integenX

# Apollo 324™ System

## PrepX™ PGM 200 DNA Library Protocol

## User Guide

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Chapter 1

# Overview

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This *PrepX PGM 200 DNA Library Protocol User Guide* provides the basic information necessary to use your Apollo 324™ System to prepare genomic DNA libraries for analysis on the Ion PGM™ Sequencer from Ion Torrent™.

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

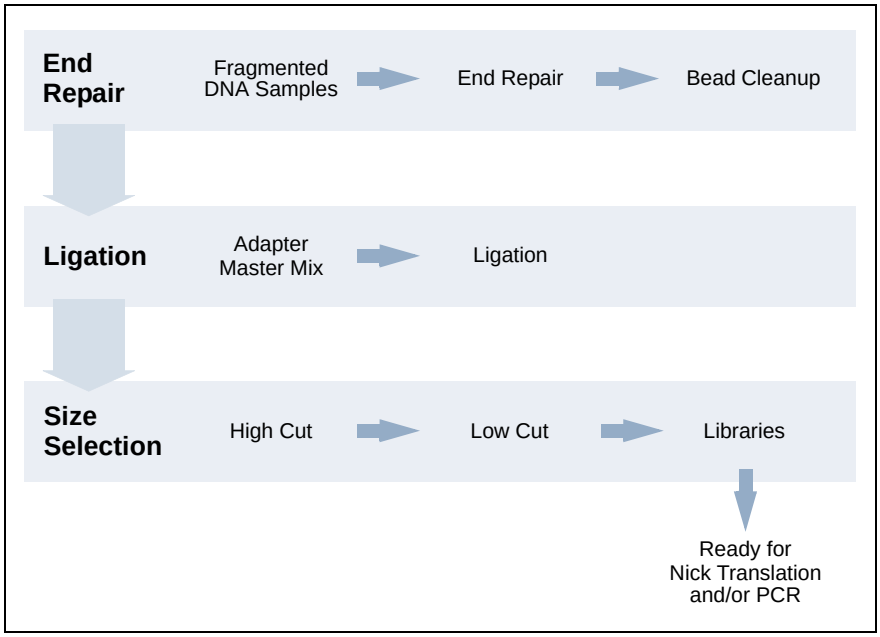
The PrepX™ PGM DNA Library reagent kit (Cat. No. 400029) provides researchers the flexibility to prepare 1 to 8 DNA libraries for sequencing on the Ion PGM Sequencer, using the 200 base pair Ion PGM 200 Sequencing Kit.

The library preparation process is completed in approximately 69 minutes, with the products ready for amplification. Up to 40 libraries can be prepared in one eight-hour work day.

# Workflow

1. On the touchscreen, press the DNA button, and then press the PrepX PGM 200 button to activate the Peltier heating/cooling units.
2. Place consumables, reagents, magnetic beads and samples in the racks on the work surface.
3. Start the protocol run, using the touchscreen interface.
4. The samples undergo the following processing:
   a. End repair
   b. Intermediary bead-based cleanup
   c. Adapter ligation
5. Final double bead-based cleanup and size selection is performed.
6. DNA libraries are now ready for nick translation and PCR enrichment. An optional XT gel cut can be performed when the run is complete. Use qPCR to quantify the libraries, as recommended by Ion Torrent. The Agilent Bioanalyzer or equivalent DNA analyzer is recommended to verify library size before and after PCR.

# Processing Schematic




**IMPORTANT:** A-tailing is not performed in this protocol. The A-tail enzyme and buffer are included in the enzyme strips, but are not used and their tubes are not pierced.

Chapter 2

# Preparing Samples and Reagents

In this chapter: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## Overview

This chapter describes how to prepare samples and reagents for the PrepX™ PGM 200 DNA 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 PGM DNA Library Kit (Cat. No. 400029). The kit provides sufficient reagents for three 8-sample runs (24 samples).

- PrepX Universal DNA Library Kit (Box Enz)
  - 24 Enzyme strips (4 tubes in each strip, yellow labeled seal)
  - Ligase enzyme (3 tubes, 10 µL each)
  - Ligase buffer (1 tube, 360 µL)
- PrepX PGM Primer and Adapter (Box Pri)
  - Primer and adapter (1 tube each)
- NaCl, 2.5M solution (1 bottle)
- Molecular biology grade water (1 bottle)

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

## Customer-Supplied Reagents and Consumables

The following reagents and consumables are recommended to run the Apollo 324 System.

| 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 |
| Eppendorf 96-well skirted microtiter plate, conical, 150 µL | 47744-122 | VWR |
| 50 mL Falcon test tube | 21008-940 | VWR |
| 15 mL Falcon test tube | 21008-929 | VWR |
| 1.5 mL DNA LoBind tube | 022431021 | Eppendorf |
| 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** | **Part Number** | **Supplier** |
| 100%, 200 proof EtOH, reagent grade | E7023 | Sigma-Aldrich |
| AMPure Beads (450 mL kit) | A63882 | Agencourt |
| Low TE buffer or 10mM Tris, pH 8.0 | various | various |
| Platinum PCR SuperMix High Fidelity | 12532-016 | Invitrogen |
| Ion Xpress™ Barcode Adapters 1-16 Kit (optional) | 4471250 | Life Technologies |

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

| Requirement | Recommended Initial Purchase | Usage per Run |
|---|---|---|
| 1.1 mL 12-tube strips (Axygen) | 1 box | 1 rack per 4 runs run |
| 0.2 mL 8-tube strips (Axygen) | 1 box | 25 runs - 200 samples |
| 0.2 mL 8-cap strips (Axygen) | 1 box | 25 runs - 200 samples |
| Eppendorf 96-well skirted microtiter plate, conical, 150 µL | 1 box | 25 runs - 200 samples |
| 50 mL Falcon test tube | n/a | varies |
| 15 mL Falcon test tube | n/a | varies |
| 1.5 mL DNA LoBind tube | n/a | varies |
| Piercing tips | 1 box of 1000 | 125 runs - 1000 samples |
| Dispensing filter tips | 1 box of 960 | 17 runs - 136 samples |
| Reservoirs | 1 box of 100 | 25 runs - 200 samples |
| Micropipette tips (20 µL, 200 µL and 1000 µL) | n/a | varies |
| Micropipettes (20 µL, 200 µL and 1000 µL) | n/a | varies |
| Safety glasses, gloves and lab coats as required in your lab | n/a | varies |

# Decontaminating the Lab Work Space and Instrument

1. Wipe the lab work surfaces with a 10% bleach solution.
2. Wipe the lab work surfaces with 70% EtOH and dry thoroughly.
3. Wipe the Apollo 324 instrument work surface with 70% EtOH and dry thoroughly.

# Preparing the Library Kit Reagents

To prepare the reagents:

1. Retrieve the PrepX PGM DNA Library Kit reagent boxes from the freezer. Only thaw the enzymes that you will be using for the run. Keep all reagents, including ligase enzyme, ligase buffer and 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.

# Using 1X AMPure XP Beads

**IMPORTANT:** This protocol uses 1X AMPure XP beads. Just before starting the protocol, you will aliquot 240 µL of the AMPure beads into each tube of an empty 8-tube strip. Do not place the beads on ice.

1. Vortex the 1X AMPure beads. Tap the tubes on benchtop to remove the beads from the tube lid.
2. Aliquot 240 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.

# Preparing 70% v/v EtOH

Combine 28 mL of 100% EtOH and 12 mL of molecular biology grade water in a sterile 50 mL Falcon tube and mix well. You will use 15 mL for each run.

# Preparing the Molecular Biology Grade Water Aliquot

Pipette 25 mL of molecular biology grade water into a sterile 50 mL Falcon tube. 15 mL will be consumed in the run.

# Preparing Samples

You must first shear the DNA sample.The size of the source for the DNA libraries should be approximately 200 to 250 bp.

## Shearing the DNA

We recommend using Covaris systems to shear the starting DNA sample. For instructions on shearing the DNA, refer to the Covaris documentation. For optimal results, use Low TE or 10mM Tris as the shearing buffer.

We recommend the following settings on the Covaris S2. These settings were tested for human and *E. coli* samples. Other genome types might require different settings.

| Setting | Value |
|---|---|
| Mode | Frequency Sweeping |
| Number of Cycles | 5 |
| Bath Temperature Limit | 15.0 °C |
| Total Process Time | 2:30 minutes |
| Treatment 1 | Duty Cycle: 10%<br>Intensity: 5<br>Cycles/Burst: 100<br>Time: 30 seconds |

## Adjusting the DNA Concentration

1. Run an Agilent high sensitivity chip analysis of the sheared DNA (diluted if required) by following the guidelines of the protocol supplied with the high sensitivity kit. The analysis will indicate the proper concentration for your samples.
2. Confirm that the shearing was successful in generating a DNA sample with a main peak between 200 and 250 bp.
3. Be sure that the concentration of the sheared DNA sample matches the protocol input concentration requirements in the range of 3.3 ng/µL to 330 ng/µL. An aliquot of 15 µL of this sample will provide 50 ng to 5 µg per lane.

   If possible, prepare the sheared DNA at the ideal recommended working concentration of 67 ng/µL.

You can now load the fragmented sample into the strip tubes.

## Preparing the Fragmented Sample

**NOTE:** Use color-coded Axygen strip tubes to ensure correct setup.

1. Pipette 15 µL of fragmented DNA sample, at a working range of 3.3 ng/µL to 330 ng/µL, 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.

2. To avoid contamination, temporarily cap the sample tubes with 8-tube 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.

# Preparing Adapter Master Mix (Non-barcoded)

Follow this procedure if you are using non-barcoded adapters.

1. Prepare the adapter master 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. Thaw, vortex, and briefly centrifuge the Ion Torrent adapter tubes.
   b. For each sample, pipette 2 µL of Ion Torrent adapter into 13 µL of molecular biology grade water to a final 15 µL total volume. Keep the mixture on ice. Pipette mix and briefly centrifuge.

**Table 2-1** Reagent volumes for adapter master mix

| Component | 1 Sample | 8 (+1) Samples |
|---|---|---|
| Molecular Biology Grade Water | 13 µL | 117 µL |
| Ion Torrent Adapter (PrepX PGM Ion Torrent Adapter) | 2 µL | 18 µL |
| **Total** | **15 µL** | **135 µL** |

2. Pipette 15 µL of the adapter master 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 Barcoded Adapters

If you are using barcoded adapters, you will prepare each adapter individually in the strip. You can choose any of the 16 Ion Xpress adapters in the kit. For product information, refer to the list of “Customer-Supplied Reagents and Consumables.”

1. Combine the adapters directly in each tube of an 8-tube strip.
   a. Thaw, vortex, and briefly centrifuge the Ion Torrent barcoded adapter tubes.
   b. For each sample, pipette 2 µL of Ion Torrent adapter P1 and 2 µL of Ion Torrent adapter [1-16] adapter into 11 µL of molecular biology grade water to a final 15 µL total volume. Keep the mixture on ice. Pipette mix and briefly centrifuge.

**Table 2-2** Reagent volumes for barcode adapters

| Component | 1 Sample |
|---|---|
| Molecular Biology Grade Water | 11 µL |
| Ion Xpress Barcode Adapter P1 | 2 µL |
| Ion Xpress Barcode Adapter [1-16] | 2 µL |
| **Total** | **15 µL** |

2. Temporarily cap the tubes and keep on ice or in the IntegenX 8-well cold block.

# Preparing Ligation Master Mix

This recipe prepares ligation master mix for one run of eight samples. See Table 2-3 for ligation master mix component volumes.

**IMPORTANT:** If you are processing fewer than eight samples in a run, follow the procedure in the section "Preparing Ligase Enzyme and Buffer for Fewer Than Eight Samples" to prevent pipetting loss of ligase enzyme and buffer.

## Preparing Ligation Master Mix for Eight Samples

1. Prepare the ligation master 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. Thaw, vortex, and briefly centrifuge the Adapter Ligase Buffer tubes.
   b. Thaw and briefly centrifuge the Adapter Ligase Enzyme tubes.
   c. Combine 108 µL of Adapter Ligase Buffer, 9 µL of Adapter Ligase Enzyme, and 18 µL of molecular biology grade water in a 1.5 mL tube. Keep the mixture on ice. Pipette mix and centrifuge briefly.

   **Table 2-3** Reagent volumes for ligation master mix

| Component | 1 Sample | 8 (+1) Samples |
|---|---|---|
| Adapter Ligase Buffer | 12 µL | 108 µL |
| Adapter Ligase Enzyme | 1 µL | 9 µL |
| Molecular Biology Grade Water | 2 µL | 18 µL |
| **Total** | **15 µL** | **135 µL** |

2. Pipette 15 µL of the ligation master mix into each tube of an 8-tube strip. Temporarily cap the tubes and keep on ice or in the IntegenX 8-well cold block.

## Preparing Ligase Enzyme and Buffer for Fewer Than Eight Samples

1. Thaw, vortex, and briefly centrifuge the Adapter Ligase Buffer tubes and Adapter Ligase Enzyme tubes.
2. Aliquot 12 µL of Adapter Ligase Buffer into each tube of an Axygen 8-tube strip.
3. Aliquot 1 µL of Adapter Ligase Enzyme into each tube of an Axygen 8-tube strip.
4. Cap the tubes and freeze for later use.
5. When you are ready to use the reagents:
   a. Thaw, vortex, and briefly centrifuge the Adapter Ligase Enzyme tubes and Adapter Ligase Buffer tubes.
   b. Pipette the 1 µL of Adapter Ligase Enzyme into the 12 µL of Adapter Ligase Buffer in each tube.
   c. Pipette 2 µL of molecular biology grade water to each tube.
   d. Temporarily cap the tubes and keep on ice or in the IntegenX 8-well cold block.

Chapter 3

# Setting Up and Running the Protocol

In this chapter:


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## 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.”

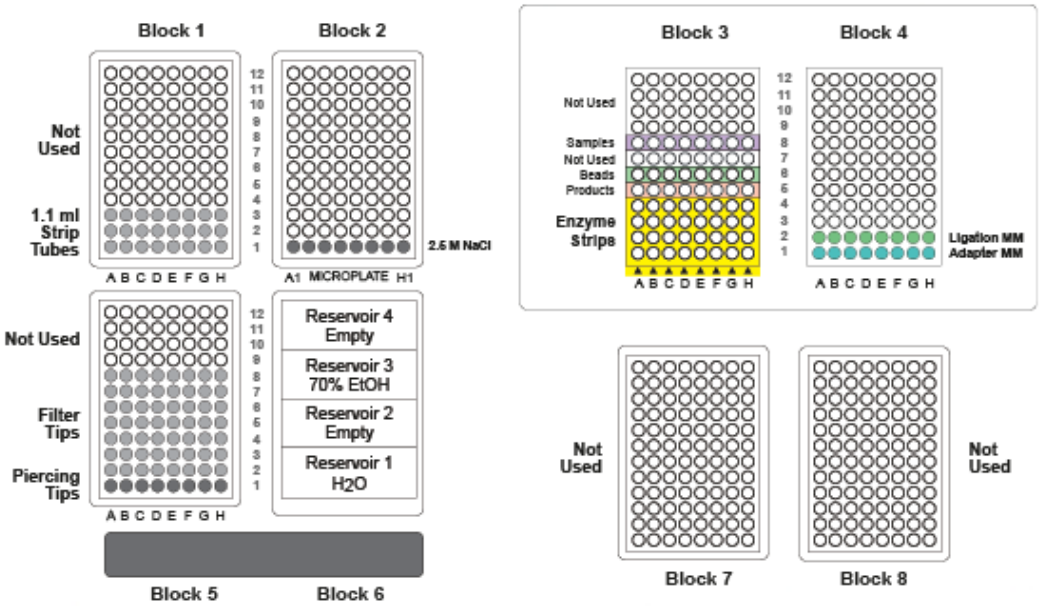

An accumulation of discarded pipette tips in the waste tip box can cause a run to fail. Be sure to open the waste tip box access door and check that the box has been emptied.

## 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. The setup windows provide guidance for setting up runs.




### 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 System 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.

   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.

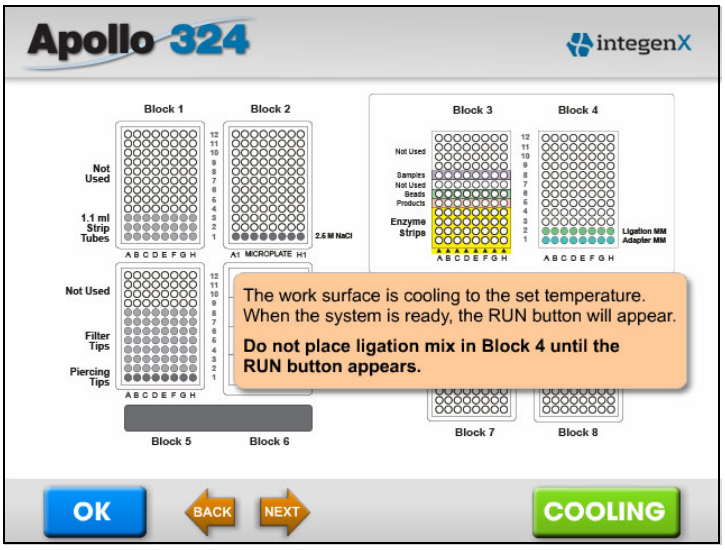
**IMPORTANT:** Pressing in the center of the window or pressing and holding any of the buttons for several seconds will initiate calibration of the touchscreen. If you accidentally do so, refer to the *Apollo 324 System User Guide* for details on calibrating the touchscreen.

2. To select the protocol, in the Start window, press **DNA**, then in the window showing the available DNA protocols, press **PrepX PGM 200**.

   The work surface setup window is displayed.

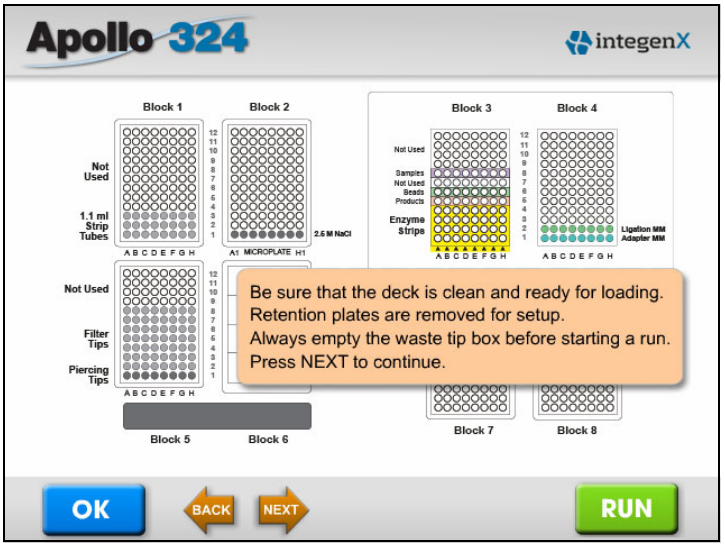
   On the instrument work surface, Block 3 remains at 18 °C, while Block 4 cools to 4 °C.

   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 Block 4 is cooling, 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."

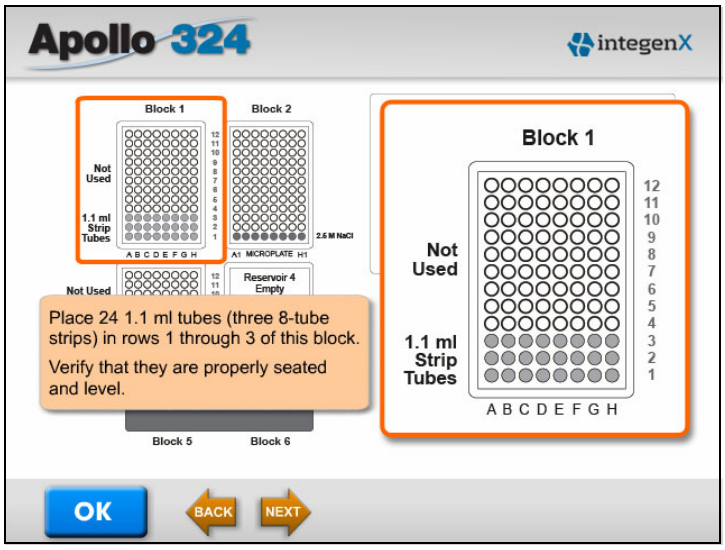
   For instructions on loading reagents and samples, see "Loading Samples and Reagents."

## Placing Consumables on the Work Surface

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

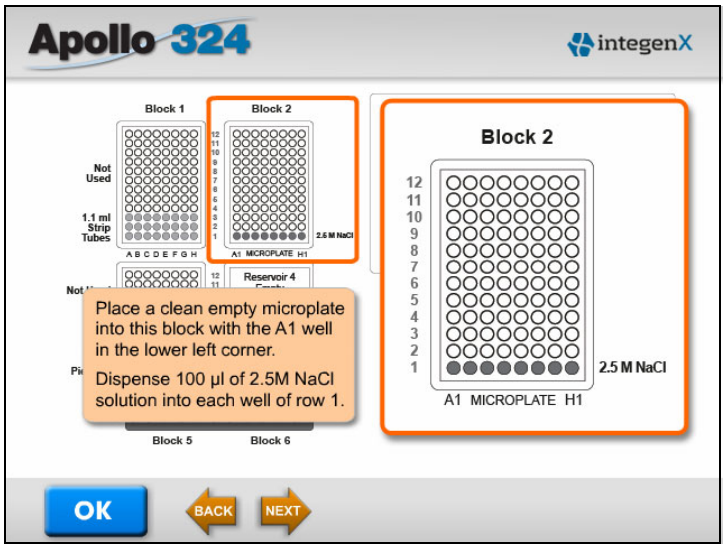
1. Place 24 1.1 mL tubes (three strips of 8-tube strips) in rows 1–3 of Block 1.

   Verify that they are properly seated and level. These tubes will be filled during the run.



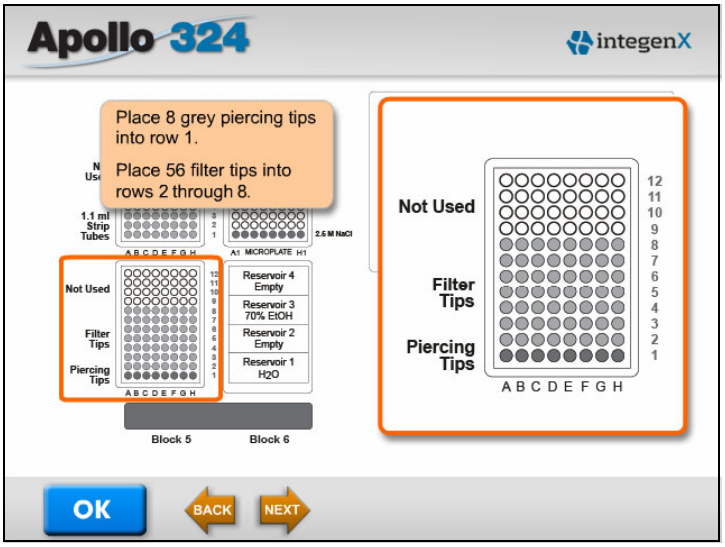

2. Place a clean, empty 96-well microtiter plate in Block 2, with the A1 well in the lower left corner.

   Dispense 100 µL of the 2.5M NaCl solution into each well of row 1.



3. Place the piercing tips and dispensing pipette tips into Block 5 as follows:
   a. Place eight grey piercing tips into row 1 of Block 5.
   b. Place 56 dispensing pipette tips into rows 2–8 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

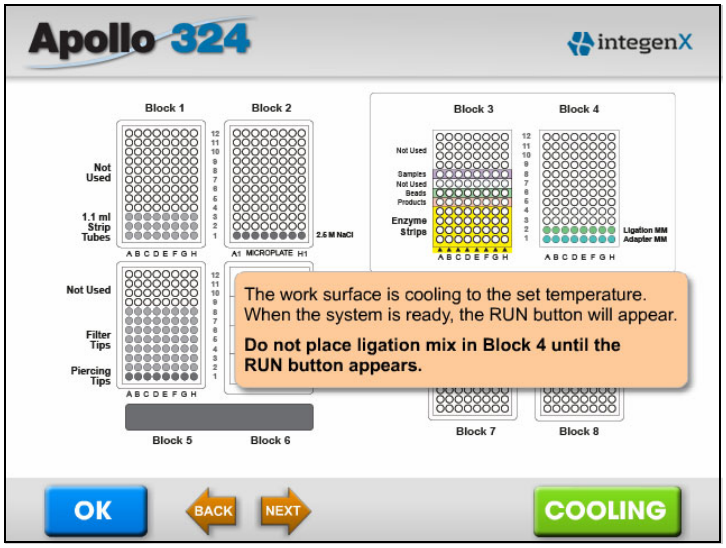
1. If you have not already done so, press **DNA** in the Start window, and then **PrepX PGM 200** in to initiate the protocol.

   The work surface setup window is displayed.

   On the instrument work surface, Block 3 remains at 18 °C, while Block 4 cools to 4 °C.

   While Block 4 is cooling, the **COOLING** button is displayed; when it reaches 4 °C, the **RUN** button appears.

   While Block 4 is cooling, you can place samples and reagents on the work surface.



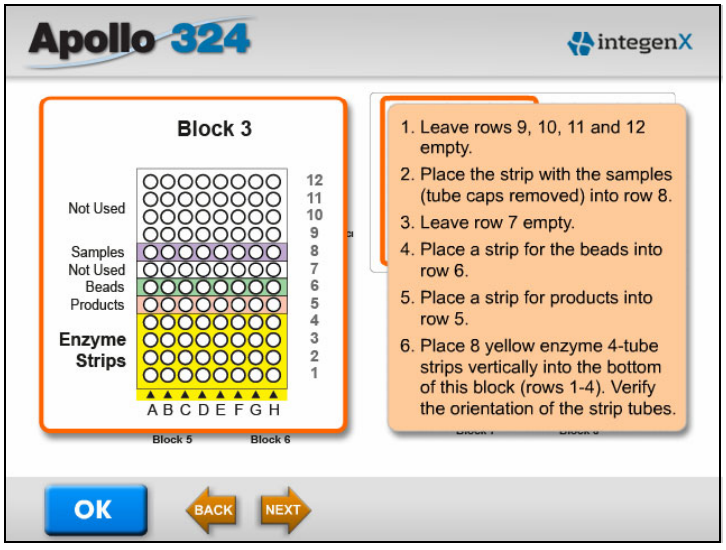

2. Load the prepared sample and reagents into Block 3 as follows:
   a. Place the 8-tube strip with the sample (tube caps removed) into row 8.
   b. Place the 8-tube strip with the 1X AMPure beads into row 6.

      **NOTE:** Do not centrifuge the bead strip before placing in row 6. Pipette out any air pockets in the tubes.
   c. Place an empty 8-tube strip for product in row 5.

      Be sure to label this strip with the date and other details about the run.
   d. Place each of the eight 4-tube enzyme strips vertically into rows 1–4 at the bottom of the block so that the arrows point toward the rear of the work surface.

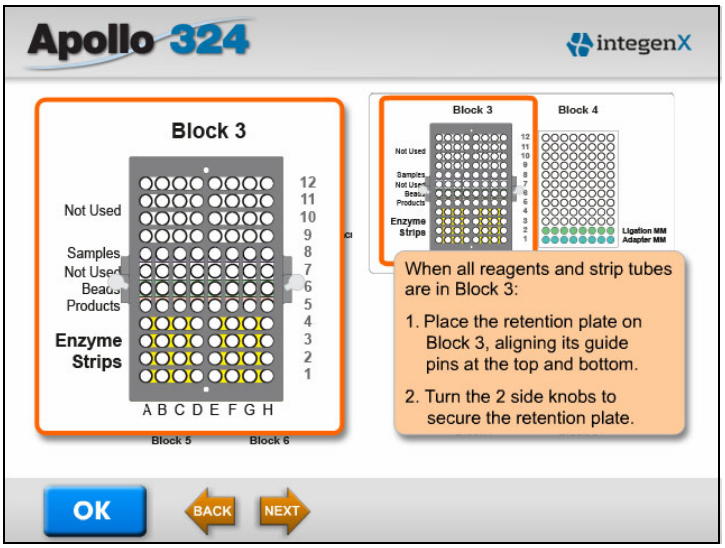
**NOTE:** Verify that all tubes are oriented and seated correctly.

**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.



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.

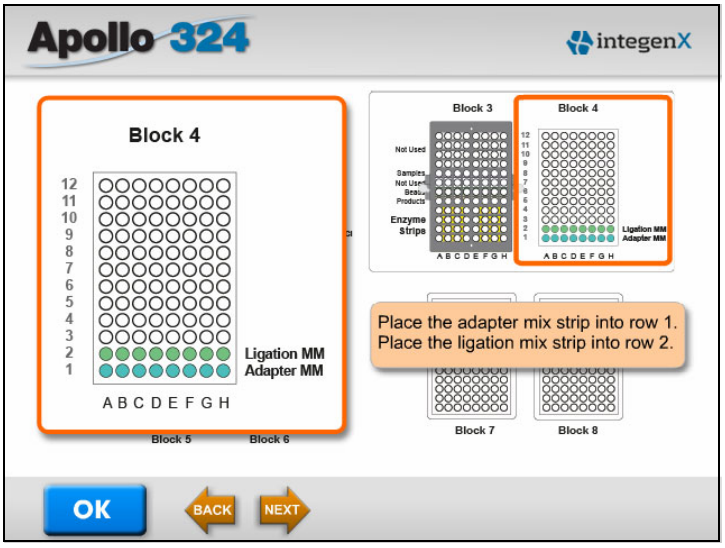
   Rotate the side knobs to lock the retention plate in place.




4. After the RUN button appears, in Block 4, place the strips with adapter master mix and ligation master mix, as follows:

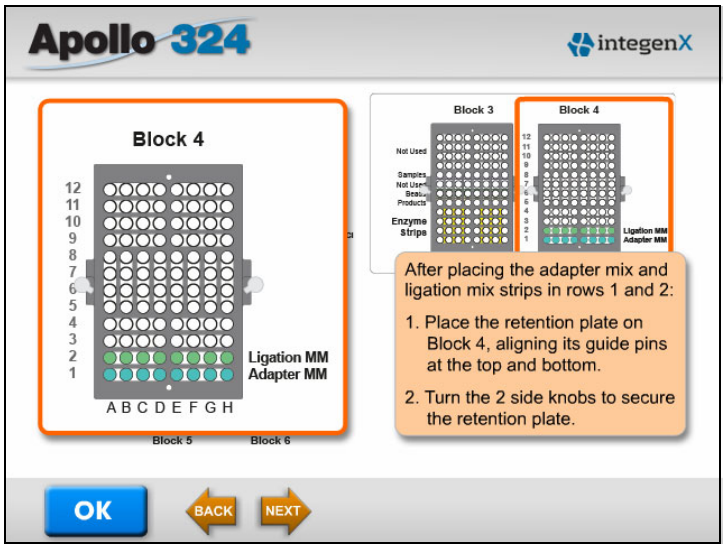
   a. Place the strip with the adapter master mix into row 1.

   b. Place the strip with the ligation master mix into row 2.

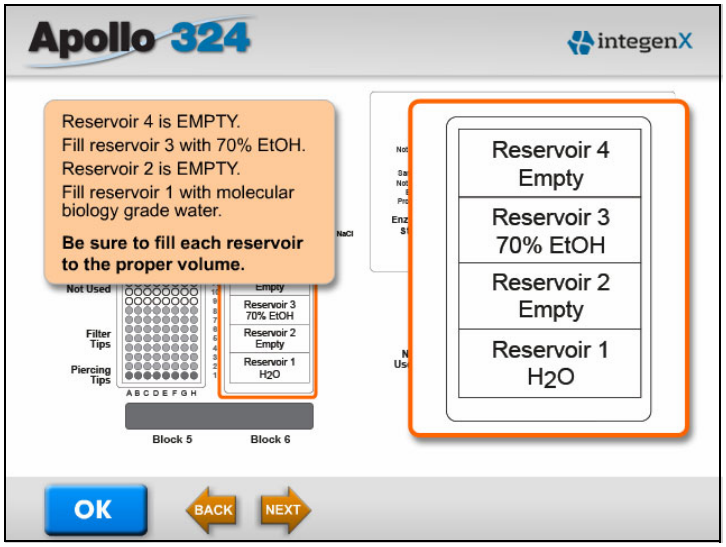


5. Place the metal retention plate over Block 4 to secure the tubes and keep them stable, aligning the guide pins at the top and bottom.

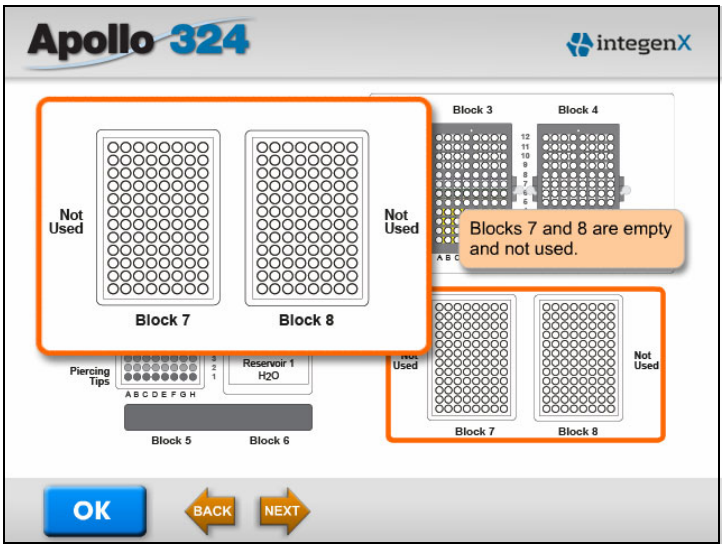
   Rotate the side knobs to lock the retention plate in place.




6. Fill the reagent reservoirs in Block 6 as follows:
   a. Dispense 15 mL of freshly prepared 70% v/v 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.”

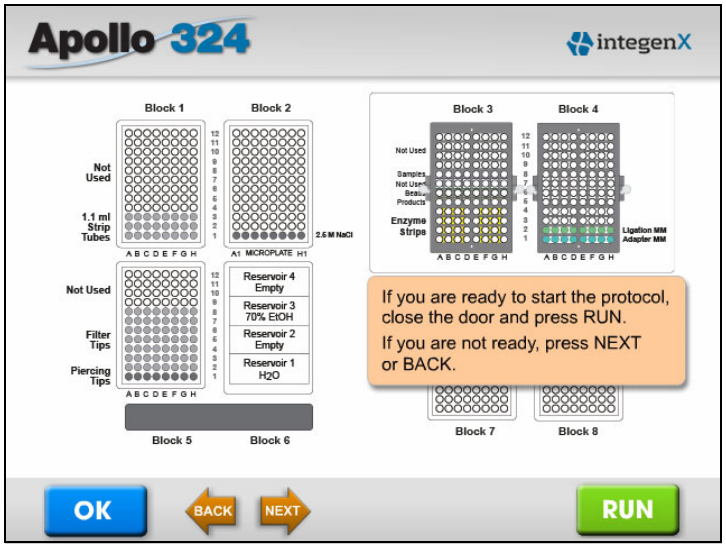
# Running the Protocol

## 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.

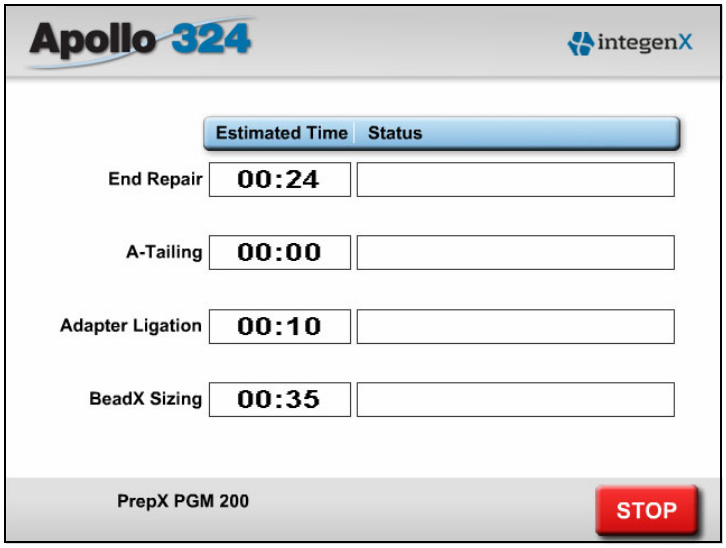
If you are ready to start the protocol, press **RUN**. If you are not ready, press **NEXT**, **BACK** or any block to review the setup and make changes to items on the work surface.




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.

DANGER

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 Libraries

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

The DNA samples are ready for library size verification and quantitation, and then nick-translation and amplification.

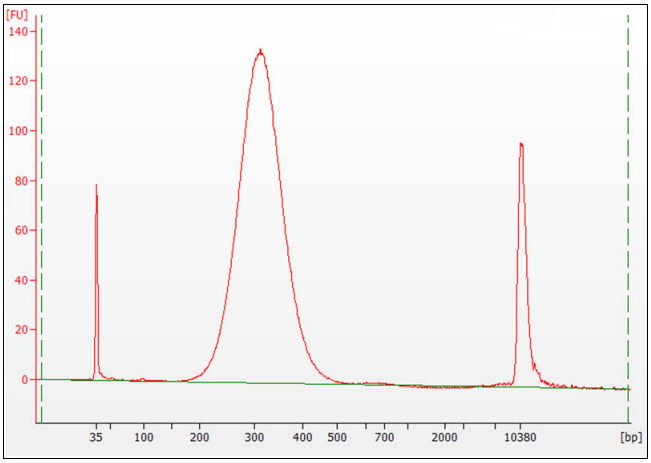
# Recommendations for PCR and Post-PCR Conditions

## Library Size Verification Using the Bioanalyzer

1. Run an Agilent high sensitivity chip of the PrepX PGM 200 DNA library by following the guidelines of the Agilent protocol supplied with the high sensitivity kit.

   **NOTE:** It may not be necessary to dilute the libraries generated from the PrepX PGM 200 DNA Library protocol.

2. Verify that the size distribution is similar to the following example, with a peak at ~300–330 bp.




   **NOTE:** You can also perform a sharper cut by using the LabChip® XT or similar instrument.

## Library Size Quantitation Using qPCR

To perform library size quantitation, use the Ion Torrent Ion Library Quantitation Kit.

**IMPORTANT:** We highly recommend that you follow the instructions in the *Ion Library Quantitation Kit User Guide*, Catalog Number 4468802.

## Nick Translation and Amplification of PrepX PGM 200 DNA Libraries

This section provides recommendations for your library enrichment PCR using the Ion Torrent™ reagents. This protocol consumes 10 µL of the 15 µL PrepX PGM 200 DNA library product.

1. Prepare the reaction mix and combine with the sample:

| Component | 1 Sample (µL) | 8 (+1) Samples (µL) |
|---|---|---|
| Platinum® PCR SuperMix High Fidelity | 200 | 1800 |
| Primer Mix | 10 | 90 |
| Water | 30 | 270 |
| **Subtotal** | **240** | **2160** |
| DNA (10 µL sample) | 10 | – |
| **Total/reaction** | **250** | – |

2. Gently mix and briefly spin the reaction mix.
3. Split the reaction into two by aliquotting 125 µL of the prepared reaction mix into each of two Axygen 8-tube strip PCR tubes.
4. Run the nick translation and PCR amplification program:

| Stage | Step | Temp (°C) | Time |
|---|---|---|---|
| Holding | Nick Translation | 72 | 20 min |
| | Denature | 95 | 5 min |
| Cycling: 7 cycles total | Denature | 95 | 15 sec |
| | Anneal | 58 | 15 sec |
| | Extend | 72 | 1 min |
| Holding | | 4 | infinite |

5. Pool the pairs of PCR samples into new 1.5 mL Eppendorf tubes.

**IMPORTANT:** Immediately proceed to bead purification to avoid degradation of your samples.

## Bead Purification of DNA Samples

Perform magnetic bead based capture to purify the DNA samples.

1. Add 375 µL AMPure beads (1.5 volumes of the sample volume) to the sample. Mix thoroughly with a pipette, ensuring that the beads and sample are mixed uniformly.
2. Centrifuge briefly, about two seconds, just enough to ensure that no volume is in the tube caps.
3. Incubate for only five minutes to capture the main PCR product.

4. Pellet the beads for about two minutes, using a magnet to capture the beads to one side of each tube. Carefully remove and discard the supernatant.
5. Wash the bead pellet as follows:
   a. Resuspend the pellet with 250 µL freshly prepared 70% EtOH, carefully bouncing it from side to side using a magnet until it is uniformly dispersed. **DO NOT VORTEX**.
   b. Re-pellet the beads for about two minutes, using a magnet to capture the beads to one side of each tube. Carefully remove and discard the supernatant.
   c. Carefully remove and discard the 70% EtOH.
6. Repeat the wash step (step 5) once, for a total of two washes.
7. After the second wash, pulse spin the tubes to re-pellet the beads. Use a magnet and a 10 µL pipette to remove as much of the remaining 70% EtOH as possible from the bottom of the tubes.
8. Allow the tubes to air dry against a magnet for approximately five minutes.

   The beads should lighten in color.
9. Resuspend the elute as follows:
   a. Dispense 10 µL of molecular biology grade water into each sample tube.
   b. Vortex for 10 seconds to resuspend the bead pellet. This might require scraping beads from the tube side walls and several aspirations with a pipette in order to ensure homogeneity.
10. Pulse spin the tubes and place against a magnet for one to two minutes, until the liquid is clear. Visually confirm that all beads are pelleted.
11. Transfer the supernatant to clean 8-tube strips and discard the remaining bead pellet.

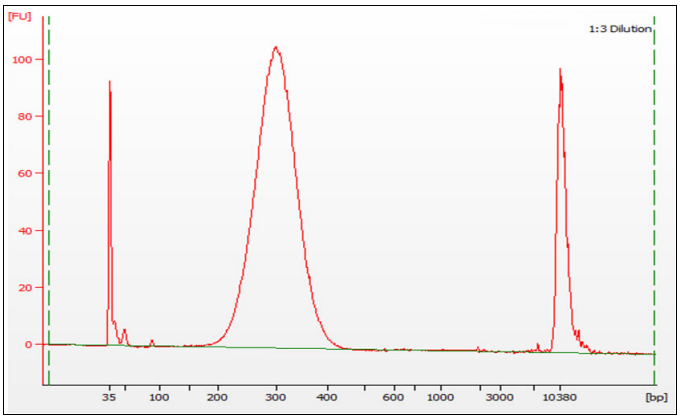
## Determining the Proper Concentration

1. Use a UV-Vis spectrophotometer to obtain a quick reading of the purified PCR samples. Perform two readings of each sample and record the concentration (ng/µL): 260/280 and 260/230.

   Always blank the spectrophotometer with molecular biology grade water prior to taking the reads.
2. Run a high sensitivity chip analysis of the PrepX PGM 200 DNA library PCR samples by following the guidelines of the protocol supplied with the high sensitivity kit. The analysis will indicate the proper concentration for your samples.

   It might be necessary to perform a dilution of the PCR samples to avoid overloading the Agilent high sensitivity chip. Typically, a 1:3 or 1:5 dilution is sufficient.

3. Verify that the enriched PCR sample has a size distribution and yield similar to the following example (peak at ~300 bp).




4. For barcoded libraries, prepare an equimolar pool of the barcoded libraries, typically at 10nM, and run an Agilent High Sensitivity Chip using 1 µL of the 10nM pool to confirm the concentration.

5. Samples are now ready to begin the Ion OneTouch™ and Ion Sequencing workflow. Follow the Ion OneTouch and Ion Sequencing protocol documentation for determining the template dilution factor for optimal sequencing results.

# 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**
**Microtiter Plate**

| Row | Description |
|---|---|
| 12 | |
| 11 | |
| 10 | |
| 9 | |
| 8 | |
| 7 | |
| 6 | |
| 5 | |
| 4 | |
| 3 | |
| 2 | |
| 1 | NaCl, 2.5M (100 µL) |

**Block 3**
**0.2 mL PCR Tubes (PCR 0)**

| Row | Material | Description |
|---|---|---|
| 12 | | Not used |
| 11 | | Not used |
| 10 | | Not used |
| 9 | | Not used |
| 8 | Samples x8 (15 µL) | Sample tube, user input |
| 7 | | Not used |
| 6 | 1X AMPure Beads | User input 1X beads |
| 5 | Product collection | Empty strip tube |
| 4 | ER Enzyme | Enzyme strip (kit) |
| 3 | ER Buffer | Enzyme strip (kit) |
| 2 | AT Enzyme (not used) | Enzyme strip (kit) |
| 1 | AT Buffer (not used) | Enzyme strip (kit) |

**Block 4**
**0.2 mL PCR Tubes (PCR 1)**

| Row | Description |
|---|---|
| 12 | |
| 11 | |
| 10 | |
| 9 | |
| 8 | |
| 7 | |
| 6 | |
| 5 | |
| 4 | |
| 3 | |
| 2 | Ligation Master Mix (15 µL) |
| 1 | Adapter Master Mix (15 µL) |

**Block 5**
**Tip Rack**

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

**Block 6**
**Reservoirs**

| Material |
|---|
| Empty |
| 70% EtOH (15 mL |
| Empty |
| $H_2O$ (15 mL) |

**Block 7**
**Empty, Not Used**

| |
|---|
| |
| |
| |
| |
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**Block 8**
**Empty, Not Used**

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