

Genotyping with the 48.48 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 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™ cycler	Biomark HD/Biomark or EP1

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

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 IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

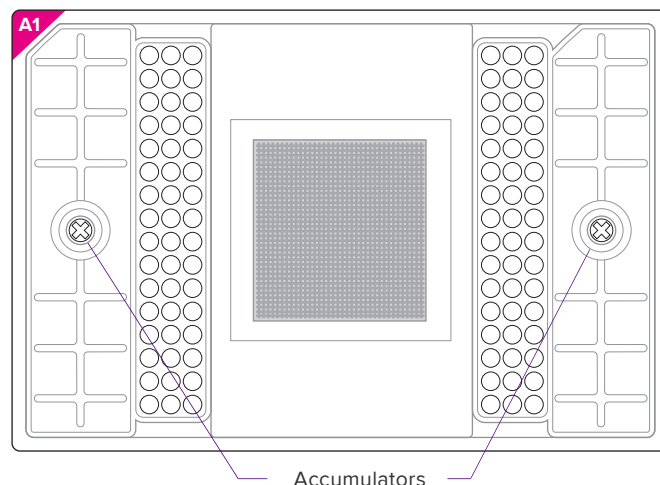
- 1 Inject control line fluid into each accumulator on the IFC.
- 2 Remove and discard blue protective film from bottom of IFC.
- 3 Place the IFC into the instrument and run the prime script:
 - Juno: **Prime 48.48 GT**
 - MX: **Prime (124x)**

Prepare 10X TaqMan® Assays

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

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Vol. Per 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	2.50
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 the SNP assay mix, call Fluidigm technical support



Prepare Sample Pre-Mix and Samples

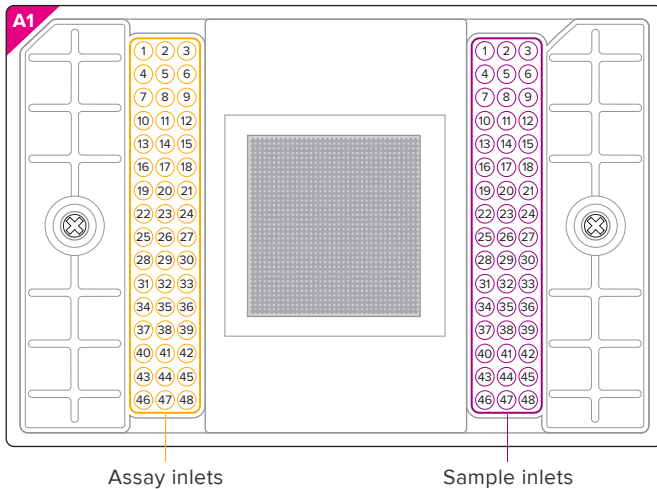
- 1 Combine components in table below to make sample pre-mix and final sample mixture.

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Sample Pre-mix for 48.48* (µL)
SAMPLE PRE-MIX			
2X GTXpress™ Master Mix (Life Technologies PN 4403311)	2.5	3.0	180.0
20X Fast GT Sample Loading Reagent (Fluidigm PN 100-7606) ●	0.25	0.3	18.0
DNA-free water	0.17	0.2	12.0
Genomic DNA (added individually to sample pre-mix)	2.08	2.5	—
Total	5.0	6.0	—

*60 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 3.5 µL of sample pre-mix for each sample.
- 3 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.

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 µL assay loading reagent, 0.25 µL ROX, and 2.25 µL water per inlet.
- For unused sample inlets, use 3.5 µL of sample 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 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 Fast	“Collect End-Point 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	Probe GT tab: Fast PCR 48.48
FC1 cycler	PCR only	GT 48X48 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 Check the status bar to verify that the lamp and the camera are ready. Be sure both are green before proceeding.
- 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

EMAIL **United States and other countries not in Europe or Asia** techsupport@fluidigm.com | **Europe** techsupporteurope@fluidigm.com | **Asia** techsupportasia@fluidigm.com
 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

For Research Use Only. Not for use in diagnostic procedures.

Information in this publication is subject to change without notice. Use standard laboratory safety protocols. **Safety data sheet information** fluidigm.com/sds **Patent and license information** fluidigm.com/legal/notice **EU's WEEE directive information** fluidigm.com/compliance | Fluidigm, the Fluidigm logo, Biomark, Dynamic Array, EP1, FC1, and Juno are trademarks or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners.
 © 2015 Fluidigm Corporation. All rights reserved. 03/2015