4-Primer Amplicon Tagging on Biomark HD

Contents

About This Protocol

This protocol provides a method to evaluate PCR amplification and PCR products using the LP 48.48 IFC (integrated fluidic circuit), AX controllers, and the Biomark™ HD system. For detailed instructions on instrument and software operation, see the IFC Controller AX User Guide (PN 68000157) and the Biomark HD Data Collection User Guide (PN 100-2451).

IMPORTANT Before using this protocol, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see Appendix E.

NOTE EvaGreen® and other DNA-binding dyes have been shown to inhibit PCR, particularly for amplicons with a percentage of GC greater than 60%.

Safety Alert Conventions

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
DANGER	Signal word that indicates more severe hazards.
WARNING	Signal word that indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the instrument user guide for the applicable pictograms and hazards pertaining to instrument usage.
DANGER	Signal word that indicates an imminent hazard that will result in severe injury or death if not avoided.
WARNING	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.
CAUTION	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
IMPORTANT	Signal word that indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Introduction

This protocol describes how to prepare libraries using the 4-primer amplicon tagging strategy using two AX controllers and a Biomark HD for thermal cycling.

The 4-primer amplicon tagging strategy uses a pair of target-specific primers and a pair of sample-specific barcode primers (a total of 4 primers) in each reaction chamber of an LP 48.48 IFC. This allows the incorporation of sample barcodes and sequencer adapters into the amplicons of a sequencer-ready library in a single amplification step.

As amplification occurs, library quantity can be detected in real time on Biomark HD using a DNA-binding dye, such as EvaGreen (as detailed in the protocol). This application is appropriate when amplifying one target per reaction chamber in the LP 48.48 IFC.

If real-time data collection is not required, master mix free of DNA-binding dye may be used.

If multiplexing is required, you can amplify up to 10 target-specific regions per reaction chamber. In this amplification strategy, sample-specific barcode primers are not recommended. An off-IFC amplification step is required to generate sequencer-ready libraries.

This protocol describes all workflows proposed here with modifications in the context of the 4-primer amplification protocol.

Materials

Kits and Reagents

Required Kit and Reagents from Fluidigm

IMPORTANT Store reagents as soon as they are received, according to manufacturer's storage recommendations.

Product Name	Source	Part Number	Storage
48.48 Access Array™ Loading Reagent Kit—10 IFCs	Fluidigm	100-1032-R	–20 °C
Access Array Barcode Library for Illumina Sequencers—384 (Single Direction)	Fluidigm	100-4876	–20 °C

* 1X Access Array Harvest Solution (Fluidigm, PN 100-1031) is not packaged for individual sale. It can be purchased in units of 10, under the name Access Array Harvest Pack, PN 100-3155, or as a component in the 48.48 Access Array Loading Reagent Kit, PN 100-1032.

Required Reagents from Other Suppliers

Product Name	Source	Part Number
FastStart™ High Fidelity PCR System, dNTPack	Sigma-Aldrich	04738292001
20X EvaGreen dye	Biotium	31000
ROX® reference dye, 50X	Thermo Scientific	12223-012
Agilent® DNA 1000 Kit	Agilent	5067-1504
Quant-iT™ PicoGreen® fluorescent assay	Thermo Fisher Scientific	P11496
PCR Certified Water	Teknova	W3330
DNA Suspension Buffer (10 mM TRIS, pH 8.0, 0.1 mM EDTA)	Teknova	T0221
Agencourt® AMPure® XP Reagent Beads	Beckman Coulter	A63880
100% ethanol	Major laboratory supplier (MLS)	—

Required Consumables

Required Consumables from Fluidigm

Product Name	Source	Part Number	Storage
LP 48.48 IFC	Fluidigm	101-1926	Room temperature
LP Control Line Fluid 48.48	Fluidigm	101-2345	Room temperature

Required Consumables from Other Suppliers

Product Name	Source	Part Number
Microcentrifuge tubes, 1.5 mL	MLS	101-1926
96- and 384-well microtiter plates	MLS	—
Adhesive seals for PCR plates	MLS	—
P2-P1000 pipette tips	Rainin	

Required Equipment

Required Equipment from Fluidigm

Product	Source	Part Number
Pre-PCR IFC Controller AX (for priming and loading)	Fluidigm	IFC-AX
Post-PCR IFC Controller AX (for harvesting)	Fluidigm	IFC-AX
Biomark HD system	Fluidigm	BMKHD-BMKHD

Required Equipment from Other Suppliers

Product	Source	Part Number
DynaMag™-2 Magnet	Thermo Fisher Scientific	123-21D
Qubit™ 4 Fluorometric Quantitation Instrument	Thermo Fisher Scientific	For example, Q33226
Agilent 2100 Bioanalyzer®	Agilent	G2939BA
Microcentrifuge	MLS	—
Vortex mixer	MLS	—
Plate centrifuge	MLS	—
96- and 384-well PCR thermal cycler	MLS	—
Single-channel P2-P1000 pipettes	Rainin	
8-channel P20 pipette	Rainin	

Sample Requirements

This protocol supports using template gDNA at 50 ng/µL.

Required Software

Fluidigm Real-Time PCR Analysis software v4.5.1 or later and Biomark Data Collection software v4.5.1 or later is required for this protocol. For software updates, go to fluidigm.com/software.

Before You Begin

IMPORTANT Read and understand the safety information in Appendix E.

For the overall success of the protocol, we recommend the following best practices.

- Protect assays from light and store in the refrigerator or freezer when not in use. Assays are light-sensitive.
- Ensure that lab consumables (tubes, tips, plates) used for the RNA handling steps are RNase-free.
- Thaw reagents at room temperature (15–30 °C) unless directed to thaw them on ice.
- Mix and centrifuge reagents as directed.
- Avoid creating bubbles when transferring reagents to the IFC.
    - Check the source plate or tube for bubbles before pipetting and pipette tips for air gaps while pipetting.
    - Pipet into the IFC inlets at an angle and always stop at the first stop on the pipette to avoid creating bubbles in the inlets. If a bubble is introduced, ensure that it floats to the top of the inlet.
    - If necessary, remove any bubbles from an IFC inlet by removing the contents of the inlet by pipette and then carefully re-pipetting the contents into the inlet.
    - To distribute the same mix into 24, 48 or 192 wells, we recommend first aliquoting reagents into an 8-well PCR tube strip to enable transfer into a 96-well PCR plate using an 8-channel pipette.

Prime the IFC

To prime the IFC, inject Control Line Fluid into each accumulator and then use the IFC Controller AX to run the prime script. For detailed instructions about injecting Control Line Fluid, see the Control Line Fluid Loading Procedure (PN 68000132). For more information about using the IFC Controller AX, see the IFC Controller AX User Guide (PN 68000157).

IMPORTANT Use the following best practices when injecting Control Line Fluid into the IFC.

- To ensure correct accumulator volume, use only syringes containing LP 48.48 Control Line Fluid.
- Be careful when removing the syringe cap to prevent drips.
- Avoid getting Control Line Fluid on the exterior of the IFC or in the inlets because this makes the IFC unusable. If this occurs, use a new IFC.
- Ensure that the 1X Access Array Harvest Solution is thawed completely to room temperature and mixed thoroughly prior to use.

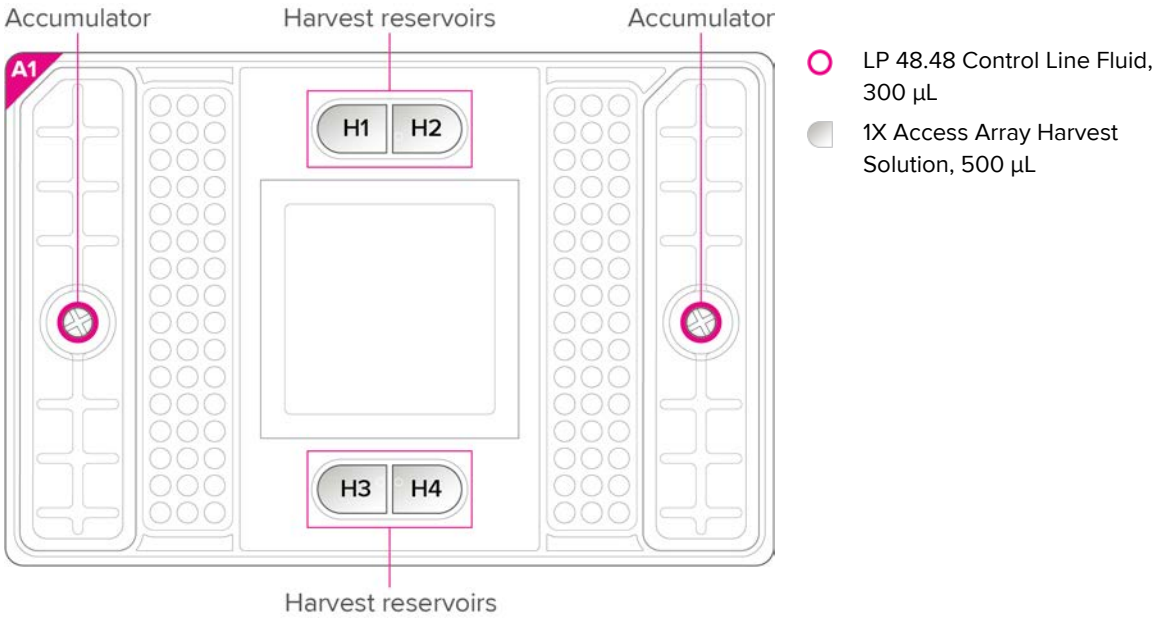


Figure 1. Priming map for the LP 48.48 IFC

1 Pull the protective tape down and away from the bottom of the IFC.

2 Inject LP 48.48 Control Line Fluid into each accumulator on the IFC (see Figure 1). Use the entire contents of one syringe in each accumulator. Holding the IFC at a 45-degree angle, insert the syringe tip into one side of the check valve, use the tip to gently press down to move the black O-ring to the side, and then release the fluid (see Figure 2).

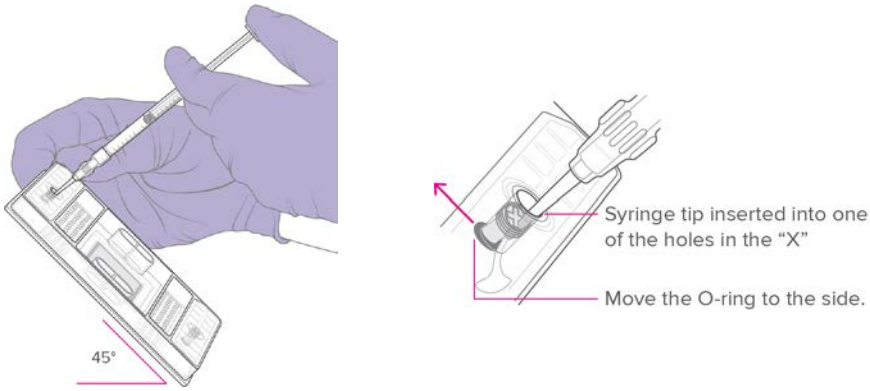


Figure 2. Injecting Control Line Fluid

3 Place the IFC on a flat surface and pipet 500 µL of 1X Access Array Harvest Solution into each of the harvest reservoirs H1, H2, and H3, and H4 (see Figure 1).

4 On the **pre-PCR** IFC Controller AX in the pre-PCR lab, press **EJECT** to move the tray out of the instrument.

5 Place the IFC on the tray, aligning the notched corner of the IFC to the A1 mark.

6 Press **LOAD CHIP** to register the barcode of the IFC and activate the script selection.

7 Select **PRIME (155x)** and **RUN SCRIPT** to prime the IFC. Priming the IFC takes approximately 10 min.

8 After the script is finished, press **EJECT** to remove the IFC.

Prepare the 20X Primer Mixes

Retrieve Reagents

Required Reagent	Preparation
20X Access Array Loading Reagent	Remove from –20 °C, thaw to room temperature.
DNA Suspension Buffer (10 mM TRIS, pH 8.0, 0.1 mM EDTA)	Remove from storage and keep at recommended temperature.
CS1/CS2 tagged target-specific primers FWD and RV	Remove from –20 °C, thaw to room temperature.

Prepare the Primer Mixes

IMPORTANT Warm up the 20X Access Array Loading Reagent to room temperature before use.

1 Briefly vortex and centrifuge the reagents before using.

2 Prepare the 20X primer solutions for 48 individual primer pairs/pools as shown in Table 1. These will be loaded into the primer inlets of the LP 48.48 IFC.

NOTE With multiplex amplification on the IFC, each well can contain up to 10 forward primers and 10 reverse primers.

Table 1. 20X Primer mixes

Component	Volume (µL)	Final Concentration
50 µM CS1-Tagged TS Forward Primer	2.0	1 µM
50 µM CS2-Tagged TS Reverse Primer	2.0	1 µM
20X Access Array Loading Reagent	5.0	1X
DNA Suspension Buffer (10 mM TRIS, pH 8.0, 0.1 mM EDTA)	91.0	—
Total	**100.0**	**—**

3 Vortex the 20X primer solutions for a minimum of 20 seconds and centrifuge for 30 seconds to bring down all components.

NOTE The final Tagged TS Forward and Reverse Primer concentrations are 1 µM in the 20X primer solution. The final TS Forward and Reverse Primer concentrations in the Access Array reaction chamber are 50 nM.

Prepare Sample Master Mix Solutions

All DNA samples and the barcode primers need to be added into the sample pre-mix individually prior to loading the sample mix solutions into the sample inlets of the LP 48.48 IFC.

Retrieve Reagents

Required Reagent	Preparation
20X Access Array Loading Reagent (Fluidigm)	If frozen, thaw to room temperature. For subsequent uses, keep at 4 °C.
FastStart High Fidelity PCR System, dNTPack (Roche pack; Sigma-Aldrich)	Remove from storage and keep at recommended temperature.
PCR Certified Water (Teknova)	
50X ROX Reference Dye (Thermo Scientific)	
20X EvaGreen Dye (Biotium)	

Prepare the Sample Pre-Mix

This protocol prepares enough sample pre-mix for 60 reactions. This is enough reagent to load one LP 48.48 IFC with 16 additional reactions to compensate for dead volume and pipetting error.

1 In a DNA-free hood, prepare the sample pre-mix as shown in Table 2:

Table 2. Sample pre-mix

Component	Volume per Reaction (µL)	Volume for 60 Reactions (µL)	Final Concentration
10X FastStart High Fidelity Reaction Buffer **with** 18 mM $MgCl_2$ (Roche)	0.50	30.0	1X
25 mM $MgCl_2$ (Roche)	0.54	32.4	2.7 mM
DMSO (Roche)	0.25	15.0	5%
10 mM PCR Grade Nucleotide Mix (Roche)	0.10	6.0	200 µM ea
5 U/µL FastStart High Fidelity Enzyme Blend (Roche)	0.05	3.0	0.05 U/µL
20X Access Array Loading Reagent (Fluidigm)	0.25	15.0	1X
50X ROX Reference Dye (Thermo Scientific)	0.05	3.0	0.5X
20X EvaGreen Dye (Biotium)	0.25	15.0	1X
PCR-Certified Water (TEKnova)	1.01	60.6	
Total	**3.0**	**180.0**	

NOTE

- Due to restrictions in volume, it is important to use the 10X FastStart High Fidelity Reaction Buffer with 18 mM MgCl2 when using ROX and EvaGreen dye in the reaction. The final MgCl2 concentration of reaction buffer and the extra MgCl2 combined will be 4.5 mM.
- If real-time data detection is not required, ROX and EvaGreen dyes may be replaced by 1.31 µL PCR Certified Water per sample.

2 Vortex the sample pre-mix for a minimum of 20 seconds and centrifuge for 30 seconds to pellet all components.

Prepare the Sample Mix

1 Prepare the sample mix solution as shown in Table 3. The table lists the components required to prepare 48 individual sample mix solutions in a 96-well plate for singleplex target-specific amplification and sample-specific barcoding in the IFC during thermal cycling.

NOTE If multiplex target-specific amplification is being performed in the IFC (≥2 primers per reaction chamber), it is not recommended to perform sample-specific

barcoding in the same reaction. In this case replace the Access Array Barcode Primer with 1 µL PCR Certified Water and perform barcoding reactions off of the IFC with each harvest pool.

Table 3. Sample mix solution

Component	Volume per Reaction (µL)	Final Concentration
Sample pre-mix	3.0	—
50 ng/µL Genomic DNA	1.0	10 ng/µL
Access Array Barcode Library for Illumina Sequencers—384 (Single Direction)	1.0	400 nM
Total	**5.0**	**—**

2 Vortex the sample mix solutions for a minimum of 20 seconds and centrifuge for 30 seconds to bring down all components.

IMPORTANT Vortex all components to ensure complete mixing.

NOTE The final Access Array Barcode Library Forward and Reverse Primers concentrations are 400 nM in the sample mix solutions.

Load the LP 48.48 IFC

IMPORTANT Use the following best practices to load assay and sample mixes on the IFC:

- Before pipetting reagents, maintain traceability by noting the orientation of the A1 corner, assay inlets, and sample inlets, as shown in Figure 1.
- When pipetting reagents onto the IFC, make sure to do so slowly to avoid creating bubbles. Air bubbles in the inlets may result in sample or amplicon dropout due to load failure.

1 Turn on the Biomark HD system (computer and instrument) and launch the Data Collection software to allow the instrument to start up and the camera to cool to the appropriate temperature.

2 Use an 8-channel pipette to load the 20X assay mixes and sample mixes into the LP 48.48 IFC, as shown in Figure 3.

   a Carefully pipet 4.0 µL of each 20X primer mix from the plate into the designated primer inlets on the IFC.

   a Carefully pipet 4.0 µL of each sample mix from the plate into the designated sample inlets of the IFC based on the predefined sample map.

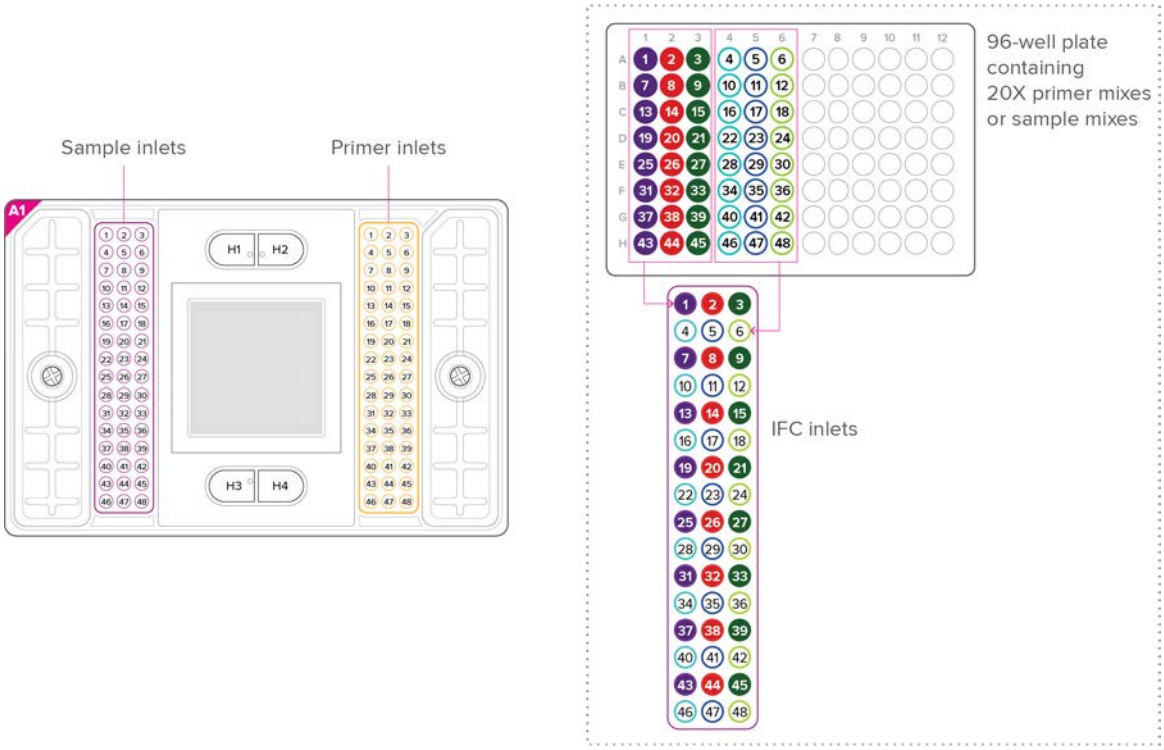


Figure 3. Primer and sample loading map

3 Insert the IFC into the **pre-PCR** IFC Controller AX in the pre-PCR lab.

4 Press **EJECT** to move the tray out of the instrument.

5 Place the IFC on the tray, aligning the notched corner of the IFC to the A1 mark.

6 Press **LOAD CHIP** to register the barcode of the IFC and activate the script selection.

7 Select **Load Mix (155x)** and **RUN SCRIPT** to load the IFC. Loading the IFC takes approximately 1 h and 30 min.

8 After the script is finished, press **EJECT** to remove the IFC from the controller and thermal cycle on Biomark HD.

IMPORTANT Start the IFC run within 60 minutes of completing the loading script.

Thermal Cycle on Biomark HD

Transfer the loaded IFC from the controller to Biomark HD. Before you begin, make sure that the Chip Definition file required to perform PCR on Biomark HD for the LP 48.48 IFC has been installed appropriately as described in Appendix C.

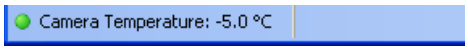
For more information about using Biomark HD, see the Biomark HD Data Collection User Guide (PN 100-2451).

1 Use clear tape to remove any dust particles or debris from the IFC surface.

2 If necessary, double-click the **Data Collection** icon on the desktop of the Biomark HD system computer to launch the software.

3 Click **Start a New Run**.

4 Confirm that the camera status indicator at the bottom of the window are green.



5 Place the IFC on the instrument tray, aligning the notched A1 corner on the IFC with the A1 on the tray, and click **Load**.

6 Complete the Chip Barcode and Type section:

a Verify IFC barcode and IFC type.

b Choose project settings (if applicable) and click **Next**.

7 Complete the Chip Run section by selecting either a new or pre-defined run.

8 Complete the Chip Run Name and Location section:

a Enter a run name or select the checkbox to use the IFC barcode as the run name.

b Select a file storage location for a new IFC run or browse to select a pre-defined run file and click Next.

9 Complete all sections of run setup in one of the following ways:

- If running the LP 48.48 IFC with detection chemistry:

  a Complete the following sections:

For...	Select
Application	Gene Expression
Assay	Single Probe

Probes	EvaGreen
Thermal protocol	AA 48x48 Standard v1.pcl

b Confirm that **Auto Exposure** is selected.

c Set to capture image at the end of slice.

d Click **Next**.

- If running the LP 48.48 IFC without detection chemistry:

a Complete the following sections:

For...	Select
Application	Thermal Only
Thermal protocol	AA NoI 48x48 Standard v1.pcl

NOTE For a description of the thermal protocol, see Appendix B.

b Confirm that **Auto Exposure** is selected.

c Click **Next**.

10 Verify the IFC run information and click **Start Run**.

The IFC run takes approximately around 2 hours when you are using the thermal protocol without image detection.

Harvest the LP 48.48 IFC

1 After the PCR has finished, move the LP 48.48 IFC from the Biomark HD instrument into the post-PCR lab for harvesting.

2 Remove the remaining fluids from the H1–H4 harvest reservoirs (see Figure 4).

3 Pipet 600 µL of fresh 1X Access Array Harvest Solution into the H1–H4 wells. (Do not use Hydration Reagent here.)

4 Pipet 2 µL of 1X Access Array Harvest Solution into each of the sample Inlets on the IFC (see Figure 4).

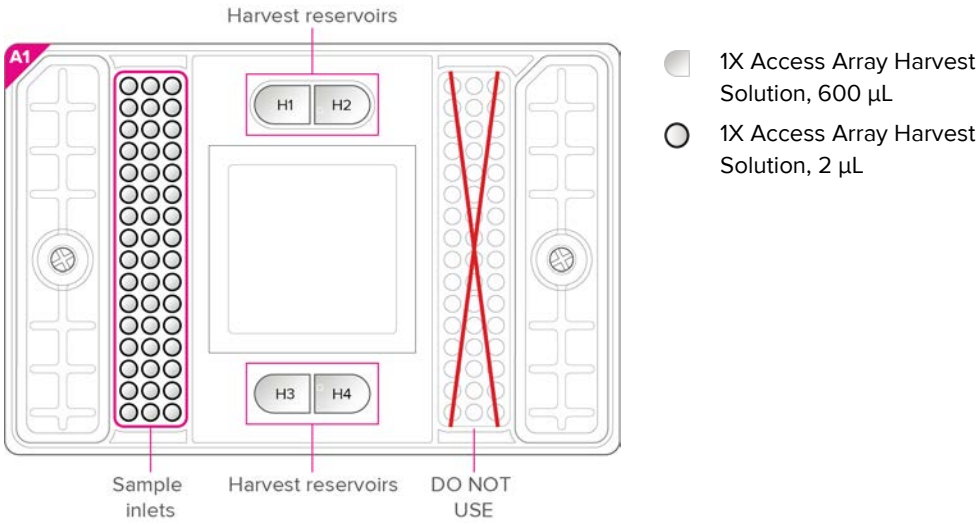


Figure 4. Pipetting map for harvesting the IFC

5 On the **post-PCR** IFC Controller AX, press **EJECT** to move the tray out of the instrument.

6 Place the IFC onto the tray, aligning the notched corner of the IFC to the A1 mark.

7 Press **LOAD CHIP** to register the barcode of the IFC and activate the script selection.

8 Select **Harvest (155x)** and **RUN SCRIPT**. This script takes approximately 1 hr and 30 min to complete.

9 After the script is finished, press **EJECT** to remove the IFC.

10 Label a 96-well plate with the LP 48.48 IFC barcode. Using an 8-channel pipette, carefully transfer 10 µL of harvested PCR products from each of the sample Inlets into columns 1–6 of the 96-well PCR plate.

11 Either store harvested material at –20 °C for future use or proceed with one of these options in the Access Array System for Illumina Sequencing Systems User Guide (PN 100-3770):

- If the multiplex amplification strategy was used on the IFC, first go to Chapter 5 (Attach Sequence Tags and Sample Barcodes), and then go to Chapter 7 (Post-PCR Amplicon Purification and Quantitation).
- If the singleplex amplification strategy was used to amplify target-specific regions already containing sample-specific barcodes and sequencer tags, then go directly to Chapter 7 (Post-PCR Amplicon Purification and Quantitation).

Analyze the Data

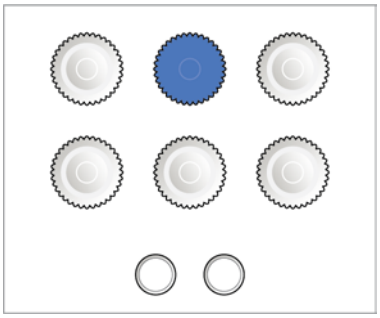
If real-time data collection was performed during library preparation, follow the steps for data analysis. For information about using the analysis software, see the Real-Time PCR Analysis User Guide (PN 68000088).

1 Launch the Fluidigm Biomark Real-Time PCR Analysis Software.

2. Click **Open a Chip Run** and then double-click the IFC run.bml file to open it in the software.
3. Click **Details Views** in the Chip Explorer pane.
4. Enter the following settings into the Analysis Settings pane:
   a. Set the Quality Threshold to **0.3**.
   b. Set the Baseline Correction to **Constant**.
   c. Set the C_t Threshold Method to **Auto (Global)**.
5. Click **Analyze** to process the data.
6. Save the data and export as a .csv file. The file can be opened in Microsoft Excel® or Word®.

Appendix A: Reagent Kit Components

48.48 Access Array Loading Reagent Kit (PN 100-1032-R)



- 2 tubes 20X Access Array Loading Reagent, 250 µL (PN 100-0883)
- 5 bottles 1X Access Array Harvest Solution, 10 mL (PN 100-1031)
- 1 bottle Hydration Reagent V.2, 5.0 mL (PN 100-7966)

Appendix B: Access Array 48x48 Thermal Cycler Protocol

The AA 48x48 Standard v1.pcl/AA NoI 48x48 Standard v1.pcl thermal cycling parameters are:

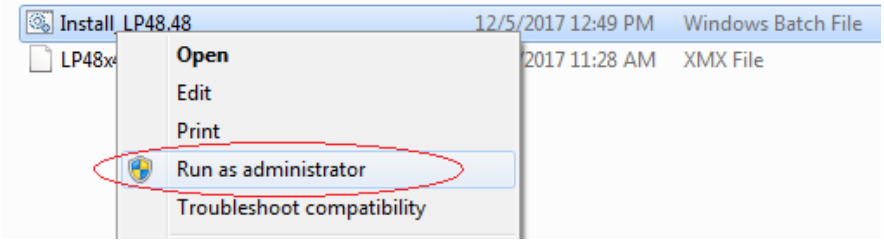
Temperature	Time	Cycles	Description	
50 °C	2 min	1	Thermal Mix	50C
70 °C	20 min			70C
95 °C	10 min	1	Hot Start	Hot Start
95 °C	15 sec	10	PCR Cycle	Denaturation
60 °C	30 sec			Annealing
72 °C	60 sec			Extension
95 °C	15 sec	2	C0t Cycle	Denaturation
80 °C	30 sec			C0t
60 °C	30 sec			Annealing
72 °C	60 sec			Extension
95 °C	15 sec	8	PCR Cycle	Denaturation
60 °C	30 sec			Annealing
72 °C	60 sec			Extension
95 °C	15 sec	2	C0t Cycle	Denaturation
80 °C	30 sec			C0t
60 °C	30 sec			Annealing
72 °C	60 sec			Extension
95 °C	15 sec	8	PCR Cycle	Denaturation
60 °C	30 sec			Annealing
72 °C	60 sec			Extension
95 °C	15 sec	5	C0t Cycle	Denaturation
80 °C	30 sec			C0t
60 °C	30 sec			Annealing
72 °C	60 sec			Extension
72 °C	3 min	1	Extension	Extension
10 °C		1	Cool Down	Cool Down

Appendix C: Installing Chip Definition File Required to Perform PCR on Biomark HD for the LP-48.48 IFC

To perform PCR on Biomark for the LP-48.48 IFC, you need to install the appropriate IFC (integrated fluidic circuit) definition file on the Biomark HD or Biomark.

Follow the instructions below to install the required file on the instruments in your lab. You will need a portable USB drive to complete installation.

1. Unzip the installation package: **101-7480.zip**
2. Insert a USB drive into the USB port of your computer.
3. Copy the unzipped folder to the USB drive.
4. Insert the USB drive into the USB port on Biomark HD or Biomark.
5. Open the folder 101-7480 in the USB drive.
6. Double-click the Install_LP48.48.bat file to install the files. If the installation fails, right-click the Install_LP48.48.bat file and select **Run as administrator.**



7. Click **Yes** to enable the batch file to modify the system files.
8. If Step 6 fails, contact your facility's IT department to enable you to modify the C:\Program Files\Fluidigm directory.
9. Remove the USB drive from Biomark HD or Biomark.
10. Close Data Collection software if it is running.
11. Start the Data Collection software.

Appendix D: Related Documents

Go to fluidigm.com/documents to download these related documents.

Title	Part Number
Quick Reference: Control Line Fluid Loading Procedure	68000132
IFC Controller AX User Guide	68000157
Biomark HD Data Collection User Guide	100-2451
Real-Time PCR Analysis User Guide	68000088

Appendix E: Safety

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Instrument Safety

For complete instrument safety information, including a full list of the symbols on the instrument, refer to the IFC Controller AX User Guide (PN 68000157) and Biomark HD Data Collection User Guide (PN 100-2451).

WARNING BIOHAZARD. If you are putting biohazardous material on the instrument, use appropriate personal protective equipment and adhere to Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines online at: cdc.gov/biosafety/publications/index.htm.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous

levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.

Disposal of Products

Used IFCs and reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

For technical support visit fluidigm.com/support.

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