Genotyping with the Juno 96.96 Genotyping IFC Using SNP Type Assays

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

Prepare the 200 nM Primer Pool for Preamplification

1. In a new 1.5-mL microcentrifuge tube, combine 2 µL of 100 µM SNP Type™ assays specific target amplification primers (100 µM STA) up to a total of 96 assays. The total volume is *Y* in Table 1.
2. In the same microcentrifuge tube, combine 2 µL of each of the 100 µM SNP Type assays locus-specific primers (100 µM LSP) up to a total of 96 assays. The total volume is *Z* in Table 1.
3. Add Dilution Reagent to the SNP Type assays:

Table 1: SNP Type assays pool

Component	Vol. (µL)	Final Conc. (nM*)
SNP Type assays specific target amplification primers (100 µM STA)	*Y* (up to 96 assays)	200.0
SNP Type assays locus-specific primers (100 µM LSP)	*Z* (up to 96 assays)	200.0
Dilution Reagent (Fluidigm PN 100-8725)	1,000 – (*Y* + *Z*)	—
Total	**1,000.0**	**—**

*Final concentration of each primer in preamplification reaction is 50 nM.

Prepare 50X Primer Mix for Each Single Assay Inlet

In a DNA-free hood, in a new 96-well plate, combine the following reagents for each assay:

Component	Vol. per 40 µL Stock	Final Conc. (µM)
SNP Type assays allele-specific primers pooled ASP1 and ASP2 Primers (100 µM ASP1/100 µM ASP2)	3.0	7.5
SNP Type assays locus-specific primers (100 µM LSP)	8.0	20.0
Dilution Reagent	29.0	—
Total	**40.0**	**—**

Prepare 2X SNP Type Assays from the 50X Primer Mix for Genotyping

In a DNA-free hood, in a new 96-well plate, combine the following reagents for each assay:

Component	Vol. per 25 µL Stock (µL)	Final Conc.
50X Primer Mix	1.0	2X
Dilution Reagent	24.0	—
Total	**25.0**	**—**

Prepare the Assay Mix

1. In a DNA-free hood, in a new 1.5-mL microcentrifuge tube labeled “Assay Pre-Mix,” combine the Juno SNP Type GT Master Mix and 60X SNP Type Reagent. (See Table 2.)
2. Label a new 96-well plate “SNP TYPE ASSAY PLATE.” In a DNA-free hood, pipet 2.5 µL of assay pre-mix into each well.
3. Pipet 2.5 µL of 2X SNP Type assay into each well of the SNP Type assay plate.
4. In unused assay or no-assay control inlets, combine 2.5 µL of assay pre-mix with 2.5 µL of Dilution Reagent.
5. Seal the plate with MicroAmp® Clear Adhesive Film, vortex it for 5 seconds, then centrifuge it at 1,000 x *g* for 1 minute.

Table 2: Assay mix

Component	Vol. per Inlet (µL)	Vol. per Inlet with Overage (µL)	Assay Mix for IFC with Overage* (µL)
ASSAY PRE-MIX			
Juno SNP Type GT Master Mix (Fluidigm PN 100-8360)	1.933	2.417	290.0
60X SNP Type Reagent (Fluidigm PN 100-3402)	0.066	0.083	10.00
2X SNP Type assays†	2.00	2.50	—
Total	**4.00**	**5.00**	**300.0**

*120 reactions

† See "Prepare 2X SNP Type Assays from the 50X Primer Mix for Genotyping".

Prepare Sample Mix

IMPORTANT Before use, thaw reagents to room temperature. Thoroughly vortex and 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 3.)
2. Label a new 96-well plate “SAMPLE PLATE,” Pipet 2.25 µL of the sample pre-mix into each well of the plate. Do not add sample pre-mix to no template control wells.
3. In a DNA sample hood, pipet 2.75 µL of genomic DNA (for high-quality human, 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.

IMPORTANT Prepare at least one no template control.

5 Seal the plate with MicroAmp Clear Adhesive Film, vortex it for 5 seconds, then centrifuge it at 1,000 x *g* for 1 minute.

Table 3: Sample mix

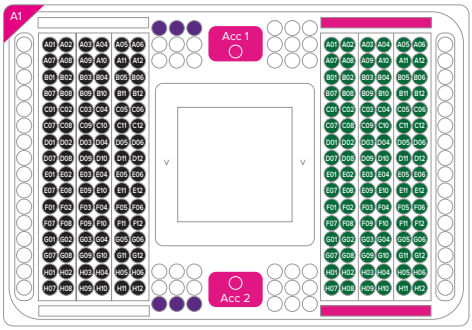
Component	Vol. per Inlet (µL)	Vol. per Inlet + Overage (µL)	Sample Mix for IFC,120 reactions (µL)
SAMPLE PRE-MIX			
Juno GT Preamp Master Mix (Fluidigm PN 100-8358)	0.800	1.00	120
Primer pool for preamplification	1.00	1.25	150
Genomic DNA	2.20	2.75	—
Total	4.00	5.00	270

Load and 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 SNP Type 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 at 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 SNP Type assay plate and pipet 4.0 µL of each assay mix into an assay inlet (black circles on 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. Start the run <60 minutes after pipetting the reagents into the IFC.

11 On the Juno scripts screen, tap the **SNP Type** tab**, Juno 96.96**, 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 lamp and 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 Select **Genotyping**, **ROX as reference**, and **SNPtype-FAM** and **SNPtype-HEX**, then click **Next**.

8 Confirm **Auto Exposure** is selected.

9 Click **Start Run**.

Use a Biomark™ or Biomark HD 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 to launch the software, and then click **Start a New Run**.

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

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

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

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

7 Select **Genotyping** as application and **ROX** as reference**.**

8 Select probe types manually. Select **SNPtype-FAM** and **SNPtype-HEX**, and then click **Next**.

9 In the GT Protocol folder, choose **GT End Point v1.pcl**, click **Open**, then click **Next**.

10 Confirm **Auto Exposure** is selected.

11 Verify the chip run information, and then click **Start Run**.

For technical support visit fluidigm.com/support

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