

Genotyping with the 96.96 IFC Using SNP Type Assays or the SNP Trace Panel

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

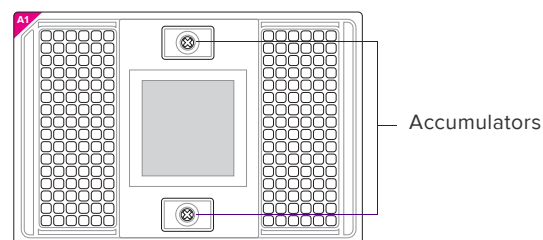
Prime	Load	Thermal-cycle (PCR) and image
Juno or HX	Juno or HX	Biomark HD/Biomark

Prime the 96.96 IFC

! IMPORTANT

- Use the 96.96 Dynamic Array™ integrated fluidic circuit (IFC) 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 IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

- Inject 150 µL of control line fluid into each accumulator.



- Remove and discard the 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) (High-precision: 139x)**

Prepare Assay Primer Mixes

- ### ! IMPORTANT
- Before use, vortex thoroughly and centrifuge all mix components, pre-mix, and final mix solutions.

Prepare each assay primer mix:

Component	Vol. (µL)	Final Conc. (µM)
Allele-specific primers 1 and 2 (100 µM ASP1 and 100 µM ASP2)	3.0	7.5
Locus-specific primers (100 µM LSP)	8.0	20.0
DNA suspension buffer	29.0	—
Total	40.0	—

Prepare 10X Assays

- In a DNA-free hood, combine 2X Assay Loading Reagent with PCR-certified water to create the assay pre-mix.
- Combine 4 µL of assay pre-mix and 1 µL of each individual assay primer mix (as prepared in “Prepare Assay Primer Mixes” in this quick reference) for a total of 5 µL 10X assay mix.

Component	Vol. per Inlet (µL)	Vol. per Inlet with Overage (µL)	Assay Pre-Mix for 96.96* (µL)
ASSAY PRE-MIX			
2X Assay Loading Reagent (Fluidigm PN 100-7611)	2.0	2.5	300.0
PCR-certified water	1.2	1.5	180.0
Assay primer mix	0.8	1.0	—
Total	4.0	5.0	—

*120 reactions for ease of pipetting

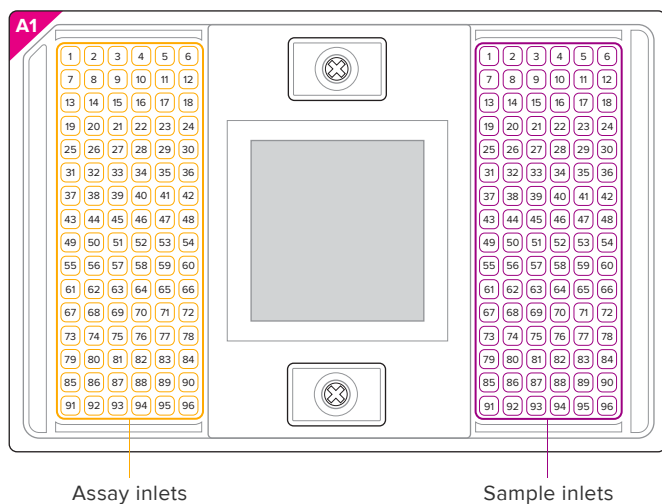
Prepare Sample Pre-Mix and Samples

- Combine the Biotium 2X Fast Probe Master Mix, 20X SNP Type™ sample loading reagent, 60X SNP Type reagent, ROX™, and PCR-certified water to make sample pre-mix.
- 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.

Component	Vol. per Inlet (µL)	Vol. per Inlet with Overage (µL)	Sample Pre-Mix for 96.96* (µL)
SAMPLE PRE-MIX			
Biotium 2X Fast Probe Master Mix (Biotium PN 31005)	2.5	3.0	360.0
20X SNP Type sample loading reagent (Fluidigm PN 100-7608) ○	0.25	0.3	36.0
60X SNP Type reagent (Fluidigm PN 100-7607) ●	0.083	0.1	12.0
ROX Reference Dye (50X) (Life Technologies PN 12223-012)	0.03	0.036	4.3
PCR-certified water	0.053	0.064	7.7
Genomic DNA	2.083	2.5	—
Total	5.0	6.0	420.0

*120 reactions for ease of pipetting

96.96 IFC Pipetting Map



Load the IFC

! IMPORTANT

- Vortex thoroughly and centrifuge all assay and sample solutions before pipetting them into 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 4.0 μL of assay pre-mix and 1.0 μL of water per inlet.
- For unused sample inlets, use 3.5 μL of sample pre-mix and 2.5 μL of water per inlet.

- 1 When the prime script has finished, remove the primed IFC from the instrument and pipet 4 μL of each assay and 5 μL of each sample into the respective inlets on the IFC (for the pipetting scheme, see the SNP Trace™ Panel User Guide (PN 100-7282).
- 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	“Collect Data”
Juno	Loading only	Load Mix 96.96 GT	“Thermal-Cycle the 96.96 IFC”
HX	Loading only	Load Mix (138x) (High-precision: 139x)	“Thermal-Cycle the 96.96 IFC”

- ! **IMPORTANT** Start the IFC run within 1 hour after loading the samples and assays.

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	SNP Type tab: PCR 96.96
FC1 cyclers	PCR only	SNPtype 96X96 v1.pcl
Biomark HD or Biomark	PCR and imaging	Continue to “Collect Data” and select SNPtype 96.96 v1 or SNPtype E 96.96 v1

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

Collect Data

- 1 Double-click the **Data Collection** icon on the desktop.
- 2 Click **Start a New Run**.
- 3 Ensure that the status indicators for the lamp (Biomark and EP1 only) and the camera are green.
- 4 Remove debris from the top of the IFC with clear tape.
- 5 Place the IFC into the instrument. Click **Load**.
- 6 Verify IFC barcode and IFC type.
- 7 Choose project settings (if applicable), then 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 and reference: **Genotyping** and **ROX**.
- 10 Select probe types: **SNPtype-FAM** and **SNPtype-HEX**.
- 11 Click **Next**.
- 12 Browse to and choose a thermal protocol:
 - Biomark HD or Biomark for end-point read only (after cycling on Juno or FC1), select **GT End Point v1**.
 - Biomark HD (fast) for thermal cycling and imaging, select **SNPtype 96.96 v1**.
 - Biomark HD or Biomark (standard) for thermal cycling and imaging, select **SNPtype E 96.96 v1**.
 - EP1, continue to the next step.
- 13 Confirm **Auto Exposure** is selected. Click **Next**.
- 14 Verify the IFC run information. Click **Start Run**.

For technical support visit fluidigm.com/support

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