

Genotyping with the 96.96 IFC Using Fast TaqMan Assays

For more information, see the SNP Genotyping Analysis User Guide (PN 68000098) and the Juno System User Guide (PN 100-7070).

Choose a Juno/IFC Controller HX Workflow

Prime	Load and Thermal-Cycle (PCR)	Image
Juno™	Juno one-step loading and PCR	Biomark™ HD/Biomark or EP1™

Prime	Load	Thermal-Cycle (PCR)	Image
Juno or HX	Juno or HX	Juno or FC1™ cyclers	Biomark HD/Biomark or EP1

Prime	Load	Thermal-Cycle (PCR) and Image
Juno or HX	Juno or HX	Biomark HD

Prime the 96.96 IFC

ⓘ IMPORTANT

- Use the 96.96 Dynamic Array™ integrated fluidic circuit (IFC) within 24 hours of opening package.
- Due to different accumulator volumes, only use 96.96 syringes with 150 µL of control line fluid.
- Control line fluid on IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

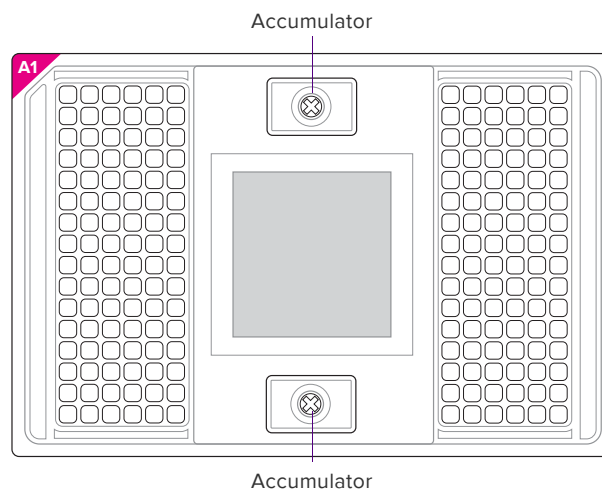
- Inject control line fluid into each accumulator on the IFC.
- Remove and discard blue protective film from bottom of IFC.
- Place the IFC into the instrument and run the prime script:
 - Juno: **Prime 96.96 GT**
 - HX: **Prime (138x)**

Prepare 10X TaqMan® Assays

In a DNA-free hood, prepare aliquots of 10X assays using volumes in the following table. Scale up appropriately for multiple runs.

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Vol. for 50 µL Stock
SNP Genotyping Assay Mix (80X*) (Life Technologies)	0.5	0.625	6.25
2X Assay Loading Reagent (Fluidigm PN 100-7611) ●	2.0	2.5	25.0
ROX™ (50X) (Life Technologies PN 12223-012)	0.2	0.25	2.5
DNA-free water	1.3	1.625	16.25
Total	4.0	5.0	50.0

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



Prepare Sample Pre-Mix and Samples

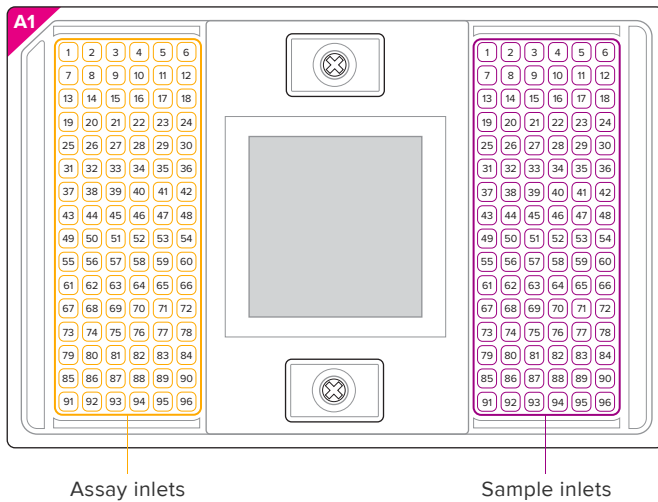
- Combine components in the following table to make sample pre-mix and final sample mixture.

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Sample Pre-Mix for 96.96* (µL)
SAMPLE PRE-MIX			
2X GTXpress™ Master Mix (Life Technologies PN 4403311)	2.5	3.0	360.0
20X Fast GT Sample Loading Reagent ● (Fluidigm PN 100-7606)	0.25	0.3	36.0
DNA-free water	0.17	0.2	24.0
Genomic DNA (added individually to sample pre-mix)	2.08	2.5	—
Total	5.0	6.0	—

*120 reactions for ease of pipetting

- 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 IFC. Aliquot 3.5 µL of the sample pre-mix for each sample.
- Remove the aliquots from the DNA-free hood and add 2.5 µL of genomic DNA to each, making a total volume of 6 µL in each aliquot.

96.96 IFC Pipetting Map



Load the IFC

! IMPORTANT

- Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.
- For unused assay inlets, use 2.5 μ L assay loading reagent, 0.25 μ L ROX and 2.25 μ L water per inlet.
- For unused sample inlets, use 3.5 μ L of sample mix and 2.5 μ L of water per inlet.

- 1 When the prime script has finished, remove the primed IFC from the instrument and pipet 4 μ L of each assay and 5 μ L of each sample into their respective inlets on the IFC.
- 2 Return the IFC to the instrument and run the load script according to operation:

Instrument	Operation	Run Script	Continue to
Juno	One-step loading and thermal cycling	One Step 96.96 Fast	“Collect End-Point Data”
Juno	Loading only	Load Mix 96.96 GT	“Thermal-Cycle the 96.96 IFC”
HX	Loading only	Load Mix (138x)	“Thermal-Cycle the 96.96 IFC”

- ! **IMPORTANT** Start IFC run within 1 hour of loading samples.

Thermal-Cycle the 96.96 IFC

Choose the instrument and run the script:

Instrument	Operation	Run Script
Juno	One-step loading and PCR	—
Juno	PCR only	Probe GT tab: Fast PCR 96.96
FC1 cycler	PCR only	GT 96x96 Fast v3.pcl

For more information about thermal cycling using FC1 cycler, see the FC1 Cycler Usage Quick Reference (PN 100-1250).

To thermal-cycle using Biomark HD, see the SNP Genotyping Analysis User Guide (PN 68000098).

Collect End-Point Data

To collect data using Biomark HD or Biomark, see the SNP Genotyping Analysis User Guide (PN 68000098).

- 1 Remove any dust particles or debris from the IFC surface.
- 2 Double-click the **Data Collection** icon on the desktop to launch the software.
- 3 Click **Start a New Run**.
- 4 Ensure that the status indicators for the lamp and the camera are green.
- 5 Place the loaded IFC into EP1. Click **Load**.
- 6 Verify IFC barcode and IFC type.
- 7 Choose project settings (if applicable). Click **Next**.
- 8 Provide a name and select a file storage location for a new IFC run, or browse to select a predefined run file. Click **Next**.
- 9 Choose the application, reference, and probes:
 - a Application type: **Genotyping**
 - b Passive reference: **ROX**
 - c Probe types: **FAM-MGB** and **VIC-MGB**
 - d Click **Next**.
- 10 Confirm **Auto Exposure** is selected. Click **Next**.
- 11 Verify the IFC run information.
- 12 Click **Start Run**.

For technical support visit fluidigm.com/support

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