Genotyping with the 192.24 IFC Using SNP Type 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 RX Workflow

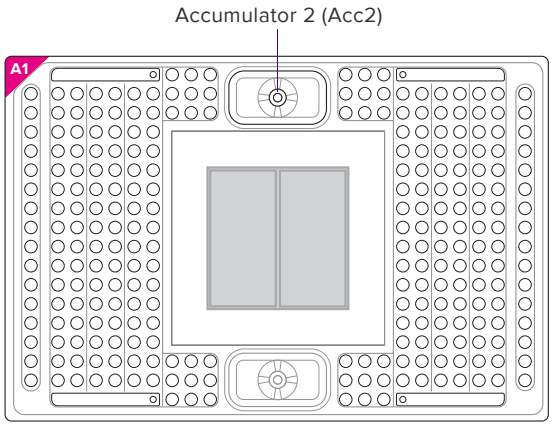
Load and thermal-cycle (PCR)		Image
Juno™ one-step loading and PCR		Biomark™ HD/Biomark or EP1™
Load	**Thermal-cycle (PCR)**	**Image**
Juno or RX	Juno or FC1™ cycler	Biomark HD/Biomark or EP1
Load	**Thermal-cycle (PCR) and image**	
Juno or RX	Biomark HD/Biomark	

Prepare the 192.24 IFC

IMPORTANT

- Use the 192.24 Dynamic Array™ integrated fluidic circuit (IFC) within 24 hours of opening the package.
- Due to different accumulator volumes, use only syringes with 150 µL of control line fluid.
- Control line fluid on the IFC or in the inlets makes IFC unusable.

1. Inject control line fluid into accumulator 2 (Acc2) on the IFC.
2. Remove and discard the blue protective film from bottom of IFC.



Prepare SNP Type Assay Mixes

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

Component	Vol. (µL)	Final Concentration (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

1. In a DNA-free hood, prepare aliquots of 10X assays using volumes in table below. Scale up appropriately for multiple runs.
2. Combine 2X Assay Loading Reagent with PCR-certified water to create the assay pre-mix.
3. Combine 3.2 µL of assay pre-mix and 0.8 µL of each individual SNP Type assay mix (as prepared in "Prepare SNP Type Assay Mixes") for a total of 4 µL 10X assay mix.

Component	Vol. per Inlet (µL)	Vol. per Inlet with Overage (µL)	Vol. for 50 µL Stock
ASSAY PRE-MIX			
2X Assay Loading Reagent (Fluidigm PN 100-7611)	1.5	2.0	25.0
PCR-certified water	0.9	1.2	15.0
SNP Type assay mix	0.6	0.8	10.0
Total	**3.0**	**4.0**	**50.0**

Prepare Sample Pre-Mix and Samples

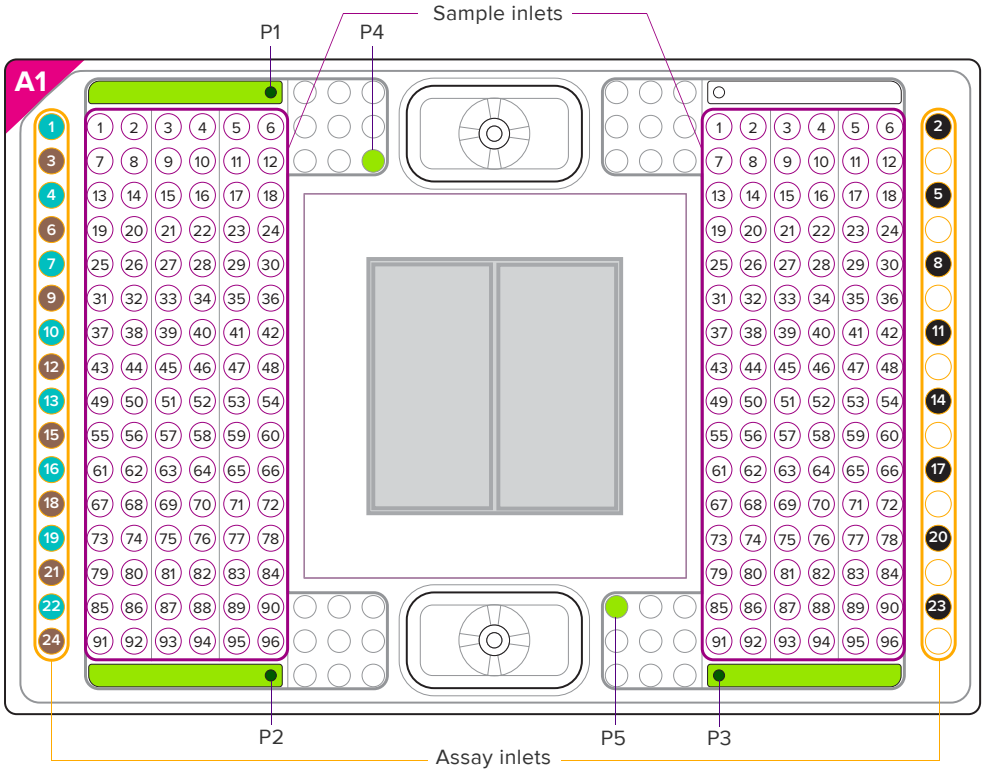
1. Combine the Biotium Fast Probe Master Mix, 20X SNP Type sample loading reagent, SNP Type reagent, ROX™, and PCR-certified water to make sample pre-mix as described in the table below.
2. Combine 2.6 µL of sample pre-mix with 1.9 µL of each genomic DNA (gDNA) to make a total of 4.525 µL of sample mix solution.

Component	Vol. per Inlet (µL)	Vol. per Inlet with Overage (µL)	Sample Pre-Mix 192.24 with Overage* (µL)
SAMPLE PRE-MIX			
Biotium 2X Fast Probe Master Mix (Biotium PN 31005)	1.492	2.25	540.0
SNP Type 20X sample loading reagent (Fluidigm PN 100-7608)	0.149	0.225	54.0
SNP Type reagent (Fluidigm PN 100-7607)	0.050	0.075	18.0
ROX (50X) (Life Technologies PN 12223-012)	0.018	0.027	6.48
PCR-certified water	0.032	0.048	11.52
Genomic DNA	1.260	1.9	—
Total	**3.0**	**4.525**	**—**

*240 reactions for ease of pipetting

192.24 IFC Pipetting Map

Place the IFC directly on the actual-size map as a guide when loading IFC.



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 bubbles into inlets.
- Make sure the interface plate on the IFC controller RX is clean and dust-free before loading the IFC. You can use clear tape to remove dust and debris.
- For unused assay inlets, use 3.2 µL of assay pre-mix and 0.8 µL of water per inlet.
- For unused sample inlets, use 2.6 µL sample pre-mix and 1.9 µL water per inlet.

1. Pipet 3 µL of each assay and 3 µL of each sample into the respective inlets on the IFC. See the 192.24 IFC pipetting map.
2. Pipet 150 µL of pressure fluid into the P1, P2 and P3 wells.
3. Pipet 20 µL of pressure fluid into the P4 and P5 wells.
4. Blot carrier surface with dry, lint-free cloth.
5. Return IFC to instrument and run load script according to operation:

Instrument	Operation	Run Script	Continue to
Juno	One-step loading and thermal cycling	**One step 192.24**	"Collect Data"
Juno	Loading only	**Load Mix 192.24 GT**	"Thermal-Cycle the 192.24 IFC"
RX	Loading only	**Load Mix (166x)**	"Thermal-Cycle the 192.24 IFC"

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

Thermal-Cycle the 192.24 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 192.24**
FC1 cycler	PCR only	**SNPtype 192X24 Fast v1**
Biomark HD or Biomark	PCR and imaging	Continue to "Collect Data" and select **SNPtype 192.24 v1** or **SNPtype E 192.24 v1**

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

Assay loading key

- First dispense of 8 assays
- Second dispense of 8 assays
- Third dispense of 8 assays
- No assays—leave empty

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 application and reference: **Genotyping** and **ROX**.
10. Select probe types: **SNPtype-FAM** and **SNPtype-HEX**. Click **Next**.
11. 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 192.24 v1**.
    - Biomark HD or Biomark (standard) for thermal cycling and imaging, select **SNPtype E 192.24 v1**.
    - EP1, continue to the next step.
12. Confirm **Auto Exposure** is selected. Click **Next**.
13. Verify the IFC run information. Click **Start Run**.

For technical support visit fluidigm.com/support

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