Genotyping with the Flex Six 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 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

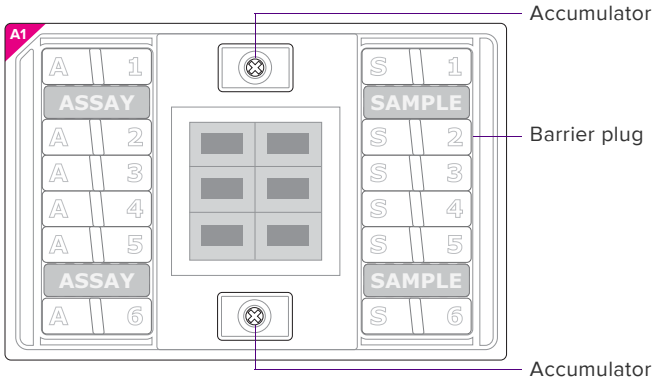
Prime the Flex Six IFC (first use only)

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



Prepare Assay Primer Mixes

Prepare each assay primer mix using the following table:

Component	Vol. (µL)	Final Conc. (µM)
Allele-specific primers 1 and 2 (100 µM ASP1 and 100 µM ASP2)	3.0	7.5
Locus-specific primers (100 µM LSP)	8.0	20.0
DNA suspension buffer	29.0	—
Total	**40.0**	**—**

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.

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*
2X Assay Loading Reagent (Fluidigm PN 100-7611)	2.0	2.5	25.0
PCR-certified water	1.2	1.5	15.0
Assay primer mix	0.8	1.0	10.0
Total	**4.0**	**5.0**	**50.0**

*10 replicates

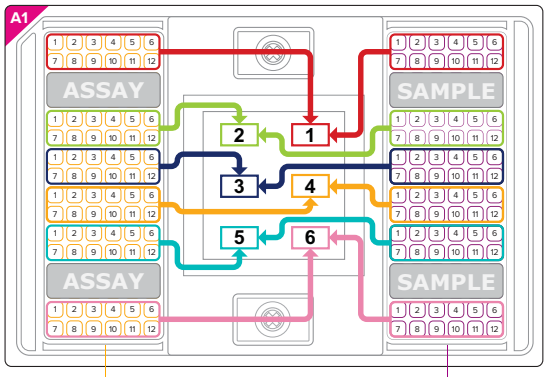
Prepare Sample Pre-Mix and Samples

1. In a DNA-free hood, combine the sample pre-mix components to make enough for your experiment (52.5 µL/partition). Scale up appropriately for multiple runs.
2. Aliquot 3.5 µL of the pre-mix for each sample.
3. 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.

Component	Vol. per inlet (µL)	Vol. per Inlet with Overage (µL)	Sample Pre-Mix for 1 Partition* (µL)
SAMPLE PRE-MIX			
Biotium Fast Probe Master Mix (2X) (Biotium PN 31005)	2.5	3.0	45.0
20X SNP Type™ Sample Loading Reagent (Fluidigm PN 100-7608)	0.25	0.3	4.5
60X SNP Type Reagent (Fluidigm PN 100-7607)	0.083	0.1	1.5
ROX™ Reference Dye (50X) (Life Technologies PN 12223-012)	0.03	0.036	0.54
PCR-certified water	0.053	0.064	0.96
DNA sample (genomic or preamplified) added individually to sample pre-mix	2.083	2.5	—
Total	**5.0**	**6.0**	**52.5**

*15 reactions for ease of pipetting.

Flex Six Partitions and Inlets



Assay inlets Sample inlets

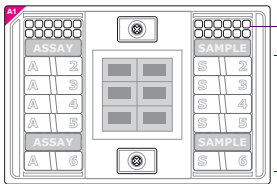
Load the IFC

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.
- All assay and sample inlets must be filled.
  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. Remove the primed IFC from Juno or HX.
2. Be sure to place barrier plugs on unused inlets to prevent pipetting into wrong inlets and to track used/unused partitions.



3. Pipet one partition at a time by removing the barrier plugs for the selected set of partition inlets.
4. Pipet 4 µL of each assay and 5 µL of each sample into their respective inlets. Do not replace barrier plugs after pipetting.
5. 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 and thermal cycling	**One Step Flex Six**	"Collect Data"
Juno	Loading only	**Load Mix Flex Six GT**	"Thermal-Cycle the Flex Six IFC"
HX	Loading only	**Load Mix (154x)**	"Thermal-Cycle the Flex Six IFC"

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	SNP Type tab: **PCR Flex Six**
FC1 cycler	PCR only	**SNPtype FLEXsix v1**
Biomark HD or Biomark	PCR and imaging	Continue to "Collect Data" and select **SNPtype Flex Six v1** or **SNPtype E Flex Six v1**

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

Collect 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. 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 Flex Six v1**
    - Biomark HD or Biomark (standard) for thermal cycling and imaging, select **SNPtype E Flex Six v1**
    - EP1, continue to the next step.
12. Confirm **Auto Exposure** is selected. Click **Next**.
13. Verify the IFC run information. Click **Start Run**.

Perform Post-Run

1. Immediately after the IFC run, return the 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 the 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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