Genotyping with the 96.96 Dynamic Array IFC Using the Advanta Sample ID Genotyping Panel

IMPORTANT Before using the Advanta™ Sample ID Genotyping Panel (PN 101-7773) with the 96.96 Dynamic Array™ IFC for Genotyping (PN BMK-M-96.96GT), read and understand the detailed instructions and safety guidelines in the SNP Genotyping Analysis User Guide (PN 68000098).

Choose a Juno/IFC Controller HX Workflow

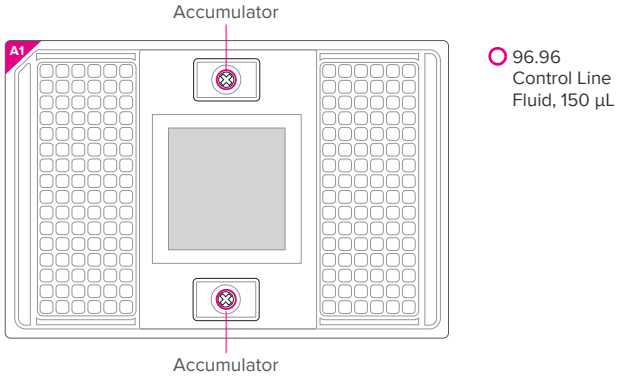
Prime Juno™	**Load and thermal-cycle (PCR)** Juno one-step loading and PCR		**Image** Biomark™ HD or EP1™
Prime Juno or HX	**Load** Juno or HX	**Thermal-cycle (PCR)** Juno or FC1™ cycler	**Image** Biomark HD or EP1
Prime Juno or HX	**Load** Juno or HX	**Thermal-cycle (PCR) and image** Biomark HD	

Prime the 96.96 IFC

IMPORTANT

- Use the 96.96 integrated fluidic circuit (IFC) within 24 hours of opening the package.
- Only use 96.96 syringes with 150 µL of Control Line Fluid (PN 89000021).
- Do not evacuate air from syringes prior to injecting Control Line Fluid.
- Avoid bending the syringe tip. Be careful when removing the syringe cap to prevent drips.
- Avoid getting Control Line Fluid on the exterior of the IFC or in the inlets because this makes the IFC unusable. If this occurs, use a new IFC.

☐ **1** Inject 150 µL of Control Line Fluid into each accumulator.



☐ **2** Remove and discard the protective film from the bottom of the IFC.

☐ **3** Place the IFC into the instrument and run the prime script:

- Juno: **Prime 96.96 GT**
- HX: **Prime (138x)**

For more information about using Juno, see the Juno System User Guide (PN 100-7070). For more information about using the IFC Controller HX, see the IFC Controller MX and IFC Controller HX User Guide (PN 68000112).

Prepare Assay Primer Mixes

IMPORTANT Before use, vortex thoroughly and centrifuge all mix components, pre-mix, and final mix solutions.

Prepare each assay primer mix:

Table 1. Assay primer mix

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 (Teknova PN T0221)	29.0	—
Total	**40.0**	**—**

Prepare 10X Assay Mixes

☐ **1** In a DNA-free hood, prepare the assay pre-mix in a new 1.5 mL microcentrifuge tube as shown in Table 2.

Table 2. Assay pre-mix

Component	Vol. per Inlet (µL)*	Assay Pre-Mix for One 96.96 IFC (µL)†
2X Assay Loading Reagent (PN 100-7611)	2.5	300.0
PCR-certified water	1.5	180.0
Total	**4.0**	**480.0**

* Includes overage
† 120 reactions for ease of pipetting

☐ **2** Pipet 58 µL of the assay pre-mix into each well of a new 8-well strip (see Figure 1).

☐ **3** Prepare 10X assay mix in a 96-well plate as shown in Table 3.

IMPORTANT For unused assay inlets, use 4.0 µL of assay pre-mix and 1.0 µL of water per inlet.

Table 3. 10X assay mix

Component	Vol. per Inlet (µL)*
Assay pre-mix (see Table 2)	4.0
Assay primer mix (see Table 1)	1.0
Total	**5.0**

* Includes overage

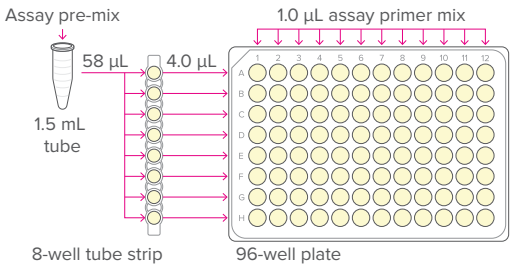


Figure 1. Preparation of sample mixes

Prepare Sample Mixes

☐ **1** In a DNA-free hood, prepare the sample pre-mix in a new 1.5 mL microcentrifuge tube as shown in Table 4.

Table 4. Sample pre-mix

Component	Vol. per Inlet (µL)*	Sample Pre-Mix for One 96.96 IFC (µL)†
Biotium 2X Fast Probe Master Mix (Biotium PN 31005)	3.0	360.0
20X SNP Type™ Sample Loading Reagent (PN 100-7608)	0.3	36.0
60X SNP Type Reagent (PN 100-7607)	0.1	12.0
ROX™ Reference Dye (50X) (Thermo Fisher Scientific PN 12223-012)	0.036	4.3
PCR-certified water	0.064	7.7
Total	**3.5**	**420.0**

* Includes overage
† 120 reactions for ease of pipetting

☐ **2** Pipet 50 µL of the sample pre-mix into each well of a new 8-well strip (see Figure 2).

☐ **3** In a DNA sample hood, prepare the sample mixes by pipetting the components shown in Table 5 into each well of a new 96-well plate as shown in the diagram in Figure 2. Use an 8-channel pipette to transfer the sample pre-mix from the 8-well strip.

IMPORTANT

- Assign at least 1 well as NTC (no template control). Do not add genomic DNA to this well. Instead, add 2.5 µL of PCR-certified water.
- For unused sample inlets, use 3.5 µL of sample pre-mix and 2.5 µL of water per inlet.

Table 5. Sample mix

Component	Vol. per Inlet (µL)*
Sample pre-mix (see Table 4)	3.5
Genomic DNA	2.5
Total	**6.0**

* Includes overage

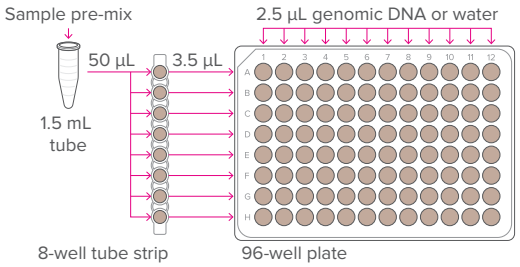


Figure 2. Preparation of sample mixes

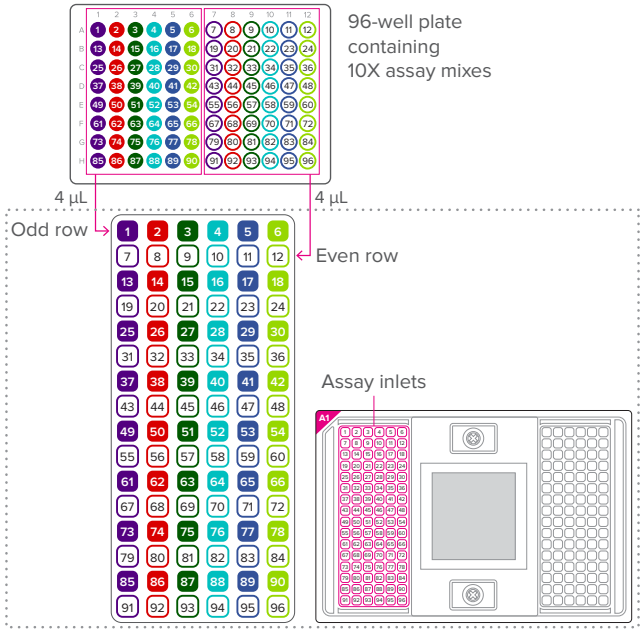
Load the IFC

IMPORTANT

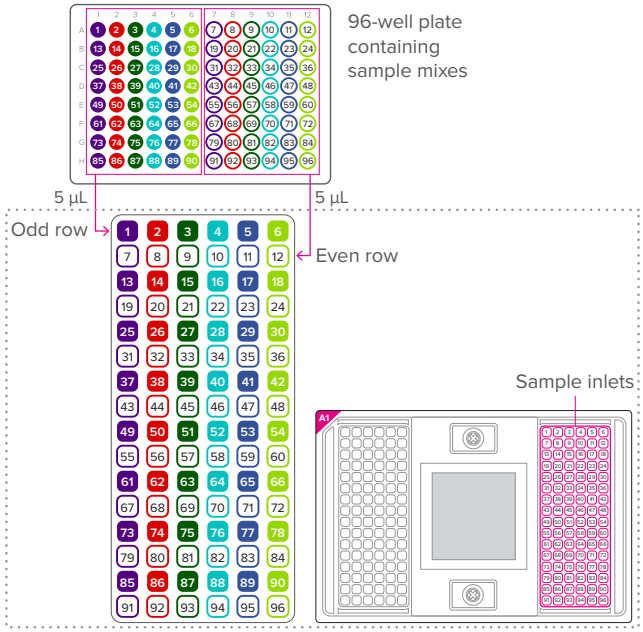
- Vortex thoroughly and centrifuge all assay and sample solutions before pipetting them into IFC inlets. Failure to do so may result in a decrease in data quality.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

☐ **1** When the prime script has finished, remove the primed IFC from the controller.

☐ **2** Pipet 4 µL of each assay mix into the assay inlets of the IFC.



☐ **3** Pipet 5 µL of each sample mix into the sample inlets of the IFC.



☐ **4** Return the IFC to the controller and run the load script according to operation:

Instrument	Operation	Run Script	Continue to
Juno	One-step loading and thermal cycling	**One Step 96.96**	Collect Data
Juno	Loading only	**Load Mix 96.96 GT**	Thermal-Cycle the 96.96 IFC
HX	Loading only	**Load Mix (138x)**	Thermal-Cycle the 96.96 IFC

IMPORTANT Start the IFC run immediately after loading the samples and assays.

Thermal-Cycle the 96.96 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 96.96**
FC1 cycler	PCR only	**SNPtype 96X96 v1.pcl**
Biomark HD	PCR and imaging	Continue to Collect Data and select **SNPtype 96.96 v1** or **SNPtype E 96.96 v1**

For more information about thermal cycling using FC1 cycler, see the FC1 Cycler User Guide (PN 100-1279).

Collect Data

For more information about using Biomark HD, see the Biomark HD Data Collection User Guide (PN 100-2451). For more information about using EP1, see the Biomark/EP1 Data Collection User Guide (PN 68000127).

☐ **1** Use clear tape to remove any dust particles or debris from the IFC surface.

☐ **2** If necessary, double-click the **Data Collection** icon on the desktop of the Biomark HD or EP1 system computer to launch the software.

☐ **3** Click **Start a New Run**.

☐ **4** Confirm that the camera status indicator and lamp status indicator (EP1 only) at the bottom of the window are green.

☐ **5** Place the IFC on the instrument tray, aligning the notched A1 corner on the IFC with the A1 on the tray, and click **Load**.

☐ **6** Complete the Chip Barcode and Type section and click **Next**.

☐ **7** Complete the Chip Run section by selecting either a new or pre-defined run.

☐ **8** Complete the Chip Run Name and Location section and click **Next**.

☐ **9** Complete the Application, Reference and Probes section and then click **Next**.

For...	Select...
Application	**Genotyping**
Passive reference	**ROX**
Assay	**Two Probes**
Probes	• **SNPtype-FAM** • **SNPtype-HEX**

☐ **10** Browse to and select the thermal protocol:

- Biomark HD for end-point read only (after cycling on Juno or FC1), select **GT End Point v1**.
- Biomark HD for thermal cycling and imaging:
  - For fast, select **SNPtype 96.96 v1**.
  - For standard, select **SNPtype E 96.96 v1**.
- EP1, continue to the next step.

☐ **11** Confirm that **Auto Exposure** is selected and click **Next**.

☐ **12** Verify the IFC run information and click **Start Run**. The IFC run takes approximately 2 hours.

☐ **13** After the run is complete, process your data using the SNP Trace™ Panel Analysis Tool in the SNP Genotyping Analysis software. For more information about using the software, see the SNP Genotyping Analysis User Guide (PN 68000098)

For technical support visit fluidigm.com/support.

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