

Fluidigm® FR48.48 SNPtype™ Genotyping Workflow Quick Reference

PN 100-3911 Rev. A1

For more information see the Fluidigm SNP Genotyping User Guide, PN 68000098

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.

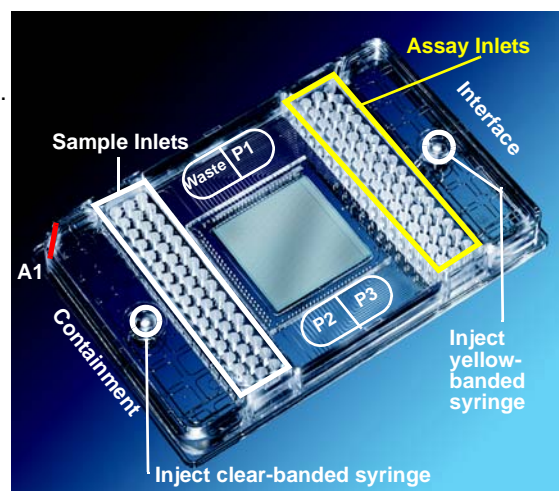
NOTE MAKE SURE THERE ARE NO BUBBLES IN THE WELLS.

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 SNPtype Assay Mixes

1 Prepare each SNPtype Assay Mix as described in the table below.

Component	Volume (µL)	Final Concentration
SNPtype Assay ASP1/ASP2 (100 µM each)	3.0	7.5 µM
SNPtype Assay LSP (100 µM each)	8.0	20.0 µM
DNA Suspension Buffer	29.0	
Total Volume	40.0	

3 Preparing 10X Assays

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

2 Combine 2X Assay Loading Reagent with PCR-certified water to create the Assay Pre-Mix.

3 Combine 4 µL of Assay Pre-Mix + 1 µL of each individual SNPtype Assay Mix (as prepared in table above) for a total of 5 µL 10X Assay Mix.

ASSAY PRE-MIX

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Volume per 50 µL Stock
2X Assay Loading Reagent (Fluidigm, PN 85000736) ●	2.0	2.5	25.0
PCR-certified water	1.2	1.5	15.0
SNPtype Assay Mix	0.8	1.0	10.0
Total Volume	4.0	5.0	50.0

4 Preparing Sample Pre-Mix and Samples

1 Combine the the Biotium Fast Probe Master Mix, 20X SNPtype Sample Loading Reagent, SNPtype Reagent, ROX and PCR-certified water to make Sample Pre-Mix as described in the table below.

2 Combine 3.5 µL of Sample Pre-Mix with 2.5 µL of each genomic DNA (gDNA) to make a total of 6 µL of Sample Mix Solution.

SAMPLE PRE-MIX

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Sample Pre-Mix for FR48.48 (µL) (60 for ease of pipetting)
Biotium 2X Fast Probe Master Mix (Biotium, PN 31005)	2.5	3.0	180.0
SNPtype 20X Sample Loading Reagent (Fluidigm, PN 100-3425) ○	0.25	0.3	18.0
SNPtype Reagent (Fluidigm, PN 100-3402)	0.083	0.1	6.0
ROX (50X) (Invitrogen, PN 12223-012)	0.03	0.036	2.2
PCR-certified water	0.053	0.064	3.8
genomic DNA	2.083	2.5	
Total Volume	5.0	6.0	210.0

5 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 μ L 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 3.5 μ L OF SAMPLE PRE-MIX AND 2.5 μ L OF WATER PER INLET. FOR UNUSED ASSAY INLETS, USE 4.0 μ L ASSAY PRE-MIX, AND 1.0 μ L WATER PER INLET.



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 μ L of each assay and 5 μ L 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 the 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.

6 Using the FC1™ Cyclar

- 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 **SNptype 48X48 Fast v1.pcl** protocol from the protocol selection window.
- 7 Press **Run**.
- 8 Once the protocol is finished, a confirmation screen appears. (During an active protocol, **Abort** will cancel the chip run.)

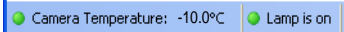


CAUTION! NEVER PRESS DOWN ON THE CHIP WHEN IT IS ON THE FC1 CYCLER.



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

7 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.

8 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

