

# Genotyping with the 192.24 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 RX Workflow

Load and thermal-cycle (PCR)		Image
Juno™ one-step loading and PCR		Biomark™ HD/Biomark or EPI™
Load	Thermal-Cycle (PCR)	Image
Juno or RX	Juno or FC1™ cyclers	Biomark HD/Biomark or EPI
Load	Thermal-Cycle (PCR) and Image	
Juno or RX	Biomark HD	

## 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 the IFC unusable.

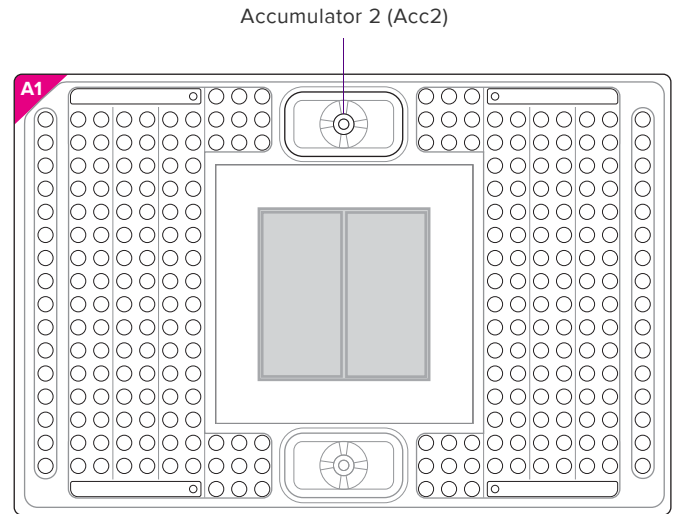
- 1 Inject control line fluid into accumulator 2 (Acc2) on the IFC.
- 2 Remove and discard the blue protective film from the bottom of the IFC.

## Prepare 10X Assays

- 1 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)	0.375	0.5	6.25
2X Assay Loading Reagent (Fluidigm PN 100-7611) ●	1.5	2.0	25.0
ROX™ (50X) (Life Technologies PN 12223-012)	0.15	0.2	2.5
DNA-free water	0.975	1.3	16.25
<b>Total</b>	<b>3.0</b>	<b>4.0</b>	<b>50.0</b>

\*For 40x SNP assay, double SNP assay mix volume and reduce the DNA-free water. For other starting concentrations, contact technical support.



## Prepare Sample Pre-Mix and Samples

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

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Sample Pre-Mix for 192.24 with Overage* (µL)
<b>SAMPLE PRE-MIX</b>			
GTXpress™ Master Mix (2X) (Life Technologies PN 4401892)	1.5	2.0	480.0
20X Fast GT Sample Loading Reagent ● (Fluidigm PN 100-7606)	0.15	0.2	48.0
DNA-free water	0.15	0.2	48.0
Genomic DNA (added individually to the sample pre-mix)	1.2	1.6	—
<b>Total</b>	<b>3.0</b>	<b>4.0</b>	<b>—</b>

\*240 reactions for ease of pipetting

- 2 In a DNA-free hood, combine the three sample pre-mix components in a 1.5 mL sterile tube—enough volume to fill an entire IFC. Aliquot 2.4 µL of this sample pre-mix for each sample.
- 3 Remove the aliquots from the DNA-free hood and add 1.6 µL of genomic DNA to each, making a total volume of 4 µL in each aliquot.

## 192.24 IFC Pipetting Map

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

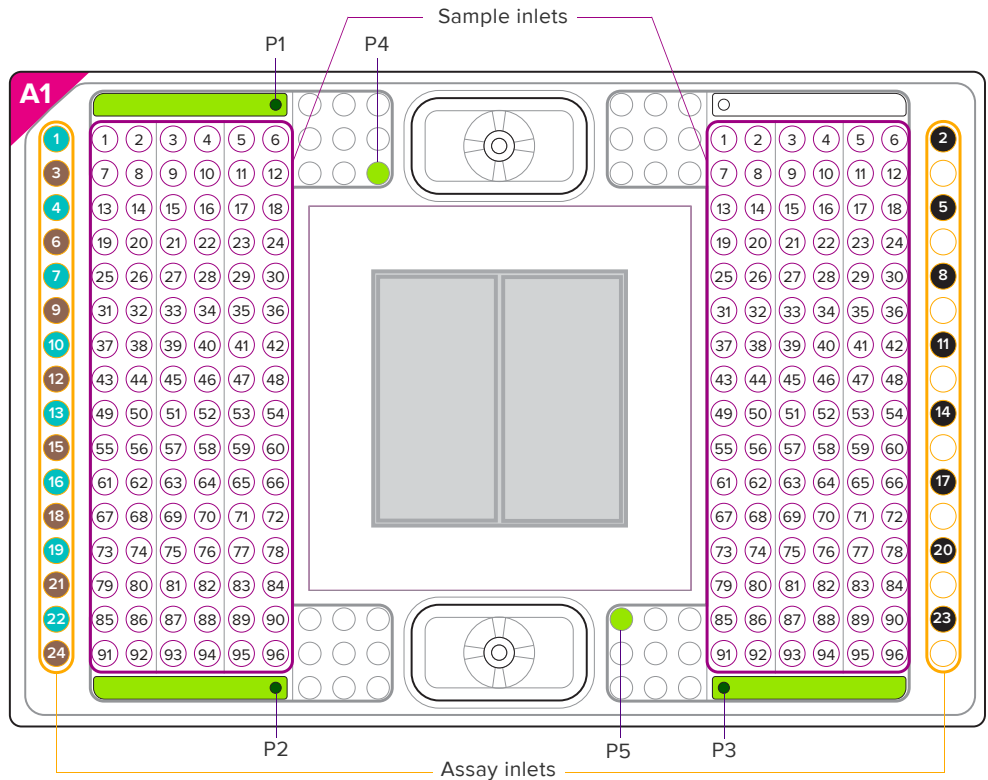
### Assay loading key

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

## Load the IFC

### ! IMPORTANT

- Vortex thoroughly and centrifuge all assay and sample solutions before pipetting 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.
- For unused assay inlets, use 2  $\mu\text{L}$  assay loading reagent, 0.2  $\mu\text{L}$  ROX™ and 1.8  $\mu\text{L}$  water per inlet.
- For unused sample inlets, use 2.4  $\mu\text{L}$  of sample mix and 1.6  $\mu\text{L}$  of water per inlet.



- 1 Pipet 3  $\mu\text{L}$  of each assay and 3  $\mu\text{L}$  of each sample into the respective inlets on the IFC.
- 2 Pipet 150  $\mu\text{L}$  of pressure fluid into the P1, P2 and P3 wells.
- 3 Pipet 20  $\mu\text{L}$  of pressure fluid into the P4 and P5 wells.
- 4 Blot carrier surface with dry, lint-free cloth.
- 5 Return the IFC to the instrument and run the load script according to the operation:

Instrument	Operation	Run Script	Continue to
Juno	One-step loading and thermal cycling	<b>One Step 192.24 Fast</b>	"Collect End-Point Data"
Juno	Loading only	<b>Load Mix 192.24 GT</b>	"Thermal-Cycle the 192.24 IFC"
RX	Loading only	<b>Load Mix (166x)</b>	"Thermal-Cycle the 192.24 IFC"

! **IMPORTANT** Start 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	Probe GT tab: <b>Fast PCR 192.24</b>
FC1 cycler	PCR only	<b>GT 192X24 Fast v1.pcl</b>

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.
- 3 Click **Start a New Run**.
- 4 Ensure that the status indicators for the lamp (Biomark and EP1 only) and the camera are green.
- 5 Place the loaded IFC into EP1.
- 6 Choose project settings (if applicable). Click **Next**.
- 7 Click **Load**.
- 8 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**.
- 9 Confirm **Auto Exposure** is selected.
- 10 Click **Start Run**.

## For technical support visit [fluidigm.com/support](http://fluidigm.com/support)

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 PHONE **United States (toll-free)** +1 866 358 4354 | **Europe** +33 1 60 92 42 40 | **Japan** +81 3 3662 2150 | **China** +86 21 3255 8368 | **All other countries** +1 650 266 6100

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