Fluidigm® FR48.48 Dynamic Array IFC Genotyping Workflow

1 Priming the FR48.48 Dynamic Array™ IFC

1 Perform system clean and clean interface plate the day before your chip run. Repeat cleaning step at the end of the day (see Cleaning QRC for more details).

CAUTION! DO NOT INJECT ANY LIQUID INTO THE WASTE INLET. IT MUST REMAIN EMPTY.

2 Inject fluid from the yellow-banded syringe into the **Interface** accumulator on the chip.

3 Pipette 300 µL of Pressure Fluid into the **P1**, **P2** and **P3** wells on the chip.

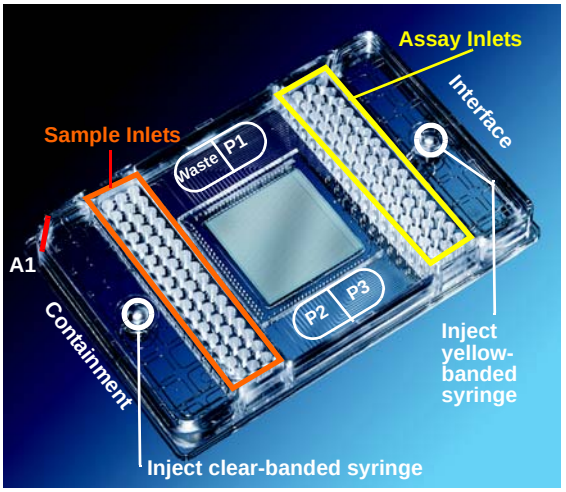
NOTE MAKE SURE THERE ARE NO BUBBLES IN THE WELLS.

4 Inject fluid from the clear-banded syringe into the **Containment** accumulator on the chip. (Only for first chip run)

5 Remove and discard the blue protective film from the bottom of the chip.

6 Place the chip into the *Load* IFC Controller WX, then run the **Prime (168x)** script to prime the chip. (Only for first chip run)

CAUTION! FOR THE FIRST USE, LOAD THE CHIP IN THE *LOAD* IFC CONTROLLER WX WITHIN 60 MINUTES OF PRIMING. FOR SUBSEQUENT USES OF THE CHIP, DO NOT PRIME THE CHIP (SKIP STEPS 4-6 ABOVE FOR THE SECOND THROUGH FIFTH USES.)



* Please note the location of the sample inlets is reversed from 48.48/96.96 Genotyping IFCs.

2 Preparing TaqMan® 10X Assays

1 In a DNA-free hood, prepare aliquots of 10X assays using volumes in table below (scale up appropriately for multiple runs).

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Volume per 50 µL Stock
SNP Genotyping Assay Mix (80x*) (Applied Biosystems)	0.5	0.625	6.25
2X Assay Loading Reagent (Fluidigm, PN 85000736)	2.0	2.5	25.0
ROX (50x) (Invitrogen, PN 12223-012)	0.2	0.25	2.5
DNA-free water	1.3	1.625	16.25
Total Volume	**4.0**	**5.0**	**50.0**

***** If you are using 40x SNP assay, double the volume of SNP assay mix and reduce the DNA-free water. For other starting concentrations of the SNP assay mix, call Fluidigm Technical Support.

3 Preparing Sample Pre-Mix and Samples

1 Combine the components in the table below to make the Sample Pre-Mix, and the final Sample Mixture.

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Sample Pre-Mix for FR48.48 (µL) (60 for ease of pipetting)
SAMPLE PRE-MIX			
GTXpress Master Mix (2x) (Applied BioSystems, PN 4401892)	2.5	3.0	180.0
20X Fast GT Sample Loading Reagent (PN 100-3065)	0.25	0.3	18.0
DNA-free water	0.25	0.3	18.0
genomic DNA (added individually to the Sample Pre-Mix)	2.0	2.4	
Total Volume	**5.0**	**6.0**	**210.0**

2 In a DNA-free hood, combine the three Sample Pre-Mix components in a 1.5 mL sterile tube—enough volume to fill an entire chip. Aliquot 3.6 µL of this Sample Pre-Mix for each sample.

3 Remove the aliquots from the DNA-free hood and add 2.4 µL of genomic DNA to each, making a total volume of 6 µL in each aliquot.

4 Loading the Chip

IMPORTANT!
- MAKE SURE YOU THOROUGHLY MIX ALL ASSAY SOLUTIONS AND ALL SAMPLES *BEFORE* PIPETTING INTO THE CHIP INLETS.
- CHECK THAT P1, P2, P3 WELLS CONTAIN 300 µL OF PRESSURE FLUID.
- CHECK THAT THE INTERFACE ACCUMULATOR CONTAINS THE FULL VOLUME FROM THE YELLOW-BANDED SYRINGE.
- CHECK THAT THE WASTE WELL IS EMPTY.
- MAKE SURE THE INTERFACE PLATE ON THE *LOAD* IFC CONTROLLER WX IS CLEAN AND DUST-FREE BEFORE LOADING THE IFC. YOU CAN USE SCOTCH TAPE TO REMOVE DUST AND DEBRIS.
- FOR UNUSED SAMPLE INLETS, USE 3.6 µL OF SAMPLE PRE-MIX AND 2.4 µL OF WATER PER INLET. FOR UNUSED ASSAY INLETS, USE 2.5 µL ASSAY LOADING REAGENT, 0.25 µL ROX, AND 2.25 µL WATER PER INLET.

4 Loading the Chip Continued ...

CAUTION! WHILE PIPETTING, DO NOT GO PAST THE FIRST STOP ON THE PIPETTE. DOING SO MAY INTRODUCE AIR BUBBLES INTO INLETS.

1. When the *Prime (168x)* script has finished, remove the primed chip from the *Load* IFC controller WX and pipette 4 µL of each assay and 5 µL of each sample into the respective inlets on the chip.
2. Tilt chip slowly to confirm volume in Interface Accumulator covers the inlet hole. (See loading map at bottom of the page for inlet hole site.)
3. Return the chip to the *Load* IFC controller WX.
4. Using the IFC Controller WX software, run the **Load Mix (168x)** script to load the samples and assays into the chip.
5. When the *Load Mix (168x)* script has finished, remove loaded chip from the *Load* IFC controller WX.
6. Remove any dust particles or debris from the chip surface.
7. Cover the inlets with tape prior to thermal cycling.

You are now ready for your chip run.

CAUTION! START THE CHIP RUN ON THE FC1 CYCLER WITHIN 4 HOURS OF LOADING THE IFC.

5 Using the FC1™ Cycler

1. Press the **Start** button.
2. Open the lid.
3. Place the chip onto the thermal cycling block (chuck) on top of the instrument by aligning the notched corner of the IFC chip to the **A1** mark.
4. Close the lid.
5. Press **Continue** to display available thermal protocols.
6. Choose **GT 48X48 Fast v1.pcl** protocol from the protocol selection window.
7. Press **Run**.

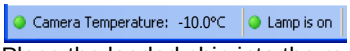
CAUTION! NEVER PRESS DOWN ON THE CHIP WHEN IT IS ON THE FC1 CYCLER.

NOTE: A STATUS SCREEN APPEARS WITH A TIME ESTIMATE FOR COMPLETION.

8. Once the protocol is finished, a confirmation screen appears. (During an active protocol, **Abort** will cancel the chip run.)

6 Using the EP1™ Reader Data Collection Software

1. Double-click the Data Collection Software icon on the desktop.
2. Click **Start a New Run**.
3. Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.



4. Place the loaded chip into the reader.
   a. Choose project settings (if applicable).
   b. Click **Next**.
5. Click **Load**.
6. Application, Reference, Probes:
   a. Select Application Type—**Genotyping**.
   b. Select Passive Reference (ROX).
   c. Select probe types.
   d. Click **Next**.
7. Confirm **Auto Exposure** is selected.
8. Click **Start Run**.

NOTE TO RUN THIS PROTOCOL AS AN END-POINT RUN ON THE BIOMARK SYSTEM, PLEASE SEE THE *SNP GENOTYPING USER GUIDE*, PN 6800098.

7 Post Chip Run

1. Remove tape covering the inlets.
2. Remove liquid from sample and assay wells with a pipette. You are now ready to Wash the FR48. See the *FR48.48 Cleaning Quick Reference*, PN 100-2228, for more information.
3. Use **System Clean** to blow out the *Load* IFC Controller WX control lines at the end of each day.

Technical Support

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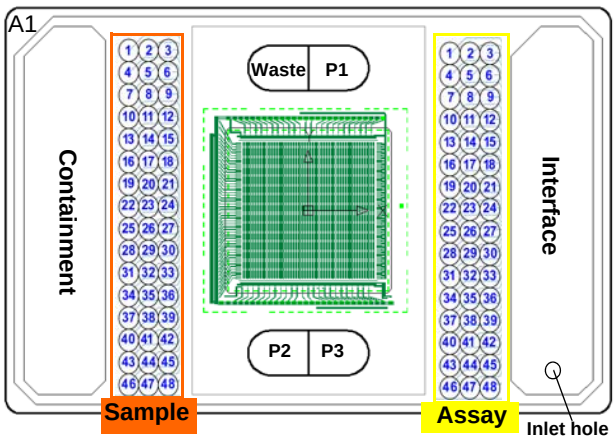
FR48.48 Dynamic Array IFC Loading Map



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