Fluidigm® 48.48 Genotyping Workflow Quick Reference

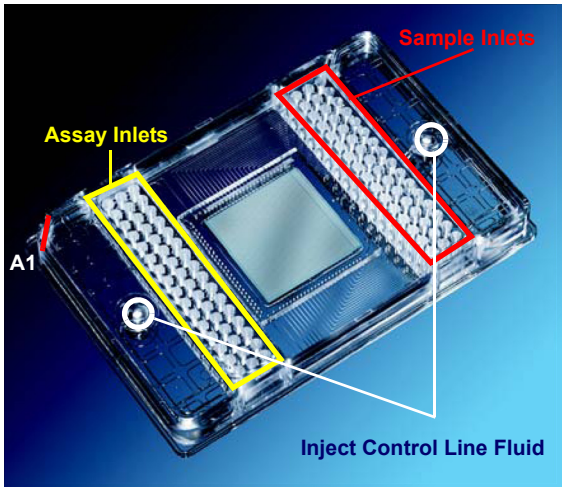
PN 68000099, Rev. E

For more information see the *BioMark Genotyping Analysis Software User Guide, PN 68000098*

1 Priming the 48.48 Dynamic Array™ IFC

CAUTION! USE THE 48.48 CHIP WITHIN 24 HOURS OF OPENING THE PACKAGE.

- DUE TO DIFFERENT ACCUMULATOR VOLUMES, ONLY USE 48.48 SYRINGES WITH 300 µL OF CONTROL LINE FLUID.
- CONTROL LINE FLUID ON THE CHIP OR IN THE INLETS MAKES THE CHIP UNUSABLE.
- LOAD THE CHIP WITHIN 60 MINUTES OF PRIMING.



1 Inject control line fluid into each accumulator on the chip.

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

3 Place the chip into the IFC (Integrated Fluidic Circuit) Controller MX, then run the **Prime (124x)** script to prime the control line fluid into the chip.

2 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).

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	2.5	25
ROX (50X) (Invitrogen, PN 12223-012)	0.2	0.25	2.5
DNA-free water	1.3	1.625	16.25
Total Volume	**4**	**5**	**50**

* 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 48.48 (µL) (60 for ease of pipetting)
SAMPLE PRE-MIX			
TaqMan® Universal PCR Master Mix (2X) (Applied BioSystems, PN 4304437)	2.5	3	180
20X GT Sample Loading Reagent (Fluidigm, PN 85000741)	0.25	0.3	18
AmpliTaq Gold® DNA Polymerase (Applied Biosystems, PN 4311806)	0.05	0.06	3.6
DNA-free water	0.1	0.12	7.2
genomic DNA (added individually to Sample Pre-Mix)	2.1	2.52	
Total Volume	**5**	**6**	

2 In a DNA-free hood, combine the four Sample Pre-Mix components in a 1.5 mL sterile tube—enough volume to fill an entire chip. Aliquot 3.48 µL of this Sample Pre-Mix for each sample.

3 Remove the aliquots from the DNA-free hood and add 2.52 µL of genomic DNA to each, making a total volume of 6 µL in each aliquot.

4 Loading the Chip

CAUTION! VORTEX THOROUGHLY AND CENTRIFUGE ALL ASSAY AND SAMPLE SOLUTIONS BEFORE PIPETTING INTO THE CHIP INLETS. FAILURE TO DO SO MAY RESULT IN A DECREASE IN DATA QUALITY.

IMPORTANT! FOR UNUSED SAMPLE INLETS, USE 3.48 µL OF SAMPLE MIX AND 2.52 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.

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 (124x)** script has finished, remove the primed chip from the IFC Controller MX and pipette 4 µL of each assay and 5 µL of each sample into the respective inlets on the chip.

2 Return the chip to the IFC Controller MX.

3 Using the IFC Controller MX software, run the **Load Mix (124x)** script to load the samples and assays into the chip.

4 When the **Load Mix (124x)** script has finished, remove loaded chip from the IFC Controller MX.

5 Remove any dust particles or debris from the chip surface.

You are now ready for your chip run.

CAUTION! **START THE CHIP RUN ON THE BIOMARK INSTRUMENT WITHIN 4 HOURS OF LOADING THE SAMPLES.**

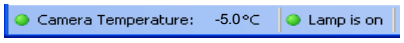
5 Using the Data Collection Software

IMPORTANT! BE SURE TO SELECT *ALL* PROBE TYPES PRESENT IN YOUR EXPERIMENT. DATA *ARE NOT* COLLECTED ON UNSPECIFIED PROBES.

1 Double-click the Data Collection Software icon on the desktop to launch the software.

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.

5 Click **Load**.

6 Verify chip barcode and chip type.

- **a** Choose project settings (if applicable).
- **b** Click **Next**.

7 Chip Run file:

- **a** Select **New** or **Predefined**.
- **b** Browse to a file location for data storage.
- **c** Click **Next**.

8 Application, Reference, Probes:

- **a** Select Application Type—**Genotyping**.
- **b** Select Passive Reference (ROX).
- **c** Select probe types.
- **d** Click **Next**.

9 Click **Browse** to find the appropriate thermal protocol file—**GT 48x48 Standard v1.pcl**.

CAUTION! **MAKE SURE THAT YOU USE A 48.48-SPECIFIC PROTOCOL.**

10 Confirm **Auto Exposure** is selected.

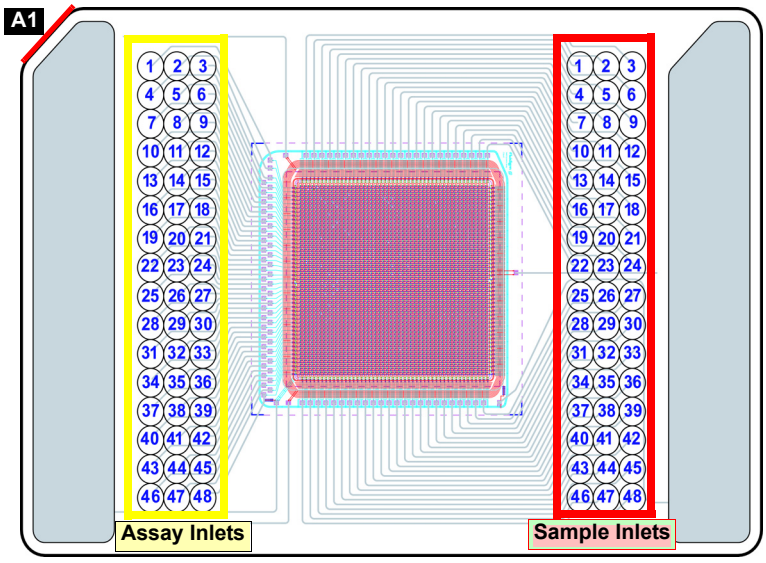
11 Click **Next**.

12 Verify that the chip run information is correct.

13 Click **Start Run**.

NOTE TO RUN THIS PROTOCOL AS AN END-POINT AND USE THE FLUIDIGM STAND-ALONE THERMAL CYCLER OR THE FLUIDIGM FC1™ CYCLER, REFER TO THE *FLUIDIGM STAND-ALONE THERMAL CYCLER USAGE QUICK REFERENCE* (PN 68000111) OR THE *FLUIDIGM FC1 CYCLER USAGE QUICK REFERENCE* (PN 100-1250), RESPECTIVELY.

Chip Pipetting Map



Technical Support

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