FLUIDIGM®

PN 100-7074 D1

Genotyping with Juno

Getting Started Guide

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About This Guide

CAUTION ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For complete safety information, see the safety appendix on page 30.

Purpose

This guide describes how to perform genotyping of low-concentration DNA with the Juno™ 96.96 Genotyping IFC (integrated fluidic circuit) on the Juno™ system. This is possible through advanced microfluidics technology that integrates preamplification and genotyping reactions of up to 96 samples and 96 genotyping assays in a single workflow on an IFC.

The IFC produces 9,216 genotypes in less than three hours using a simple workflow with minimal hands-on time. Samples are loaded into individual inlets of the Juno 96.96 Genotyping IFC, then distributed across multiple reaction chambers in nanoliter-volume aliquots. With high-quality samples, detecting the specific targets requires thermal cycling for preamplification and PCR for genotyping on the instrument.

After genotyping is performed on the Juno system, the IFC is scanned on the EP1™ system or the Biomark™ HD system to collect genotyping data for later analysis.

How to Use This Guide

The chapters in this guide are organized according to assay type. Refer to the appropriate chapter to run the Juno 96.96 Genotyping IFC on the Juno system.

For detailed instructions on instrument and software operation, refer to the Juno System User Guide (PN 100-7070).

Safety Alert Conventions

This guide uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

Fluidigm follows the United Nations Globally Harmonized System (GHS) for communicating chemical hazard information. GHS provides a common means of classifying chemical hazards and a standardized approach to chemical label elements and safety data sheets (SDSs). Key elements include:

- Pictograms that consist of a symbol on a white background within a red diamond-shaped frame. Refer to the individual SDS for the applicable pictograms and warnings pertaining to the chemicals being used.

- Signal words that alert the user to a potential hazard and indicate the severity level. The signal words used for chemical hazards under GHS:

  DANGER Indicates more severe hazards.

  WARNING Indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this guide uses the following indicators:

- Pictograms that consist of a symbol on a white background within a black triangle-shaped frame.

- Signal words that alert the user to a potential hazard and indicate the severity level. The signal words used for instrument hazards:

  DANGER Indicates an imminent hazard that will result in severe injury or death if not avoided.

  WARNING Indicates a potentially hazardous situation that could result in serious injury or death.

  CAUTION Indicates a potentially hazardous situation that could result in minor or moderate personal injury.

  IMPORTANT Indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm Corporation, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Getting Started

Workflow

	Reagent Handling	Automated Steps	Estimated Times
1	Prepare preamplification and genotyping assay and sample mixes		30–60 minutes
2	Pipet preamplification, genotyping mixes, and control line fluid into the IFC		10–20 minutes
3		Run a script to preamplify and genotype the DNA	2.5 hours (TaqMan protocol); 3.5 hours (SNP Type protocol)
4		Perform genotyping analysis on EP1 or Biomark systems	5–10 minutes

Best Practices

- Use good laboratory practices to minimize contamination of samples. Use a new pipette tip for every new sample. Whenever possible, separate pre- and post-PCR activities. Dedicate laboratory materials to designated areas.
- Unless otherwise specified, thaw reagents at room temperature, then use them at room temperature. Store reagents at their specified storage temperatures. (See “Required Kit Contents” on page 8.)
- Vortex reagents for 20 seconds, and then centrifuge reagents for 2 seconds before use.

Related Documentation

Go to fluidigm.com/documents

Chapter 1: Product Information

Required Kit Contents

The kits include the reagents required for preparing 10 IFCs to use on the Juno system. For suggested kits, see “Suggested Kits” on page 28.

IMPORTANT

- Do not pipet reagents from the TaqMan and SNPType assay kits into the same IFC. Use a different IFC for each kit. Do not mix reagents from different kits.
- Unless otherwise specified, thaw reagents at room temperature, then use them at room temperature. Store reagents at their specified storage temperatures. Vortex reagents for 20 seconds, then centrifuge reagents for 2 seconds before use.

TaqMan Assay Kit

Box	Component	Cap Color	Quantity	Volume per Tube (mL)	Storage
Juno Genotyping Kit for 10 IFCs (PN 100-8362)	Juno GT Preamp Master Mix	Light purple	1 tube	1.35	–20 °C
	Dilution Reagent	Natural	2 tubes	1.7	
	Probe GT Master Mix	Gold	2 tubes	1.6	
	Juno GT Flux Fluid	Purple	1 tube	0.9	
	Juno 96.96 Genotyping IFC—10 IFCs	—	10 IFCs	—	Room temperature
	Juno 96.96 GT Control Line Fluid	—	2 boxes; 20 syringes/box	—	

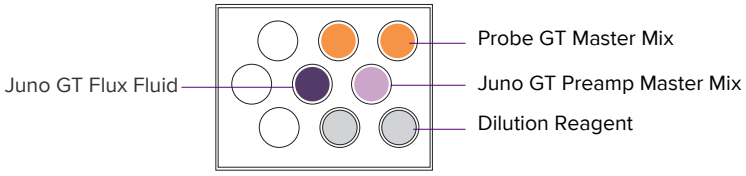


Figure 1. Juno™ Genotyping Kit for 10 IFCs (PN 100-8362).

SNP Type Assay Kit

Box	Component	Cap Color	Quantity	Volume per Tube or Bottle	Storage
Juno SNP Type Genotyping Kit for 10 IFCs (PN 100-8364)	Juno GT Preamp Master Mix	Light purple	1 tube	1.35 mL	–20 °C
	Juno SNP Type GT Master Mix	Light blue	2 tubes	1.6 mL	
	60X SNP Type Reagent	Amber	2 tubes	70 µL	
	Juno GT Flux Fluid	Purple	1 tube	1.0 mL	
	Dilution Reagent	Natural	• 2 bottles • 1 tube	• 3.7 mL • 1.7 mL	
	Juno 96.96 Genotyping IFC—10 IFCs	—	10 IFCs	—	Room temperature
	Juno 96.96 GT Control Line Fluid	—	2 boxes; 20 syringes/ box	—	

Figure 2. Juno™ SNP Type Genotyping Kit for 10 IFCs (PN 100-8364).

Required Reagents

TaqMan Assays

Product Name	Company	Part Number
20X, 40X, or 80X TaqMan® genotyping assays	Thermo Fisher Scientific	—

SNP Type Assays

Product Name	Company	Part Number
SNP Type assays specific target amplification Primers (100 µM STA)	Fluidigm	—
SNP Type assays ASP1/ASP2 (100 µM each)	Fluidigm	—
SNP Type assays LSP (100 µM each)	Fluidigm	—

Suggested Reagents

Product Name	Company	Part Number
UltraPure™ DNase/RNase-Free Distilled Water	Thermo Fisher Scientific	10977-015

Required Consumables

Product Name	Company	Part Number
Juno 96.96 Genotyping IFC: • Juno 96.96 Genotyping IFC • Juno 96.96 Genotyping IFC, 10 Pack	Fluidigm	 100-6499 100-8365
Disposable microcentrifuge tubes, polypropylene, 1.5 mL	Major laboratory supplier (MLS)*	—
96-well PCR plates	MLS†	—
MicroAmp® Clear Adhesive Film	Thermo Fisher Scientific	4306311

* Recommended: VWR® Slick Disposable Microcentrifuge Tubes, Polypropylene, 1.5 mL (VWR PN 20170-666)

† Recommended: TempPlate® semi-skirted 96-well PCR plates (USA Scientific PN 1402-9700)

Required Equipment

Product Name	Company	Part Number
Juno system, including system software version v3.1 or later, instrument, software, MX Interface Plate, Interface Plate Loading Fixture, Cleaning Plate, and Barrier Tape Applicator and Adapter	Fluidigm	101-6455
For Juno 96.96 Genotyping IFC: SX Interface Plate	Fluidigm	100-6368
Vortexer	MLS	—
Pipettes (P2, P20, P200, P1000) and appropriate low-retention tips	MLS	—
8-channel pipettes and appropriate low-retention tips	MLS	—
Microcentrifuge	MLS	—

Suggested Equipment

Product Name	Company	Part Number
Two biocontainment hoods (DNA hood and DNA-free hood) to prevent DNA contamination of lab and samples	MLS	—

Required Software

- Fluidigm Data Collection software v4.2 or later
- Fluidigm SNP Genotyping Analysis software v4.2 or later

IFC Type and Related Scripts

Barcode (prefix)	Scripts	Description
180x	Juno 96.96 Fast	Preamplification and genotyping of samples by TaqMan assays (180x)
180x	Juno 96.96	Preamplification and genotyping of samples by SNP Type assay (180x).

Chapter 2: Genotyping with the Juno 96.96 Genotyping IFC Using TaqMan Assays

Prepare Assay and Sample Mixes

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, labeled 1.5-mL microcentrifuge tube, combine 2 µL of each 20X TaqMan genotyping assay up to a total of 96 assays. The total volume of assays is 2*Y* in Table 1, where *Y* is the number of assays used. Each assay is at a final concentration of 0.2X in the primer pool.

3 Add Dilution Reagent to the 20X TaqMan assays:

Table 1. Prepare the primer pool for preamplification

Component	Volume (µL)	Final Concentration
20X TaqMan genotyping assays, 18 µM*	2*Y* (up to 96 assays)	180 nM (0.2X)
Dilution Reagent	200 – 2*Y*	—
Total	**200.0**	**—**

* See step 1.

The final concentration of each primer in the preamplification reaction is 45 nM.

NOTE The volume can be adjusted proportionally based on the number of samples to be amplified.

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 or DNase-free water	9.0	—
Total	**10.0**	**—**

Prepare the Assay Mix

1 Label a new 96-well plate, "TAQMAN ASSAY PLATE." In a DNA-free hood, pipet 2.5 µL of Probe GT Master Mix into each well. (See Table 2.)

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. (See "Prepare 2X TaqMan Assays for Genotyping".)

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 it for 5 seconds, then centrifuge it at 1,000 x *g* for 1 minute.

Table 2. Assay mix

Component	Volume (µL)
Probe GT Master Mix	2.5
2X TaqMan assays*	2.5
Total	**5.0**

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

Obtain the Minimum Required Genomic DNA

For high-quality human samples, the minimum DNA required is 2.5 ng/µL in 2.75 µL. Larger genomes require higher concentrations of genomic DNA.

Prepare the Sample Mix

1 In a DNA-free hood, in a new 1.5-mL microcentrifuge tube labeled "Sample Pre-Mix," combine the Juno GT Preamp Master Mix and the 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. Skip wells that are for no template controls. 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 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 it for 5 seconds, then centrifuge it at 1,000 x *g* for 1 minute.

Table 3. 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	0.8	1.00	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**

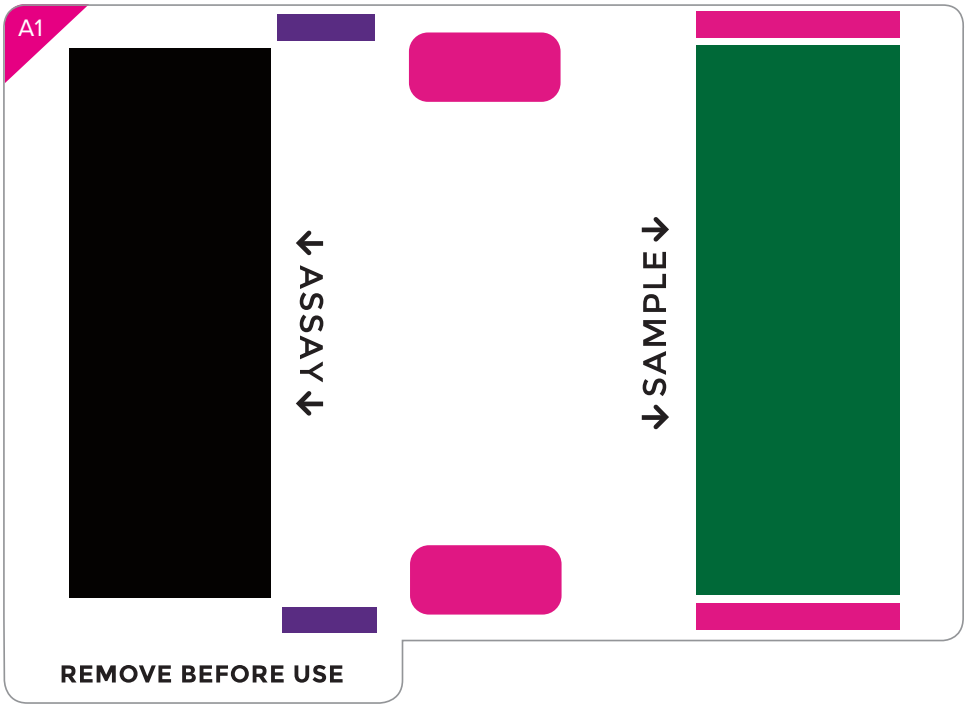
* 120 reactions for ease of pipetting

† See "Prepare the Primer Pool for Preamplification" on page 13.

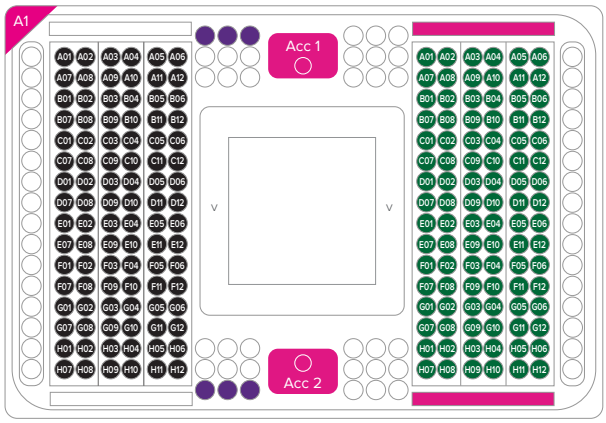
IMPORTANT Do not go past the first stop on the pipette. Doing so may introduce air bubbles into the inlets. To avoid bubbles, pipet 4.0 µL into each inlet from the 5.0 µL overage volume.

Load and Run the IFC on Juno

1 Review the loading map, which is affixed to the bottom of every new Juno 96.96 Genotyping IFC. The loading map is a general guide to show you how to pipet samples, assays, flux fluid, and control line fluid:



2 Review the pipetting map, which provides specific instructions for pipetting reagents in the IFC. Pipet reagents from the TaqMan assay plate and the sample plate to the IFC. On the pipetting map, each inlet is labeled with the plate well location of the sample or assay to be pipetted into that inlet:



Key			
Load 1		**Load 2**	
	Juno 96.96 GT Control Line Fluid		Assay mix, 4.0 µL
	Juno GT Flux Fluid, 15 µL		Sample mix, 4.0 µL
	Juno 96.96 GT Control Line Fluid	—	Empty

Figure 1. Pipetting map for the Juno 96.96 Genotyping IFC

3 Ensure that the notched corner of the IFC (“A1”) is at the top left.

4 Load an entire syringe of Juno 96.96 GT Control Line Fluid in Acc1 and a second syringe in Acc2. (See pink squares on the pipetting map.) To ensure correct accumulator volume, only use syringes containing Juno 96.96 GT Control Line Fluid.

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

IMPORTANT Carefully dispense control line fluid into the reservoirs. If control line fluid comes into contact with the sample inlets, use a new IFC.

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

7 Unseal the TaqMan assay plate and pipet 4.0 µL of each assay mix into an assay inlet. (See black circles on the pipetting map and “Prepare the Assay Mix” on page 14.)

8 Unseal the sample plate and pipet 4.0 µL of each sample mix into a sample inlet. (See green circles on the pipetting map and “Prepare the Sample Mix” on page 15.)

IMPORTANT Do not go past the first stop on the pipette. Doing so may introduce air bubbles into the inlets. Pipet 4.0 µL from the 5.0 µL overage volume to ensure that no air bubbles enter the inlet.

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

10 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.

11 Ensure that the SX interface plate (silver label) is installed in the instrument. [See the Juno System User Guide (PN 100-7070).]

12 Place the IFC into the Juno instrument, then start the run <60 minutes after pipetting the reagents into the IFC.

13 On the Juno Scripts screen, tap the **Probe GT** tab, **Juno 96.96 Fast**, then **Run**. It takes ~2.7 hours to complete.

The script contains these thermal cycling protocols:

Cycles	Temperature	Time
Hot start	95 °C	2 min
14	95 °C	15 sec
	60 °C	4 min

Cycles	Temperature	Time
Hot start	95 °C	2 min
45	95 °C	2 sec
	60 °C	20 sec

14 After the run is finished, tap **EJECT** to eject the IFC.

IMPORTANT After a run, perform an end-point read of the IFC in ≤1 hour. Do not leave the IFC overnight in the instrument. Doing so will adversely affect the reaction.

Perform Genotyping Analysis on the Samples

Refer to the appropriate document:

- SNP Genotyping User Guide (PN 68000098)
- Biomark HD Data Collection User Guide (PN 100-2451)
- Biomark/EP1 Data Collection User Guide (PN 68000127).

Chapter 3: Genotyping with the Juno 96.96 Genotyping IFC Using SNP Type Assays

Prepare Assay and Sample Mixes

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 4.

2 In the same microcentrifuge tube, combine 2 µL of 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 4.

3 Add Dilution Reagent to the SNP Type assays:

Table 4. Pool SNP Type assays

Component	Volume (µL)	Final Concentration (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	1,000 – (*Y* + *Z*)	—
Total	**1,000.0**	**—**

* The final concentration of each primer in the preamplification reaction is 50 nM.

NOTE

- Volume can be adjusted proportionally based on the number of samples to be amplified.
- You can store the pooled SNP Type STA assays at –20 °C for 1 year or ≤10 freeze-thaw cycles, whichever is shorter.

Prepare 2X SNP Type Assays

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	Volume per 40 µL Stock (µL)	Final Concentration (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***	—

* A 40.0-µL volume is sufficient for 40, 2X SNP Type assays.

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	Volume per 25 µL Stock (µL)	Final Concentration
50X Primer Mix*	1.0	2X
Dilution Reagent	24.0	—
Total	**25.0†**	—

* See “Prepare 50X Primer Mix for Each Single Assay Inlet”.

† A 25.0-µL volume is sufficient for 10 IFC runs.

NOTE You can store the 2X SNP Type assays at –20 °C for up to one week.

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 to prepare the assay pre-mix. (See Table 5.)

2 Label a new 96-well plate "SNP TYPE ASSAY PLATE." In a DNA-free hood, pipet 2.5 µL of the 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 5 Assay mix

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Assay Mix for IFC with Overage* (µL)
ASSAY PRE-MIX			
Juno SNP Type GT Master Mix	1.933	2.417	290.0
60X SNP Type Reagent	0.066	0.083	10.00
2X SNP Type assays†	2.00	2.5	—
Total	**4.00**	**5.00**	**300**

* 120 reactions for ease of pipetting

† See "Prepare 2X SNP Type Assays" on page 21.

Obtain the Minimum Required Genomic DNA

For high-quality human samples, the minimum DNA required is 2.5 ng/µL. Larger genomes require higher concentrations of genomic DNA.

Prepare the Sample Mix

1 In a DNA-free hood, in a new microcentrifuge tube labeled "Sample Pre-Mix," combine the Juno GT Preamp Master Mix and the primer pool for preamplification to prepare the sample pre-mix. (See Table 6.)

2 Label a new 96-well plate "SAMPLE PLATE," and then 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.

IMPORTANT Prepare at least one no template control.

3 In a DNA sample hood, pipet 2.75 µL of genomic DNA 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 it for 5 seconds, then centrifuge it at 1,000 x *g* for 1 minute:

Table 6 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	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

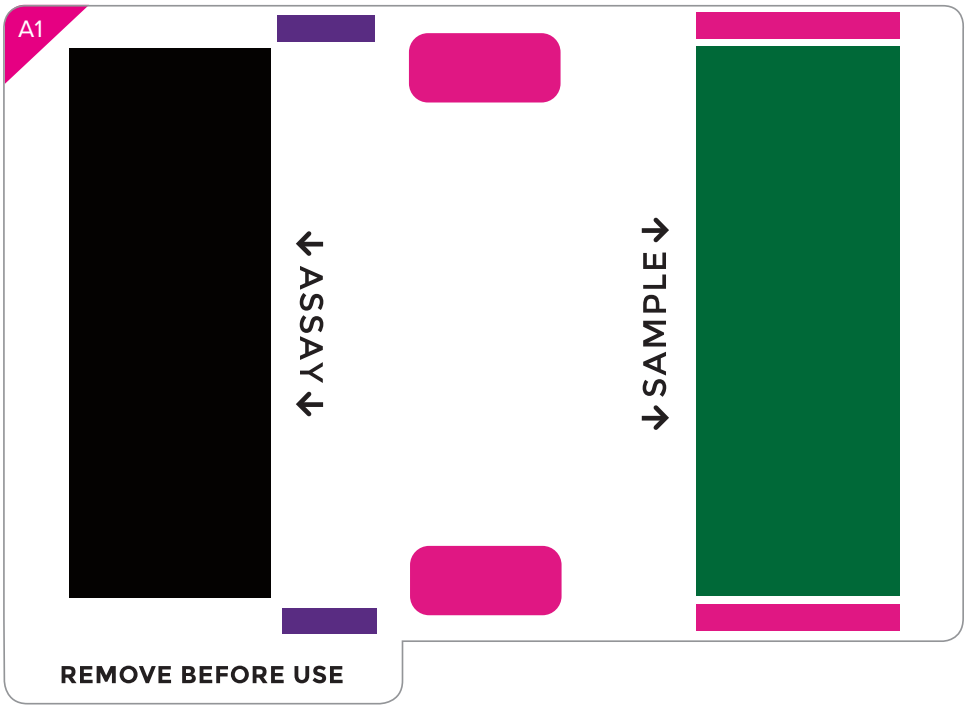
* 120 reactions for ease of pipetting

† See "Prepare the 200 nM Primer Pool for Preamplification" on page 20.

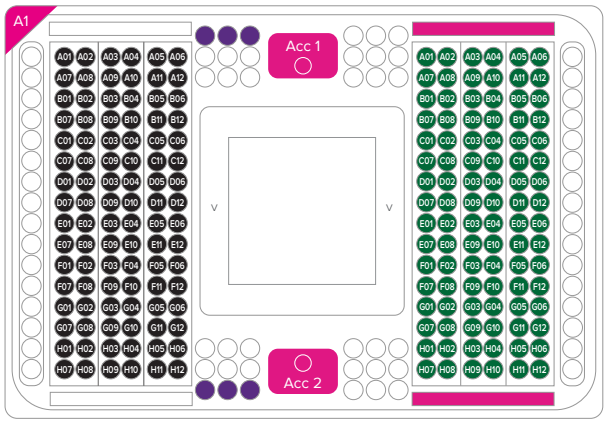
IMPORTANT Do not go past the first stop on the pipette. Doing so may introduce air bubbles into the inlets. To avoid bubbles, pipet 4.0 µL into each inlet from the 5.0 µL overage volume.

Load and Run the IFC on Juno

1 Review the loading map, which is affixed to the bottom of every new Juno 96.96 Genotyping IFC. The loading map is a general guide to show you how to pipet samples, assays, and control line fluid:



2 Review the pipetting map, which provides specific instructions for pipetting reagents in the IFC. Pipet reagents from the SNP Type assay plate and the sample plate to the IFC. On the pipetting map, each inlet is labeled with the plate well location of the sample or assay to be pipetted into that inlet:



Key			
Load 1		**Load 2**	
	Juno 96.96 GT Control Line Fluid		Assay mix, 4.0 µL
	Juno GT Flux Fluid, 15 µL		Sample mix, 4.0 µL
	Juno 96.96 GT Control Line Fluid	—	Empty

3 Ensure that the notched corner of the IFC (“A1”) is at the top left.

4 Load an entire syringe of Juno 96.96 GT Control Line Fluid in Acc1 and a second syringe in Acc2. (See the pink squares on the pipetting map.) To ensure correct accumulator volume, only use syringes containing Juno 96.96 GT Control Line Fluid.

5 Load an entire syringe of Juno 96.96 GT Control Line Fluid into each of the two reservoirs. (See the long pink rectangles on the right side of the pipetting map.)

IMPORTANT Carefully dispense control line fluid into the reservoirs. If control line fluid comes into contact with the sample inlets, use a new IFC.

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

7 Unseal the SNP Type assay plate and pipet 4.0 µL of each assay mix into an assay inlet. (See the black circles on the pipetting map and “Prepare the Assay Mix” on page 22.)

8 Unseal the sample plate and pipet 4.0 µL of each sample mix into a sample inlet. (See the green circles on the pipetting map and “Prepare the Sample Mix” on page 23.)

IMPORTANT Do not go past the first stop on the pipette. Doing so may introduce air bubbles into the inlets. Pipet 4.0 µL from the 5.0 µL overage volume to ensure that no air bubbles enter the inlet.

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

10 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.

11 Ensure that the SX interface plate (silver label) is installed in the instrument. [See the Juno System User Guide (PN 100-7070).]

12 Place the IFC into the Juno instrument, then start the run <60 minutes after pipetting the reagents into the IFC.

13 On Juno Scripts screen, tap the **SNP Type** tab**, Juno 96.96**, then **Run**. It takes 3 hours and 20 minutes to complete.

The script contains these thermal cycling protocols:

Table 7 Multiplex STA

Cycles	Temperature	Time
Hot start	95 °C	2 min
14	95 °C	15 sec
	60 °C	4 min

Table 8 SNP Type genotyping

Cycles	Temperature	Time
Hot start	95 °C	10 min
1	95 °C	15 sec
	64 °C	45 sec
	72 °C	15 sec
1	95 °C	15 sec
	63 °C	45 sec
	72 °C	15 sec
1	95 °C	15 sec
	62 °C	45 sec
	72 °C	15 sec
1	95 °C	15 sec
	61 °C	45 sec
	72 °C	15 sec
39	95 °C	15 sec
	60 °C	45 sec
	72 °C	15 sec

14 After the run is finished, tap **EJECT** to eject the IFC from the instrument.

IMPORTANT After a run, do not leave the IFC overnight in the instrument. Doing so will adversely affect the reaction.

Perform Genotyping Analysis on the Samples

Refer to the appropriate document:

- SNP Genotyping User Guide (PN 68000098)
- Biomark HD Data Collection User Guide (PN 100-2451)
- Biomark/EP1 Data Collection User Guide (PN 68000127).

Appendix A: Suggested Kits

Reagents and IFCs are available separately.

IMPORTANT Unless otherwise specified, thaw reagents at room temperature, then use them at room temperature. Store reagents at their specified storage temperatures. Vortex reagents for 20 seconds, then centrifuge reagents for 2 seconds before use.

TaqMan Assay Kit

Box	Component	Cap Color	Quantity	Volume per Tube or Bottle (mL)	Storage
Juno Genotyping Reagent Kit for 10 IFCs (PN 100-8361)	Juno GT Preamp Master Mix	Light purple	1 tube	1.35	–20 °C
	Dilution Reagent	Natural	2 tubes	1.7	
	Probe GT Master Mix	Gold	2 tubes	1.6	
	Juno GT Flux Fluid	Purple	1 tube	0.9	

SNP Type Assay Kit

Box	Component	Cap Color	Quantity	Volume per Tube or Bottle	Storage
Juno SNP Type Genotyping Reagent Kit (PN 100-8363)	Juno GT Preamp Master Mix	Light purple	1 tube	1.35 mL	–20 °C
	Juno SNP Type GT Master Mix	Light blue	2 tubes	1.6 mL	
	60X SNP Type Reagent	Amber	2 tubes	70 µL	
	Juno GT Flux Fluid	Purple	1 tube	1.0 mL	
	Dilution Reagent	Natural	• 2 bottles • 1 tube	• 3.7 mL • 1.7 mL	

Suggested Reagents to Use with TaqMan Assay and SNP Type Assay Kits

Box	Component	Cap Color	Quantity	Volume per Tube or Bottle (mL)	Storage
Dilution Reagent (PN 100-8726)	Dilution Reagent	Natural	1 bottle	25	–20 °C
Juno GT IFC and Control Line Fluid Kit (PN 100-8583)	Juno 96.96 Genotyping IFC—10 IFCs	—	10 IFCs	—	Room temperature
	Juno 96.96 GT Control Line Fluid	—	2 boxes; 20 syringes/ box	—	
Juno 96.96 Genotyping IFC (PN 100-8365)	Juno 96.96 Genotyping IFC	—	10 IFCs	—	Room temperature
Juno 96.96 Genotyping IFC (PN 100-6499)	Juno 96.96 Genotyping IFC	—	1 IFC	—	Room temperature
Juno 96.96 GT Control Line Fluid (PN 100-8574)	Juno 96.96 GT Control Line Fluid	—	20 syringes	—	Room temperature

Appendix B: Safety

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Instrument Safety

WARNING Do not modify this device. Unauthorized modifications may create a safety hazard.

CAUTION HOT SURFACE The Juno thermal cycler chuck gets hot and can burn your skin. Use caution when working near the chuck.

CAUTION PINCH HAZARD. The Juno door and tray can pinch your hand. Make sure your fingers, hand, shirtsleeves are clear of the door and tray when loading or ejecting an integrated fluidic circuit (IFC).

WARNING BIOHAZARD. If you are putting biohazardous material on the instrument, use appropriate personal protective equipment and adhere to *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) from the Centers for Disease Control and Prevention and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines at: cdc.gov/biosafety/publications/index.htm

For a full list of the symbols on the instrument, refer to the Juno System User Guide (PN 100-7070).

Electrical Safety

WARNING ELECTRICAL HAZARD. Electrical shock can result if the Juno instrument is operated without its protective covers.

WARNING ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Chemical Safety

Read and comprehend all safety data sheets (SDSs) by chemical manufacturers before you use, store, or handle any chemicals or hazardous materials.

Wear personal protective equipment (gloves, safety glasses, fully enclosed shoes, lab coats) when handling chemicals.

Do not inhale fumes from chemicals. Use adequate ventilation, and return caps to bottles immediately after use.

Check regularly for chemical spills or leaks. Follow SDS recommendations for cleaning up spills or leaks.

Disposal of Products

Used IFCs should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

Do not dispose of this product in unsorted municipal waste. This equipment may contain hazardous substances that could affect health and the environment. Use appropriate take-back systems when disposing of materials and equipment.

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For technical support visit
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