

# SNP Genotyping

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# About this User Guide

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## How to Use This Guide

The following chapters provide information about the analysis software and protocols for SNP genotyping on the Biomark™, Biomark HD, or EP1 systems.

## Safety Alert Conventions

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

### Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
<b>DANGER</b>	Signal word that indicates more severe hazards.
<b>WARNING</b>	Signal word that indicates less severe hazards.

## Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the instrument user guide for the applicable pictograms and hazards pertaining to instrument usage.
<b>DANGER</b>	Signal word that indicates an imminent hazard that will result in severe injury or death if not avoided.
<b>WARNING</b>	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.
<b>CAUTION</b>	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
<b>IMPORTANT</b>	Signal word that 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, either alone or as part of this system, go to [fluidigm.com/sds](http://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.

## Related Documents

This document is intended to be used in conjunction with these related documents:

- *Fluidigm Biomark HD Data Collection Software User Guide* (PN 100-2451)
- *Fluidigm Biomark / EP1 Data Collection Software User Guide* (PN 68000127)
- *Fluidigm® SNP Trace™ Panel User Guide* (PN 100-7282)
- *Fluidigm IFC Controller Setup Quick Reference* (PN 68000117)
- *Fluidigm IFC Controller Usage Quick Reference* (PN 68000126)
- *Fluidigm Control Line Fluid Loading Procedure Quick Reference* (PN 68000132)
- *Fluidigm 48.48 Genotyping Workflow Quick Reference* (PN 68000099)
- *Fluidigm 48.48 Fast Genotyping Workflow Quick Reference* (PN 100-3882)
- *Fluidigm 48.48 SNPtype Genotyping Workflow Quick Reference* (PN 100-3910)
- *Fluidigm 96.96 Genotyping Workflow Quick Reference* (PN 68000129)
- *Fluidigm 96.96 Fast Genotyping Workflow Quick Reference* (PN 100-3909)
- *Fluidigm 96.96 SNPtype Genotyping Workflow Quick Reference* (PN 100-3912)
- *Fluidigm 192.24 Genotyping Workflow Quick Reference* (PN 100-3184)
- *Fluidigm 192.24 SNPtype Genotyping Workflow Quick Reference* (PN 100-3913)



# System Overview

1

## Genotyping Overview

Genotyping involves the analysis of variations in genomes across individual organisms. These variations often take the form of single nucleotide changes, known as single nucleotide polymorphisms, or SNPs, that can determine the characteristics and ultimate health of the organism. In SNP genotyping studies, the DNA sequences of a group of individuals are analyzed, often using a form of polymerase chain reaction (PCR), to determine which individuals have particular SNP profiles. Statistical analysis is then performed to determine whether a SNP or group of SNPs can be associated with a particular characteristic.

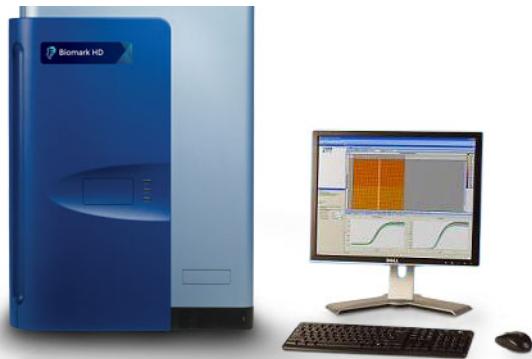
**NOTE:** To genotype your samples with the SNP Trace™ Panel, a set of SNPs, such as highly polymorphic SNPs, gender SNPs, ethnicity-specific or continental-specific SNPs, refer to the *Fluidigm® SNP Trace™ Panel User Guide* (PN 100-7282).

## System Components for Genotyping

**IMPORTANT:** Update all instrument firmware and analysis software to version 4 (v4) or later.

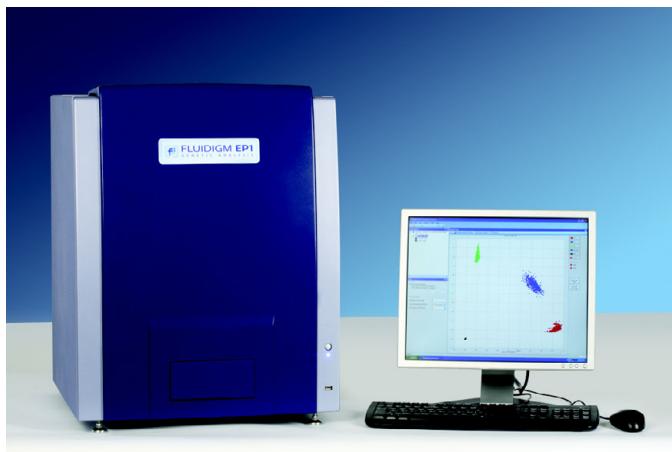
### Biomark™ System Components

Biomark™ Systems include an internal thermal cycler, flat panel monitor, keyboard, and mouse. (The Biomark™ HD System is pictured.)



## EP1™ Reader Components

The EP1™ Reader is an endpoint detection system that includes a flat panel monitor, keyboard, and mouse.



## FC1™ Cycler

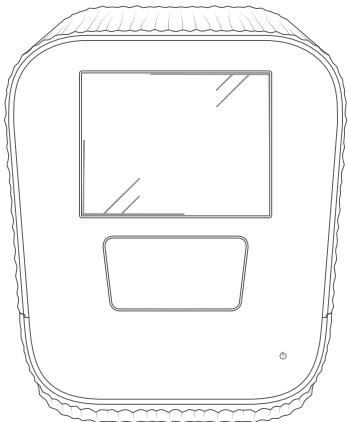
The FC1™ Cycler is a compact single-bay instrument that is used to thermal cycle IFCs.



## Controllers for IFCs

### Juno

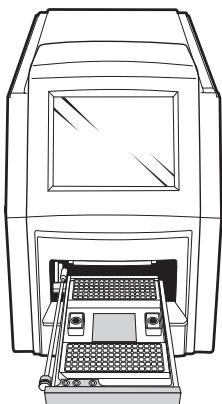
Juno™ is a universal controller that primes, loads, and thermal cycles assay-sample mixes.



The controllers were designed specifically to work with specific chips:

- **IFC Controller HX** (pictured)—for priming and loading the Flex Six™ Genotyping IFC and the 96.96 Genotyping IFC.
- **IFC Controller MX**—for priming and loading the 48.48 Dynamic Array™ IFC.
- **IFC Controller RX**—for loading the 192.24 Genotyping IFC.

The IFC Controller MX primes and loads the 12.765 Digital Array™ IFC, the 48.770 Digital Array™ IFC, and the qPCR 37K™ IFC.



**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (not an FC1 Cycler or the Biomark HD System), see Appendix D for usage instructions or contact Fluidigm Technical Support for a Personal Card, which includes SNP Type™ scripts specific to the SATC.

## Integrated Fluidic Circuit (IFC) Components

Although chip architecture varies, the essential components, such as sample and assay wells and accumulators are common to all.

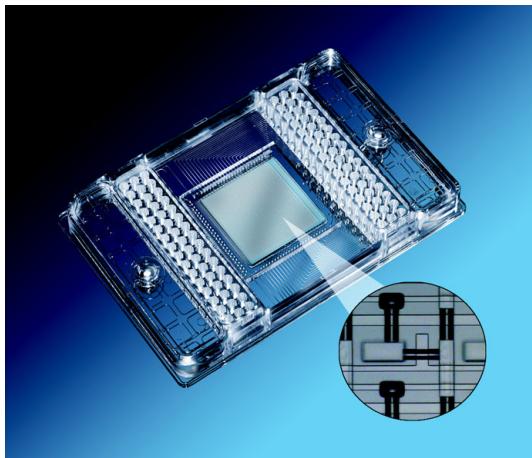
### Flex Six™ Genotyping IFC



The Fluidigm Flex Six™ Genotyping IFC addresses the requirement for substantial variation in sample and assay numbers during target selection while allowing complete use of the IFC. It utilizes a completely new architecture which incorporates six 12 X 12 partitions that can be organized in any configuration, in up to six separate experimental runs. This new IFC adjusts to customers' experimental needs during target selection and

largely eliminates the need for microplate-based experiments.

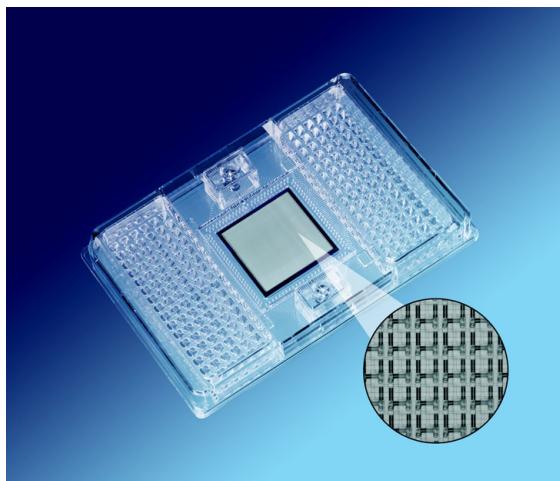
### 48.48 Dynamic Array™ IFC



The Fluidigm 48.48 Dynamic Array™ IFC is an efficient solution for large-scale, real-time qPCR. The key to this efficiency is the matrix of channels, chambers, and integrated valves finely patterned into layers of silicone. This material is gas permeable, allowing the blindfill of fluids into valve-delimited chambers. The valves partition samples and reagents and allow them to be systematically combined into 2,304 assays.

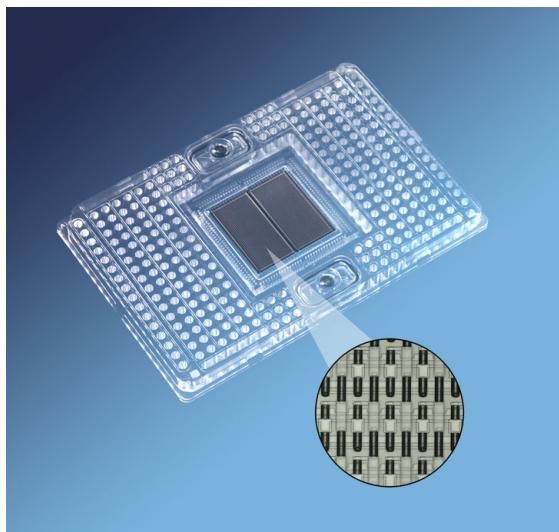
The significance of this approach to operational efficiency is immense. Managing a gene expression study involving 2,000 samples against a set of 48 genes would require 1,000 96-well plates as compared to 42 Dynamic Array™ IFCs. Managing the same study would require 192,000 steps on microplates but only 4,032 liquid-transfer steps on the chips. Comparative time required to complete such a study would typically involve 100 days on plates but just 4 1/2 days on chips. In addition, the running cost is reduced by half or more.

## 96.96 Dynamic Array™ IFC



The Fluidigm 96.96 Dynamic Array™ IFC is similar to the 48.48 Dynamic Array™ IFC but with high throughput. On one side of the frame are 96 wells to accept the samples and, on the other, 96 wells to accept the probe and primer pairs. Once in the wells, the components are pressurized into the chip using an IFC controller. The components are then systematically combined into 9,216 parallel reactions.

## 192.24 Genotyping IFC



The Fluidigm 192.24 Genotyping IFC provides a solution for targeted high sample throughput SNP genotyping. It is designed to genotype 192 samples against 24 assays in a single run and incorporates innovative design features to greatly increase sample throughput. Once in the wells, the components are pressurized into the chip using an IFC Controller. The components are then systematically combined into 4,608 parallel reactions.

## Workflow for Standard IFCs

The simplicity of running experiments is illustrated in the process below. For more detail, see *Fluidigm 48.48 Genotyping Workflow Quick Reference* (PN 68000099), *Fluidigm 96.96 Genotyping Workflow Quick Reference* (PN 68000129), or *Fluidigm 192.24 Genotyping Workflow Quick Reference* (PN 100-3184).

- 1 Prime the genotyping IFC (Integrated Fluidic Circuit) (192.24 Dynamic Array IFC does not require priming).
- 2 Add the samples and assays to the IFC.
- 3 Load and mix samples and assays in the IFC Controller.
- 4 Perform PCR on the Biomark System or FC1 Cycler.
- 5 Collect images.

## Workflow for Reusable IFCs

The simplicity of running experiments is illustrated in the workflow below.

- 1 Prime the IFC in the IFC Controller (only required for first use).
- 2 Add samples and assays to the IFC.
- 3 Load the IFC in the *Load* IFC Controller.
- 4 Perform PCR on the Biomark System or FC1 Cycler.
- 5 Collect images.
- 6 Perform cleaning of the IFC in the *Clean* IFC Controller and reuse.

## What You Need for Experiments

This section describes the materials that you need to perform your experiments including the reagents we support and sample requirements. If you deviate from the recommended procedures and material quantities, call Technical Support for help administering your experiments.

In addition, you need the following:

- Biomark or Biomark HD System or EP1 Reader with FC1 Cycler
- IFC Controller MX, HX, or RX (depending on IFC type)
- 48.48 Dynamic Array IFC-Genotyping, 96.96 Dynamic Array IFC-Genotyping or 192.24 Dynamic Array IFC-Genotyping
- Reagents

**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (not an FC1 Cycler or the Biomark™ HD System), please see Appendix D for usage instructions or contact Fluidigm Technical Support for a Personal Card, which includes SNP Type™ scripts specific to the SATC.

## Supported Reagents

Fluidigm currently supports Life Technologies TaqMan® reagents and Fluidigm SNP Type™ reagents.

### TaqMan® Assays and Reagents

Life Technologies provides TaqMan® reagents for targeted and genome-wide discovery of single nucleotide polymorphisms (SNPs), copy number variants, and other genetic variations.

The necessary reagents for TaqMan genotyping are as follows:

<b>Store at -20 °C</b>
<ul style="list-style-type: none"> <li>• TaqMan® Pre-Designed SNP Genotyping Assays (Life Technologies, PN 4351374)</li> <li>• AmpliTaq Gold® DNA Polymerase (Life Technologies, PN 4311806)*</li> <li>• 2X Assay Loading Reagent (Fluidigm, PN 85000736)</li> <li>• ROX reference dye 50X (Life Technologies, PN 12223-012)</li> <li>• Genomic DNA</li> </ul>
<b>Store at 4 °C</b>
<ul style="list-style-type: none"> <li>• TaqMan® Universal PCR Master Mix (Life Technologies, 4304437)**</li> <li>• GT Sample Loading Reagent (Fluidigm PN 85000741)***</li> <li>• 96.96 GT PCR Dilution Reagent (PN 100-9977)</li> </ul>
<b>Store at room temperature</b>
<ul style="list-style-type: none"> <li>• Deionized DNA-free, DNase-free, RNase-free water</li> <li>• TE: 10 mM Tris HCl, 0.1 mM EDTA, pH 8.0 (Teknova PN T0221) (also known as DNA Suspension Buffer).</li> </ul>

\* For reusable IFCs, do not use AmpliTaq Gold  
\*\*For reusable IFCs or fast thermal cycling applications, do not use TaqMan Universal PCR mix, use TaqMan® GTxpress™ Master Mix instead.  
\*\*\* For reusable IFCs or fast thermal cycling applications, do not use GT Sample Loading Reagent, use Fast Sample Loading Reagent (Fluidigm, PN 100-3065) instead.

TaqMan protocols are included in Appendix A of this manual.

## SNP Type™ Assays and Reagents

Fluidigm SNP Type Assays are an allele-specific PCR detection system. It offers a low-cost, high-quality solution for SNP genotyping and are designed to work seamlessly with the EP1™, Biomark, or the Biomark™ HD System. It also provides the advantages of minimal experiment setup time and optimized protocols for Dynamic Array integrated fluidic circuits (IFCs).

Assays are provided in three separate oligo plates per 96 assays. All oligos are provided in nuclease-free water.

- Allele-Specific Primers (ASP)—mixed in equal molar ratios and normalized to 100 µM in 100 µL for small; 200 µL for medium; 500 µL for large (each primer)
- Specific Target Amplification (STA) primer—individual primers normalized to 100 µM in 100 µL for small; 200 µL for medium; 500 µL for large (each primer)
- Locus-Specific Primer (LSP)—individual primers normalized to 100 µM in 100 µL for small; 200 µL for medium; 500 µL for large (each primer)

SNP Type Assays use the same probe set in every reaction to enable low startup and low running costs, which are critical for high sample throughput applications such as production-scale SNP genotyping and SNP confirmatory studies. SNP Type Assays have been developed to provide excellent results on Dynamic Array IFCs and are formatted to work directly with existing workflows.

In conjunction with the FC1 Cycler, SNP Type Assays permit fast thermal cycling, allowing faster time-to-results and thereby greatly increasing sample throughput.

Protocols for genotyping with SNP Type Assays can be found in appendix C of this user guide.

The necessary reagents for SNP Type genotyping are as follows:

Store at -20 °C
<ul style="list-style-type: none"><li>• Biotium Fast Probe Master Mix (Biotium, PN 31005)</li><li>• Qiagen 2X Multiplex PCR Master Mix (Qiagen, PN 206143)</li><li>• SNP Type Genotyping Reagent Kit 48.48 (Fluidigm, PN 100-4135) This kit is sufficient for ten 48.48 Dynamic Array IFCs.<ul style="list-style-type: none"><li>- 1 Assay Loading Reagent, 2X (1.5 mL)</li><li>- 1 SNP Type Sample Loading Reagent, 20X (250 µL)</li><li>- 1 SNP Type Reagent, 60X (70 µL)</li></ul></li><li>• SNP Type Genotyping Reagent Kit 96.96 (Fluidigm, PN 100-4134) This kit is sufficient for ten 96.96 Dynamic Array IFCs.<ul style="list-style-type: none"><li>- 2 Assay Loading Reagent, 2X (1.5 mL)</li><li>- 2 SNP Type Sample Loading Reagent, 20X (250 µL)</li><li>- 2 SNP Type Reagent, 60X (70 µL)</li></ul></li><li>• SNP Type Genotyping Reagent Kit 192.24 (Fluidigm, PN 100-4136) This kit is sufficient for ten 192.24 Dynamic Array IFCs.<ul style="list-style-type: none"><li>- 1 Assay Loading Reagent, 2X (1.5 mL)</li><li>- 2 SNP Type Sample Loading Reagent, 20X (250 µL)</li><li>- 2 SNP Type Reagent, 60X (70 µL)</li><li>- 2 Pressure Fluid syringes (3.5 mL each)</li></ul></li><li>• SNP Type Assays<ul style="list-style-type: none"><li>- SNP Type Assay Allele-Specific Primers (ASP) Plate (100 µM ASP1/100 µM ASP2)</li><li>- SNP Type Assay Locus-Specific Primer (LSP) plate (100 µM)</li><li>- SNP Type Assay Specific-Target Amplification (STA) primer plate (100 µM)</li></ul></li><li>• Genomic DNA</li></ul>
Store at Room Temperature
<ul style="list-style-type: none"><li>• DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA) (Teknova, PN T0221)</li><li>• PCR-certified water</li><li>• 20 Control line fluid syringes (300 µL each) (from Fluidigm SNP Type Genotyping Reagent Kit 48.48)</li><li>• 20 Control line fluid syringes (150 µL each) (from Fluidigm SNP Type Genotyping Reagent Kit 96.96)</li><li>• 10 Control line fluid syringes (160 µL each) (from Fluidigm SNP Type Genotyping Reagent Kit 192.24)</li></ul>

## Supported Detection Reagents

We support the following genotyping detection reagents with the System.

### Probe Types

For TaqMan

- FAM-MGB
- VIC-MGB

For SNP Type

- SNPtype-FAM
- SNPtype-HEX

### Additional Probe Types

Fluidigm does not support other probe types at this time, however, additional probe types may be run with the System using the following guidelines:

Fluorophores With...		
Excitation Wavelengths		Emission Wavelengths
between 465 and 505 nm	And	between 500 and 550 nm
between 510 and 550 nm	And	between 540 and 600 nm

## PCR Master Mixes

Standard TaqMan genotyping protocols use TaqMan® Universal PCR Master Mix (2X) (Life Technologies PN 4304437). Fast TaqMan genotyping protocols use TaqMan® GTxpress™ Master Mix. SNP Type genotyping protocols use Biotium Fast Probe Master Mix (Biotium, PN 31005). If you choose to use master mixes other than those listed above, you may have to alter the protocol described in this manual. Contact Fluidigm Technical Support for additional information.

**IMPORTANT:** You must use a passive reference (ROX).

# Sample Requirements

## DNA Quality

- The DNA should have a 260:280 ratio between 1.5 and 1.8.
- Prior to use on an IFC, check the integrity of your DNA on a system such as the Agilent 2100 Bioanalyzer.
- For human DNA, use a concentration of  $\geq 60 \text{ ng}/\mu\text{L}$ . If you intend to use lesser concentrations, contact Fluidigm Technical Support for advice.
- If the DNA concentration is  $< 60 \text{ ng}/\mu\text{L}$ , we recommend specific target amplification (STA) (see “[SNP Type Assays for SNP Genotyping on the 48.48 or 96.96 Dynamic Array IFCs](#)” on page 132 or “[SNP Type Assays for SNP Genotyping on the 192.24 Dynamic Array IFC](#)” on page 141). The minimum recommended sample concentration for STA is  $10 \text{ ng}/\mu\text{L}$ .
- We recommend performing specific target amplification (STA) on DNA derived from species with genomes significantly larger than human size.

## DNA Storage

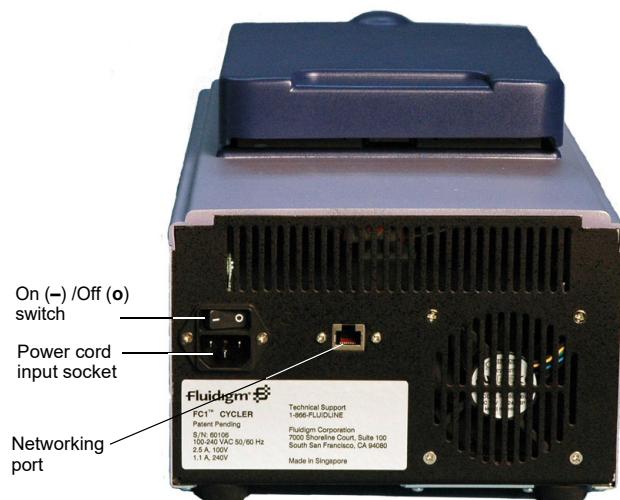
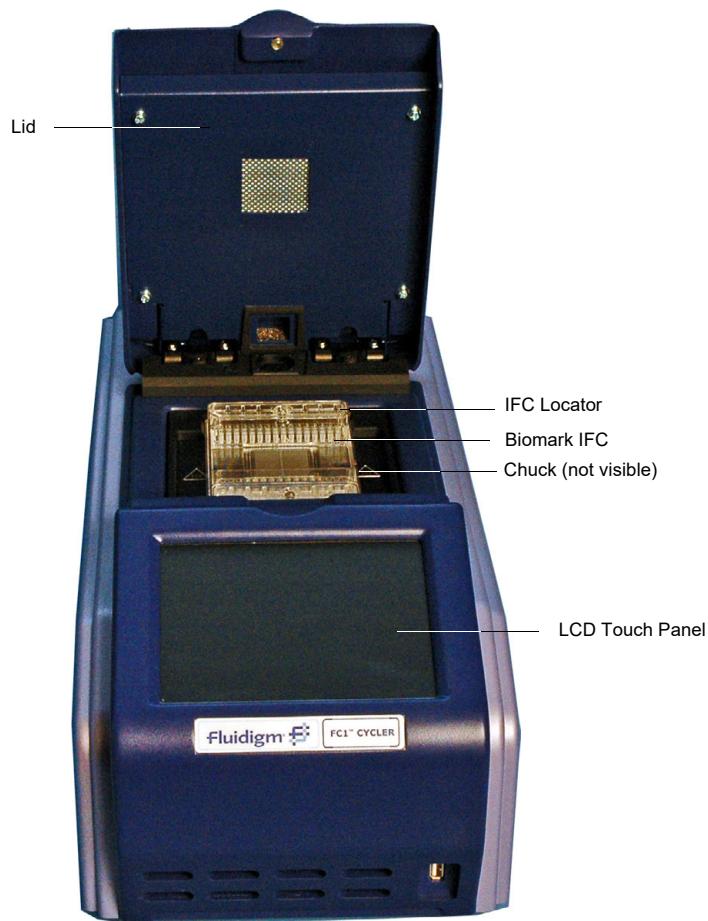
Avoid multiple freeze-thaw cycles by storing DNA at  $4^\circ\text{C}$ . For longer storage, aliquots may be stored at  $-20^\circ\text{C}$ .

## No Template Control (NTC)

The Fluidigm® SNP Genotyping Analysis Software requires at least one NTC to perform auto-calling. The NTC or negative control is used to normalize the data against background. The NTC contains Biotium Fast Probe Master Mix, 20X SNP Type™ Sample Loading Reagent, 60X SNP Type™ Reagent, and ROX. The NTC has water or buffer substituted for the template (DNA sample).

## Using the Fluidigm FC1™ Cycler

**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (not an FC1 Cycler or the Biomark™ HD System), please see Appendix D for usage instructions or contact Fluidigm Technical Support for a Personal Card, which includes SNP Type™ scripts specific to the SATC.



## Powering On the FC1 Cycler

**NOTE:** The FC1 Cycler uses a touch-screen interface, therefore all interactions with the application occur by touching the screen.

- 1 If you are using a powerstrip, turn it ON.
- 2 Press the switch at the back of the FC1 Cycler to the ON position.

**NOTE:** Initialization may take up to 1 minute to complete.

- 3 At completion of power up and instrument initialization, the Fluidigm FC1 Cycler home screen appears.

## Login

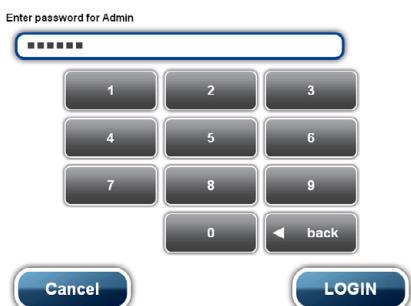
- 1 Press **Login** button to log in.



- 2 Default password for the following users are:

Admin: use **123456**

User: no password



**NOTE:** For information on setting passwords, see the Fluidigm FC1 Cycler User Guide, PN 100-1279.

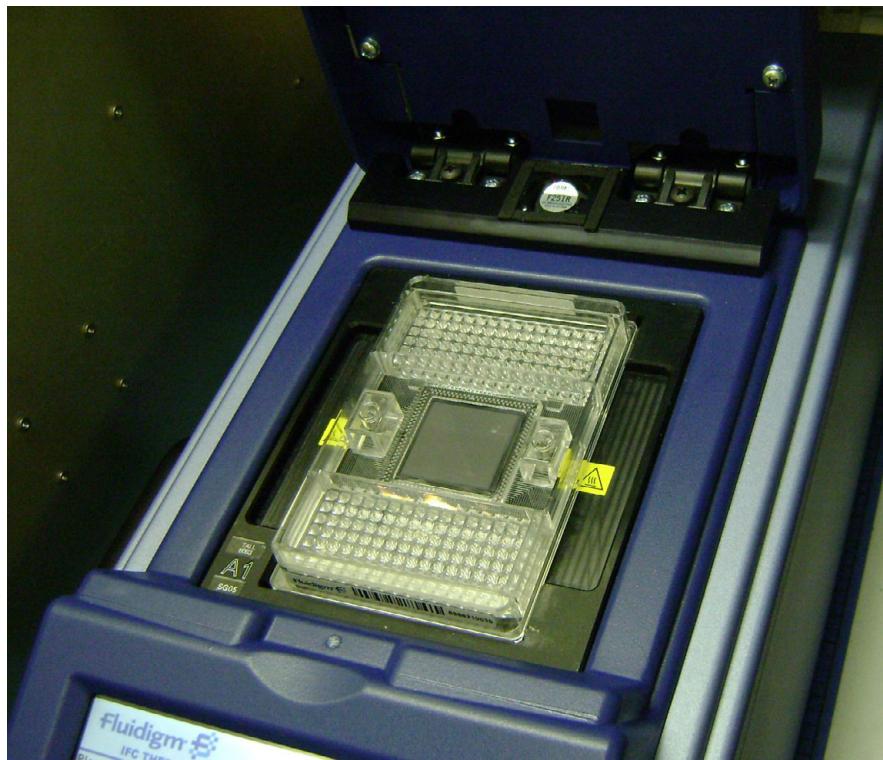
## Prepare IFC for Thermal Cycling

- 1 Remove the protective film from the bottom of the IFC.



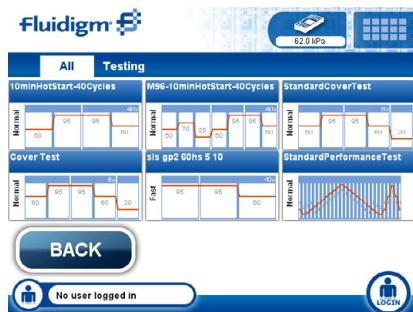
## Running a Protocol

- 1 Press the Start button.
- 2 Open the lid.
- 3 Place the IFC onto the thermal cycling block (chuck) on top of the instrument by aligning the notched corner of the IFC to the A1 mark.

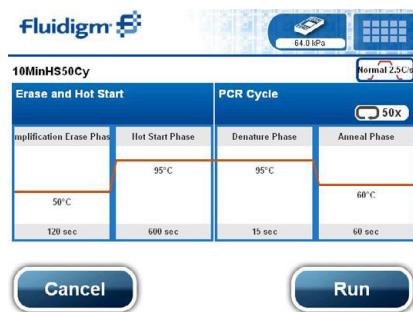


- 4 Close the lid.
- 5 Press Continue to display available thermal protocols.

**6 Choose a protocol to run from the protocol selection window.**



**7 Press Run.**



**CAUTION! HOT SURFACE HAZARD.** Never press down on the integrated fluidic circuit (IFC) when it is on the thermal cycler chuck. If you encounter a vacuum problem, turn off the system, allow it to cool down, and remove the IFC. Clean the bottom of the IFC and/or chuck surface with a lint-free cloth and 70% isopropyl alcohol.

**NOTE:** A status screen appears with a time estimate for completion.

**8 Once the protocol is finished, a confirmation screen appears. (During an active protocol, **Abort** will cancel the chip run.)**

## Cleaning Protocol

It is important to keep the thermal chuck surface clean. Any grease or debris will impact the thermal contact between the chuck and IFC. Turn off system prior to cleaning the chuck.

- 1 Turn system Off.  
Let system cool down.
- 2 Use a lint-free cloth sprayed with 70% isopropyl alcohol to gently wipe the chuck.



**CAUTION! HOT SURFACE HAZARD.** Make sure the chuck has had time to cool. It can get very hot and cause burn injury.

## Troubleshooting

Error Message	Possible Solutions
The screen is blank	Check power connection at the wall outlet, power strip and instrument inlet.
Error while loading the IFC, no vacuum	Verify the protective film has been removed from the bottom of the IFC. Verify the IFC is properly seated. Make sure the chuck surface and bottom of the IFC are clean. Follow cleaning protocol above.

# Using the Genotyping Analysis Software

2

## Launching the Genotyping Analysis Software

**IMPORTANT:** Update all instrument firmware and analysis software to version 4 (v4) or later.



Double-click the **Genotyping Analysis software** icon on the desktop.



You can either **Create a New Chip Run** or **Open a Chip Run**.

**NOTE:** To genotype your samples with the SNP Trace™ Panel, a set of SNPs, such as highly polymorphic SNPs, gender SNPs, ethnicity-specific or continental-specific SNPs, refer to the *Fluidigm SNP Trace™ Panel User Guide* (PN 100-7282).

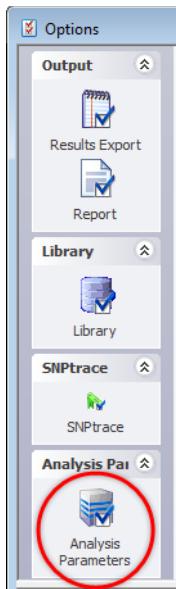
## Customizing the Analysis Parameters

You can customize the confidence threshold parameters before you open a chip run. This change will apply only to chip runs that have not been previously analyzed.

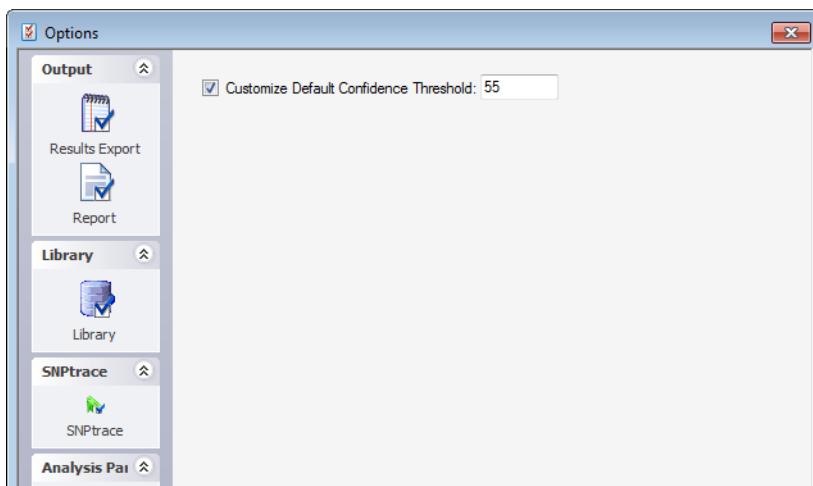
**IMPORTANT:** If you customize the Analysis Parameters on a shared workstation, this will cause default setting to be changed for all users. Please use caution.

After launching the SNP Genotyping Analysis software:

- 1 Select Tools > Options.
- 2 In the Options dialog box, click Analysis Parameters.



- 3 Check the checkbox for Customize Confidence Threshold, then enter the desired setting. Unchecking the box will use the original default settings.



## Open a Chip Run

- 1 Click Open a Chip Run.
- 2 Navigate to the run directory or run folder for the run that you want to analyze.
- 3 Double-click the chip run file you want (.bml extension).

## Annotate Samples Using Sample Setup

Sample annotation is the process of matching sample information to the sample source plate used in your test. When you annotate, you provide the sample name and type (unknown, control, or NTC), and the location of samples in the IFC.

You can annotate samples by cutting and pasting information from a table in a Microsoft® Excel® file, creating a new sample setup, or by importing sample information that is contained in a .plt file or a .csv file (see “[Create a .csv file to Import Sample or Assay Information for Annotation](#)” on page 53):

- “[Annotate Samples Using a Microsoft® Excel® File](#)”
- “[Annotate Samples by Creating a New Sample Setup](#)” on page 35
- “[Annotate Samples by Importing a .plt or a .csv file](#)” on page 38

### Annotate Samples Using a Microsoft® Excel® File

For quick annotation, you can copy and paste an entire plate or inlet (16 row x 6 column) format from a Microsoft® Excel® file with sample names or sample types arranged in either 96-well plate or inlet format.

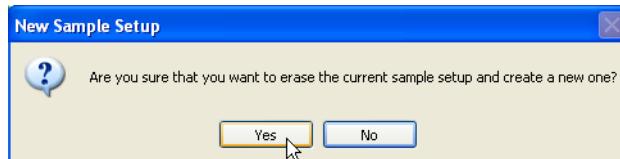
- 1 Ensure that you can designate an NTC in your sample set using the sample type annotation. The NTC is used for normalization and is required for analysis of a new run.
- 2 Create a Microsoft® Excel® file with the layout of the samples. For example: **96-Well Format**

S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12
S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36
S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48
S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60
S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71	S72
S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83	S84
S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95	NTC

## Inlet Format

S01	S02	S03	S04	S05	S06
S07	S08	S09	S10	S11	S12
S13	S14	S15	S16	S17	S18
S19	S20	S21	S22	S23	S24
S25	S26	S27	S28	S29	S30
S31	S32	S33	S34	S35	S36
S37	S38	S39	S40	S41	S42
S43	S44	S45	S46	S47	S48
S49	S50	S51	S52	S53	S54
S55	S56	S57	S58	S59	S60
S61	S62	S63	S64	S65	S66
S67	S68	S69	S70	S71	S72
S73	S74	S75	S76	S77	S78
S79	S80	S81	S82	S83	S84
S85	S86	S87	S88	S89	S90
S91	S92	S93	S94	S95	S96

- 3 Copy (or select the cells, then press **Ctrl-C**) the entire 96-well or inlet layout in the file.
- 4 Click **Sample Setup** in the Chip Explorer pane.
- 5 Click **New**. If the sample plate has been set up previously, an alert opens:



- 6 Click **Yes**.
- 7 Select the Container type and Container format:



### Container type dropdown menu options:

- **SBS Plate**: import custom plate with annotations that you have already completed from a previously saved run.

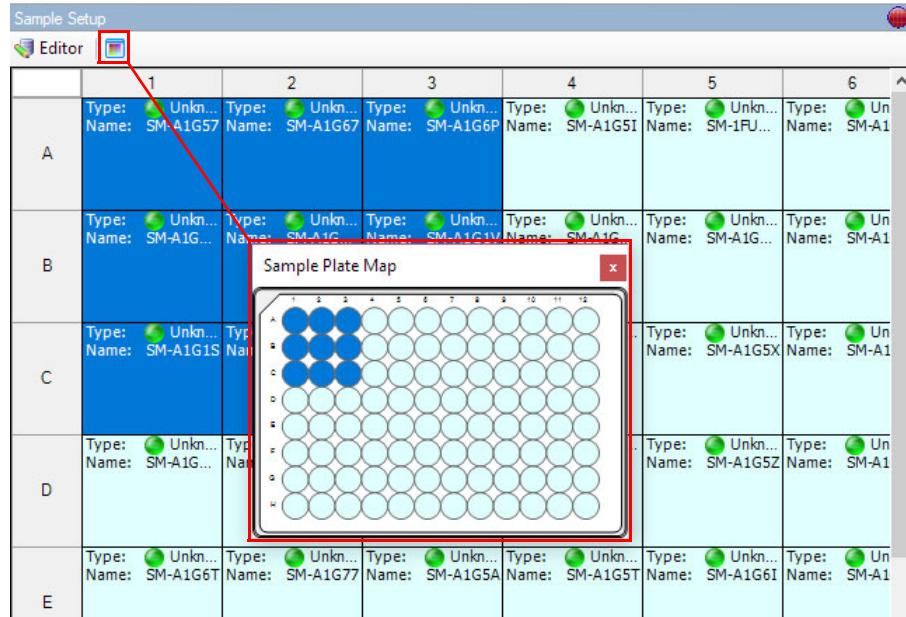
Or,

- **Sample Inlets**: select sample inlets to add annotations for the current run.

**Container format**: SBS96 is the default setting. If you choose Sample Inlets for type, format is inactivated. Use SBS384 for 192.24 Dynamic Array IFCs.

- 8 Click **OK**.

- 9 In the Genotyping Analysis Software, highlight all of the cells by using one of the following:
- Click and drag.
  - Press **Ctrl** while clicking individual cells.
  - Click the corner square to select all cells.
- 10 OPTIONAL: Click the Map icon . The map opens and shows selected cell(s) relative to the entire detector plate.



**NOTE:** If you selected SBS Plate as your container type, clicking the Map icon opens a detector plate map. If you selected assay detector Inlets, clicking the Map icon opens an assay detector inlet map.

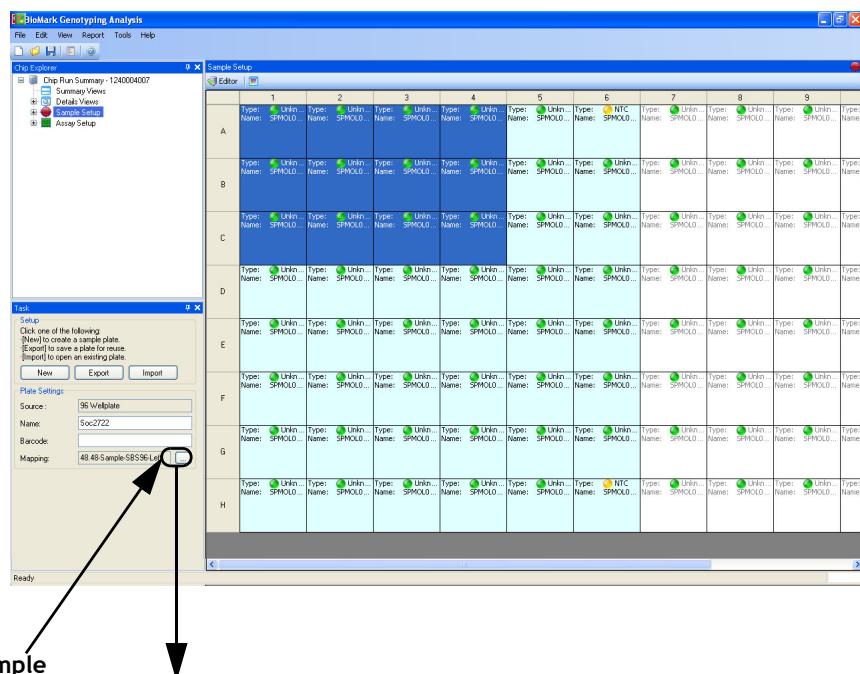
**11 Proceed as follows:**

- If you clicked the corner square to select all cells, right-click the same, upper-left corner cell again, then select **Paste** (or left-click the cell, then press **Ctrl-V**). The Data Item Selection dialog box displays.
- If you selected cells by another method, proceed to the next step.

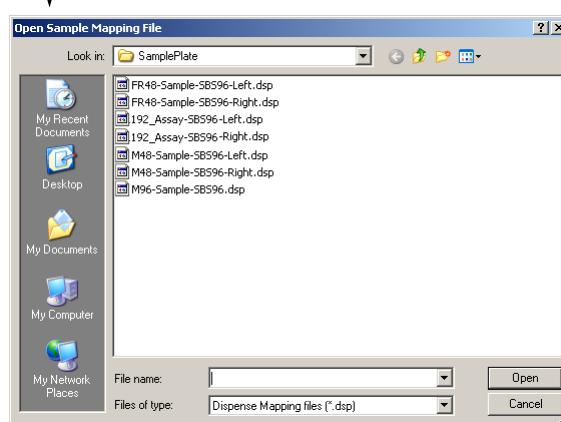
**12** From the drop-down menu in the dialog box, select **Sample Name**, then click **Accept**. The cells in Sample Setup are populated with the sample information.

**13 Proceed as follows:**

- If you selected the Sample Inlets container type, skip to step **14**.
- If you selected the **SBS Plate** container type, click the **Sample Mapping** button:



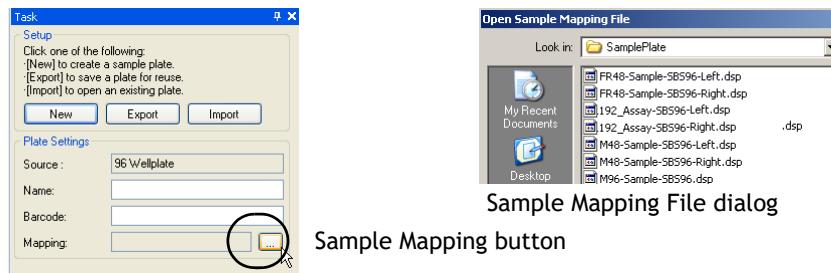
Click the **Sample Mapping** button to open the mapping file.



**13** From the file, select either **Left** or **Right** dispense map.

**NOTE:** If you are analyzing a:

- 48.48 chip run, select *M48-Sample-SBS96 (left or right).dsp*
- 96.96 chip run, select *M96-Sample-SBS96.dsp*
- 192.24 chip run, select *192-Sample-SBS384 (left or right).dsp*, *192-Sample-SBS96 (left or right).cdsp*, or *192-Sample-SBS96 (Even or Odd).cdsp*



**14** In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Sample Summary Views**, then click **Analyze**. The highlighted cells reflect the changes.

**15 (Optional)** To save the new sample setup, click **File > Save**. You can also export the new setup by clicking **Export** in the Task pane and saving the setup as a **.plt** file.

**NOTE:** If you exit the application without saving, a warning appears.

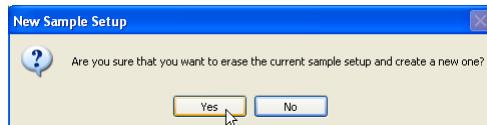
### Annotate Samples by Creating a New Sample Setup

**1** Ensure that you can designate an NTC in your sample set using the sample type annotation. The NTC is used for normalization and is required for analysis of a new run.

**NOTE:** If you did not include an NTC in the experimental setup, you may be able to analyze the current run with another SNP Trace run (see “[Importing Multiple Chip Runs](#)” on page 55).

**2** Click **Sample Setup** in the Chip Explorer pane.

**3** Click **New**. If the sample plate has been set up previously, an alert opens:



**4** Click **Yes**.

## 5 Select the Container type and Container format:



### Container type dropdown menu options:

- *SBS Plate*: import custom plate with annotations that you have already completed from a previously saved run.

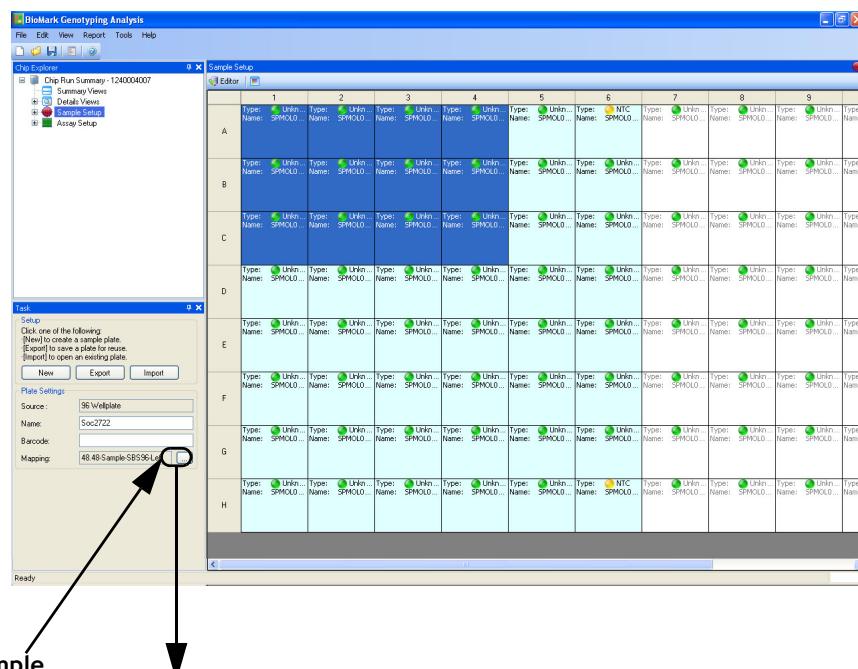
Or,

- *Sample Inlets*: select sample inlets to add annotations for the current run.

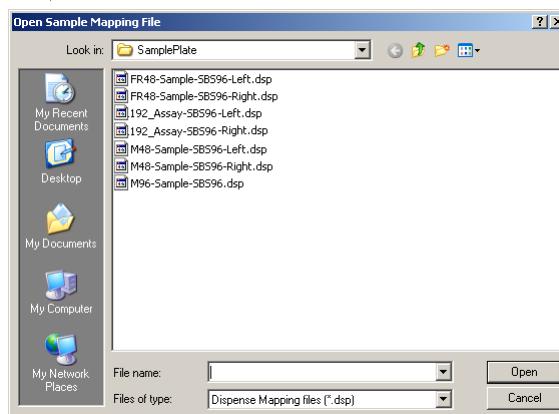
**Container format:** SBS96 is the default setting. If you choose Sample Inlets for type, format is inactivated.

## 6 Click OK.

## 7 Click the Sample Mapping button:



Click the Sample Mapping button to open the mapping file.



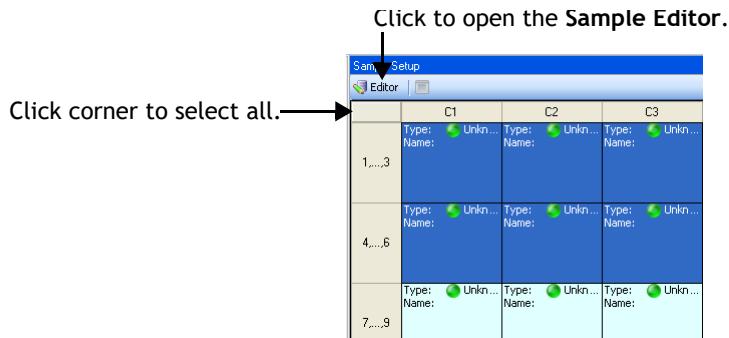
### 13 From the file, select either Left or Right dispense map.

**NOTE:** If you are analyzing a:

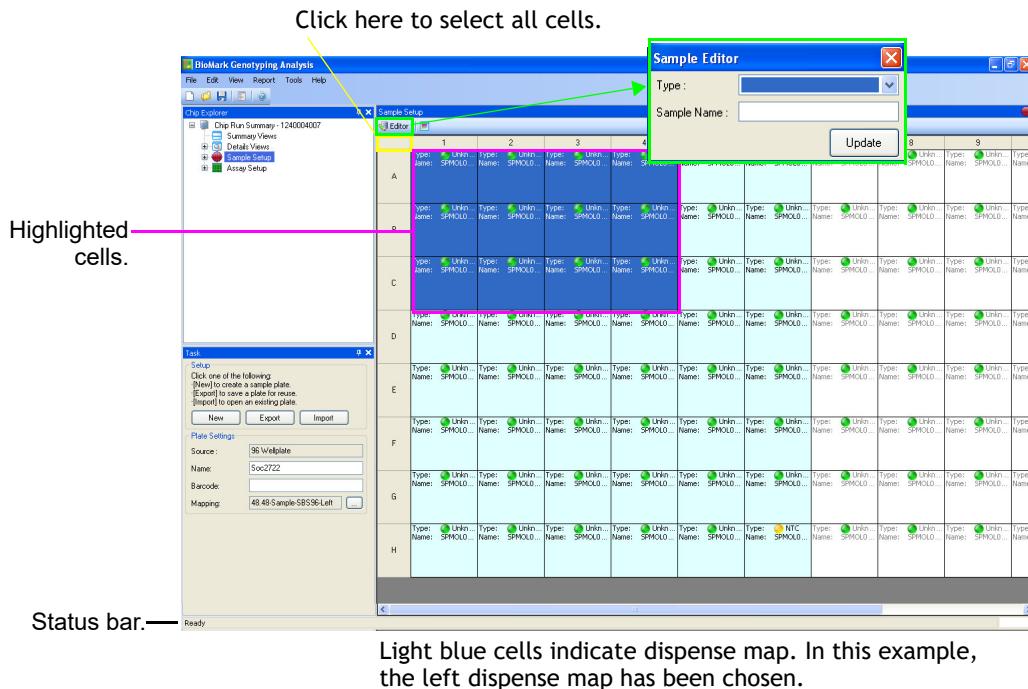
- 48.48 chip run, select *M48-Sample-SBS96 (left or right).dsp*
- 96.96 chip run, select *M96-Sample-SBS96.dsp*
- 192.24 chip run, select *192-Sample-SBS384 (left or right).dsp*, *192-Sample-SBS96 (left or right).cdsp*, or *192-Sample-SBS96 (Even or Odd).cdsp*

### 8 Highlight the cells to annotate using one of the following:

- Click and drag.
- Press **Ctrl** while clicking individual cells.
- Click the corner square to select all cells.



### 9 To manually add or edit information, click **Editor**:



- a Select Unknown, NTC or Control from the Sample Editor menu:



- b Type the sample name.  
c Click **Update**. The highlighted cells reflect the changes.

10 (Optional) To save the new sample setup, click **File > Save**. You can also export the new setup by clicking **Export** in the Task pane and saving the setup as a .plt file.

**NOTE:** If you exit the application without saving, a warning appears.

11 In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Sample Summary Views**, then click **Analyze**.

## Annotate Samples by Importing a .plt or a .csv file

### Annotating Samples by Importing a .plt file

If you have previously exported a .plt file containing sample annotation information, you can import the saved annotation information to a new run.

- 1 Click **Sample Setup** in the Chip Explorer pane.
- 2 Click **Import** in the Task pane.
- 3 Select **.plt** from the drop-down menu, browse to the saved file, then click **Open**.

The sample annotation information will fill into the Sample Setup pane.

4 (Optional) To save the new sample setup, click **File > Save**. You can also export the new setup by clicking **Export** in the Task pane and saving the setup as a .plt file.

**NOTE:** If you exit the application without saving, a warning appears.

5 In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Sample Summary Views**, then click **Analyze**.

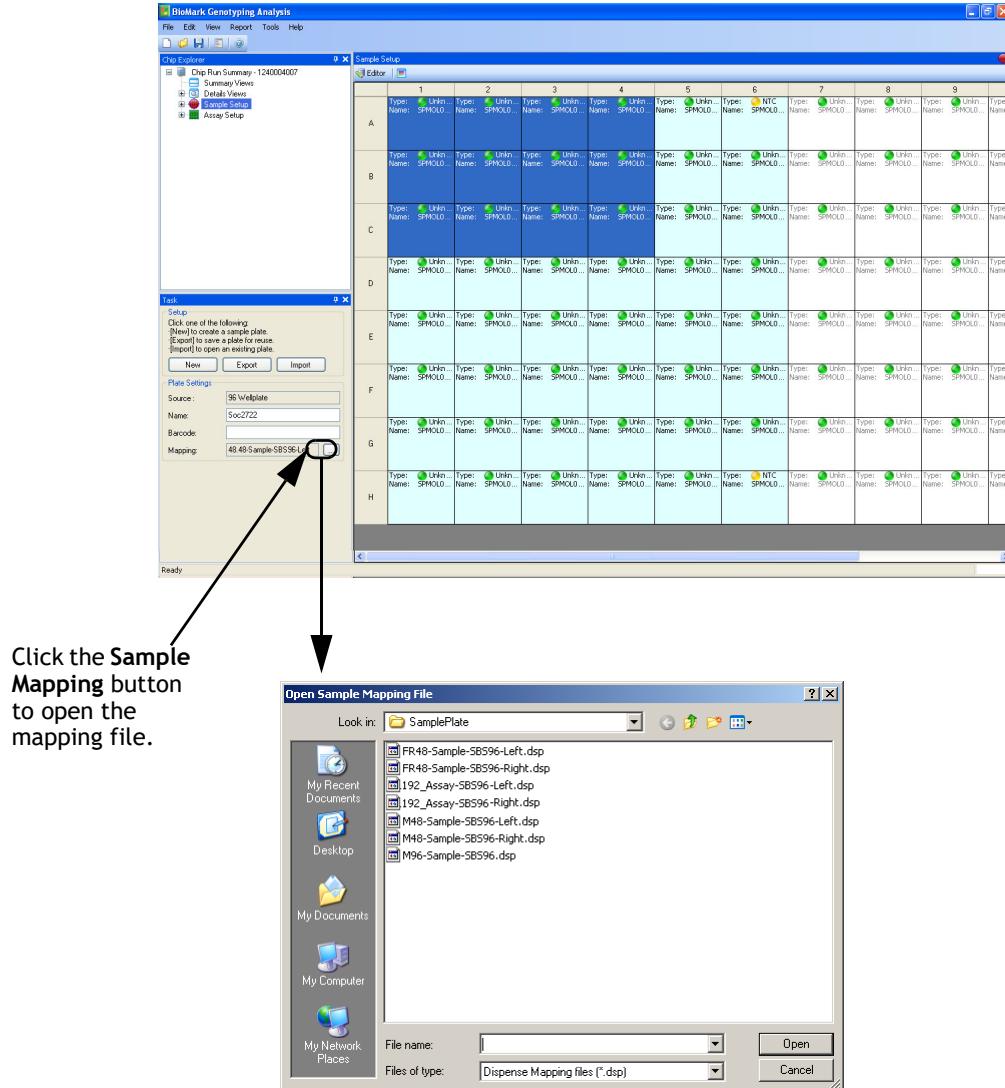
### Annotating Samples by Importing a .csv file

- 1 Ensure that you have created a .csv file to import (see “[Create a .csv file to Import Sample or Assay Information for Annotation](#)” on page 53).
- 2 Click **Sample Setup** in the Chip Explorer pane.
- 3 Click **Import** in the Task pane.
- 4 Select **.csv** from the drop-down menu, browse to the saved file, then click **Open**.

The sample annotation information will fill into the Sample Setup pane.

**NOTE:** If you did not include an NTC in the experimental setup, you may be able to analyze the current run with another SNP Trace run (see “[Importing Multiple Chip Runs](#)” on page 55).

**5 If you are importing a .csv file for a plate-based layout, click the Sample Mapping button:**



**6 From the file, select either Left or Right dispense map.**

**NOTE:** If you are analyzing a:

- 48.48 chip run, select *M48-Sample-SBS96 (left or right).dsp*
- 96.96 chip run, select *M96-Sample-SBS96.dsp*
- 192.24 chip run, select *192-Sample-SBS384 (left or right).dsp*, *192-Sample-SBS96 (left or right).cdsp*, or *192-Sample-SBS96 (Even or Odd).cdsp*

**7 (Optional)** To save the new sample setup, click **File > Save**. You can export the new setup by clicking **Export** in the Task pane and saving the setup as a .csv file.

**NOTE:** If you exit the application without saving, a warning appears.

**8** In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Sample Summary Views**, then click **Analyze**.

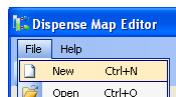
## Using the Dispense Map Editor

Use the **Dispense Map Editor** to set dispensing map parameters and to record custom load maps for future use. After recording your loading sequence, you can save it and play it back anytime.

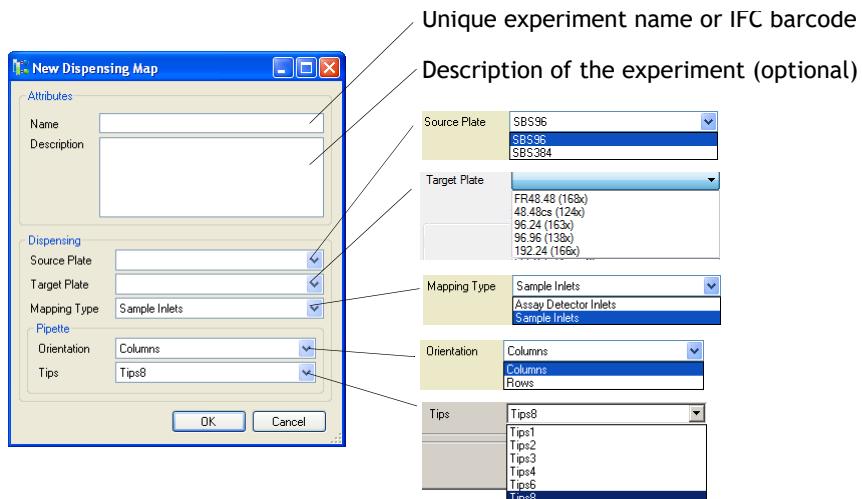
**1** Click **Tools > Dispense Map Editor**.



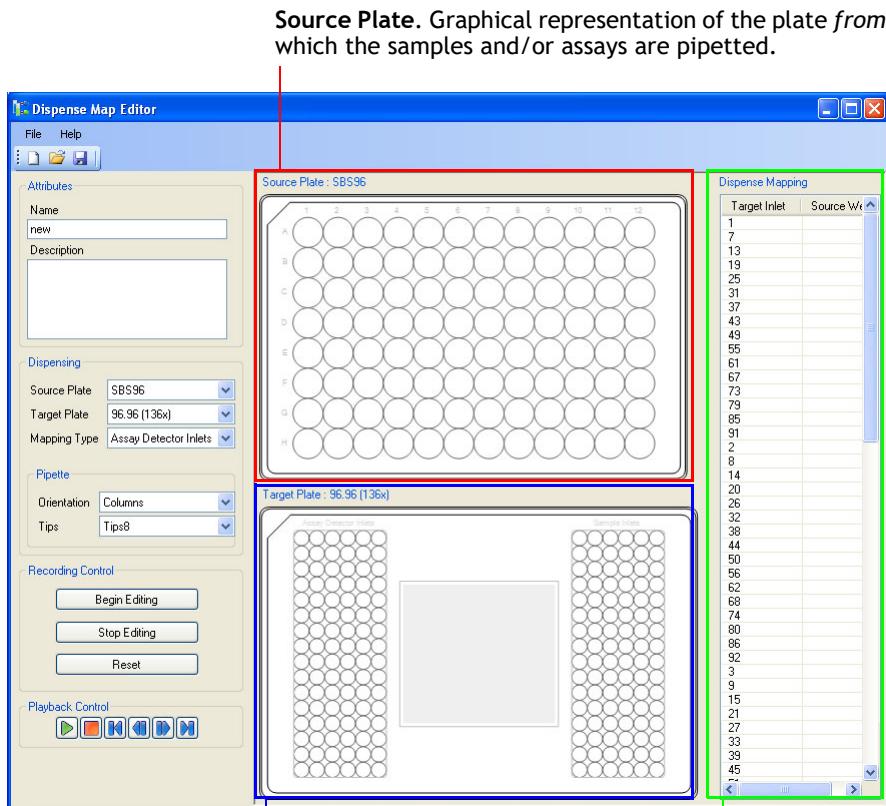
**2** Click **File > New**.



**3** Complete the **New Dispense Map** dialog using the following as a guide.



**4** Click **OK** to open the new dispense map in the **Dispense Map Editor**.



**Source Plate.** Graphical representation of the plate from which the samples and/or assays are pipetted.

**Dispense Map.** This table show you where the samples and assays are on the IFC.

## 5 Click the Begin Editing button in the Recording Control pane.



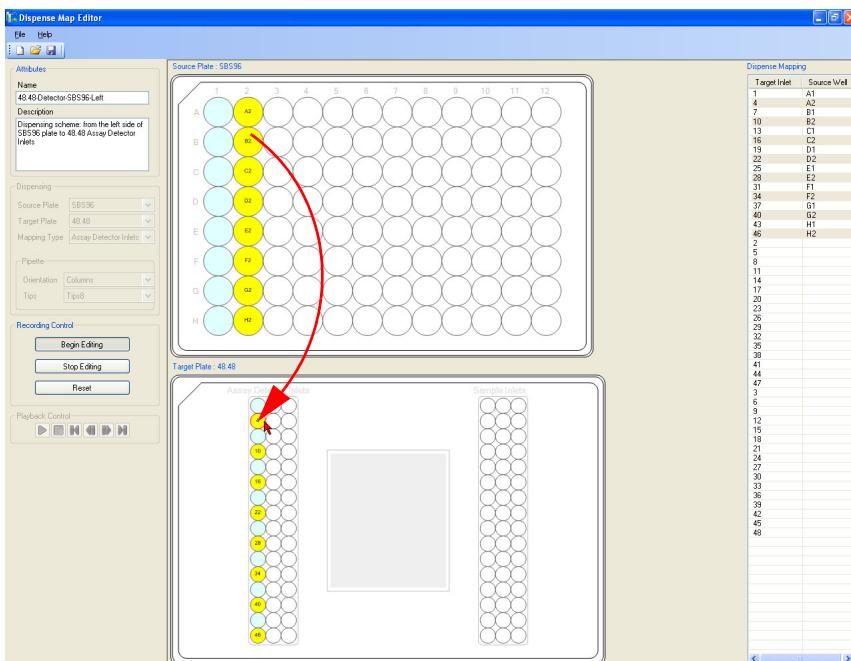
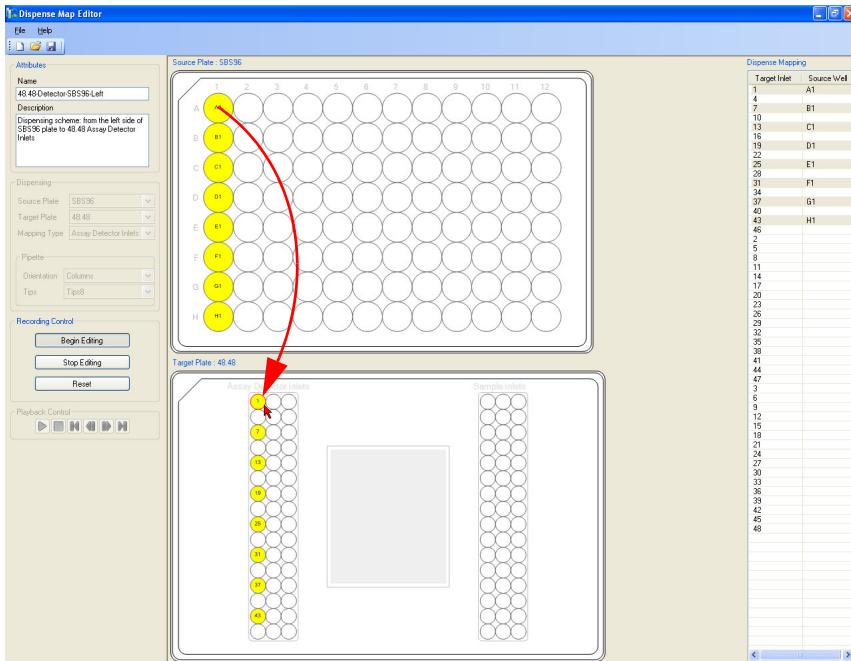
- Click the first cell from the Source Plate. Then, click the location in the Target Plate.
- Continue clicking appropriate cells (that is to say, from the Source Plate to the Target Plate) until your custom loading map has been recorded.

**NOTE:** When you click Begin Editing, the dispensing pane becomes inactive.

## 6 Click Stop Editing.

Refer to the following two graphics as an example of custom loading and how it looks as you proceed.

## Using the Genotyping Analysis Software



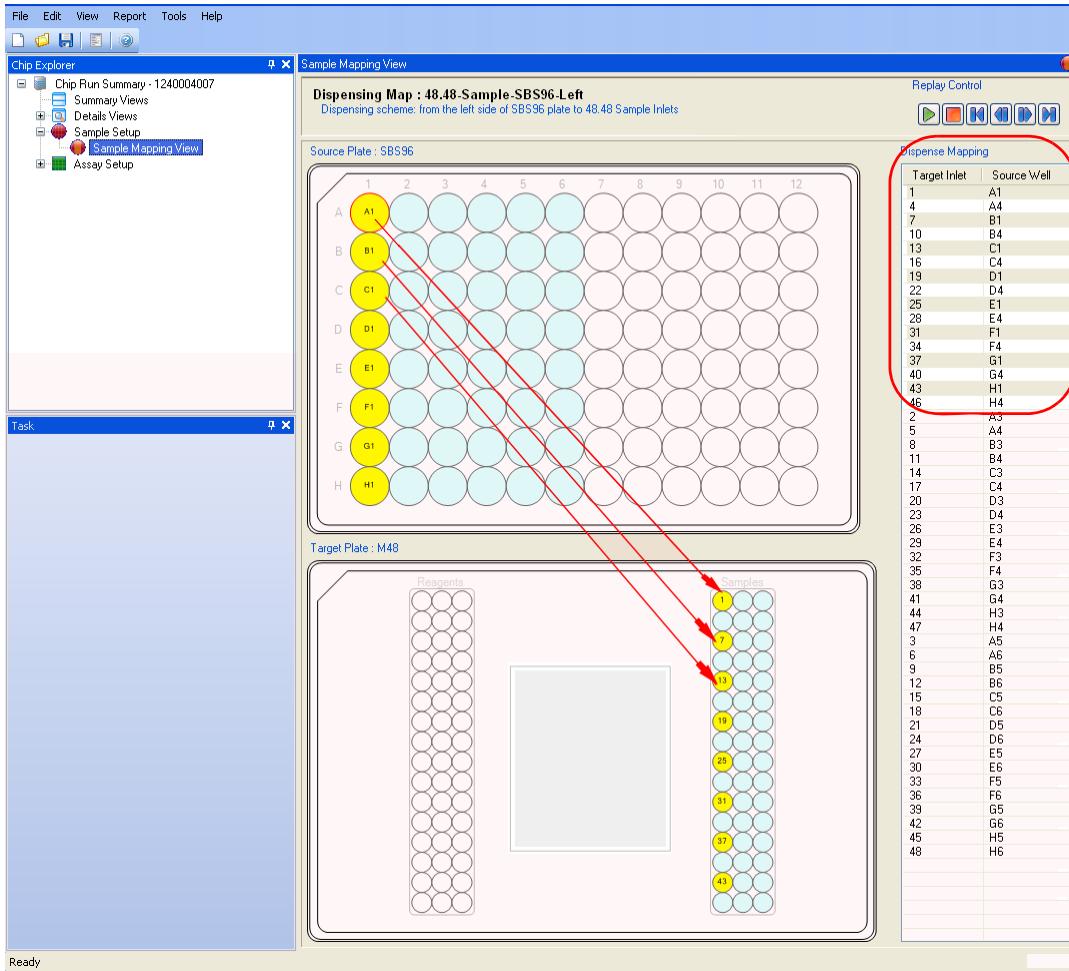
Review the loading pattern you have recorded by clicking the green arrow button in the playback control pane.



# Using Sample Mapping

After setting up the sample plate, view and/or record the loading pattern in the Sample Mapping Viewer.

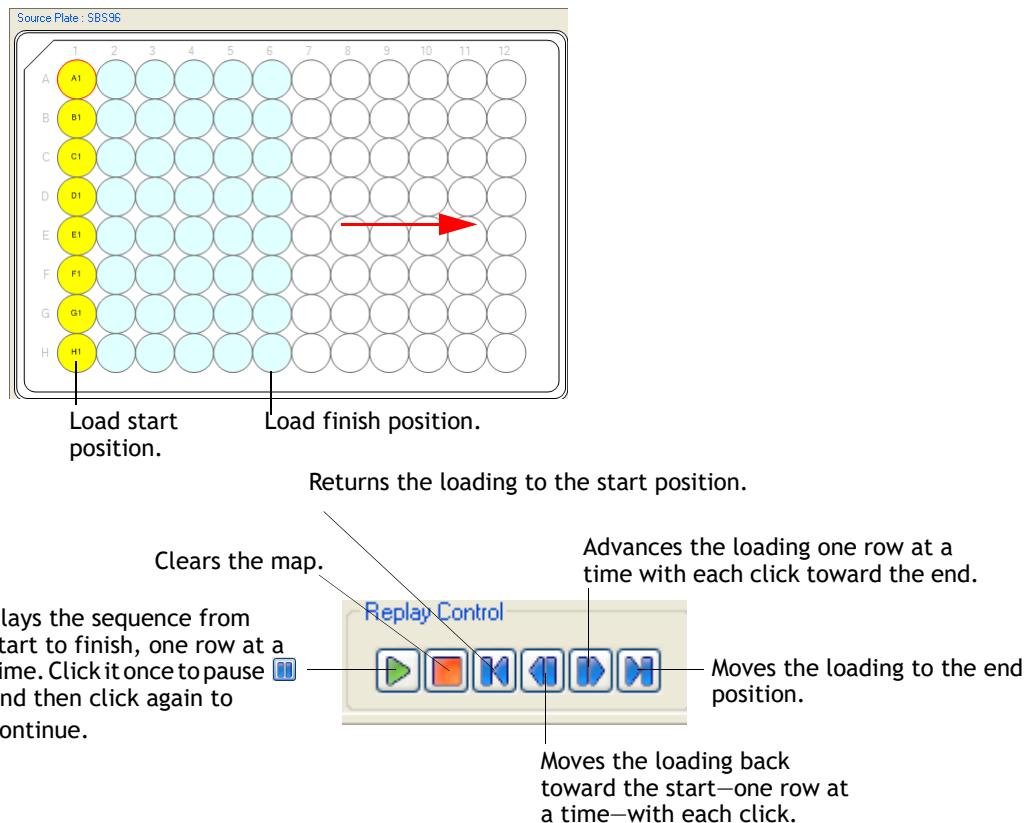
- 1 Click Sample Mapping View in the chip explorer pane.
- 2 Click a cell in the Source Plate to see where it loads on the Target Plate (see below).



# Using the Replay Control

Use the replay controls to show you where and in what sequence the Target Plate receives the samples from the Source Plate.

When you click Play, the yellow highlight moves sequentially from row 1 to row 6.



## Annotate Assays Using Assay Setup

Assay annotation is the process of matching assay information to the assay source plate that you are using in your test. When you annotate, you provide the Assay Name, Allele X Name, Allele Y Name, and the location of the assays in the IFC.

You can annotate assays by cutting and pasting information from a table in a Microsoft® Excel® file, creating a new assay setup, or by importing sample information that is contained in a .plt file or a .csv file (see “[Create a .csv file to Import Sample or Assay Information for Annotation](#)” on page 53):

- “[Annotate Assays Using a Microsoft® Excel® File](#)” on page 45
- “[Annotate Assays by Creating a New Assay Setup for Plate-Based Formats](#)” on page 48
- “[Annotate Assays by Importing a .plt or a .csv file](#)” on page 51

**NOTE:** Fluidigm will provide a design.xls Microsoft® Excel® file with the assay information. The format of the file is:

####STP##O1\_Design.xls

where #### = 4 digit unique identifier, STP = SNP Type, and ## = year. For example: 1845STP13O1\_Design.xls.

### Annotate Assays Using a Microsoft® Excel® File

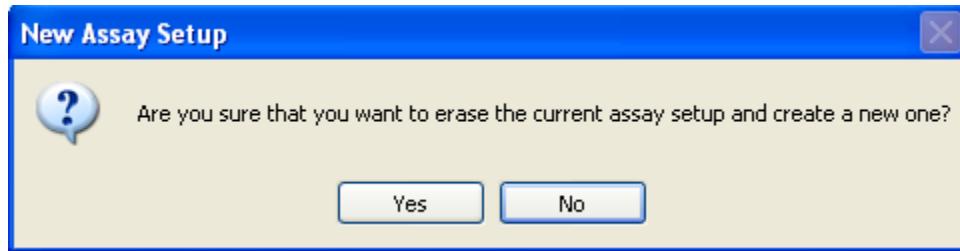
For quick annotation, you can copy and paste an entire plate format from a Microsoft® Excel® file with assay and allele names arranged in either 96-well plate or inlet format. As an option, you can copy and paste or inlet (16 row x 6 column) format.

- 1 Create a Microsoft® Excel® file with the layout of the assays. For example:  
**96-Well Plate Format**

1	2	3	4	5	6	7	8	9	10	11
hu1	hu2	hu3	hu4	hu5	hu7	hu9	hu11	hu12	hu14	hu15
hu17	hu18	hu20	hu21	hu22	hu23	hu24	hu25	hu26	hu27	hu28
hu31	hu32	hu33	hu34	hu35	hu36	hu37	hu38	hu39	hu41	hu42
hu45	hu46	hu47	hu48	hu49	hu51	hu52	hu53	hu54	hu55	hu56
hu59	hu61	hu62	hu63	hu64	hu65	hu66	hu68	hu69	hu70	hu71
hu73	hu74	hu75	hu76	hu78	hu79	hu80	hu81	hu82	hu83	hu84
hu86	hu87	hu88	hu89	hu90	hu91	hu92	hu93	hu95	hu96	hu98Y
hu107X	hu109X	hu111Y	hu200	hu201	hu202	hu203	hu204	hu205	hu207	hu208

- 2 Copy (or select the cells, then press Ctrl-C) the entire 96-well or inlet layout in the file.
- 3 Click **Assay Setup** in the Chip Explorer pane.

- 4 Click **New**. If the sample plate has been set up previously, an alert opens:



- 5 Click **Yes**.



**Container type menu options:**

- **SBS Plate**: import custom plate with annotations that you have already completed from a previously saved run.

Or,

- **Assay Inlets**: select assay inlets to add annotations for the current run.

**Container format**: no choices, only 96 default. If you choose Assay Inlets for type above, format is inactivated.

- 6 Select container type and container format.

- 7 Click **OK**.

- 8 In the Genotyping Analysis Software, highlight all of the cells by using one of the following:

- Click and drag.
- Press **Ctrl** while clicking individual cells.
- Click the corner square to select all cells.

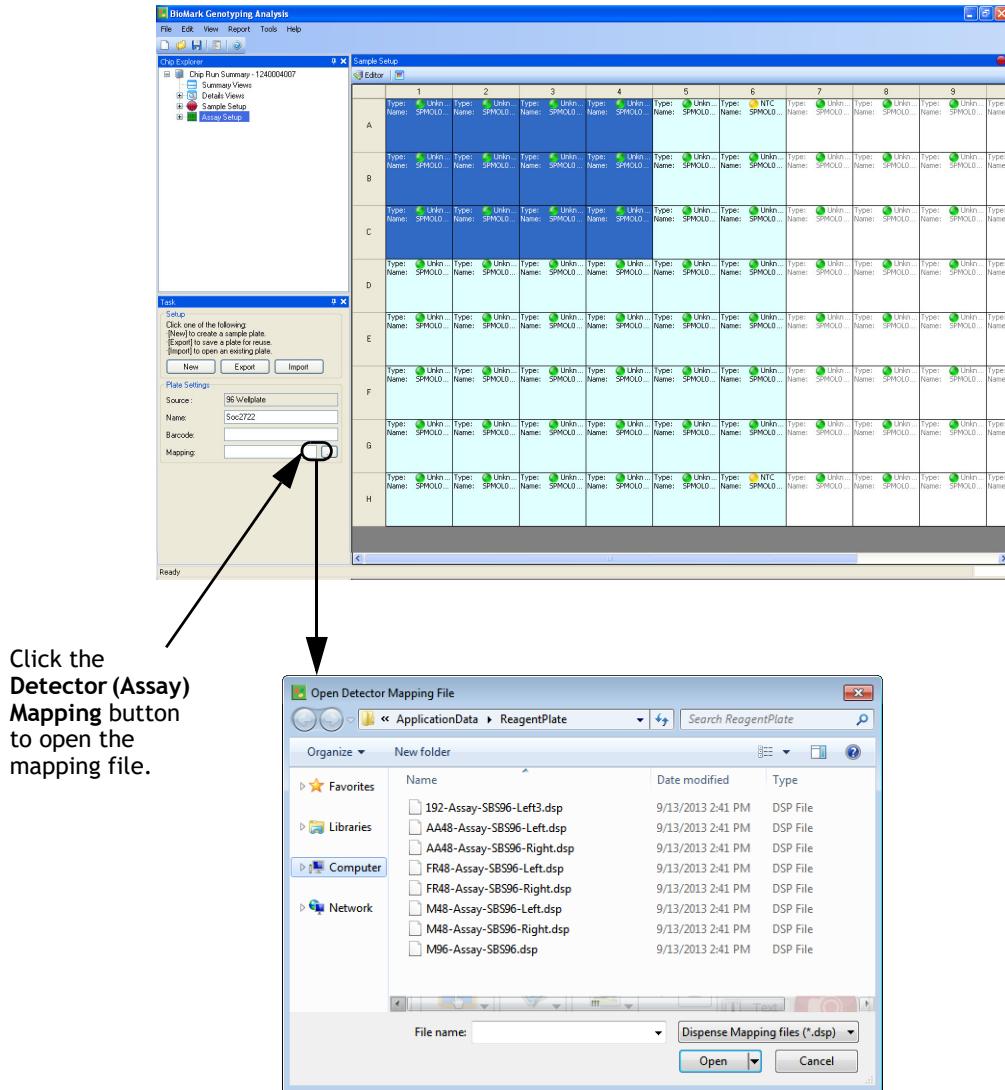
- 9 Proceed as follows:

- If you clicked the corner square to select all cells, right-click the same, upper-left corner cell again, then select **Paste** (or left-click the cell, then press **Ctrl-V**). The Data Item Selection dialog box displays.
- If you selected cells by another method, proceed to the next step.

- 10 From the drop-down menu in the dialog box, select **Assay Name**, then click **Accept**. The cells in Assay Setup are populated with the assay information.

- 11 (*Optional*) Repeat step 10 to import the Allele X Name and Allele Y Name.

## 12 Click the Detector (Assay) Mapping button:



## 13 From the file, select either Left or Right dispense map.

**NOTE:** If you are analyzing a:

- 48.48 chip run, select *M48-Assay-SBS96 (left or right).dsp*
- 96.96 chip run, select *M96-Assay-SBS96.dsp*
- 192.24 chip run, select *192-Assay-SBS96-Left3.dsp*

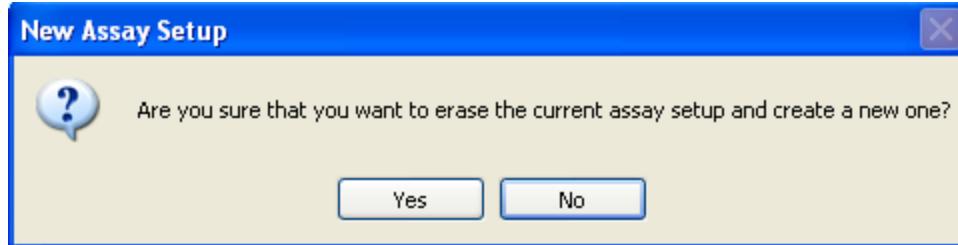
**14 (Optional)** To save the new assay setup, click **File > Save**. You can export the new setup by clicking **Export** in the Task pane and saving the setup as a .plt file.

**NOTE:** If you exit the application without saving, a warning appears.

**15** In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Assay Summary Views**, then click **Analyze**.

## Annotate Assays by Creating a New Assay Setup for Plate-Based Formats

- 1 Click **Assay Setup**.
- 2 In the Task pane, click **New**. If the assay plate has been set up previously, the **New Assay Setup** dialog box opens:



- 3 If necessary, click **Yes**.



**Container type menu options:**

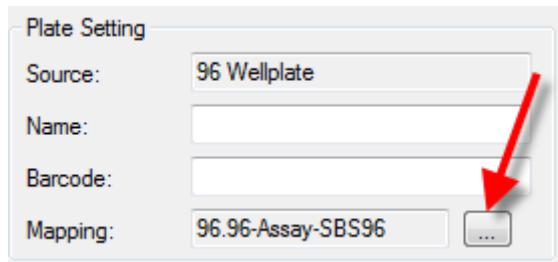
- *SBS Plate*: import custom plate with annotations that you have already completed from a previously saved run.

Or,

- *Assay Inlets*: select assay inlets to add annotations for the current run.

**Container format:** no choices, only 96 default. If you choose Assay Inlets for type above, format is inactivated.

- 4 Select container type and container format.
- 5 Click **OK**.
- 6 In the Task pane, click the **Detector (Assay) Mapping** button:



- 7 From the file, select either **Left** or **Right** dispense map.

**NOTE:** If you are analyzing a:

- 48.48 chip run, select *M48-Assay-SBS96 (left or right).dsp*
- 96.96 chip run, select *M96-Assay-SBS96.dsp*
- 192.24 chip run, select *192-Assay-SBS96-Left3.dsp*

- 8 Highlight the cells to annotate using one of the following:
  - Click and drag.
  - Press **Ctrl** while clicking individual cells.

- Click the corner square to select all cells.

Click corner to select all. →

	C1	C2	C3
1....3	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...
4....6	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...
7....9	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...
10....12	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...
13....15	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...	Type: Unkn... Name: Unkn...

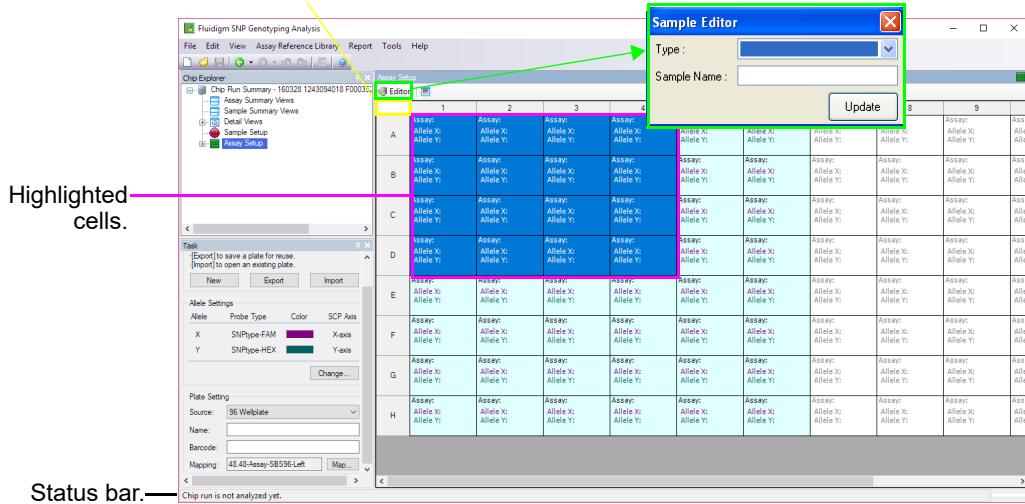
- 9 OPTIONAL: Click the Map icon . The map opens and shows selected cell(s) relative to the entire detector plate.

The screenshot shows the 'Assay Setup' window with the 'Editor' tab selected. The main area displays a grid of 7 rows (A-F) and 6 columns (1-6). Each cell contains assay information: Assay ID, Allele X, and Allele Y. A red arrow points from the 'Map' icon in the toolbar to a callout box labeled 'Detector Plate Map' which shows a 12x12 grid of wells, indicating the relative position of the selected cells.

**NOTE:** If you selected SBS Plate as your container type, clicking the Map icon opens a detector plate map. If you selected assay detector Inlets, clicking the Map icon opens an assay detector inlet map.

## 9 To manually add or edit assay information, click **Editor**:

Click here to select all cells.



Light blue cells indicate dispense map. In this example, the left dispense map has been chosen.

## 10 In the Assay Editor dialog box, enter Assay Name and (optional) Allele X Name and (optional) Allele Y Name, then click **Update**.

**NOTE:** The Allele X and Allele Y annotations can be switched in the final call by adding “>” at the beginning of the allele name. The switched annotations are shown in the Converted column in the experiment information table in the Details view.

Experiment Information										Results			
Chamber	SNP Assay and Allele Names		Sample	Type	Call Information	Confidence	Final	Converted					
S06-A63	rs76432344 >C	>T	NA11275	Unknown	YY	99.95	YY	T:T					
S06-A62	hu11Y	A	C	NA11275	Unknown	YY	100.00	YY	C:C				
S06-A61	hu15	G	T	NA11275	Unknown	YY	100.00	YY	T:T				
S06-A72	rs2358996 >A	>G	NA11275	Unknown	XY	99.99	XY	G:A					
S06-A71	rs3108237	G	A	NA11275	Unknown	XY	99.99	XY	G:A				
S06-A70	rs2236277	C	T	NA11275	Unknown	XX	100.00	XX	C:C				
S06-A69	rs27529	A	G	NA11275	Unknown	YY	100.00	YY	G:G				
S06-A68	rs2074265	>A	>C	NA11275	Unknown	XX	100.00	XX	A:A				
S06-A67	rs3753886	>G	>T	NA11275	Unknown	XY	99.96	XY	T:G				
S06-A78	rs2292745	>A	>G	NA11275	Unknown	YY	99.99	YY	G:G				
S06-A77	rs5965201	A	G	NA11275	Unknown	XY	99.88	XY	A:G				
S06-A76	rs3736757	>A	>G	NA11275	Unknown	XY	100.00	XY	G:A				
S06-A75	rs3750390	>T	>C	NA11275	Unknown	YY	100.00	YY	C:C				

## 11 (Optional) To save the new assay setup, click **File > Save**. You can export the new setup by clicking **Export** in the Task pane and saving the setup as a .plt file.

**NOTE:** If you exit the application without saving, a warning appears.

## 12 In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Assay Summary Views**, then click **Analyze**.

## Annotate Assays by Importing a .plt or a .csv file

### Annotating Assays by Importing a .plt file

If you have previously exported a .plt file containing assay annotation information, you can import the saved annotation information to a new run.

- 1 Click **Assay Setup** in the Chip Explorer pane.
- 2 Click **Import** in the Task pane.
- 3 Select **.plt** from the drop-down menu, browse to the saved file, then click **Open**.  
The assay annotation information will fill into the Assay Setup pane.
- 4 (*Optional*) To save the new assay setup, click **File > Save**. You can also export the new setup by clicking **Export** in the Task pane and saving the setup as a .plt file.  
**NOTE:** If you exit the application without saving, a warning appears.
- 5 In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Assay Summary Views**, then click **Analyze**.

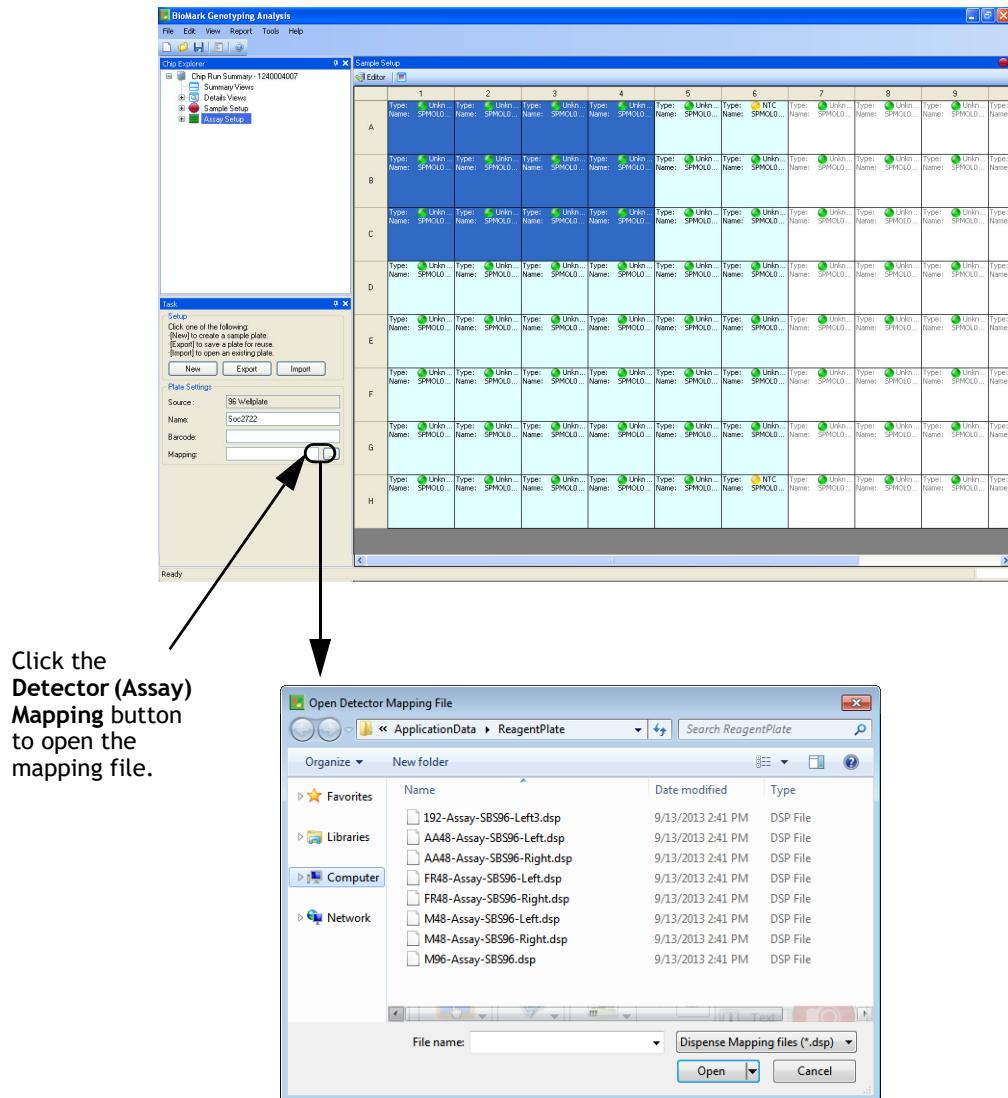
### Annotating Assays by Importing a .csv file

- 1 Ensure that you have created a .csv file to import (see “[Create a .csv file to Import Sample or Assay Information for Annotation](#)” on page 53).
- 2 Click **Assay Setup** in the Chip Explorer pane.
- 3 Click **Import** in the Task pane.
- 4 Select **.csv** from the drop-down menu, browse to the saved file, then click **Open**.

The assay annotation information will fill into the Assay Setup pane.

**NOTE:** If you did not include an NTC in the experimental setup, you may be able to analyze the current run with another SNP Trace run (see “[Importing Multiple Chip Runs](#)” on page 55).

## 5 Click the Detector (Assay) Mapping button:



## 6 From the file, select either Left or Right dispense map.

**NOTE:** If you are analyzing a:

- 48.48 chip run, select *M48-Assay-SBS96 (left or right).dsp*
- 96.96 chip run, select *M96-Assay-SBS96.dsp*
- 192.24 chip run, select *192-Assay-SBS96-Left3.dsp*

**7 (Optional)** To save the new assay setup, click **File > Save**. You can export the new setup by clicking **Export** in the Task pane and saving the setup as a .csv file.

**NOTE:** If you exit the application without saving, a warning appears.

**8** In the Chip Explorer pane of the Fluidigm SNP Genotyping Analysis software, click **Assay Summary Views**, then click **Analyze**.

## Create a .csv file to Import Sample or Assay Information for Annotation

- 1 Browse to the template:  
C:\Program Files (x86)\Fluidigm\BioMarkGenotypingAnalysis\ApplicationData\FileFormats
- 2 Open the appropriate template and tab:

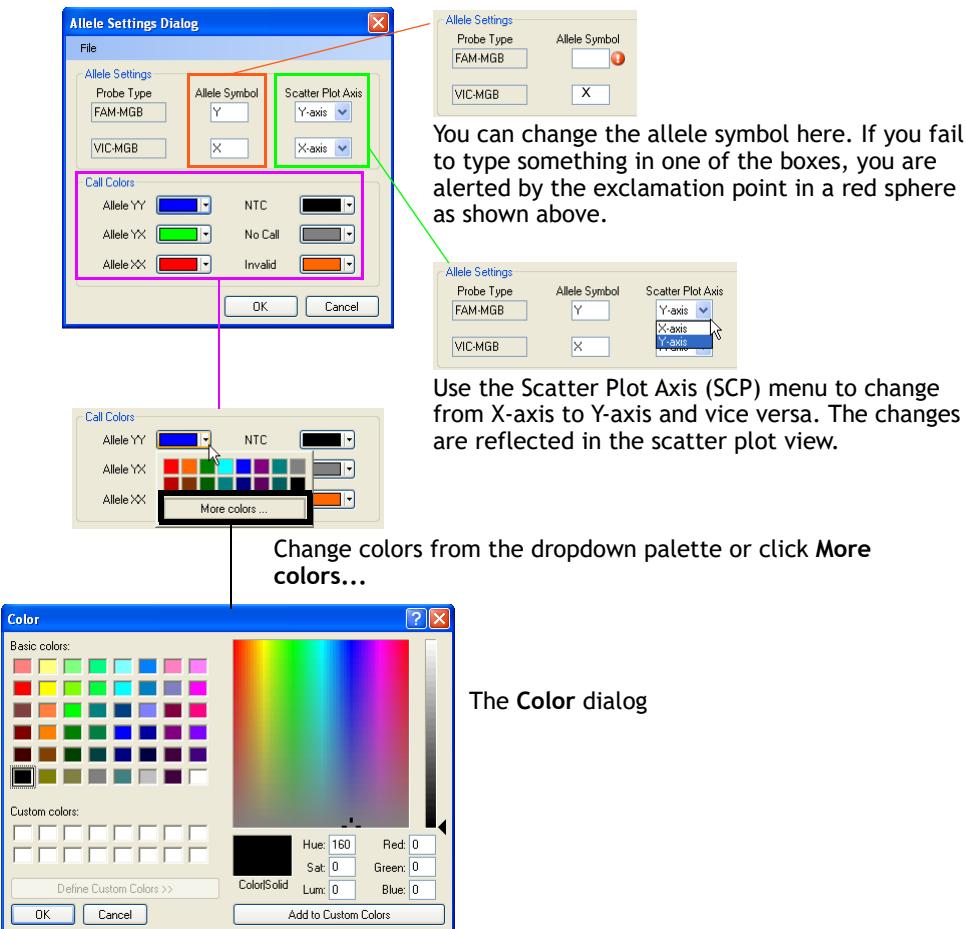
		Enter Sample Information	Enter Assay Information
Template		BioMark Sample Template.xls	BioMark GenoTyping Assay Template.xls
Tab	Sample information by <i>plate well location</i>	SBS96 for 48.48 or 96.96	SBS96 for All
	Sample information by <i>inlet number</i>	96.96 inlets	96.96 inlets

- 3 On the template spreadsheet, enter the sample or assay information
- 4 Keep the tab open, then save the file as a .csv file with a unique filename.

## Changing Allele Settings

**Change...**

- 1 Ensure that you have clicked **Assay Setup** in the Chip Explorer pane.
- 2 To change Click **Change** button in the Task pane under Allele Settings.
- 3 Using the following as a guide when changing allele settings.



## Advanced User Operations

Below are additional ways to load chip runs into the analysis software. These are generally used by advanced users.

### Create a New Chip Run

- 1 Click **Create a New Chip Run**.
- 2 Follow the steps in the wizard to create a new chip run.
- 3 Proceed to “[Annotate Assays Using Assay Setup](#)” on page 45.
- 4 Proceed to “[Annotate Samples Using Sample Setup](#)” on page 31.

## New From Current Chip Run

To create a new chip run template using data from an analyzed IFC that includes sample and assay settings, perform the following:

- 1 Open an *analyzed* and annotated chip run (.bml file extension).
- 2 Click File > New From Current Chip Run.



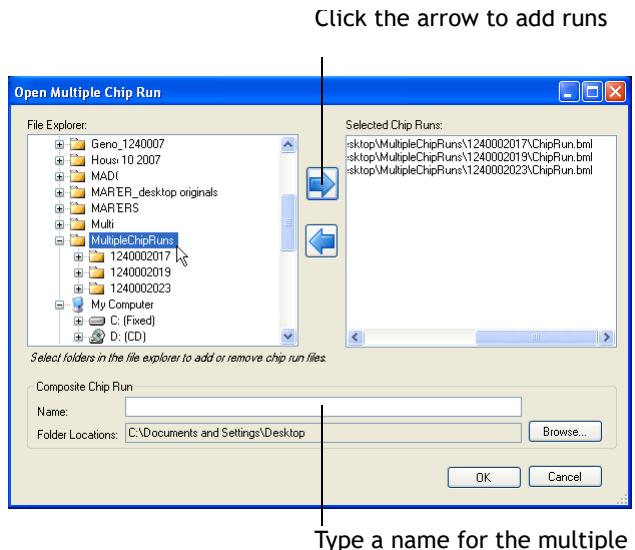
- 3 Open an *unanalyzed* and annotated chip run to create a run based on the analyzed run.
- 4 Type a name for the new chip run or, type the barcode number and then check **Use Barcode as Chip Run Name**.
- 5 Click **Browse** to navigate to a desired save location.
- 6 Click **OK** and the new chip run opens.
- 7 You can perform additional edits to the setup within the Genotyping software or save the file to be opened in the Data Collection software to run the new IFC.

## Importing Multiple Chip Runs

You can import multiple chip runs to increase data points. This can be done to combine multiple 48.48, 96.96 or 192.24 Dynamic Array IFCs.

**NOTE:** If multiple IFCs contain the same samples and assays, to ensure consistent annotation, you can annotate the samples/assays of the first IFC, and export the .plt file. Then, import the same .plt file to annotate the other chip runs.

- 1 Click File > Open Multiple Chip Runs.



Type a name for the multiple run

- 2 Click the folder containing the chip runs that you want to combine.
- 3 Click the arrow to move the selected chip runs in the folder to the other pane. Alternately, expand the multiple chip run folder and choose specific .bml run files, clicking the arrow for each file that you want to add to the other pane. Alternatively, you can select files from various folders.
- 4 Type a name.
- 5 Click **Browse**.



- 6 Navigate to a location to store the data.
- 7 If you are combining endpoint reads from same system and want to use only the raw intensity when combining the IFC runs, select the **Use the Raw Intensity Only** checkbox.
- 8 Click **OK**.

## Finding Corners Manually (if required)

**NOTE:** If the chamber finding algorithm cannot locate the four corner cells of the IFC during the first analysis, the following error message will appear.



If this occurs, you can manually set the corners and then analyze the IFC.

- 1 Click OK.

The Set Corners of the Chambers Area dialog box appears.

- 2 Zoom in to see the corner cells.

**NOTE:** If you cannot see the four corner cells, adjust the Contrast slider. If an insufficient amount of ROX dye (due to an error in reaction set-up or sample loading), it will be difficult to see the corner cells.

If no ROX is present, the corner cells are very dark. You may have to count the number of rows and columns (96 down, 96 across for the 96.96 IFC, for example) to make sure you are placing cross hairs correctly.

- 3 Position the corners of the red box at the perimeter of the IFC image.

- 4 Make sure each corner is placed on the outer edges of each corner cell (see below).

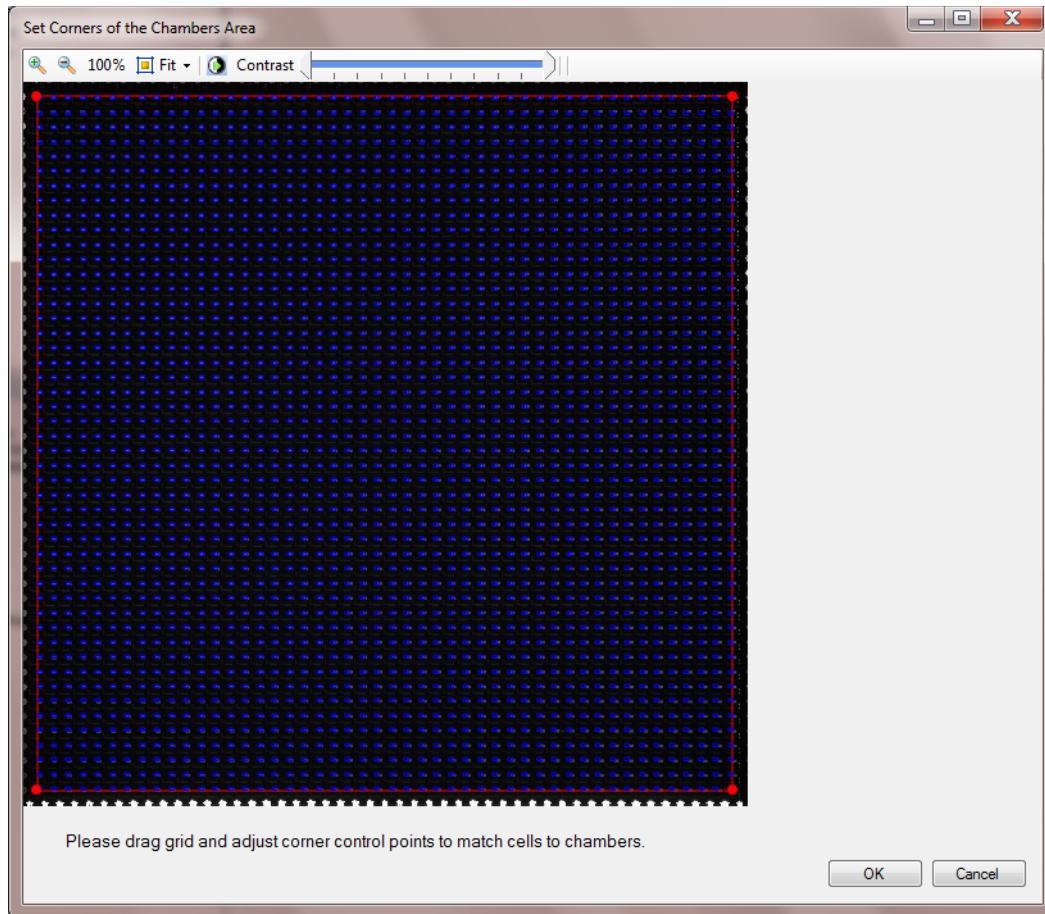


**NOTE:** You may not see the hydration chambers along the left and bottom edge of the image

- 5 Click Done.

**NOTE:** If no ROX is present, the corner cells are very dark. You may have to count the number of rows and columns (48 down, 48 across for the 48.48 IFC for example) to make sure you are placing cross hairs correctly.

- 6 You can drag the red box corners to the corner chamber locations to match the cells to chambers.



## Forced Manual Corner Find

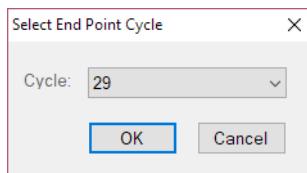
If the automated manual corner find results are not satisfactory, you can perform a forced manual corner find by pressing the **CTRL** key and simultaneously clicking on the **Analyze** button.

## Save a Real-Time IFC Run as an Endpoint IFC Run

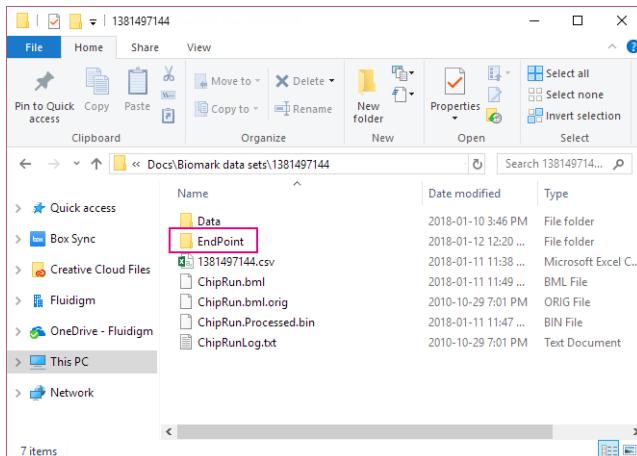
- 1 You can save a real-time IFC run as an endpoint run by selecting **File > Save as End Point Chip Run**.



- 2 Select the PCR cycle you want to use for the endpoint run, and then click OK.



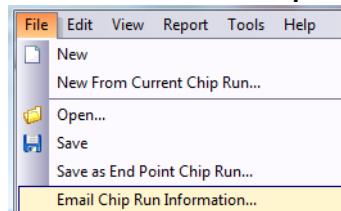
- 3 The IFC run will be saved in a new folder named **EndPoint** in the original IFC run folder.



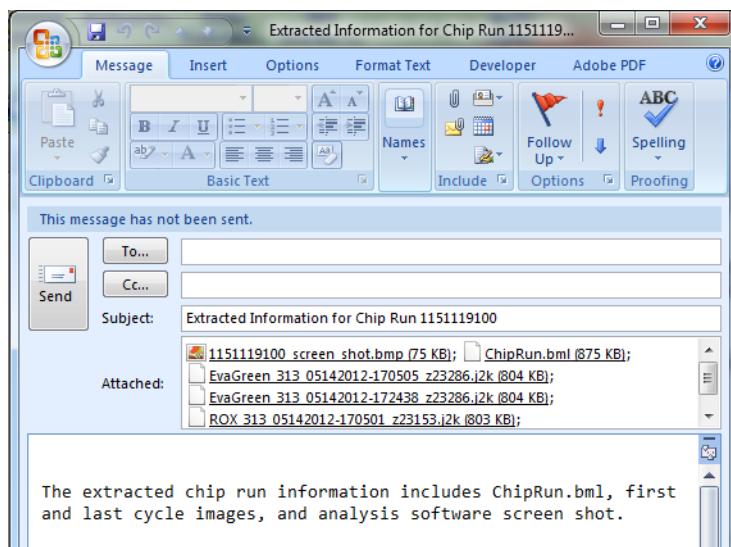
## Email Chip Run Information

Pertinent chip run information can be shared via email with co-workers, collaborators, Fluidigm Technical Support, etc.

- 1 Open the chip run you wish to share.
- 2 Go to **File > Email Chip Run Information.**



The software creates an email with attachments including: the chip run .bml file, first and last images and an analysis screenshot.



- 3 Enter the email address(es) of the people you want to send the information to.
- 4 Click **Send**.



# Viewing Run Data in the Analysis Software

3

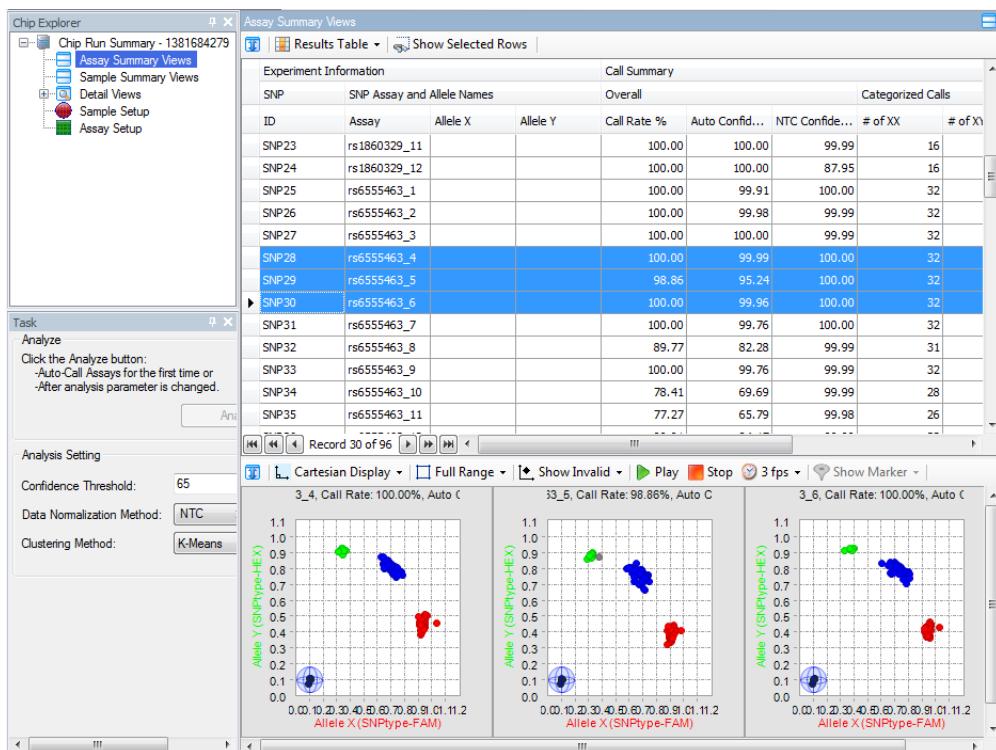
## Viewing Data in Summary Views

You can select either Assay Summary Views or Sample Summary Views in the Chip Explorer.

Assay Summary Views allow you to view where the center of plotted samples are relative to the assay selected. Sample Summary View allows you to view the center of plotted assays relative to the sample selected.

### Assay Summary Views

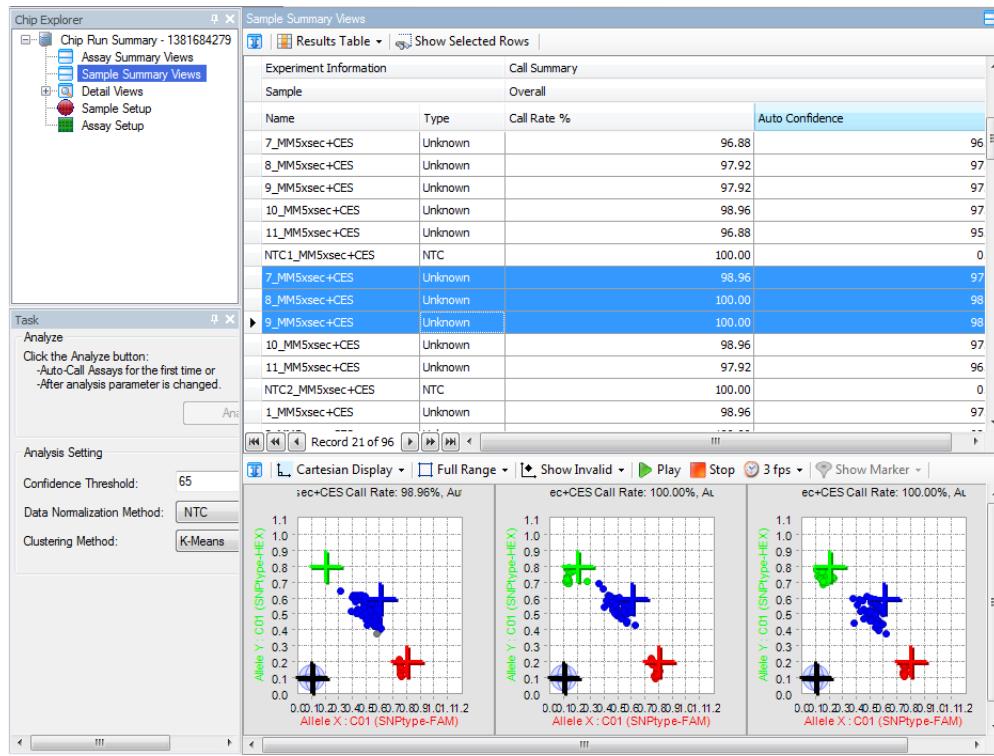
- 1 Click Assay Summary Views.
- 2 Select SNP assays of interest to view scatter plots below.



The scatter plots display where samples are relative to each assay selected.

## Sample Summary Views

- 1 Click Sample Summary Views.
- 2 Select samples of interest to view scatter plots below.



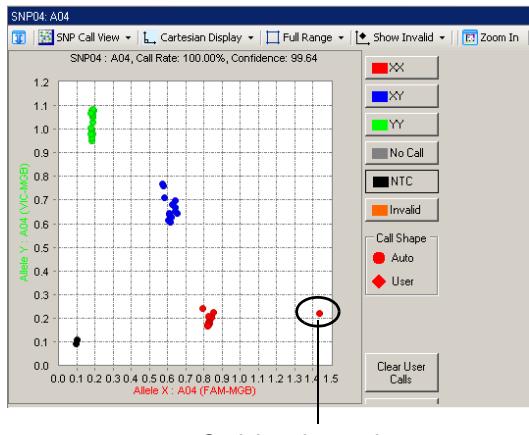
The scatter plots display where the center of plotted assays are relative to each sample selected.

## Using Cartesian Display/Polar Display

You can view the Scatter Plot data as a Cartesian Display or a Polar Display by clicking the **Cartesian/Polar** toggle button for individual SNPs and Summary View.

## Cartesian Display

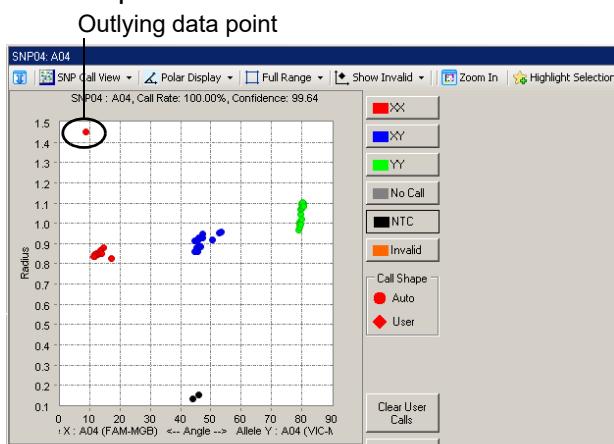
This is a standard view format for plotted results.



Outlying data point

## Polar Display

For example:



## Changing the Confidence Threshold

You can change the number in the Confidence Threshold text box (0-100) to reflect your desired level of confidence in the genotype assignment of data points for a particular SNP assay. It is calculated by examining the distance of the point to the center of the cluster it belongs to, along with the distances to the centers of the clusters it does not belong to. If a point is close to the center of the cluster it belongs to compared with the other cluster centers, it will have a higher confidence level.

If a data point call is less than the threshold number you set, the data point call is changed to No Call. The default confidence threshold setting is 65.

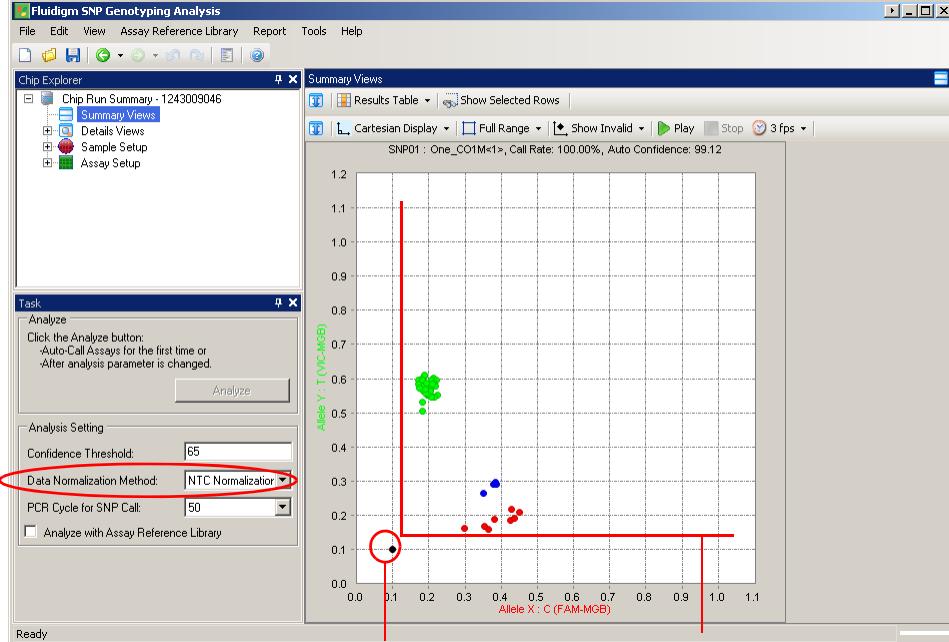
## Data Normalization Method

Under **Task**, you can set the Data Normalization Method to “NTC”, “None”, or “SNPtype Normalization”.

- **NTC** stands for “No Template Control” normalization and is the default setting for chip runs that do not use Fluidigm SNP Type Assays. This option makes viewing assays on the plotted graphs easier, because it normalizes the position of the no template control cells. The no template control cells are aligned to the  $x = 0.1$  and  $y = 0.1$  location on the plotted graph. It also normalizes the intensities of the assays so that they are roughly plotted in a square.
- **None** does not normalize the NTC cells nor does it normalize the assays.

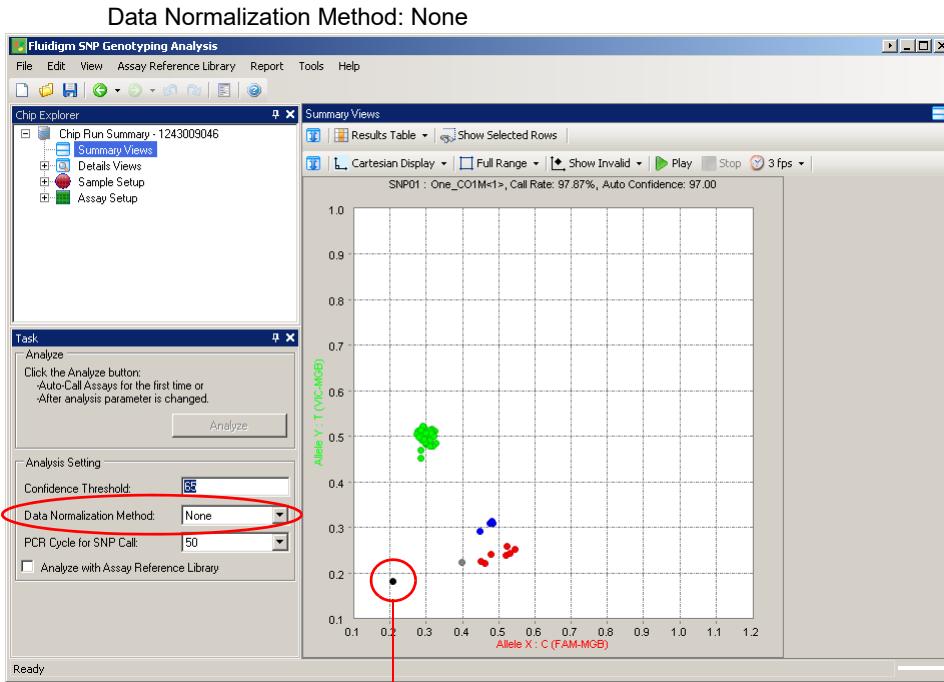
See examples below of NTC normalized data and non-normalized data.

Data Normalization Method: NTC



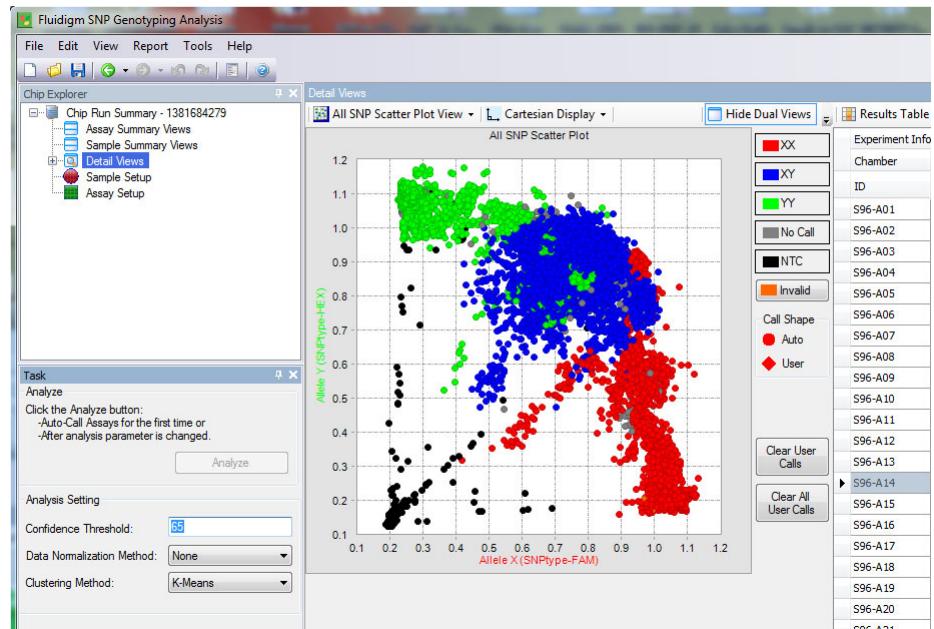
Notice the black “no template control” cells are shifted to the  $x = 0.1$  and  $y = 0.1$  location on the plotted graph

The assays intensities are plotted roughly in a square



Note the no template control cells are *not* plotted at the  $x = .1$ ,  $y = .1$  position.

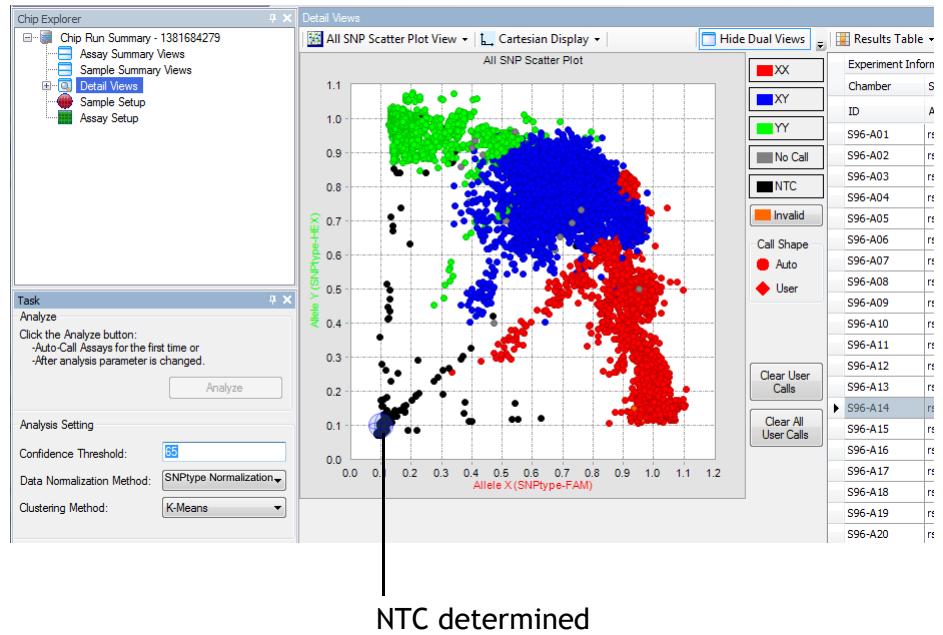
Below is a scatter plot view of a chamber with an unusable NTC (floating). “None” is selected as the normalization method.



- **SNPtype Normalization** is the default option for chip runs using Fluidigm SNP Type Assays. This option determines a global NTC setting from the NTCs across the chip run. In cases where there are no NTCs defined, this option will estimate the location of the NTC.

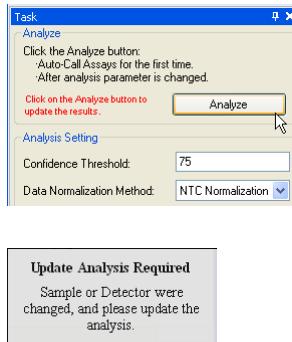
**NOTE:** SNPtype Normalization will not work on chip runs using TaqMan assays.

If you re-analyze that same chamber with the “SNPtype Normalization” method, the algorithm applies the averaged NTC location (global NTC), and the results in this chamber are now usable.



## Analyze

Be sure to click the **Analyze** button every time you change a parameter (see graphic below). A prompt in red text displays when you need to click the **Analyze** button again. A popup also displays.



In this example, changing the confidence threshold to 75 generates the red text prompt.

**IMPORTANT:** If you do not click **Analyze**, your changes are not applied to the data.

## Detail Views

When you click **Detail Views**, four options are available on the drop-down menu in the Detail Views pane:

- “[Results Table View](#)”
- “[All SNP Scatter Plot View](#)” on page 73
- “[Image View](#)” on page 74
- “[Call Map View](#)” on page 77

Each view opens data for the entire IFC.

### Results Table View

Click **Results Table View** to see the default view which includes data from the entire IFC, each row representing a chamber on the IFC.

**NOTE:** Select a single row or multiple rows by pressing shift and/or Ctrl keys to highlight them. Then, click Show Selected Rows to bring them to the top of the table. The selected rows remain highlighted:

Click Detail Views to open default view

ID	Assay	Allele X	Allele Y	Name	Type	Auto	Confidence	Comments
S96-A01	rs921933_1			NTC2_MM5x...	NTC	NTC	100	
S96-A02	rs921933_2			NTC2_MM5x...	NTC	NTC	100	
S96-A03	rs921933_3			NTC2_MM5x...	NTC	NTC	100	
S96-A04	rs921933_4			NTC2_MM5x...	NTC	NTC	100	
S96-A05	rs921933_5			NTC2_MM5x...	NTC	NTC	100	
S96-A06	rs921933_6			NTC2_MM5x...	NTC	NTC	100	
S96-A07	rs921933_7			NTC2_MM5x...	NTC	NTC	100	
S96-A08	rs921933_8			NTC2_MM5x...	NTC	NTC	100	
S96-A09	rs921933_9			NTC2_MM5x...	NTC	NTC	100	
S96-A10	rs921933_10			NTC2_MM5x...	NTC	NTC	100	
S96-A11	rs921933_11			NTC2_MM5x...	NTC	NTC	100	
S96-A12	rs921933_12			NTC2_MM5x...	NTC	NTC	100	
S96-A13	rs1860329_1			NTC2_MM5x...	NTC	NTC	100	
S96-A14	rs1860329_2			NTC2_MM5x...	NTC	NTC	100	
S96-A15	rs1860329_3			NTC2_MM5x...	NTC	NTC	100	
S96-A16	rs1860329_4			NTC2_MM5x...	NTC	NTC	100	
S96-A17	rs1860329_5			NTC2_MM5x...	NTC	NTC	100	
S96-A18	rs1860329_6			NTC2_MM5x...	NTC	NTC	100	
S96-A19	rs1860329_7			NTC2_MM5x...	NTC	NTC	100	
S96-A20	rs1860329_8			NTC2_MM5x...	NTC	NTC	100	

### Adding User Defined Comments

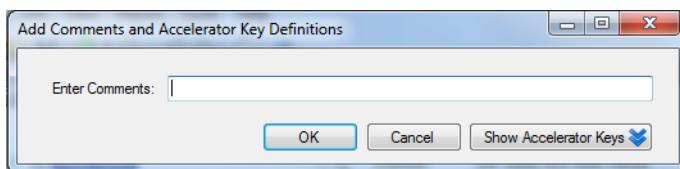
You can add comments to each chamber in the Detail Views. In addition, macros can be set up to apply common comments to multiple chambers.

#### 1 Click on the Detail Views

- 2 Select Results Table view.
- 3 Click on sample you wish to add a comment to.
- 4 Click on the **Comments** button on the top toolbar:

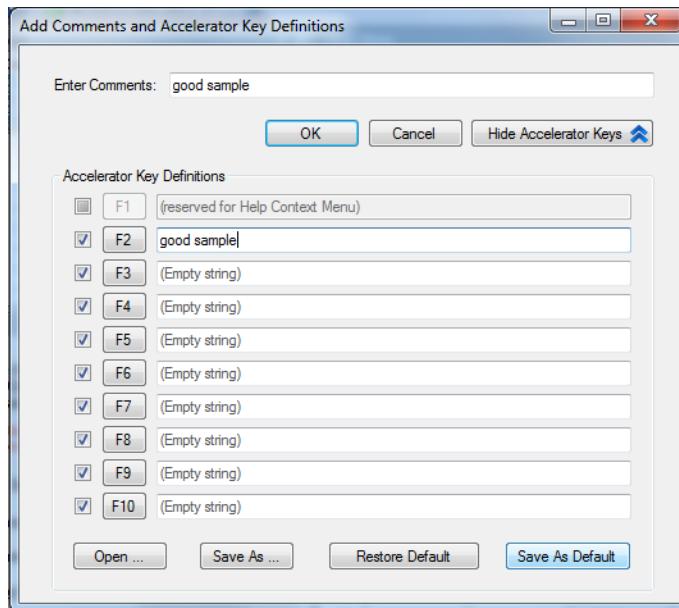
Experiment Information				Results			
Chamber	SNP Assay and Allele Names			Comments		Call Information	
ID	Assay	Allele X	Allele Y	Name	Type	Auto	Confidence
S03-A48	rs2238213_12			3_MM5xsec...	Unknown	XY	100
S03-A54	rs6559417_6			3_MM5xsec...	Unknown	YY	100
S03-A53	rs6559417_5			3_MM5xsec...	Unknown	YY	100
S03-A52	rs6559417_4			3_MM5xsec...	Unknown	YY	100
S03-A51	rs6559417_3			3_MM5xsec...	Unknown	YY	100
S03-A50	rs6559417_2			3_MM5xsec...	Unknown	YY	100
S03-A49	rs6559417_1			3_MM5xsec...	Unknown	No Call	
S03-A60	rs6559417_12			3_MM5xsec...	Unknown	YY	100
S03-A59	rs6559417_11			3_MM5xsec...	Unknown	YY	100
S03-A58	rs6559417_10			3_MM5xsec...	Unknown	YY	100

The Add Comments and Accelerator Key Definitions dialog box appears.



- 5 Enter a comment, such as “good sample”.
- 6 Click **OK** to apply to the first sample. You see the comment appear in the Comments column of the Results table.
- 7 (Optional) Click the Show Accelerator Keys button to enable adding a common comment to multiple samples.
- 8 Enter a comment that will be used frequently, “good sample,” for example, in the Enter Comments box.

- 9 To save and display commonly used comments with a keyboard shortcut, select the preferred Accelerator Key and repeat the comment. F2 was used in this example:

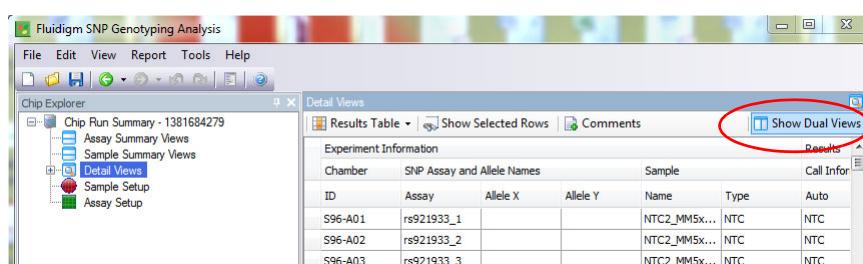


- 10 Click the **Save As Default** button.
- 11 Select chambers you wish to add this comment to and simply press the F2 key.

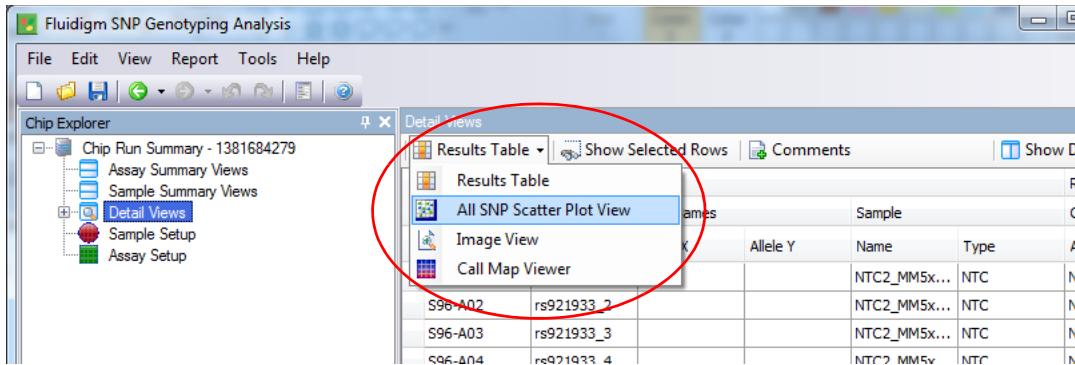
## Using Show Dual Views

The Show Dual Views button allows two primary views side-by-side. Any two of four views can be displayed side-by-side. For example, a Results Table View and an All SNP Scatter View:

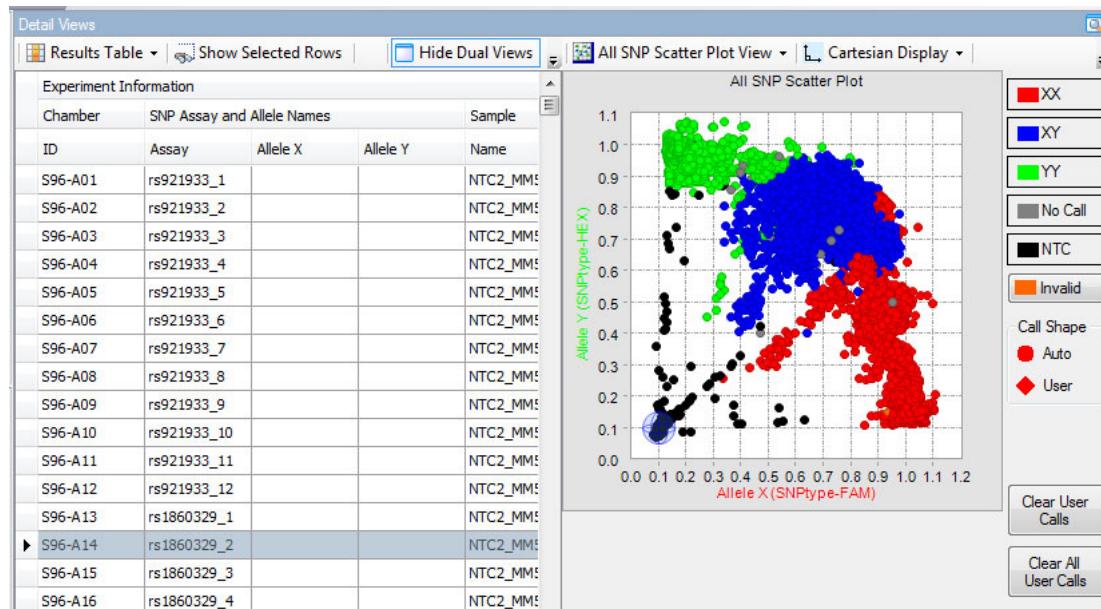
- 1 From the Analysis View, click the **Show Dual Views** button.



- 2 Select which type of primary view you'd like the software to display.



The primary view displays:



**3** Click Hide Dual Views to go back to only one primary view, if desired.

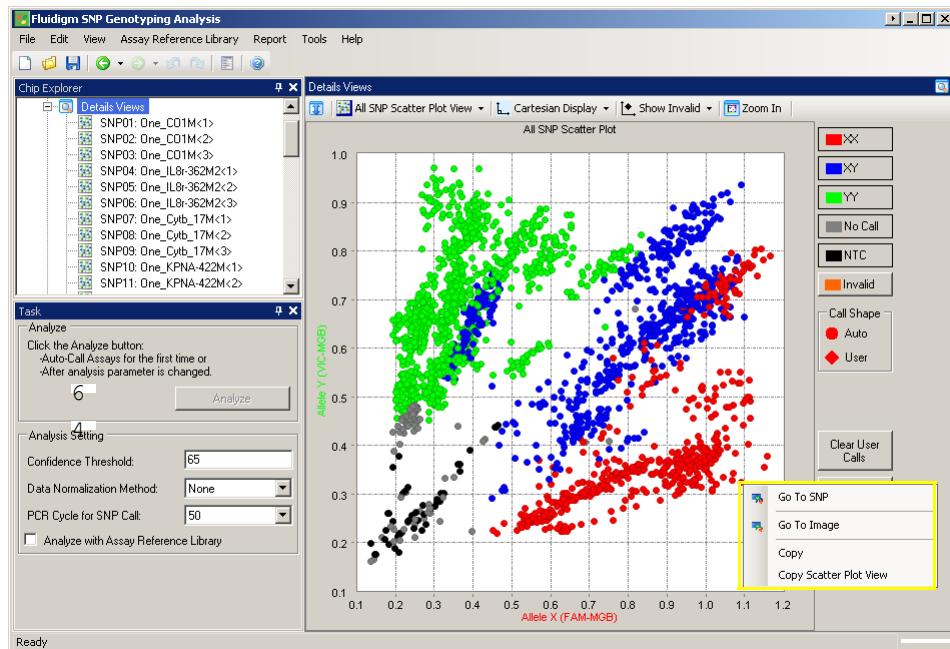
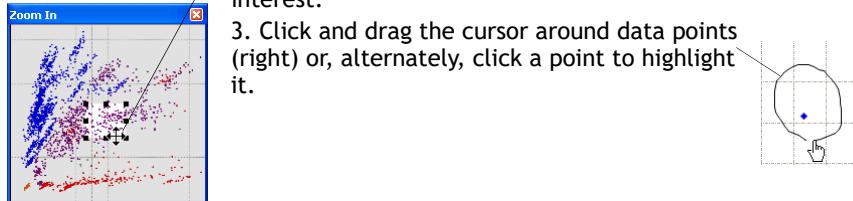
## All SNP Scatter Plot View

The All SNP Scatter Plot View option shows all SNP data from the IFC in one scatter plot. Use the zoom to enlarge areas of interest.

1. Click **Zoom In** to display the zoom map (left).

2. Position your cursor over the highlighted square, click and drag the square to the area of interest.

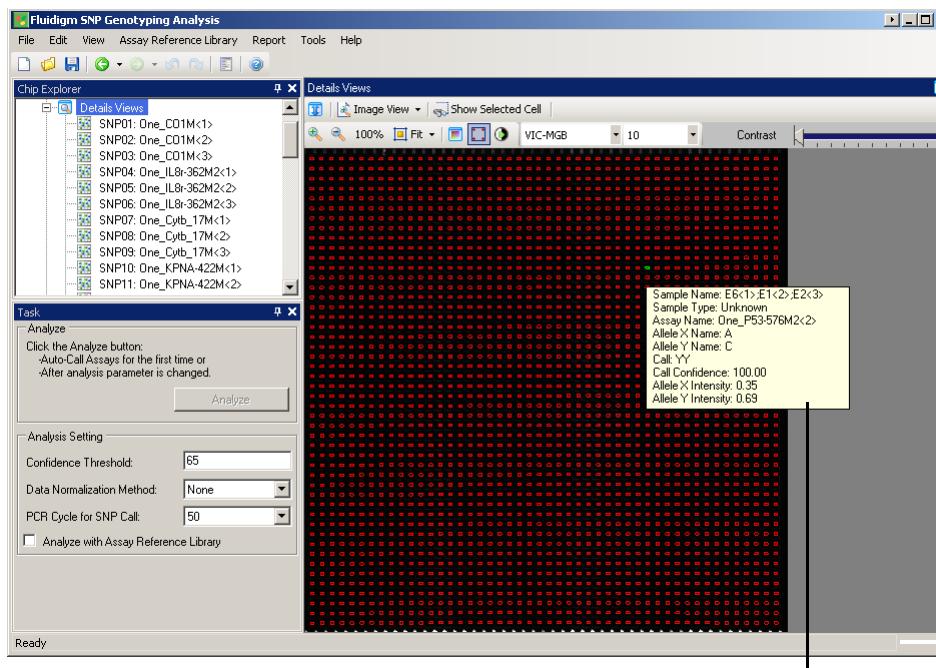
3. Click and drag the cursor around data points (right) or, alternately, click a point to highlight it.



**NOTE:** To change a call of a SNP, right-click a data point in scatter plot view then click Go to SNP and change the call in the Detail Views of the scatter plot for that SNP. For more information on changing calls, proceed to “Changing Calls” on page 88.

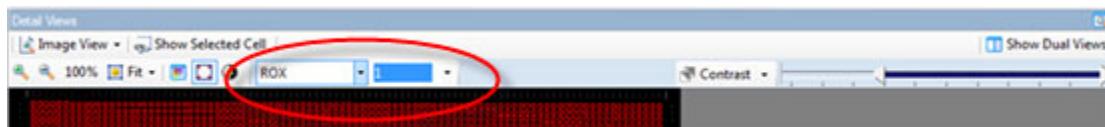
## Image View

The **Image View** option shows the reaction chambers on the IFC.

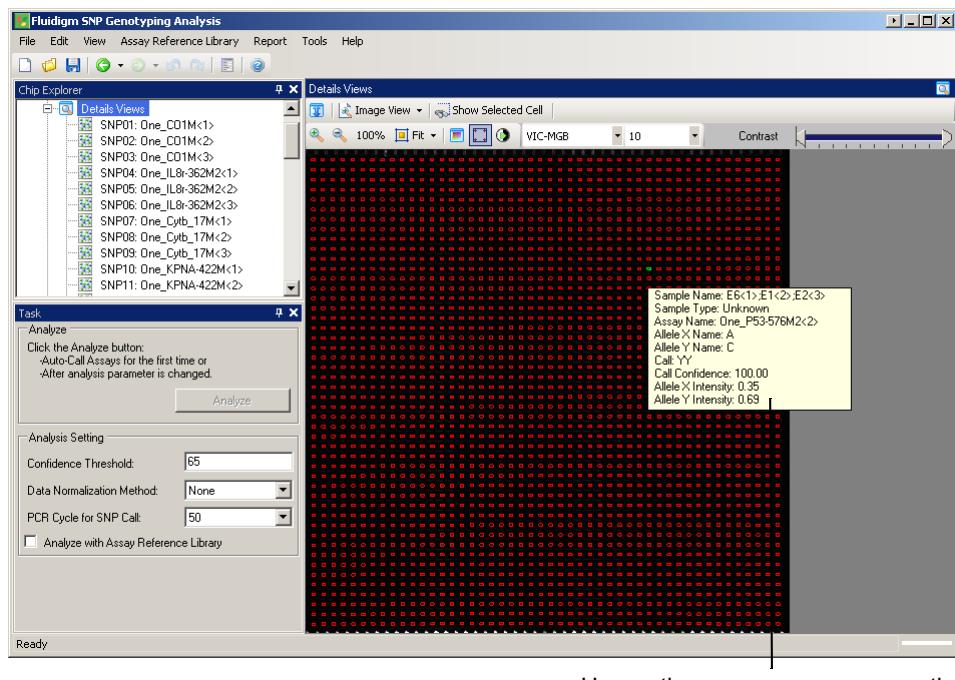


Hover the cursor over a reaction chamber to display details

2 Select the dye and cycle number:



3 (Optional) Adjust the contrast (see “[Contrast \(Auto or Manual\)](#)” on page 76):

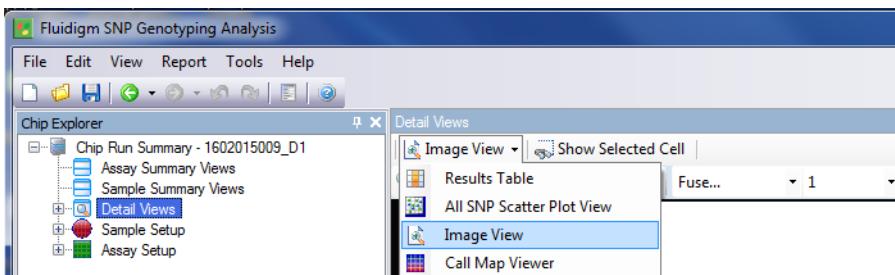


Hover the cursor over a reaction chamber to display details

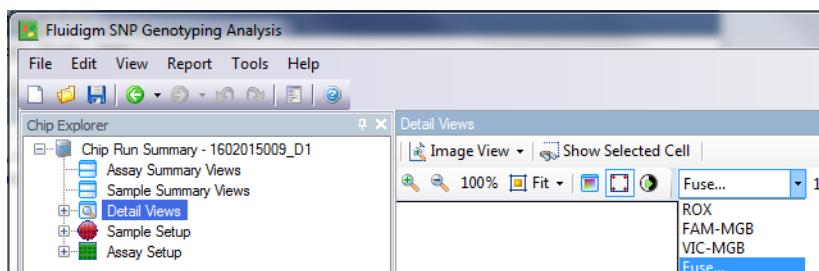
## Fused Image View

If the chip run you are analyzing has more than one color, the Fuse option will be available in the dye selection drop-down menu. This allows you to see the difference in intensity across multiple dyes in a single image.

- 1 Click on Detail Views.
- 2 Select Image View.

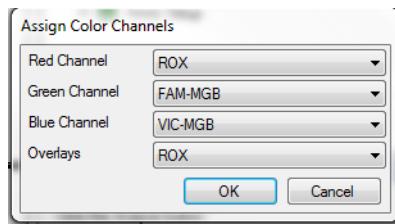


- 3 From the dye drop-down menu, select Fuse.



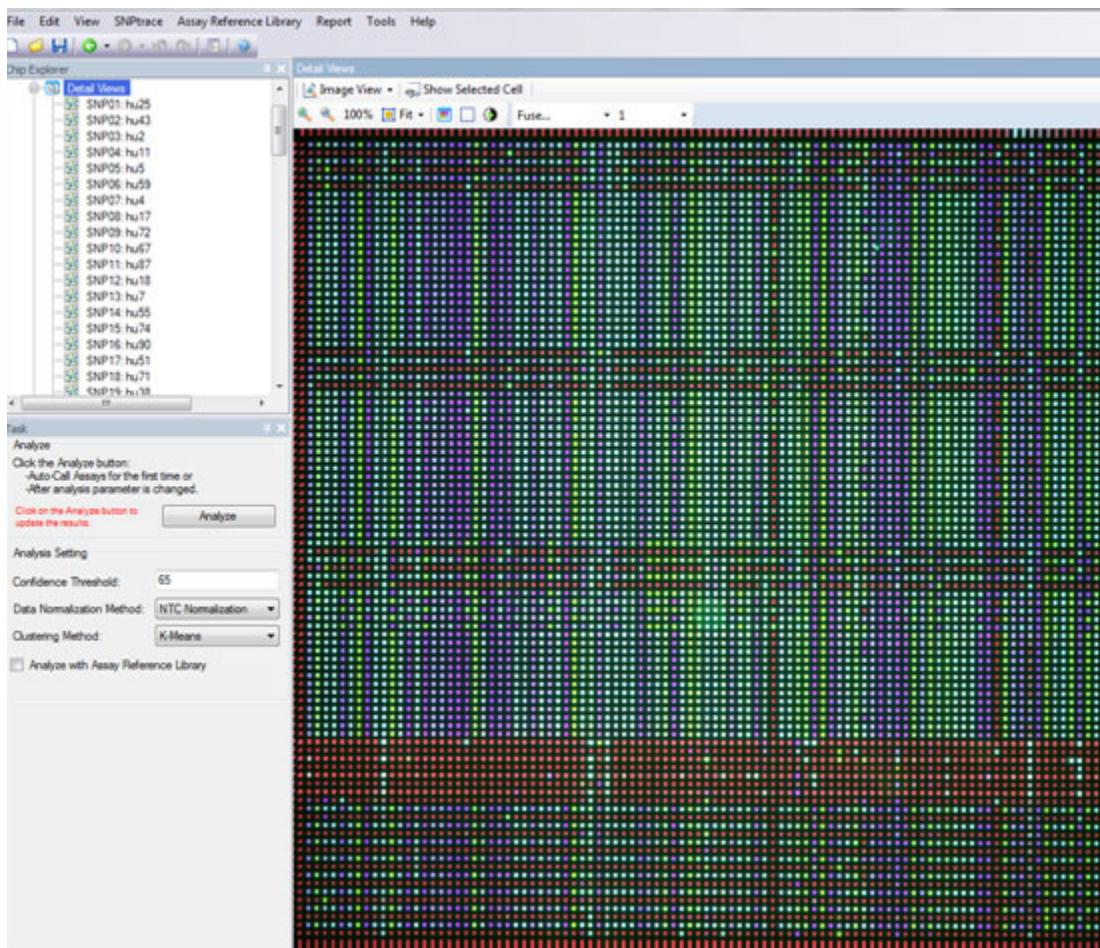
The Assign Color Values dialog box appears.

- 4 Select a color for each dye.
- 5 Select the dye you wish to overlay on top of the other.



- 6 Click OK.

A fused image displays:



- 7 You can select **Fuse** again to change any parameter.

## Contrast (Auto or Manual)

You can apply contrast adjustments to all the dyes at once or to each dye individually by selecting **All** or **Individual** from the Contrast drop-down menu.



To adjust image contrast:

- Click the Auto-Contrast icon.
- Or,
- Move the contrast sliders by placing your cursor over a slider, then click and drag.

## Call Map View

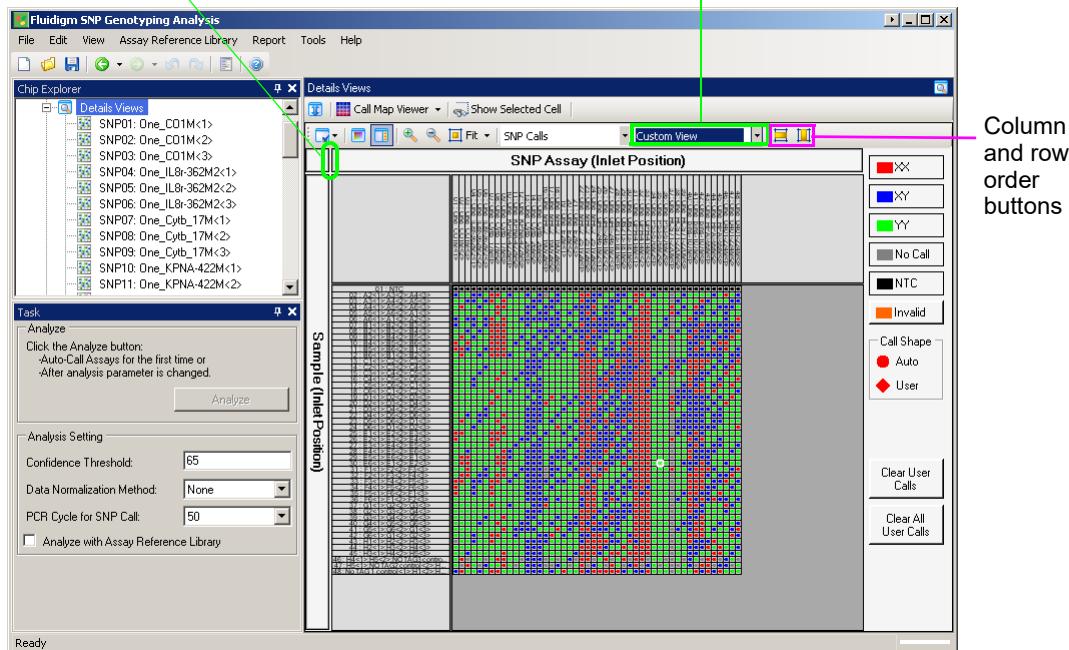
The Call Map View shows the location and genotype call for each SNP and sample on the IFC:

Click and drag the column border to expand it.

Switch from Inlet-based View to Chip-based View to Custom View.

Inlet-based View shows a map of the IFC based on the delivery of assays and samples from the loading inlets on the IFC.

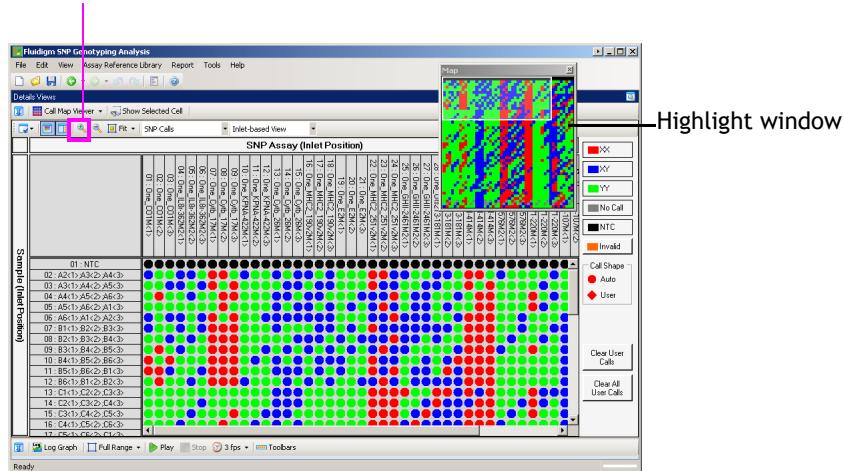
Chip-based View shows the actual physical position of the reaction on the IFC. Custom View allows the user to define the layout of the Call Map View. It begins with the same layout as the Inlet-based View, but the user can select which rows and columns should be visible and the order of them through the column and row order buttons next to the layout selector.



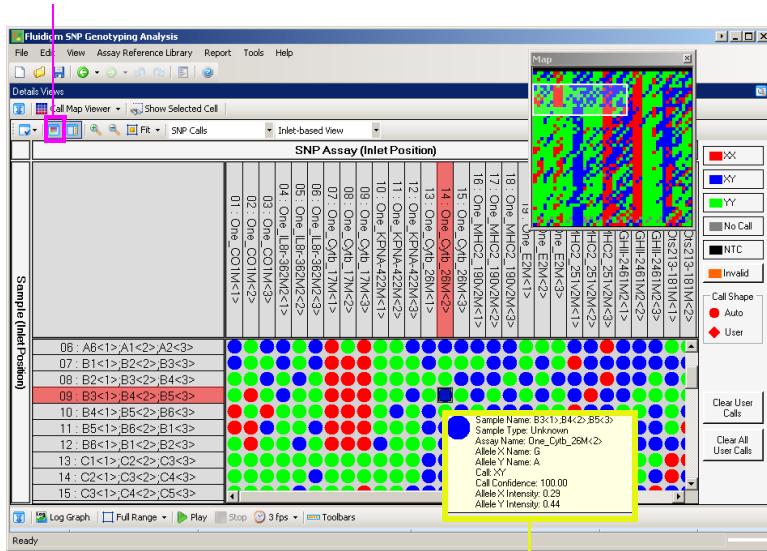
## Using the Call Map View Zoom

You can also use the call map view zoom feature in Image View (see “[Image View](#)” on page 74).

1. Click the Map icon to display the map.



2. Repeatedly click the Zoom in icon to enlarge the individual cells as shown below.

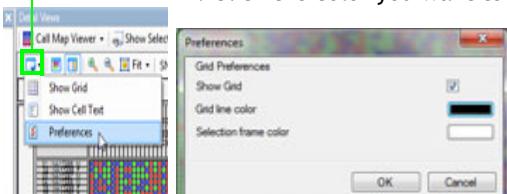


Hover over cells for more information.

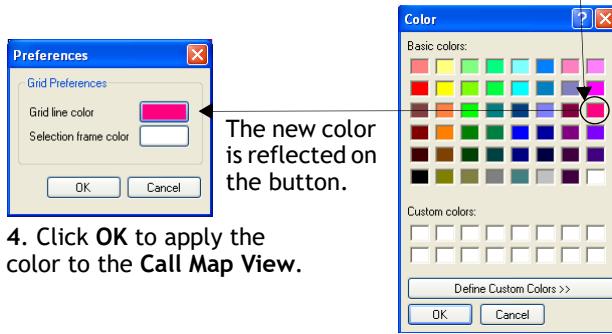
## Changing the Call Map View Color Scheme

1. Go to Grid Preferences > Preferences.

2. Click the color you want to change.

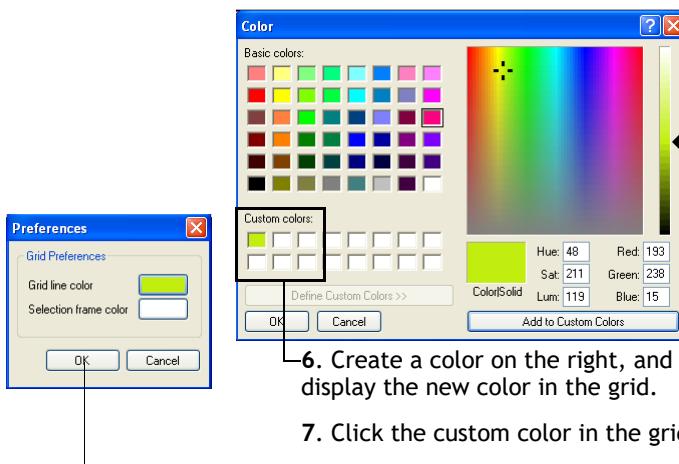


3. Click a basic color and then click OK.



4. Click OK to apply the color to the Call Map View.

5. Alternately, click Define Custom Colors to expand color choices.



6. Create a color on the right, and click Add to Custom Colors to display the new color in the grid.

7. Click the custom color in the grid and click OK.

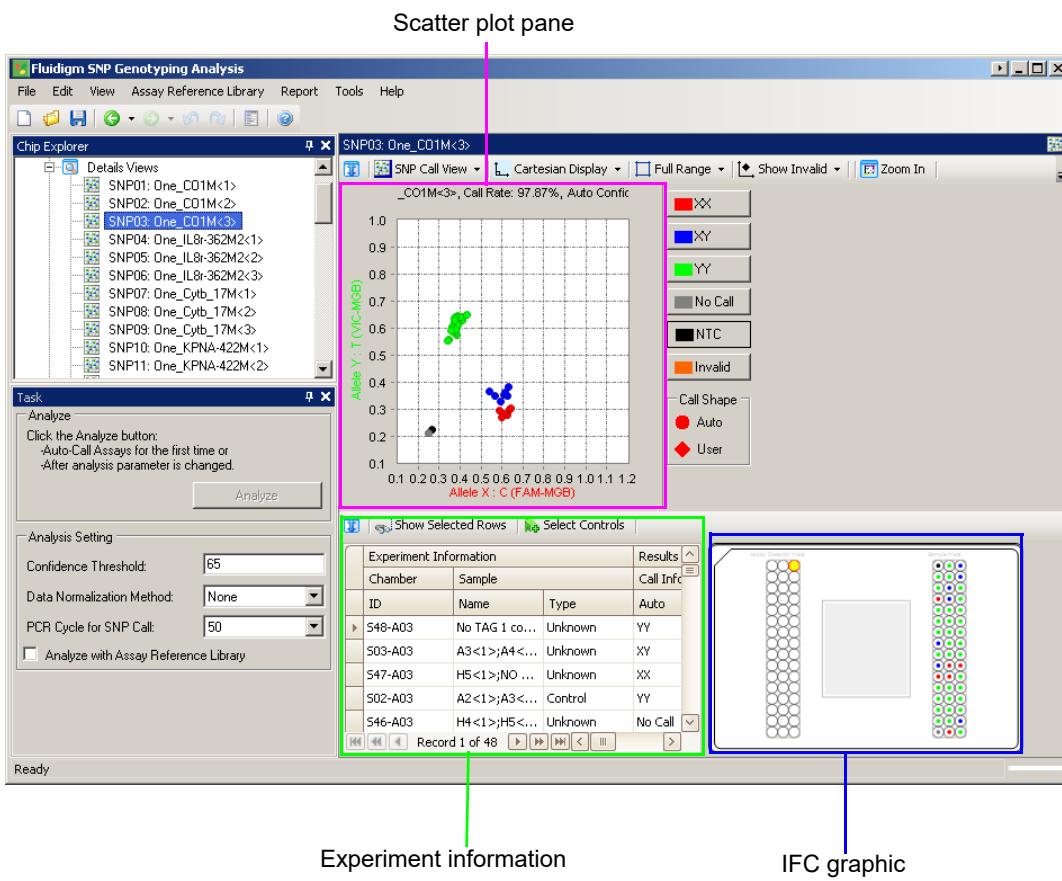
8. Click OK to apply the color to the Call Map View.

## Viewing Individual SNP Data

Single SNP data points are shown simultaneously in the scatter plot pane, the **Experiment Information** pane, and the IFC graphic.

To display SNP data points, click a single SNP data point under expanded Detail Views.

**NOTE:** Click  to expand the display of the Scatter Plot pane or the Experiment Information pane to the entire window.

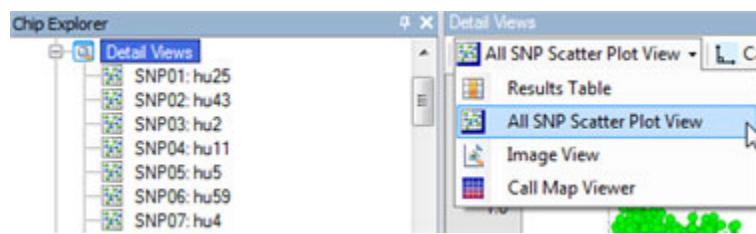


## SNP Call View

The SNP Call View shows specific SNP data as a scatter plot. Individual SNP Call Views for each assay are available by expanding the Detail Views in the Chip Explorer pane.

To display the SNP Call View:

- 1 Click “+” to expand Detail Views:



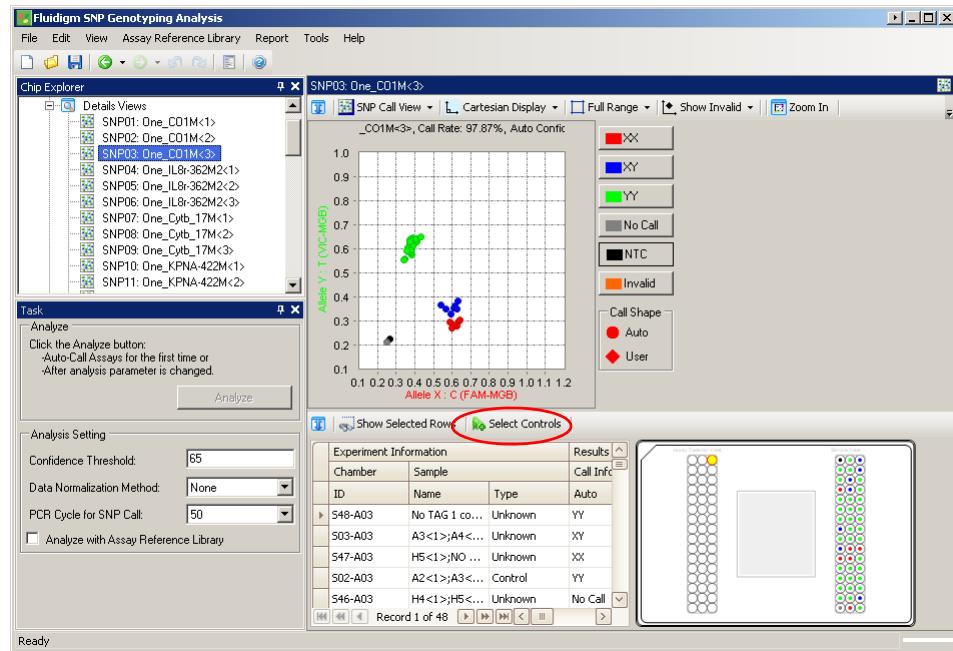
- 2 Select a SNP under Detail Views:



NOTE: Click to expand the active pane. Click it again to return to the default view.

## Select Control

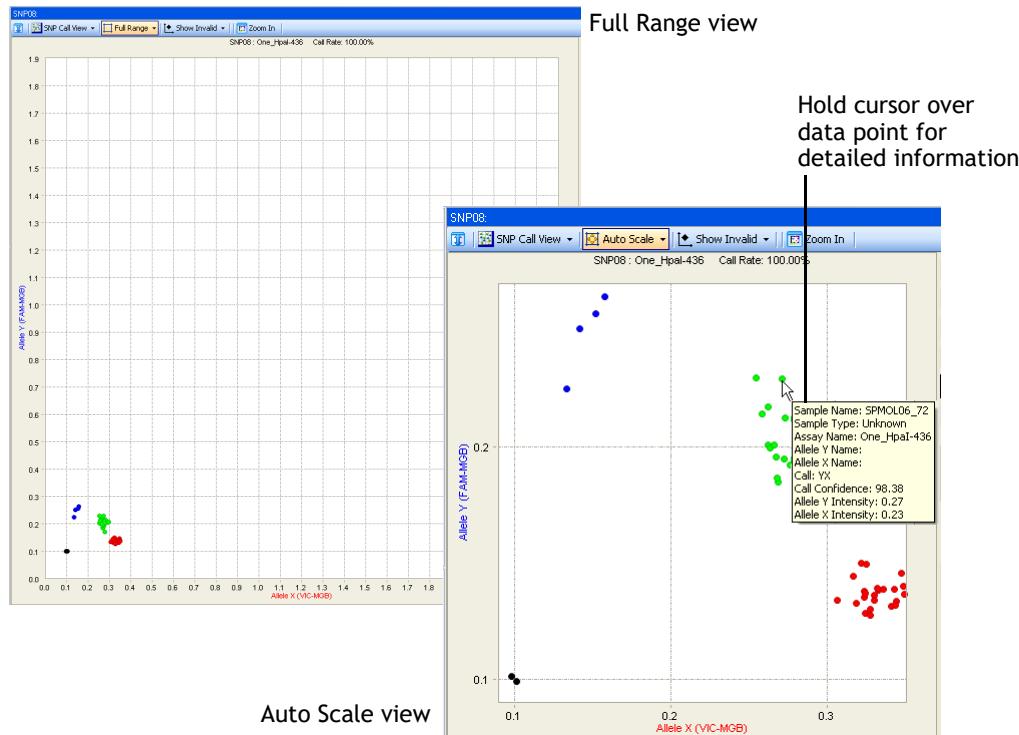
If you annotate any samples as “Control” types, a **Select Controls** button displays:



## Auto Scale/Full Range Toggle in SNP Call View

Auto Scale determines the best zoom factor to present all data points in the scatter plot. If invalid points are hidden, they are not used in determining the zoom range (see “[Show/Hide Invalid in SNP Call View](#)” on page 88).

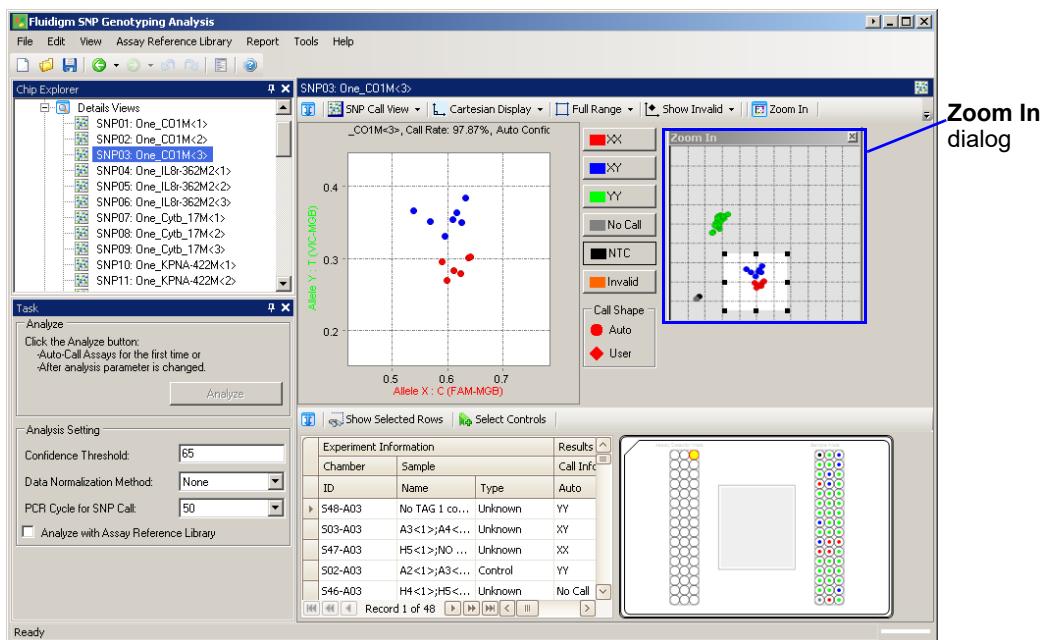
Full Range shows the data points without zoom.



## Using Zoom In

The **Zoom In** feature allows you to zoom in on an area of interest.

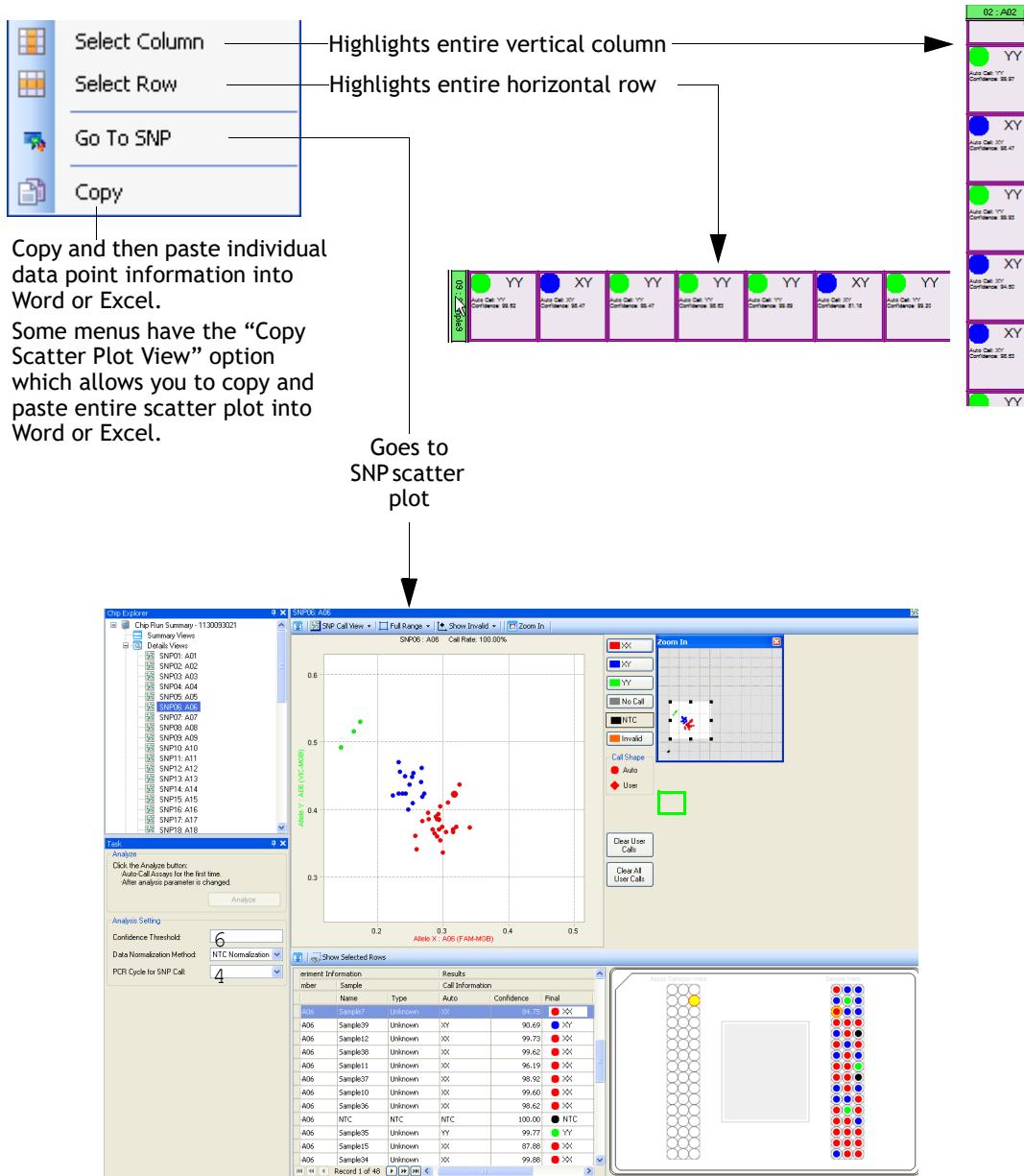
- 1 Click the **Zoom In** tab.



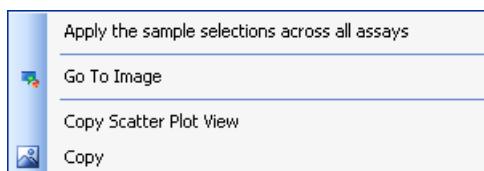
- 2 You can position the white box around an area of interest, and the new view will display under **SNP Call View**.

## Using the Right-Click Menus

Right-click a point of interest to open an options menu. Right-click menus throughout the application vary. The right-click menu on a sample point in Call Map Viewer and Image View:



If you right-click on a sample point in each SNP Call View:



## Changing Calls

You can only change the call of a SNP in Detail Views and SNP Call View (to change to views, see “[Detail Views](#)” on page 69). To change a current call, highlight a data point or circle data points with a cursor. Call choices are color coded in the legend. You can use the Undo Call and Redo Call buttons to revert a call to its previous state.

**Default SNP Call View.**

1. Click Auto Scale to enlarge the scatter plot.  
Drag the highlight window to an area of interest.

2. Click the data point of interest. Alternately, if the cluster is still too tight to clearly distinguish the data point, click Zoom In and position and resize the highlight window over the cluster containing the data point.

3. Ensure you have located the correct data point by placing your cursor over it to display relevant information.

4. Click the data point to activate it—when active, the data point is larger.  
  
Auto calls are circles and are made by the software  
User calls are diamonds and are set manually

5. Select the appropriate call from the legend—in this example below, Invalid is selected and the data point now reflects that change.

Right-click a data point for menu options:  
 —Apply the sample selections across all assays—see how data points perform across all of the other assays  
 —Go To Image—takes you to the Image View  
 —Copy—copies the data point information; can be pasted into Word or Excel  
 —Copy Scatter Plot View—copies a picture of the current scatter plot; can be pasted into a Microsoft® Word® or Microsoft® Excel® file

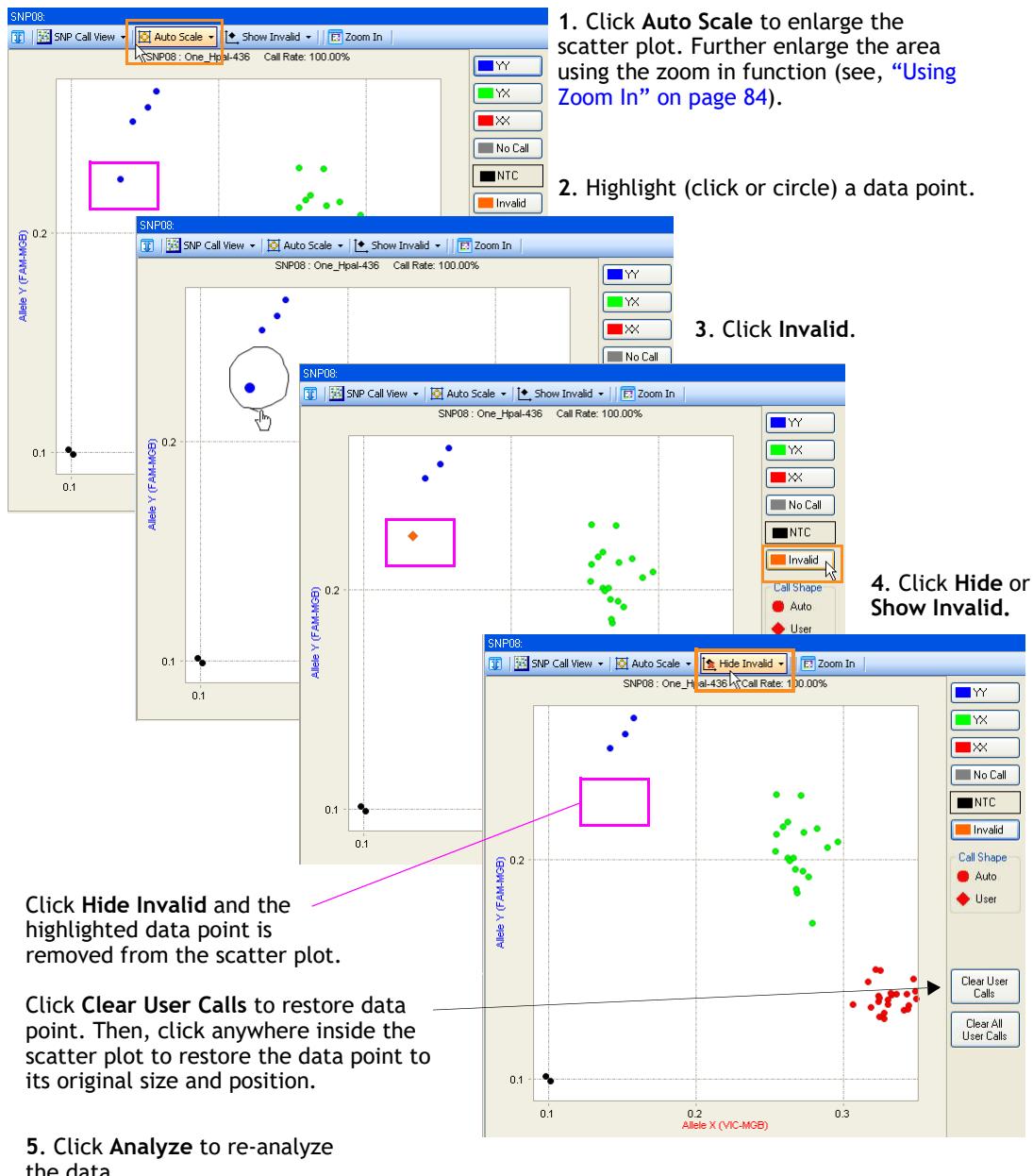
6. Click Analyze to update the analysis.

Clear User Calls restores individual points. Click anywhere inside the scatter plot to restore data point to its original size, color, and position.  
 Clear All User Calls restores all data points.

## Show/Hide Invalid in SNP Call View

You can hide invalid samples to exclude them from viewing. You can manually mark individual samples invalid by changing the sample name to “Invalid” or you can mark all samples for a specific assay invalid by changing the assay name to “Invalid.”

Bad chambers are automatically marked “Invalid” instead of “No Call” so they can be hidden by selecting **Hide Invalid**.



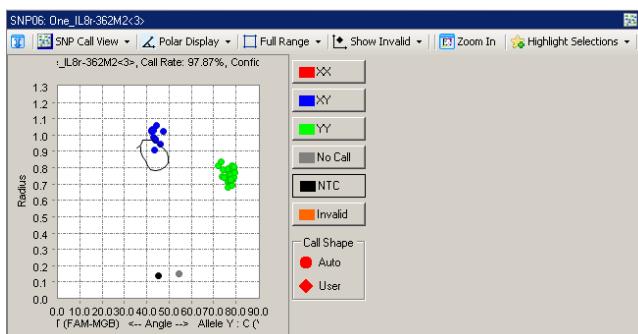
**NOTE:** An “Invalid” data point is not used by the clustering algorithm. A “No Call” (an ambiguous call) is used by the clustering algorithm.

## Highlight Selections

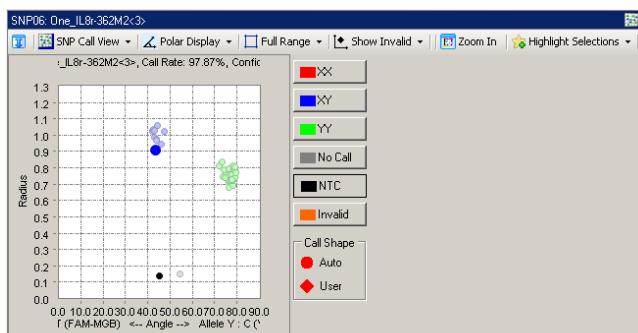
The Highlight Selections/No Highlight Selections toggle allows you to highlight points of interest:

- **Highlight points of interest:** Select **Highlight Selections**, then click a single data point or draw a circle around a group of data points. The selected data points enlarge, and the colors of unselected data points diminish.
- **Do not highlight points of interest:** Select **No Highlight Selections**, then click a single data point or draw a circle around a group of data points. The selected data points enlarge, but the unselected data points remain their original size and in full color.

Hold down your left mouse button and draw a circle around a point or points of interest:

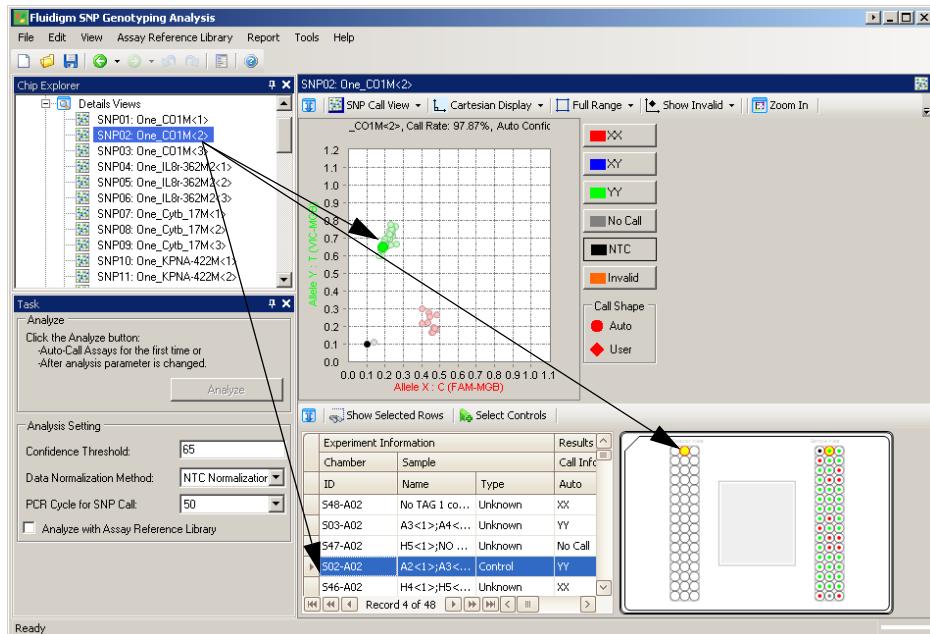


The dots for the selected points are enlarged.



## Experiment Information Pane

In the screenshot below, note that the highlighted data point is shown relative to its position on the scatter plot and its sample and assay sources on the IFC.

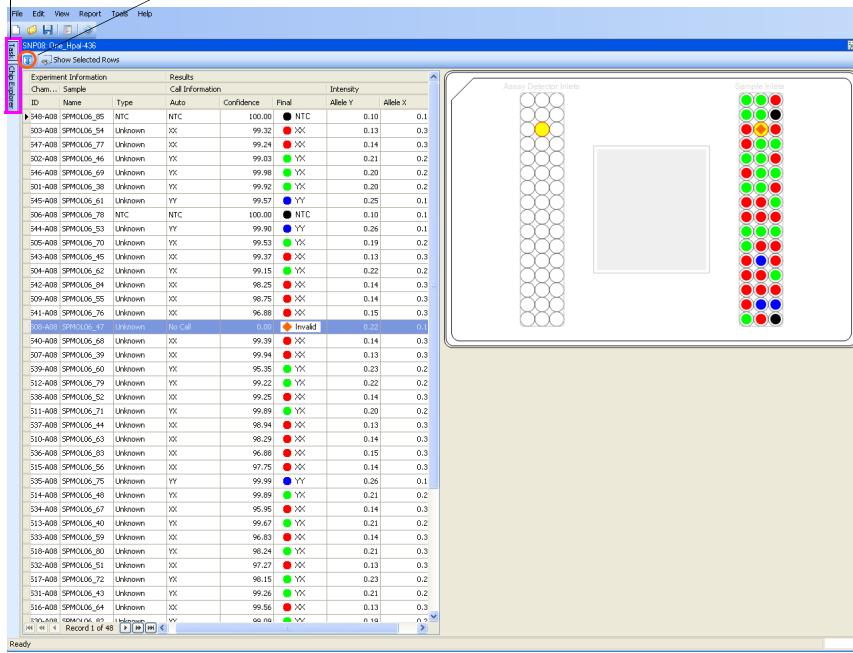


## Expanding the Experiment Information Pane

Click to expand the pane vertically. For additional space, use the push-pins to collapse surrounding panes. An expanded pane with unused panes ‘pinned’ to the left side is shown above.

Push-pinned panes concealed along border

Experiment Information pane expanded vertically

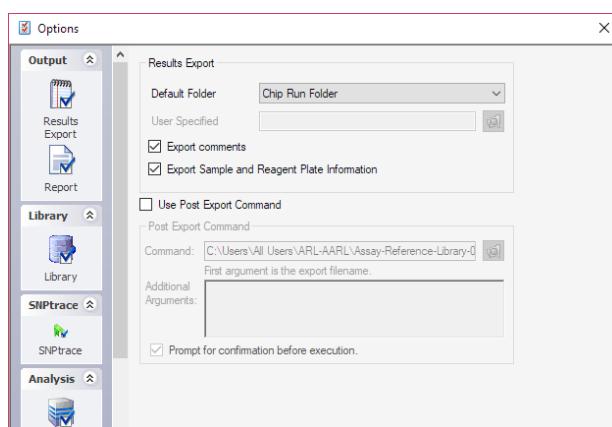


## Using the Tool Menu Options

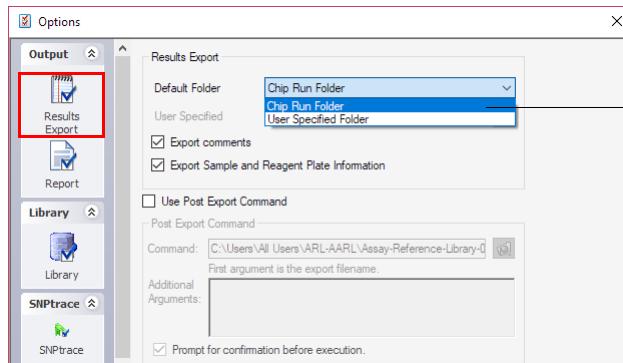
Set export, report and Assay Reference Library parameters using Options.

### Results Export

1 Click Tools > Options.



## 2 Click Results Export.



Choose a destination from the **Default Folder** menu. The browse icon is activated when you choose **User Specified Folder**.

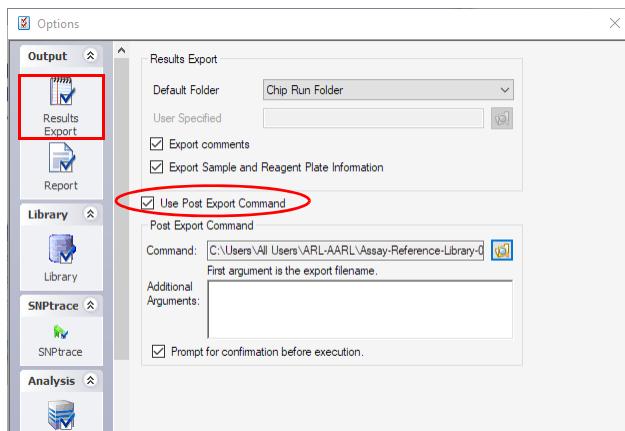


- 3 Use the default export destination or browse to another location.
- 4 Click **Apply**.
- 5 [Optional] Select **Export Sample and Reagent Plate Information** to include the sample and reagent plate names and barcodes in the report.
- 6 [Optional] If you are not changing the Report (graphs) options, click **OK**.

## Post Export Command

If you want to export your results to an integrated Assay Reference Library system use the Post Export Command.

- 1 Go to Tools > Options.
- 2 Check the **Use Post Export Command** option.

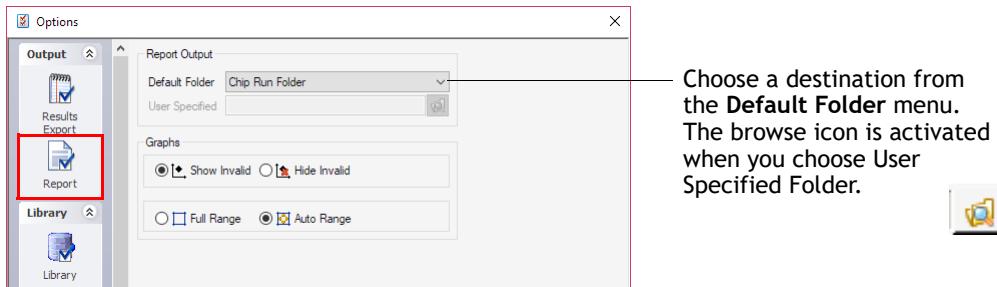


- 3 Then, browse to the Assay Reference Library system in the Command box.
- 4 Click **Apply**.
- 5 Click **OK**.

The data will be exported as a .csv file to the Assay Reference Library system you selected.

## Report

- 1 Click Tools > Options.
- 2 Click Report to open options.

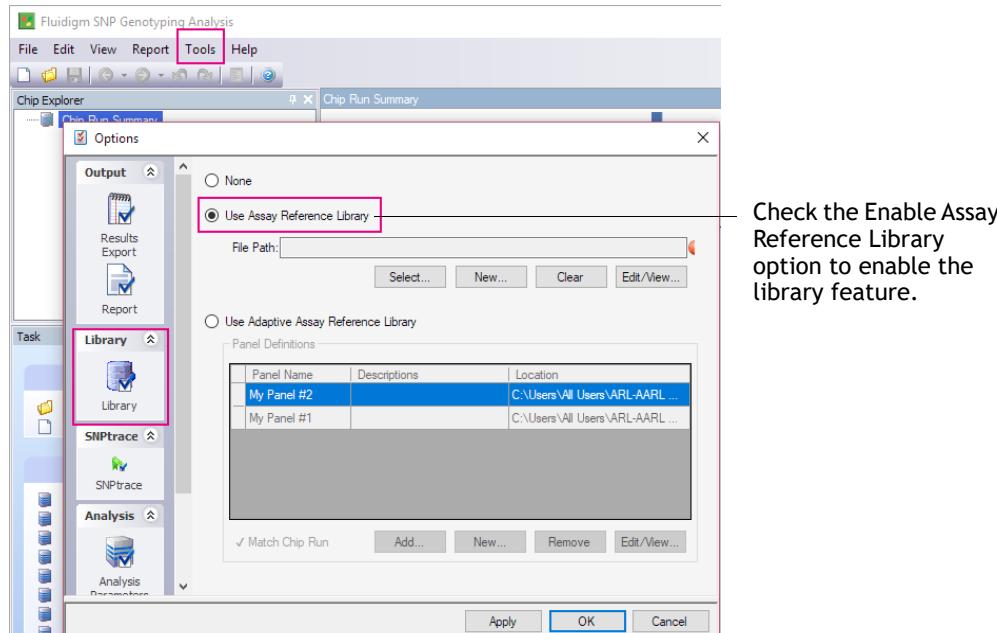


- 3 Use the default export destination or browse to another location.
- 4 Select graph options. When you export data that includes graphs or scatter plots, they reflect the options you choose here.
- 5 Click **Apply**, and then click **OK**.

## Library

**NOTE:** See *Chapter 4* in this manual for more information about Assay Reference Library.

- 1 Click Tools > Options.
- 2 Click Library on the Options dialog to see Assay Reference Library options.

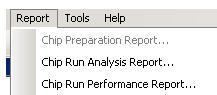


**NOTE:** If the Use Assay Reference Library is not selected and the currently open chip run has not been analyzed with an Assay Reference Library before, the Assay Reference Library-related GUI features are hidden.

- 3 Enter (or browse to) the location where you would like to store your Assay Reference Library.
- 4 Click **Apply**.
- 5 Click **OK**.

## Generating Reports

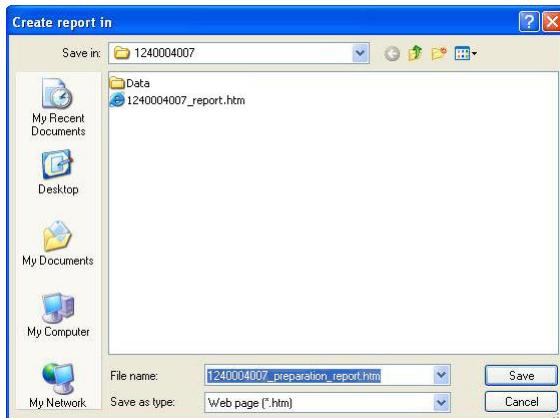
There are three different reports available depending on the type of chip run being opened: Chip Run Preparation Report, Chip Run Analysis Report and Chip Run Performance Report.



### Chip Run Preparation Report

This report is available if you open an *unanalyzed* chip run file or, if you create a new chip run file. The report shows dispense mapping information, and is saved as an .htm file.

- 1 Click **Report > Chip Preparation Report**.



The report name defaults to the IFC barcode number with the report type.

- 2 Click **Save** to save the Chip Preparation Report as an .htm file. The report opens automatically after you save it.

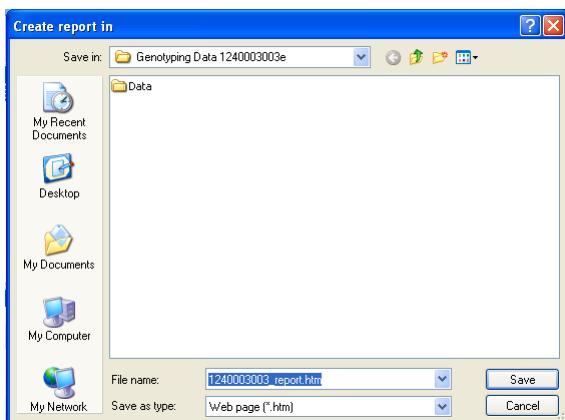
- 3 An example of a Chip Preparation Report is shown below.



## Chip Analysis Report

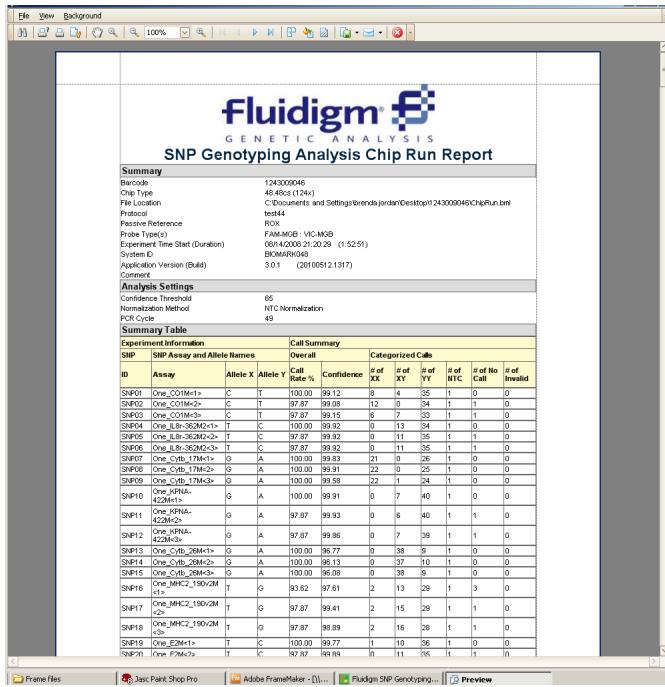
This report is available when an analyzed chip run is opened or, when an unanalyzed chip run is analyzed. The report shows analysis data, and is saved as an .htm file.

- 1 Click **Report > Chip Run Analysis Report**. A Preview window of the Chip Analysis Report displays in a new window.  
The default report name (File name) is the chip run barcode.
- 2 Click **Save** to save the Chip Run Analysis Report as an .htm file:

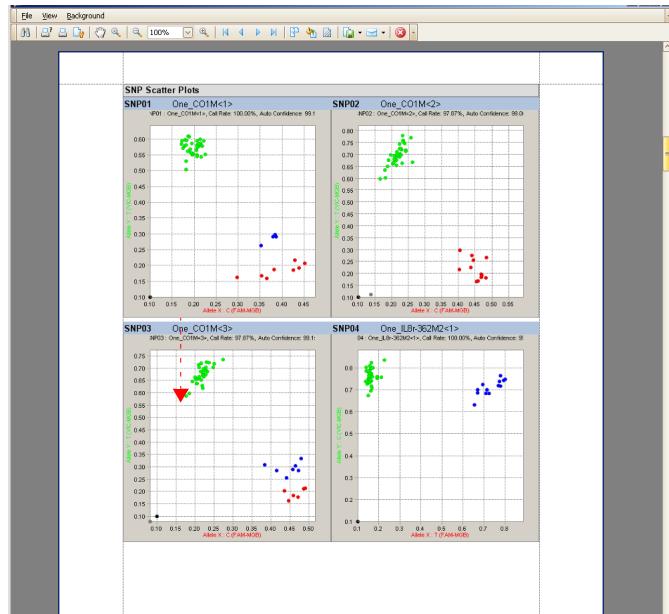


The report opens automatically after you save it.

An example of a Chip Run Analysis Report is shown below. The report includes individual scatter plots for each of the 48 reactions.



Data for each of the 48 reactions.

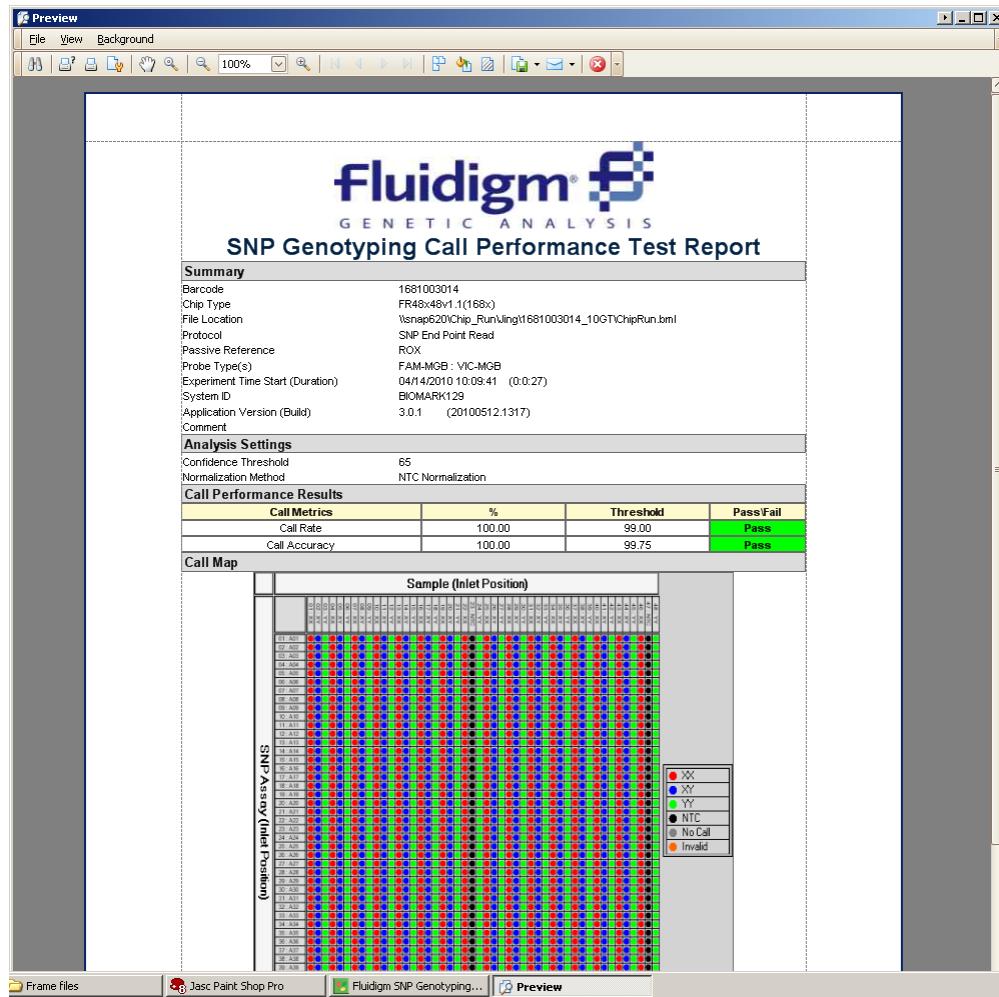


Individual scatter plots for each of the 48 reactions.

# Chip Run Performance Report

This report is designed to test the call performance of an IFC with a specific setup.

**NOTE:** The report results will not be accurate if the chip setup is not correct. Please contact Fluidigm Technical Support with any questions.

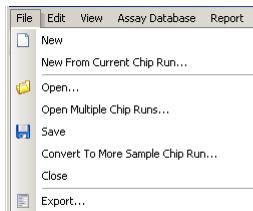


## Export IFC Run Data

You can export IFC run data in a comma-separated value format (.csv) that can be viewed in programs such as Microsoft Excel.

**NOTE:** The raw data output is used by advanced users to develop alternative analysis methods. This export option will report the fluorescence intensity values for each reaction chamber for each dye and for each PCR cycle (for real-time protocols). Additionally, dose meter data is exported, which is used for normalization when IFC runs are combined, or for normalization when the Assay Reference Library or Adaptive Assay Reference Library is used. For detailed information, contact Fluidigm Technical Support.

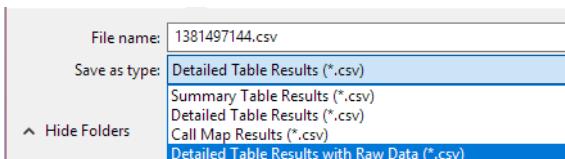
**1 Click File > Export.**



**2 Enter a name for the file.**

**3 Select the type of view . You can export data from:**

- Summary table view
- Detailed table view
- Call Map View
- Detailed table view, with raw data



**4 Click Save.**

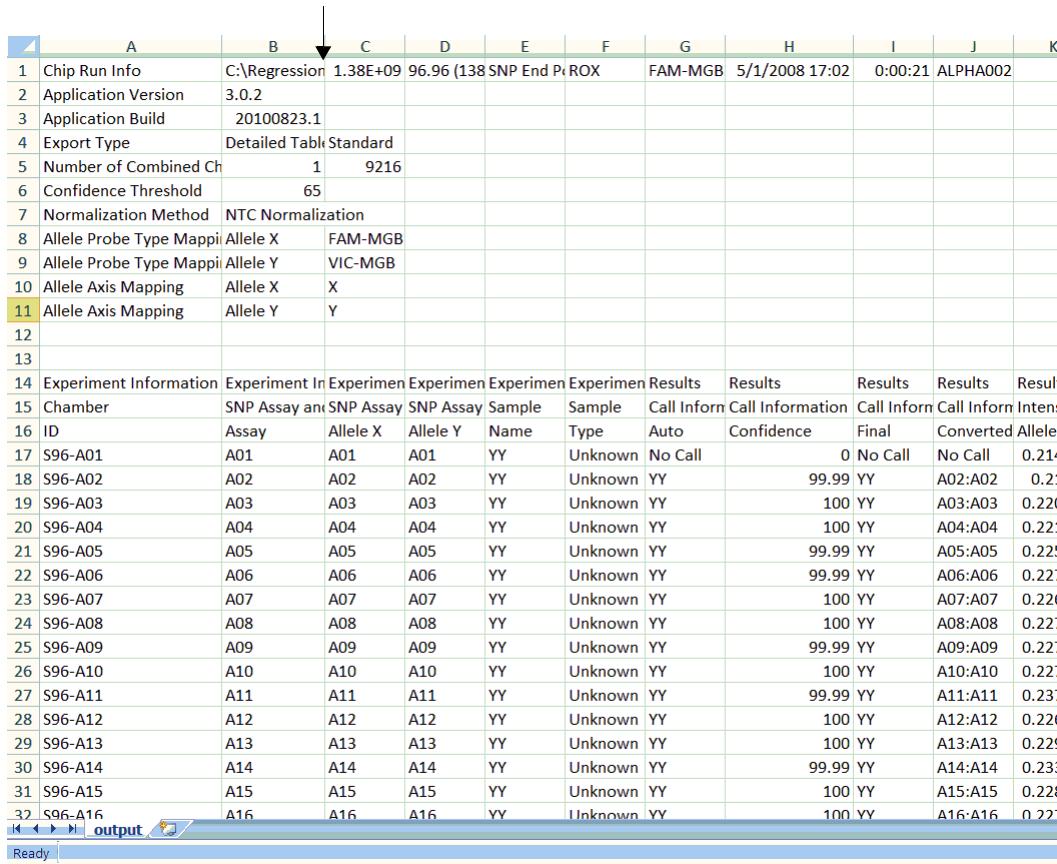
The file is saved in the same folder as the IFC run (.bml) file.

**5 To open the saved data, navigate to the .csv file.**

**6 Double-click the .csv file to open it in a program such as Microsoft® Excel.**

Below is an example of exported data (Detailed Table Results) in a .csv file, opened in an Microsoft® Excel spreadsheet.

Double-click between columns to expand them.



	A	B	C	D	E	F	G	H	I	J	K
1	Chip Run Info	C:\Regression	1.38E+09	96.96 (138 SNP End P ROX		FAM-MGB	5/1/2008 17:02	0:00:21	ALPHA002		
2	Application Version	3.0.2									
3	Application Build	20100823.1									
4	Export Type	Detailed Tabl Standard									
5	Number of Combined Ch	1	9216								
6	Confidence Threshold	65									
7	Normalization Method	NTC Normalization									
8	Allele Probe Type Mappi	Allele X	FAM-MGB								
9	Allele Probe Type Mappi	Allele Y	VIC-MGB								
10	Allele Axis Mapping	Allele X	X								
11	Allele Axis Mapping	Allele Y	Y								
12											
13											
14	Experiment Information	Experiment In	Experimen	Experimen	Experimen	Results	Results	Results	Results	Results	Result
15	Chamber	SNP Assay	and SNP Assay	SNP Assay	Sample	Sample	Call Inform	Call Information	Call Inform	Call Inform	Intens
16	ID	Assay	Allele X	Allele Y	Name	Type	Auto	Confidence	Final	Converted	Allele
17	S96-A01	A01	A01	A01	YY	Unknown	No Call		0 No Call	No Call	0.214
18	S96-A02	A02	A02	A02	YY	Unknown	YY		99.99 YY	A02:A02	0.21
19	S96-A03	A03	A03	A03	YY	Unknown	YY		100 YY	A03:A03	0.220
20	S96-A04	A04	A04	A04	YY	Unknown	YY		100 YY	A04:A04	0.221
21	S96-A05	A05	A05	A05	YY	Unknown	YY		99.99 YY	A05:A05	0.225
22	S96-A06	A06	A06	A06	YY	Unknown	YY		99.99 YY	A06:A06	0.227
23	S96-A07	A07	A07	A07	YY	Unknown	YY		100 YY	A07:A07	0.226
24	S96-A08	A08	A08	A08	YY	Unknown	YY		100 YY	A08:A08	0.227
25	S96-A09	A09	A09	A09	YY	Unknown	YY		99.99 YY	A09:A09	0.227
26	S96-A10	A10	A10	A10	YY	Unknown	YY		100 YY	A10:A10	0.227
27	S96-A11	A11	A11	A11	YY	Unknown	YY		99.99 YY	A11:A11	0.237
28	S96-A12	A12	A12	A12	YY	Unknown	YY		100 YY	A12:A12	0.226
29	S96-A13	A13	A13	A13	YY	Unknown	YY		100 YY	A13:A13	0.229
30	S96-A14	A14	A14	A14	YY	Unknown	YY		99.99 YY	A14:A14	0.233
31	S96-A15	A15	A15	A15	YY	Unknown	YY		100 YY	A15:A15	0.228
32	S96-A16	A16	A16	A16	YY	Unknown	YY		100 YY	A16:A16	0.227



# Using the Assay Reference Library and the Adaptive Assay Reference Library

4

## Introduction

The Assay Reference Library and the Adaptive Assay Reference Library are features that advanced users can use to analyze a single IFC run against a known reference library. The Assay Reference Library and the Adaptive Assay Reference Library are especially helpful when performing clustering analysis. The performance of the reference library depends on adding only well-curated and well-analyzed IFC runs (runs that have been annotated correctly and had outliers removed). All the assays for either library must contain data for all the relevant genotypes.

There are two types of assay reference library:

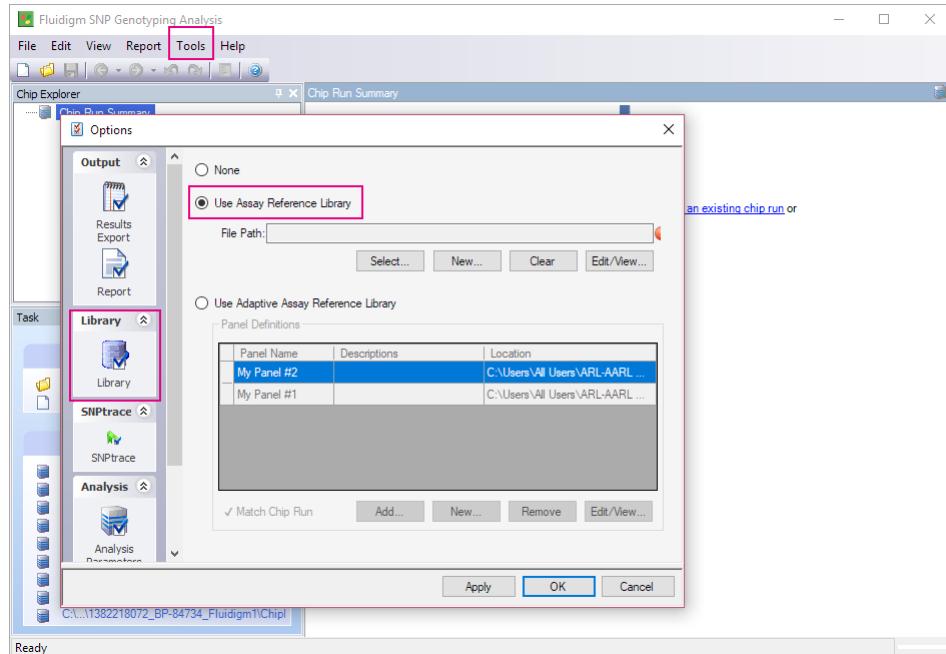
- **Assay Reference Library:** Contains assay cluster information from IFC runs for multiple systems, IFC types, and assay set. When the Assay Reference Library is enabled, the IFC runs are analyzed using the library data that matches the system, IFC type, and assay. The Assay Reference library is best for poor-performing assays that are difficult to auto-call, and it requires consistent assay performance over time.
- **Adaptive Assay Reference Library:** Contains assay cluster information from IFC runs for a specific system, IFC type, and SNP Type™ assay set. Using the adaptive library reduces the batch-to-batch, lot-to-lot fluorescence intensity variations in SNP Type assays. The Adaptive Assay Reference Library is made up of separate panel definitions that contain IFC run data. When the Adaptive Assay Reference Library is enabled, the IFC runs are analyzed using the panel definition that matches the system, IFC type, and assay set. The Adaptive Assay Reference Library is ideal for a fixed SNP Type assay set for medium- and large-size projects that require multiple different lots of reagents.

## Enable the Assay Reference Library or Adaptive Assay Reference Library

Before you can use the Assay Reference Library or Adaptive Assay Reference Library, you must first select the type of library you want to use.

To enable the Assay Reference Library:

- 1 Select Tools > Options.
- 2 Click Library on the Options dialog box to see Assay Reference Library options.



### 3 Select Use Assay Reference Library.

**IMPORTANT:** If you select **None**, the GUI features that relate to the Assay Reference Library are hidden.

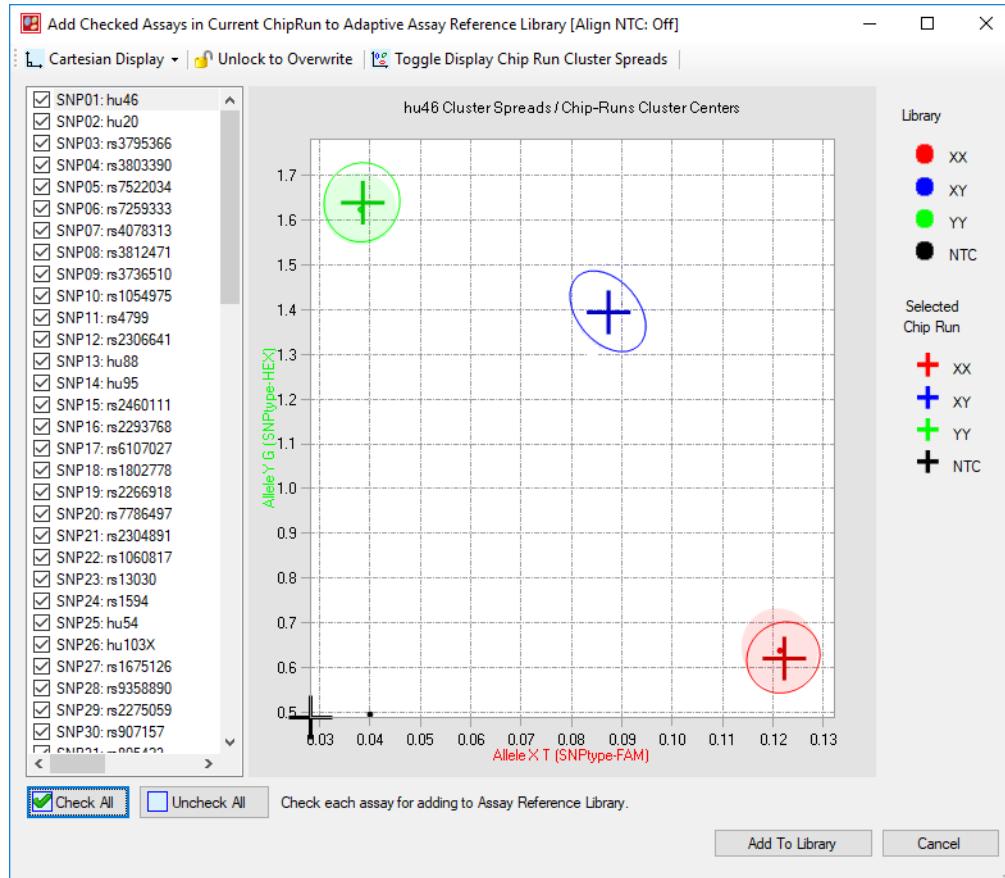
- 4 Click **Apply**. You can either select and use an existing library or create a new one:
  - To use an existing library, click **Select**, and then browse to and select the existing library.
  - To create a new library, click **New**, enter (or browse to) the location where you want to store your assay, and enter a name for your library. Add assays to your library by using the Import Assays Wizard (see [page 105](#)) or adding an IFC run to the library (see [page 107](#)).

#### To enable the Adaptive Assay Reference Library:

- 1 Create a combined IFC run with assays from the same lot. Ensure that all of the genotype clusters are present for all of the assays.

**NOTE:** Combined IFC runs provide enough data to show all relevant (three) genotypes (clusters).

- 2 Select **Tools > Options**, and then click **Library**.
- 3 Click **New**, browse to the location where you want to store your Adaptive Assay Reference Library files, and then enter a name for the adaptive library file.
- 4 (Optional) Enter a description for the panel definition.
- 5 Click **Next**, and then select the assays you want to add to your library.
  - To add assays individually, select them in the left pane.
  - To add all of the assays, select **Check All** at the bottom of the list.



- 6 When you are finished selecting assays (all assays), click **Add To Library**.

## Assay Reference Library Manager or Adaptive Assay Reference Library Manager

To view and manage your reference library, open the Library options by selecting **Tools > Options**, and then selecting **Library**. Click the **Edit/View** button to open the Assay Reference Library Manager or the Adaptive Assay Reference Library Manager. From there, you can view individual reference libraries based on the system and specific IFC type.

**NOTE:** Only one instance of the Assay Reference Library Manager or the Adaptive Assay Reference Library Manager can be open at a time.



See the callouts in Figure 1 for a description of the various components of the reference library manager. The Adaptive Assay Reference Manager adds a field for the panel name and description above the Search bar, as shown in Figure 2.

The File menu has a sub-menu item **Import Assays**. If you click on this it launches the Import Assays Wizard. You can also export reference library data to a .csv file.

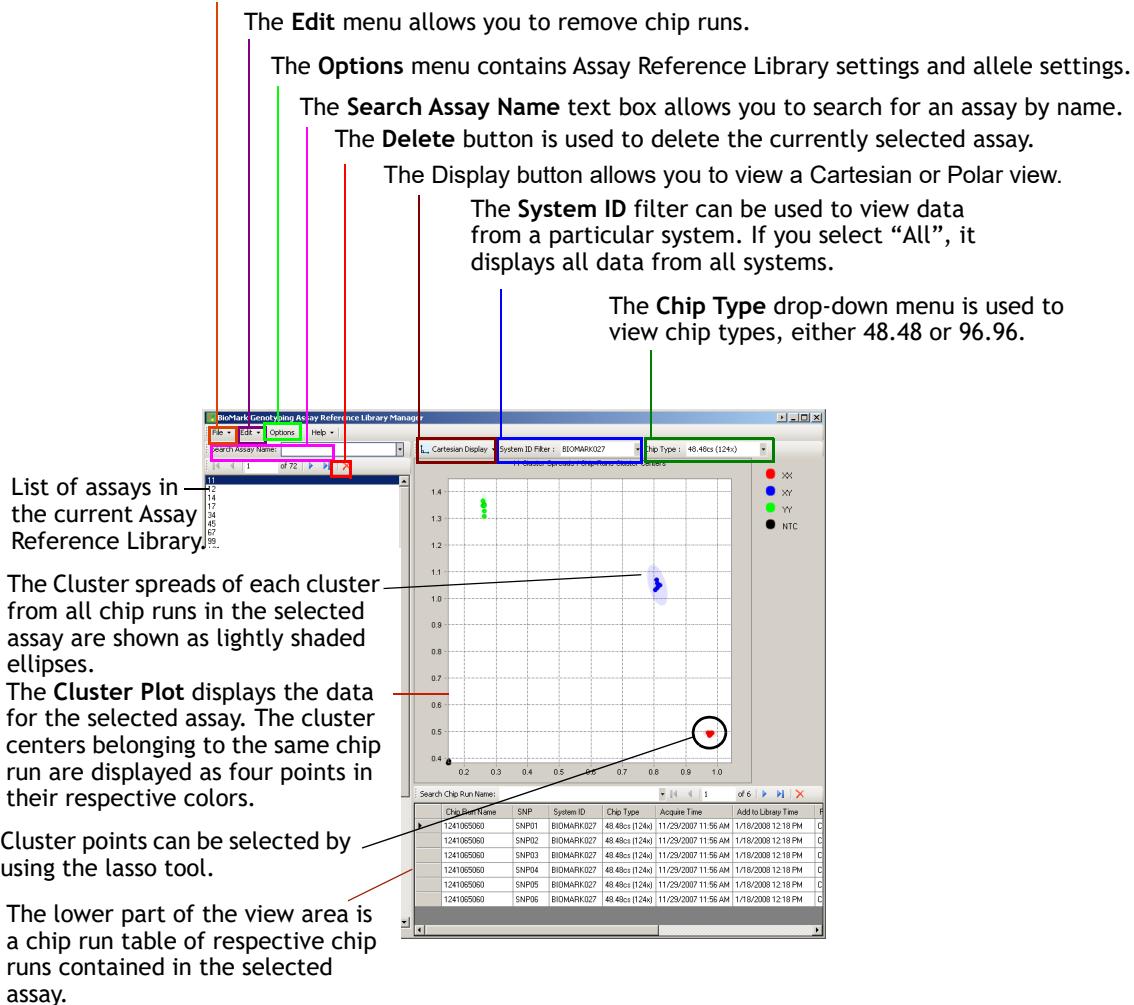


Figure 1. Assay Reference Library Manager

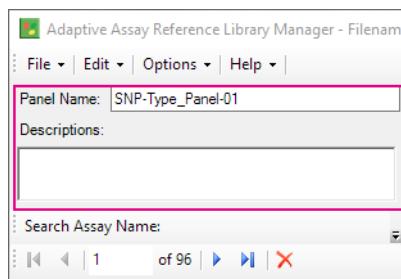


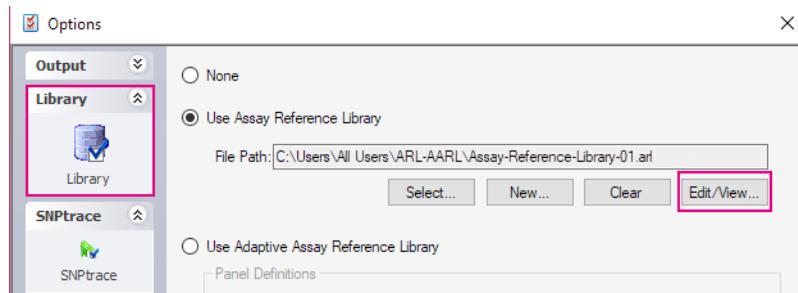
Figure 2. Additional fields in the Adaptive Assay Reference Manager

## Import Assays Wizard

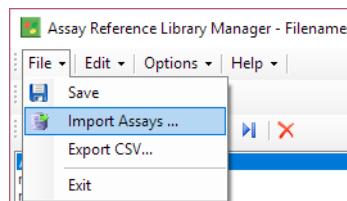
**NOTE:** This feature is not available for the Adaptive Assay Reference Library.

To import assays to the Assay Reference Library, use the Import Assays Wizard in the Assay Reference Library Manager.

- 1 Open the Assay Reference Library Manager by selecting **Tools > Options**, selecting, and then clicking **Edit/View**.

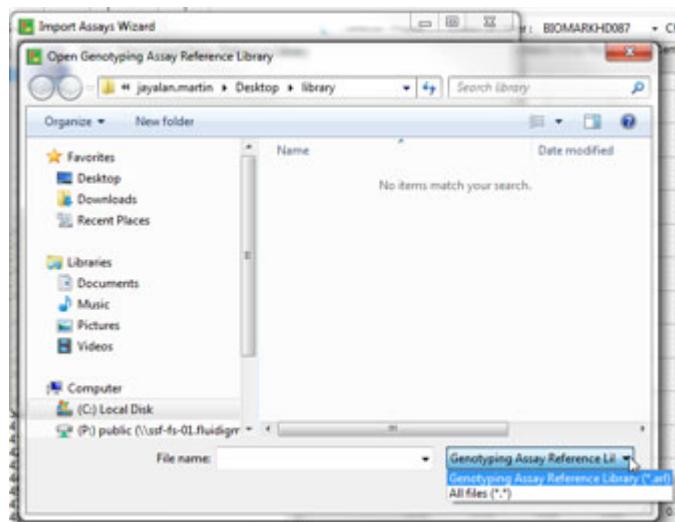


- 2 Open the **Import Assays Wizard** by selecting **File > Import Assays** from the Assay Reference Library Manager window.



- 3 Enter (or browse to) an Assay Reference Library that you want to open:

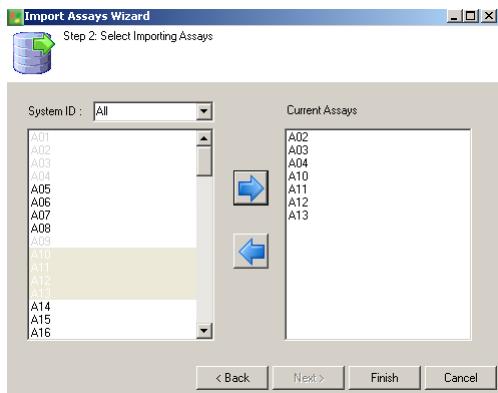
**NOTE:** The Assay Reference Library has an .arl extension.



- 4 Click **Next** to open a second Import Assays Wizard page.
- 5 Select Assays from the reference library listed on the left side of the dialog box and click the forward arrow to move them to the Current Assays box on the right side.

**6 Click Finish.**

The selected assays are added to the current Assay Reference Library and the wizard closes.

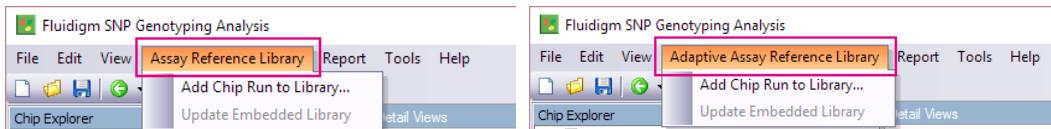


# Assay Reference Library Menu or Adaptive Assay Reference Library Menu

Use the Assay Reference Library menu or the Adaptive Assay Reference Library menu to access the tools for adding IFC runs or updating the embedded library.

**NOTE:** The reference library menu only appears when a reference library is selected in Library options.

**NOTE:** The menu name reflects whether you enabled the Assay Reference Library or the Adaptive Assay Reference Library.



## Add Chip Run to Library

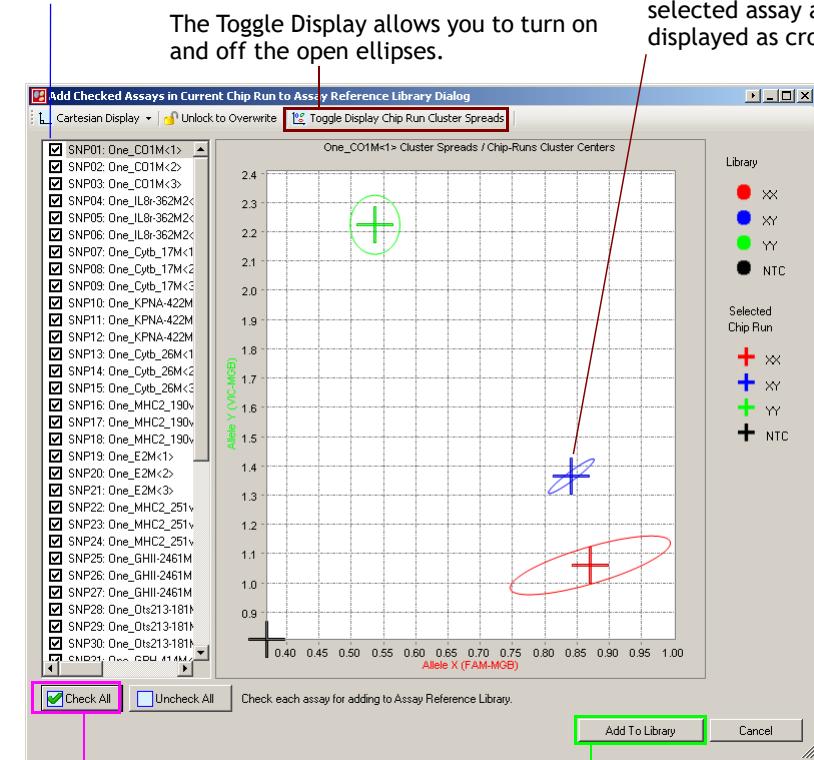
- 1 Select **Add Chip Run to Library** on the Assay Reference Library or Adaptive Assay Reference Library menu to open the Add Checked Assays in Chip Run to Assay

**NOTE:** If the assay reference library file is read-only, an error message appears and the dialog box does not open

**NOTE:** If you enabled the Adaptive Assay Reference Library, the IFC run will be added to the panel definition that matches system ID, IFC type, and assay set.

- 2 Select the assays that you want to add to the reference library individually on the left pane. To add all of the assays, select **Check All** at the bottom of the list.

Select specific Assays you want to add to library.



Or, click Check All to select all assays to add to library.

Cluster centers of the selected assay are displayed as crosses.

This adds checked assays to library.

**NOTE:** To cancel adding the assays to the reference library, click **Cancel** on the loading bar.

**NOTE:** If you enabled the Adaptive Assay Reference Library, review all the assays and ensure that the clusters relatively match those already in the library. The main purpose of adding IFC runs to the adaptive library is to add genotypes that are not present in the library.

If an assay in the current IFC run has been added to the currently selected reference library before, the checkbox for that assay is replaced with a lock icon to protect against accidental overwriting. To remove this protection, click the **Unlock to Overwrite** button to toggle the overwriting state.

Unlock to Overwrite toggle

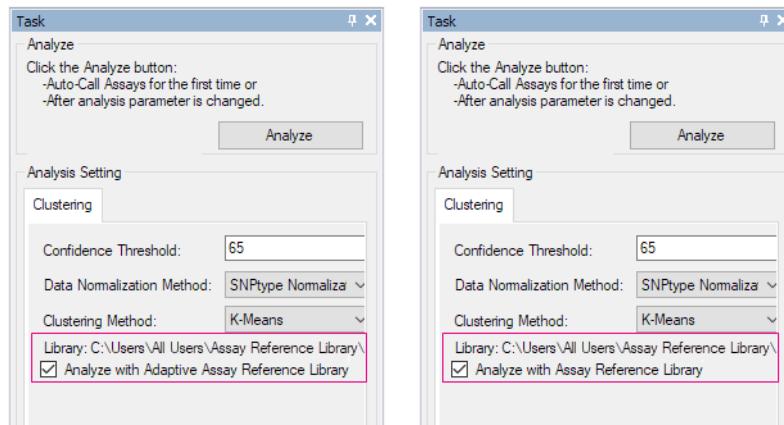


# Use the Assay Reference Library or the Adaptive Assay Reference Library to Analyze an IFC Run

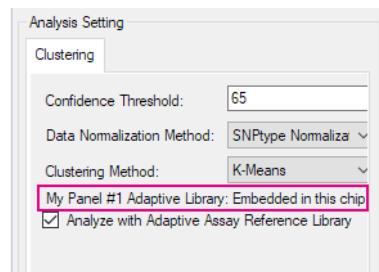
**IMPORTANT:** An individual Assay Reference Library can only be built from IFC runs of a single IFC type (for example, 48.48 or 96.96) that were run on a single system.

**IMPORTANT:** Each panel definition in the Adaptive Assay Reference Library can only be built from IFC runs of a single IFC type (for example, 48.48 or 96.96), using a specific set of SNP Type assays, and that were run on a single system.

- 1 In the Chip Explorer select either **Summary Views**, **Assay Views**, or **Detail Views**.
- 2 In the Task pane, check **Analyze with Assay Reference Library** or **Analyze with Adaptive Assay Reference Library**, depending on which library type is enabled. This allows a new IFC run to be analyzed against the currently selected assay reference library. The currently selected assay reference library is shown above the checkbox.



After the IFC run is analyzed against a reference library, a snapshot of the library is stored with the IFC run. The label below the checkbox changes from “Library: <path to library>” to “<library name>: Embedded in this chip run.” This means that the next time you click Analyze, the system uses the embedded assay reference library snapshot to cluster and label the data instead of using the currently selected assay reference library. Repeatedly clicking Analyze causes the IFC run to be re-analyzed with the embedded snapshot of the library.

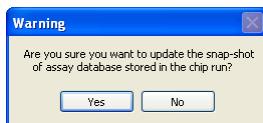


## Update Embedded Assay Reference Library

To analyze against the current assay reference library instead of the embedded one, select **Assay Reference Library > Update Embedded Assay Reference Library**. This re-analyzes the operation and updates the stored embedded library snapshot in the IFC run with the current assay reference library.

The Update Embedded Assay Reference Library feature allows you to update the embedded assay reference library or snapshot of an existing analyzed assay reference library. In other words, you can work with a segment (snapshot) of an assay reference library and then synchronize it to the greater library when you are finished.

- 1 Select **Assay Reference Library or Adaptive Assay Reference Library > Update Embedded Assay Reference Library**. A warning message displays:



- 2 Click **Yes** to update the embedded assay library.

## View Run Data with the Assay Reference Library or the Adaptive Assay Reference Library

You can use the same components of the SNP Genotyping Analysis software to view run data when the IFC runs were analyzed using either the Assay Reference Library or the Adaptive Assay Reference Library. See Chapter 3 for more information about viewing run data.

If you have used the Assay Reference Library or the Adaptive Assay Reference Library to analyze an IFC run against a reference library, there are two additional columns on the Summary Views table: Clustering Method and Concordance ([Figure 3](#)).

- The Clustering Method column displays only a scatter plot icon if the SNP has not been analyzed with an assay reference library. This column displays both a scatter plot icon and an Assay Reference Library icon if the SNP has been analyzed against an assay reference library.
- The Concordance column displays the consistency between the results when a library is used and when it is not used. The number is high for well-separated clusters and low for assays that do not have good cluster separation.

Two additional columns appear if Assay Reference Library has been used.

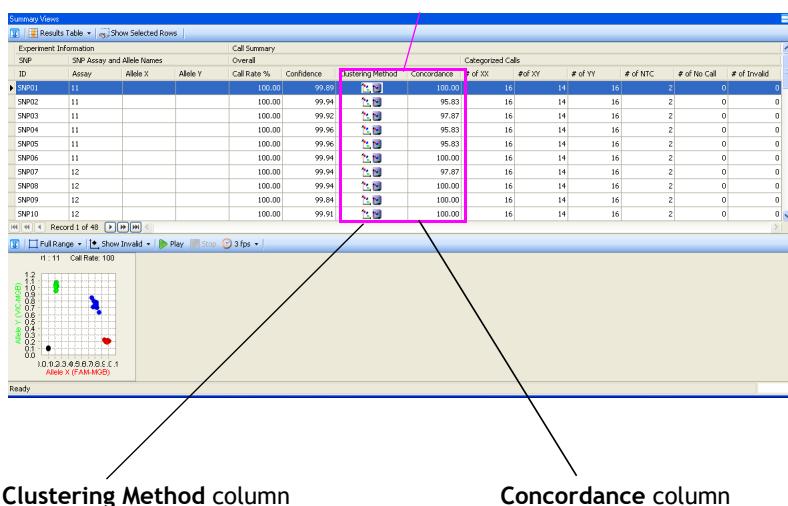


Figure 3. Clustering Method and Concordance columns in the Sample Views pane

The Assay Setup and Sample Setup views are the same as when no reference library is used. Refer to sections “[Annotate Assays Using Assay Setup](#)” on page 45 and “[Annotate Samples Using Sample Setup](#)” on page 31 to set up assays and samples when the reference libraries are used.

**NOTE:** Samples require at least one NTC-normalized point.

If you used the Adaptive Assay Reference Library to analyze an IFC run, there are three additional columns on the Assay Summary View results table for panel matching results: Assay Score, Overall Mean, and % Matched.

- **Assay Score** represents how well the clusters for an individual assay match the data in the reference library by measuring how well the centers of auto-called clusters match the centers of the reference clusters in the scatter plot.
  - A score of 1.00 indicates a perfect match.
  - A score of 0.50 indicates that one of the clusters lie between two reference clusters.
  - A score of 0 indicates that the cluster center of an auto-called cluster matches a reference cluster of a wrong genotype.

**NOTE:** The matching algorithm only uses assays with auto calls that match the reference clusters (for example, a matching score greater than 0.5). For the assays which are not used in the matching process, their matching scores are assigned -1.00 in the Assay Score column.

- **% Matched** is the percent of assays for which the Assay Scores are greater than 0.5.

- **Overall Mean** is the average of the Assay Scores for the assays with scores greater than 0.5.

The Overall Mean and % Matched values provide a way to check the matching quality of the IFC run. The % Matched score is dependent on the assay set and samples (that is, how many assays have three clusters that match the ones in the reference library). For an IFC run with good matching quality, the Overall Mean should be greater than 0.8. The ideal ranges of these two values should be established at the beginning of the project.

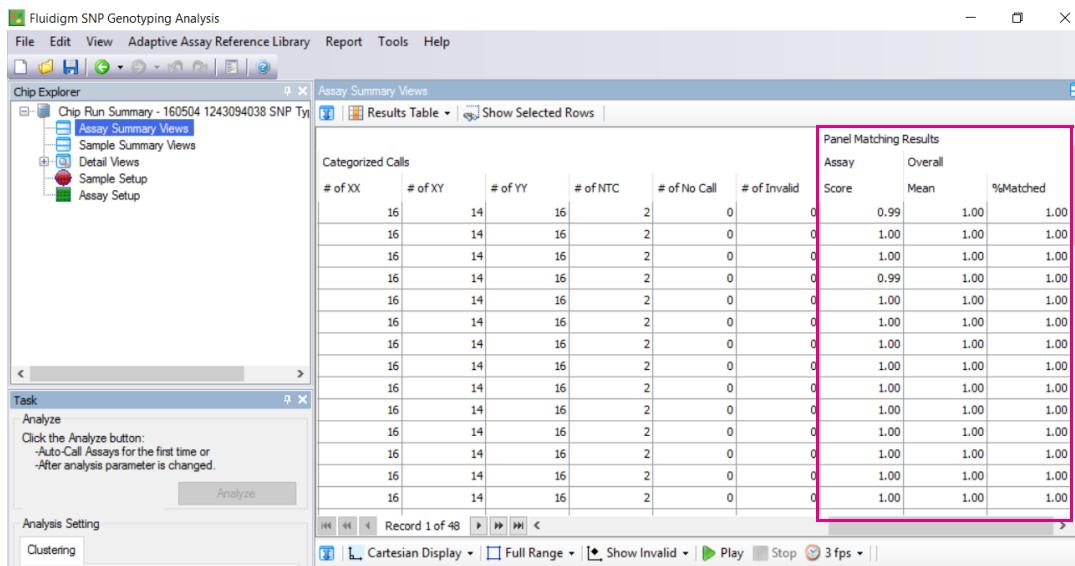


Figure 4. Panel Matching Results for the Assay Summary Results Table (Adaptive Assay Reference Library)

These additional datapoints appear in the results table for all new IFC runs analyzed against the Adaptive Assay Reference Library. If an IFC run was analyzed prior to installing v4.4.1 or later of the SNP Genotyping Analysis software and you want the panel matching results to be included in the Assay Summary Results Table, remove the existing analysis data by deleting the chiprun.processed.bin file from the run folder and performing a new analysis of the IFC run.

# TaqMan® Assays for SNP Genotyping on the Dynamic Array IFCs

---

A

## Introduction

The following document describes the protocol for using Life Technologies TaqMan® assays on 48.48, 96.96 and 192.24 Dynamic Array Integrated Fluidic Circuits (IFCs) for SNP genotyping.

## Required Reagents

### Standard SNP Genotyping

- TaqMan Universal Master Mix (Life Technologies, PN 4304437)
- 20X GT Sample Loading Reagent (Fluidigm, PN 85000741) *or*
- AmpliTaq Gold® DNA Polymerase (Life Technologies, PN 4311806)
- **For 96.96 IFCs:** 96.96 GT PCR Dilution Reagent (PN 100-9977)  
**For 48.48 IFCs:** DNA-free water

### Fast Genotyping

- TaqMan® GTxpress™ Master Mix (2x) (Life Technologies, PN 4401892)
- 20X Fast GT Sample Loading Reagent (Fluidigm, PN 100-3065)

### Both Standard and Fast Genotyping

- SNP Genotyping Assay Mix (80X) Life Technologies)
- 2X Assay Loading Reagent (Fluidigm, PN 85000736)
- 50X ROX™ reference dye (Life Technologies, PN 12223-012)
- Genomic DNA
- DNA-free water

## Required Equipment

- Fluidigm FC1 Cycler or the Biomark™ HD System
- EP1 Reader or Biomark or Biomark HD system
- IFC Controller MX, HX or RX
- 96-well plates
- 48.48, 96.96 or 192.24 Dynamic Array IFCs (Fluidigm)

## Required Consumables

- P2-P1000 pipette tips (Rainin recommended)

## Software Requirements

SNP Genotyping Analysis Software and Fluidigm Data Collection Software.

# Protocol for SNP Genotyping on 48.48 and 96.96 Dynamic Array IFCs

## Priming the 48.48 or 96.96 Dynamic Array IFC

**IMPORTANT:** Use the IFCs within 24 hours of opening the package.

**IMPORTANT:** Due to different accumulator volumes:

- only use 48.48 syringes with 300 µL of control line fluid for 48.48 Dynamic Array IFCs, and
- only use 96.96 syringes with 150 µL of control line fluid for 96.96 Dynamic Array IFCs.

**IMPORTANT:** Control line fluid on the IFC or in the inlets makes the IFC unusable.

**IMPORTANT:** Load the IFC within 60 minutes of priming.

- 1 Inject control line fluid into each accumulator on the IFC.
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 For 48.48 IFCs: Place into the IFC Controller MX, then run the **Prime (124x)** script to prime the control line fluid into the IFC.  
For 96.96 IFCs: Place into the IFC Controller HX, then run the **Prime (138x)** script to prime the control line fluid into the IFC.

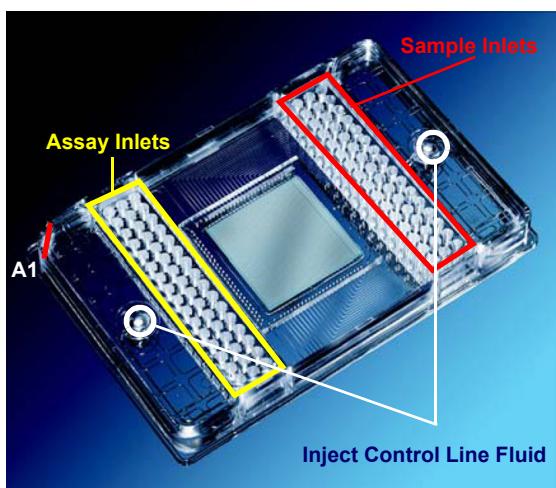


Figure 1. 48.48 Dynamic Array IFC

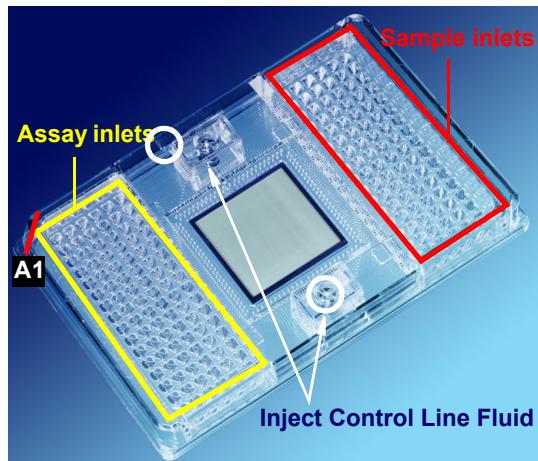


Figure 2. 96.96 Dynamic Array IFC

## Preparing the 10X Assays

In a DNA-free hood, prepare aliquots of 10X assays using volumes in the table below (scale up appropriately for multiple runs).

Component	Volume per Inlet ( $\mu\text{L}$ )	Volume per Inlet with Overage ( $\mu\text{L}$ )	Volume per 50 $\mu\text{L}$ Stock
SNP Genotyping Assay Mix (80X*) (Life Technologies)	0.5	0.625	6.25
2X Assay Loading Reagent (Fluidigm, PN 85000736) 	2.0	2.5	25.0
ROX reference dye (50X) (Life Technologies, PN 12223-012)	0.2	0.25	2.5
DNA-free water	1.3	1.625	16.25
<b>Total Volume</b>	<b>4.0</b>	<b>5.0</b>	<b>50.0</b>

\* If you are using 40X SNP assays, double the volume of SNP assay mix and reduce the DNA-free water. For other starting concentrations of SNP assay mix, call Fluidigm Technical Support.

## Preparing Sample Pre-Mix and Sample Mixes

**NOTE:** Ensure that all components are thawed and thoroughly mixed before use.

- 1 Combine the components in the table below to make the Sample Pre-Mix and the final Sample Mixture.

Component	Volume per Inlet ( $\mu\text{L}$ )	Volume per Inlet with Overage ( $\mu\text{L}$ )	Sample Pre-Mix for 48.48 ( $\mu\text{L}$ ) (60 reactions for ease of pipetting)	Sample Pre-Mix for 96.96 ( $\mu\text{L}$ ) (120 reactions for ease of pipetting)
TaqMan® Universal PCR Master Mix (2X) (Life Technologies, PN 4304437)	2.5	3.0	180.0	360.0
20X GT Sample Loading Reagent (Fluidigm, PN 85000741)	0.25	0.3	18.0	36.0
AmpliTaq Gold® DNA Polymerase (Life Technologies, PN 4311806)	0.05	0.06	3.6	7.2
<b>For 96.96 IFCs:</b> 96.96 GT PCR Dilution Reagent (PN 100-9977)	0.1	0.12	7.2	14.4
<b>For 48.48 IFCs:</b> DNA-free water				
Genomic DNA (added individually to Sample Pre-Mix)	2.1	2.52	—	—
<b>Total</b>	<b>5.0</b>	<b>6.0</b>	<b>208.8</b>	<b>417.6</b>

- 2 In a DNA-free hood, combine the four Sample Pre-Mix components in a 1.5 mL sterile tube—enough volume to fill an entire IFC. Aliquot 3.48  $\mu\text{L}$  of Sample Pre-Mix for each Sample.
- 3 Remove the aliquots from the DNA-free hood and add 2.52  $\mu\text{L}$  of genomic DNA to each, making a total volume of 6  $\mu\text{L}$  in each aliquot.

**NOTE:** The Fluidigm® SNP Genotyping Analysis Software requires at least one NTC to perform auto-calling. The NTC or negative control is used to normalize the data against background. The NTC contains Biotium Fast Probe Master Mix, 20X SNP Type™ Sample Loading Reagent, 60X SNP Type™ Reagent, and ROX. The NTC has water or buffer substituted for the template (DNA sample).

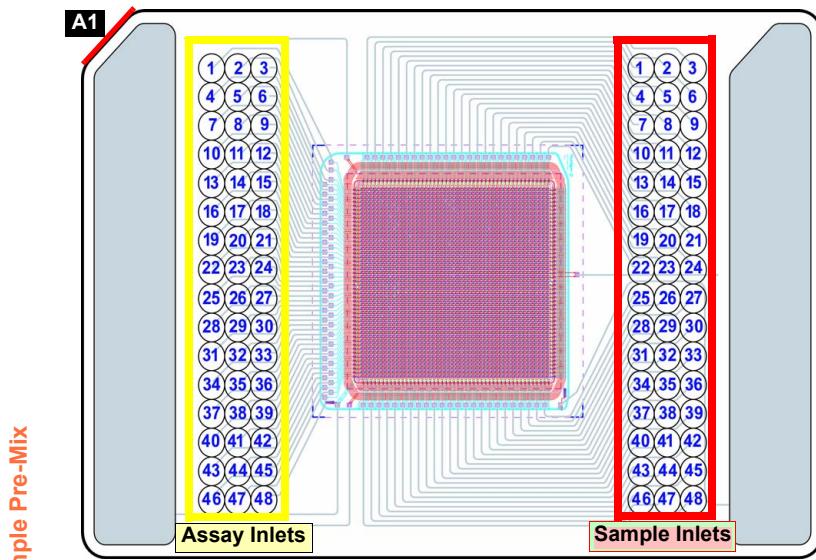


Figure 3. 48.48 Dynamic Array IFC Pipetting Map

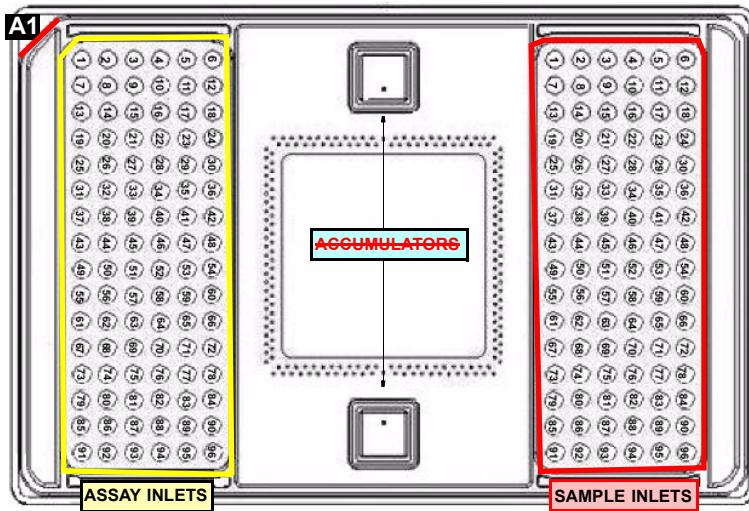


Figure 4. 96.96 Dynamic Array IFC Pipetting Map

## Loading the Dynamic Array IFC

**IMPORTANT:** Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.

**IMPORTANT:** For unused sample inlets, use 3.48 µL of sample mix and 2.52 µL of water per inlet. For unused assay inlets, use 2.5 µL assay loading reagent, 0.25 µL ROX reference dye and 2.25 µL water per inlet.

**IMPORTANT:** While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

- 1 When the Prime (124x or 138x) script has finished, press **Eject** to remove the primed IFC from the IFC Controller MX or HX.
- 2 Pipet 4 µL of each assay into each assay inlet on the IFC.
- 3 Pipet 5 µL of sample into each sample inlet on the IFC.
- 4 Place the IFC into the IFC Controller MX or HX.
- 5 For 48.48: Run the **Load Mix (124x)** script to load the samples and assays into the IFC.  
For 96.96: Run the **Load Mix (138x)** script to load the samples and assays into the IFC.
- 6 Press **Eject** to remove the loaded IFC from the IFC Controller MX or HX.
- 7 Remove any dust particles or debris from the IFC surface.

You are now ready for your chip run.

## Using the Data Collection Software

- 1 Double-click the Data Collection Software icon on the desktop to launch the software.
- 2 Click **Start a New Run**.
- 3 Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.  

- 4 Place the IFC into the reader.
- 5 Click **Load**.
- 6 Verify IFC barcode and IFC type.
  - a Choose project settings (if applicable).
  - b Click **Next**.
- 7 Chip Run file:
  - a Select **New** or **Predefined**.
  - b Browse to a file location for data storage.
  - c Click **Next**.
- 8 Application, Reference, Probes:
  - a Select Application Type: **Genotyping**.
  - b Select Passive Reference: **ROX**.
  - c Select probe types.
  - d Click **Next**.
- 9 Click **Browse** to find the appropriate thermal protocol file.

– For 48.48 IFC: **GT 48x48 Standard v1.pcl**

Segment	Type	Temperature (°C)	Duration (seconds)	Ramp Rate	Cycles
1	UNG	50	120	Normal 2°C/s	1
2	Hot Start	95	600	Normal 2°C/s	1
3	Denaturation	95	15	Normal 2°C/s	40
4	Annealing	60	60		

– For 96.96 IFC: **GT 96x96 Standard v1.pcl**

Segment	Type	Temperature (°C)	Duration (seconds)	Ramp Rate	Cycles
1	Thermal Mix	50	120	Normal 2°C/s	1
2	Thermal Mix	70	1,800	Normal 2°C/s	1
3	Thermal Mix	25	600	Normal 2°C/s	1
4	UNG and Hot Start	50	120	Normal 2°C/s	1
5	UNG and Hot Start	95	600	Normal 2°C/s	1
6	PCR Cycle	95	15	Normal 2°C/s	40
7	PCR Cycle	60	60		

10 Confirm Auto Exposure is selected.

11 Click Next.

12 Verify the chip run information.

13 Click Start Run.

# Protocol for SNP Genotyping on 192.24 Dynamic Array IFCs

## Preparing the 192.24 Dynamic Array IFC

**IMPORTANT:** Use the IFCs within 24 hours of opening the package.

**IMPORTANT:** Due to different accumulator volumes: only use syringes with 150 µL of control line fluid.

**IMPORTANT:** Control line fluid on the IFC or in the inlets makes the IFC unusable.

- 1 Inject control line fluid into each accumulator on the IFC.
- 2 Remove and discard the protective film from the bottom of the IFC.

## Preparing the 10X Assays

In a DNA-free hood, prepare aliquots of 10X assays using volumes in the table below (scale up appropriately for multiple runs).

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Volume per 50 µL Stock
SNP Genotyping Assay Mix (80X*) (Life Technologies)	0.375	0.5	6.25
2X Assay Loading Reagent (Fluidigm, PN 85000736) 	1.5	2.0	25.0
ROX reference dye (50X) (Life Technologies, PN 12223-012)	0.15	0.2	2.5
DNA-free water	0.975	1.3	16.25
<b>Total</b>	<b>3.0</b>	<b>4.0</b>	<b>50.0</b>

\* If you are using 40X SNP assays, double the volume of SNP assay mix and reduce the DNA-free water. For other starting concentrations of SNP assay mix, call Fluidigm Technical Support.

## Preparing Sample Pre-Mix and Sample Mixes

**NOTE:** Ensure that all components are thawed and thoroughly mixed before use.

- 1 Combine the components in the table below to make the Sample Pre-Mix and the final Sample Mixture.

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Sample Pre-Mix for 192.24 (µL) (240 reactions for ease of pipetting)
TaqMan® GTxpress™ Master Mix (2X) (Life Technologies, PN 4401892)	1.5	2.0	480.0
20X Fast GT Sample Loading Reagent (Fluidigm, PN 100-3065)	0.15	0.2	48.0
DNA-free water	0.15	0.2	48.0
Genomic DNA (added individually to Sample Pre-Mix)	1.2	1.6	—
<b>Total</b>	<b>3.0</b>	<b>4.0</b>	<b>576.0</b>

- 2 In a DNA-free hood, combine the four Sample Pre-Mix components in a 1.5 mL sterile tube—enough volume to fill an entire IFC. Aliquot 2.4 µL of Sample Pre-Mix for each Sample.
- 3 Remove the aliquots from the DNA-free hood and add 1.6 µL of genomic DNA to each, making a total volume of 4 µL in each aliquot.

## Important Notes About Loading the 192.24 Chip

**IMPORTANT:** Thoroughly vortex and centrifuge all assay solutions and all samples *before* pipetting into the IFC inlets.

**IMPORTANT:** For unused sample inlets, use 2.4 µL of sample mix and 1.6 µL of water per inlet. For unused assay inlets, use 2 µL assay loading reagent, 0.2 µL ROX reference dye and 1.8 µL water per inlet.

**IMPORTANT:** Make sure the interface plate on the IFC Controller RX is clean and dust free before loading the IFC. You can use Scotch® tape to remove dust and debris.

**IMPORTANT:** While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

- 1 Pipet 3 µL of each assay and 3 µL of each sample into the respective inlets on the IFC.
- 2 Pipet 150 µL of Pressure Fluid into the P1, P2 and P3 wells.
- 3 Pipet 20 µL of Pressure Fluid into the P4 and P5 wells.
- 4 Blot carrier surface with a dry, lint-free cloth.
- 5 Place the IFC into the *Load* IFC Controller RX.
- 6 Using the IFC Controller RX software, run the **Load Mix (166x)** script to load the sample and assays into the IFC.

- 7 When the *Load Mix (166x)* script has finished, eject the loaded IFC from the Load IFC Controller RX.
  - 8 Remove any dust particles or debris from the IFC surface.
- You are now ready for your chip run.
- IMPORTANT:** Start the chip run on the FC1 Cycler within four hours of loading the IFC.

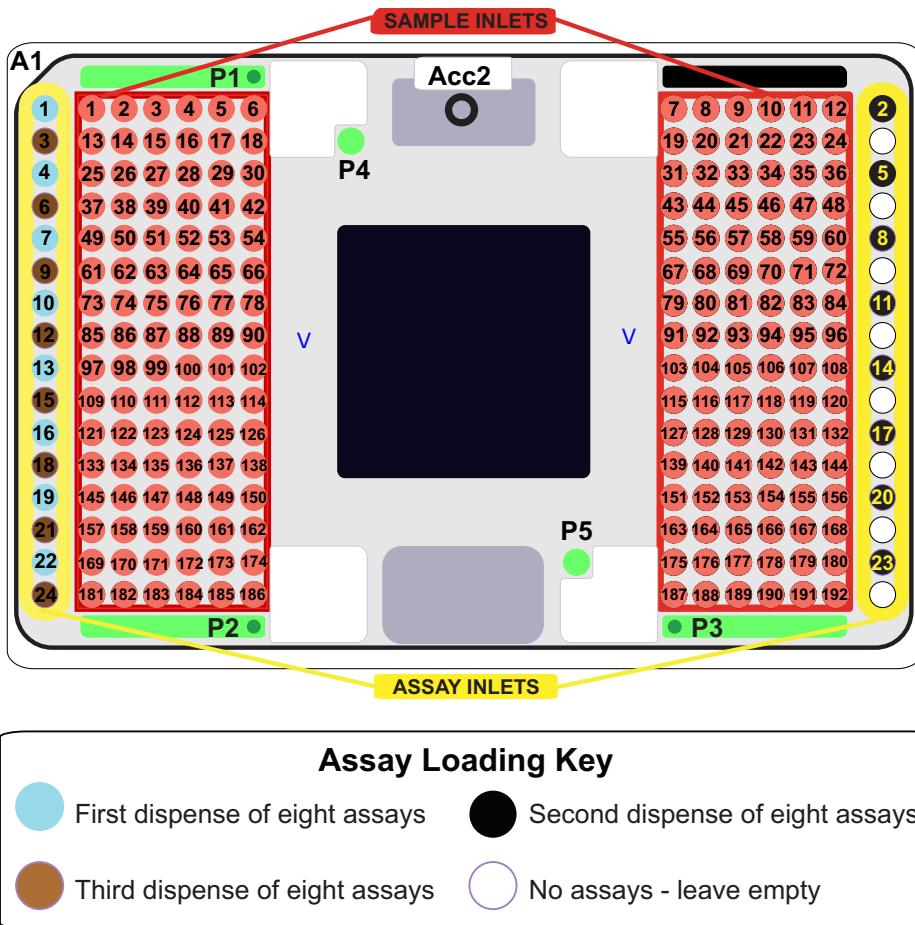


Figure 5. 192.24 Dynamic Array IFC Pipetting Map

## Using the FC1 Cycler

- 1 Press the **Start** button.
- 2 Open the lid.
- 3 Place the IFC onto the thermal cycling block (chuck) on the top of the instrument by aligning the notched corner of the IFC to the **A1** mark.
- 4 Close the lid.
- 5 Press **Continue** to display available thermal protocols.

- 6 Choose the **GT 192x24 Fast v1.pcl** protocol from the protocol selection window.

- 7 Press **Run**.



**CAUTION!** HOT SURFACE HAZARD. Never press down on the integrated fluidic circuit (IFC) when it is on the thermal cycler chuck. If you encounter a vacuum problem, turn off the system, allow it to cool down, and remove the IFC. Clean the bottom of the IFC and/or chuck surface with a lint-free cloth and 70% isopropyl alcohol.

**NOTE:** A status screen appears with a time estimate for completion.

- 8 Once the protocol is finished, a confirmation screen appears. (During an active protocol, **Abort** will cancel the chip run.)

## Using the EP1 Reader Data Collection Software

- 1 Double-click the Data Collection Software icon on the desktop.
- 2 Click **Start a New Run**.
- 3 Check the status bar to verify that the lamp and camera are ready. Make sure both are green before proceeding.
- 4 Place the loaded IFC into the reader.
  - a Choose project settings (if applicable).
  - b Click **Next**.
- 5 Click **Load**.
- 6 Application, Reference, Probes:
  - a Select Application Type—**Genotyping**.
  - b Select Passive Reference (ROX reference dye).
  - c Select probe types.
  - d Click **Next**.
- 7 Confirm **Auto Exposure** is selected.
- 8 Click **Start Run**.



# Convert Regular Chip Run to a More Samples Chip Run

B

## Accessing More Samples Templates

### Set Up More Samples

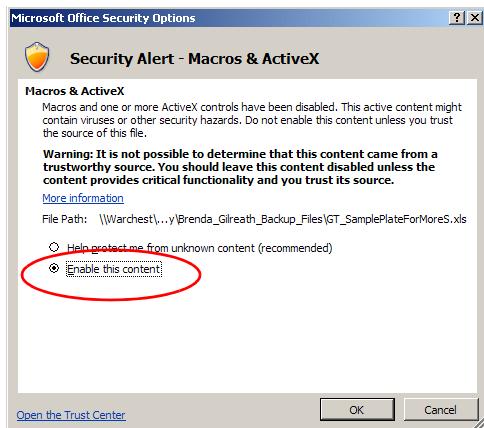
The More Samples feature requires special sample and detector setup. Fluidigm provides setup templates in the Microsoft® Excel® file type. You can use the following workflow to set up your samples and detectors and convert them to .csv files and then annotate sample plates and detector plates in the analysis software.

### Sample Setup

- 1 On your Biomark™ System, Biomark™ HD System, or EP1™ system computer, go to C:\Program Files\Fluidigm\BiomarkGenotypingAnalysis\ApplicationData\FileFormats.
- 2 Open the file labeled “SamplePlateDefinitionForMoreS”.
  - You may have to enable Active X. To do so:
    - Click on the Options tab.



- Select “Enable this content” from the Microsoft Office Security Options dialog box.



- Click OK.
- 3 Edit the Microsoft Excel template to match your experiment.  
4 Click Create Plate CSV File button.

The screenshot shows an Excel spreadsheet with two main sections: 'Sample Plate 1' and 'Sample Plate 2'. Both sections have columns labeled 1 through 12. Row 12 is labeled 'Tag ID:'. In Sample Plate 1, row 13 has values 1 and 2. Rows 14 through 21 are labeled A through H respectively. In Sample Plate 2, row 24 has values 1 and 2. Rows 25 through 32 are labeled A through H respectively. Between the two plates, there is a button labeled 'Create Plate CSV File' which is circled in red. The entire spreadsheet is set against a light blue background.

C12	A	B	C	D	E	F	G	H	I	J	K	L	M
	Clear Definition			Number of Tags:									
1				2									
2													
3													
12	Tag ID:												
13		1	2	3	4	5	6	7	8	9	10	11	12
14	A												
15	B												
16	C												
17	D												
18	E												
19	F												
20	G												
21	H												
22													
23	Tag ID:												
24		1	2	3	4	5	6	7	8	9	10	11	12
25	A												
26	B												
27	C												
28	D												
29	E												
30	F												
31	G												
32	H												

A new tab labeled “CSV file” will be added to the Excel file (see screenshot below).

- Open the new tab and double check your annotations.

The screenshot shows a Microsoft Excel spreadsheet titled "BioMark Sample Format V1.0". The top row contains configuration parameters: File Format (BioMark Sample Format V1.0), Sample Plate Name (Tagged Samples), Barcode ID, Description (Tagged Sample Definition), Plate Type (SBS96), Well Location, Sample Name, Sample Concentration, and Sample Type. Below this is a table with rows for A01 and A02. At the bottom, there are tabs for "Tagged Samples" and "CSV File", with the "CSV File" tab being highlighted. Two specific buttons are circled in red: the "Save to a CSV file" button in the top right corner and the "CSV File" tab at the bottom.

1	File Format	BioMark Sample Format V1.0
2	Sample Plate Name	Tagged Samples
3	Barcode ID	
4	Description	Tagged Sample Definition
5	Plate Type	SBS96
6		
7	Well Location	Sample Name
8	A01	
9	A02	

- Click **Save to a CSV file** button to save the file and select a convenient location for future retrieval.

## Assay Setup

- Next, open the file labeled “AssayPlateDefinitionForMoreS”.

The screenshot shows a Microsoft Excel spreadsheet titled "AssayPlateDefinitionForMoreS". It features four distinct sections: 
 

- TAG Layout:** Rows 5-14, columns 1-12. Headers A-H are in row 5, and columns 1-12 are numbered 1-12.
- Assay Names:** Rows 17-26, columns 1-12. Headers A-H are in row 17, and columns 1-12 are numbered 1-12.
- Allele X Names:** Rows 28-37, columns 1-12. Headers A-H are in row 28, and columns 1-12 are numbered 1-12.
- Allele Y Names:** Rows 39-48, columns 1-12. Headers A-H are in row 39, and columns 1-12 are numbered 1-12.

 Each section has a header row (e.g., "TAG Layout", "Assay Names") and a data row (e.g., "A", "B", "C", "D", "E", "F", "G", "H").

- Edit the Microsoft Excel file to match your experiment.
  - Click **Create Plate CSV File** button.
- A new tab labeled “CSV file” will be added to the Excel file.
- Open the new tab and double check your annotations.
  - Click **Save to a CSV file** button to save the file and select a convenient location for future retrieval.

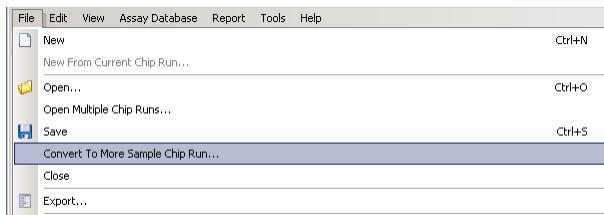
## Import the Plates

Import the Sample plate files.

- 1 Open the SNP Genotyping Analysis software.
- 2 Open a chip run that you wish to annotate.
- 3 Select **Sample Setup** in the Chip Explorer.
- 4 Click **Import** in the Task pane.
- 5 Browse to the location where you saved your sample plate.
- 6 Click **Open**.

Import the Assay plate files.

- 1 Open the SNP Genotyping Analysis software.
- 2 Open a chip run that you wish to annotate.
- 3 Select **Assay Setup** in the Chip Explorer.
- 4 Click **Import** in the Task pane.
- 5 Browse to the location where you saved your assay plate.
- 6 Click **Open**.
- 7 Go to the Analysis view and click the **Analyze** button on the Task pane.
- 8 Go to **File > Convert to More Samples Chip Run...**



Below is an example of a more samples chip run.





# SNP Type™ Assays for SNP Genotyping on the Dynamic Array™ IFCs

C

## Introduction

The following document describes the protocol for using SNP Type assays on 48.48, 96.96 or 192.24 Dynamic Array Integrated Fluidic Circuits (IFCs) for SNP genotyping. Please refer to Appendix B for standard protocols not covered in this appendix.

Assays are provided in three separate oligo plates per 96 assays. All oligos are provided in nuclease-free water.

- Allele-Specific Primers (ASP)—mixed in equal molar ratios and normalized to 100 µM in 100 µL for small; 200 µL for medium; 500 µL for large (each primer)
- Specific Target Amplification (STA) primer—individual primers normalized to 100 µM in 100 µL for small; 200 µL for medium; 500 µL for large (each primer)
- Locus-Specific Primer (LSP)—individual primers normalized to 100 µM in 100 µL for small; 200 µL for medium; 500 µL for large (each primer)

## Required Reagents

- Biotium Fast Probe Master Mix (Biotium, PN 31005)
- Qiagen 2X Multiplex PCR Master Mix (Qiagen, PN 206143)
- DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA) (Teknova, PN T0221)
- SNP Type Genotyping Reagent Kit 48.48 (Fluidigm, PN 100-4135)  
This kit is sufficient for ten 48.48 Dynamic Array IFCs.
  - 1 Assay Loading Reagent, 2X (1.5 mL) (Fluidigm, PN 85000736)
  - 1 SNP Type Sample Loading Reagent, 20X (250 µL) (Fluidigm, PN 100-3425)
  - 1 SNP Type Reagent, 60X (70 µL) (Fluidigm, PN 100-3402)
  - 20 Syringes of control line fluid (300 µL each)
- SNP Type Genotyping Reagent Kit 96.96 (Fluidigm, PN 100-4134)  
This kit is sufficient for ten 96.96 Dynamic Array IFCs.
  - 2 Assay Loading Reagent, 2X (1.5 mL) (Fluidigm, PN 85000736)
  - 2 SNP Type Sample Loading Reagent, 20X (250 µL) (Fluidigm, PN 100-3425)
  - 2 SNP Type Reagent, 60X (70 µL) (Fluidigm, PN 100-3402)
  - 20 Syringes of control line fluid (150 µL each) (Fluidigm, PN 89000021)
- SNP Type Genotyping Reagent Kit 192.24 (Fluidigm, PN 100-4136)  
This kit is sufficient for ten 192.24 Dynamic Array IFCs.
  - 1 Assay Loading Reagent, 2X (1.5 mL) (Fluidigm, PN 85000736)

- 2 SNP Type Sample Loading Reagent, 20X (250 µL) (Fluidigm, PN 100-3425)
- 2 SNP Type Reagent, 60X (70 µL) (Fluidigm, PN 100-3402)
- 10 Syringes of control line fluid (160 µL each)
- 2 Pressure Fluid syringes (3.5 mL each)
- SNP Type Assays
  - SNP Type Assay Allele-Specific Primers (ASP) Plate (100 µM ASP1/100 µM ASP2)
  - SNP Type Assay Locus-Specific Primer (LSP) plate (100 µM)
  - SNP Type Assay Specific-Target Amplification (STA) primer plate (100 µM)
- 50X ROX (Life Technologies, PN 12223-012)
- Genomic DNA
- PCR-certified water

## Required Equipment

- Fluidigm FC1 Cycler or the Biomark™ HD System
- EP1 Reader
- IFC Controller MX, IFC Controller HX, or IFC Controller RX
- 96-well plates
- 48.48 Dynamic Array IFC (Fluidigm, PN BMK-M-48.48) *or*
  - 96.96 Dynamic Array IFC (Fluidigm, PN BMK-M-96.96 GT) *or*
    - 192.24 Dynamic Array IFC (Fluidigm, PN BMK-M-192.24 GT)

**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (SATC) (not an FC1 Cycler or the Biomark™ HD System), please contact Fluidigm Technical Support for a Personal Card, which includes SNP Type scripts specific to the SATC.

## Software Requirements

SNP Genotyping Analysis Software v.3.1.1 or higher and Fluidigm Data Collection Software v.3.1.1 or higher is required for this protocol.

## SNP Type Assays for SNP Genotyping on the 48.48 or 96.96 Dynamic Array IFCs

**NOTE:** Specific target amplification (STA) is highly recommended for all plant samples and any potentially low quality DNA. The STA protocol described on [page 133](#) is not required for SNP Type assays if the samples are of high quality and appropriate concentration (60 ng/µL of human genome size equivalent).

If you want to perform STA, we recommend the minimum sample concentration to be 10 ng/µL.

If you do not want to perform STA, proceed to [page 134](#)

## Preparing the 10X SNP Type Specific Target Amplification (STA) Primer Pool for 48 assays

Prepare the primer pool as described in the following table.

Component	Volume ( $\mu$ L)	Final Concentration
100 $\mu$ M SNP Type Assay STA Primer (for each of 48 assays)	2 ( $x 48 = 96$ total)	500.0 nM
100 $\mu$ M SNP Type Assay LSP (for each of 48 assays)	2 ( $x 48 = 96$ total)	500.0 nM
DNA Suspension Buffer	208.0	—
<b>Total</b>	<b>400.0</b>	—

## Preparing the 10X SNP Type Specific Target Amplification (STA) Primer Pool for 96 assays

Prepare the primer pool as described in the following table.

Component	Volume ( $\mu$ L)	Final Concentration
100 $\mu$ M SNP Type Assay STA Primer (for each of 96 assays)	2 ( $x 96 = 192$ total)	500.0 nM
100 $\mu$ M SNP Type Assay LSP (for each of 96 assays)	2 ( $x 96 = 192$ total)	500.0 nM
DNA Suspension Buffer	16.0	—
<b>Total</b>	<b>400.0</b>	—

## Performing STA

- 1 In a DNA-free hood, prepare aliquots of STA Pre-Mix using volumes in the table below (scale up appropriately for multiple runs).
- 2 In a 96-well PCR plate, combine 3.75 µL STA Pre-Mix with 1.25 µL of genomic DNA and mix well.

<b>STA Pre-Mix</b>	<b>Component</b>	<b>Volume (µL)</b>	<b>STA Pre-Mix for 48.48 with Overage (µL) (60 reactions for ease of pipetting)</b>	<b>STA Pre-Mix for 96.96 with Overage (µL) (120 reactions for ease of pipetting)</b>
	Qiagen 2X Multiplex PCR Master Mix (Qiagen, PN 206143)	2.5	150.0	300.0
	10X SNP Type STA Primer Pool	0.5	30.0	60.0
	PCR-certified water	0.75	45.0	90.0
	Genomic DNA	1.25	—	—
	<b>Total</b>	<b>5.0</b>	<b>225.0</b>	<b>450.0</b>

- 3 Thermal cycle using the following protocol:

	<b>Hold</b>	<b>14 Cycles</b>	
Temperature	95°C	95°C	60°C
Time	15 minutes	15 seconds	4 minutes

- 4 Dilute the STA products 1:100 in DNA Suspension Buffer.
- 5 Store diluted STA products at -20°C until ready to proceed.

## Preparing SNP Type Assay Mixes

- 1 Thaw the SNP Type™ Assay Mixes at room temperature.
- 2 Ensure the assays are sealed, then vortex the plates for a minimum of 20 seconds and centrifuge the plates for 30 seconds.
- 3 In a 96-well plate, combine the reagents in this order:

<b>Component</b>	<b>Volume (µL)</b>	<b>Final Concentration</b>
SNP Type Assay ASP1/ASP2 (100 µM each)	3.0	7.5 µM
SNP Type Assay LSP (100 µM)	8.0	20.0 µM
DNA Suspension Buffer	29.0	—
<b>Total</b>	<b>40.0</b>	<b>—</b>

## Preparing 10X Assays

- 1 In a DNA-free hood, prepare aliquots of 10X assays using volumes in table below (scale up appropriately for multiple runs).
- 2 Combine 2X Assay Loading Reagent with PCR-certified water to create the Assay Pre-Mix.
- 3 Combine 4 µL of Assay Pre-Mix + 1 µL of each individual SNP Type Assay Mix (as prepared in table above) for a total of 5 µL 10X Assay Mix.

Component	Volume per Inlet (µL)	Assay Pre-Mix for 48.48 with Overage (µL) (60 reactions for ease of pipetting)	Assay Pre-Mix for 96.96 with Overage (µL) (120 reactions for ease of pipetting)
2X Assay Loading Reagent (Fluidigm, PN 85000736)	2.5	150.0	300.0
PCR-certified water	1.5	90.0	180.0
SNP Type Assay Mix	1.0	—	—
<b>Total</b>	<b>5.0</b>	<b>240</b>	<b>480</b>

**NOTE:** Excess 10X Assays can be stored at -20°C for up to three weeks.

## Preparing Sample Pre-Mix and Sample Mixes

**NOTE:** Ensure that all components are thawed and thoroughly mixed before use.

- 1 Combine the Biotium Fast Probe Master Mix, 20X SNP Type Sample Loading Reagent, SNP Type Reagent, ROX and PCR-certified water to make the Sample Pre-Mix as described in the table below.
- 2 Combine 3.5 µL of Sample Pre-Mix with 2.5 µL of each genomic DNA (gDNA) to make a total of 6 µL of Sample Mix solution.

**NOTE:** If using STA, add 2.5 µL of 1:100 diluted STA product instead of gDNA. If STA is not used, the recommended DNA concentration is the genome copy number equivalent to 60 ng/µL or higher of human DNA.

- 3 Vortex the Sample Mix for a minimum of 20 seconds, and centrifuge for at least 30 seconds to spin down all components.

Sample Pre-Mix

Component	Volume per Inlet ( $\mu\text{L}$ )	Sample Pre-Mix for 48.48 ( $\mu\text{L}$ ) (60 reactions for ease of pipetting)	Sample Pre-Mix for 96.96 ( $\mu\text{L}$ ) (120 for ease of pipetting)
Biotium 2X Fast Probe Master Mix (Biotium, PN 31005)	3.0	180.0	360.0
20X SNP Type Sample Loading Reagent (Fluidigm, PN 100-3425)	0.3	18.0	36.0
60X SNP Type Reagent (Fluidigm, PN 100-3402)	0.1	6.0	12.0
ROX (Life Technologies, PN 12223-012)	0.036	2.2	4.3
PCR-certified water	0.064	3.8	7.7
Genomic DNA	2.5	—	—
<b>Total</b>	<b>6.0</b>	<b>210.0</b>	<b>420.0</b>

**NOTE:** In order for the analysis software to perform the auto-calling function, samples require at least one NTC normalized point.

## Priming and Loading the Dynamic Array IFC

**NOTE:** See the Appendix B for complete running instructions.

- 1 Inject control line fluid into each accumulator on the IFC (see Figure 1).
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 Place the IFC into the IFC Controller MX (for the 48.48 Dynamic Array IFC) or the IFC Controller HX (for the 96.96 Dynamic Array IFC).
- 4 For the 48.48 Dynamic Array IFC: Run the **Prime (124x)** script.
  - For the 96.96 Dynamic Array IFC: Run the **Prime (138x)** script.
- 5 When the prime script is complete, press **Eject** to remove the primed IFC from the IFC Controller MX or HX.
- 6 Pipet 4  $\mu\text{L}$  of appropriate 10X Assay Mix into each assay inlet on the IFC (see Figures 1 and 2).
- 7 Pipet 5  $\mu\text{L}$  of appropriate Sample Mix into each sample inlet on the IFC (see Figures 1 and 2).
- 8 Place the IFC into the IFC Controller MX (for the 48.48 Dynamic Array IFC) or the IFC Controller HX (for the 96.96 Dynamic Array IFC).
- 9 For the 48.48 Dynamic Array IFC: Run the **Load Mix (124x)** script to load the samples and assays into the IFC.

- For the 96.96 Dynamic Array IFC: Run the **Load Mix (138x)** script to load the samples and assays into the IFC.
- 10 Press **Eject** to remove the loaded IFC from the IFC Controller MX or HX.
- 11 Place the IFC onto the FC1 Cycler or the Biomark™ HD System.
- 12 For the 48.48 Dynamic Array IFC: Run thermal cycling protocol **SNP Type 48x48 v1:**

Thermal Cycling Conditions	Cycles	Temperature	Time
Hot Start	1	95 °C	5 min
Touchdown (from 64.0 °C–61.0 °C, dropping 1 °C per cycle)	1	95 °C 64 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 63 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 62 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 61 °C 72 °C	15 sec 45 sec 15 sec
	34	95 °C 60 °C 72 °C	15 sec 45 sec 15 sec
Cool	1	25 °C	10 sec

**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (SATC) (not an FC1 Cycler or the Biomark™ HD System), please contact Fluidigm Technical Support for a Personal Card, which includes SNP Type scripts specific to the SATC.

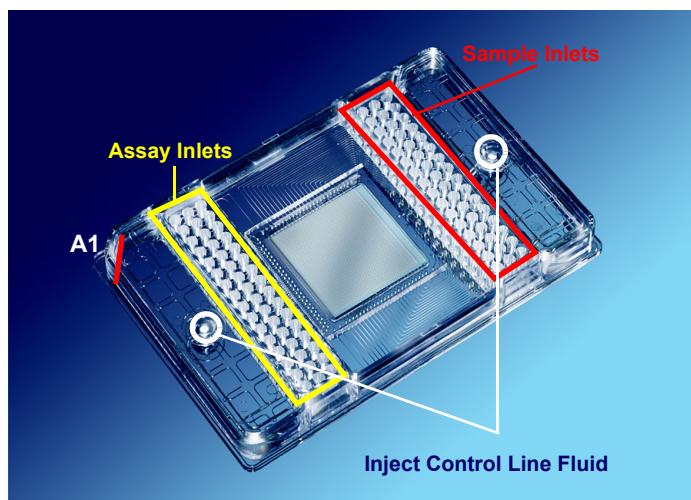


Figure 1. 48.48 Dynamic Array IFC assay and sample inlets

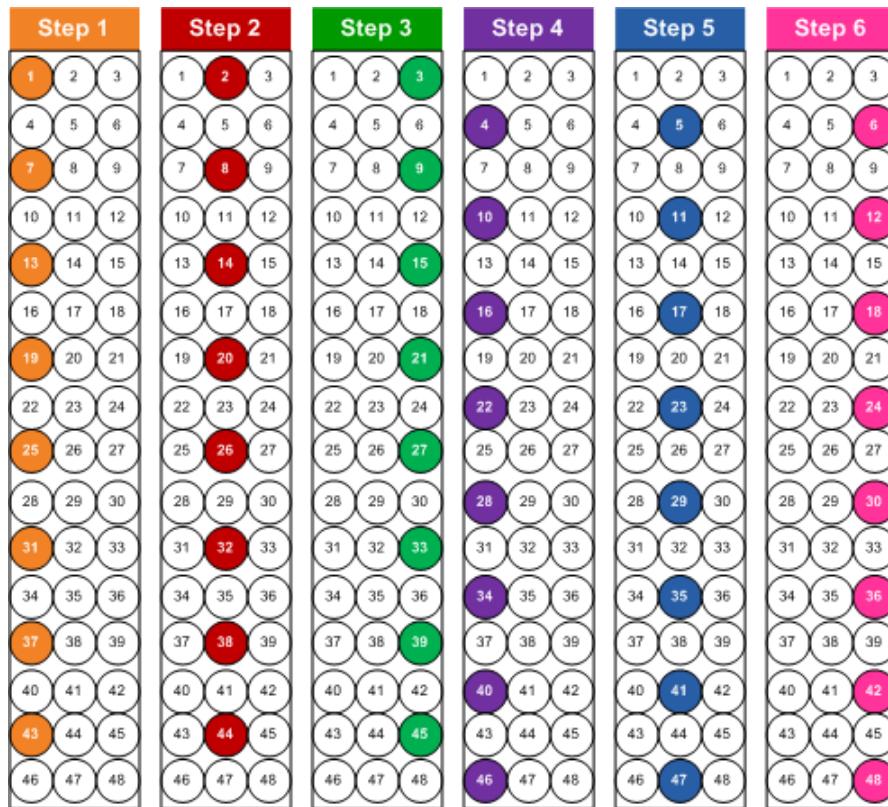


Figure 2. 48.48 Dynamic Array IFC pipetting schema

- For the 96.96 Dynamic Array IFC: Run thermal cycling protocol **SNPtype 96x96 v1:**

Thermal Cycling Conditions	Cycles	Temperature	Time
Thermal Mix	1	70 °C 25 °C	30 min 10 min
Hot Start	1	95 °C	5 min
Touchdown (from 64.0 °C–61.0 °C, dropping 1 °C per cycle)	1	95 °C 64 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 63 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 62 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 61 °C 72 °C	15 sec 45 sec 15 sec
Additional PCR cycles	34	95 °C 60 °C 72 °C	15 sec 45 sec 15 sec
Cool	1	25 °C	10 sec

**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (SATC) (not an FC1 Cycler or the Biomark™ HD System), please contact Fluidigm Technical Support for a Personal Card, which includes SNP Type scripts specific to the SATC.

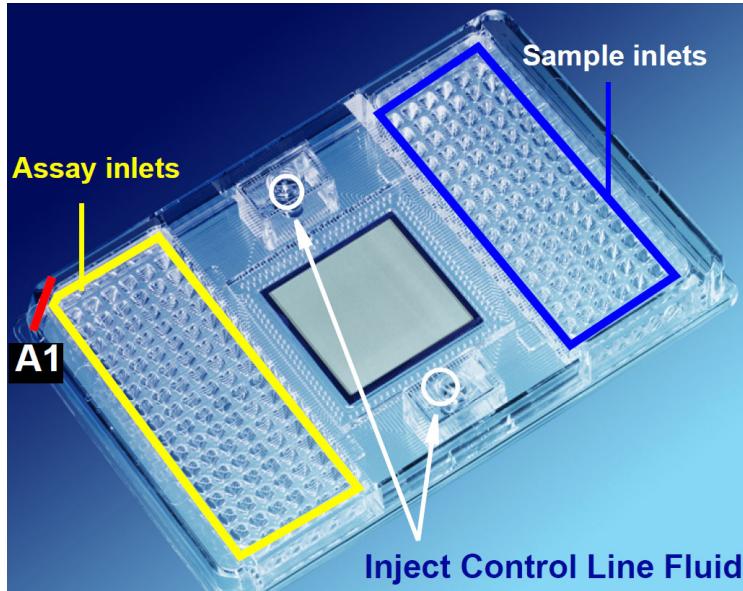


Figure 3. 96.96 Dynamic Array IFC assay and sample inlets

## Using the Data Collection Software

- 1 Double-click the Data Collection Software icon on the desktop to launch the software.
- 2 Click **Start a New Run**.
- 3 Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.
- 4 Place the IFC into the reader.
- 5 Click **Load**.
- 6 Verify IFC barcode and IFC type.
  - a Choose project settings (if applicable).
  - b Click **Next**.
- 7 Chip Run file:
  - a Select **New or Predefined**.
  - b Browse to a file location for data storage.
  - c Click **Next**.
- 8 Application, Reference, Probes:
  - a Select Application Type: **Genotyping**.
  - b Select Passive Reference: **ROX**.
  - c Select probe types.

NOTE: Choose **SNPtype-FAM** and **SNPtype-HEX** for SNP Type assays.

- d Click **Next**.
- 9 Confirm **Auto Exposure** is selected.
- 10 Click **Next**.
- 11 Verify the chip run information.
- 12 Click **Start Run**.

# SNP Type Assays for SNP Genotyping on the 192.24 Dynamic Array IFC

**NOTE:** Specific target amplification (STA) is highly recommended for all plant samples and any potentially low quality DNA. The STA protocol described on [page 141](#) is not required for SNP Type assays if the samples are of high quality and of appropriate concentration (60 ng/ $\mu$ L of human genome size equivalent).

If you want to perform STA, we recommend the minimum sample concentration to be 10 ng/ $\mu$ L.

If you do not wish to perform STA, proceed to [page 142](#).

## Preparing the 10X SNP Type Specific Target Amplification (STA) Primer Pool

Prepare the primer pool as described in the following table.

Component	Volume ( $\mu$ L)	Final Concentration
100 $\mu$ M SNP Type Assay STA Primer (for each of 24 assays)	2 (x 24 = 48 total)	500.0 nM
100 $\mu$ M SNP Type Assay LSP (for each of 24 assays)	2 (x 24 = 48 total)	500.0 nM
DNA Suspension Buffer	304.0	—
<b>Total</b>	<b>400.0</b>	—

## Performing STA

- 1 In a DNA-free hood, prepare aliquots of STA Pre-Mix using volumes in the table below (scale up appropriately for multiple runs).
- 2 In a 96-well PCR plate, combine 3.75  $\mu$ L STA Pre-Mix with 1.25  $\mu$ L of genomic DNA and mix well.

STA Pre-Mix

Component	Volume ( $\mu$ L)	STA Pre-Mix for 192.24 with Overage ( $\mu$ L) (220 for ease of pipetting)
Qiagen 2X Multiplex PCR Master Mix (Qiagen, PN 206143)	2.5	550.0
10X SNP Type STA Primer Pool	0.5	110.0
PCR-certified water	0.75	165.0
Genomic DNA	1.25	—
<b>Total</b>	<b>5.0</b>	<b>825.0</b>

- 3 Thermal cycle using the following protocol:

	<b>Hold</b>	<b>14 Cycles</b>	
Temperature	95°C	95°C	60°C
Time	15 minutes	15 seconds	4 minutes

- 4 Dilute the STA products 1:100 in DNA Suspension Buffer.
- 5 Store diluted STA products at -20°C until ready to proceed.

## Preparing SNP Type Assay Mixes

- 1 Prepare each SNP Type Assay Mix as described in the table below.

<b>Component</b>	<b>Volume (µL)</b>	<b>Final Concentration</b>
SNP Type Assay ASP1/ASP2 (100 µM each)	3.0	7.5 µM
SNP Type Assay LSP (100 µM)	8.0	20.0 µM
DNA Suspension Buffer	29.0	—
<b>Total</b>	<b>40.0</b>	<b>—</b>

## Preparing 10X Assays

- 1 In a DNA-free hood, prepare aliquots of 10X assays using volumes in table below (scale up appropriately for multiple runs).
- 2 Combine 2X Assay Loading Reagent with PCR-certified water to create the Assay Pre-Mix.
- 3 Combine 3.2 µL of Assay Pre-Mix + 0.8 µL of each individual SNP Type Assay Mix (as prepared in table above) for a total of 4 µL 10X Assay Mix.

<b>Component</b>	<b>Volume per Inlet (µL)</b>	<b>Assay Pre-Mix for 192.24 with Overage (µL) (30 reactions for ease of pipetting)</b>
2X Assay Loading Reagent (Fluidigm, PN 85000736)	2.0	60.0
PCR-certified water	1.2	36.0
SNP Type Assay Mix	0.8	—
<b>Total</b>	<b>4.0</b>	<b>—</b>

**NOTE:** Excess 10X Assays can be stored at -20°C for up to three weeks.

## Preparing Sample Pre-Mix and Sample Mixes

**NOTE:** Ensure that all components are thawed and thoroughly mixed before use.

- 1 Combine the Biotium Fast Probe Master Mix, 20X SNP Type Sample Loading Reagent, SNP Type Reagent, ROX and PCR-certified water to make the Sample Pre-Mix as described in the table below.
- 2 Combine 2.6 µL of Sample Pre-Mix with 1.9 µL of each genomic DNA (gDNA) to make a total of 4.5 µL of Sample Mix solution.

**NOTE:** If using STA, add 1.9 µL of 1:100 diluted STA product instead of gDNA. If STA is not used, the recommended DNA concentration is the genome copy number equivalent to 60 ng/µL or higher of human DNA.

- 3 Vortex the Sample Mix for a minimum of 20 seconds, and centrifuge for at least 30 seconds to spin down all components.

Component	Volume per Inlet (µL)	Sample Pre-Mix for 192.24 (µL) (240 for ease of pipetting)
Biotium 2X Fast Probe Master Mix (Biotium, PN 31005)	2.25	540.0
20X SNP Type Sample Loading Reagent (Fluidigm, PN 100-3425)	0.225	54.0
60X SNP Type Reagent (Fluidigm, PN 100-3402)	0.075	18.0
ROX (Life Technologies, PN 12223-012)	0.027	6.48
PCR-certified water	0.048	11.52
Genomic DNA	1.9	—
<b>Total</b>	<b>4.5</b>	<b>630.0</b>

**NOTE:** In order for the analysis software to perform the auto-calling function, samples require at least one NTC normalized point.

## Priming and Loading the Dynamic Array IFC

**NOTE:** See the *Fluidigm 192.24 Genotyping Workflow Quick Reference* (PN 100-3184) for complete running instructions.

- 1 Inject control line fluid into accumulator 2 (Acc2).
- 2 Pipet 3 µL of appropriate 10X Assay Mix into each assay inlet on the IFC (see Figures 1 and 2).

- 3 Pipet 3 µL of appropriate Sample Mix into each sample inlet on the IFC (see Figures 1 and 2).
- 4 Remove and discard the protective film from the bottom of the IFC.
- 5 Pipet 150 µL of pressure fluid into P1, P2 and P3. Tilt IFC so that the pressure fluid covers the entire well.
- 6 Pipet 20 µL of pressure fluid into P4 and P5.
- 7 Place the IFC into the IFC Controller RX.
- 8 Run the **Load Mix (166x)** script to load the samples and assays into the IFC.
- 9 Press **Eject** to remove the loaded IFC from the IFC Controller RX.
- 10 Place the IFC onto the FC1 Cycler or the Biomark™ HD System.
- 11 Run thermal cycling protocol **SNPtype 192x24 v1**:

Thermal Cycling Conditions	Cycles	Temperature	Time
Hot Start	1	95 °C	5 min
Touchdown (from 64.0 °C–61.0 °C, dropping 1 °C per cycle)	1	95 °C 64 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 63 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 62 °C 72 °C	15 sec 45 sec 15 sec
	1	95 °C 61 °C 72 °C	15 sec 45 sec 15 sec
	34	95 °C 60 °C 72 °C	15 sec 45 sec 15 sec
Cool	1	25 °C	10 sec

**NOTE:** If you are using a Fluidigm Stand-Alone Thermal Cycler (SATC) (not an FC1 Cycler or the Biomark™ HD System), please contact Fluidigm Technical Support for a Personal Card, which includes SNP Type scripts specific to the SATC.

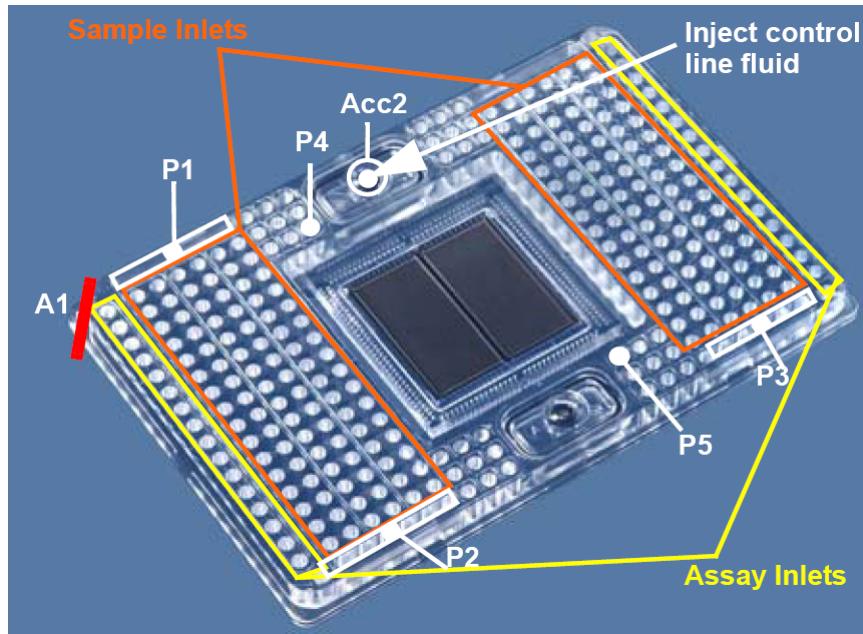


Figure 4. 192.24 Dynamic Array IFC assay and sample inlets

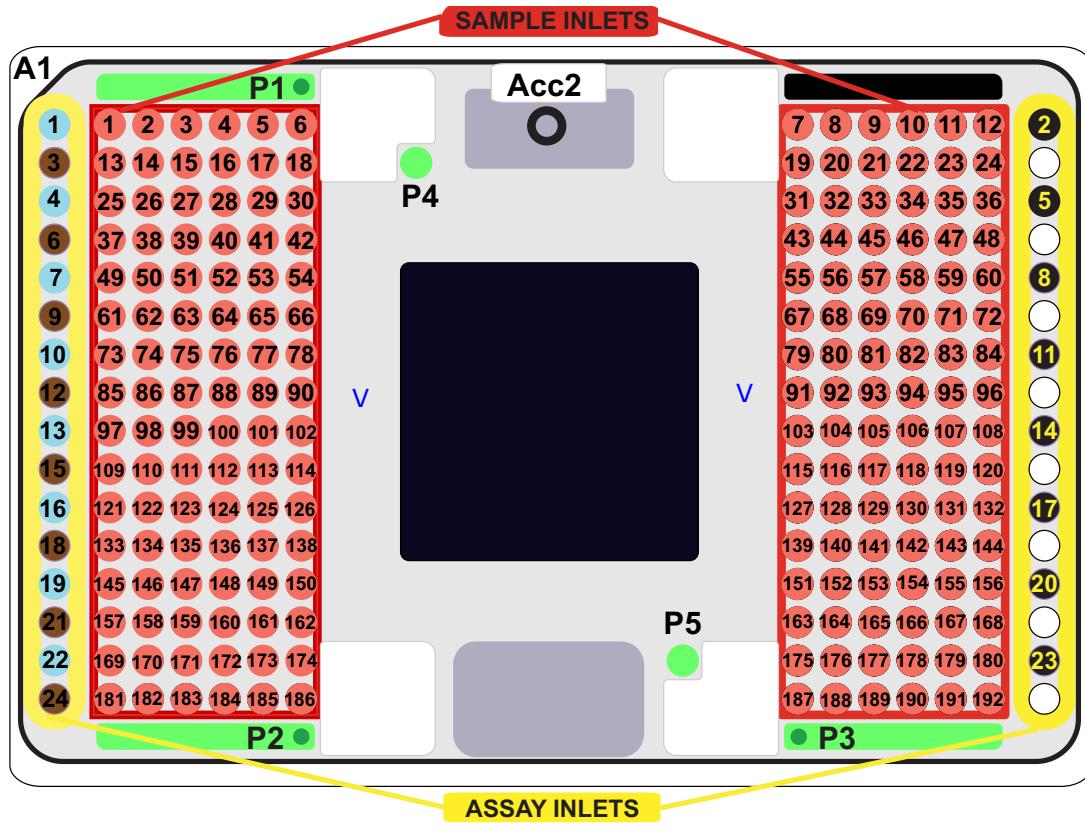


Figure 5. 192.24 Dynamic Array IFC Assay Loading Key

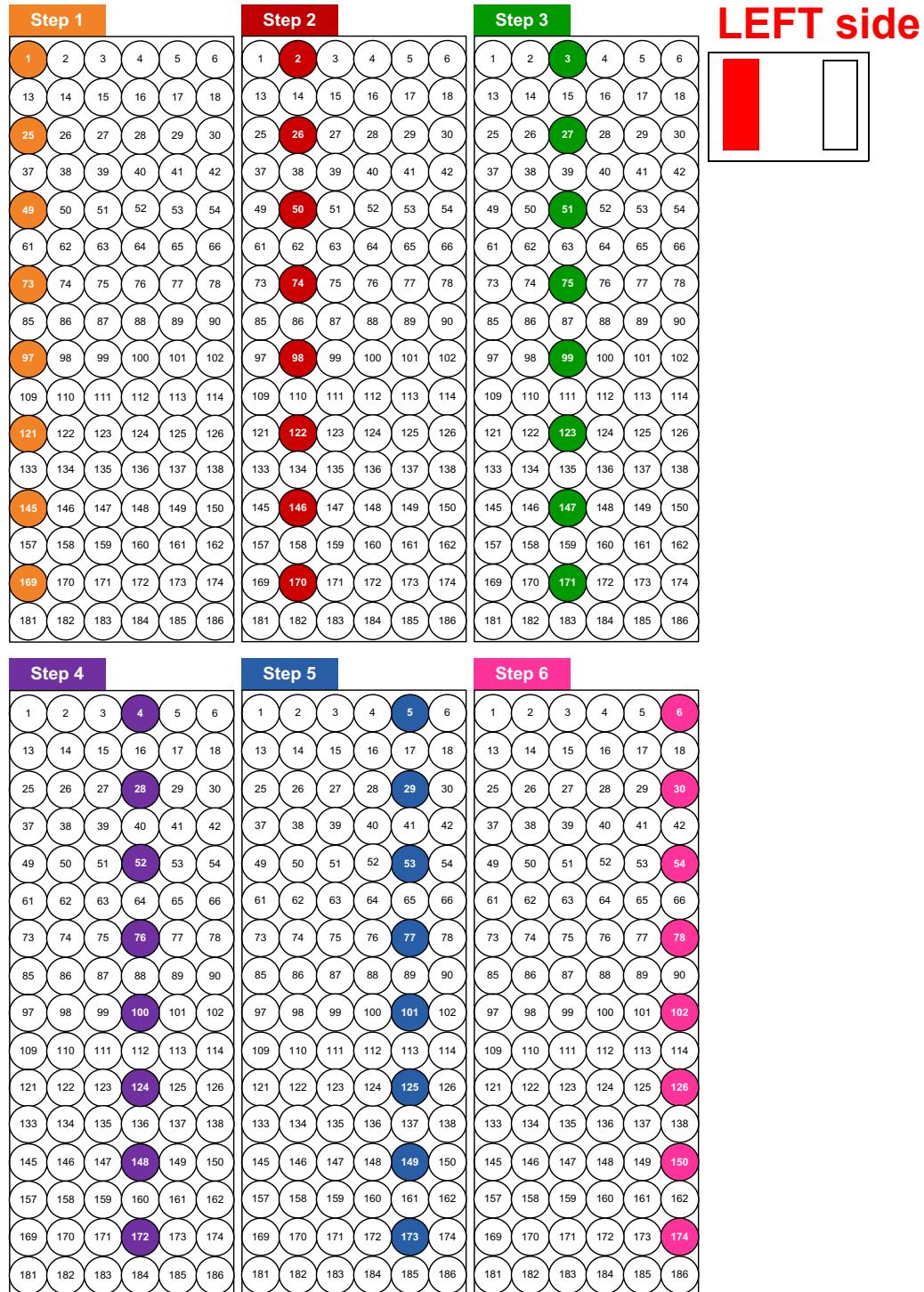
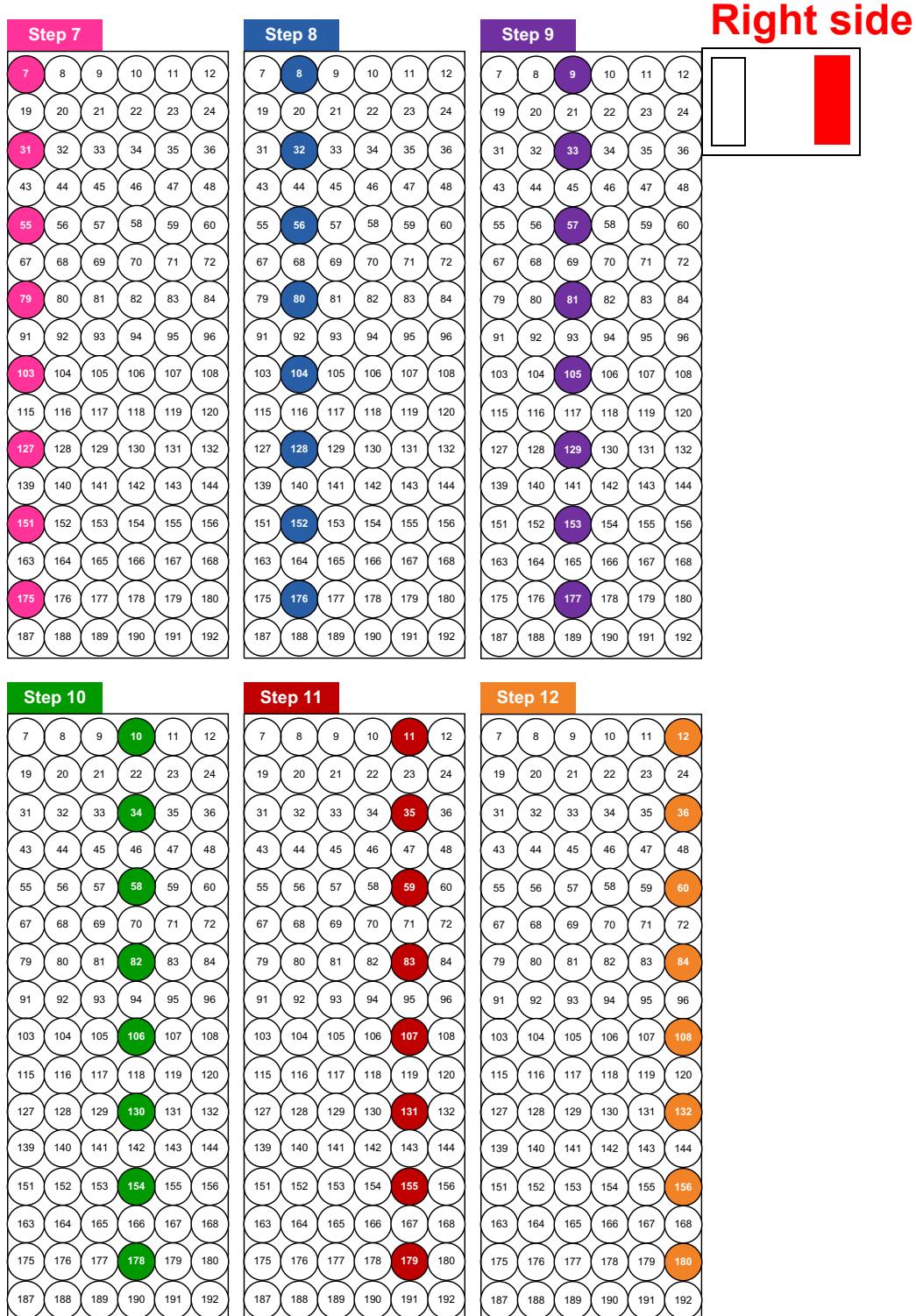


Figure 6. First six sample dispense steps on the left side of the 192.24



**Figure 7. Next six sample dispense steps on the right side of the 192.24**

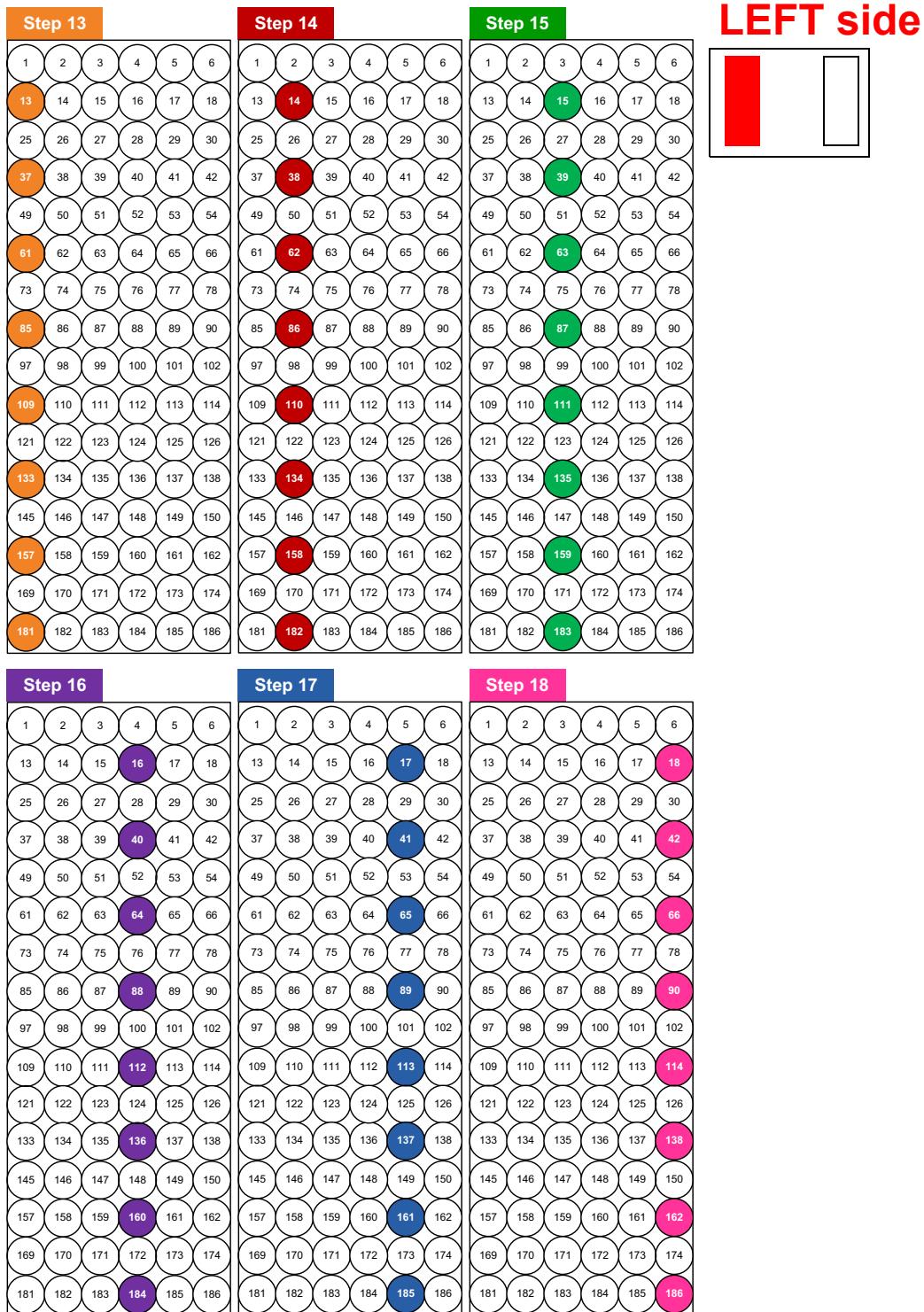


Figure 8. Next six sample dispense steps on the left side of the 192.24

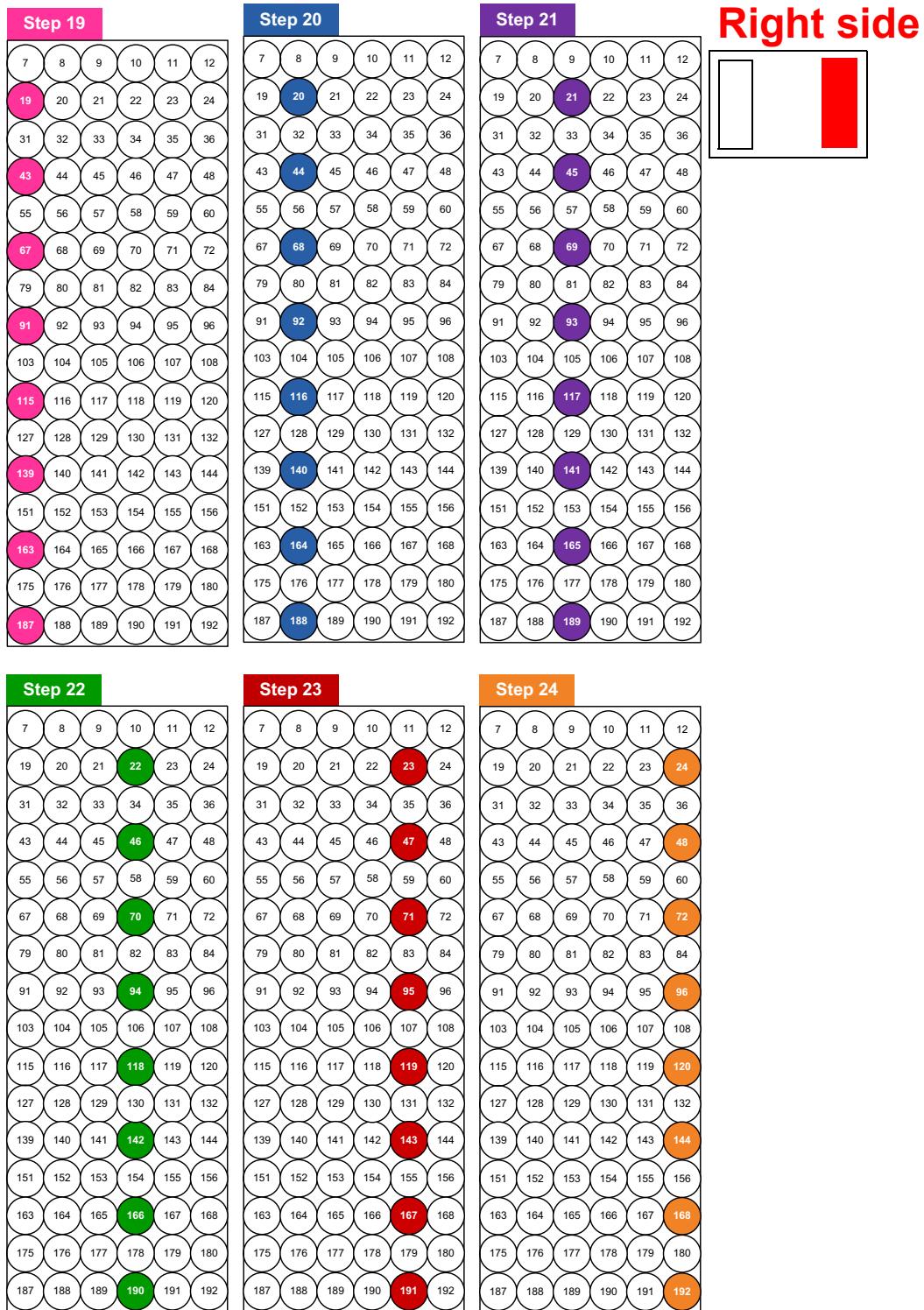


Figure 9. Last six sample dispense steps on the right side of the 192.24

## Using the Data Collection Software

- 1 Double-click the Data Collection Software icon on the desktop to launch the software.
- 2 Click **Start a New Run**.
- 3 Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.
- 4 Place the IFC into the reader.
- 5 Click **Load**.
- 6 Verify IFC barcode and IFC type.
  - a Choose project settings (if applicable).
  - b Click **Next**.
- 7 Chip Run file:
  - a Select **New or Predefined**.
  - b Browse to a file location for data storage.
  - c Click **Next**.
- 8 Application, Reference, Probes:
  - a Select Application Type: **Genotyping**.
  - b Select Passive Reference: **ROX**.
  - c Select probe types.

NOTE: Choose **SNPtype-FAM** and **SNPtype-HEX** for SNP Type assays.

- d Click **Next**.
- 9 Confirm **Auto Exposure** is selected.
- 10 Click **Next**.
- 11 Verify the chip run information.
- 12 Click **Start Run**.



# Fast Genotyping Using TaqMan Assays and the FC1 Cycler on the Biomark HD System

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## Introduction

This protocol is intended for use with the Fluidigm FC1™ Cycler or the Biomark™ HD System and either 48.48 or 96.96 Dynamic Array™ Integrated Fluidic Circuits (IFCs). Following this protocol will reduce the cycling time to approximately 30 minutes for the 48.48 Dynamic Array IFC and approximately 70 minutes for the 96.96 Dynamic Array IFC.

Achieving these fast cycling times requires the Fluidigm FC1 Cycler or Biomark HD System, a new thermal cycling program and updated chemistry. The FC1 Cycler allows the user to select the ramp rate. For this protocol, use the Fast ramp rate of 5.5 °C/sec.

We recommend the use of the TaqMan® GTxpress™ Master Mix from Life Technologies. This master mix has been optimized for use with fast thermal cycling protocols and it is also suitable for use with standard cycling protocols. The TaqMan® GTxpress™ Master Mix does not contain dUTP or UNG. Therefore, the UNG step has been eliminated from the cycling program. This master mix also uses an alternative Hot Start method to reduce the time to reactivate the enzyme.

## Required Reagents

- TaqMan Genotyping Assays (Life Technologies)
- TaqMan® GTxpress™ Master Mix (Life Technologies, PN 4401892)
- DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA) (Teknova, PN T0221)
- 2X Assay Loading Reagent (Fluidigm, PN 85000736)
- 20X Fast GT Sample Loading Reagent (Fluidigm, PN 100-3065)
- ROX (50X) (Life Technologies, PN 12223-012)
- DNA-free water

## Required Equipment

- Fluidigm FC1 Cycler or Biomark HD System
- IFC Controller MX (for the 48.48 Dynamic Array IFC) or IFC Controller HX (for the 96.96 Dynamic Array IFC)
- EP1™ Reader or Biomark™ System for end-point reads (if the FC1 Cycler is used)

## Software Requirements

SNP Genotyping Analysis Software v.3.0.2 or higher and Fluidigm Data Collection Software v.3.0.2 or higher is required for this advanced development protocol.

## Preparing 10X Assays

- 1 In a DNA-free hood, prepare aliquots of 10X assays using volumes in the table below (scale up appropriately for multiple runs).

Component	Volume per Inlet ( $\mu$ L)	Volume per Inlet with Overage ( $\mu$ L)	Volume per 50 $\mu$ L Stock
SNP Genotyping Assay Mix (80X*) (Life Technologies)	0.5	0.625	6.25
2X Assay Loading Reagent (Fluidigm, PN 85000736) 	2	2.5	25
ROX (50X) (Life Technologies, PN 12223-012)	0.2	0.25	2.5
DNA-free water	1.3	1.625	16.25
<b>Total</b>	<b>4</b>	<b>5</b>	<b>50</b>

# Preparing Sample Pre-Mix and Samples

**NOTE:** Ensure that all components are thawed and thoroughly mixed before use.

- 1 Prepare a Sample Pre-Mix solution containing the TaqMan® GTxpress™ Master Mix and 20X Fast GT Sample Loading Reagent sufficient for the number and type of chips to be run.

The following table provides the component amounts for one 48.48 or one 96.96 IFC.

Component	Volume per Inlet ( $\mu$ L)	Volume per Inlet with Overage ( $\mu$ L)	Sample Pre-Mix for 48.48 ( $\mu$ L) (60 for ease of pipetting)	Sample Pre-Mix for 96.96 ( $\mu$ L) (120 for ease of pipetting)
TaqMan® GTxpress™ Master Mix	2.5	3.0	180.0	360.0
20X Fast GT Sample Loading Reagent	0.25	0.3	18.0	36.0
DNA-free water	0.17	0.2	12.0	24.0
Genomic DNA	2.08	2.5		
<b>Total</b>	<b>5</b>	<b>6</b>	<b>210.0</b>	<b>420.0</b>

These volumes include some overage to account for pipetting error.

- 2 In a DNA-free hood, combine the two Sample Pre-Mix components in a 1.5 mL sterile tube--enough volume to fill an entire IFC. Aliquot 3.5  $\mu$ L of the Sample Pre-Mix for each sample.
- 3 Remove the aliquots from the DNA-free hood and add 2.5  $\mu$ L of gDNA to each, to make a total volume of 6  $\mu$ L in each aliquot.

# Priming and Loading the Dynamic Array IFC

**IMPORTANT:** Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.

- 1 Inject control line fluid into each accumulator on the IFC.
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 Place the IFC into the IFC Controller MX (for the 48.48 Dynamic Array IFC) or the IFC Controller HX (for the 96.96 Dynamic Array IFC).
- 4 Run the **Prime (124x)** script for the 48.48 Dynamic Array IFC or  
- **Prime (138x)** script for the 96.96 Dynamic Array IFC.

- 5 When the prime script is complete, remove the primed IFC from the IFC Controller.
- IMPORTANT:** While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.
- 6 Pipet 4 µL of Assay Mix into each assay inlet on the IFC.
- 7 Pipet 5 µL of Sample Mix into each sample inlet on the IFC.
- 8 Place the IFC into the IFC Controller MX (for the 48.48 Dynamic Array IFC) or the IFC Controller HX (for the 96.96 Dynamic Array IFC).
- 9 Run the **Load Mix (124x)** script (for the 48.48 Dynamic Array IFC) or the **Load Mix (138x)** script (for the 96.96 Dynamic Array IFC) to load the samples and assays into the IFC.
- 10 When the Load Mix script has finished, remove the loaded IFC from the IFC Controller.
- 11 Remove any dust particles or debris from the IFC surface using scotch tape.  
You are now ready for your chip run.

## Fast Thermal Cycling the Dynamic Array IFC on the FC1 Cycler

- 1 Press the **START** button.
- 2 Open the lid.
- 3 Place the IFC onto the thermal cycling block (chuck) on top of the instrument by aligning the notched corner of the IFC to the **A1** mark.
- 4 Close the lid.
- 5 Press **CONTINUE** to display available thermal protocols.
- 6 Choose the appropriate protocol:
  - a For the 48.48 Dynamic Array IFC: **GT 48x48 Fast v3.pcl**.
  - b For the 96.96 Dynamic Array IFC: **GT 96x96 Fast v3.pcl**.
- 7 Press **Run**.

The IFC is now ready for imaging on the EP1 Reader or the Biomark HD System.

## Fast Thermal Cycling the Dynamic Array IFC on the Biomark HD

- 1 Remove the protective film from the bottom of the IFC, if this was not done previously and start the Biomark HD Data Collection Software if this was not done earlier. Refer to the *Fluidigm® BioMark™ HD Data Collection Software v3.0 User Guide* (PN 100-2451) for details on using the Biomark HD instrument.
- 2 Click **Start New Chip Run**.
- 3 Place the IFC into the loading position.

- 4 Click **Load**.
- 5 Verify IFC barcode and IFC type:
  - a Choose project settings (if applicable)
  - b Click **Next**.
- 6 Chip run file:
  - a Select **New or Predefined**.
  - b Browse to a file location for data storage.
  - c Click **Next**.
- 7 Application, Reference, Probes:
  - a Select Application Type—**Genotyping**
  - b Select Passive Reference: **ROX**.
  - c Select probe: **Manually**.
  - d Probe 1: **FAM-MGB**.
  - e Probe 2: **VIC-MGB**.
  - f Click **Next**.
- 8 Click **Browse** to find the thermal cycling protocol file. These are found in the GT folder. Cycling conditions are shown in the thermal cycling table below.
  - a For the 48.48 Dynamic Array IFC: **GT 48x48 Fast v3.pcl**.
  - b For the 96.96 Dynamic Array IFC: **GT 96x96 Fast v3.pcl**.

	<b>48x48 GT</b>		<b>96x96 GT</b>		<b># of Cycles</b>
<b>Step</b>	<b>Time</b>	<b>Temp</b>	<b>Time</b>	<b>Temp</b>	
<b>Hot Mix</b>	N/A	N/A	30 min	70°C	
	N/A	N/A	10 min	25°C	
<b>Hot Start</b>	120s	95°C	120s	95°C	
<b>Denature</b>	2s	95°C	2s	95°C	45
<b>Anneal/Extend</b>	20s	60°C	20s	60°C	

- 9 Confirm **Auto Exposure** is selected.
- 10 Click **Next**.
- 11 Verify the chip run information.
- 12 Click **Start Run**.

## Using the Data Collection Software for the EP1 Reader for an End-Point Read

- 1 Double-click the EP1 Data Collection Software icon on the desktop to launch the software.
- 2 Click **Start a New Run**.
- 3 Confirm that the camera and lamp are at temperature.
- 4 Place the IFC into the EP1 Reader.
- 5 Click **Load**.
- 6 Verify IFC barcode and IFC type.
  - a Choose project settings (if applicable).
  - b Click **Next**.
- 7 Chip Run file:
  - a Select **New or Predefined**.
  - b Browse to a file location for data storage.
  - c Click **Next**.
- 8 Application, Reference, Probes:
  - a Select Application Type—**Genotyping**.
  - b Select Passive Reference: **ROX**.
  - c Select probe: **Manually**.
  - d Probe 1: **FAM-MGB**.
  - e Probe 2: **VIC-MGB**.
  - f Click **Next**.
- 9 Confirm **Auto Exposure** is selected.
- 10 Click **Next**.
- 11 Verify the chip run information.
- 12 Click **Start Run**.

## Using the Data Collection Software for the Biomark System and Biomark HD System for an End-Point Read

- 1 Double-click the Biomark Data Collection Software icon on the desktop to launch the software.
- 2 Click **Start a New Run**.
- 3 Confirm that the camera and lamp are at temperature.
- 4 Place the IFC into the Biomark.
- 5 Click **Load**.

- 6 Verify IFC barcode and IFC type.**
  - a Choose project settings (if applicable).**
  - b Click Next.**
- 7 Chip Run file:**
  - a Select New or Predefined.**
  - b Browse to a file location for data storage.**
  - c Click Next.**
- 8 Application, Reference, Probes:**
  - a Select Application Type--Genotyping.**
  - b Select Passive Reference: ROX.**
  - c Select probe: Manually.**
  - d Probe 1: FAM-MGB**
  - e Probe 2: VIC-MGB.**
  - f Click Next.**
- 9 Click Browse to find the thermal cycling protocol file:**
  - a For the 48.48 Dynamic Array IFC: GT End Point v1.pcl.**
  - b For the 96.96 Dynamic Array IFC: GT End Point v1.pcl.**
- 10 Confirm Auto Exposure is selected.**
- 11 Click Next.**
- 12 Verify the chip run information.**
- 13 Click Start Run.**



# Assay- and Sample-Loading on the Flex Six™ IFC

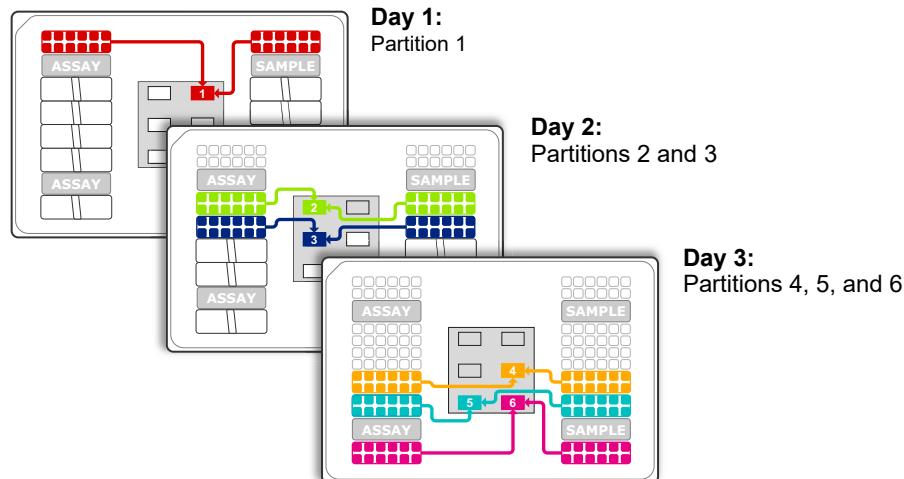
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## Overview of the Flex Six IFC

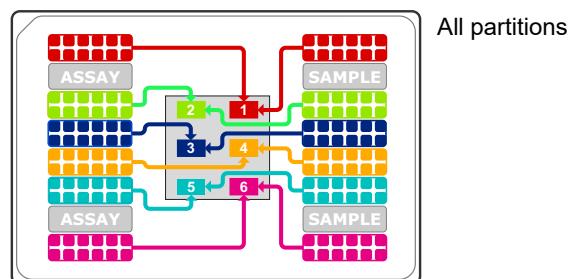
The Fluidigm® Flex Six™ Genotyping IFC provides substantial flexibility in sample and assay numbers during target selection and genotyping while allowing complete use of the IFC. The six 12 X 12 partitions can be organized in any configuration in up to six separate experimental runs.

**Note:** The chemistry (for example, SNPtype or TaqMan) for an experimental run can differ from run to run. However, all partitions in use for a single experimental run must use the same chemistry.

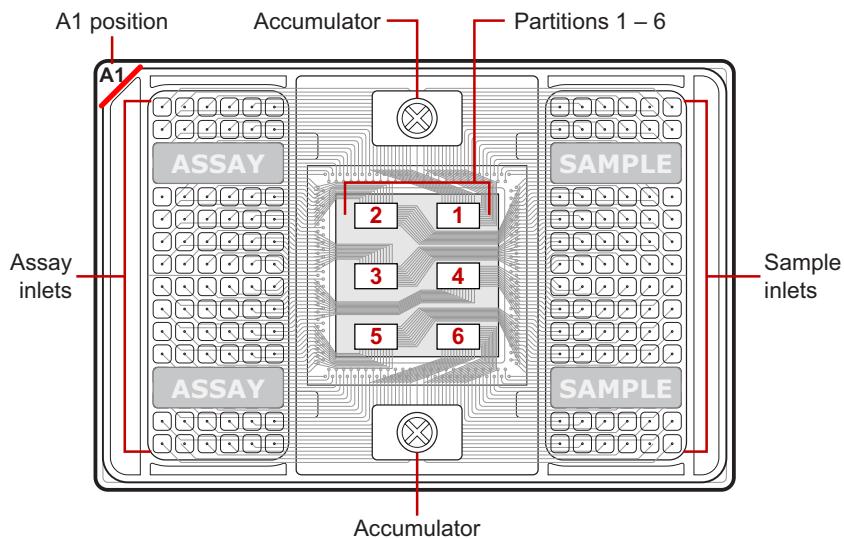
- **Sequential Runs.** You can run any combination of experimental partitions sequentially. As a simple example, you can run a single partition, store the IFC, and then run additional partitions after storing the IFC again until all six partitions are used or until the IFC passes its 90-day expiration date:



- **Parallel Runs.** You can run experimental partitions in parallel. Each partition can contain an independent experiment, can be grouped together to form tiled experiments, or can be a mixture of both.



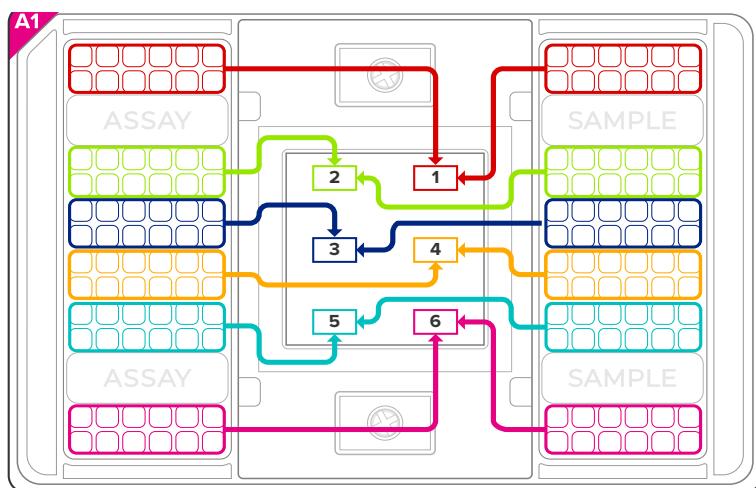
## Components of the Flex Six IFC



### Partitions and Inlets

- There are a total of **six** independent partitions in each Flex Six IFC (partitions 1-6).
- Each partition has a 12 X 12 format (12 assay and 12 sample inlets).
- Each partition can be run independently as a separate experimental run (at different times or on different days) or simultaneously (up to six partitions per run).

**IMPORTANT:** At minimum, all 12 assay inlets and all 12 sample inlets for an active partition must be filled.



The recommended volume of fluid in each inlet for the Flex Six Genotyping IFC is 4 µL for assays and 5 µL for samples. In general, only one No Template Control (NTC, a negative control) per partition is required for genotyping studies.

## Barcodes

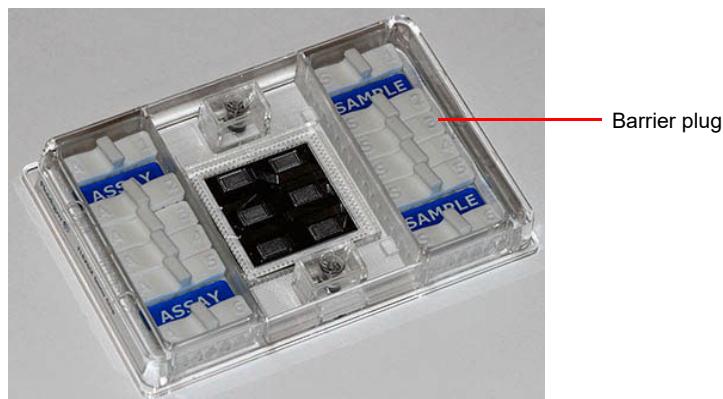
Each Flex Six IFC has a unique barcode. The first three digits of the barcode determine which applications are listed in the Applications menu in the software:

- 153x - Flex Six Gene Expression (GE) IFC
- 154x - Flex Six Genotyping (GT) IFC

**IMPORTANT:** Do not combine GE and GT runs in the same IFC. Each IFC type is for a specific purpose.

## Barrier Plugs

Barrier plugs allow tracking of which partitions are used and prevent pipetting into the wrong wells. Do not discard the barrier plugs.

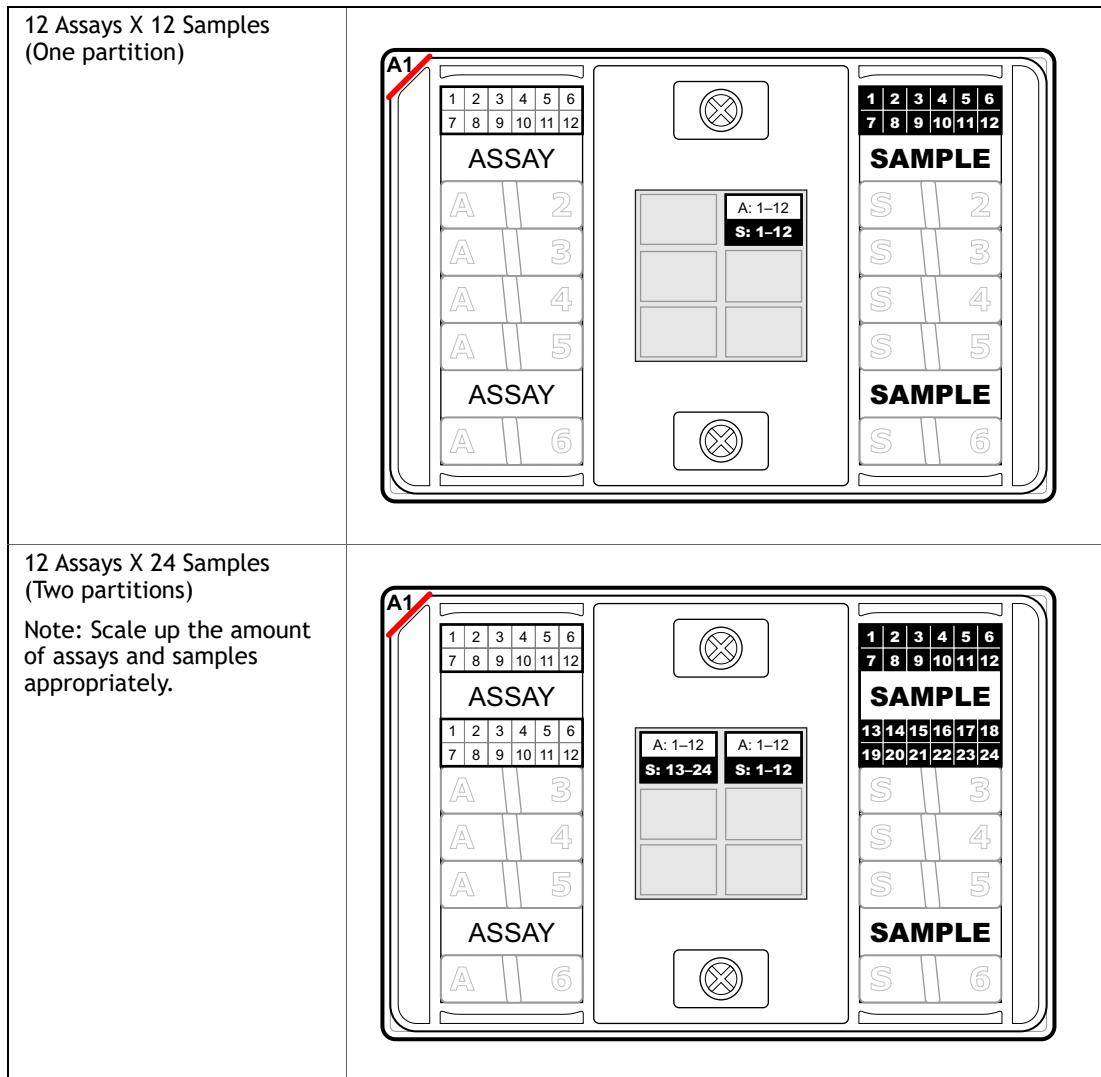


- Each plug is labeled according to its location (A for assay or S for sample, plus a number between 1 and 6).
- You can write on the plugs to denote which partitions have been used.
- Do not interchange the locations of the barrier plugs.
- During an experimental run, leave barrier plugs on the unused inlets of the IFC.
- When an IFC is being stored, ensure that there are barrier plugs on all unused inlets to eliminate dust or other contaminants in the inlets and to serve as visual aids for tracking partitions that can be used at a later date.

## Configuring an Experiment

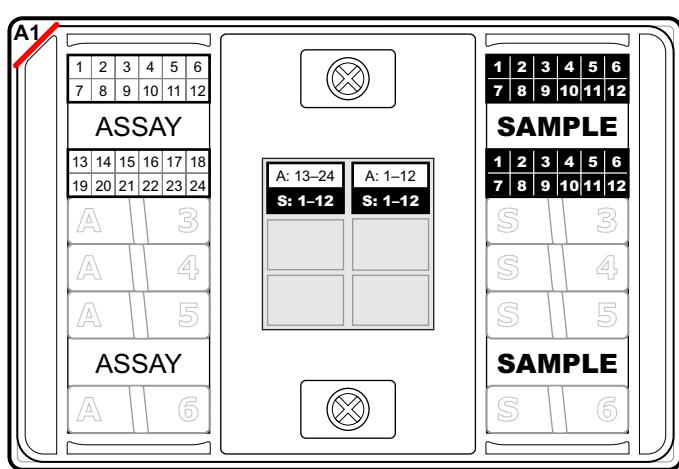
You can configure experiments in several ways.

**Note:** The chemistry (for example, SNPtype or TaqMan) for an experimental run can differ from run to run. However, all partitions in use for a single experimental run must use the same chemistry.



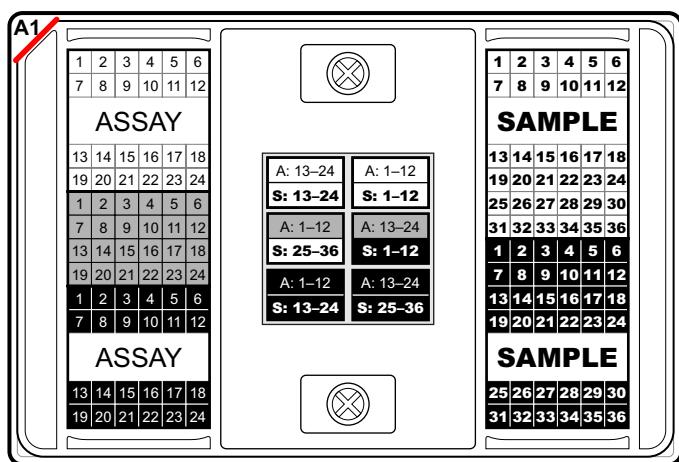
24 Assays X 12 Samples  
(Two partitions)

Note: Scale up the amount  
of assays and samples  
appropriately.



24 Assays X 36 Samples  
(Six partitions)

Note: Scale up the amount  
of assays and samples  
appropriately.



# Flex Six IFC Workflows

## Required Reagents

Reagent	Part number	Store at ...
ROX	Life Technologies, PN 12223-012	-20 °C
Genomic DNA (long-term storage)	—	
DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA)	TEKnova, PN T0221	Room temperature
PCR Certified Water	TEKnova, PN W3330	
2 Control Line Fluid Syringes (150 µL each)	—	
<b>For SNPtype™ Assays</b>		
<ul style="list-style-type: none"> <li>Biotium Fast Probe Master Mix (2X)</li> <li>2X Assay Loading Reagent</li> <li>20X SNPtype Sample Loading Reagent</li> <li>60X SNPtype Reagent</li> </ul>	Biotium, PN 31005 Fluidigm, PN 100-5359 Fluidigm, PN 100-7445 Fluidigm, PN 100-3402	-20 °C
<b>For TaqMan® Assays</b>		
<b>Fast</b>	Life Technologies, PN 4401892	4 °C
	Fluidigm, PN 100-7444	-20 °C
<b>Standard</b>	Life Technologies, PN 4304437	4 °C
	Fluidigm, PN 100-7443	-20 °C
	Life Technologies, PN 4311806	

## Required Equipment

- IFC Controller HX
- FC1™ Cycler, Biomark System, or Biomark HD System for thermal cycling

**Note:** If you are using a Fluidigm Stand-Alone Thermal Cycler (SATC) (not an FC1 Cycler, Biomark System, or Biomark HD System), please contact Fluidigm Technical Support for a Personal Card, which includes SNPtype scripts specific to the SATC.

- EP1™ System, Biomark System, or Biomark HD System for data collection
- Microcentrifuge
- Vortex mixer
- Plate centrifuge
- Single-channel P2-P1000 pipettes (Rainin recommended)

## Required Firmware and Software

- Fluidigm Data Collection Software v4 or later
- Fluidigm SNP Genotyping Analysis Software v4 or later
- IFC Controller HX
  - Firmware 53 or later
  - Software 2.6 or later
- FC1 Cycler
  - Firmware 25 or later
  - Software 1.5 or later

# Flex Six IFC SNPtype Assays Genotyping Workflow

## Prime the Flex Six IFC

**IMPORTANT:** Use the Flex Six integrated fluidic circuit (IFC) within three months of opening the package. Load the IFC within 60 minutes of priming on first use.

**IMPORTANT:** Dripping control line fluid on the chip or in the inlets makes the IFC unusable.

**Note:** It is not necessary to evacuate air from the syringe prior to injection of control line fluid.

**Note:** You only need to prime the IFC on the **first run**. On subsequent use, skip this step.

For information on how to inject control line fluid, see the *Fluidigm® Control Line Fluid Loading Procedure Quick Reference* (PN 68000132).

For information on using the IFC Controller HX, see the *IFC Controller MX-HX User Guide* (PN 68000112).

During the **first usage** of each Flex Six IFC, prime the new Flex Six IFC:

- 1 Using the included syringes, inject 150 µL of control line fluid into each accumulator on the IFC. **Do not** remove the barrier plugs until you load the IFC.
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 Place the IFC into the IFC Controller HX, then run the **Prime (154x)** script. This should take about 15 minutes.

## Prepare Assay Primer Mixes

**Note:** When preparing for an experiment that uses more than one partition, scale up the amount of assay primer mix appropriately.

Prepare each Assay Primer Mix using volumes in Table 1.

**Table 1** Assay Primer Mix for SNPtype Assays Genotyping

Component	Volume (µL)	Final Concentration
Allele-Specific Primers 1 and 2 (100 µM ASP1 and 100 µM ASP2)	3.0	7.5 µM
Locus-Specific Primers (100 µM LSP)	8.0	20.0 µM
DNA Suspension Buffer	29.0	—
<b>Total</b>	<b>40.0</b>	—

## Prepare 10X Assays

**IMPORTANT:** Due to the small pipetting volumes necessary for preparing a single assay mix, preparing a 10X assay stock is recommended. Unused 10X Assays can be stored at -20°C for up to three weeks.

**Note:** When preparing for an experiment that uses more than one partition, scale up the amount of assays appropriately.

In a DNA-free hood, prepare aliquots of 10X assays using volumes in Table 2.

**Table 2** 10X assays for SNPtype Assays Genotyping

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Volume for 50 µL Stock (µL) (10 replicates)
2X Assay Loading Reagent (Fluidigm, PN 100-5359)	2.0	2.5	25.0
PCR Certified Water	1.2	1.5	15.0
Assay Primer Mix	0.8	1.0	10.0
<b>Total Volume</b>	<b>4.0</b>	<b>5.0</b>	<b>50.0</b>

## Preparing Sample Pre-Mix and Samples

**Note:** When preparing for an experiment that uses more than one partition, scale up the amount of samples appropriately.

Combine components in Table 3 to make the Sample Pre-Mix and final Sample Mixture in a 96-well plate, tubes, or tube strips.

- 1 In a DNA-free hood, combine the **Sample Pre-Mix** components to make enough for your experiment (52.5 µL/partition). Aliquot 3.5 µL of the pre-mix for each sample.
- 2 Remove the aliquots from the DNA-free hood and add 2.5 µL of each DNA sample (genomic\* or preamplified) to make a total of 6 µL of Sample Mix Solution. (\* Genomic DNA must be ≥60 ng/µL of human genome size equivalent.)

**Table 3** Sample Pre-Mix and Sample Mixture for SNPtype Assays Genotyping

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Sample Pre-Mix for 1 Partition (µL) (15 reactions for ease of pipetting)
Biotium Fast Probe Master Mix (2X) (Biotium, PN 31005)	2.5	3.0	45.0
20X SNPtype™ Sample Loading Reagent (Fluidigm, PN 100-7445)	0.25	0.3	4.5
60X SNPtype™ Reagent (Fluidigm, PN 100-3402)	0.083	0.1	1.5
ROX Reference Dye (50X) (Life Technologies, PN 12223-012)	0.03	0.036	0.54
PCR Certified Water	0.053	0.064	0.96
DNA sample (genomic or preamplified) (Added individually to Sample Pre-Mix)	2.083	2.5	—
<b>Total Volume</b>	<b>5.0</b>	<b>6.0</b>	<b>52.5</b>

## Load the IFC

**IMPORTANT:** Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into IFC inlets. Failure to do so may result in a decrease in data quality.

**IMPORTANT:** While pipetting, do not go past the first stop on the pipette. Doing so may introduce bubbles into inlets, which can cause load failures.

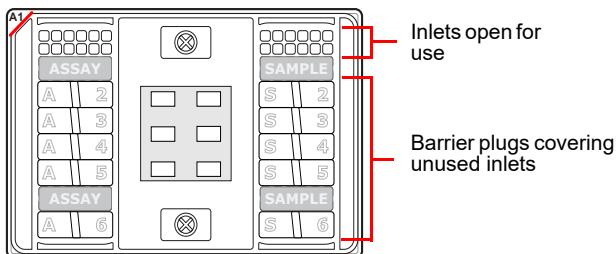
**IMPORTANT:** At minimum, all 12 assay inlets and all 12 sample inlets for a partition must be filled.

- For unused assay inlets in active partitions, prepare 2.5 µL Assay Loading Reagent, and 2.5 µL water per inlet.
- For unused sample inlets in active partitions, prepare 3.5 µL Sample Pre-Mix and 2.5 µL water per inlet.

**Note:** To facilitate automatic genotype calling, a minimum of 24 samples is recommended for clustering analysis. It may be possible to combine a single partition with a previous run, if consistency has been maintained with respect to assays used, thermal conditions and instrument used for data collection.

To load the Flex Six IFC:

- 1 Make sure barrier plugs are placed on unused inlets to mitigate pipetting into the wrong inlets and to track used/unused partitions.



- 2 Pipet one partition at a time by removing the barrier plugs for the selected set of partition inlets.
- 3 Pipet **4 µL of each assay and 5 µL of each sample** into their respective inlets.  
**Do not replace the barrier plugs after pipetting.**
- 4 Place the IFC in the IFC Controller HX.
- 5 Using the IFC Controller HX software, run the **Load Mix (154x)** script to load the samples and assays into the IFC. This should take 50 minutes.
- 6 When the **Load Mix (154x)** script has finished, remove the loaded IFC from the IFC Controller HX.  
**Do not replace barrier plugs after loading.**

You are now ready for data collection on the Biomark, Biomark HD, or EP1 System with FC1 Cycler.

## Using the FC1 Cycler

- 1 Press the **Start** button, open the lid, and place IFC onto the thermal cycling block (chuck) on top of the instrument, aligning the notched corner of the IFC to the A1 mark.



**CAUTION! HOT SURFACE HAZARD.** Never press down on the integrated fluidic circuit (IFC) when it is on the thermal cycler chuck. If you encounter a vacuum problem, turn off the system, allow it to cool down, and remove the IFC. Clean the bottom of the IFC and/or chuck surface with a lint-free cloth and 70% isopropyl alcohol.

- 2 Close the lid, press **Continue** to display available thermal protocols, select **SNPtype Flex Six v1**, then press **Run**.

A status screen appears with a time estimate for completion. Once the protocol is finished, a confirmation screen appears. Continue to data collection using the EP1 Reader.

## Data Collection Using the Biomark or Biomark HD System or the EP1 Reader

### Setting Up a Tracking File

If this is the first time you are running a Flex Six IFC after installing the Data Collection software or after restarting the computer, you will need to set up a tracking file. After the tracking file is set up initially, the software creates a directory and stores all Flex Six data in this location.

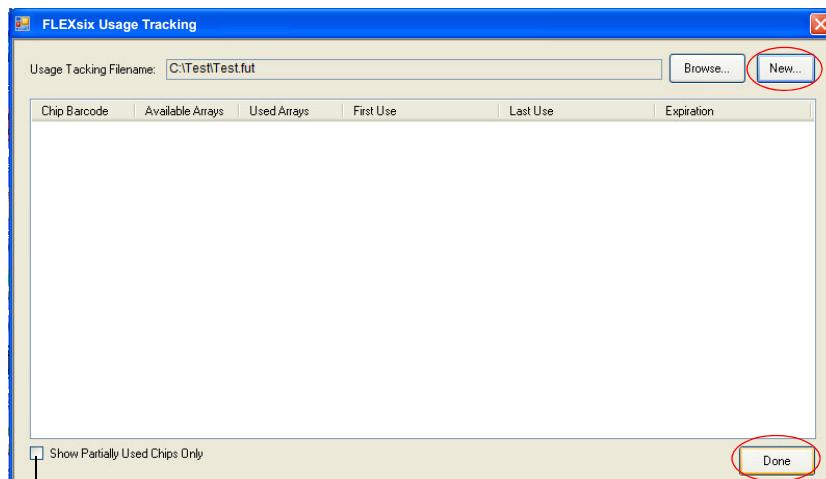
**Note:** If the directory containing tracking files changes, the Data Collection software will no longer be able to track IFC usage.

**Note:** If you have multiple Biomark Systems, you should create the tracking file in a location that can be accessed by all the Biomark Systems. Only usage tracking files should be stored this way; we do not recommend storing chip run data on a network.

- 1 On the Home page, select Tools > FLEXsix Usage Tracking.



- 2 Click **New** to create a new tracking file, or you can select an existing tracking file by using the **Browse** button.



Removes completely-used IFCs from the list.

- 3 If you are creating a new tracking file, enter a name for the file and navigate to the desired storage location.
- 4 Click **Done**.

## Run the Flex Six IFC

- 1 Double-click the Data Collection Software icon on the desktop to launch, then click **Start a New Run**.
- 2 Remove debris from the top of the IFC with clear tape, place the loaded IFC into the Biomark System or EP1 Reader, then click **Load**.
- 3 Verify the IFC barcode and type, choose project settings (if applicable), then click **Next**.
- 4 Select the partitions you wish to run.
- 5 Select the Application, Reference, Probes:
  - a Select Application Type: **Genotyping**.
  - b Select Passive Reference: **ROX**.
  - c Select probe types: **SNPtype-FAM** and **SNPtype-HEX**.
  - d Click **Next**.
- 6 Select a protocol:
  - If using a Biomark or Biomark HD for thermal cycling and image capture (end-point read only), select:
    - For Biomark HD only (fast): **SNPtype FLEXsix v1**

Type			Temperature	Time
Ramp Rate: Fast 5.5 °C/s	Thermal Mix		25 °C	30 min
			70 °C	60 min
	Hot Start		95 °C	5 min
	PCR Cycle	1 Cycle	Denaturation	95 °C
			Annealing	64 °C
			Extension	72 °C
	PCR Cycle	1 Cycle	Denaturation	95 °C
			Annealing	63 °C
			Extension	72 °C
	PCR Cycle	1 Cycle	Denaturation	95 °C
			Annealing	62 °C
			Extension	72 °C
	PCR Cycle	1 Cycle	Denaturation	95 °C
			Annealing	61 °C
			Extension	72 °C
	PCR Cycle	34 Cycles	Denaturation	95 °C
			Annealing	60 °C
			Extension	72 °C
End Point		Capture	20 °C	30 s

- For Biomark or Biomark HD (standard): **SNPtype E FLEXsix v1**

Type			Temperature	Time	
Ramp Rate: Normal 2 °C/s	Thermal Mix		25 °C	30 min	
			70 °C	60 min	
	Hot Start		95 °C	5 min	
	PCR Cycle	1 Cycle	Denaturation	95 °C	15 s
			Annealing	64 °C	45 s
			Extension	72 °C	15 s
	PCR Cycle	1 Cycle	Denaturation	95 °C	15 s
			Annealing	63 °C	45 s
			Extension	72 °C	15 s
	PCR Cycle	1 Cycle	Denaturation	95 °C	15 s
			Annealing	62 °C	45 s
			Extension	72 °C	15 s
	PCR Cycle	1 Cycle	Denaturation	95 °C	15 s
			Annealing	61 °C	45 s
			Extension	72 °C	15 s
	PCR Cycle	34 Cycles	Denaturation	95 °C	15 s
			Annealing	60 °C	45 s
			Extension	72 °C	15 s
End Point		Capture	20 °C	30 s	

- If using a Biomark or Biomark HD for end-point read only (after cycling on the FC1), select: **GT End Point v1**

Type	Temperature	Time
Ramp Rate: Normal 2 °C/s	Acquisition	
	20 °C	1 s

- If using an EP1 Reader, continue to the next step.

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

## Post Chip Run

The Flex Six IFC requires a Post Run Process to ensure the 90-day life time. Run the five-minute Post Run script immediately after data collection on the Biomark, Biomark HD, or EP1 Reader, prior to any storage of the IFC.

- 1 Immediately after data collection on the Biomark, Biomark HD, or EP1 Reader, take out the IFC, load it into the IFC Controller HX, then run the **Post Run (154x)** script to relax the valves. This takes approximately five minutes.
- 2 You can now put the barrier plugs back into the used inlets. Remember to label the used barrier plugs so that you have a record of which partitions/inlets have been used.

## Store a Used Flex Six IFC

**IMPORTANT:** Use the entire IFC within **90 days of first use**.

**IMPORTANT:** After storage, you can load any unused partitions without the need to re-prime the used IFC.

Store the IFC at room temperature and protect from dust until the next use.

- The IFC can be stored at room temperature on the bench top or in a drawer. It is not necessary to store the IFC in the dark, inside the silver wrapper, or inside the box.
- Lay the IFC flat with inlets facing up when storing. Do not store the IFC on its side or upside-down.
- After a run, put the barrier plugs back into the used inlets. Remember to label used barrier plugs so that you have a record of which partitions/inlets have been used.
- Between runs, be certain that the barrier plugs are in their proper positions for all unused partitions and that the IFC remains free of dust.

# Flex Six IFC TaqMan Fast/Standard Genotyping Workflow

## Prime the Flex Six IFC

**IMPORTANT:** Use the Flex Six integrated fluidic circuit (IFC) within three months of opening the package. Load the IFC within 60 minutes of priming on first use.

**IMPORTANT:** Dripping control line fluid on the chip or in the inlets makes the IFC unusable.

**Note:** It is not necessary to evacuate air from the syringe prior to injection of control line fluid.

**Note:** You only need to prime the IFC on the **first run**. On subsequent use, skip this step.

For information on how to inject control line fluid, see the *Fluidigm® Control Line Fluid Loading Procedure Quick Reference* (PN 68000132).

For information on using the IFC Controller HX, see the *IFC Controller MX-HX User Guide* (PN 68000112).

During the **first usage** of each Flex Six IFC, prime the new Flex Six IFC:

- 1 Using the included syringes, inject 150 µL of control line fluid into each accumulator on the IFC. **Do not** remove the barrier plugs until you load the IFC.
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 Place the IFC into the IFC Controller HX, then run the **Prime (154x)** script. This should take about 15 minutes.

## Prepare 10X Assays

**IMPORTANT:** Due to the small pipetting volumes necessary for preparing a single assay mix, preparing a 10X assay stock is recommended. Unused 10X Assays can be stored at -20°C for up to three weeks.

In a DNA-free hood, prepare aliquots of 10X assays using volumes in Table 4.

**Table 4** 10X assays for SNPtype Assays Genotyping

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Volume for 50 µL Stock (µL) (10 replicates)
2X Assay Loading Reagent (Fluidigm, PN 100-5359)	2.0	2.5	25.0
ROX Reference Dye (50X) (Life Technologies, PN 12223-012)	0.2	0.25	2.5
PCR Certified Water	1.3	1.625	16.25
SNP Genotyping Assay Mix (80X <sup>1</sup> ) (Life Technologies)	0.5	0.625	6.25
<b>Total Volume</b>	<b>4.0</b>	<b>5.0</b>	<b>50.0</b>

1. If you are using 40X SNP assay, double the volume and reduce the PCR Certified Water. For other starting concentrations of the SNP assay mix, call Fluidigm Technical Support.

## Preparing Sample Pre-Mix and Samples

**Note:** When preparing for an experiment that uses more than one partition, scale up the amount of samples appropriately.

Combine components in Table 5 to make the Sample Pre-Mix and final Sample Mixture in a 96-well plate, tubes, or tube strips.

- 1 In a DNA-free hood, combine the **Sample Pre-Mix** components to make enough for your experiment (52.5 µL/partition). Aliquot 3.5 µL of the pre-mix for each sample.
- 2 Remove the aliquots from the DNA-free hood and add 2.5 µL of each DNA sample (genomic\* or preamplified) to make a total of 6 µL of Sample Mix Solution. (\* Genomic DNA must be  $\geq 60$  ng/µL of human genome size equivalent.)

**Table 5** Sample Pre-Mix and Sample Mix for TaqMan Fast/Standard Genotyping

FAST Chemistry Component	Volume per Inlet ( $\mu\text{L}$ )	Volume per Inlet with Overage ( $\mu\text{L}$ )	Sample Pre-Mix for 1 Partition ( $\mu\text{L}$ ) (15 reactions for ease of pipetting)
TaqMan® GTxpress Master Mix (2X) (Life Technologies, PN 4403311)	2.5	3.0	45.0
20X Fast GT Sample Loading Reagent (Fluidigm, PN 100-7444) 	0.25	0.3	4.5
PCR Certified Water	0.17	0.2	3.0
DNA sample (genomic or preamplified) (Added individually to Sample Pre-Mix)	2.08	2.5	—
<b>Total Volume</b>	<b>5.0</b>	<b>6.0</b>	<b>52.5</b>

STANDARD Chemistry Component	Volume per Inlet ( $\mu\text{L}$ )	Volume per Inlet with Overage ( $\mu\text{L}$ )	Sample Pre-Mix for 1 Partition ( $\mu\text{L}$ ) (15 reactions for ease of pipetting)
TaqMan® Universal PCR Master Mix (2X) (Life Technologies, PN 4304437)	2.5	3.0	45.0
20X GT Sample Loading Reagent (Fluidigm, PN 100-7443) 	0.25	0.3	4.5
AmpliTaq Gold® DNA Polymerase (Life Technologies, PN 4311806)	0.05	0.06	0.9
PCR Certified Water	0.12	0.14	2.1
DNA sample (genomic or preamplified) (Added individually to Sample Pre-Mix)	2.08	2.5	—
<b>Total Volume</b>	<b>5.0</b>	<b>6.0</b>	<b>52.5</b>

## Load the IFC

**IMPORTANT:** Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into IFC inlets. Failure to do so may result in a decrease in data quality.

**IMPORTANT:** While pipetting, do not go past the first stop on the pipette. Doing so may introduce bubbles into inlets, which can cause load failures.

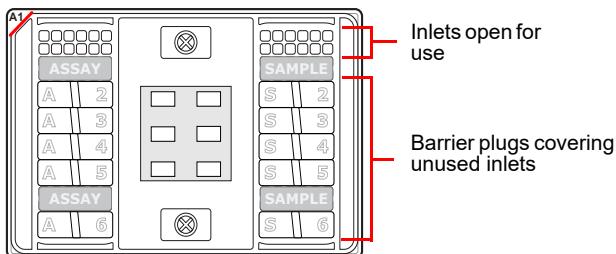
**IMPORTANT:** At minimum, all 12 assay inlets and all 12 sample inlets for a partition must be filled.

- For unused assay inlets in active partitions, prepare 2.5  $\mu\text{L}$  Assay Loading Reagent, 0.25  $\mu\text{L}$  ROX, and 2.25  $\mu\text{L}$  water per inlet.
- For unused sample inlets in active partitions, prepare 3.5  $\mu\text{L}$  Sample Pre-Mix and 2.5  $\mu\text{L}$  water per inlet.

**Note:** To facilitate automatic genotype calling, a minimum of 24 samples is recommended for clustering analysis. It may be possible to combine a single partition with a previous run, if consistency has been maintained with respect to assays used, thermal conditions and instrument used for data collection.

To load the Flex Six IFC:

- 1 Make sure barrier plugs are placed on unused inlets to mitigate pipetting into the wrong inlets and to track used/unused partitions.



- 2 Pipet one partition at a time by removing the barrier plugs for the selected set of partition inlets.
- 3 Pipet **4 µL of each assay and 5 µL of each sample** into their respective inlets.  
**Do not** replace the barrier plugs after pipetting.
- 4 Place the IFC in the IFC Controller HX.
- 5 Using the IFC Controller HX software, run the **Load Mix (154x)** script to load the samples and assays into the IFC. This should take 50 minutes.
- 6 When the **Load Mix (154x)** script has finished, remove the loaded IFC from the IFC Controller HX.  
**Do not** replace barrier plugs after loading.

You are now ready for data collection on the Biomark, Biomark HD, or EP1 System with FC1 Cycler.

## Using the FC1 Cycler (End-point Read only)

- 1 Press the **Start** button, open the lid, and place IFC onto the thermal cycling block (chuck) on top of the instrument, aligning the notched corner of the IFC to the A1 mark.



**CAUTION! HOT SURFACE HAZARD.** Never press down on the integrated fluidic circuit (IFC) when it is on the thermal cycler chuck. If you encounter a vacuum problem, turn off the system, allow it to cool down, and remove the IFC. Clean the bottom of the IFC and/or chuck surface with a lint-free cloth and 70% isopropyl alcohol.

- 2 Close the lid, press **Continue** to display available thermal protocols, select **GT FLEXsix Fast v1** (fast chemistry) or **GT FLEXsix Standard v1** (standard chemistry), then press **Run**.

A status screen appears with a time estimate for completion. Once the protocol is finished, a confirmation screen appears. Continue to data collection using the EP1 Reader.

## Data Collection Using the Biomark or Biomark HD System or the EP1 Reader

### Setting Up a Tracking File

If this is the first time you are running a Flex Six IFC after installing the Data Collection software or after restarting the computer, you will need to set up a tracking file. After the tracking file is set up initially, the software creates a directory and stores all Flex Six data in this location.

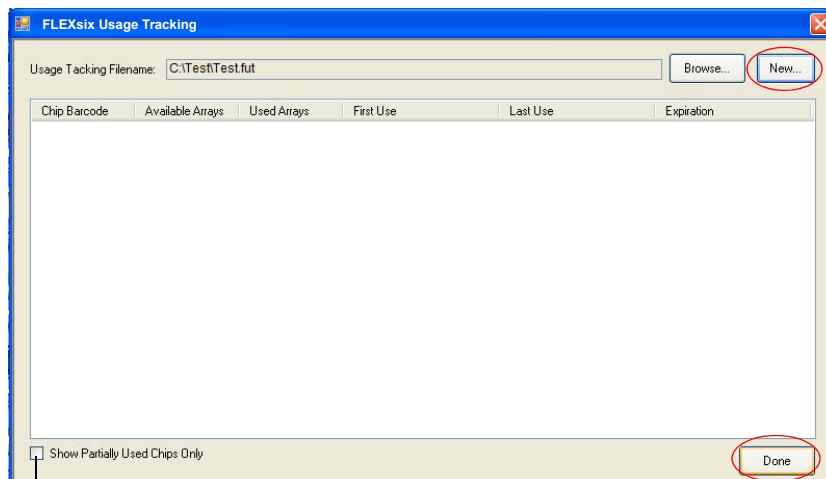
**Note:** If the directory containing tracking files changes, the Data Collection software will no longer be able to track IFC usage.

**Note:** If you have multiple Biomark Systems, you should create the tracking file in a location that can be accessed by all the Biomark Systems. Only usage tracking files should be stored this way; we do not recommend storing chip run data on a network.

- 1 On the Home page, select Tools > FLEXsix Usage Tracking.



- 2 Click **New** to create a new tracking file, or you can select an existing tracking file by using the **Browse** button.



Removes completely-used IFCs from the list.

- 3 If you are creating a new tracking file, enter a name for the file and navigate to the desired storage location.
- 4 Click **Done**.

## Run the Flex Six IFC

- 1 Double-click the Data Collection Software icon on the desktop to launch, then click **Start a New Run**.
- 2 Remove debris from the top of the IFC with clear tape, place the loaded IFC into the Biomark System or EP1 Reader, then click **Load**.
- 3 Verify the IFC barcode and type, choose project settings (if applicable), then click **Next**.
- 4 Select the partitions you wish to run.
- 5 Select the Application, Reference, Probes:
  - a Select Application Type: **Genotyping**.
  - b Select Passive Reference: **ROX**.
  - c Select probe types **FAM-MGB** and **VIC-MGB**.
  - d Click **Next**.
- 6 Select a protocol:
  - If using a Biomark or Biomark HD for thermal cycling and image capture (end-point read only), select:
    - For Biomark HD only (fast): **GT FLEXsix Fast v1**

		Type	Temperature	Time	
Ramp Rate: Fast 5.5 °C/s	Thermal Mix		25 °C	30 min	
			70 °C	60 min	
	Hot Start		95 °C	2 min	
	PCR Cycle	1 Cycle	Denaturation	95 °C	2s
			Annealing	60 °C	20 s

- For Biomark HD only (fast): **SNPtype FLEXsix v1**

		Type	Temperature	Time	
Ramp Rate: Standard 2 °C/s	Thermal Mix		25 °C	30 min	
			70 °C	60 min	
	UNG and Hot Start		UNG	50 °C	2 min
			Hot Start	95 °C	10 min
	PCR Cycle	1 Cycle	Denaturation	95 °C	15 s
			Annealing	60 °C	1 min

- For Biomark or Biomark HD (standard): **GT FLEXsix Standard v1**

		Type	Temperature	Time
Ramp Rate: Normal 2 °C/s	Acquisition	20 °C	1 s	

- If using an EP1 Reader, continue to the next step.

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

## Post Chip Run

The Flex Six IFC requires a Post Run Process to ensure the 90-day life time. Run the five-minute Post Run script immediately after data collection on the Biomark, Biomark HD, or EP1 Reader, prior to any storage of the IFC.

- 1 Immediately after data collection on the Biomark, Biomark HD, or EP1 Reader, take out the IFC, load it into the IFC Controller HX, then run the **Post Run (154x)** script to relax the valves. This takes approximately five minutes.
- 2 You can now put the barrier plugs back into the used inlets. Remember to label the used barrier plugs so that you have a record of which partitions/inlets have been used.

## Store a Used Flex Six IFC

**IMPORTANT:** Use the entire IFC within 90 days of first use.

**IMPORTANT:** After storage, you can load any unused partitions without the need to re-prime the used IFC.

Store the IFC at room temperature and protect it from dust until the next use.

- The IFC can be stored at room temperature on the bench top or in a drawer. It is not necessary to store the IFC in the dark, inside the silver wrapper, or inside the box.
- Lay the IFC flat with inlets facing up when storing. Do not store the IFC on its side or upside-down.
- After a run, put the barrier plugs back into the used inlets. Remember to label used barrier plugs so that you have a record of which partitions/inlets have been used.
- Between runs, be certain that the barrier plugs are in their proper positions for all unused partitions and that the IFC remains free of dust.

## General Safety

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

- Use the appropriate personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves, according to your laboratory safety practices.
- 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 the instruments. Unauthorized modifications may create a safety hazard.



**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), a publication 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 online at [cdc.gov/biosafety/publications/index.htm](http://cdc.gov/biosafety/publications/index.htm).



**CAUTION!** The FC1™ thermal cycler chuck gets hot and can burn your skin. Use caution when working near the chuck.

For a full list of the symbols on the instrument, refer to the Biomark™ HD Data Collection Software User Guide (PN 100-2451) or the Biomark™/EP1™ Data Collection Software User Guide (PN 68000127).

## Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.

## Disposal of Products

Used IFCs and reagents 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.

Learn more at [fluidigm.com/compliance](http://fluidigm.com/compliance)







For technical support visit  
[fluidigm.com/support](http://fluidigm.com/support).