

Genotyping with the 48.48 IFC Using SNP Type Assays

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

Choose a Juno/IFC Controller MX 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 MX	Juno or MX	Juno or FC1™ cyclers	Biomark HD/Biomark or EP1

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

Prime the 48.48 IFC

! IMPORTANT

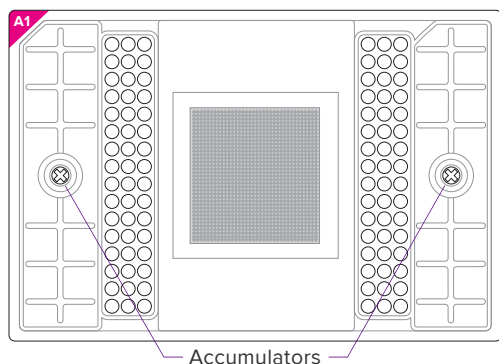
- Use the 48.48 Dynamic Array™ integrated fluidic circuit (IFC) within 24 hours of opening package.
- Due to different accumulator volumes, only use 48.48 syringes with 300 µL of control line fluid.
- Control line fluid on the IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

- Inject control line fluid into each accumulator on the IFC.
- Remove and discard the blue protective film from bottom of IFC.
- Place the IFC in the instrument and run the prime script:
 - Juno: **Prime 48.48 GT**
 - MX: **Prime (124x)**

Prepare SNP Type Assay Mixes

- Prepare each SNP Type™ assay mix as described in the following table.

Component	Vol. (µL)	Final Conc. (µM)
SNP Type assay ASP1/ASP2 (100 µM each)	3.0	7.5
SNP Type assay LSP (100 µM each)	8.0	20.0
DNA suspension buffer	29.0	—
Total	40.0	—



Prepare 10X Assays

- In a DNA-free hood, prepare aliquots of 10X assays using volumes in the table below. Scale up appropriately for multiple runs.
- Combine the 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 SNP Type assay mix (as shown in the following table) for a total of 5 µL 10X assay mix.

Component	Vol. per Inlet (µL)	Vol. per Inlet with Overage (µL)	Vol. per 50 µL Stock
ASSAY PRE-MIX			
2X Assay Loading Reagent (Fluidigm PN 100-7611) ●	2.0	2.5	25.0
PCR-certified water	1.2	1.5	15.0
SNP Type assay mix	0.8	1.0	10.0
Total	4.0	5.0	50.0

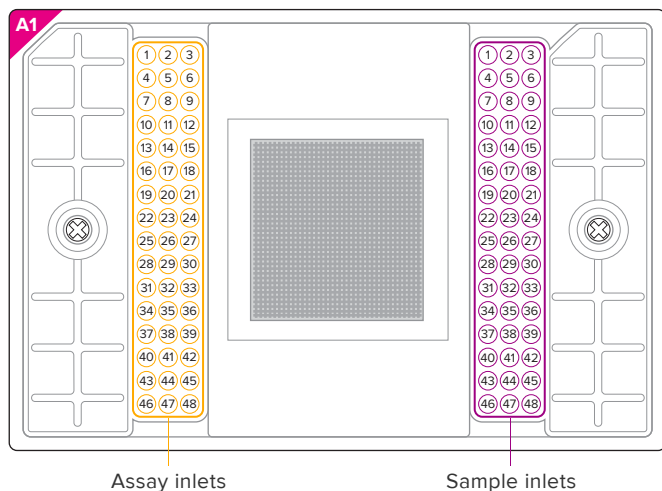
Prepare Sample Pre-Mix and Samples

- Combine the Biotium Fast Probe Master Mix, 20X SNP Type sample loading reagent, SNP Type reagent, ROX™, and PCR-certified water to make sample pre-mix as described in the table below.
- 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 48.48* (µL)
SAMPLE PRE-MIX			
Biotium 2X Fast Probe Master Mix (Biotium PN 31005)	2.5	3.0	180.0
SNP Type 20X sample loading reagent (Fluidigm PN 100-7608) ○	0.25	0.3	18.0
SNP Type reagent (Fluidigm PN 100-7607)	0.083	0.1	6.0
ROX™ (50X) (Life Technologies 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	5.0	6.0	210.0

*60 reactions for ease of pipetting

IFC Pipetting Map



Load the IFC

! IMPORTANT

- Vortex thoroughly and centrifuge all assay and sample solutions before pipetting them 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 4.0 μ L of assay loading reagent and 1.0 μ L of water.
- For unused sample inlets, use 3.5 μ L of sample mix and 2.5 μ L of DNA-free 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 48.48	“Collect Data”
Juno	Loading only	Load Mix 48.48 GT	“Thermal-Cycle the 48.48 IFC”
MX	Loading only	Load Mix (124x)	“Thermal-Cycle the 48.48 IFC”

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

Thermal-Cycle the 48.48 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 48.48
FC1 cycler	PCR only	SNPtype 48X48 v1.pcl
Biomark HD or Biomark	PCR and imaging	Continue to “Collect Data” and select SNPtype 48.48 v1 or SNPtype E 48.48 v1

For more information about thermal cycling using the FC1 cycler, 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 48.48 v1**.
 - Biomark HD or Biomark (standard) for thermal cycling and imaging, select **SNPtype E 48.48 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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