

# Fluidigm® 96.96 Genotyping Workflow Quick Reference

PN 68000129, Rev. C

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

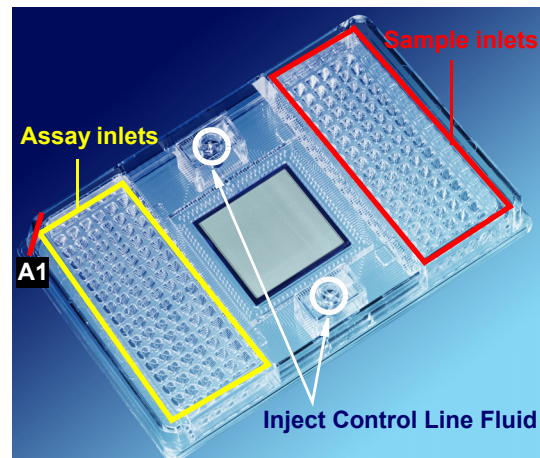
## 1 Priming the 96.96 Dynamic Array™ IFC



**CAUTION!** USE THE 96.96 CHIP WITHIN 24 HOURS OF OPENING THE PACKAGE.

- DUE TO DIFFERENT ACCUMULATOR VOLUMES, ONLY USE 96.96 SYRINGES WITH 150 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 HX, then run the **Prime (138x)** 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 for 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
<b>Total Volume</b>	<b>4</b>	<b>5</b>	<b>50</b>

\*If you are using 40X SNP assay, double the volume 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 96.96 (µL)
(120 for ease of pipetting)			
TaqMan® Universal PCR Master Mix (2X) (Applied Biosystems, PN 4304437)	2.5	3	360
20X GT Sample Loading Reagent (Fluidigm, PN 85000741) ●	0.25	0.3	36
AmpliTaQ Gold® DNA Polymerase (Applied Biosystems, PN 4311806 )	0.05	0.06	7.2
DNA-free water	0.1	0.12	14.4
genomic DNA (added individually to Sample Pre-mix)	2.1	2.52	
<b>Total Volume</b>	<b>5</b>	<b>6</b>	

SAMPLE PRE-MIX

- 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 the 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 (138x)** script has finished, remove the primed chip from the IFC Controller HX 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 HX.
- 3 Using the IFC Controller HX software, run the **Load Mix (138x)** script to load the samples and assays into the chip.
- 4 When the **Load Mix (138x)** script has finished, remove loaded chip from the IFC Controller HX.
- 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 IMMEDIATELY AFTER 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.

● Camera Temperature: -5.0°C ● Lamp is on

- 4 Place the 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**.

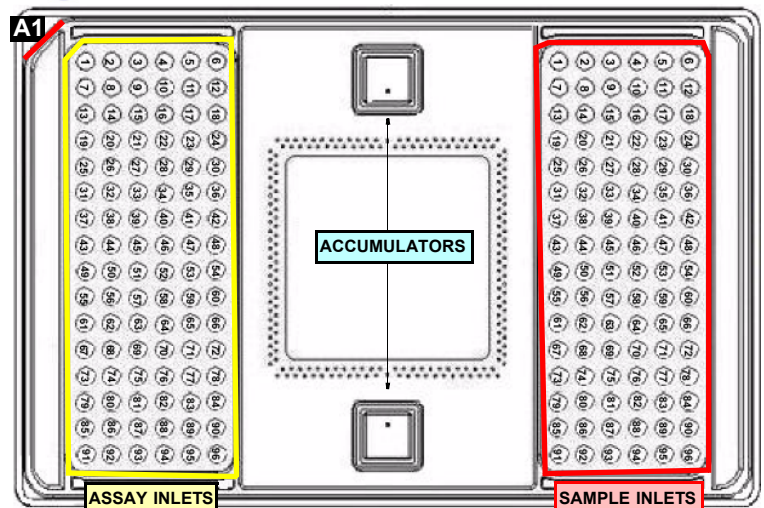
- 9 Click **Browse** to find the appropriate thermal protocol file—**GT 96x96 Standard v1.pcl**.

**CAUTION!** MAKE SURE THAT YOU USE A 96.96-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

### TELEPHONE

Within the United States: 1-866-358-4354  
Outside the United States: 1-650-266-6100

### EMAIL

techsupport@fluidigm.com

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