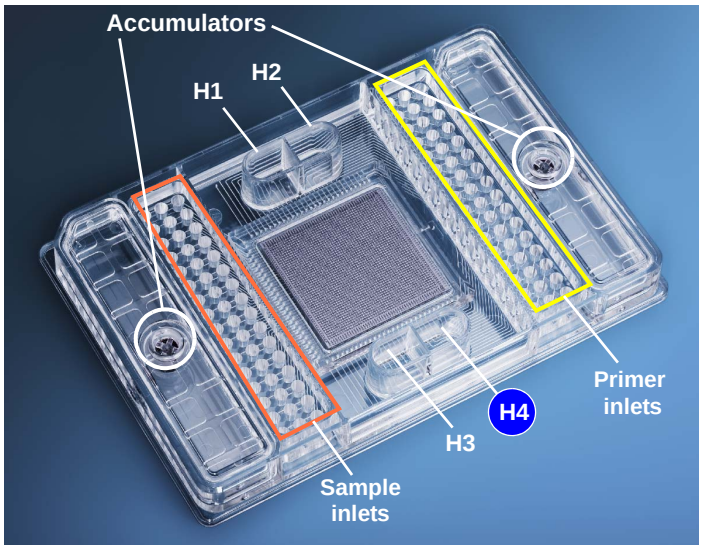
Access Array™ IFC 2-Primer Workflow

For more information, see the user guides for the Access Array System (PN 100-3770, PN 100-5024, PN 68000158).



Note: Please note the location of the sample inlets is different from 48.48 Gene Expression or Genotyping IFCs.

PRIMING THE ACCESS ARRAY

CAUTION!

- Use the Access Array IFC within 24 hours of opening the package.
- Due to different accumulator volumes, use only 48.48 syringes with 300 µL of control line fluid (PN 89000020).
- Control line fluid on the IFC or in the inlets makes the IFC unusable.
- Load the IFC within 60 minutes of priming.

IMPORTANT! Be certain that the reagents 1X Access Array Harvest Solution and 1X Access Array Hydration Reagent v2 are thawed completely to room temperature and mixed thoroughly prior to use.

1. Inject control line fluid into each accumulator on the IFC.
2. Add **500 µL** of 1X Access Array Harvest Solution (PN 100-1031) to the H1, H2, and H3 wells on the IFC.
3. Add **500 µL** of 1X Access Array Hydration Reagent v2 (blue cap, Fluidigm, PN 100-7966) to the H4 well on the IFC.

IMPORTANT! Hydration Reagent v2 ensures uniform harvest volumes.

4. Remove and discard the blue protective film from the bottom of the IFC.
5. Place the IFC into the *Pre-PCR* IFC Controller AX located in the Pre-PCR Lab and run script **Prime (151x)**.

PREPARING 20X PRIMER SOLUTIONS

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	8.0
50 µM CS2-Tagged TS Reverse Primer	8.0
20X Access Array Loading Reagent (PN 100-0883) ◯	5.0
PCR Certified Water	79.0
Total Volume	**100.0**

Vortex 20X Primer Solutions for 20 seconds and centrifuge for 30 seconds.

Note: The final concentration of each primer is 4 µM in the 20X Primer Solution and 200 nM in the PCR reaction.

PREPARING SAMPLES

1. **Prepare Pre-Sample Master Mix**: In a DNA-free hood, combine the components listed below from the FastStart High Fidelity PCR System, dNTP pack (Roche, 04 738 292 001), with 20X Access Array Loading Reagent and PCR certified water in a 1.5 mL sterile tube (sufficient volume for one IFC).

Component	Volume per reaction (µL)	Pre-Sample Master Mix for 48.48 (µL) (60 for ease of pipetting)
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
5 U/µL FastStart High Fidelity Enzyme Blend	0.05	3.0
20X Access Array Loading Reagent (PN 100-0883) ◯	0.25	15.0
PCR Certified Water	1.95	117.0
Total	**4.0**	**240.0**

2. Vortex Pre-Sample Master Mix for 20 seconds and centrifuge for 30 seconds before preparing Sample Mix.
3. **Prepare Samples:** For each sample, in an individual microtube or in a 96-well PCR plate, prepare the following solution:

Component	Volume per reaction (µL)
Pre-Sample Master Mix (from above step)	4.0
50 ng/µL Genomic DNA	1.0
Total	**5.0**

4 Vortex Samples for 20 seconds and centrifuge for 30 seconds after all samples are prepared.

LOADING SAMPLES

CAUTION!

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

1 Pipet **4 µL** of 20X Primer Solution into each of the Primer Inlets.

2 Pipet **4 µL** of Sample Mix into each of the Sample Inlets.

3 Place the IFC into the *Pre-PCR* IFC Controller AX in the Pre-PCR Lab and run script **Load Mix (151x)**.

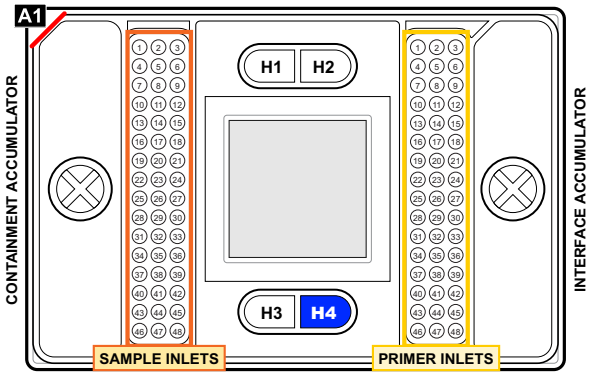


Figure 1 IFC Load Map

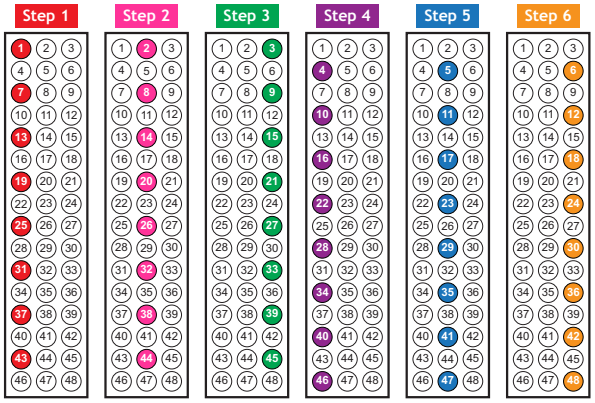


Figure 2 IFC Pipetting Scheme

THERMAL CYCLING THE IFC

Place the IFC onto one of the following and run PCR by selecting the protocol specified below.

- For the **Fluidigm FC1™ Cycler**, choose the **AA 48x48 Standard v1** protocol. See the *Fluidigm FC1 Cycler Usage Quick Reference,* PN 100-1250, for more information.
- For the **Fluidigm Stand-Alone Thermal Cycler**, choose the **AA48v1** protocol. See the *Fluidigm Stand-Alone Thermal Cycler Usage Quick Reference*, PN 68000111, for more information.
- For the **BioMark™ or BioMark™ HD System**, contact Technical Support.

HARVESTING THE IFC

1 After PCR has finished, move Access Array IFC into the Post-PCR Lab for harvesting.

2 Remove remaining fluids from the H1-H4 wells.

3 Pipet **600 µL** of 1X Access Array Harvest Solution into the H1-H4 wells. (Do not use the hydration reagent here.)

4 Pipet **2 µL** of 1X Access Array Harvest Solution into each of the Sample Inlets on the IFC.

5 Place the IFC into the *Post-PCR* IFC Controller AX located in the Post-PCR Lab and run script **Harvest v5 (151x).**

Note: **Harvest v5 (151x)** is a script update from **Harvest (151x)** and is available on the Fluidigm website. For assistance, contact Technical Support.

6 When the **Harvest v5 (151x)** script has finished, remove the IFC from the *Post-PCR* IFC Controller.

7 Label a 96-well plate using the barcode number on the Access Array IFC. Carefully transfer the harvested samples into columns 1-6 of the pre-labeled 96-well PCR plate. Follow the same pipetting pattern you used to transfer samples from the 96-well plate to the IFC. (For the pipetting scheme, see Figure 2.)

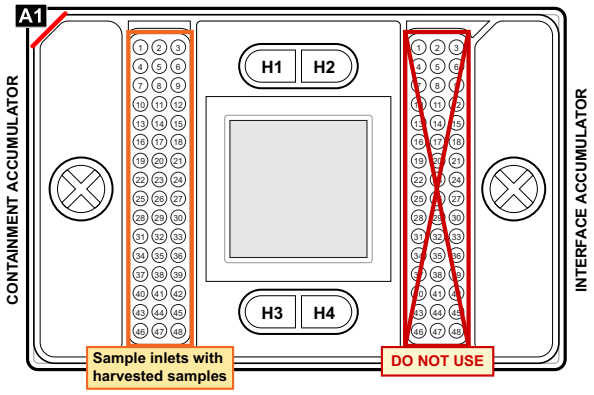


Figure 3 IFC Harvest Map

Technical Support

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