4-Primer Amplicon Tagging with the LP 48.48 IFC

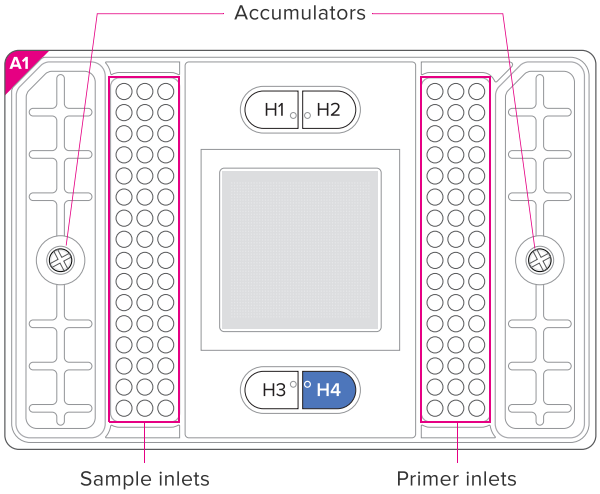
IMPORTANT

- Before using this document, read and understand the detailed instructions and safety guidelines in the Access Array™ System for Illumina® Sequencing Systems User Guide (PN 100-3770) or the Access Array System for the Ion Torrent™ PGM™ Sequencing System User Guide (PN 100-5024).
- This quick reference requires the use of updated scripts for FC1 (v1.6 or later) and IFC Controller AX (v2.8 or later) that can be downloaded from fluidigm.com/software.

Prime the IFC

IMPORTANT

- Use the LP 48.48 integrated fluidic circuit (IFC) or the Access Array 48.48 IFC within 24 hours of opening the package.
- Due to different accumulator volumes, only use 48.48 syringes with 300 µL of Control Line Fluid (LP 48.48: PN 100-2345; Access Array 48.48: PN 89000020).
- Control Line Fluid on IFC or in the inlets makes the IFC unusable.
- Load the IFC within 60 minutes of priming.
- Be certain that the 1X Access Array Harvest Solution and 1X Access Array Hydration Reagent v2 are thawed completely to room temperature and mixed thoroughly prior to use.



NOTE The location of the sample inlets is different from 48.48 Gene Expression and Genotyping IFCs.

1. Inject Control Line Fluid into each accumulator on the IFC.
2. Add 500 µL of 1X Access Array Harvest Solution (Fluidigm, PN 100-1031) into the H1–H3 wells of the IFC.
3. Add 500 µL of Access Array Hydration Reagent v2 (● blue cap, Fluidigm, PN 100-7966) into the H4 well of the IFC.

   NOTE 1X Access Array Harvest Solution (Fluidigm, PN 100-1031) can be substituted for Access Array Hydration Reagent v2 (blue cap, Fluidigm, PN 100-7966) in the H4 well.
4. Remove and discard the protective film from bottom of the IFC.
5. In the pre-PCR lab, load the IFC into the **pre-PCR** IFC Controller AX.
6. Prime the LP 48.48 IFC. Select **Prime (155x)** and **Run Script**.

NOTE For Access Array 48.48 IFCs, select **Prime (151x)** and **Run Script**. If your instrument does not contain the Prime (155x) script, download the latest version of the IFC Controller AX instrument software from fluidigm.com/software.

Prepare the 20X Primer Solution

IMPORTANT Be certain that the 20X Access Array Loading Reagent is thawed completely to room temperature and mixed thoroughly prior to use.

Component	Volume (µL)
50 µM CS1-tagged TS forward primer*	2.0
50 µM CS2-tagged TS reverse primer	2.0
20X Access Array Loading Reagent (PN 100-0883) ○	5.0
PCR-certified water	91.0
Total	**100.0**

*Fluidigm Access Array target-specific primers (PN ASY-AA) are provided as mixed forward and reverse primers at 50 µM per primer. If using Fluidigm assays, pipet 2 µL of mixed primers and adjust water to 93 µL.

Vortex the 20X primer solution for 20 seconds and centrifuge for 30 seconds.

NOTE The final concentration of each primer is 1 µM in the 20X primer solution and 50 nM in the PCR reaction.

Prepare the Sample Pre-Mix Solution

1. In a DNA-free hood, combine the following components from the FastStart™ High Fidelity PCR System, dNTPack (Sigma-Aldrich, PN 04738292001), with 20X Access Array Loading Reagent and PCR-certified water in a 1.5 mL sterile tube (sufficient volume for one IFC):

Component	Volume (µL)	Volume per IFC* (µL)
PCR-certified water	0.95	57.0
10X FastStart High Fidelity Reaction Buffer without $MgCl_2$	0.5	30.0
25 mM $MgCl_2$	0.9	54.0
DMSO	0.25	15.0
10 mM PCR Grade Nucleotide Mix	0.1	6.0
20X Access Array Loading Reagent (PN 100-0883) ○	0.25	15.0
5 U/µL FastStart High Fidelity Enzyme Blend	0.05	3.0
Total	**3.0**	**180.0**

*60 reactions for ease of pipetting into a single LP 48.48 IFC or Access Array 48.48 IFC

2. Vortex the sample pre-mix solution for 20 seconds and centrifuge for 30 seconds before preparing the sample mix solution.

Prepare the Sample Mix Solution

Primers containing a unique, sample-specific barcode must be added to each sample.

1 Combine the following components in a 96-well PCR plate to prepare 48 individual sample mix solutions where each well receives a uniquely barcoded primer pair:

Component	Volume (µL)
Sample pre-mix solution	3.0
50 ng/µL genomic DNA	1.0
2 µM Access Array Barcode Primers*	1.0
Total	**5.0**

*Select 48 unique barcodes from either library: Access Array Barcode Library for Illumina Sequencers–384 (Single direction; PN 100-4876) or Access Array Barcode Library for the Ion Torrent PGM™ Sequencer–96 (Bidirectional; PN 100-4911).

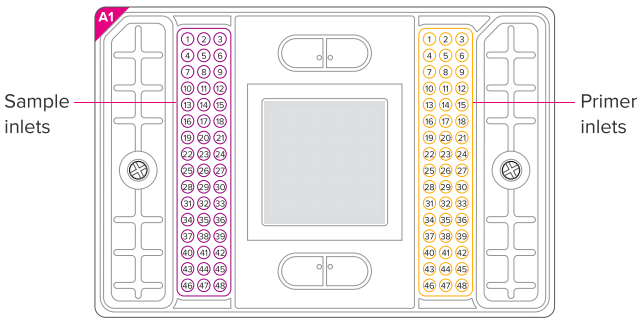
2 Vortex for 20 seconds and centrifuge for 30 seconds.

Load the IFC

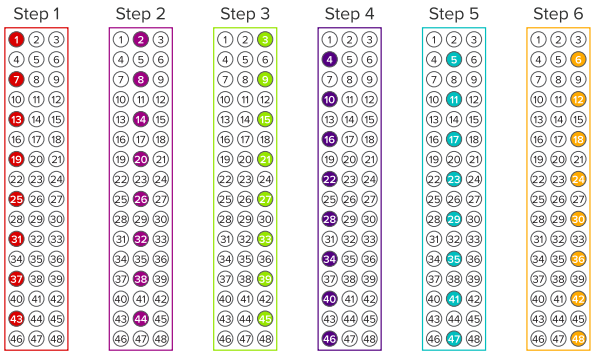
IMPORTANT

- Check IFC orientation before pipetting reagents into inlets.
- While pipetting, do not go past the first stop on the pipette. Doing so might introduce air bubbles into the inlets.

Loading Map for LP 48.48 IFC or Access Array 48.48 IFC



Pipetting Scheme



1 Pipet 4 µL of 20X primer solution into each primer inlet.

2 Pipet 4 µL of sample mix solution into each sample inlet.

IMPORTANT All inlets must be filled. If you have less than 48 primer sets or samples, prepare mixes as follows: Use water in place of tagged primers in the 20X primer solution and use water in place of the genomic DNA and barcode primers in the sample mix solution.

3 Load the LP 48.48 IFC. Select **Load Mix (155x)** and **Run Script**.

NOTE For Access Array 48.48 IFCs, select **Load Mix v7 (151x)** and **Run Script.** Load Mix v7 (151x) is a script update from Load Mix (151x). If your instrument does not contain the Load Mix v7 (151x) script, download the latest version of the IFC Controller AX instrument software from fluidigm.com/software.

Thermal-Cycle the IFC

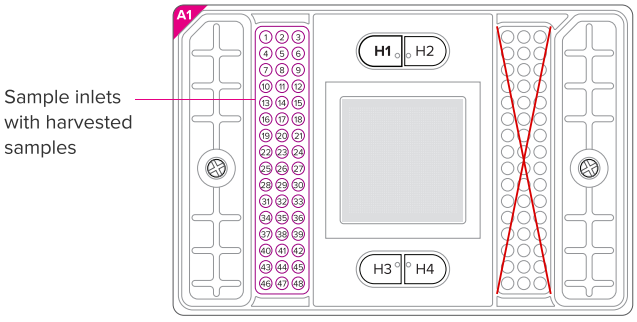
Choose the thermal-cycling instrument and run the protocol:

Instrument	Run Protocol
FC1™ cycler*	**AA 48x48 Standard v1**
Stand-alone thermal cycler (SATC)†	**AA48v1**

*See the FC1 Cycler Usage Quick Reference (100-1250).

†See the Stand-Alone Thermal Cycler Quick Reference (68000111).

Harvest the IFC



1 In the post-PCR lab, replace H1–H4 with 650 µL of 1X Access Array Harvest Solution. (Do not use the Hydration Reagent v2.)

2 Pipet 2 µL of 1X Access Array Harvest Solution into each of the sample inlets on the IFC.

3 Load the IFC into the **post-PCR** IFC Controller AX.

4 Harvest the LP 48.48 IFC. Select **Harvest (155x)** and **Run Script**.

NOTE

- For Access Array 48.48 IFCs, select **Harvest v7 (151x)** and **Run Script**. Harvest v7 (151x) is a script update from Harvest v5 (151x). If your instrument does not contain the Harvest v7 (151x) script, download the latest version of the IFC Controller AX instrument software from fluidigm.com/software.
- Label a 96-well plate using the barcode number on the IFC. Transfer the harvested samples into columns 1–6 of the pre-labeled 96-well PCR plate. See Pipetting Scheme.

5 Continue with your library preparation as described in the Post-PCR Amplicon Purification and Quantitation chapter of the Illumina (PN 100-3770) or Ion Torrent (PN 100-5024) user guide.

For technical support visit fluidigm.com/support.

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