Genotyping with the Juno 96.96 IFC Using TaqMan Assays

For safety information and complete details, refer to the Genotyping with Juno Getting Started Guide (PN 100-7074).

Prepare the Primer Pool for Preamplification

1. If necessary, adjust the concentration of TaqMan® genotyping assays with DNase-free water to 18 µM (20X).
2. In a new microcentrifuge tube, combine 2 µL of each 20X TaqMan Genotyping Assay (18 µM) up to a total of 96 assays, then add Dilution Reagent to reach a total volume of 200 µL in order to prepare 0.2X final concentration for each assay.

Prepare 2X TaqMan Assays for Genotyping

1. If necessary, adjust the concentration of TaqMan genotyping assays with DNase-free water to 18 µM (20X).
2. In a new 96-well plate, dilute the 20X TaqMan genotyping assays in Dilution Reagent or DNase-free water to a final concentration of 2X for each assay:

Component	Volume (µL)	Final Concentration
20X TaqMan genotyping assays	1.0	2X
Dilution Reagent (Fluidigm PN 100-8725) or DNase-free water	9.0	—
Total	**10.0**	**—**

Prepare the Assay Mix

1. Label a new 96-well plate "TAQMAN ASSAY PLATE," then, in a DNA-free hood, pipet 2.5 µL of Probe GT Master Mix into each well. (See Table 1).
2. In a DNA-free hood, pipet 2.5 µL of 2X TaqMan assays into a well of the TaqMan assay plate for each assay.
3. In unused assay inlets, combine 2.5 µL of Probe GT Master Mix with 2.5 µL DNase-free water.
4. Seal the plate with MicroAmp® Clear Adhesive Film, vortex the plate for 5 seconds and centrifuge the plate at 1,000 x *g* for 1 minute.

Table 1: Assay mix

Component	Volume (µL)
Probe GT Master Mix (Fluidigm PN 100-8359)	2.5
2X TaqMan assays*	2.5
Total	**5.0**

*See "Prepare 2X TaqMan Assays for Genotyping".

Prepare Sample Mix

IMPORTANT Before use, thaw reagents to room temperature. Then thoroughly vortex then centrifuge all mix components, pre-mix, and final mix solutions.

1. In a DNA-free hood, in a new 1.5-mL microcentrifuge tube labeled "Sample Pre-Mix," combine Juno™ GT Preamp Master Mix and primer pool for preamplification to prepare the sample pre-mix (see Table 2).
2. Label a new 96-well plate "SAMPLE PLATE," and pipet 2.25 µL of the sample pre-mix into each well. Do not add sample pre-mix to no template control wells.

IMPORTANT Prepare at least one no template control.

3. In a DNA sample hood, pipet 2.75 µL of genomic DNA (for high-quality human genomic DNA, use ≥2.5 ng/µL) into the appropriate wells of the sample plate.
4. In a DNA sample hood, pipet 5.00 µL of Dilution Reagent, into each no template control well.
5. Seal the plate with MicroAmp® Clear Adhesive Film, vortex the plate for 5 seconds, then centrifuge the plate at 1,000 x *g* for 1 minute.

Table 2: Sample mix

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Sample Mix for IFC with Overage (µL)
SAMPLE PRE-MIX			
Juno GT Preamp Master Mix (Fluidigm PN 100-8358)	0.8	1.0	120.0
Primer pool for preamplification*	1.0	1.25	150.0
Genomic DNA	2.2	2.75	—
Total	**4.0**	**5.0**	**270.0**

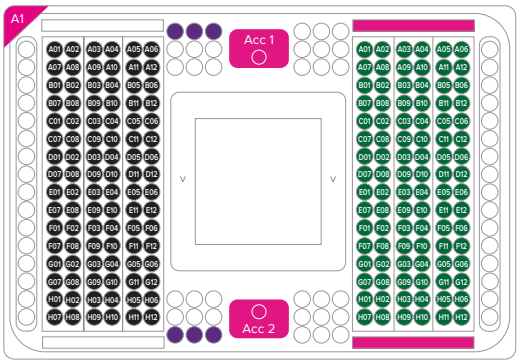
*See "Prepare the Primer Pool for Preamplification".

Load, and then Run the IFC on the System

IMPORTANT

- To ensure correct accumulator volume, only use syringes containing Juno™ 96.96 GT Control Line Fluid.
- If control line fluid comes into contact with the sample inlets, use a new IFC.
- Vortex thoroughly and centrifuge all assay and sample mixes before pipetting into IFC inlets. Failure to do so may result in decreased data quality.
- To ensure that no air bubbles enter an inlet, do not go past the first stop on the pipette.

1 Review the pipetting map for exact locations to pipet reagents into the IFC. Pipet reagents from the TaqMan assay plate and the sample plate to the IFC according to the 96-well plate locations shown on the pipetting map:



2 Ensure that the notched corner of the IFC ("A1") is at the top left, and view the loading map on the bottom of the IFC.

3 Load an entire syringe of Juno 96.96 GT Control Line Fluid in Acc1 and a second syringe in Acc2 (pink squares on the pipetting map).

4 Load an entire syringe of Juno 96.96 GT Control Line Fluid into a reservoir and a second syringe into the second reservoir (long pink rectangles on the right side of the pipetting map).

5 Pipet 15 µL of Juno™ GT Flux Fluid into each of the six ports (purple circles on the pipetting map).

6 Unseal the TaqMan assay plate and pipet 4.0 µL of each assay mix into an assay inlet (black circles on the pipetting map).

7 Unseal the sample plate and pipet 4.0 µL of each sample mix into a sample inlet (green circles on the pipetting map).

8 Pull the sticker front tab down and away from the IFC to gently peel off the loading map. Do not invert the IFC.

9 If necessary, remove any bubbles from an IFC inlet by removing the contents by pipette and then carefully re-pipetting the contents into the inlet.

10 Ensure that the SX interface plate (silver label) is installed on the instrument.

11 Start the run <60 minutes after pipetting the reagents into the IFC. On the Juno Scripts screen, tap the **Probe GT** tab, **Juno 96.96 Fast**, and then **Run**.

12 After the IFC is finished, tap **EJECT** to eject the IFC, then perform an end-point read of the IFC in ≤60 minutes. Do not leave the IFC in the instrument overnight.

Use EP1™ for End-Point Reads

1 Remove any dust particles or debris from the IFC surface.

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

3 Click **Start a New Run**.

4 Ensure that the status indicators for the lamp and the camera are green.

5 Place the loaded IFC into the reader, choose project settings (if applicable), then click **Next** and then **Load**.

6 Provide a name and select a file storage location for a new IFC run, or browse to select a predefined run file. Click **Next**.

7 For TaqMan assays, select **Genotyping**, **FAM,** and **VIC** or the appropriate dye, then click **Next**.

8 Confirm **Auto Exposure** is selected, then click **Start Run**.

Use a Biomark for End-Point Reads

1 Remove any dust particles or debris from the IFC surface.

2 Double-click the Biomark™ or Biomark HD Data Collection software icon on the desktop to launch the software.

3 Click **Start a New Run**.

4 Ensure that the status indicators for the Biomark lamp and the camera are green.

5 Place the IFC into the system. Click **Load**.

6 Verify IFC barcode and IFC type. Choose project settings (if applicable), then click **Next**.

7 Provide a name and select a file storage location for a new chip run or browse to select a predefined run file. Click **Next**.

8 Select **Genotyping** as the application and **ROX** as the reference.

9 Select probe types manually. For TaqMan assays, select **FAM** and **VIC** or the appropriate dye, then click **Next**.

10 Choose the protocol: navigate to the GT Protocol folder, choose **GT End Point v1.pcl**, click **Open**, then click **Next**.

11 Confirm **Auto Exposure** is selected, verify chip run information, and then click **Start Run**.

For technical support visit fluidigm.com/support

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