

# 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

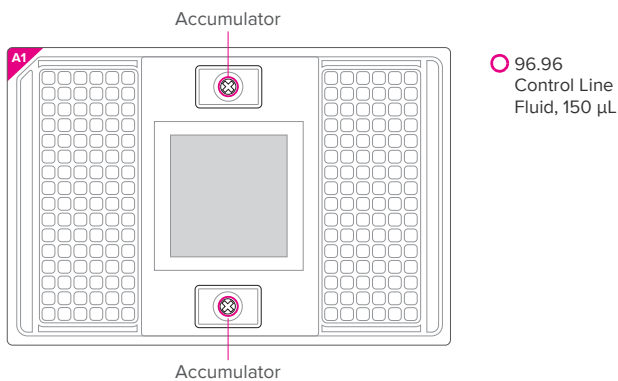
Prime	Load and thermal-cycle (PCR)	Image	
Juno™	Juno one-step loading and PCR	Biomark™ HD or EP1™	
Prime	Load	Thermal-cycle (PCR)	Image
Juno or HX	Juno or HX	Juno or FC1™ cycler	Biomark HD or EP1
Prime	Load	Thermal-cycle (PCR) and image	
Juno or HX	Juno or HX	Biomark HD	

## 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  $\mu\text{L}$  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.

- Inject 150  $\mu\text{L}$  of Control Line Fluid into each accumulator.



- Remove and discard the protective film from the bottom of the IFC.
- 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. ( $\mu\text{L}$ )	Final Conc. ( $\mu\text{M}$ )
Allele-specific primers 1 and 2 (100 $\mu\text{M}$ ASP1 and 100 $\mu\text{M}$ ASP2)	3.0	7.5
Locus-specific primers (100 $\mu\text{M}$ LSP)	8.0	20.0
DNA Suspension Buffer (Teknova PN T0221)	29.0	—
<b>Total</b>	<b>40.0</b>	<b>—</b>

## Prepare 10X Assay Mixes

- 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 ( $\mu\text{L}$ )*	Assay Pre-Mix for One 96.96 IFC ( $\mu\text{L}$ )†
2X Assay Loading Reagent (PN 85000736)	2.5	300.0
PCR-certified water	1.5	180.0
<b>Total</b>	<b>4.0</b>	<b>480.0</b>

\* Includes overage

† 120 reactions for ease of pipetting

- Pipet 58  $\mu\text{L}$  of the assay pre-mix into each well of a new 8-well strip (see Figure 1).
- Prepare 10X assay mix in a 96-well plate as shown in Table 3.
 

**IMPORTANT** For unused assay inlets, use 4.0  $\mu\text{L}$  of assay pre-mix and 1.0  $\mu\text{L}$  of water per inlet.

Table 3. 10X assay mix

Component	Vol. per Inlet ( $\mu\text{L}$ )*
Assay pre-mix (see Table 2)	4.0
Assay primer mix (see Table 1)	1.0
<b>Total</b>	<b>5.0</b>

\* Includes overage

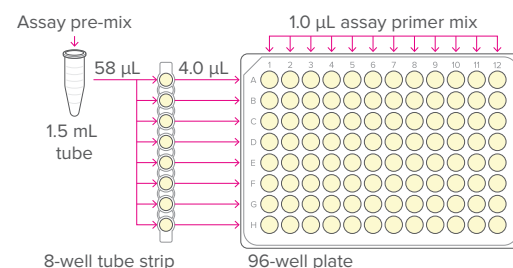


Figure 1. Preparation of assay mixes

## 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 100-3425)	○	0.3	36.0
60X SNP Type Reagent (PN 100-3402)	●	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
<b>Total</b>		<b>3.5</b>	<b>420.0</b>

\* 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
<b>Total</b>	<b>6.0</b>

\* Includes overage

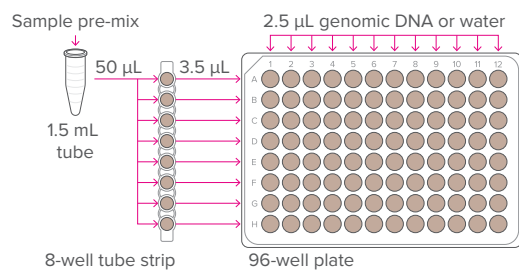


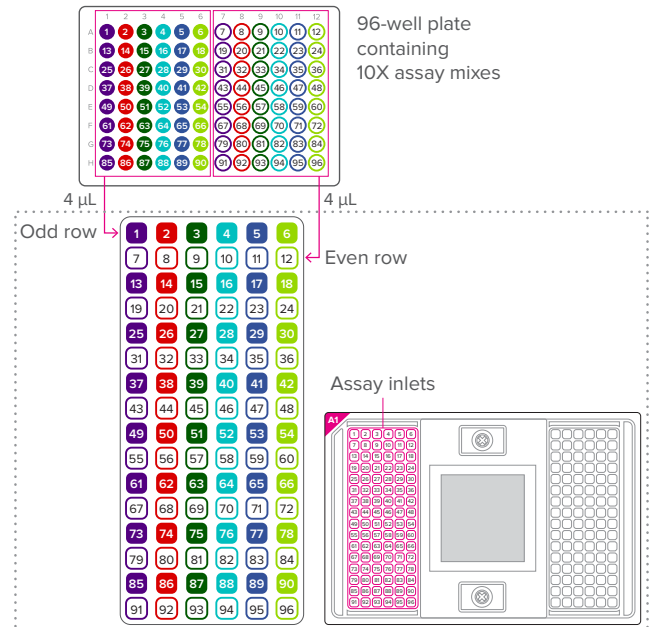
Figure 2. Preparation of sample mixes

## Load the IFC

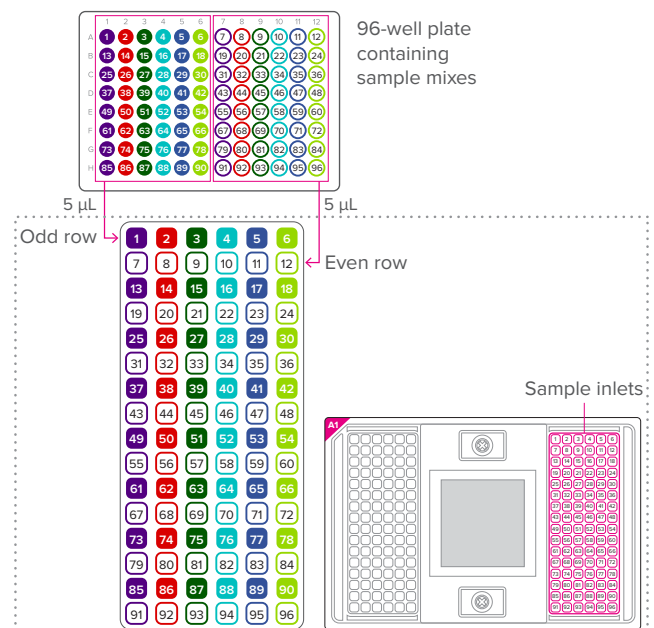
### IMPORTANT

- 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	<b>One Step 96.96</b>	<a href="#">Collect Data</a>
Juno	Loading only	<b>Load Mix 96.96 GT</b>	<a href="#">Thermal-Cycle the 96.96 IFC</a>
HX	Loading only	<b>Load Mix (138x)</b>	<a href="#">Thermal-Cycle the 96.96 IFC</a>

**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: <b>PCR 96.96</b>
FC1 cycler	PCR only	<b>SNPtype 96X96 v1.pcl</b>
Biomark HD	PCR and imaging	Continue to <a href="#">Collect Data</a> and select <b>SNPtype 96.96 v1</b> or <b>SNPtype E 96.96 v1</b>

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	<b>Genotyping</b>
Passive reference	<b>ROX</b>
Assay	<b>Two Probes</b>
Probes	<ul style="list-style-type: none"> <li>• <b>SNPtype-FAM</b></li> <li>• <b>SNPtype-HEX</b></li> </ul>

- 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](http://fluidigm.com/support).**

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