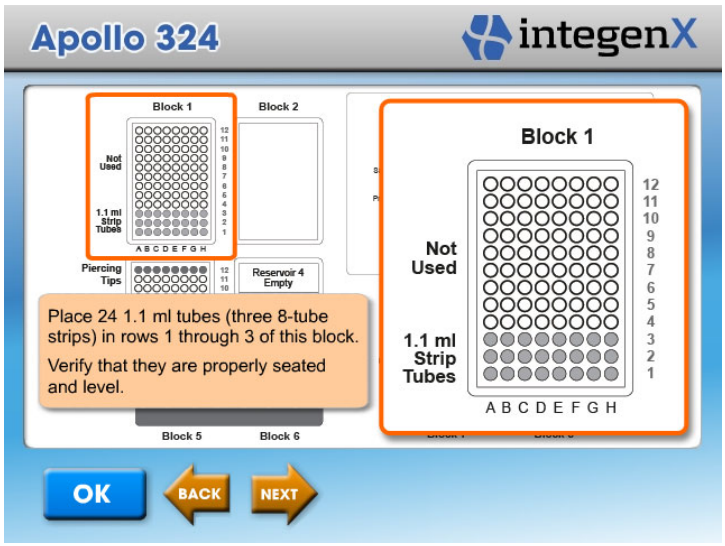
   NOTE: You can place the consumables (tubes, plates, reservoirs and tips) on the work surface before starting the software and loading the samples and reagents. For details, see "Placing Consumables on the Work Surface."

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

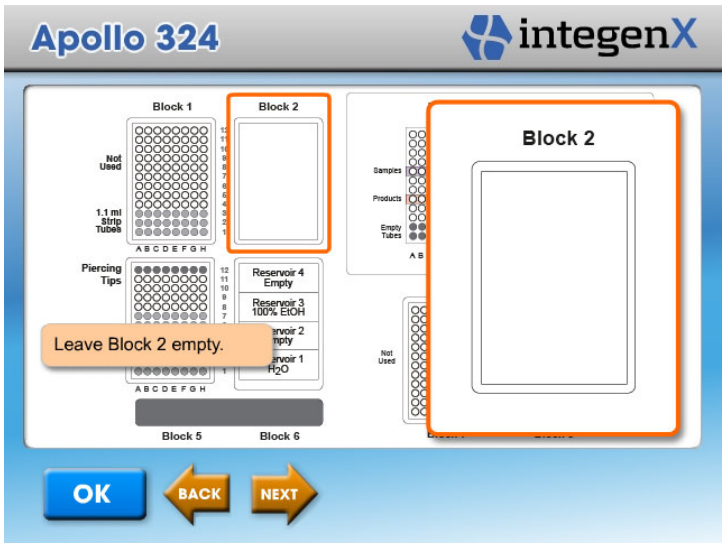
Placing Consumables on the Work Surface

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

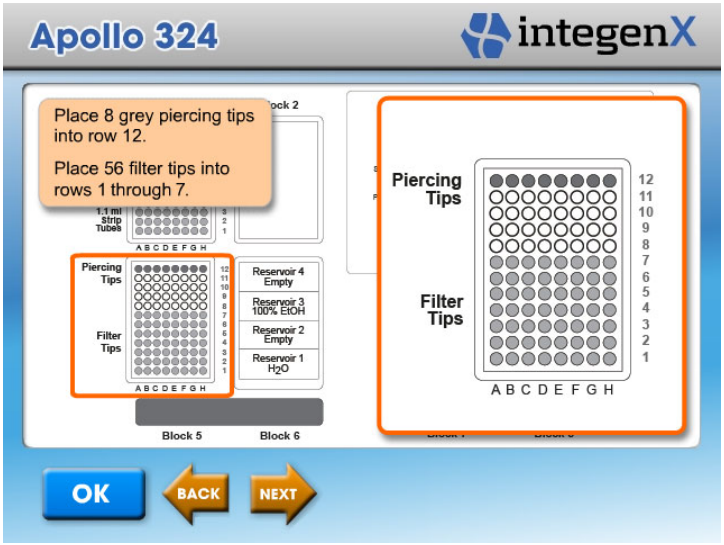
1. Place 24 1.1 mL tubes (three strips of 8-tube strips) in rows 1–3 of Block 1.
   Verify that they are properly seated and level. These tubes will be filled during the run.



2. Leave Block 2 empty.



3. Place the piercing tips and dispensing filter tips into Block 5 as follows:
   a. Place 8 grey piercing tips into rows 12 of Block 5.
   b. Place 56 dispensing filter tips into rows 1–7 of Block 5.



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. You will fill these later.

   Make sure that the reservoirs are seated properly.

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

6. Verify that the waste tip box is empty.

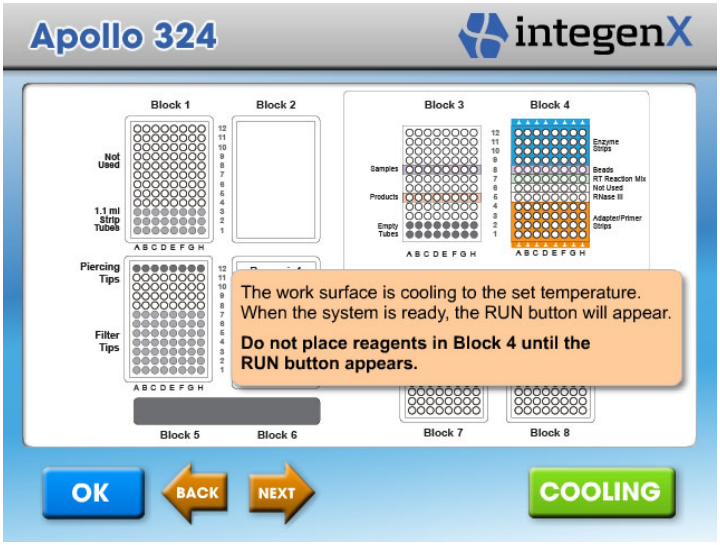
Loading Samples and Reagents

1. If you have not already done so, press the **PrepX mRNA Library** button in the Start window to initiate the protocol.

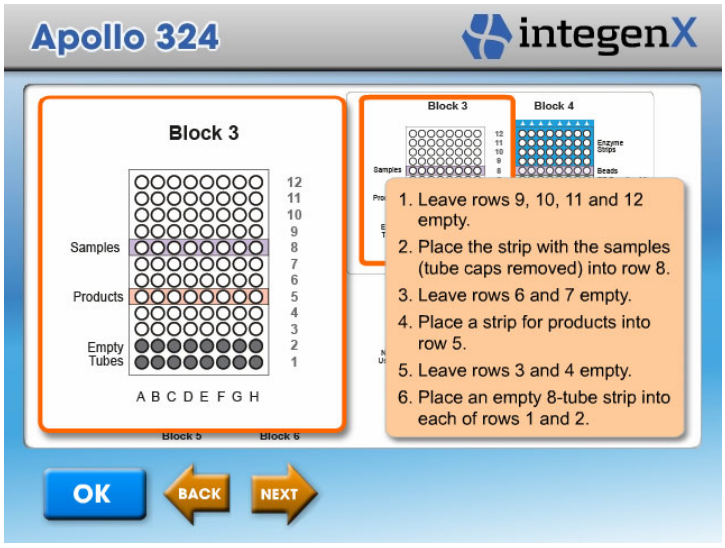
   The work surface setup window is displayed.

   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.



2. Load Block 3 as follows:
   a. Leave rows 9–12 empty.
   b. Place the 8-tube strip with the mRNA samples (tube caps removed) into row 8.
   c. Leave rows 6 and 7 empty.
   d. Place an empty 8-tube strip for receiving products in row 5.
      Be sure to label this strip with the date and other details about the run.
   e. Leave rows 3 and 4 empty.
   f. Place empty 8-tube strips into rows 1 and 2.

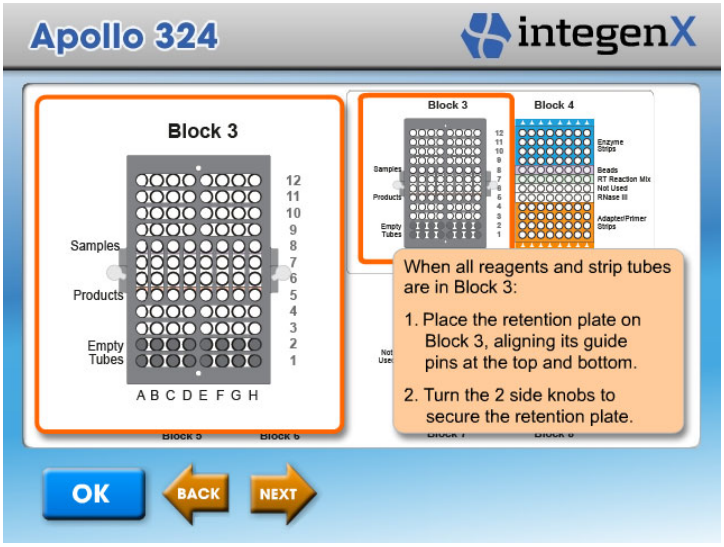


NOTE: Verify that all tubes are seated correctly.

CAUTION 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 (except for the tubes containing 1X AMPure beads).

3. Place the metal retention plate over Block 3 to secure the tubes and keep them stable, aligning the guide pins at the top and bottom.

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



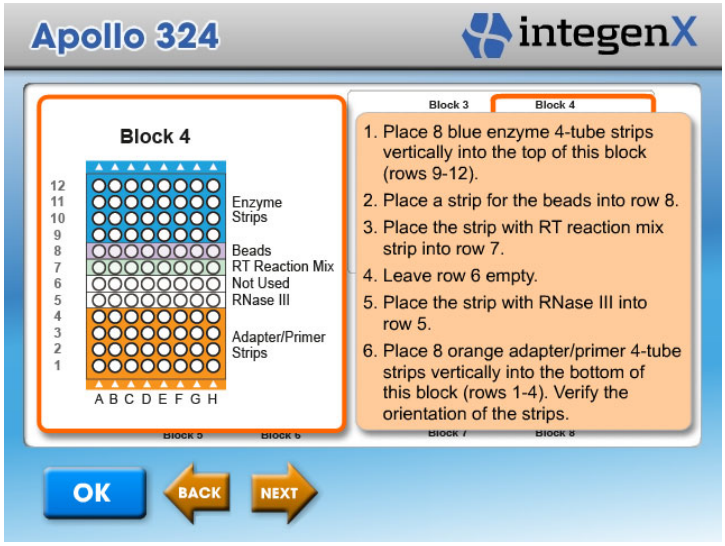
4. After the RUN button appears, place reagents in Block 4 as follows:
   a. Place each of the eight 4-tube enzyme/buffer strips (blue) vertically into rows 9–12 at the top of the block so that the arrows point up toward the rear of the work surface.
   b. Place the 8-tube strip with the 1X AMPure beads into row 8.

      NOTE: Do not centrifuge the bead strip before placing in row 8. Pipette out any air pockets in the tubes.

   c. Place the strip with RT reaction mix into row 7.
   d. Leave row 6 empty.
   e. Place the strip with RNase III enzyme mix into row 5.
   f. Place each of the eight 4-tube adapter/primer strips (orange) vertically into rows 1–4 at the bottom of the block so that the arrows point up toward the rear of the work surface

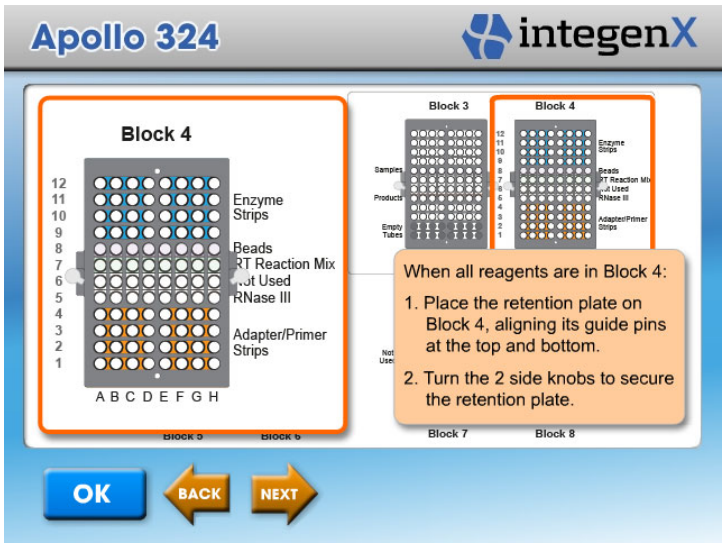
   NOTE: Verify that all tubes are oriented and seated correctly.

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.

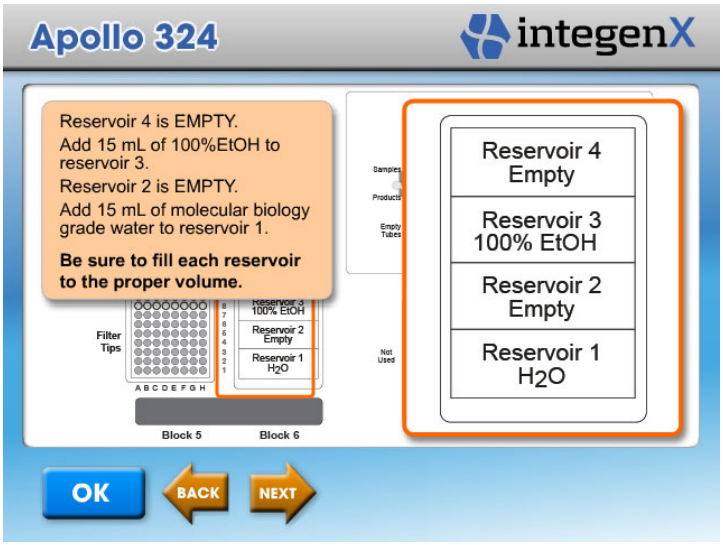


5. Place the metal retention plate over Block 4 to secure the tubes and keep them stable, aligning the guide pins at the top and bottom.

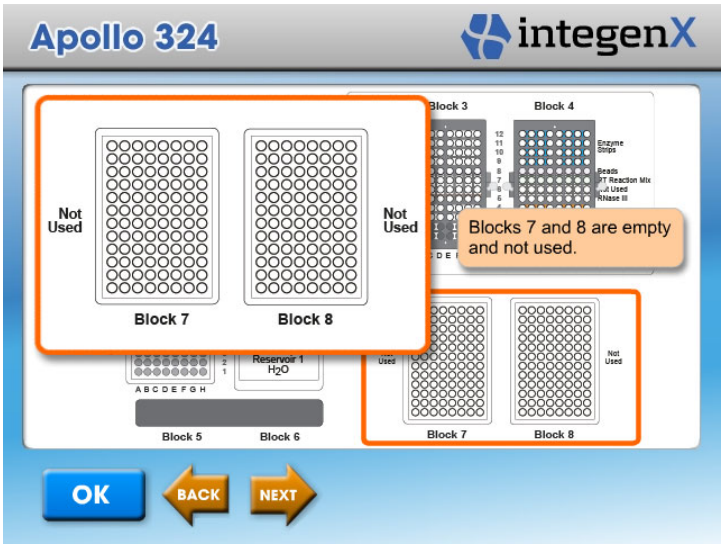
   Rotate the side knobs to lock the retention plate in place.



6. Fill the reagent reservoirs in Block 6 as follows:
   a. Dispense 15 mL of 100% EtOH into Reservoir 3.
   b. Dispense 15 mL of molecular biology grade water into Reservoir 1.
   c. Leave Reservoir 2 and Reservoir 4 empty; they are not used in this protocol



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



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

   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.”

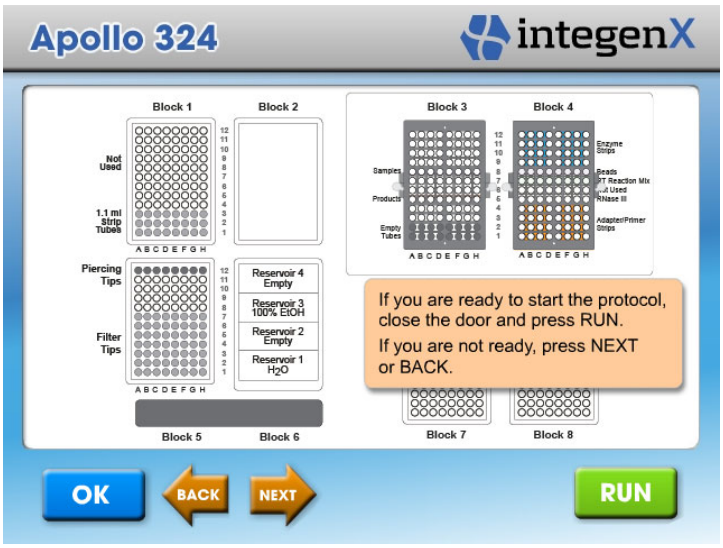
Running the Protocol

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.

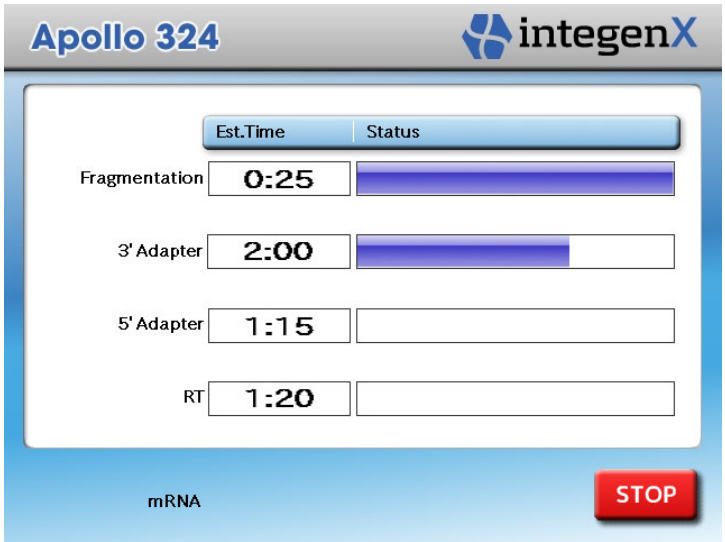
If you are ready to start the protocol, press **RUN**. If you are not ready, press **NEXT**, **BACK** or any block to review the setup and make changes to items on the work surface.



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



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.

Press **OK** to display the Start window. At this point, the door unlocks.

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.

Retrieving and Handling the Processed cDNA

1. Open the door and remove the 8-tube product strip (in row 5, Block 3).
2. Cap the processed samples immediately and store them on ice or at -20 °C.
3. Remove and discard used reagents and consumables.

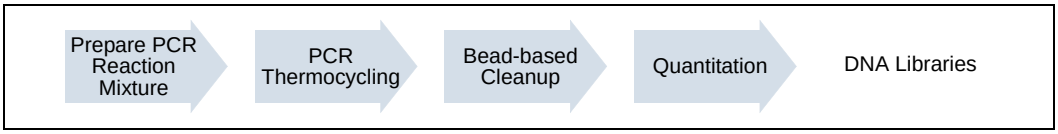
The cDNA samples are now ready for PCR processing.

Preparing DNA Libraries from cDNA

Use the following PCR method to prepare the DNA libraries from cDNA.

Workflow

1. Prepare the PCR reaction mixture
2. PCR thermocycling
3. Bead-based cleanup
4. Quantitation



Preparing the PCR Reaction Mixture

IMPORTANT: For a list of the Index 1–12 Primers to use, see Appendix A, "Index Primers and Sequences."

Thawing Reagents

1. Remove the following from -20 °C:
   - LongAmp Hot Start Taq 2X Master Mix
   - SR Primer
   - Index I Primer or Index 1–12 Primers
2. Thaw at room temperature.

Preparing the Reaction Mixture

Use the following table as a guide for PCR Master Mix volumes.

IntegenX routinely uses index primers (1–12), reconstituted to 10µM, added individually.

Table 3-1 Reagent volumes for the PCR Master Mix

Reagent	Volume per reaction (µL)	Per 8 (+1) Reaction Volume (µL)
cDNA sample from Apollo 324	20	192
LongAmp Hot Start Taq 2X Master Mix	25	240
SR Primer	2.5	24
Index 1–12 Primers (10µM)	2.5	Added individually
Total Volume	**50**	**–**

Thermocycling the Reaction Mixture

1. Thermocycle the PCR reactions as follows:
   - For starting samples of 50–250 ng of polyA mRNA use 12 PCR cycles.
   - For starting samples of 0.1–50 ng of polyA mRNA, use 15 PCR cycles.

Table 3-2 PCR thermocycling conditions

Reagent	Temperature °C	Time (seconds)	Number of cycles
Initial denaturation (hot start)	94	60	1
Denaturation	94	10	12 (50–250 ng mRNA) 15 (0.1–50 ng mRNA)
Annealing	60	30	
Extension	65	30	
Final extension	65	420	1
Hold	4	infinite	infinite

IMPORTANT: Freeze the samples if you are not processing them immediately.

Bead Cleanup of PCR Sample Products

1. Prepare 70% EtOH in a 15 mL Falcon tube, using the following volumes:

Table 3-3 70% EtOH preparations

Reagent	Volume (mL)
100% EtOH	3.5
Molecular biology grade water	1.5
Total	5

2. Calculate the volumes of 1X AMPure XP beads and 70% EtOH for post-PCR product cleanup, using the following table as a guide:

Table 3-4 Bead and EtOH volumes for bead cleanup

Reagent	Volume per reaction (µL)	Volume per 8 (+1) reactions (µL)
Post-PCR sample	50	450
1X AMPure XP beads	90	810
70% EtOH	200	1800

3. Remove the 1X AMPure beads from the refrigerator and allow to warm to room temperature.
4. Thoroughly resuspend the 1X AMPure beads and dispense 90 µL of the beads to each 50 µL of post-PCR product.
5. Mix by vortexing to ensure uniformity.
6. Incubate the mix of 1X AMPure XP beads and post-PCR product at room temperature for only five minutes to capture the main PCR product.

7. Place the tubes on a magnetic stand and pellet the beads for two minutes, until the solution is clear.
8. Carefully aspirate and discard the supernatant.
9. Wash the bead pellet as follows:
    a. Add 200 µL of freshly prepared 70% EtOH to the pellet.
    b. Mix briefly by vortexing.
    c. Centrifuge the tubes briefly.
    d. Place the tubes on a magnetic stand and recapture the beads for about two minutes.
    e. Carefully aspirate and discard the supernatant.
10. Repeat the wash step (step 9) once, for a total of two washes.
11. After the second wash, recapture the beads and remove as much of the remaining supernatant as possible from the tube.
12. Air dry the bead pellet for at least five minutes (and no longer than 10 minutes).

    The pellet is completely dry when cracks appear. **DO NOT ALLOW THE SAMPLE TO DRY ANY FURTHER**. At this point, the PCR product is ready for elution.
13. Elute the PCR product from the beads as follows:
    - For starting samples of 50–250 ng of polyA mRNA:
        a. Dispense **15** µL of molecular biology grade water into each sample tube.
        b. Cap the tube, vortex and centrifuge briefly.
    - For starting samples of 0.1–50 ng of polyA mRNA:
        a. Dispense **5** µL of molecular biology grade water into each sample tube.
        b. Cap the tube, vortex and centrifuge briefly.
14. Place the tubes on a magnetic stand and pellet the beads in each tube for five minutes until the liquid is clear. Visually confirm that all beads are pelleted.
15. Transfer the purified PCR product (supernatant) to a clean tube and discard the remaining bead pellet.

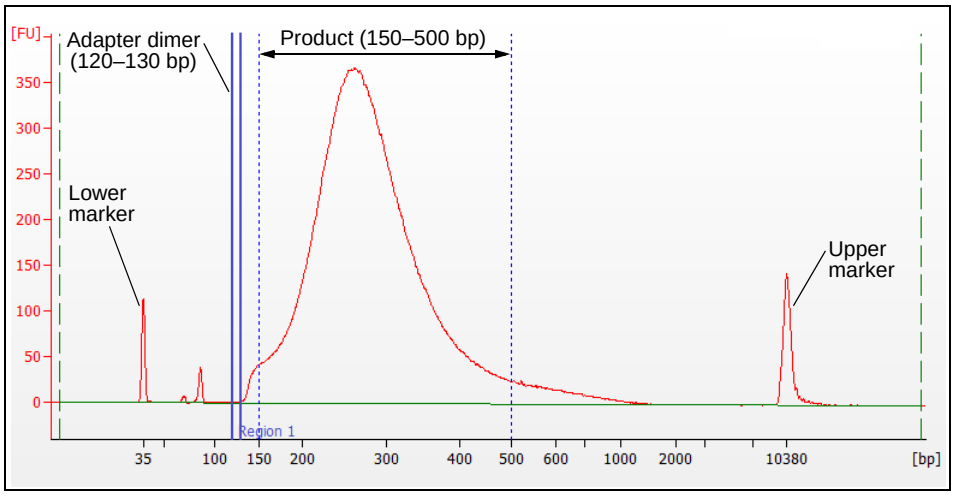
Quantitation of PCR product

Load the Agilent® High Sensitivity PCR chip according to manufacturer's specifications.

Table 3-5 Quality specifications for Bioanalyzer output

Specification	Bioanalyzer Output
Library size distribution	150–500 bp
Adapter dimer (120–130 bp)	<5%

The following post-PCR Bioanalyzer output is an example of a representative mRNA library preparation from 50 ng of human brain polyA mRNA.



After quantitation, follow the Illumina® recommendations for preparing sequencing reactions.

Reagent and Reaction Locations

Block 1
Axygen 1.1 mL Tubes

Row	Description
12	
11	
10	
9	
8	
7	
6	
5	
4	
3	1.1 mL tubes
2	1.1 mL tubes
1	1.1 mL tubes

Block 2
Empty, Not Used

Row	Description
12	
11	
10	
9	
8	
7	
6	
5	
4	
3	
2	
1	

Block 3
0.2 mL PCR Tubes (PCR 0)

Row	Material	Description
12		Not used
11		Not used
10		Not used
9		
8	Samples x8 (15 µL)	Sample tube, user input
7		
6		
5	Product collection (20 µL)	Empty strip tube
4		
3		
2	5’ adapter reaction	Empty strip tube
1	3’ adapter reaction	Empty strip tube

Block 4
0.2 mL PCR Tubes (PCR 1)

Row	Material	Description
12	3’ Ligation buffer	Enzyme strip (kit)
11	3’ Ligation enzyme	Enzyme strip (kit)
10	5’ Ligation buffer	Enzyme strip (kit)
9	5’ Ligation enzyme	Enzyme strip (kit)
8	1X AMPure XP Beads	User input 1X AMPure XP Beads
7	RT reaction mix (31 µL)	RT reaction mix, user input
6		
5	RNase III (4 µL)	RNase III and buffer, user input
4	5’ Adapter	Adapter/primer strip (kit)
3	SR RT Primer	Adapter/primer strip (kit)
2	3’ SR Adapter	Adapter/primer strip (kit)
1	Nuclease-free water	Adapter/primer strip (kit)

Block 5
Tip Rack

Row	Description
12	Piercing tip
11	
10	
9	
8	
7	Pipette tip
6	Pipette tip
5	Pipette tip
4	Pipette tip
3	Pipette tip
2	Pipette tip
1	Pipette tip

Block 6
Reservoirs

Material
Empty
100% EtOH (15 mL
Empty
H_2O (15 mL)

Block 7
Empty, Not Used

Block 8
Empty, Not Used

Appendix A

Index Primers and Sequences

In this appendix:

This appendix lists the Index primer sequences and the Index sequences for this PrepX mRNA protocol on the Apollo 324™ System.

For details on preparing the PCR reaction mixture, see "Preparing the PCR Reaction Mixture" in Chapter 3.

Index 1–12 Primer Sequences for Illumina

The following are the Index 1–12 primer sequences for PrepX™ mRNA library preparation for Illumina®.

Index 1 Primer

5´-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 2 Primer

5´-CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 3 Primer

5´-CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 4 Primer

5´-CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 5 Primer

5´-CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 6 Primer

5´-CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 7 Primer

5´-CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 8 Primer

5´-CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 9 Primer

5´-CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 10 Primer

5´-CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 11 Primer

5´-CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index 12 Primer

5´-CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´

Index Sequences

Index	Sequence
Index 1	ATCACG
Index 2	CGATGT
Index 3	TTAGGC
Index 4	TGACCA
Index 5	ACAGTG
Index 6	GCCAAT
Index 7	CAGATC
Index 8	ACTTGA
Index 9	GATCAG
Index 10	TAGCTT
Index 11	GGCTAC
Index 12	CTTGTA

Index

R

S

T

W

IntegenX Inc.
5720 Stoneridge Drive, Suite 300
Pleasanton, CA 94588

925.701.3400

support@integenX.com

www.IntegenX.com

Part Number P037497, Rev. A
9/2012