Genotyping with the 48.48 IFC Using Standard 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/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 IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

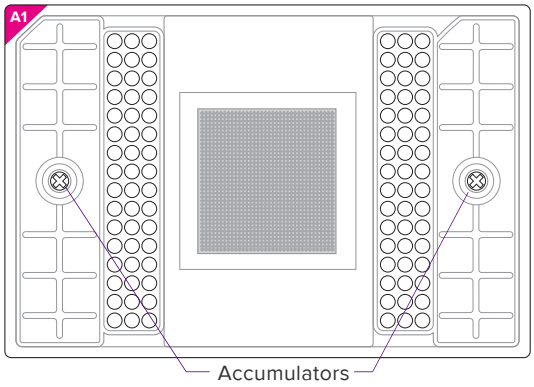
1. Inject control line fluid into each accumulator on the IFC.
2. Remove and discard blue protective film from bottom of IFC.
3. Place the IFC into the instrument and run the prime script:
   - Juno: **Prime 48.48 GT**
   - MX: **Prime (124x)**

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 85000736)	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, contact Fluidigm technical support.



Prepare Sample Pre-Mix and Samples

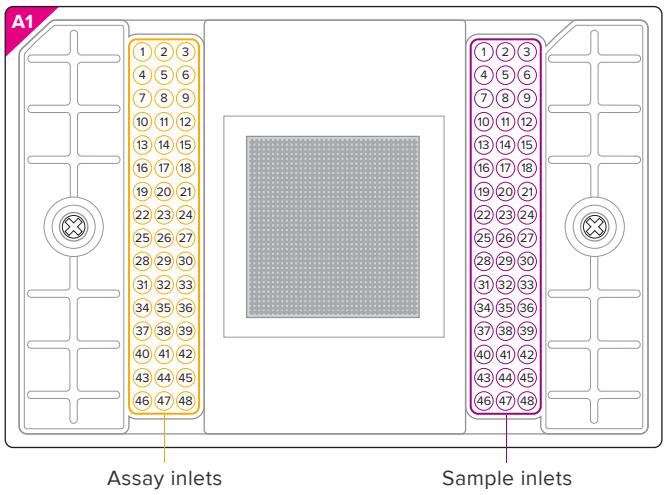
1. Combine components in table below to make sample pre-mix and final sample mixture.

Component	Vol. per inlet (µL)	Vol. per inlet with overage (µL)	Sample pre-mix for 48.48* (µL)
SAMPLE PRE-MIX			
TaqMan® Universal PCR Master Mix (2X) (Life Technologies, PN 4304437)	2.5	3.0	180.0
20X GT Sample Loading Reagent (Fluidigm, PN 85000741)	0.25	0.3	18.0
AmpliTaq Gold® DNA Polymerase (Life Technologies, PN 4311806)	0.05	0.06	3.6
DNA-free water	0.1	0.12	7.2
Genomic DNA (added individually to sample pre-mix)	2.1	2.52	—
Total	5.0	6.0	—

*60 reactions for ease of pipetting

2. In a DNA-free hood, combine the four sample pre-mix components in a 1.5 mL sterile tube—enough volume to fill an entire IFC. Aliquot 3.48 µL of the sample pre-mix for each sample.
3. Remove the aliquots from the DNA-free hood and add 2.52 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 3.48 µL of sample mix and 2.52 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 Juno or MX, then run the script according to operation:

Instrument	Operation	Run script	Continue to
Juno	One-step loading and thermal cycling	**One Step 48.48**	"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: **PCR 48.48**
FC1 cycler	PCR only	Continue to "Collect End-Point Data" and select **GT 48X48 Standard v1.pcl**

To run this protocol as an end-point read using the FC1 cycler or the Fluidigm Stand-alone Thermal Cycler, see the FC1 Cycler Usage Quick Reference (PN 100-1250) or the Stand-alone Thermal Cycler Usage Quick Reference (PN 68000111), respectively.

Collect End-Point Data

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 Ensure that the status indicators for the lamp (Biomark and EP1 only) and the camera are green.

5 Place the loaded IFC into the instrument. Click **Load**.

6 Verify IFC barcode and IFC type.

7 Choose project settings (if applicable). 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, reference, and probes:

a Application type: **Genotyping**

b Passive reference: **ROX**

c Probe types: **FAM-MGB** and **VIC-MGB**

d Click **Next**.

10 Browse to and choose the thermal protocol:

- Biomark HD or Biomark for end-point read only (after cycling on Juno or FC1), select **GT End Point v1**
- Biomark HD or Biomark for both thermal cycling and image capture (end-point read only), select **GT 48X48 Standard v1.pcl**
  Be sure to use a 48.48-specific protocol.
- EP1, continue to the next step.

11 Confirm **Auto Exposure** is selected. Click **Next**.

12 Verify the IFC run information. Click **Start Run**.

For technical support visit fluidigm.com/support

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