Standard TaqMan Genotyping

Choose a Juno/IFC Controller HX Workflow

Prime	Load and Thermal-Cycle (PCR)	Image	Post-Run
Juno™	Juno one-step loading and PCR	Biomark™ HD or Biomark or EP1™	Juno or HX

Prime	Load	Thermal-Cycle (PCR)	Image	Post-Run
Juno or HX	Juno or HX	Juno or FC1™ cycler	Biomark HD or Biomark or EP1	Juno or HX

Prime	Load	Thermal-Cycle (PCR) and Image	Post-Run
Juno or HX	Juno or HX	Biomark HD or Biomark	Juno or HX

Prime the Flex Six IFC (First Use Only)

Once the IFC is primed, skip these steps on subsequent use.

IMPORTANT

- Use the Flex Six™ integrated fluidic circuit (IFC) within three months of opening the package.
- Control line fluid on IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

1. Using the included syringes, inject 150 µL of control line fluid into each accumulator. Do not remove the barrier plugs until you load the IFC.
2. Remove and discard the blue protective film from the bottom of the IFC.
3. Place the IFC into Juno or HX.
4. Choose the instrument and run the prime script:
   - Juno: **Prime Flex Six GT**
   - HX: **Prime (154x)**

Prepare 10X Assays

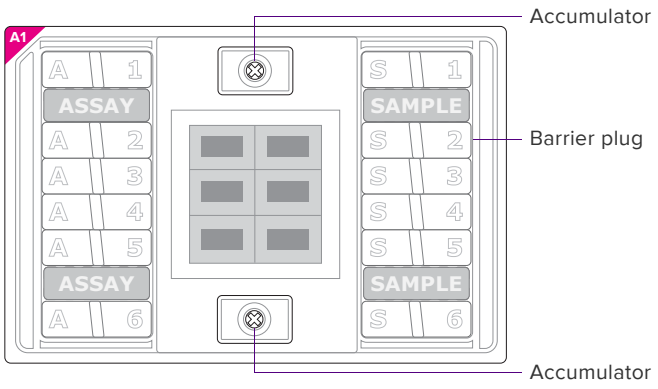
We recommend preparing 10X assay stock, due to the small pipetting volumes needed to prepare a single assay mix. Unused 10X assays can be stored at –20 °C for up to three weeks.

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*
2X Assay Loading Reagent (Fluidigm, PN 100-7611)	2.0	2.5	25.0
ROX™ Reference Dye (50X) (Life Technologies, PN 12223-012)	0.2	0.25	2.5
PCR-certified water	1.3	1.625	16.25
SNP Genotyping Assay Mix (80X†) (Life Technologies)	0.5	0.625	6.25
Total	**4.0**	**5.0**	**50.0**

*10 replicates

† If you are using 40X SNP assay, double the volume and reduce the PCR-certified water. For other starting concentrations of the SNP assay mix, call Fluidigm technical support.



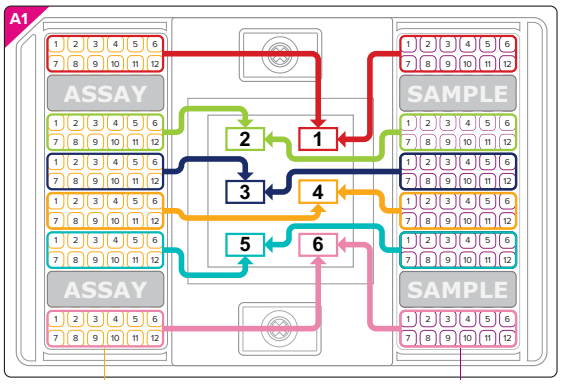
Standard: Prepare Sample Pre-Mix and Samples

1. In a DNA-free hood, combine the sample pre-mix components for either fast or standard chemistry (scale up appropriately for multiple runs) to make enough for your experiment (52.5 µL/partition). Aliquot 3.5 µL of the pre-mix for each sample.
2. Remove the aliquots from the DNA-free hood and add 2.5 µL of each DNA sample (genomic or preamplified) to make a total of 6 µL of sample mix solution. Genomic DNA must be ≥60 ng/µL of human genome size equivalent.

Standard chemistry component	Vol./ Inlet (µL)	Vol./Inlet with Overage (µL)	Sample Pre-Mix for 1 Partition* (µL)
SAMPLE PRE-MIX (**STANDARD**)			
TaqMan® Universal PCR Master Mix (2X) (Life Technologies, PN 4304437)	2.5	3.0	45.0
20X GT Sample Loading Reagent (Fluidigm PN 100-7612)	0.25	0.3	4.5
AmpliTaq Gold® DNA Polymerase (Life Technologies, PN 4311806)	0.05	0.06	0.9
PCR-certified water	0.12	0.14	2.1
DNA sample (genomic or preamplified) added individually to sample pre-mix	2.08	2.5	—
Total	**5.0**	**6.0**	**52.5**

*15 reactions for ease of pipetting

Flex Six Partitions and Inlets



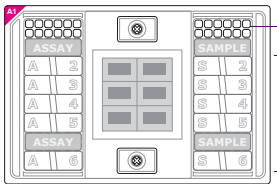
Standard: Load the IFC

Each Flex Six IFC has a total of six independent partitions (1–6 above). Each partition has a 12 × 12 format (12 assay inlets and 12 sample inlets) and can be run independently as a separate experimental run at different times or on different days or simultaneously.

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.
- At minimum, all 12 assay inlets and all 12 sample inlets for a partition must be filled.
  For unused assay inlets in active partitions, prepare 2.5 µL assay loading reagent, 0.25 µL ROX, and 2.25 µL water per inlet.
  For unused sample inlets in active partitions, prepare 3.5 µL sample pre-mix and 2.5 µL water per inlet.

1 Be sure barrier plugs are placed on unused inlets to prevent pipetting into the wrong inlets and to track used/unused partitions.



2 Pipet one partition at a time by removing the barrier plugs for the selected set of partition inlets.

3 Pipet 4 µL of each assay and 5 µL of each sample into their respective inlets. Do not replace the barrier plugs after pipetting.

4 Return the IFC to the instrument and run the load script according to operation. After the run, do not replace the barrier plugs.

Instrument	Operation	Run Script	Continue to
Juno	One-step loading & thermal cycling	**One Step Flex Six**	"Standard: Collect End-Point Data"
Juno	Loading only	**Load Mix Flex Six GT**	"Standard: Thermal-Cycle the Flex Six IFC"
HX	Loading only	**Load Mix (154x)**	"Standard: Thermal-Cycle the Flex Six IFC"

Standard: Thermal-Cycle the Flex Six 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 Flex Six**
FC1 cycler	PCR only	**GT FLEXsix Standard v1**
Biomark HD or Biomark	PCR and imaging	Continue to "Fast: Collect End-Point Data" and select **GT FLEXsix Standard v1**

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

Standard: Collect End-Point Data

1 Double-click the **Data Collection** icon on the desktop.

IMPORTANT If this is your first time running a Flex Six IFC, set up a tracking file: select **Tools** > **FLEXsix Usage Tracking**. Click **New**, enter a filename, and select a location. Click **Done**.

2 Click **Start a New Run**.

3 Remove debris from the top of the IFC with clear tape.

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.

6 Choose project settings (if applicable) and click **Next.** Click **Load**.

7 Verify the IFC barcode and type, choose project settings (if applicable), then click **Next.**

8 Select the partitions you wish to run.

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 a thermal protocol:

- Biomark or Biomark HD for end-point read only (after cycling on the FC1), select **GT End Point v1**
- Biomark HD or Biomark (standard) for thermal cycling and image capture (real-time), select **GT FLEXsix Standard v1**
- EP1, continue to the next step.

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

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

Post-Run

1 Immediately after IFC run, return IFC to Juno or HX and run the post-run script to relax the valves:

- Juno: **Post Run Flex Six GT**
- HX: **Post Run (154x)**

2 Put the barrier plugs back into used inlets. Label used barrier plugs to record which partitions/inlets were used.

3 Store the IFC at room temperature and protect from dust.

For technical support visit fluidigm.com/support

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