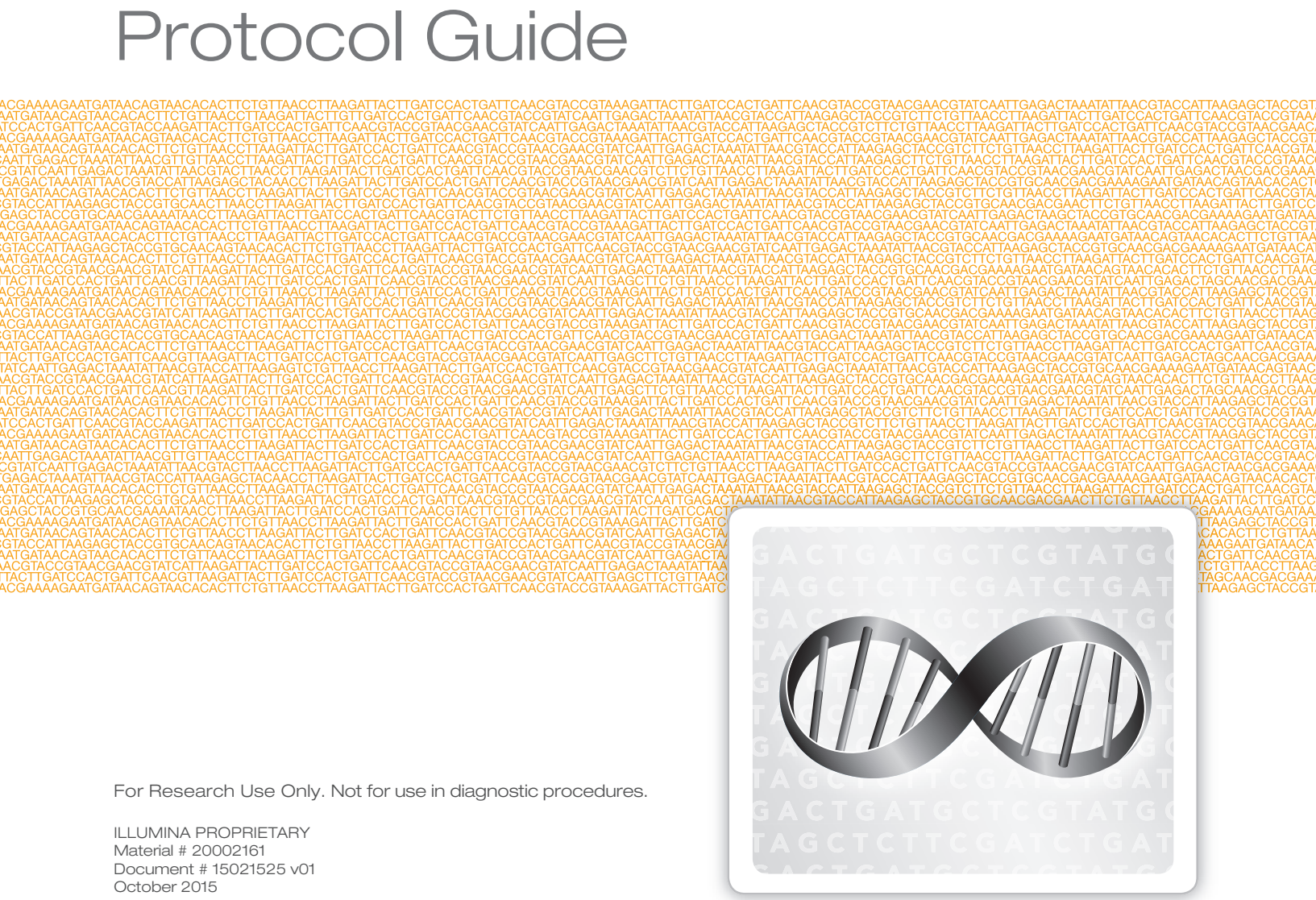
illumina®

Infinium® HD FFPE Assay Protocol Guide



For Research Use Only. Not for use in diagnostic procedures.

ILLUMINA PROPRIETARY
Material # 20002161
Document # 15021525 v01
October 2015

Revision History

Part #	Revision	Date	Description of Change
15019519	01	October 2015	• Corrected the temperature of the hybridization oven in the ***Make the MSA5 Plate*** and ***Incubate DNA*** sections to 37°C in both the Manual and Automated chapters. • Updated the total volume of PB1 required depending on the number of alignment fixtures being used.
15019519	C	May 2012	Indicated that up to 24 BeadChips can be washed with 550 ml of PB1.
15019519 Catalog # WG-901-2001	B	April 2011	Corrected reagent tube quantities from 1 tube per 96 samples to 2 tubes per 96 samples in the Consumables tables for the ***Make the MSA5 Plate***, ***Fragment DNA***, and ***Precipitate DNA*** sections in both the Manual and Automated chapters.
15019519 Catalog # WG-901-2001	A	March 2011	Initial release.

Table of Contents

Revision History iii
Table of Contents v
List of Tables vii

Chapter 1 Overview 1

Introduction to Infinium HD FFPE Assay 2
Audience and Purpose 3
Infinium HD FFPE Assay 4
Illumina Infinium BeadChips 9
Illumina Lab Protocols 10
Tracking Tools 11
Tecan GenePaint 14
Imaging Systems 15
GenomeStudio Integrated Informatics Platform 16

Chapter 2 Manual Protocol 17

Introduction to Infinium HD FFPE Manual Protocol 18
Infinium HD FFPE Manual Workflow 19
Equipment, Materials, and Reagents 20
Make the MSA5 Plate 24
Incubate DNA (Post-Amp) 27
Fragment DNA (Post-Amp) 28
Precipitate DNA (Post-Amp) 30
Resuspend DNA (Post-Amp) 34
Hybridize to BeadChip (Post-Amp) 37
Wash BeadChips (Post-Amp) 53
Extend and Stain (XStain) BeadChip 64
Image BeadChip 84
Illumina GenomeStudio 85

Chapter 3 Automated Protocol 87

Introduction to Infinium HD FFPE Automated Protocol 88
Infinium HD FFPE Automated Workflow 89
Equipment, Materials, and Reagents 90
Make the MSA5 Plate 94
Incubate DNA (Post-Amp) 99

Fragment DNA (Post-Amp) 100
Precipitate DNA (Post-Amp) 105
Resuspend DNA (Post-Amp) 112
Hybridize to BeadChip (Post-Amp) 117
Wash BeadChips (Post-Amp) 134
Extend and Stain (XStain) BeadChip 145
Image BeadChip 177
Illumina GenomeStudio 178

Appendix A System Controls 179

Infinium HD FFPE Assay System Controls 180

Index 181

Technical Assistance 183

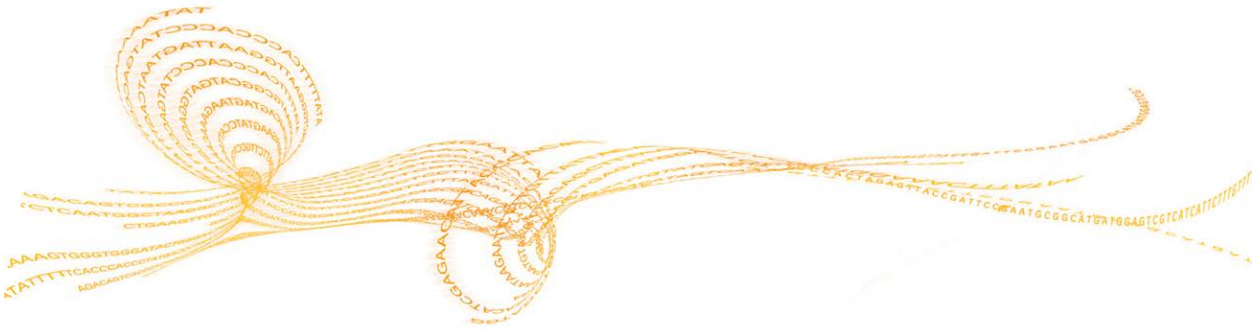
List of Tables

Table 1 Sample Sheet Guidelines 11
Table 2 AutoLoader, AutoLoader2, and AutoLoader2x Features 15
Table 3 User-Supplied Equipment 20
Table 4 Illumina-Supplied Equipment 20
Table 5 User Supplied Materials 20
Table 6 Illumina-Supplied Reagents 22
Table 7 Scan Settings for Infinium HD FFPE 84
Table 8 User-Supplied Equipment 90
Table 9 Illumina-Supplied Materials 90
Table 10 User-Supplied Materials 91
Table 11 Illumina-Supplied Reagents 92
Table 12 List of Reactions 155
Table 13 Scan Settings for Infinium HD FFPE 177
Table 14 Illumina General Contact Information 183
Table 15 Illumina Customer Support Telephone Numbers 183

Overview

Chapter 1

Introduction to Infinium HD FFPE Assay 2
Audience and Purpose 3
Infinium HD FFPE Assay 4
Illumina Infinium BeadChips 9
Illumina Lab Protocols 10
Tracking Tools 11
Tecan GenePaint 14
Imaging Systems 15
GenomeStudio Integrated Informatics Platform 16



Introduction to Infinium HD FFPE Assay

Used in conjunction with the Infinium® HD FFPE Restore Kit, Illumina's® Infinium HD FFPE Assay revolutionizes DNA analysis of degraded FFPE samples by streamlining sample preparation and enabling high multiplexing. Using Infinium I and Infinium II probe designs and dual color channel approach, the Infinium HD FFPE Assay scales DNA analysis from 3,000 up to several hundred thousand SNPs and CNV markers per sample, dependent only on the number of features (bead types) on the array.

The Infinium HD FFPE Assay accomplishes this unlimited multiplexing by combining whole-genome amplification (WGA) sample preparation with direct, array-based capture and enzymatic scoring of the SNP loci. Locus discrimination or copy number (CNV) determination is provided by a combination of high beadtype representation per feature, sequence-specific hybridization capture and array-based, single-base primer extension. In the case of the Infinium II probe design, the 3' end of the primer is positioned directly adjacent to the SNP site, or the non-polymorphic site in the case of non-polymorphic probes. In the case of the Infinium I probe design, the 3' end of the primer overlaps with the SNP site. If there is a perfect match, extension occurs and signal is generated. If there is a mismatch, extension does not occur and no signal is generated.

Allele-specific single base extension of the primer incorporates a biotin-labeled nucleotide or a dinitrophenyl-labeled nucleotide. C and G nucleotides are biotin-labeled; A and T nucleotides are dinitrophenyl-labeled. Signal amplification of the incorporated label further improves the overall signal-to-noise ratio of the assay.

The Infinium HD FFPE Assay offers:

- High multiplexing
- High call rate and accuracy
- Single tube amplification—single chip—no PCR
- Minimal risk of carryover contamination
- Low DNA input—200 ng per sample. Only 100 ng of original FFPE DNA is needed as input into the Infinium HD FFPE Restore kit protocol. A resulting 200 ng from the restore kit is then input into the Infinium FFPE assay.
- Walk-away automation using Tecan Genesis or Freedom EVO Robots and Tecan GenePaint system
- Compatibility with both Illumina iScan™ and HiScan™ Systems
- Multiple-Sample (12-) BeadChip format

Audience and Purpose

This guide is for laboratory technicians running the Infinium HD FFPE Assay. The guide documents the laboratory protocols associated with the assay. Follow all the protocols in the order shown.

Chapter 2 Manual Protocol explains how to run the assay manually in the lab.

Chapter 3 Automated Protocol explains how to automate the protocol with the aid of the Tecan 8-tip robot.

Important Note

Before following any of the procedures in this guide, read the *Infinium Lab Setup and Procedures Guide*, which explains how to equip and run an Infinium HD FFPE Assay laboratory. The guide includes important information on the following topics:

- Prevention of amplification product contamination
- Safety precautions
- Equipment, materials, and reagents
- Standard lab procedures
- Robot use
- BeadChip imaging
- System maintenance
- GenomeStudio controls
- Troubleshooting

The instructions apply equally to all Infinium BeadChips provided by Illumina. All the Infinium HD FFPE documentation assumes that you have already set up the laboratory space and are familiar with the standard procedures and safety precautions.

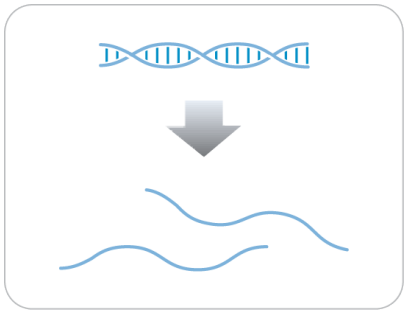
Infinium HD FFPE Assay

This section describes and illustrates the assay protocol. The assay requires only 100 ng of original FFPE sample. A resulting 200ng from the restore kit is then input into the Infinium FFPE assay.

Amplify DNA (Pre-Amp)

The DNA samples are denatured and neutralized to prepare them for amplification.

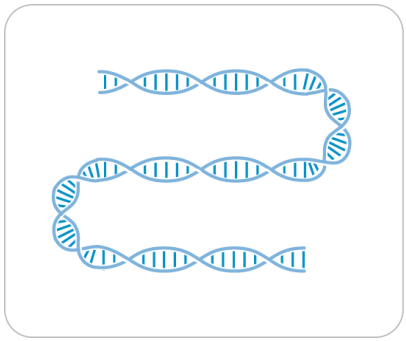
Figure 1 Denaturing and Neutralizing DNA



Incubate DNA (Post-Amp)

The denatured DNA is isothermally amplified in an overnight step. The whole-genome amplification uniformly increases the amount of the DNA sample by several thousand-fold without significant amplification bias.

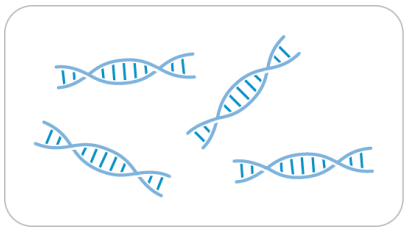
Figure 2 Incubating DNA to Amplify



Fragment DNA (Post-Amp)

A controlled enzymatic process fragments the amplified product. The process uses endpoint fragmentation to prevent overfragmentation.

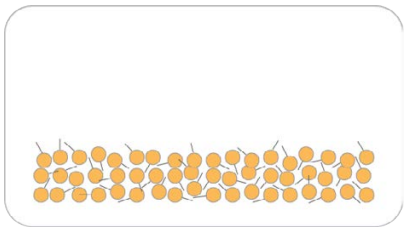
Figure 3 Fragmenting DNA



Precipitate DNA (Post-Amp)

After an isopropanol precipitation, centrifugation at 4°C collects the fragmented DNA.

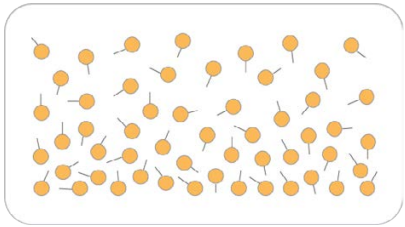
Figure 4 Precipitating DNA



Resuspend DNA (Post-Amp)

The precipitated DNA is resuspended in Hybridization Buffer.

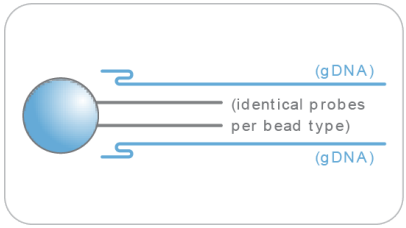
Figure 5 Resuspending DNA



Hybridize to BeadChip (Post-Amp)

Samples are applied to a BeadChip and separated by an IntelliHyb seal (or gasket). The loaded BeadChip is incubated overnight in the Illumina Hybridization Oven. The amplified and fragmented DNA samples anneal to locus-specific 50-mers during hybridization.

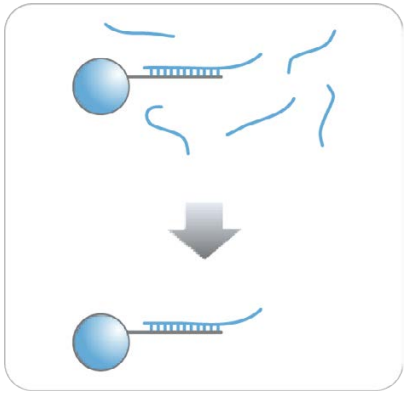
Figure 6 Hybridize to BeadChip



Wash BeadChip (Post-Amp)

Unhybridized and nonspecifically hybridized DNA is washed away and the BeadChip is prepared for staining and extension.

Figure 7 Washing BeadChip



Extend and Stain (XStain) BeadChip (Post-Amp)

Single-base extension of the oligos on the BeadChip, using the captured DNA as a template, incorporates detectable labels on the BeadChip and determines the genotype call for the sample. XStain occurs in a capillary flow-through chamber.

Figure 8 Extending and Staining BeadChip

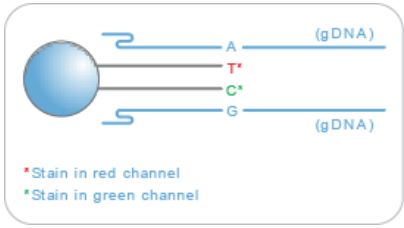


Image BeadChip (Post-Amp)

The Illumina HiScan or iScan System scans the BeadChip, using a laser to excite the fluorophore of the single-base extension product on the beads. The scanner records high-resolution images of the light emitted from the fluorophores.

Figure 9 Imaging BeadChip

Illumina Infinium BeadChips

Illumina Infinium BeadChips are sophisticated silicon-based array devices. An IntelliHyb seal separates the sample sections of the slide so that you can run multiple samples simultaneously.

Each individual sample section holds oligonucleotide probe sequences that are attached to beads assembled into the microwells of the BeadChip substrate. Because the microwells outnumber the distinct bead types, multiple copies of each bead type are present in the array. This built-in redundancy improves robustness and measurement precision. The BeadChip manufacturing process includes hybridization-based quality controls of each array feature, allowing consistent production of high-quality, reproducible arrays.

Illumina Lab Protocols

Illumina lab protocols are designed to promote efficiency and minimize the risk of contamination. The *Infinium Lab Setup and Procedures Guide* documents standard operating procedures and tools for an Infinium Assay lab and explains how to set up and maintain separate pre- and post-amplification areas. Familiarize yourself with this guide before performing any Infinium assays.

Chapter 2 Manual Protocol and Chapter 3 Automated Protocol show how to perform the assay protocol with clearly divided pre- and post-amplification processes using a manual and automated process respectively.

Tracking Tools

Illumina provides the following tools for sample tracking and guidance in the lab:

- **Experienced User Cards** to guide you through the protocols. There are separate sets of cards for the manual and automated processes.
- **Lab Tracking Form** to map DNA samples to BeadChips and record the barcode of each reagent and plate used in the protocol.
- **Sample sheet template** to record information about your samples for later use in data analysis.

All these documents are available for printing and reference at www.illumina.com/documentation.

Sample Sheet

Illumina recommends that you create a sample sheet to track your samples and assay effectively. The GenomeStudio application uses the sample sheet later for data analysis. For instructions on data analysis, see the *GenomeStudio Genotyping User Guide*.

Create your sample sheet according to the guidelines provided in the table.

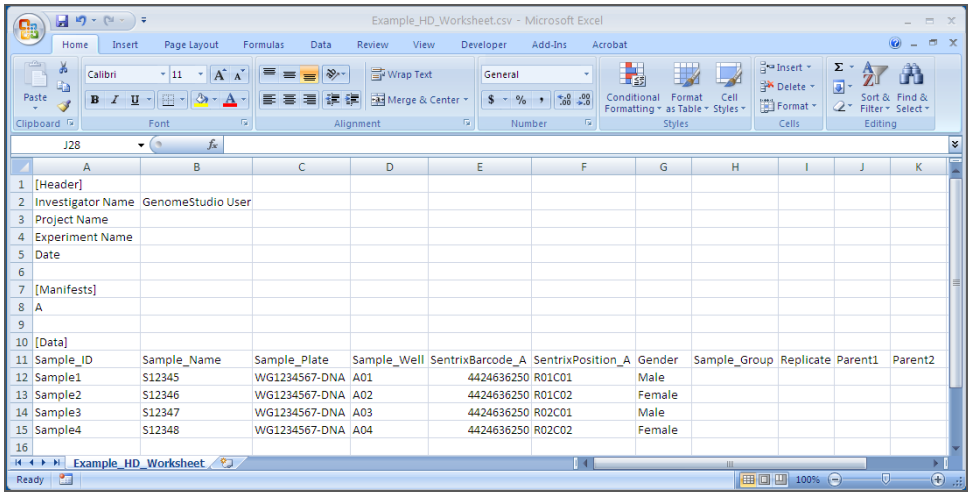
Table 1 Sample Sheet Guidelines

Column Heading	Description	Optional (O) or Required (R)
Sample_ID	Unique identifier for the sample.	R
Sample_Name	Name of the sample. Used only for display in the table.	O
Sample_Plate	The barcode of the sample plate for this sample. Used only for display in the table.	O
Sample_Well	The sample plate well for this sample. Used only for display in the table.	O
SentrixBarcode_A	The barcode of the array product (BeadChip) to which this sample was hybridized, for Manifest A.	R

Column Heading	Description	Optional (O) or Required (R)
SentrixPostion_A	The position within the array product to which this sample was hybridized, for the manifests in your project.	R
Gender	Male, Female, or Unknown.	O
Sample_Group	A group, if any, to which this sample belongs.	O
Replicate	The Sample_ID of a replicate to this sample. Used in reproducibility error calculations.	O
Parent1	The Sample_ID of the first parent.	O
Parent2	The Sample_ID of the second parent.	O
Notes	Your sample sheet header can contain whatever information you choose. Your sample sheet can contain any number of columns you choose. Your sample sheet must be in a comma-delimited (.csv) file format. Commas in the sample sheet are not allowed. Save the sample sheet under any name you wish; for example, the user-defined experiment name.	

The following figure provides an example of the sample sheet format. Product documentation includes an electronic, read-only sample sheet template file (Sample Sheet Template.csv) that you can copy and use from www.illumina.com/documentation.

Figure 10 Sample Sheet Example



	A	B	C	D	E	F	G	H	I	J	K
1	[Header]										
2	Investigator Name	GenomeStudio User									
3	Project Name										
4	Experiment Name										
5	Date										
6											
7	[Manifests]										
8	A										
9											
10	[Data]										
11	Sample_ID	Sample_Name	Sample_Plate	Sample_Well	SentrixBarcode_A	SentrixPosition_A	Gender	Sample_Group	Replicate	Parent1	Parent2
12	Sample1	S12345	WG1234567-DNA	A01	4424636250	R01C01	Male				
13	Sample2	S12346	WG1234567-DNA	A02	4424636250	R01C02	Female				
14	Sample3	S12347	WG1234567-DNA	A03	4424636250	R02C01	Male				
15	Sample4	S12348	WG1234567-DNA	A04	4424636250	R02C02	Female				
16											

Tecan GenePaint

The Infinium HD FFPE Assay uses the GenePaint automated slide processor on the Tecan to process BeadChips. The GenePaint system employs a capillary gap flow-through chamber to enable reagent entrapment and exchange over the active surface of the BeadChip. Washing, blocking, extension, and signal amplification happen simply by adding reagents to the flow cell. Addition of a new reagent displaces the entrapped reagent from the flow cell. For maximum flexibility, these additions can be performed either manually or via the Tecan Genesis or Tecan Freedom Evo robots. The optional automated robotic processing and single-use reagent tube barcoding assure maximum consistency from slide to slide.

CAUTION

Do not run any other programs or applications while using the Tecan robot. Your computer and the robot can lock up and stop a run.

Imaging Systems

BeadChips are imaged using either the Illumina HiScan System, iScan System, or BeadArray Reader. All of these are two-channel high-resolution laser imagers that scan BeadChips at two wavelengths simultaneously and create an image file for each channel (i.e., two per array).

The iScan Control Software (or BeadScan, for BeadArray Reader), determines intensity values for each bead type and creates data files for each channel. GenomeStudio uses this data file in conjunction with the oligo pool manifest file (*.opa), individual bead pool map (*.bpm), or manifest file (*.bgx) to analyze the data from the assay.

Loading and unloading the iScan System or BeadArray Reader can be automated with the optional AutoLoader or AutoLoader2 respectively or AutoLoader 2x for the HiScan System. All AutoLoaders support unattended processing by placing BeadChip carriers in the imaging system's tray, so that it can scan the BeadChips. Features include those listed below:

Table 2 AutoLoader, AutoLoader2, and AutoLoader2x Features

Feature	AutoLoader	AutoLoader2	AutoLoader2x
Integrated with iScan Control Software		•	•
Integrated with BeadScan Software	•		
Email alert system	•	•	•
Single-reader or dual-reader configuration	•	•	•
Number of BeadChips supported per carrier	2	4	4
Number of carriers processed at a time	20	48	48

NOTE
The HumanOmniExpress-FFPE BeadChip cannot be scanned on the BeadArray Reader.

GenomeStudio Integrated Informatics Platform

Illumina GenomeStudio is an integrated data analysis software platform that provides a common environment for analyzing data obtained from Illumina microarray and sequencing technologies. Within this common environment, or framework, the Illumina GenomeStudio software modules allow you to perform application-specific analyses. The Illumina GenomeStudio Genotyping Module, included with your Illumina Infinium Assay system, is an application for extracting genotyping data from intensity data files (*.idat files) collected from systems such as the Illumina HiScan System. For information on the latest software offerings, including software for applications such as cytogenetics, visit www.illumina.com.

Data analysis features of the GenomeStudio Genotyping Module include:

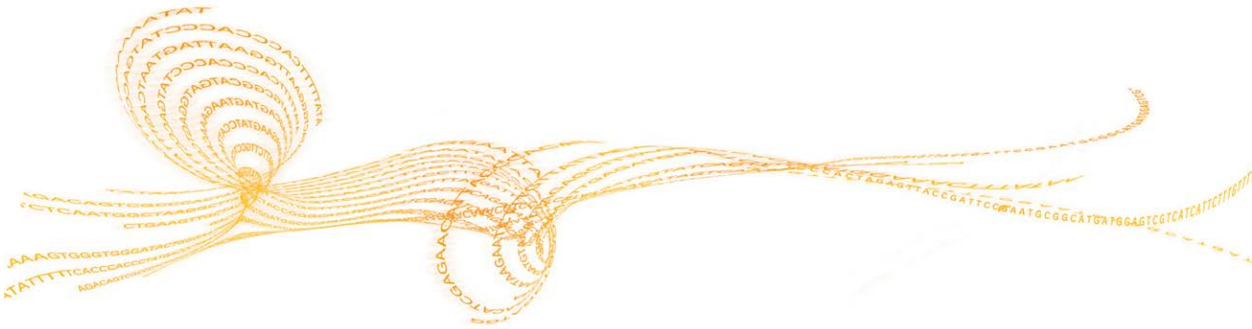
- Choice of assay analysis within a single application
- Data tables for information management and manipulation
- Plotting and graphing tools
- Whole-genome display of sample data in the IGV (Illumina Genome Viewer)
- Data visualization of one or more samples in the ICB (Illumina Chromosome Browser)
- Data normalization
- Custom report file formats
- Genotype calling
- Clustering
- Detection of LOH (loss of heterozygosity)
- Analysis of structural variation including CNV (copy number variation)

For feature descriptions and instructions on using the GenomeStudio platform to visualize and analyze genotyping data, see the *GenomeStudio Framework User Guide* and the *GenomeStudio Genotyping User Guide*.

Manual Protocol

Chapter 2

Introduction to Infinium HD FFPE Manual Protocol 18
Infinium HD FFPE Manual Workflow 19
Equipment, Materials, and Reagents 20
Make the MSA5 Plate 24
Incubate DNA (Post-Amp) 27
Fragment DNA (Post-Amp) 28
Precipitate DNA (Post-Amp) 30
Resuspend DNA (Post-Amp) 34
Hybridize to BeadChip (Post-Amp) 37
Wash BeadChips (Post-Amp) 53
Extend and Stain (XStain) BeadChip 64
Image BeadChip 84
Illumina GenomeStudio 85



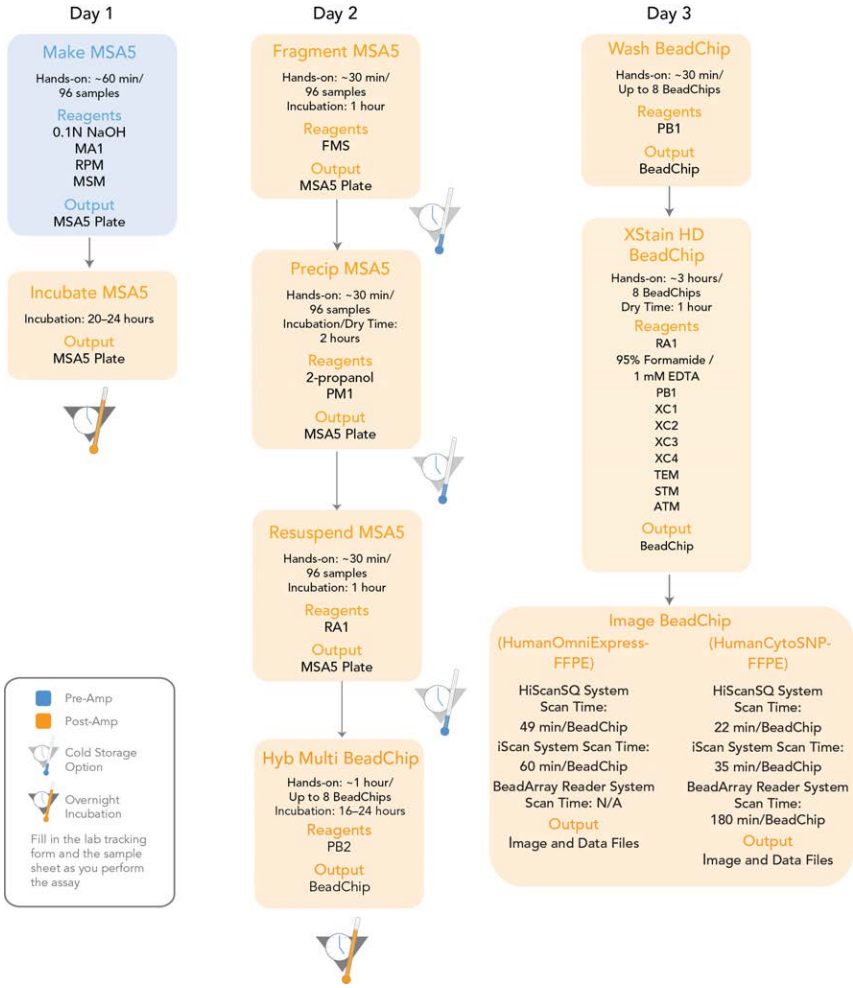
Introduction to Infinium HD FFPE Manual Protocol

This chapter describes pre- and post-amplification manual laboratory protocols for the Infinium HD FFPE Assay. Follow the protocols in the order shown.

Infinium HD FFPE Manual Workflow

The following figure graphically represents the Infinium HD FFPE Assay manual workflow for the Infinium HumanOmniExpress-FFPE and HumanCytoSNP-FFPE BeadChips. These protocols describe the procedure for preparing 96 DNA samples.

Figure 11 Infinium HD FFPE Assay Manual Workflow



Equipment, Materials, and Reagents

These materials are specific to the manual Infinium HD FFPE Assay. For a list of other equipment, materials, and reagents needed in an Infinium HD FFPE Assay lab, see the *Infinium Lab Setup and Procedures Guide*.

User-Supplied Equipment

Table 3 User-Supplied Equipment

Item	Catalog #
Forceps	VWR Catalog # 25601-008
Auto-desiccator cabinet (Optional—allows scanning of BeadChips up to three days after processing)	VWR Catalog # 74950-342

Illumina-Supplied Equipment

Table 4 Illumina-Supplied Equipment

Item	Catalog #
Multi-Sample BeadChip Alignment Fixture	Illumina part # 218528

User-Supplied Materials

Table 5 User Supplied Materials

Item	Catalog #
Robots	Tecan
Tube vortexer	N/A
Tube rack	VWR, International

Item	Catalog #
Combination optical tachometer/stroboscope	Cole-Parmer; cat# A-87700-06; www.coleparmer.com
Microtiter plate centrifuge with g-force range 8–3000 × g, for dedicated pre- and post-amp use	N/A
Adaptor for centrifuge plates and tubes	N/A
Pipettes (two separate sets)	2 each of P-20, P-200, and P-1000
8-channel precision pipettes (two separate sets)	50 µL to 300uL
Stop watch/timer	N/A
Forceps	VWR International; cat# 25601-008; www.vwr.com
Powder-free gloves (two separate stocks)	N/A
Lab coats (separate pre- and post-amp)	N/A
Safety glasses (two separate stocks)	N/A
15 ml conical tubes	N/A
96-well 0.2 ml skirted microplates	MJ Research; cat# MSP-9601; www.mjr.com
0.8 ml storage plate (MIDI plate), conical well bottom	Abgene; cat#AB-0765; www.abgene.com
Heat Sealing foil sheets, Thermo-Seal	Abgene; cat# AB-0559; www.abgene.com
96-well cap mats (piercable, nonautoclavable)	Abgene; cat# AB-0566; www.abgene.com
Absorbent pads	N/A
Kimwipes®	N/A
Mild detergent, such as Alconox® Powder	VWR, International; cat# 21835-032; www.vwr.com

Item	Catalog #
Detergent	N/A
Aerosol filter pipette tips (two separate stocks)	20 µl, 200 µl, 1000 µl
Disposable pipetting troughs	VWR; cat# 21007-970
Reservoir, full, 150 ml	Beckman Coulter, Inc.; cat# 372784
Reservoir, half, 75 ml	Beckman Coulter, Inc.; cat# 372786; www.beckman.com
Reservoir, quarter, 40 ml	Beckman Coulter, Inc.; cat#372790; www.beckman.com
Reservoir frame	Beckman Coulter, Inc.; cat# 372795; www.beckman.com

Illumina-Supplied Materials

- WG#-MSA5 barcode labels
- WG#-DNA barcode labels

Illumina-Supplied Reagents

Table 6 Illumina-Supplied Reagents

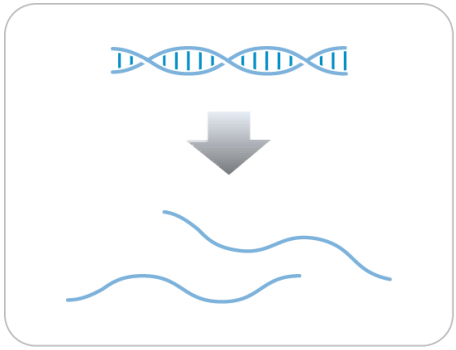
Item	Part #
ATM—Anti-Stain Two-Color Master Mix	11208317
FMS—Fragmentation solution	11203428
MA1—Multi-Sample Amplification 1 Mix	11202880
RPM—Random Primer Mix	15010230
MSM—Multi-Sample Amplification Master Mix	11203410

Item	Part #
PB1—Reagent used to prepare BeadChips for hybridization	11291245
PB2—Humidifying buffer used during hybridization	11191130
PM1—Precipitation solution	11292436
RA1—Resuspension, hybridization, and wash solution	11292441
STM—Superior Two-Color Master Mix	11288046
TEM—Two-Color Extension Master Mix	11208309
XC1—XStain BeadChip solution 1	11208288
XC2—XStain BeadChip solution 2	11208296
XC3—XStain BeadChip solution 3	11208392
XC4—XStain BeadChip solution 4	11208430

Make the MSA5 Plate

This process prepares your restored FFPE samples for DNA amplification. MA1 is first added to the MSA5 plate and then 0.1N NaOH is added to denature the DNA samples. The RPM reagent neutralizes the sample. Lastly, MSM is added to the plate.

Figure 12 Denaturing and Neutralizing DNA



Estimated Time

Hands-on time:

- 45 minutes for 48 samples
- 60 minutes for 96 samples

Consumables

Item	Quantity	Storage	Supplied By
MA1	2 tubes (per 96 samples)	-15° to -25°C	Illumina
RPM	2 tubes (per 96 samples)	-15° to -25°C	Illumina
MSM	2 tubes (per 96 samples)	-15° to -25°C	Illumina

Item	Quantity	Storage	Supplied By
0.1N NaOH	15 ml (per 96 samples)	2° to 8°C	User
MSA5 plate with restored DNA samples	1 plate	-15° to -25°C	User

CAUTION
Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

Preparation

- In preparation for the Incubate MSA5 process, preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- Thaw MA1, RPM, and MSM tubes to room temperature. Gently invert at least 10 times to mix contents.
- Thaw DNA samples to room temperature.
- In the Sample Sheet, enter the Sample_Name and Sample_Plate for each Sample_Well.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - DNA plate barcode
  - MSA5 plate barcode(s)
  - MA1 tube barcode(s)
  - RPM tube barcode(s)
  - MSM tube barcode(s)

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Steps to Make the MSA5 Plate

1 On the lab tracking form, record the original DNA sample ID for each well in the MSA5 plate.

2 Dispense 20 µl MA1 into the MSA5 plate wells containing sample.

NOTE
To ensure optimal performance, place the tips against the top edge of the wells. Use this technique for all subsequent dispensing steps.

3 Dispense 4 µl 0.1N NaOH into each well of the MSA5 plate that contains MA1 and sample.

4 Seal the MSA5 plate with a 96-well cap mat.
Orient the cap mat so that A1 on the cap matches A1 on the plate. To prevent evaporation and spills, which could lead to assay variability and cross-contamination, ensure that all 96 caps are securely seated.

5 Vortex the plate at 1600 rpm for 1 minute.

6 Pulse centrifuge to 280 xg.

7 Incubate for 10 minutes at room temperature.

8 Carefully remove the cap mat.
When you remove a cap mat, set it aside, upside down, in a safe location for use later in the protocol.

9 Dispense 68 µl RPM into each well of the MSA5 plate containing sample.

10 Dispense 75 µl MSM into each well of the MSA5 plate containing sample.

11 Reseal the MSA5 plate with the cap mat.
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

12 Vortex the sealed MSA5 plate at 1600 rpm for 1 minute.

13 Pulse centrifuge to 280 xg.

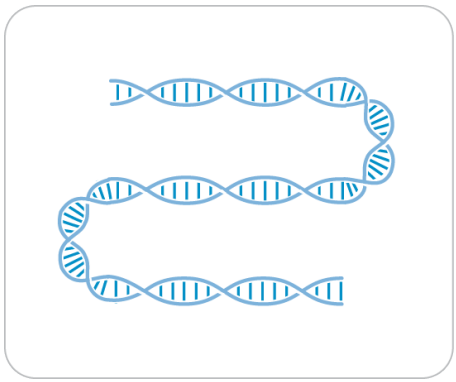
14 Discard unused reagents in accordance with facility standards.

15 Proceed immediately to *Incubate the MSA5 Plate*.

Incubate DNA (Post-Amp)

This process incubates the MSA5 plate for 20–24 hours at 37°C in the Illumina Hybridization Oven. The process uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used when in the Infinium HD FFPE Assay.

Figure 13 Incubating DNA to Amplify



Estimated Time

Incubation time: 20–24 hours

Steps to Incubate the MSA5 Plate

OVERNIGHT INCUBATION

Incubate MSA5 plate in the Illumina Hybridization Oven for at least 20 hours but no more than 24 hours at 37°C.

1 Record the start and stop times on the lab tracking form.

NOTE

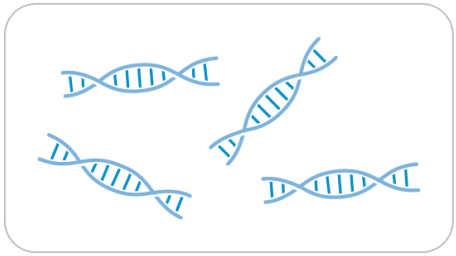
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

2 Proceed to the next step, *Fragment DNA (Post-Amp)*.

Fragment DNA (Post-Amp)

This process enzymatically fragments the amplified DNA samples. An endpoint fragmentation is used to prevent overfragmentation.

Figure 14 Fragmenting DNA



Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FMS	1 tube (per 96 samples)	-25°C to -15°C	Illumina

Preparation

1. Preheat the heat block with the midi plate insert to 37°C.
2. Thaw FMS tubes to room temperature.
3. Gently invert the FMS tubes at least 10 times to mix contents.
4. Remove the MSA5 plate from the Illumina Hybridization Oven.
5. On the lab tracking form, record:
   - Date/Time
   - Operator

- FMS tube barcodes

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Steps to Fragment the MSA5 Plate

1. Pulse centrifuge the plate to 280 × g.
2. Carefully remove the cap mat.
3. Add 50 µl FMS to each well containing sample.
4. Seal the MSA5 plate with the 96-well cap mat.

CAUTION
Orient the cap mat so that A1 on the cap matches A1 on the plate. To prevent evaporation and spills, which could lead to assay variability and cross-contamination, make sure that all 96 caps are securely seated in the wells.

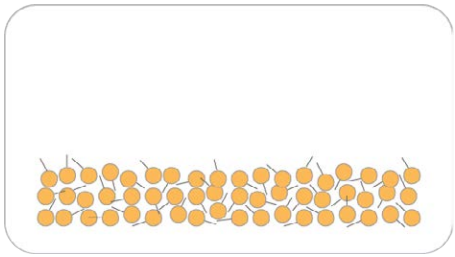
5. Vortex the plate at 1600 rpm for 1 minute.
6. Pulse centrifuge the plate to 280 × g.
7. Place the sealed plate on the 37°C heat block for 1 hour.
8. Record the start and stop times on the lab tracking form.
9. Discard unused reagents in accordance with facility standards.
10. Do either of the following:
    - Continue to the next step, *Precipitate DNA (Post-Amp)*. Leave plate in 37°C heat block until setup is complete. Do not leave the plate in the 37°C heat block for longer than 2 hours.
    - If you do not plan to proceed to the next step within the next 4 hours, store the sealed MSA5 plate at -25°C to -15°C for no more than 24 hours.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Precipitate DNA (Post-Amp)

Add PM1 and 2-propanol to the MSA5 plate to precipitate the DNA samples.

Figure 15 Precipitating DNA



Estimated Time

Hands-on time: ~30 minutes

Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	2 tubes (per 96 samples)	2°C to 8°C	Illumina
100% 2-propanol	30 ml (per 96 samples)	Room temperature	User

CAUTION
Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

Preparation

1 Do either of the following:
- If you froze the MSA5 plate after fragmentation, thaw it to room temperature, then pulse centrifuge the plate to 280 × g.

- If you proceeded immediately from *Fragment the* MSA5 *Plate*, leave the plate in the 37°C heat block until setup is complete.

2 Preheat heat block to 37°C.

3 Thaw PM1 to room temperature. Gently invert at least 10 times to mix contents.

4 Remove the 96-well cap mat.

▸ On the lab tracking form, record:

- Date/Time
- Operator
- PM1 tube barcodes
- 2-propanol lot number and date opened

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Steps to Precipitate the MSA5 Plate

1 Add 100 µl PM1 to each MSA5 plate well containing sample.

2 Seal the plate with the cap mat.

3 Vortex the plate at 1600 rpm for 1 minute.

4 Incubate at 37°C for 5 minutes.

5 Pulse centrifuge at 280 × g for 1 minute.

NOTE
Set the centrifuge at 4°C in preparation for the next centrifuge step.

6 Carefully remove the cap mat and discard it.

7 Add 300 µl 100% 2-propanol to each well containing sample.

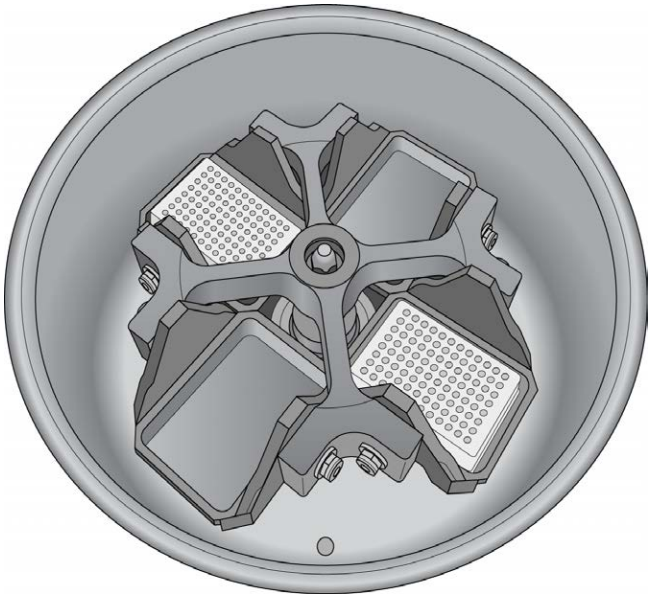
8 Carefully seal the MSA5 plate with a new, ***dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.

9 Invert the plate at least 10 times to mix contents thoroughly.

10 Incubate at 4°C for 30 minutes.

11 Place the sealed MSA5 plate in the centrifuge opposite another plate of equal weight.

Figure 16 Sealed MSA5 Plate and Plate of Equal Balance in Centrifuge



12 Centrifuge at 3000 × g at 4°C for 20 minutes. Immediately remove the MSA5 plate from centrifuge.

CAUTION
Perform the next step immediately to avoid dislodging the blue pellet. If any delay occurs, repeat the 20-minute centrifugation before proceeding.

13 Remove the cap mat and discard it.

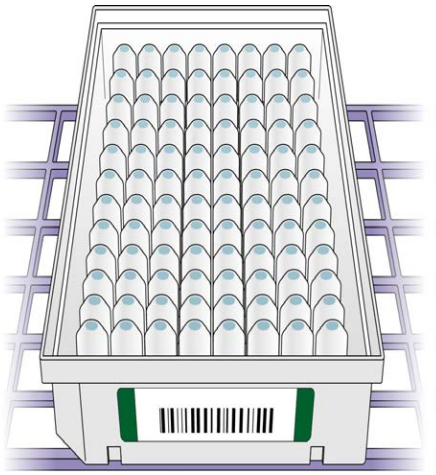
14 Quickly invert the MSA5 plate and drain the liquid onto an absorbent pad to decant the supernatant. Then smack the plate down on a dry area of the pad, avoiding the liquid that was drained onto the pad.

15 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

CAUTION
Keep the plate inverted. To ensure optimal performance, do not allow supernatant in wells to pour into other wells.

16 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
You can expect to see blue pellets at the bottoms of the wells.

Figure 17 Uncovered MSA5 Plate Inverted for Air Drying



CAUTION
Do not overdry the pellet. Pellets that are overdried are difficult to resuspend. Poorly resuspended samples lead to poor data quality.

17 Enter the start and stop times on the lab tracking form.

18 Discard unused reagents in accordance with facility standards.

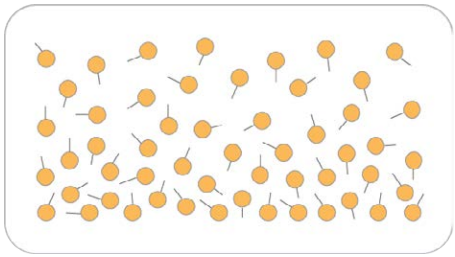
19 Do either of the following:
- Continue to the next step, *Resuspend DNA (Post-Amp).*
- If you do not plan to proceed to the next step immediately, seal the MSA5 plate with a new cap mat and store it at -25°C to -15°C.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Resuspend DNA (Post-Amp)

Add RA1 to the MSA5 plate to resuspend the precipitated DNA samples.

Figure 18 Resuspending DNA



Estimated Time

Hands-on time: ~30 minutes

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	30 µl per sample well	-25°C to -15°C	Illumina

NOTE

Pour out only the recommended volume of RA1 needed for the suggested number of samples listed in the consumables table. Additional RA1 is used later in the **XStain BeadChip** step.

WARNING

This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

1 If you stored the MSA5 plate at -25°C to -15°C, thaw it to room temperature. Remove the cap mat and discard it.

2 Preheat the Illumina Hybridization Oven to 48°C.

3 Turn on the heat sealer to preheat. Allow 20 minutes.

4 RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that can be present.

5 On the lab tracking form, record:
- Date/Time
- Operator
- RA1 bottle barcodes

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Use Fresh RA1 Reagent for Each Step

It is important to use fresh RA1 for each protocol step in the assay where it is required. RA1 that has been stored properly and has not been dispensed for use in either the XStain or Resuspension step is considered fresh RA1. After RA1 has been poured out into a reservoir and exposed to room temperature air for extended periods of time, it is no longer fresh.

To make best use of RA1, only pour out the amount needed for the current step. If you plan to perform additional assay steps requiring RA1 that same day, then leave the remaining thawed reagent in the original, closed bottle at room temperature until it is needed. Otherwise, follow the standard RA1 storage procedures described in this assay guide for next-day processing and prolonged storage conditions.

Steps to Resuspend the MSA5 Plate

1 Add 30 µl RA1 to each well of the MSA5 plate containing a DNA pellet. Reserve any leftover reagent for the Hybridization and XStain steps.

2 Apply a foil heat seal to the MSA5 plate by firmly and evenly holding the heat sealer sealing block down for 3 seconds.

3 Immediately remove the MSA5 plate from the heat sealer and forcefully roll the rubber plate sealer over the plate until you can see all 96 well indentations through the foil. Repeat application of the heat sealer if all 96 wells are not defined.

4 Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.

5 Vortex the plate at 1800 rpm for 1 minute.

6 Pulse centrifuge to 280 × g.

NOTE
If you store the pellets at -25°C to -15°C for extended periods of time after the precipitate process, you might need to repeat the vortexing and centrifugation in the previous steps until the pellets are resuspended.

7 Discard unused reagents in accordance with facility standards.

8 Do either of the following:

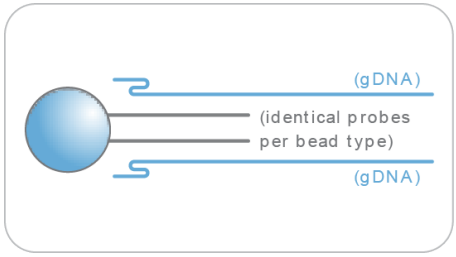
- Continue to the next step, *Hybridize to BeadChip (Post-Amp)*. If you plan to do so immediately, it is safe to leave the MSA5 plate at room temperature for up to 1 hour.
- If you do not plan to proceed to the next step immediately, store the sealed MSA5 plate at -25°C to -15°C for no more than 24 hours. Store RA1 at -25°C to -15°C.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Hybridize to BeadChip (Post-Amp)

In this process, you dispense the fragmented and resuspended DNA samples onto BeadChips. Place the DNA-loaded BeadChips into the Hyb Chamber inserts, and then place the inserts into the Hyb Chambers. Incubate the Hyb Chambers in the Illumina Hybridization Oven for 16–24 hours at 48°C.

Figure 19 Hybridize to BeadChip



Estimated Time

Hands-on time:

- 12x1 HD BeadChip: ~40 minutes for 8 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	2 tubes	Room temperature	Illumina
BeadChips	8		Illumina
Hyb Chambers	2		Illumina

Item	Quantity (per 96 Samples)	Storage	Supplied By
Hyb Chamber gaskets	2		Illumina
Hyb Chamber inserts	8		Illumina
EtOH	330 ml		User

CAUTION
Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

Preparation

1 If frozen, thaw MSA5 plate to room temperature, and then pulse centrifuge the MSA5 plate to 280 × g.

2 Preheat the heat block to 95°C.

3 Prepare the Illumina Hybridization Oven as follows:
   a Preheat the oven to 48°C:
      — Press the "F" button one time to change the display to **TSET**.
      — Press the "S" button to enter the set-temperature mode, and then use the Increment/Decrement dial to set the oven to 48°C.
      — Press the "S" button again to set 48°C as the temperature.
   b Set the rocker speed to 5:
      — Press the "F" button twice until **SPd** is indicated on the display.
      — Press the "S" button to enter the rocker speed mode.
      — Use the Increment/Decrement dial to set the rocker speed to "5".
      — Press the "S" button again.

4 Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.

5 On the lab tracking form, record:
   • Date/Time
   • Operator

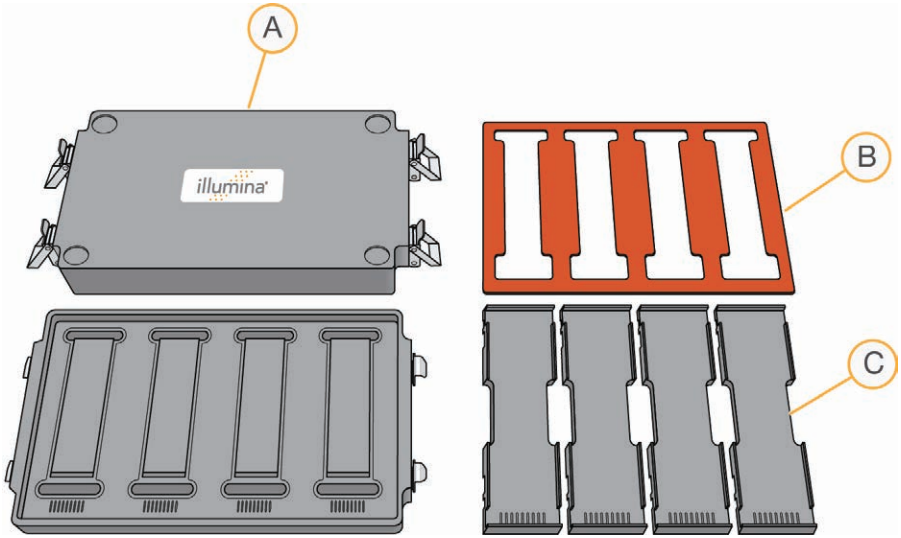
- PB2 tube lot number

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Assemble the Hybridization Chambers

1. Place the resuspended MSA5 plate on the heat block to denature the samples at 95°C for 20 minutes.
2. Remove the BeadChips from 2°C to 8°C storage, leaving the BeadChips in their plastic bags and mylar packages until you are ready to begin hybridization.
3. During the 20-minute incubation, prepare the Hyb Chambers.
   Place the following items on the benchtop for use in this procedure:

Figure 20 BeadChip Hyb Chamber Components



A BeadChip Hyb Chambers
B Hyb Chamber Gaskets

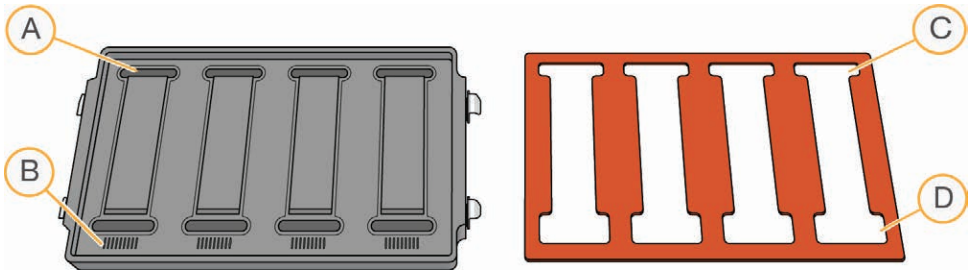
C Hyb Chamber Inserts

NOTE
To ensure optimal results from Hyb Chambers, keep the Hyb Chamber lids and bases together. Adopt a labeling convention that keeps each Hyb Chamber base paired with its original lid. Check Hyb Chamber lid-base pairs regularly to make sure that the fit remains secure. Check hinges regularly for any signs of abnormal wear or loose fittings. It is important that the hinges provide adequate clamping strength to ensure an airtight seal between the lid and the base. Record the Hyb Chamber that was used for each BeadChip, so that Hyb Chambers can be investigated and evaluated in the event of sample evaporation or other lab processing anomalies.

a Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers.

— Match the wider edge of the Hyb Chamber Gasket to the barcode-ridge side of the Hyb Chamber.

Figure 21 Hyb Chamber and Gasket



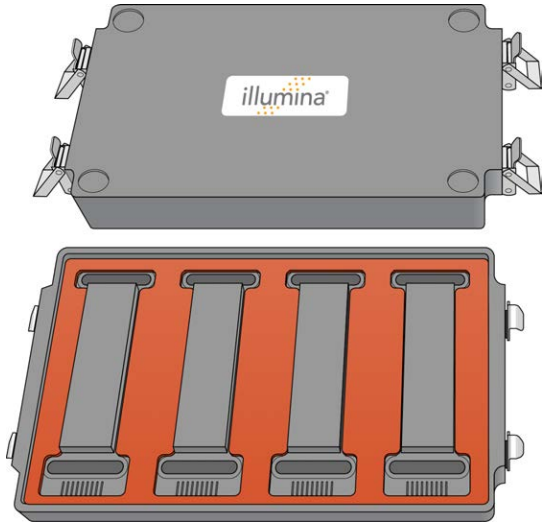
A Reservoirs
B Barcode Ridges
C Narrower Edges
D Wider Edges

— Lay the gasket into the Hyb Chamber, and then press it down all around.

Figure 22 Placing Gasket into Hyb Chamber

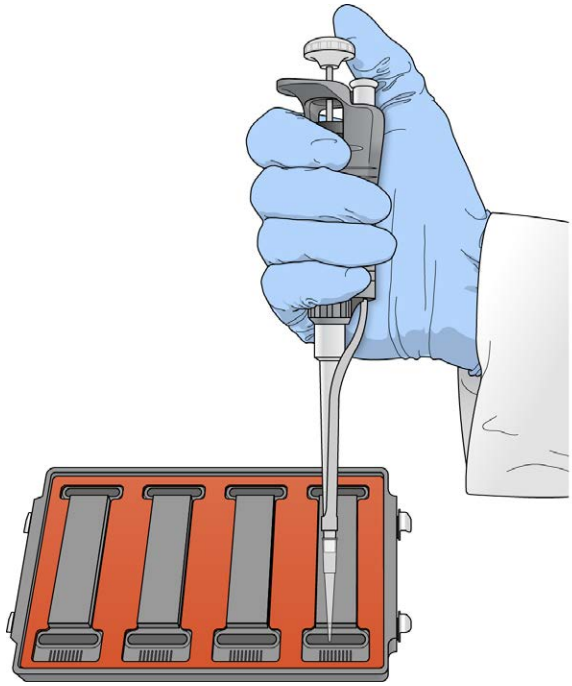
— Make sure that the Hyb Chamber gaskets are properly seated.

Figure 23 Hyb Chamber with Gasket in Place



b Dispense 400 µl PB2 into the humidifying buffer reservoirs in the Hyb Chambers.

Figure 24 Dispensing PB2 into Hyb Chamber Reservoir



WARNING

Do not replace PB2 in the Hyb Chamber with RA1. RA1 decreases the stringency and can negatively affect sample call rates and logRdev. PB2 is formulated to produce the appropriate amount of humidity within the Hyb Chamber environment to prevent sample from evaporating during hybridization.

c After you fill the Hyb Chamber reservoirs with PB2, place the lid on the Hyb Chamber right away to prevent evaporation. It is not necessary to lock the lid.

d Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample. Load BeadChips into the Hyb Chamber within 1 hour.

NOTE

You can also prepare the Hyb Chambers later, during the 30-minute cool down.

4 After the 20-minute incubation, remove the MSA5 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.

5 After the 30-minute cool down, pulse centrifuge the MSA5 plate to 280 × g. Remove the foil seal.

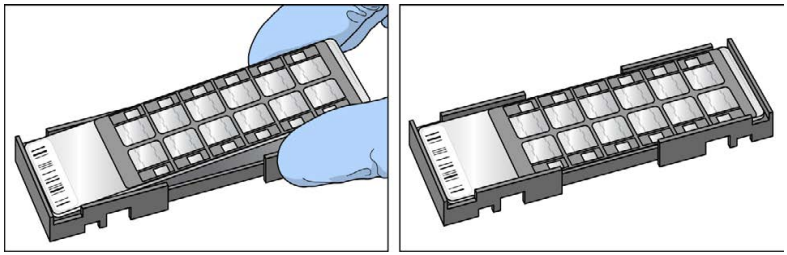
Load BeadChip

1 Just before loading DNA samples, remove all BeadChips from their ziplock bags and mylar packages.

CAUTION
Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Do not hold the BeadChip by the sides near the sample inlets. Avoid contacting the beadstripe area and sample inlets.

2 Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the Hyb Chamber insert.

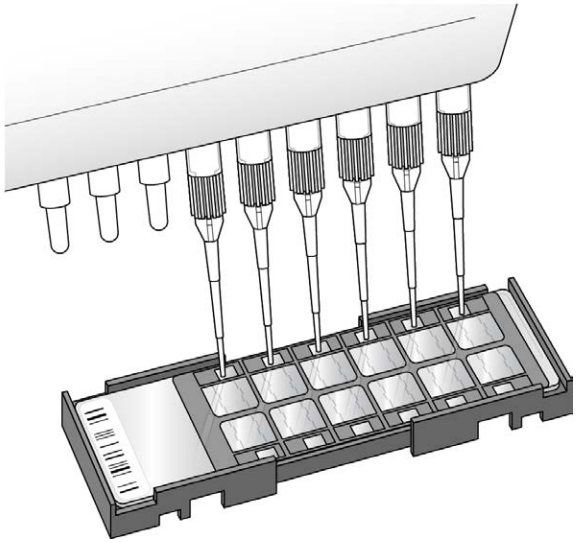
Figure 25 Placing BeadChips into Hyb Chamber Inserts



NOTE
For an alternative DNA loading protocol, see *Load BeadChip (Alternate Method)* at the end of the *Hybridize Multi BeadChip* section.

3 Using a multi-channel precision pipette, dispense 15 µl of each DNA sample onto the appropriate BeadChip section.

Figure 26 Loading Samples



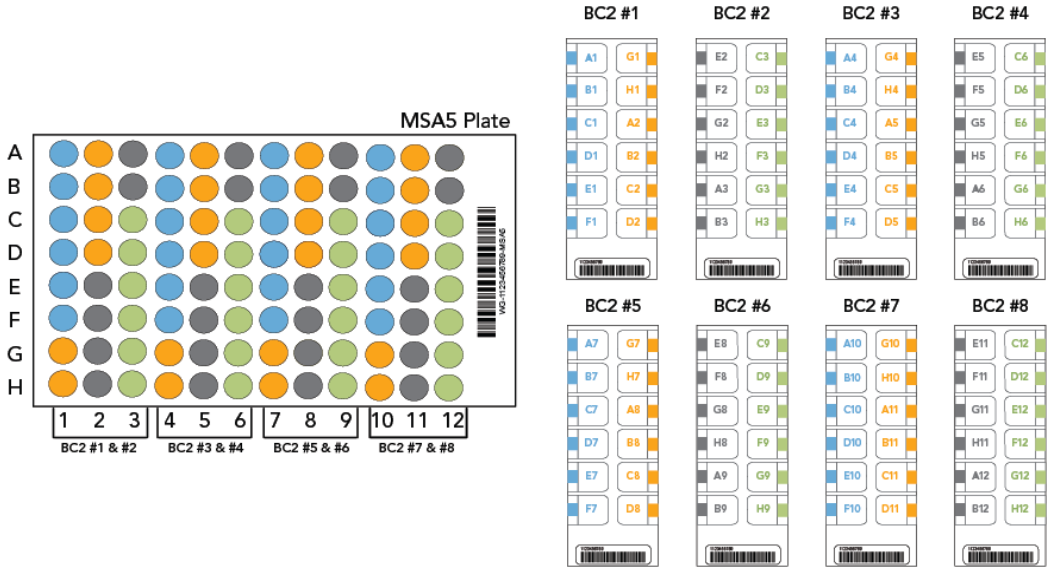
a Load samples A1–F1 from the MSA5 plate into the left side of the BeadChip. Make sure that the pipette tip is in the sample inlet prior to dispensing.

b Load samples in G1 and H1 from the MSA5 plate into the top two inlets of the right side of the BeadChip.

c Load samples A2–D2 into the remaining four inlets on the right side of the BeadChip.

d Continue in this manner, following the color-coded sections shown below, until all samples are loaded.

Figure 27 Distributing Sample in MSA5 Plate



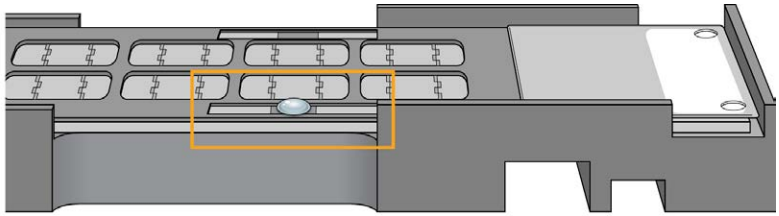
4 On the lab tracking form, record the BeadChip barcode for each group of samples.

5 In the sample sheet's Sentrix ID column, record the sample ID and position on the BeadChip. Please see the Sample Section Naming Diagram in the Lab Tracking Form.

6 After loading all DNA onto the BeadChip, wait for the sample to disperse over the entire surface.

7 Inspect the loading port to see if a large bolus of liquid remains.
Excess sample volume in the BeadChip loading port helps prevent low-intensity areas resulting from evaporation.

Figure 28 Bolus of Liquid at Loading Port



If no excess liquid is visible, it is acceptable to add additional sample from the leftover volume in the amplification plate until there is a large bolus around the loading port.

NOTE
Do not top off with RA1 (sample hyb buffer) as this will dilute the sample.

8 Record the top-off activity on the lab tracking form.

9 Heat-seal any residual sample in the MSA5 plate with foil, and store at -25°C to -15°C. Store at -80°C if you do not plan to use the sample again within 24 hours.

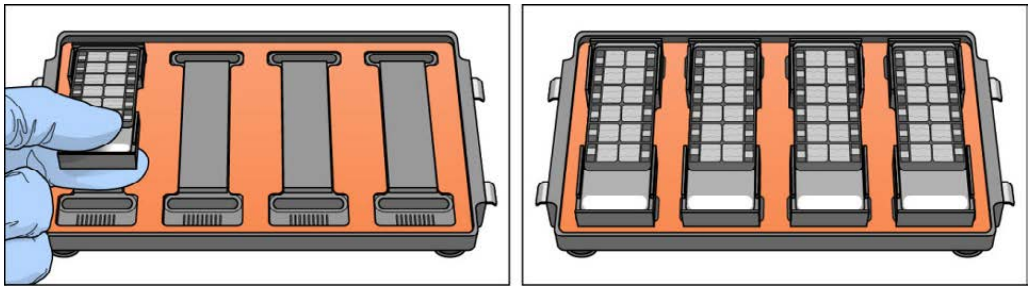
Set up BeadChip for Hybridization

CAUTION
For optimal performance, take care to keep the Hyb Chamber inserts containing BeadChips steady and level when lifting or moving. Avoid shaking and always keep parallel to the lab bench. Do not hold by the sides near the sample inlets.

1 Load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chamber. Position the barcode end over the ridges indicated on the Hyb Chamber.

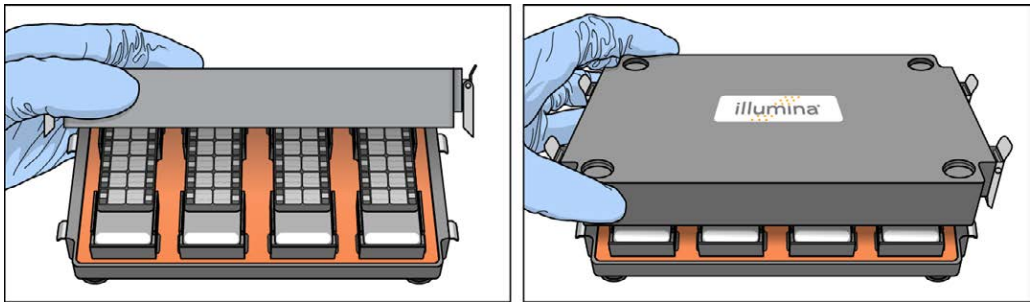
WARNING
Keep Hyb Chambers at room temperature when you load the BeadChips. Do not place the Hyb Chambers in the Illumina Hybridization Oven before loading the BeadChips. If you heat the PB2 and then open the Hyb Chamber to add BeadChips, some of the PB2 evaporates, leading to a change in the osmolality of PB2 and an imbalance in the vapor pressure between PB2 and RA1.

Figure 29 Placing Hyb Chamber Inserts into the Hyb Chamber



2 Place the back side of lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts.

Figure 30 Seating Lid onto Hyb Chamber



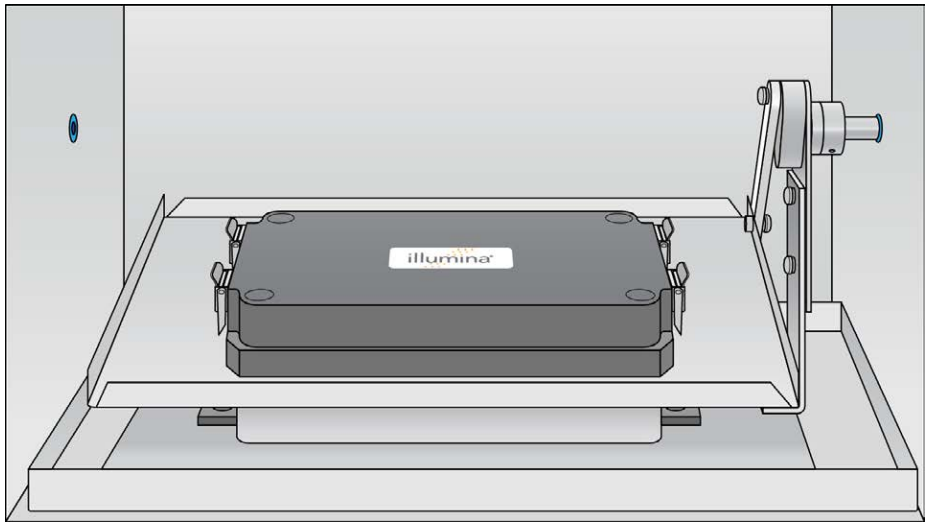
3 Close the clamps on both sides of the Hyb Chamber so that the lid is secure and even on the base (no gaps). It is best to close the clamps in a kitty-corner fashion, closing first the top left clamp, then the bottom right, then the top right followed by the bottom left.

NOTE
Keep the Hyb Chamber steady and level when moving it or transferring it to the Illumina Hybridization Oven.

4 Place the Hyb Chamber in the 48°C Illumina Hybridization Oven with the clamps on the left and right sides of the oven and the Illumina logo facing you.

CAUTION
After loading the BeadChips into the Hyb Chambers, place the Hyb Chambers into the Illumina Hybridization Oven immediately. Do not modify the hybridization environment by adding additional fixtures or humidifying elements. Leave the Hyb Chambers in the oven at the correct orientation and temperature until hybridization is complete. Changes to the hybridization environment can have unexpected effects on data quality.

Figure 31 Hyb Chamber Correctly Placed in Hyb Oven



NOTE

If you are stacking multiple Hyb Chambers in the Illumina Hybridization Oven, fit the feet of the top Hyb Chamber into the matching indents on the lid of the Hyb Chamber below it. The fitted feet and lid hold the Hyb Chambers in place while they are rocking. You can stack up to 3 Hyb Chambers per row for a maximum of 6 Hyb Chambers total in the Illumina Hybridization Oven.

Figure 32 Two Hyb Chambers Correctly Placed in Hyb Oven

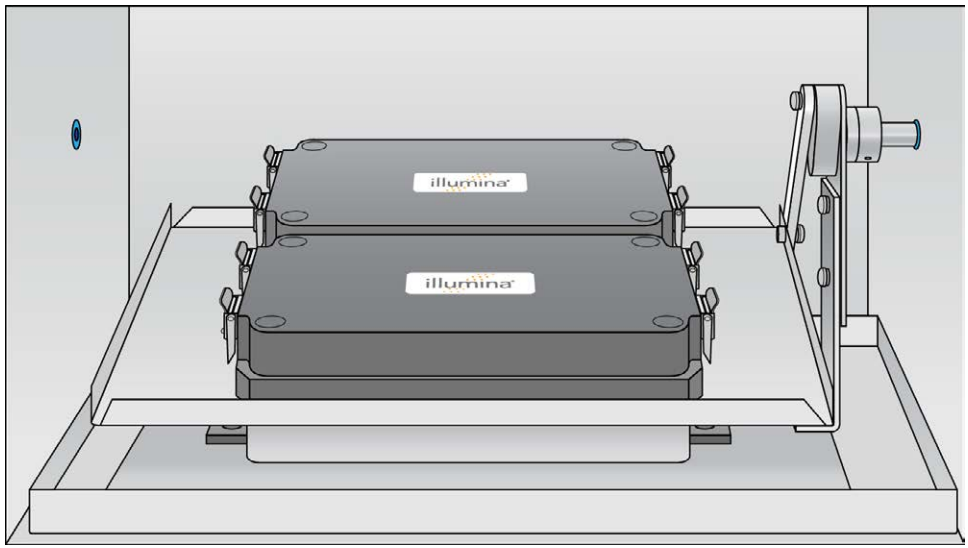
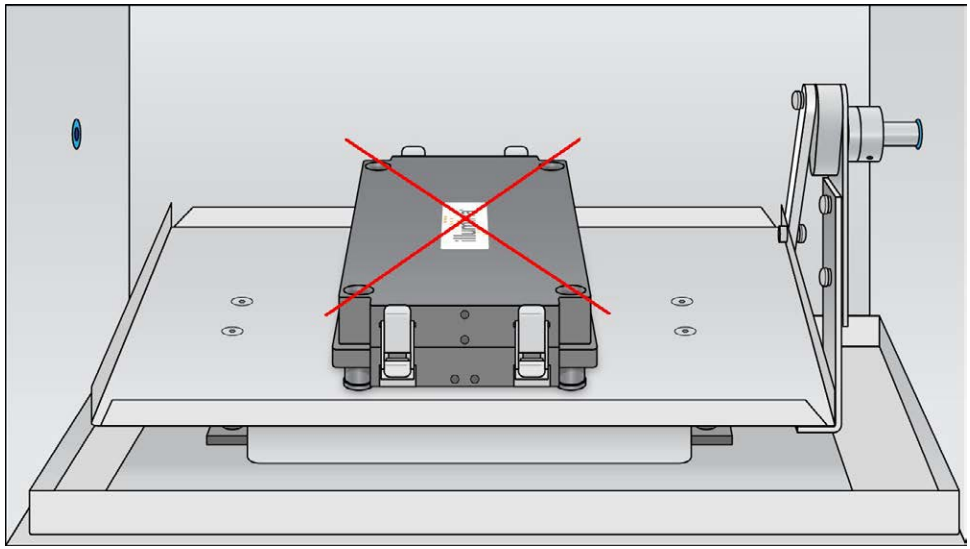


Figure 33 Incorrectly Placed Hyb Chamber



5 [Optional] Start the rocker, setting the speed to 5.

OVERNIGHT INCUBATION
Incubate at 48°C for at least 16 hours but no more than 24 hours.

6 Enter the start and stop times on the lab tracking form.

7 Place RA1 into the freezer at -25°C to -15°C for use the next day.

8 Proceed to *Wash BeadChips (Post-Amp)* after the overnight incubation.

Resuspend XC4 Reagent for XStain BeadChip

Keep the XC4 in the bottle in which it was shipped until you are ready to use it. In preparation for the XStain protocol, follow these steps to resuspend the XC4 reagent.

1 Add 330 ml 100% EtOH to the XC4 bottle, for a final volume of 350 ml.
Each XC4 bottle has enough solution to process up to 24 BeadChips.

2 Shake the XC4 bottle vigorously to ensure complete resuspension. After it is resuspended, use XC4 at room temperature.
You can store it at 2°C to 8°C for 2 weeks if unused.

Load BeadChip (Alternate Method)

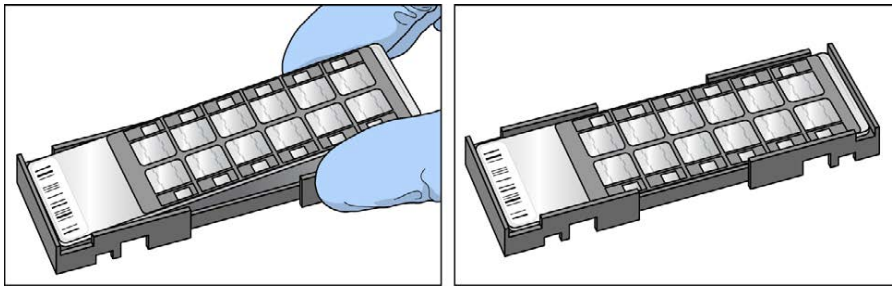
This section describes an alternative method of manually loading the DNA by rows instead of columns from the MSA5 plate. For the original method of loading DNA samples, see *Load BeadChip* on page 43.

1 Just before loading DNA samples, remove all BeadChips from their ziplock bags and mylar packages.

CAUTION
Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Do not hold the BeadChip by the sides near the sample inlets. Avoid contacting the beadstripe area and sample inlets.

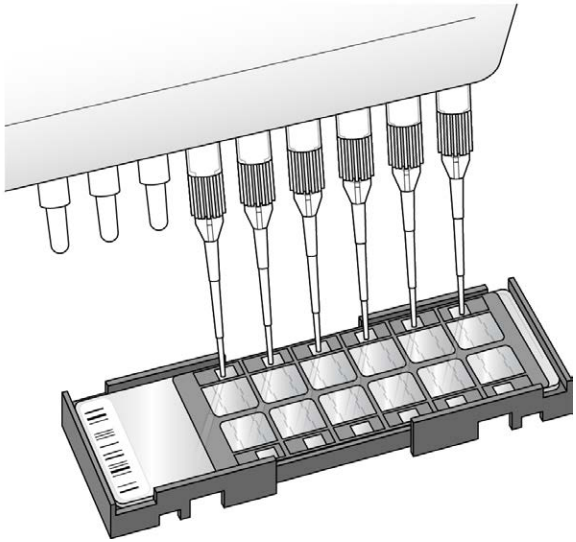
2 Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the Hyb Chamber insert. Repeat for each BeadChip.

Figure 34 Placing BeadChips into Hyb Chamber Inserts



3 Using a multi-channel precision pipette, dispense 15 µl of each DNA sample onto the appropriate BeadChip section.

Figure 35 Loading Samples



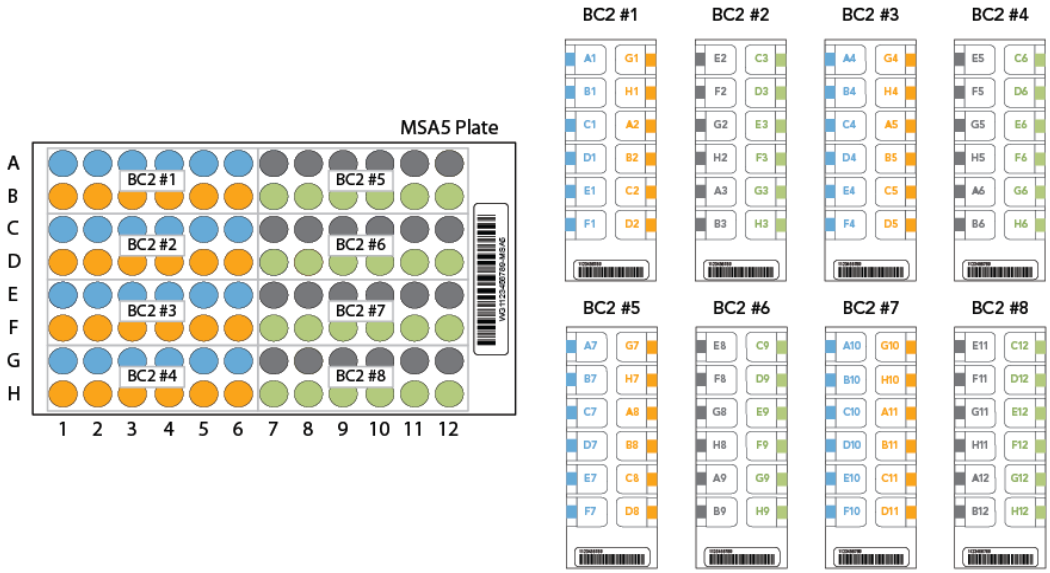
a Load samples A1–A6 from the MSA5 plate to the left side of the BeadChip. Make sure that the pipette tip is in the sample inlet prior to dispensing.

b Load samples B1–B6 from the MSA5 plate to the right side of the BeadChip.

c Continue in this manner for Rows C through H.

d When you finish the left half of the plate, move to the right half of the plate. Load samples A7–A12 to the left side of the BeadChip and samples B7–B12 to the right side of the BeadChip. Continue in this manner for rows C through H.

Figure 36 Alternate Distributing Sample in MSA5 Plate



4 On the lab tracking form, record the BeadChip barcode for each group of samples.

5 In the sample sheet, record the sample ID and position on the BeadChip in the Sentrix ID column. Please see the Sample Section Naming Diagram in the Lab Tracking Form.

6 Visually inspect all sections of the BeadChips to ensure the DNA sample covers all of the sections of each bead stripe. Record any sections that are not completely covered on the lab tracking form.

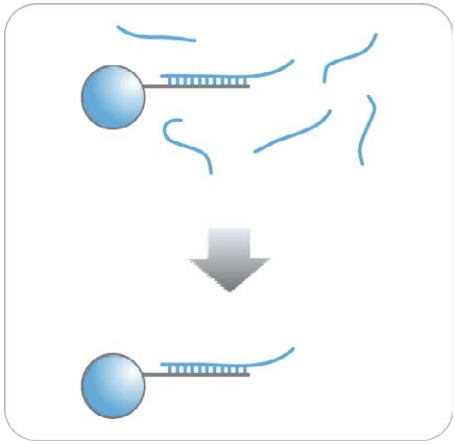
7 Heat-seal residual sample in the MSA5 plate with foil and store at -15 to -25°C. Store at -80°C if you do not plan to use it again within 24 hours.

Wash BeadChips (Post-Amp)

In this procedure, the BeadChips are prepared for the XStain process.

Remove the cover seals from the BeadChips and wash the BeadChips in 2 separate PB1 reagent washes. Then, assemble the BeadChips into flow-through chambers under the PB1 buffer.

Figure 37 Washing BeadChip



Estimated Time

- 20 minutes for 4 BeadChips
- 30 minutes for 8 BeadChips

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml for 1 alignment fixture 700 ml for 2 alignment fixtures 850 ml for 3 alignment fixtures	Room temperature	Illumina
Multisample BeadChip alignment fixture	1 (per 8 BeadChips)		Illumina
Te-Flow flow-through chambers, with black frames, spacers, glass back plates, and clamps	1 (per BeadChip)		Illumina
Wash dish	2 (up to 8 BeadChips)		Illumina
Wash rack	1 (up to 8 BeadChips)		Illumina

CAUTION

Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

WARNING

This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

1 Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 30 minutes before opening.

2 Have ready on the lab bench:
   a Two wash dishes:
      — Containing 200 ml PB1, and labeled as such
   b Multi-Sample BeadChip Alignment Fixture
      — Using a graduated cylinder, fill with 150 ml PB1
   c Te-Flow flow-through chamber components:
      — Black frames
      — Spacers (separated for ease of handling)
      — Clean glass back plates as directed in the *Infinium Lab Setup and Procedures Guide*.
      — Clamps

3 On the lab tracking form, record:
   - Date/Time
   - Operator
   - PB1 bottle barcode

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Steps to Wash BeadChips

1 Attach the wire handle to the rack and submerge the wash rack in the wash dish containing 200 ml PB1.

Figure 38 Wash Rack in Wash Dish Containing PB1

2 Remove the Hyb Chamber inserts from the Hyb Chambers.

3 Remove each BeadChip from the Hyb Chamber insert.

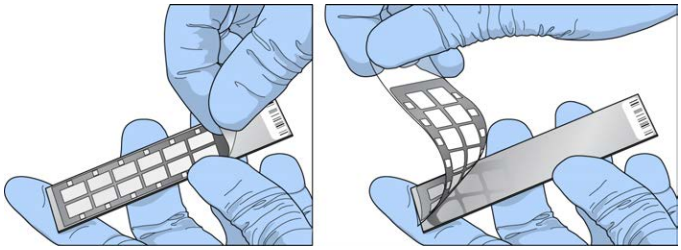
4 Remove the cover seal from each BeadChip.

NOTE
To make sure that no solution splatters on you, Illumina recommends removing the cover seal over an absorbent cloth or paper towels, preferably in a hood.

a Using powder-free gloved hands, hold the BeadChip securely and by the edges in one hand. Avoid contact with the sample inlets. Make sure that the barcode is facing up and closest to you, and that the top side of the BeadChip is angled slightly away from you.

b Remove the entire seal in a single, continuous motion. Start with a corner on the barcode end and pull with a continuous upward motion away from you and towards the opposite corner on the top side of the BeadChip.

Figure 39 Removing the Cover Seal



c Discard the cover seal.

CAUTION
Do not touch the arrays!

5 Immediately and carefully slide each BeadChip into the wash rack, making sure that the BeadChip is submerged in the PB1.

Figure 40 Submerging BeadChips in Wash Dish Containing PB1

6 Repeat steps 4 through 5 until all BeadChips (a maximum of 8) are transferred to the submerged wash rack.

7 After all BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

8 Move the wash rack to the other wash dish containing clean PB1. Make sure the BeadChips are submerged.

9 Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

10 When you remove the BeadChips from the wash rack, inspect them for remaining residue.

NOTE
Residue that can adversely affect results is sometimes left on BeadChips after seals are removed. If there is residue left on the BeadChips after the second PB1 wash, use a 200 µl pipette tip for each BeadChip and slowly and carefully scrape off the residues outward (away) from the bead-sections under PB1. Use a new pipette tip for each BeadChip. Then, continue with the protocol.

11 For each additional set of 8 BeadChips:

a Assemble the flow-through chambers for the first 8 BeadChips, as described in *Assemble Flow-Through Chambers* on page 58.

b Repeat the wash steps in this section to wash the next set of 8 BeadChips.

Assemble Flow-Through Chambers

1 If you have not done so, fill the BeadChip alignment fixture with 150 ml PB1.
If you plan to process more than 4 BeadChips, this 150 ml of PB1 can be reused for an additional set of 4 BeadChips. Use 150 ml of fresh PB1 for every additional set of 8 BeadChips.

2 For each BeadChip to be processed, place a black frame into the BeadChip alignment fixture prefilled with PB1.

Figure 41 Placing Black Frames into BeadChip Alignment Fixture

3 Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the alignment fixture.

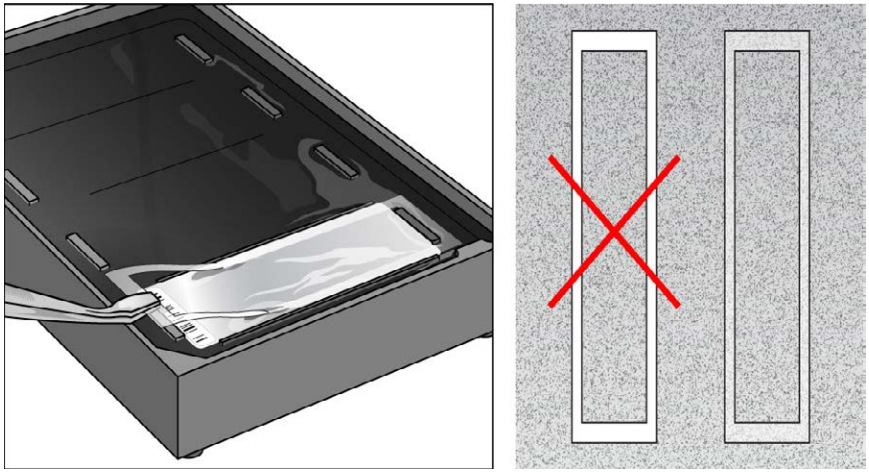
NOTE
Inspect the surface of each BeadChip for residue left by the seal. Use a pipette tip to remove any residue under buffer and be careful not to scratch the bead area.

Figure 42 Placing BeadChip into Black Frame on Alignment Fixture

4 Place a clear spacer onto the top of each BeadChip. Use the alignment fixture grooves to guide the spacers into proper position.

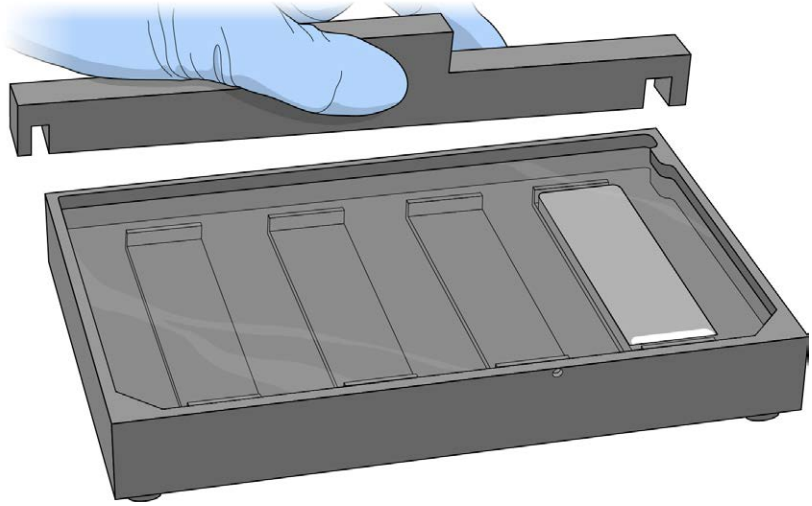
NOTE
Be sure to use the clear plastic spacers, not the white ones.

Figure 43 Placing Clear Plastic Spacer onto BeadChip



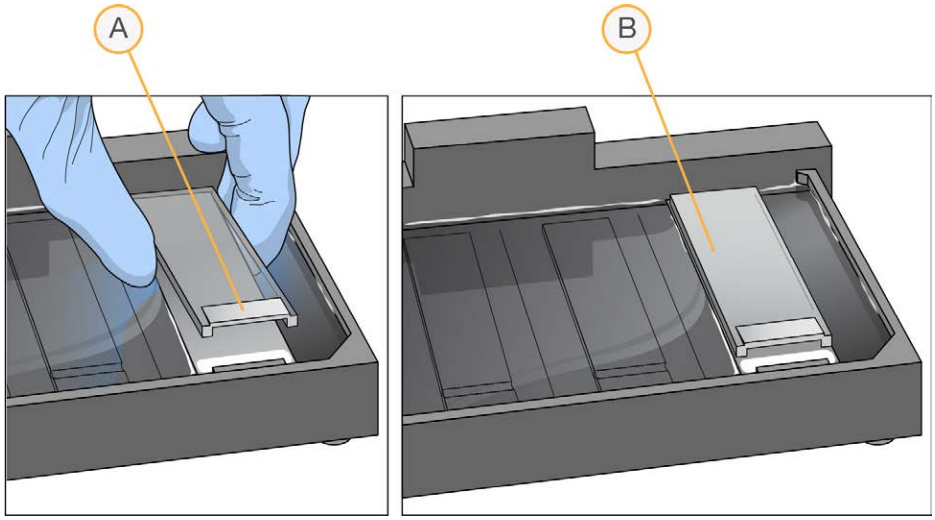
5 Place the alignment bar onto the alignment fixture.
The groove in the alignment bar fits over the tab on the alignment fixture.

Figure 44 Placing Alignment Bar onto Alignment Fixture



6 Place a clean glass back plate on top of the clear spacer covering each BeadChip. The plate reservoir is at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface.

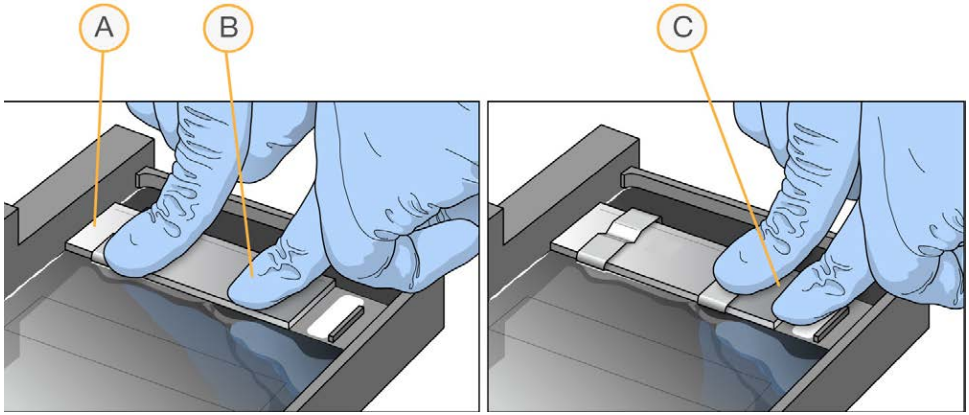
Figure 45 Placing Glass Back Plate onto BeadChip



A Reservoir at Barcode End of Glass Back Plate
B Glass Back Plate in Position

7 Attach the metal clamps to the flow-through chambers as follows:
   a Gently push the glass back plate against the alignment bar with one finger.
   b Place the first metal clamp around the flow-through chamber so that the clamp is approximately 5 mm from the top edge.
   c Place the second metal clamp around the flow-through chamber at the barcode end, approximately 5 mm from the reagent reservoir.

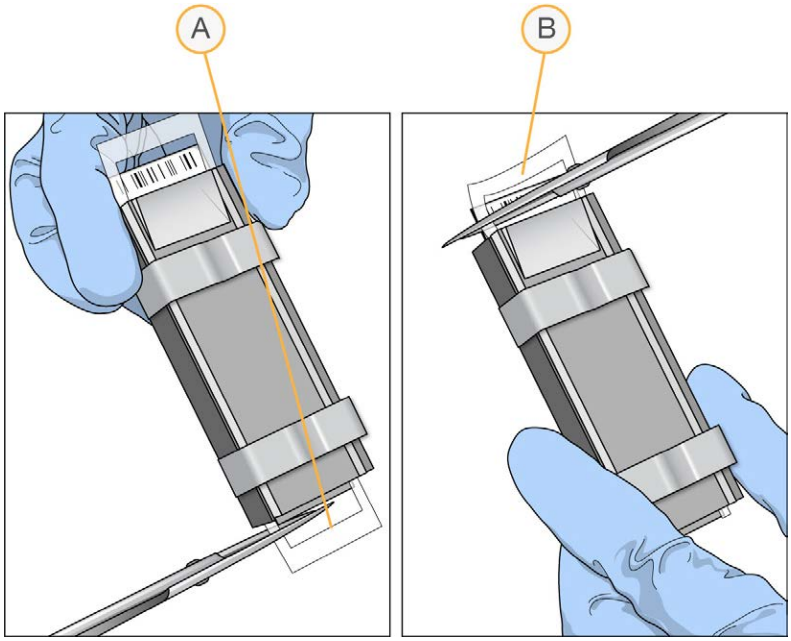
Figure 46 Securing Flow-Through Chamber Assembly with Metal Clamps



A One Stripe Shows Between First Clamp and Alignment Bar
B Glass Back Plate Pressed Against Alignment Bar
C No Stripes Show Between Second Clamp and Barcode

8 Using scissors, trim the ends of the clear plastic spacers from the flow-through chamber assembly. Slip scissors up over the barcode to trim the other end.

Figure 47 Trimming Spacer Ends from Flow-Through Chamber Assembly



A Trim Spacer at Non-Barcode End of Flow-Through Chamber
B Trim Spacer at Barcode End of Flow-Through Chamber

9 ***Immediately*** wash the Hyb Chamber reservoirs with DiH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains in the Hyb Chamber reservoir.

CAUTION
It is important to wash the Hyb Chamber reservoirs immediately and thoroughly to make sure that no traces of PB2 remain in the wells.

10 Discard unused reagents in accordance with facility standards.

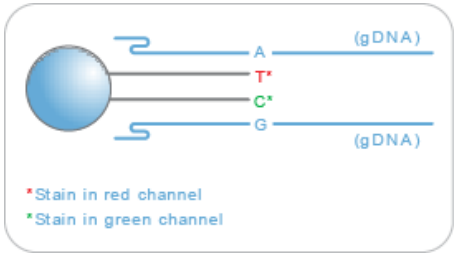
11 Proceed to the next step, *Extend and Stain (XStain) BeadChip (Post-Amp)*.

CAUTION
Place all assembled flow-through chambers on the lab bench in a horizontal position while you perform the preparation steps for XStain BeadChip. Do not place the flow-through chambers in the chamber rack until all necessary steps are completed.

Extend and Stain (XStain) BeadChip

In this process, you use RA1 reagent to wash away unhybridized and non-specifically hybridized DNA sample. Dispense TEM reagent into the Flow-Through Chambers to extend the primers hybridized to DNA on the BeadChip. This reaction incorporates labeled nucleotides into the extended primers. 95% formamide/1 mM EDTA is added to remove the hybridized DNA. After neutralization using the XC3 reagent, the labeled extended primers undergo a multi-layer staining process on the Chamber Rack. Next, you disassemble the Flow-Through Chambers and wash the BeadChips in the PB1 reagent, coat them with XC4, and then dry them.

Figure 48 Extending and Staining BeadChip



Estimated Time

Hands-on time: ~3 hours for 8 BeadChips

Dry time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	10 ml for 1-8 BeadChips 20 ml for 9-16 BeadChips 30 ml for 17-24 BeadChips	-25°C to -15°C	Illumina

Item	Quantity	Storage	Supplied By
XC1	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
XC2	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
TEM	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
XC3	50 ml for 1-8 BeadChips 100 ml for 9-16 BeadChips 150 ml for 17-24 BeadChips	Room temperature	Illumina
STM (Make sure that all STM tubes indicate the same stain temperature on the label)	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
ATM	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
PB1	310 ml for 1-8 BeadChips 285 ml for 9-24 BeadChips	Room temperature	Illumina
XC4	310 ml for 1-8 BeadChips 285 ml for 9-24 BeadChips	Room temperature	Illumina
Alconox Powder Detergent	as needed	Room temperature	User
EtOH	as needed	Room temperature	User

Item	Quantity	Storage	Supplied By
95% formamide/1 mM EDTA	15 ml for 1-8 BeadChips 17 ml for 9-16 BeadChips 25 ml for 17-24 BeadChips	-25°C to -15°C	User

CAUTION

Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

NOTE

It is important to use fresh RA1 for each protocol step in the assay where it is required. RA1 that has been stored properly and has not been dispensed for use in either the XStain or Resuspension step is considered fresh RA1. After RA1 has been poured out into a reservoir and exposed to room temperature air for extended periods of time, it is no longer fresh.

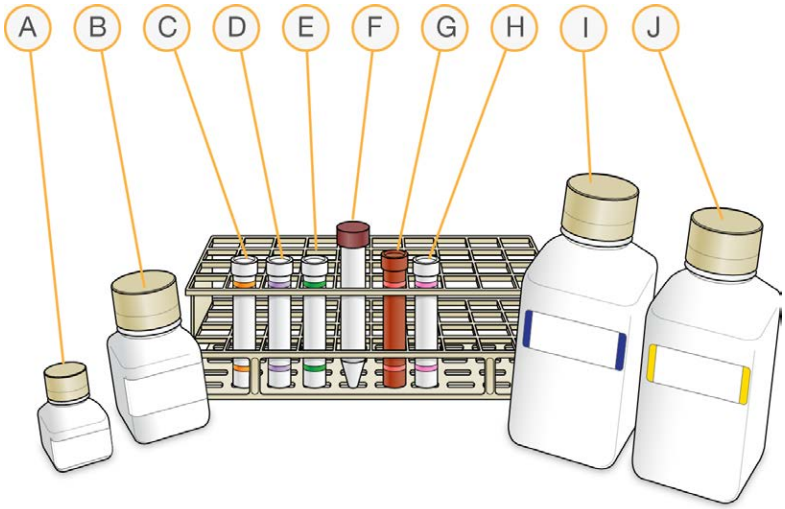
WARNING

This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

1. RA1 is shipped and stored at -25°C to -15°C. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that may be present.
2. Place all reagent tubes in a rack in the order in which they are used. If frozen, allow them to thaw to room temperature, and then gently invert the reagent tubes at least 10 times to mix contents.

Figure 49 XStain BeadChip Reagent Tubes and Bottles



A RA1
B XC3
C XC1
D XC2
E TEM
F 95% Formamide / 1mM EDTA
G STM
H ATM
I PB1
J XC4

3 Make sure that the water circulator is filled to the appropriate level.

4 Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.

5 Remove bubbles trapped in the Chamber Rack.

6 Test several locations on the Chamber Rack, using the Illumina Temperature Probe. All locations should be at 44°C ± 0.5°C.

7 Dispense all bottled reagents into disposable reservoirs, as they are needed.

8 On the lab tracking form, record:

- Date/Time
- Operator
- RA1 barcode
- XC3 barcode
- XC1 barcodes
- XC2 barcodes
- TEM barcodes
- STM barcodes
- ATM barcodes
- PB1 barcode
- XC4 barcodes

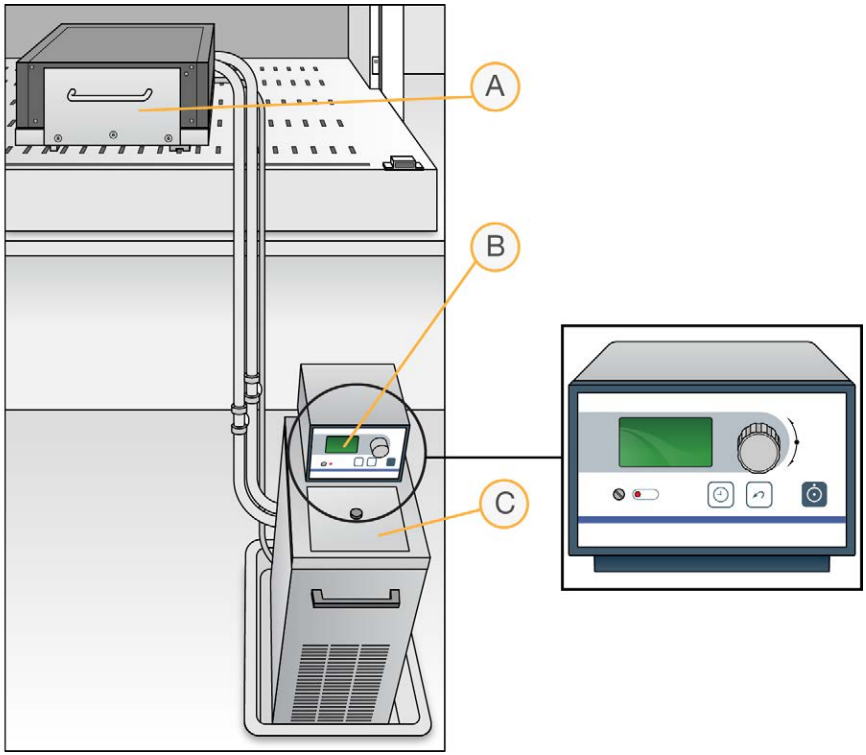
NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Set Up Chamber Rack

1. Make sure that the water circulator reservoir is filled with water to the appropriate level. See the *VWR Operator Manual*, VWR part # 110-229.
2. Turn on the water circulator and set it to a temperature that brings the chamber rack to 44°C at equilibrium.
   This temperature can vary depending on facility ambient conditions.

Figure 50 Water Circulator Connected to Chamber Rack



A Chamber Rack
B Water Circulator with Programmable Temperature Controls
C Reservoir Cover

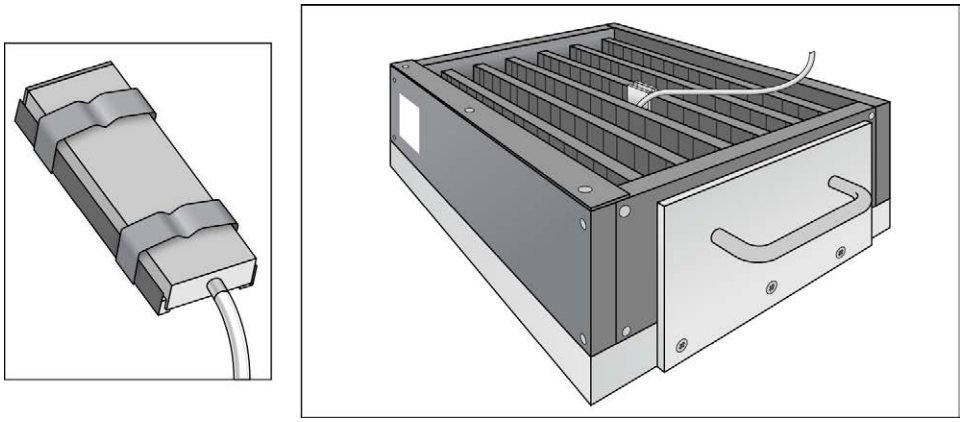
3 Confirm the temperature using the temperature probe for the chamber rack. The temperature displayed on the water circulator LCD screen might differ from the actual temperature on the chamber rack.

4 Make sure that you remove bubbles trapped in the chamber rack ***each time*** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.

5 Use the Illumina Temperature Probe in several locations to make sure that the chamber rack is at 44°C. Make sure that all locations are at 44°C ± 0.5°C.

NOTE
Do not leave the temperature probe in the first 3 rows of the chamber rack. Reserve this space for BeadChips.

Figure 51 Illumina Temperature Probe and Temperature Probe in Chamber Rack



6 For accurate temperature measurement, make sure that the Illumina Temperature Probe is touching the base of the chamber rack.

Single-Base Extension

CAUTION
The remaining steps must be performed without interruption.

1 When the Chamber Rack reaches 44°C, quickly place each Flow-Through Chamber assembly into the Chamber Rack.
For 4 BeadChips, place the Flow-Through Chambers in every other position, starting at 1, in the first row of the Chamber Rack. For larger numbers of BeadChips, fill all positions in the first row, then the second and third.

2 Make sure that each Flow-Through Chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.

3 On the lab tracking form, record the Chamber Rack position for each BeadChip.

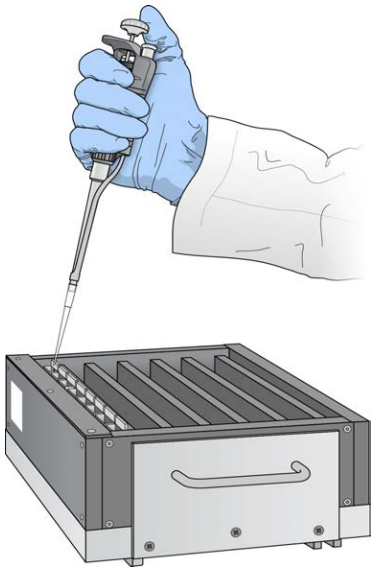
4 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

CAUTION
Do not allow pipette tips to contact BeadChip surface. Touch off in the reservoir of the glass back plate.

5 Into the reservoir of each Flow-Through Chamber, dispense:

a 150 µl RA1. Incubate for 30 seconds. Repeat 5 times.

Figure 52 Dispensing RA1 into Each Flow-Through Chamber



CAUTION
Pipette tip must not contact BeadChip surface.

b 450 µl XC1. Incubate for 10 minutes.

c 450 µl XC2. Incubate for 10 minutes.

d 200 µl TEM. Incubate for 15 minutes.

e 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat one time.

f Incubate 5 minutes.

g Begin ramping the Chamber Rack temperature to the temperature indicated on the STM tube.

h 450 µl XC3. Incubate for 1 minute. Repeat one time.

6 Wait until the Chamber Rack reaches the correct temperature.

Stain BeadChip

1. If you plan to image the BeadChip immediately after the staining process, turn on the scanner now to allow the lasers to stabilize.
2. Into the reservoir of each Flow-Through Chamber, dispense:
   a. 250 µl STM. Incubate for 10 minutes.
   b. 450 µl XC3. Incubate for 1 minute. Repeat one time.
   c. Wait 5 minutes.
   d. 250 µl ATM. Incubate for 10 minutes.
   e. 450 µl XC3. Incubate for 1 minute. Repeat one time.
   f. Wait 5 minutes.
   g. 250 µl STM. Incubate for 10 minutes.
   h. 450 µl XC3. Incubate for 1 minute. Repeat one time.
   i. Wait 5 minutes.
   j. 250 µl ATM. Incubate for 10 minutes.
   k. 450 µl XC3. Incubate for 1 minute. Repeat one time.
   l. Wait 5 minutes.
   m. 250 µl STM. Incubate for 10 minutes.
   n. 450 µl XC3. Incubate for 1 minute. Repeat one time.
   o. Wait 5 minutes.
3. Immediately remove the Flow-Through Chambers from the Chamber Rack and place horizontally on a lab bench at room temperature.

Wash and Coat 8 BeadChips

Before starting the Wash and Coat process, read these important notes:

- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates before use.
- In preparation for XC4 BeadChip coating, wash the tube racks and wash dishes thoroughly before and after use. Rinse with DI water. Immediately following wash, place racks and wash dishes upside down on a wash rack to dry.

- Place Kimwipes in 3 layers on the lab bench. Place a tube rack on top of these Kimwipe layers. Do not place on absorbent lab pads. You will place the staining rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

Equipment Needed

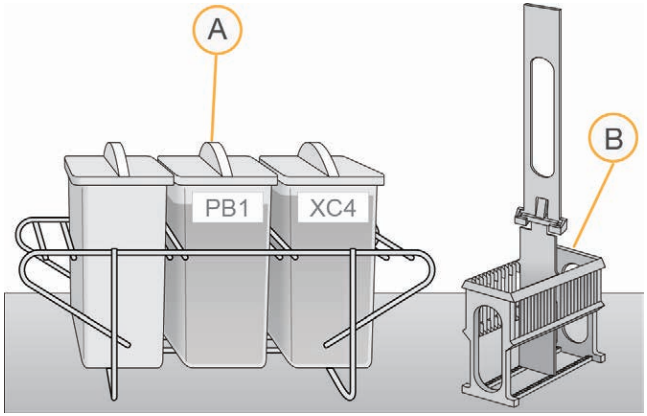
Place the following items on the bench:

- 1 staining rack
- 1 vacuum desiccator
- 1 tube rack
- Self-locking tweezers
- Large Kimwipes
- Vacuum hose

Steps

1. Set up 2 top-loading wash dishes, labeled "PB1" and "XC4".
2. To indicate the fill volume before filling wash dishes with PB1 and XC4, pour 310 ml water into the wash dishes and mark the water level on the side. Empty the water from the wash dish. Marking the level enables you to pour reagent directly from the PB1 and XC4 bottles into the wash dishes, minimizing contaminant transfer from labware to wash dishes.

Figure 53 PB1 and XC4 Wash Dishes with Staining Rack

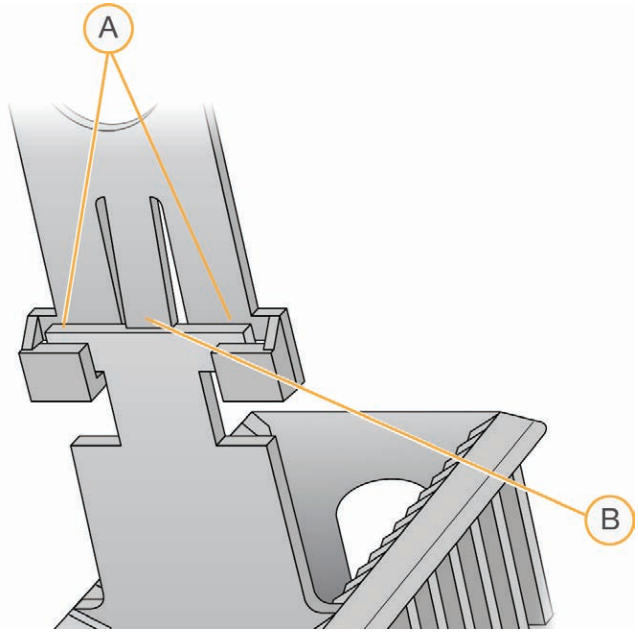


A Wash Dishes
B Staining Rack

3 Pour 310 ml PB1 into the wash dish labeled "PB1."

4 Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing towards*** you. This orientation makes it easier and safer to remove the BeadChips. Let the staining rack sit in the wash dish. You will use it to carry the BeadChips after disassembling the flow-through chambers.

Figure 54 Staining Rack Locking Arms and Tab



A Locking Arms
B Tab

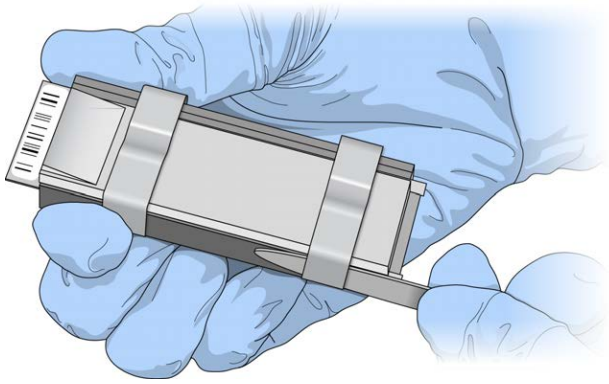
CAUTION
If the staining rack handle is not correctly oriented, the BeadChips can be damaged when you remove the staining rack handle before removing the BeadChips.

5 One at a time, disassemble each flow-through chamber:

a Use the dismantling tool to remove the 2 metal clamps.

CAUTION
It is important to use the dismantling tool to avoid chipping the glass back plates.

Figure 55 Removing the Metal Clamps from Flow-Through Chamber



b Remove the glass back plate.

c Set the glass back plate aside. When you finish the XStain HD BeadChip protocol, clean the glass back plates as described in the *Infinium Lab Setup and Procedures Guide*.

d Remove the spacer. To avoid damaging the stripes on the BeadChip, pull the spacer out so that the long sides slide along the sides of the BeadChip.

e Remove the BeadChip.

CAUTION
Do not touch the face of the BeadChips. Handle them by the barcode end or by the edges.

6 Place the BeadChips in the staining rack while it is submerged in PB1. Put 4 BeadChips above the staining rack handle and 4 below the handle. Make sure that the BeadChip barcodes ***face away*** from you and that the locking arms on the handle ***face towards*** you.

If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting each BeadChip.

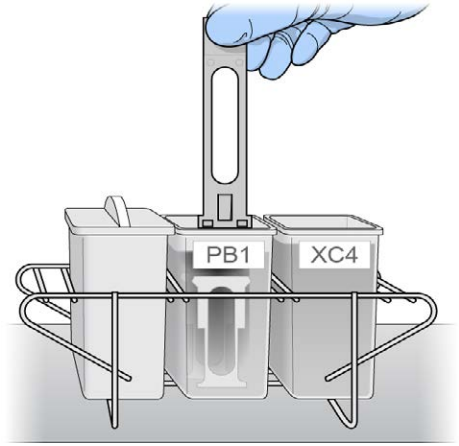
7 Make sure that the BeadChips are submerged.

CAUTION
Do not allow the BeadChips to dry. Submerge each BeadChip in the wash dish as soon as possible.

8 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

Figure 56 Washing BeadChips in PB1



9 Allow the BeadChips to soak for an additional 5 minutes.

CAUTION
Do not leave the BeadChips in the PB1 for more than 30 minutes.

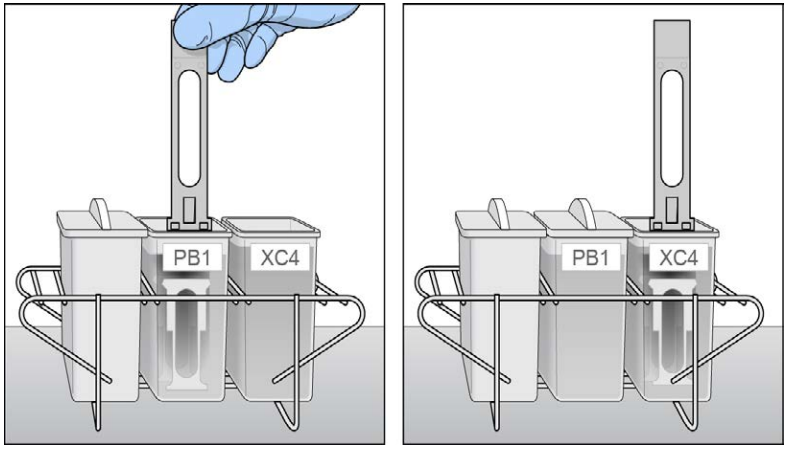
10 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until dissolved.

11 Pour 310 ml XC4 into the dish labeled "XC4," and cover the dish to prevent any lint or dust from falling into the solution.

CAUTION
Do not let the XC4 sit for longer than 10 minutes.

12 Remove the staining rack from the PB1 dish and place it directly into the wash dish containing XC4. For proper handling and coating, The barcode labels on the BeadChips must ***face away*** from you; the locking arms on the handle must ***face towards*** you.

Figure 57 Moving BeadChips from PB1 to XC4



13 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

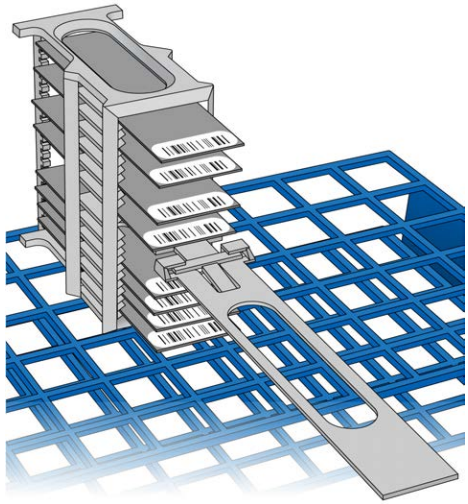
14 Allow the BeadChips to soak for an additional 5 minutes.

CAUTION
Use XC4 only one time. To process subsequent BeadChips, use a new, clean wash dish with fresh XC4.

15 Prepare 1 additional tube rack per 8 BeadChips (Illumina-provided from VWR catalog # 60916-748) that fits the internal dimensions of vacuum desiccator.

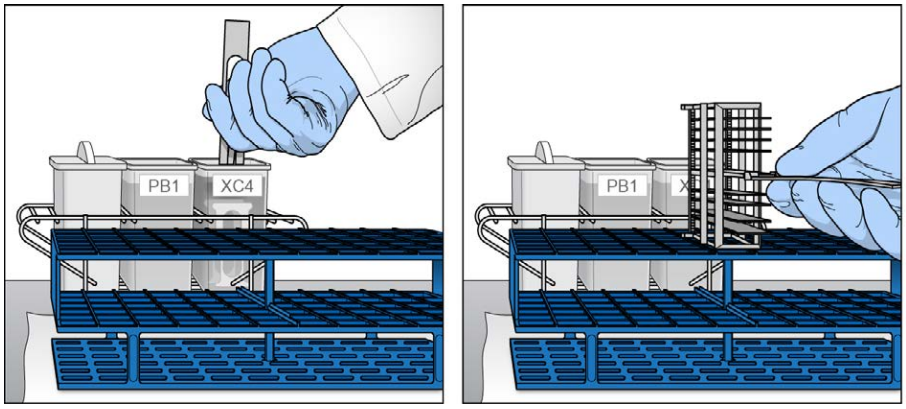
16 Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes ***face up***, and the locking arms and tabs ***face down***.

Figure 58 Staining Rack in Correct Orientation



To ensure uniform coating, place the staining rack on the center of the tube rack, avoiding the raised edges.

Figure 59 Moving the Staining Rack from XC4 to Tube Rack



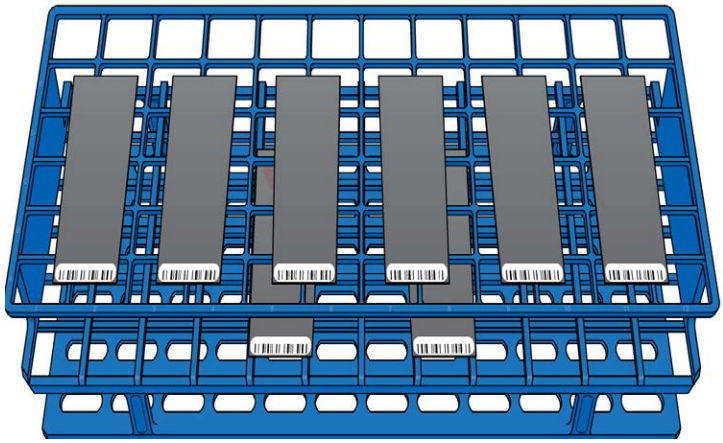
17 For each of the top 4 BeadChips, working top to bottom:

a Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

NOTE
The XC4 coat is slippery and makes the BeadChips difficult to hold. The self-locking tweezers grip the BeadChip firmly and help prevent damage.

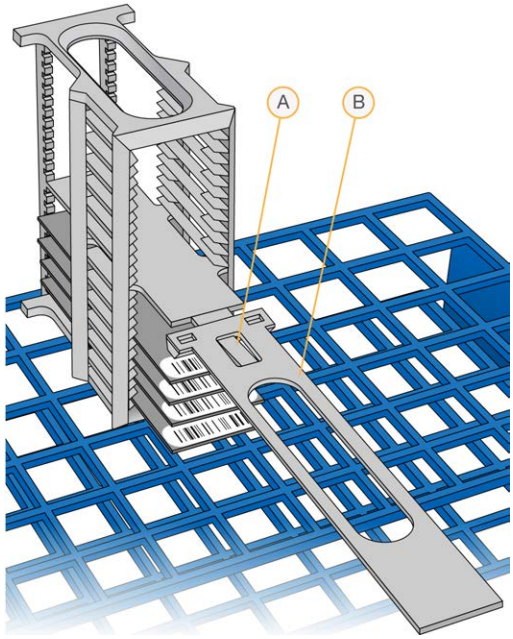
b Place each BeadChip on a tube rack with the barcode ***facing up and towards*** you.

Figure 60 BeadChips on Tube Rack



18 Holding the top of the staining rack in position, gently remove the staining rack handle by grasping the handle between the thumb and forefinger. Push up the tab with your thumb and push the handle away from you (unlocking the handle), then pull up the handle and remove.

Figure 61 Removing Staining Rack Handle



A Tab
B Handle

19 Remove the remaining BeadChips to the tube rack, with 6 BeadChips on top of the rack and 2 BeadChips on the bottom. Make sure that the barcode ends are towards you, and the BeadChips are completely horizontal.

CAUTION
To prevent wicking and uneven drying, do not allow the BeadChips to rest on the edge of the tube rack or to touch each other while drying.

20 Place the tube rack in the vacuum desiccator. Each desiccator can hold 1 tube rack (8 BeadChips).

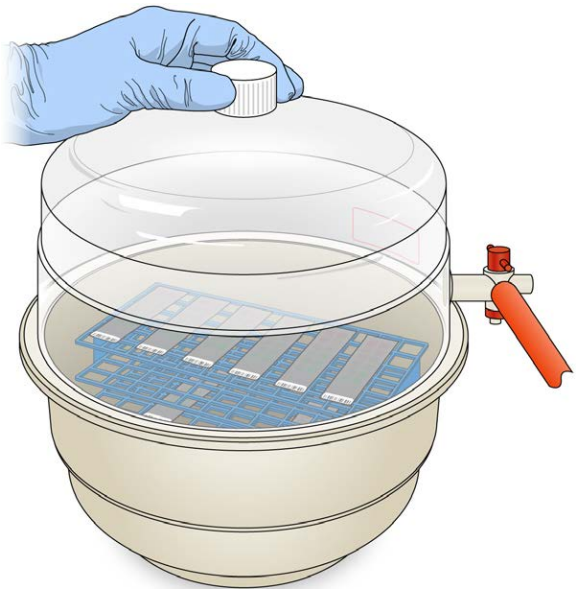
CAUTION
Make sure that the vacuum valve is seated tightly and securely.

21 Remove the red plug from the three-way valve before applying vacuum pressure.

22 Start the vacuum, using at least 675 mm Hg (0.9 bar).

23 To make sure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.

Figure 62 Testing Vacuum Seal



24 Dry under vacuum for 50–55 minutes.
Drying times can vary according to room temperature and humidity.

25 Release the vacuum by turning the handle very slowly.

WARNING
Make sure that air enters the desiccator very slowly to avoid disturbing the contents. Improper use of the vacuum desiccator can result in damage to the BeadChips, especially if you remove the valve plug while a vacuum is applied. For detailed vacuum desiccator instructions, see the documentation included with the desiccator.

26 Store the desiccator with the red valve plug in the 3-way valve of the desiccator to stop accumulation of dust and lint within the valve port.

27 Touch the borders of the BeadChips (**do not touch the stripes**) to make sure that the etched, barcoded sides are dry to the touch.

28 If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom 2 BeadChips are most likely to have some excess.
   a Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
   b Wipe along the underside of the BeadChip 5 or 6 times, until the surface is clean and smooth.

CAUTION
Do ***not*** touch the stripes with the wipe or allow EtOH to drip onto the stripes.

29 Clean the glass back plates. For instructions, see the *Infinium Lab Setup and Procedures Guide*.

30 Discard unused reagents in accordance with facility standards.

31 Do either of the following:
   - Proceed to *Image BeadChip (Post-Amp)*.
   - Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Image the BeadChips within 72 hours.

Image BeadChip

Follow the instructions in the *iScan System User Guide* or *HiScanSQ System User Guide* to scan your BeadChips.

Use the appropriate scan setting for your BeadChip, as outlined in the following table:

Table 7 Scan Settings for Infinium HD FFPE

BeadChip	Scan Setting Name
CytoSNP-FFPE	Infinium HD
HumanOmniExpress-FFPE	Infinium NXT

Illumina GenomeStudio

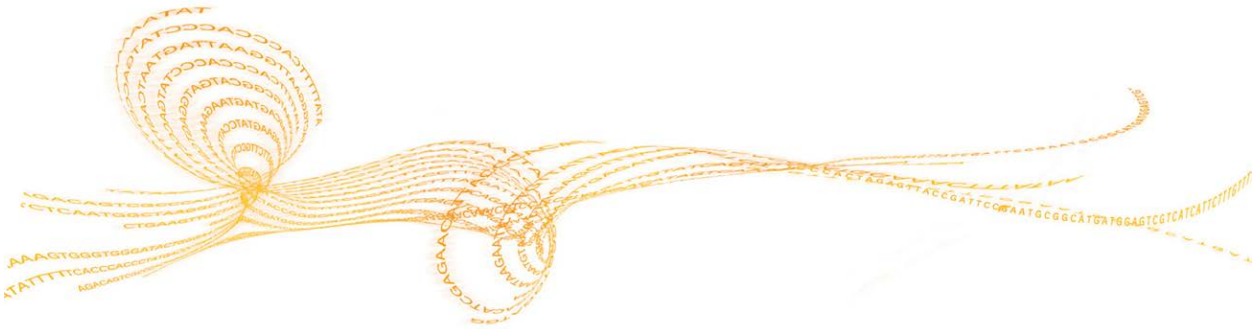
The Illumina GenomeStudio Genotyping Module, included with your Illumina Infinium Assay system, is an application for extracting genotyping data from intensity data files (*.idat files) collected from systems such as the Illumina HiScan System.

For feature descriptions and instructions on using the GenomeStudio platform to visualize and analyze genotyping data, see the *GenomeStudio Framework User Guide* and the *GenomeStudio Genotyping User Guide*.

Automated Protocol

Chapter 3

Introduction to Infinium HD FFPE Automated Protocol 88
Infinium HD FFPE Automated Workflow 89
Equipment, Materials, and Reagents 90
Make the MSA5 Plate 94
Incubate DNA (Post-Amp) 99
Fragment DNA (Post-Amp) 100
Precipitate DNA (Post-Amp) 105
Resuspend DNA (Post-Amp) 112
Hybridize to BeadChip (Post-Amp) 117
Wash BeadChips (Post-Amp) 134
Extend and Stain (XStain) BeadChip 145
Image BeadChip 177
Illumina GenomeStudio 178



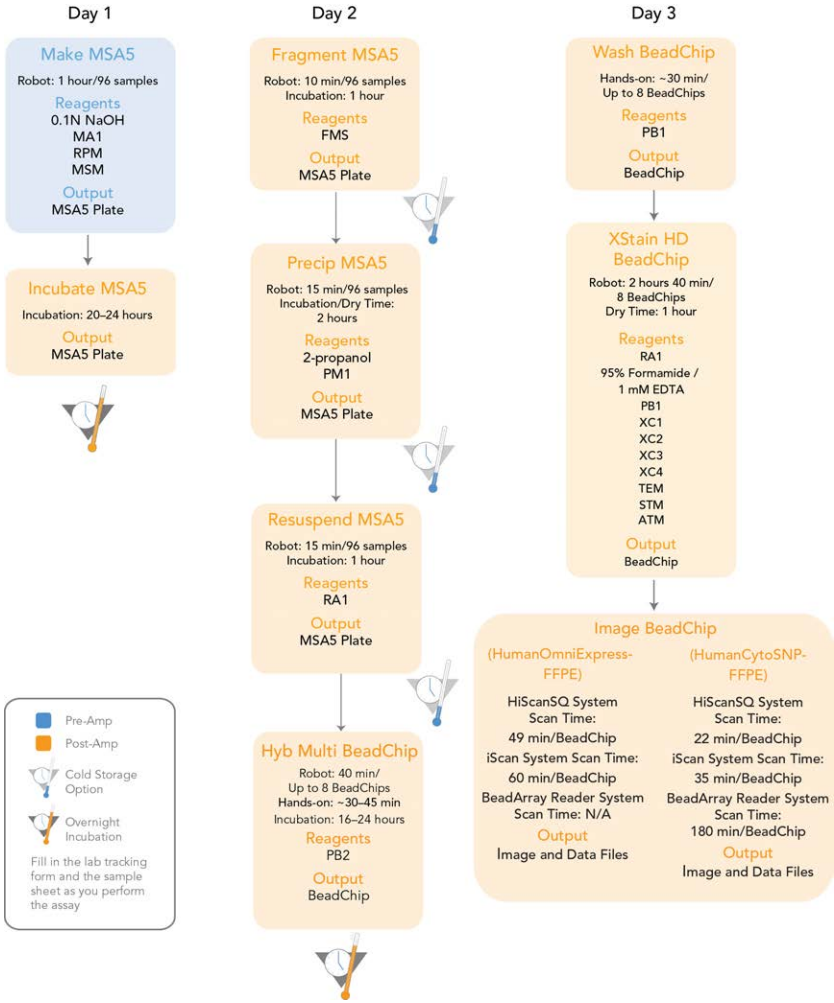
Introduction to Infinium HD FFPE Automated Protocol

This chapter describes pre- and post-amplification automated laboratory protocols for the Infinium HD FFPE Assay. Follow the protocols in the order shown.

Infinium HD FFPE Automated Workflow

The following figure graphically represents the Infinium HD FFPE Assay automated workflow for 8 BeadChips. These protocols describe the procedure for preparing 96 DNA samples.

Figure 63 Infinium HD FFPE Assay Automated Workflow



Equipment, Materials, and Reagents

These materials are specifically required for the automated Infinium HD FFPE Assay. For a list of other equipment, materials, and reagents needed in an Infinium HD FFPE Assay lab, see the *Infinium Assay Lab Setup and Procedures Guide*.

User-Supplied Equipment

Table 8 User-Supplied Equipment

Item	Suggested Vendor
Vacuum desiccator (1 per 8 BeadChips processed simultaneously)	VWR International catalog # 24988-197, www.vwr.com
Vacuum tubing	VWR International catalog # 62995-335, www.vwr.com
2 Tecan eight-tip robots (one for pre- and one for post-amp processes)	• SC-30-401 (110V) - North America and Japan • SC-30-402 (220V) - EU and Asia Pacific (Except Japan)
Carboy > 10 L, 2 per robot, pre-amp	
Forceps	VWR International catalog # 25601-008, www.vwr.com
Auto-desiccator cabinet (Optional—allows scanning of BeadChips up to three days after processing)	VWR International, Catalog # 74950-342, www.vwr.com

Illumina-Supplied Equipment

Table 9 Illumina-Supplied Materials

Item	Catalog or Part #
Multi-Sample BeadChip Alignment Fixture	Part # 218528

Item	Catalog or Part #
Robot BeadChip Alignment Fixture (6)	Part # 222691
Robot Tip Alignment Guide-B inserts	Part # 11294913
Robot Tip Alignment Guide-B	Two-piece: Catalog # SE-104-1002, Part# 11294201 One-piece: Catalog # SE-104-1006, Part# 11323260

User-Supplied Materials

Table 10 User-Supplied Materials

Item	Suggested Vendor
96-well, black, flat-bottom Fluotrac 200 plates	Greiner, catalog # 655076 www.gbo.com
Aluminum foil	
Foil adhesive seals (Microseal "F")	MJ Research, Catalog # MSF-1001, www.mjr.com
Reservoir, full, 150 ml	Beckman Coulter, catalog # 372784, www.beckmancoulter.com
Reservoir, half, 75 ml	Beckman Coulter, catalog # 372786, www.beckmancoulter.com
Reservoir, quarter, 40 ml	Beckman Coulter, catalog # 372790, www.beckmancoulter.com
Reservoir frames, 2 (per TECAN)	Beckman Coulter, catalog # 372795, www.becmancoulter.com

Item	Suggested Vendor
Tube racks for vacuum desiccator (1 for every 8 BeadChips to be processed simultaneously; must fit internal dimensions of vacuum desiccator)	VWR catalog # 66023-526, www.vwr.com
Vacuum source (greater than 508 mm Hg (0.68 bar)	
Vacuum gauge for vacuum desiccator (recommended)	

Illumina-Supplied Materials

- WG#-MSA5 barcode labels
- WG#-DNA barcode labels

Illumina-Supplied Reagents

Table 11 Illumina-Supplied Reagents

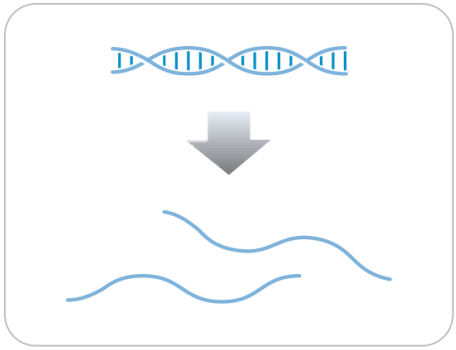
Item	Part #
ATM—Anti-Stain Two-Color Master Mix	11208317
FMS—Fragmentation solution	11203428
MA1—Multi-Sample Amplification 1 Mix	11202880
RPM—Random Primer Mix	15010230
MSM—Multi-Sample Amplification Master Mix	11203410
PB1—Reagent used to prepare BeadChips for hybridization	11291245
PB2—Humidifying buffer used during hybridization	11191130

Item	Part #
PM1—Precipitation solution	11292436
RA1—Resuspension, hybridization, and wash solution	11292441
STM—Superior Two-Color Master Mix	11288046
TEM—Two-Color Extension Master Mix	11208309
XC1—XStain BeadChip solution 1	11208288
XC2—XStain BeadChip solution 2	11208296
XC3—XStain BeadChip solution 3	11208392
XC4—XStain BeadChip solution 4	11208430

Make the MSA5 Plate

This process prepares your restored FFPE samples for DNA amplification. MA1 is first added to the MSA5 plate and then 0.1N NaOH is added to denature the DNA samples. The RPM reagent neutralizes the sample. Lastly, MSM is added to the plate.

Figure 64 Denaturing and Neutralizing DNA



Estimated Time

Robot time:

- 30 minutes for 48 samples
- 60 minutes for 96 samples

Consumables

Item	Quantity	Storage	Supplied By
MA1	2 tubes (per 96 samples)	-15° to -25°C	Illumina
RPM	2 tubes (per 96 samples)	-15° to -25°C	Illumina
MSM	2 tubes (per 96 samples)	-15° to -25°C	Illumina

Item	Quantity	Storage	Supplied By
0.1N NaOH	15 ml (per 96 samples)	2° to 8°C	User
MSA5 plate with restored DNA samples	1 plate	-15° to -25°C	User

NOTE
Thaw all reagents completely at room temperature and allow to equilibrate. After thawed, gently invert each tube several times to mix the reagent thoroughly. Pulse centrifuge each tube to 280 × g to eliminate bubbles and collect reagent at the bottom of the tube.

Preparation

1. In preparation for the Incubate MSA5 process, preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
2. Thaw MA1, RPM, and MSM tubes to room temperature. Gently invert at least 10 times to mix contents.
3. If stored in a freezer thaw DNA samples to room temperature.
4. In the Sample Sheet, enter the Sample_Name and Sample_Plate for each Sample_Well.
5. On the lab tracking form, record:
   - Date/Time
   - Operator
   - Robot
   - Batch number
   - Number of samples (48 or 96)
   - MSA5 plate barcode(s)
   - MA1 tube barcode(s)
   - RPM tube barcode(s)
   - MSM tube barcode(s)

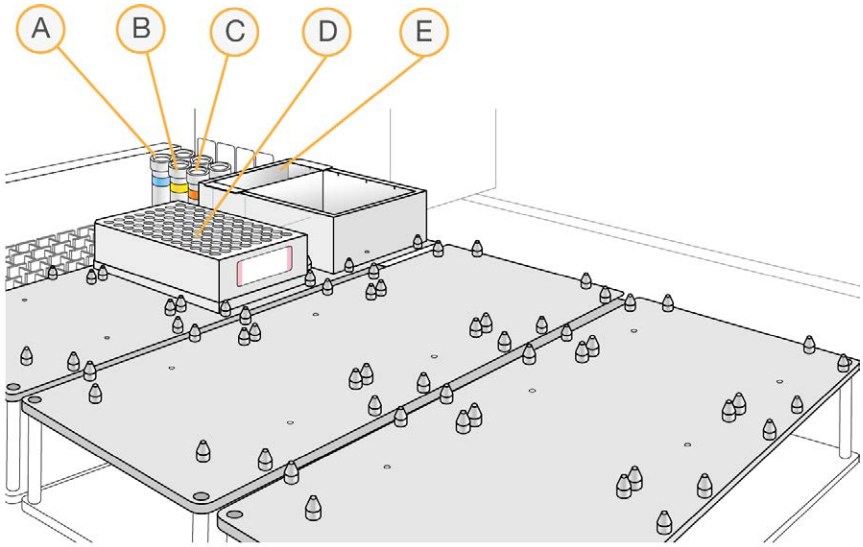
NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Prepare Robot

For instructions on preparing the robot for use in a protocol see the *Infinium Assay Lab Setup and Procedures Guide*.

Refer to the figure shown below throughout this protocol. Note that all of the barcodes face to the right.

Figure 65 Eight-Tip Robot (Make MSA5 Setup)



A MA1 Tube
B RPM Tube
C MSM Tube
D MSA5 Plate
E NaOH in Quarter Reservoir

Steps to Make the MSA5 Plate

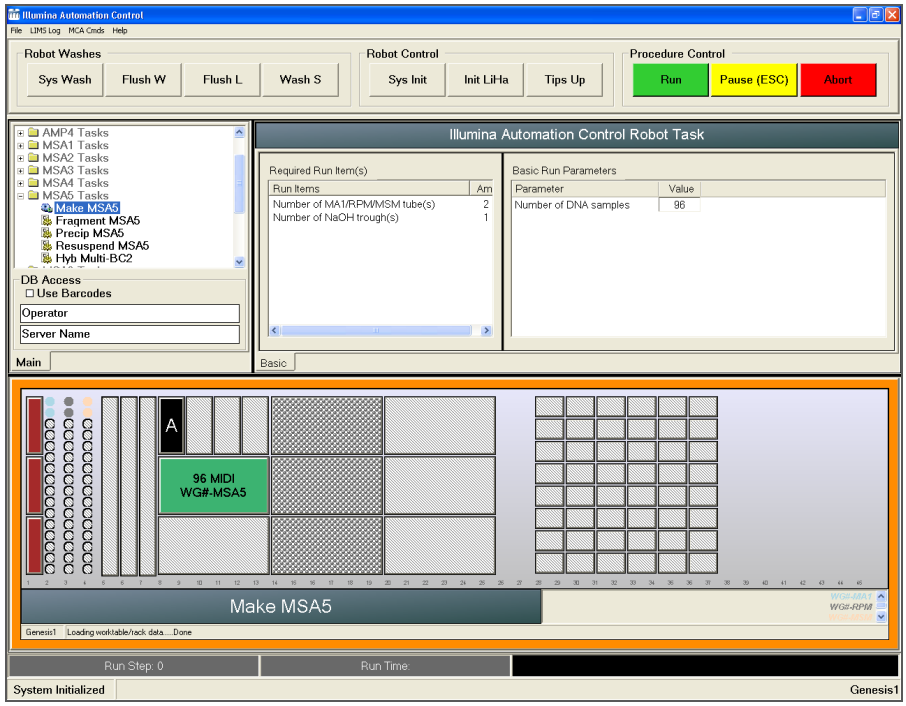
1 At the robot PC, select **MSA5 Tasks** | **Make MSA5**.

2 Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of DNA samples** (48 or 96) that are in the plate.
You can process up to 96 DNA samples per robot run.

NOTE
If you are running 48 or less samples, enter 48 in the Basic Run Parameters. Enter 96 if you are running 49-96 samples.

The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

Figure 66 Make MSA5 Screen



3 Remove caps from MA1, RPM, and MSM tubes, then place the tubes in the robot standoff tube rack according to the bed map.

4 Vortex the sealed MSA5 plate at 1600 rpm for 1 minute.

5 Pulse centrifuge to 280 xg.

6 Add 15 ml NaOH to the quarter reservoir, then place the reservoir on the robot bed according to the bed map.

7 Place the MSA5 plate on the robot bed according to the bed map.

8 On the lab tracking form, record the plate positions on the robot bed.

9 Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

10 At the robot PC, click **Run**.

11 When prompted, remove the MSA5 plate and seal it with a cap mat.

12 Vortex the sealed MSA5 plate at 1600 rpm for 1 minute.

13 Pulse centrifuge to 280 xg.

14 Remove the cap mat.
When you remove a cap mat, set it aside, upside down, in a safe location for use later in the protocol.

15 Place the MSA5 plate back on the robot bed in its original position and click **OK** to start the 10-minute incubation.
The Wait for reaction time message appears. The robot PC sounds an alert and displays a message when the process is complete. Click **OK** in the message box.

16 Remove the MSA5 plate from the robot bed and seal with the 96-well cap mat.
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

17 Vortex the sealed MSA5 plate at 1600 rpm for 1 minute.

18 Pulse centrifuge to 280 xg.

19 Record the location of DNA samples on the lab tracking form.

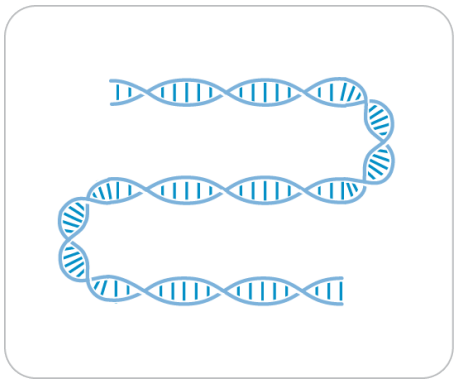
20 Discard unused reagents in accordance with facility standards.

21 Proceed immediately to *Incubate the MSA5 Plate*.

Incubate DNA (Post-Amp)

This process incubates the MSA5 plate for 20–24 hours at 37°C in the Illumina Hybridization Oven. The process uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used when in the Infinium HD FFPE Assay.

Figure 67 Incubating DNA to Amplify



Estimated Time

Incubation time: 20–24 hours

Steps to Incubate MSA5 Plate

OVERNIGHT INCUBATION

Incubate MSA5 plate in the Illumina Hybridization Oven for at least 20 hours but no more than 24 hours at 37°C.

1 Record the start and stop times on the lab tracking form.

NOTE

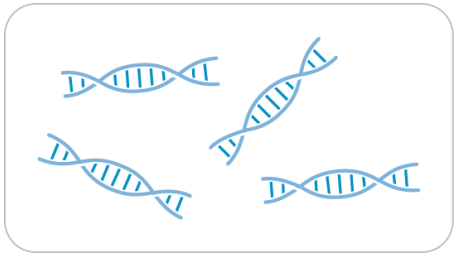
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

2 Proceed to the next step.

Fragment DNA (Post-Amp)

This process enzymatically fragments the amplified DNA samples. An endpoint fragmentation is used to prevent overfragmentation.

Figure 68 Fragmenting DNA



Estimated Time

Robot time:

- 5 minutes for 48 samples
- 10 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FMS	2 tubes (per 96 samples)	-25°C to -15°C	Illumina

NOTE
Thaw all reagents completely at room temperature and allow to equilibrate. After thawed, gently invert each tube several times to mix the reagent thoroughly. Pulse centrifuge each tube to 280 × g to eliminate bubbles and collect reagent at the bottom of the tube.

Preparation

1. Preheat the heat block with the midi plate insert to 37°C.
2. Thaw FMS tubes to room temperature. Gently invert at least 10 times to mix contents.
3. Remove the MSA5 plate from the Illumina Hybridization Oven.
4. If you plan to Resuspend the MSA5 plate today, remove the RA1 from the freezer to thaw.

▶ On the lab tracking form, record:

- Date/Time
- Operator
- Robot
- FMS tube barcodes

NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

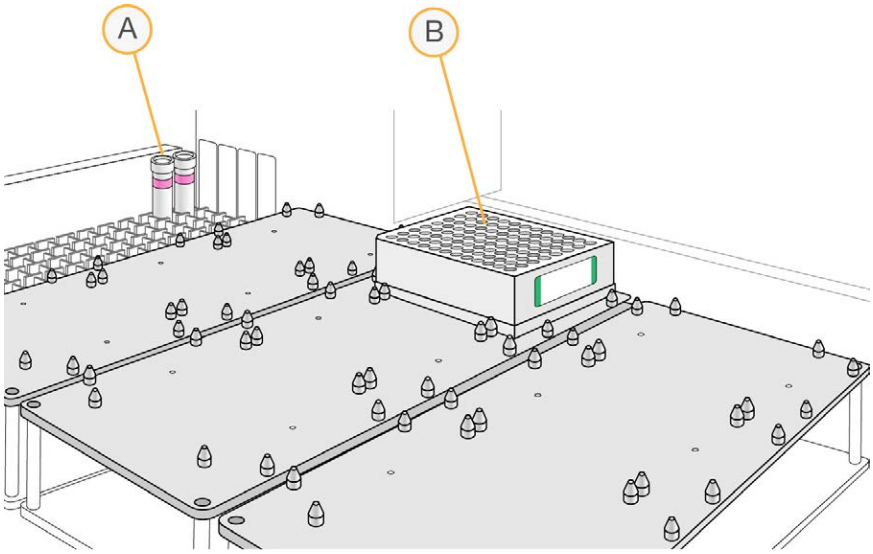
Prepare Robot

For instructions on preparing the robot for use in a protocol, see the *Infinium Assay Lab Setup and Procedures Guide*.

CAUTION
Do not run any other programs or applications while using the Tecan robot. Your computer and the robot might lock up and stop a run.

Refer to the following figure throughout this protocol.

Figure 69 Tecan 8-Tip Robot (Fragment MSA5 Setup)



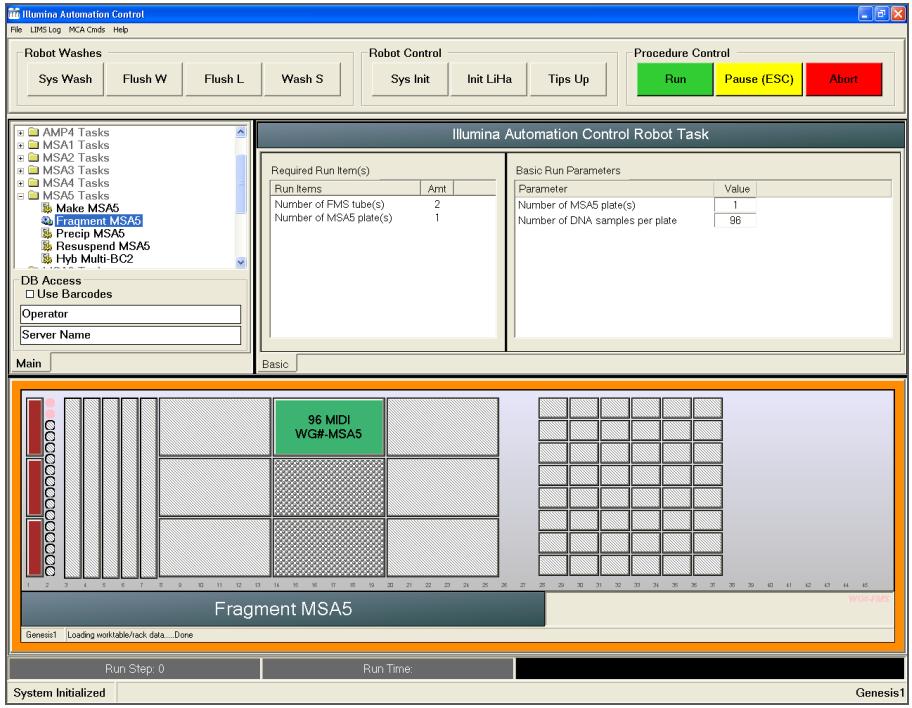
A FMS tubes
B MSA5 plate

Steps to Fragment the MSA5 Plate

1. Pulse centrifuge the MSA5 plate to 280 × g.
2. Remove the cap mat.
3. At the robot PC, select **MSA5 Tasks** | **Fragment MSA5**.

4 Make sure that the **Use Barcodes** checkbox is cleared. In the **Basic Run Parameters** pane, change the value for **Number of MSA5 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

Figure 70 Fragment MSA5 Screen



5 Place the MSA5 plate on the robot bed according to the bed map.

6 Place FMS tubes in the robot tube rack according to the bed map. Remove the cap.

7 On the lab tracking form, record the plate positions on the robot bed.

Start the Robot

1 At the robot PC, click **Run**.
The robot PC sounds an alert and displays a message when the process is done.

2 When the robot finishes, click **OK** in the message box.

3 Remove the MSA5 plate from the robot bed and seal it with a cap mat.

4 Vortex at 1600 rpm for 1 minute.

5 Pulse centrifuge to 280 × g.

6 Place the sealed plate on the 37°C heat block for 1 hour.

7 Record the start and stop times on the lab tracking form.

8 Discard unused reagents in accordance with facility standards.

9 Do one of the following:

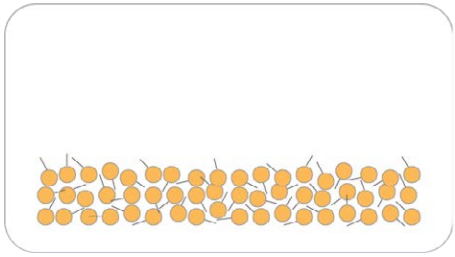
- Proceed to *Precipitate DNA (Post-Amp)*. Leave plate in 37°C heat block until you have completed the preparatory steps. Do not leave the plate in the 37°C heat block for longer than 2 hours.
- If you do not plan to proceed to the next step within the next 4 hours, store the sealed MSA5 plate at -25°C to -15°C for more than 24 hours.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Precipitate DNA (Post-Amp)

PM1 and 2-propanol are added to the MSA5 plate to precipitate the DNA samples.

Figure 71 Precipitating DNA



Estimated Time

Robot time:

- 10 minutes for 48 samples
- 20 minutes for 96 samples

Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	2 tubes (per 96 samples)	2°C to 8°C	Illumina
100% 2-propanol	30 ml (per 96 samples)	Room temperature	User

NOTE
Thaw all reagents completely at room temperature and allow to equilibrate. After thawed, gently invert each tube several times to mix the reagent thoroughly. Pulse centrifuge each tube to 280 × g to eliminate bubbles and collect reagent at the bottom of the tube.

Preparation

1 Preheat the heat block to 37°C.

2 If you froze the MSA5 plate overnight, thaw it to room temperature, then pulse centrifuge to 280 × g.

3 Thaw PM1 to room temperature. Gently invert at least 10 times to mix contents.

▸ On the lab tracking form, record:

- Date/Time
- Operator
- Robot
- PM1 tube barcodes
- 2-propanol lot number and date opened

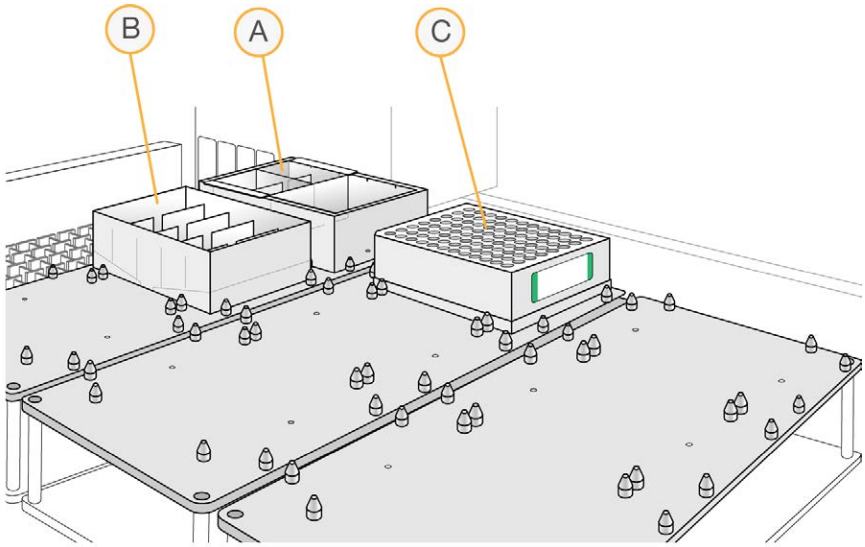
NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Prepare Robot

For instructions on preparing the robot for use in a protocol, see the *Infinium Assay Lab Setup and Procedures Guide*.

Refer to the following figure throughout this protocol. Barcodes face to the right.

Figure 72 Tecan 8-Tip Robot (Precip MSA5 Setup)



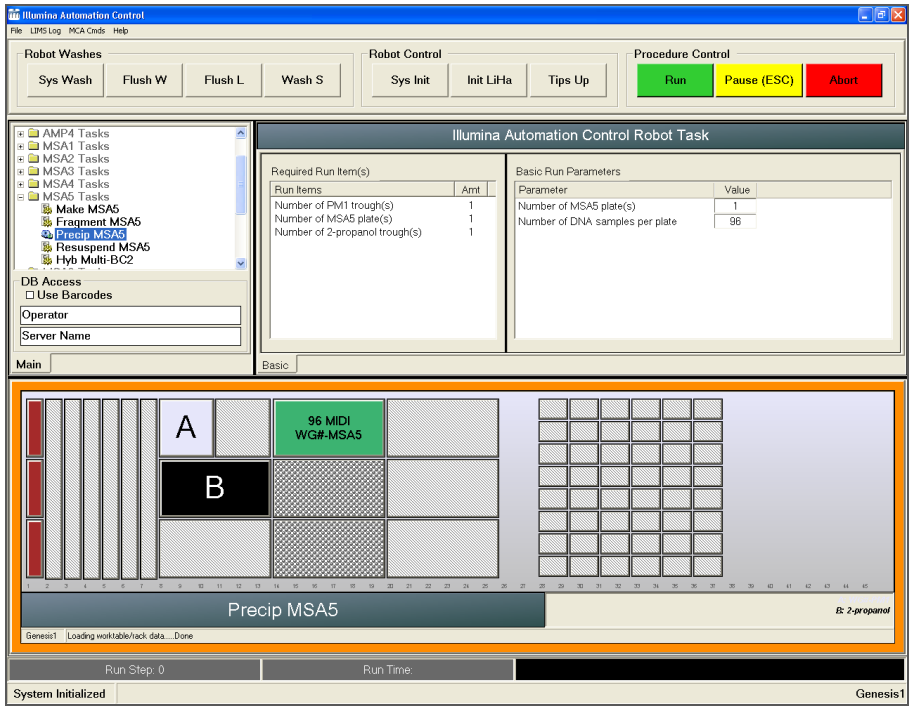
A PM1 in Half Reservoir
B 2-propanol in full Reservoir
C MSA5 Plate

Steps to Precipitate the MSA5 Plate

1 At the robot PC, select **MSA5 Tasks | Precip MSA5**.

2 Make sure that the **Use Barcodes** checkbox is cleared. In the **Basic Run Parameters** pane, change the value for **Number of MSA5 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

Figure 73 Precip MSA5 Screen



3 Pulse centrifuge the sealed MSA5 plate at 280 × g.

4 Remove the cap mat and place the MSA5 plate on the robot bed according to the bed map.

5 Place a half reservoir in the reservoir frame, according to the robot bed map, and add 1 tube PM1.

6 Place a full reservoir in the reservoir frame, according to the robot bed map, and add 30 ml 2-propanol.

7 In the lab tracking form, record the plate positions on the robot bed.

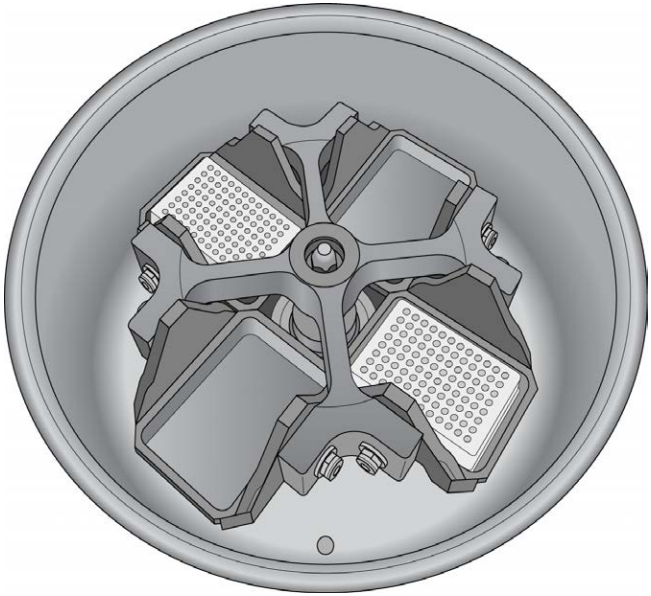
8 Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. At the robot PC, click **Run**.
   The robot PC sounds an alert and opens a message when the process is complete.
2. When prompted, remove the MSA5 plate from the robot bed. Do not click **OK** in the message box yet.
3. Seal the MSA5 plate with the same cap mat removed earlier.
4. Vortex the sealed plate at 1600 rpm for 1 minute.
5. Incubate at 37°C for 5 minutes.
6. Pulse centrifuge to 280 × g.

   NOTE
   Set the centrifuge to 4°C in preparation for the next centrifuge step.
7. Remove the cap mat and discard it.
8. Place the MSA5 plate back on the robot bed according to the bed map.
9. Click **OK** in the message box.
   The robot PC sounds an alert and opens a message when the process is complete.
10. Click **OK** in the message box. Remove the MSA5 plate from the robot bed and carefully seal with a ***new, dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.
11. Invert the plate at least 10 times to mix contents thoroughly.
12. Incubate at 4°C for 30 minutes.
13. Place the sealed MSA5 plate in the centrifuge opposite another plate of equal weight.

Figure 74 Sealed MSA5 Plate and Plate of Equal Balance in Centrifuge



14 Centrifuge at 3000 × g at 4°C for 20 minutes. Immediately remove the MSA5 plate from centrifuge.

CAUTION
Perform the next step immediately to avoid dislodging the blue pellet. If any delay occurs, repeat the 20-minute centrifugation before proceeding.

15 Remove the cap mat and discard it.

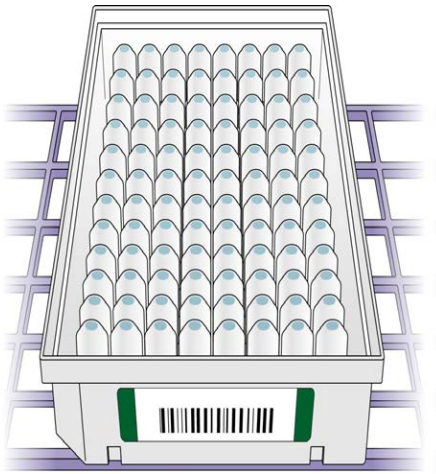
16 Quickly invert the MSA5 plate and drain the liquid onto an absorbent pad to decant the supernatant. Then smack the plate down on a dry area of the pad, avoiding the liquid that was drained onto the pad.

17 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

CAUTION
Keep the plate inverted. To ensure optimal performance, do not allow supernatant in wells to pour into other wells.

18 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
You can expect to find blue pellets at the bottoms of the wells.

Figure 75 Uncovered MSA5 Plate Inverted for Air Drying



CAUTION
Do not overdry the pellet. Pellets that are overdried are difficult to resuspend. Poorly resuspended samples lead to poor data quality.

19 Record the start and stop times on the lab tracking form.

20 Discard unused reagents in accordance with facility standards.

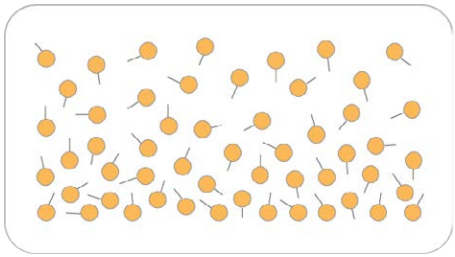
21 Do either of the following:
- Proceed to *Resuspend DNA (Post-Amp)*.
- If you do not plan to proceed to the next step immediately, seal the MSA5 plate with a new cap mat and store at -25°C to -15°C for no more than 24 hours.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Resuspend DNA (Post-Amp)

RA1 is added to the MSA5 plate to resuspend the precipitated DNA samples.

Figure 76 Resuspending DNA



Estimated Time

Robot time:

- 10 minutes for 48 samples
- 15 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	7 ml per 96 samples	-25°C to -15°C	Illumina

NOTE

Pour out only the recommended volume of RA1 needed for the suggested number of samples listed in the consumables table. Additional RA1 is used later in the XStain BeadChip step.

WARNING

This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

1. RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that might be present.
2. If you stored the MSA5 plate at -25°C to -15°C, thaw it to room temperature. Remove the cap mat and discard it.
3. Preheat the Illumina Hybridization Oven to 48°C.
4. Preheat the heat sealer. Allow 20 minutes.

▸ On the lab tracking form, record:

- Date/Time
- Operator
- Robot
- RA1 bottle barcodes

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Use Fresh RA1 Reagent for Each Step

It is important to use fresh RA1 for each protocol step in the assay where it is required. RA1 that has been stored properly and has not been dispensed for use in either the XStain or Resuspension step is considered fresh RA1. After RA1 has been poured out into a reservoir and exposed to room temperature air for extended periods of time, it is no longer fresh.

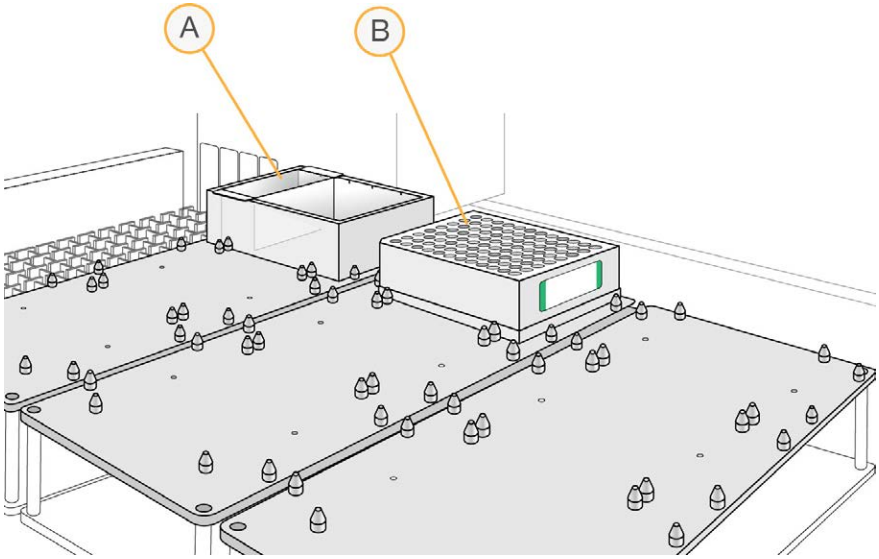
To make best use of RA1, only pour out the amount needed for the current step. If you plan to perform additional assay steps requiring RA1 that same day, then leave the remaining thawed reagent in the original, closed bottle at room temperature until it is needed. Otherwise, follow the standard RA1 storage procedures described in this assay guide for next-day processing and prolonged storage conditions.

Prepare Robot

For instructions on preparing the robot for use in a protocol, see the *Infinium Assay Lab Setup and Procedures Guide*.

Refer to the following figure throughout this protocol. Barcodes face to the right.

Figure 77 Tecan 8-Tip Robot (Resuspend MSA5 Setup)



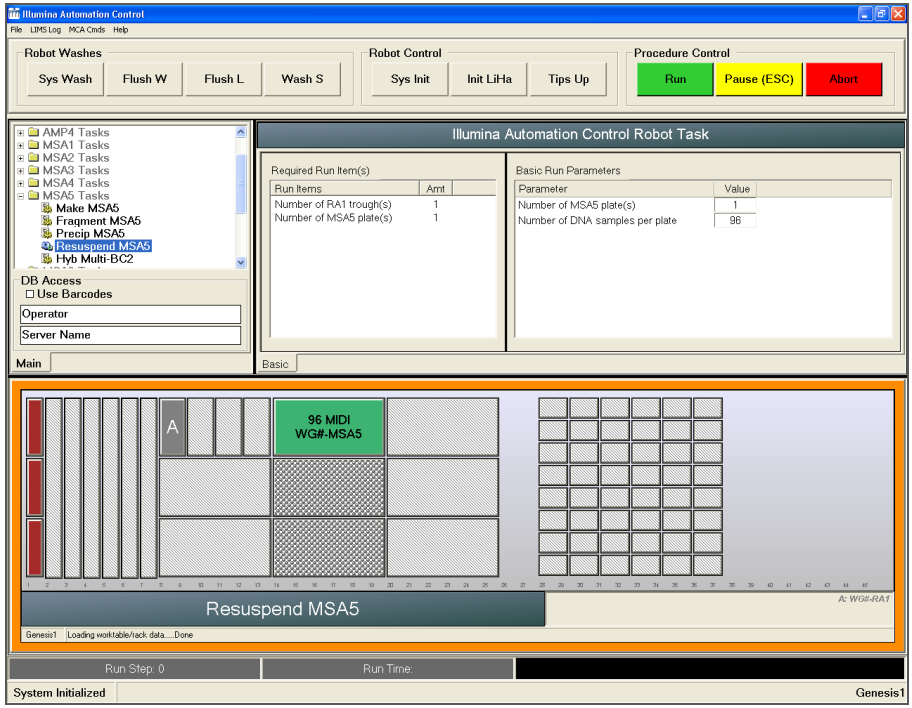
A RA1 in Quarter Reservoir
B MSA5 Plate

Steps to Resuspend the MSA5 Plate

1 At the robot PC, select **MSA5 Tasks** | **Resuspend MSA5**.

2 In the **Basic Run Parameters** pane, change the value for **Number of MSA5 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

Figure 78 Resuspend MSA5 Screen



3 Place the MSA5 plate on the robot bed according to the bed map.

4 Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add 7 ml RA1 for 96 samples.

5 In the lab tracking form, record the plate positions on the robot bed and RA1 barcodes.

6 Make sure that all items are placed correctly on the robot bed, that all caps and seals have been removed, and that all barcodes face to the right.

Start the Robot

1 At the robot PC, click **Run**.
The robot PC sounds an alert and opens a message when the process is complete.

2 Click **OK** in the message box. Remove the MSA5 plate from the robot bed.

3 Apply a foil seal to the MSA5 plate by firmly holding the heat sealer block down for 3 full seconds.

4 Immediately remove the MSA5 plate from the heat sealer and forcefully roll the rubber plate sealer over the plate until you can see all 96 well indentations through the foil. Repeat application of the heat sealer if all 96 wells are not defined.

5 Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.

6 Record the start and stop times on the lab tracking form.

7 Vortex the plate at 1800 rpm for 1 minute.

8 Pulse centrifuge to 280 × g.

NOTE
If you stored the DNA pellets at -25°C to -15°C for more than 72 hours, you might need to revortex and centrifuge until the pellets are resuspended.

9 Discard unused reagents in accordance with facility standards.

10 Do either of the following:

- Proceed to *Hybridize to BeadChip (Post-Amp)*. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
- If you do not plan to proceed to the next step immediately, store the sealed MSA5 plate at -25°C to -15°C for no more than 24 hours. Store at -80°C if storing for more than 24 hours. Store RA1 at -25°C to -15°C.

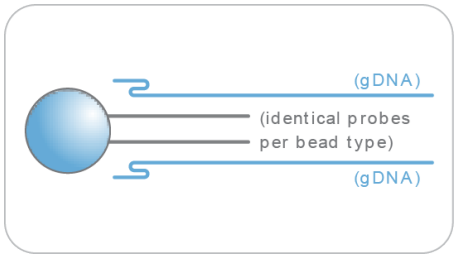
SAFE STOPPING POINT
Now is a good stopping point in the process.

Hybridize to BeadChip (Post-Amp)

In this process, the fragmented and resuspended DNA samples are dispensed onto the BeadChips. DNA-loaded BeadChips are placed into Hyb Chamber Inserts that are placed inside the Hyb Chambers.

When the DNA samples have been loaded into the flow-through chambers, incubate the chambers for 16–24 hours at 48°C in the Illumina Hybridization Oven. Hybridization occurs during the incubation period. Each sample is hybridized to an individual section of the BeadChip.

Figure 79 Hybridizing DNA to BeadChip



Estimated Time

Robot time:

- 12x1 HD BeadChip: ~40 minutes for 8 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	2 tubes	Room temperature	Illumina
BeadChips	8		Illumina
Hyb chambers	2		Illumina

Item	Quantity (per 96 Samples)	Storage	Supplied By
Hyb chamber gaskets	2		Illumina
Hyb chamber inserts	8		Illumina
Robot BeadChip alignment fixtures	4		Illumina
Robot Tip Alignment Guide-B (one-piece guide)	4		Illumina
Robot Tip Alignment Guide-B (for multipiece guide)	8		Illumina
Robot Tip Alignment Guide-B (for multipiece guide)	4		Illumina
1% aqueous Alconox solution	As needed		User

NOTE
Thaw all reagents completely at room temperature and allow to equilibrate. After thawed, gently invert each tube several times to mix the reagent thoroughly. Pulse centrifuge each tube to 280 × g to eliminate bubbles and collect reagent at the bottom of the tube.

Preparation

1 If frozen, thaw MSA5 plate to room temperature, and then pulse centrifuge the MSA5 plate to 280 × g.

2 Preheat the heat block to 95°C.

3 Prepare the Illumina Hybridization Oven as follows:
   a Preheat the oven to 48°C:
      — Press the "F" button one time to change the display to **TSET**.
      — Press the "S" button to enter the set-temperature mode, and then use the Increment/Decrement dial to set the oven to 48°C.
      — Press the "S" button again to set 48°C as the temperature.
   b Set the rocker speed to 5:

- Press the "F" button twice until **SPd** is indicated on the display.
- Press the "S" button to enter the rocker speed mode.
- Use the Increment/Decrement dial to set the rocker speed to "5".
- Press the "S" button again.

4 Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.

5 On the lab tracking form, record:

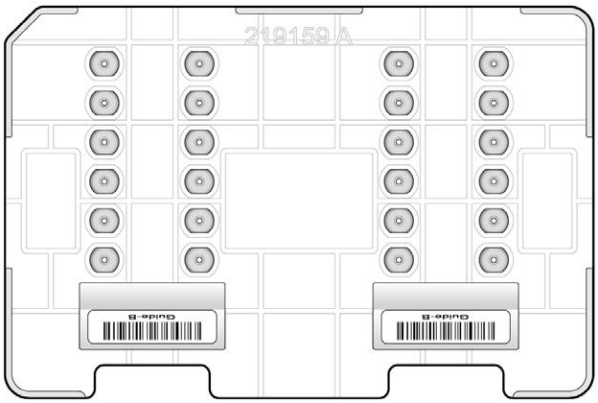
- Date/Time
- Operator
- PB2 tube lot number

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Prepare the Robot Tip Alignment Guide

1 Make sure that you have the correct Robot Tip Alignment Guide for the Infinium assay you are running. The barcode says **Guide-B**.

Figure 80 One-Piece Guide-B Robot Tip Alignment Guide



2 Wash and dry the entire one-piece Robot Tip Alignment Guide. See *Wash Robot Tip Alignment Guide* at the end of the *Hybridize to BeadChip* steps for washing instructions.

3 Place the assembled Robot Tip Alignment Guides on the lab bench until it is time to place them on the robot bed.

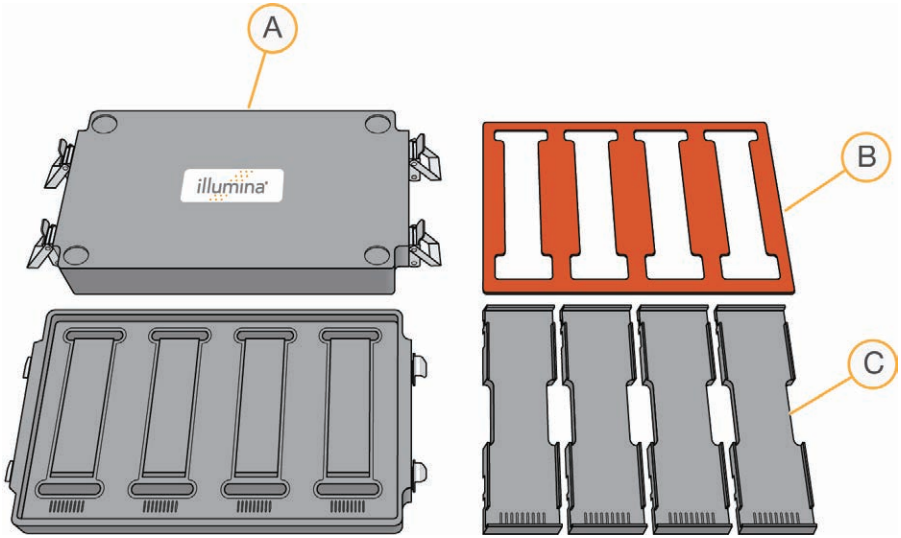
Assemble the Hybridization Chambers

1 Place the resuspended MSA5 plate on the heat block to denature the samples at 95°C for 20 minutes.

2 Remove the BeadChips from 2°C to 8°C storage, leaving the BeadChips in their plastic bags and mylar packages until you are ready to begin hybridization.

3 During the 20-minute incubation, prepare the Hyb Chambers.
Place the following items on the benchtop for use in this procedure:

Figure 81 BeadChip Hyb Chamber Components



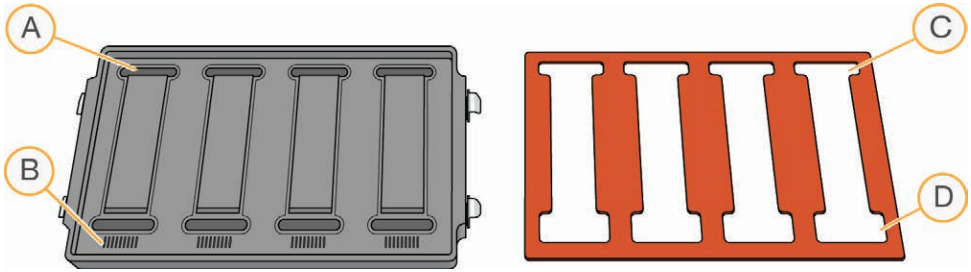
A BeadChip Hyb Chambers
B Hyb Chamber Gaskets
C Hyb Chamber Inserts

NOTE
To ensure optimal results from Hyb Chambers, keep the Hyb Chamber lids and bases together. Adopt a labeling convention that keeps each Hyb Chamber base paired with its original lid. Check Hyb Chamber lid-base pairs regularly to make sure that the fit remains secure. Check hinges regularly for any signs of abnormal wear or loose fittings. It is important that the hinges provide adequate clamping strength to ensure an airtight seal between the lid and the base. Record the Hyb Chamber that was used for each BeadChip, so that Hyb Chambers can be investigated and evaluated in the event of sample evaporation or other lab processing anomalies.

a Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers.

— Match the wider edge of the Hyb Chamber Gasket to the barcode-ridge side of the Hyb Chamber.

Figure 82 Hyb Chamber and Gasket



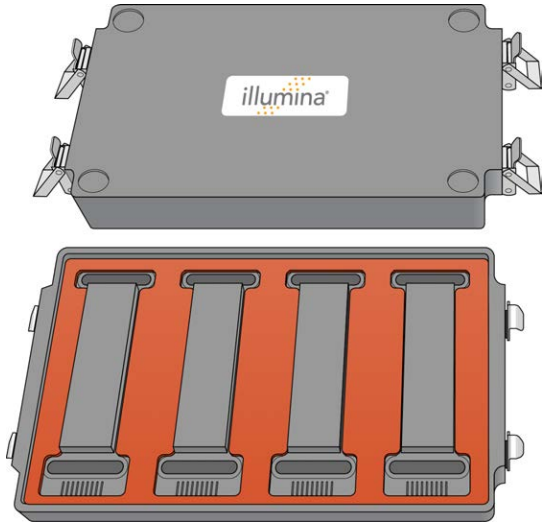
A Reservoirs
B Barcode Ridges
C Narrower Edges
D Wider Edges

— Lay the gasket into the Hyb Chamber, and then press it down all around.

Figure 83 Placing Gasket into Hyb Chamber

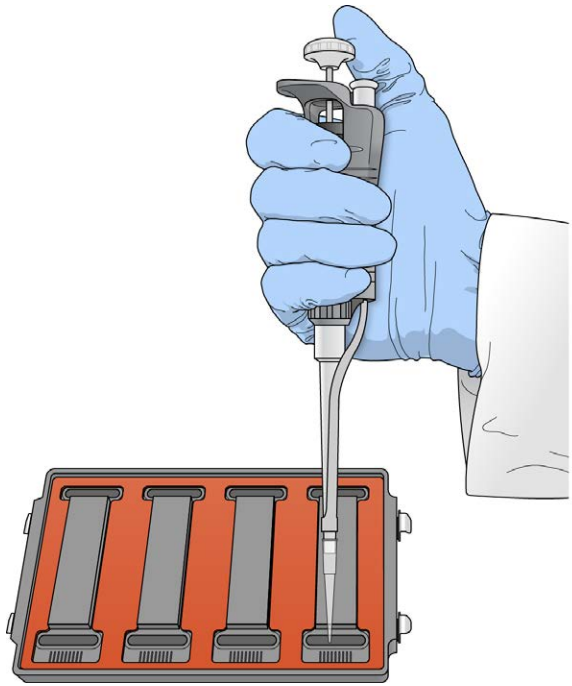
— Make sure that the Hyb Chamber gaskets are properly seated.

Figure 84 Hyb Chamber with Gasket in Place



b Dispense 400 µl PB2 into the humidifying buffer reservoirs in the Hyb Chambers.

Figure 85 Dispensing PB2 into Hyb Chamber Reservoir



WARNING

Do not replace PB2 in the Hyb Chamber with RA1. RA1 decreases the stringency and can negatively affect sample call rates and logRdev. PB2 is formulated to produce the appropriate amount of humidity within the Hyb Chamber environment to prevent sample from evaporating during hybridization.

c After you fill the Hyb Chamber reservoirs with PB2, place the lid on the Hyb Chamber right away to prevent evaporation. It is not necessary to lock the lid.

d Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample. Load BeadChips into the Hyb Chamber within 1 hour.

NOTE

You can also prepare the Hyb Chambers later, during the 30-minute cool down.

4 After the 20-minute incubation, remove the MSA5 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.

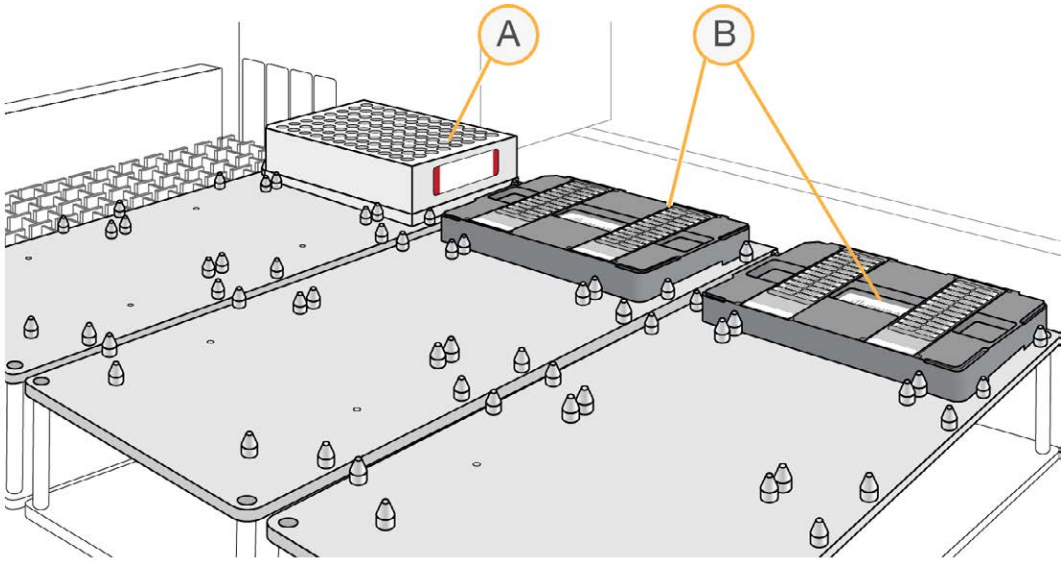
5 After the 30-minute cool down, pulse centrifuge the MSA5 plate to 280 × g. Remove the foil seal.

Prepare the Robot

For instructions on preparing the robot for use in a protocol, see the *Infinium Assay Lab Setup and Procedures Guide*.

Refer to the following figure throughout this protocol. Barcodes face to the right.

Figure 86 Placing Alignment Fixtures and MSA5 Plate onto Robot Bed



A MSA5 Plate
B Robot BeadChip Alignment Fixtures

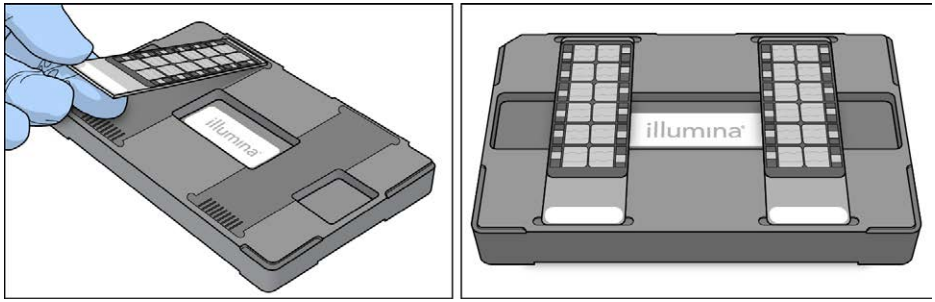
Load BeadChips

1 Remove all BeadChips from their ziplock bags and mylar packages.

CAUTION
Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Do not hold the BeadChip by the sides near the sample inlets. Avoid contacting the beadstripe area and sample inlets.

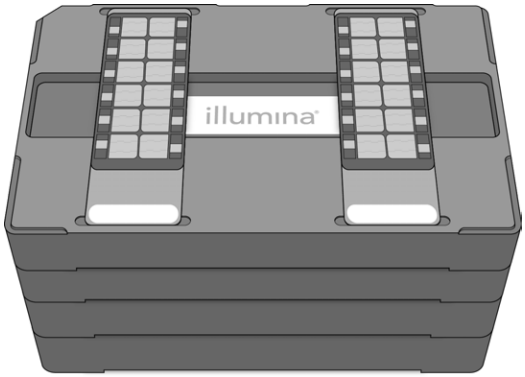
2 Place BeadChips into the Robot BeadChip Alignment Fixtures with the barcode end aligned to the ridges on the fixture.

Figure 87



3 Stack the Robot BeadChip Alignment Fixtures and carry them to the robot.

Figure 88 Four Stacked Robot BeadChip Alignment Fixtures

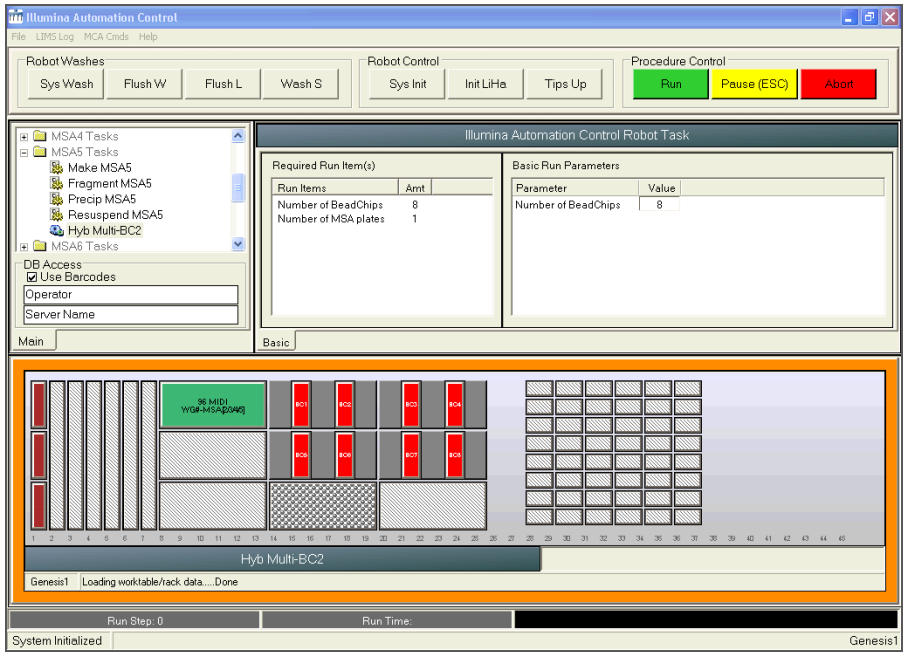


4 At the robot PC, select **MSA5 Tasks | Hyb Multi-BC2**.

5 Choose the appropriate BeadChip from the BeadChip Selection dialog box.

6 In the Basic Run Parameters pane, change the value for **Number of MSA5 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.
The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

Figure 89 Hyb Multi-BC Screen



7 Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map.

8 On the lab tracking form, record the plate position on the robot bed, BeadChip serial numbers, and BeadChip positions.

9 Pulse centrifuge the MSA5 plate to 280 xg.

10 Place the MSA5 plate onto the robot bed according to the bed map. Remove the foil seal.

Start the Robot

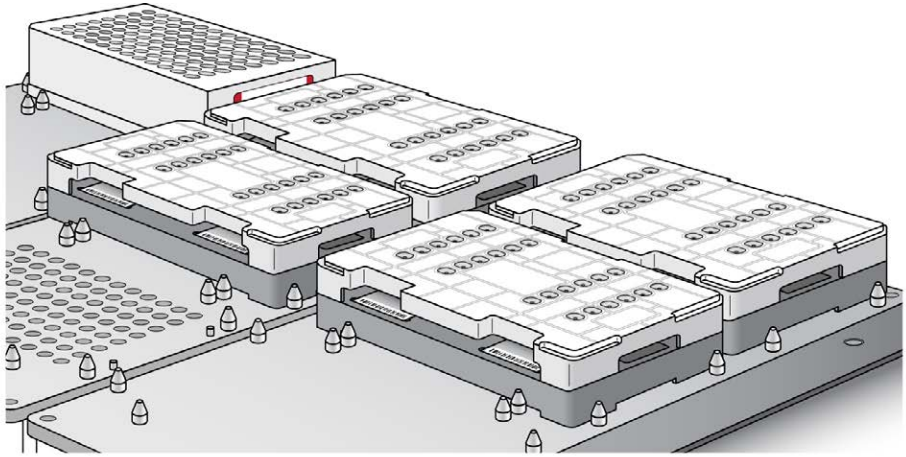
1 At the robot PC, click **Run**.

2 Place the Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture, with the Guide-B barcode upside down and facing away from you. Push both the

Robot Tip Alignment Guide and Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed.

3 At the robot PC, click **OK** to confirm that you have placed the Robot Tip Alignment Guide on top of the Robot BeadChip alignment fixture. The robot scans the barcode on the Robot Tip Alignment Guide to confirm that the correct tip guide is being used.

Figure 90 Robot Tip Alignment Guides on Robot Bed



The robot dispenses sample to the BeadChips.
The robot PC sounds an alert and opens a message when the process is complete.

4 Click **OK** in the message box.

5 Carefully remove the Robot BeadChip alignment fixtures from the robot bed and visually inspect all sections of the BeadChips. Make sure that DNA sample covers all the sections of each bead stripe. Record any sections that are not completely covered.

Set up BeadChip for Hybridization

1 Make sure that the Illumina Hybridization Oven is set to 48°C.

WARNING

Keep Hyb Chambers at room temperature when you load the BeadChips. Do not place the Hyb Chambers in the Illumina Hybridization Oven before loading the BeadChips. If you heat the PB2 and then open the Hyb Chamber to add BeadChips, some of the PB2 evaporates, leading to a change in the osmolality of PB2 and an imbalance in the vapor pressure between PB2 and RA1.

CAUTION

Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Do not hold the BeadChip by the sides near the sample inlets. Avoid contacting the beadstripe area and sample inlets.

2 Carefully remove each BeadChip from the Robot BeadChip alignment fixtures when the robot finishes.

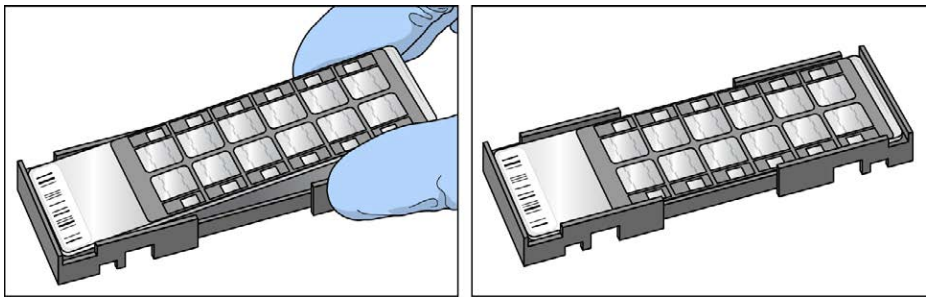
CAUTION

For optimal performance, take care to keep the Hyb Chamber inserts containing BeadChips steady and level when lifting or moving. Avoid shaking and always keep parallel to the lab bench. Do not hold by the sides near the sample inlets.

3 Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.

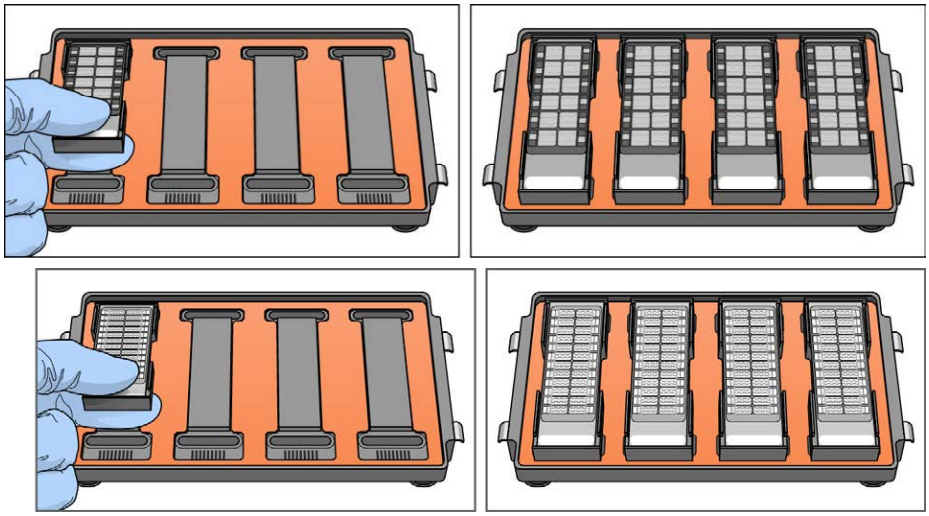
4 Carefully place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the insert.

Figure 91 Matching the Barcode End to the Insert Fixture



5 Load the Hyb Chamber inserts containing loaded BeadChips inside the Illumina Hyb Chamber. Position the barcode over the ridges indicated on the Hyb Chamber.

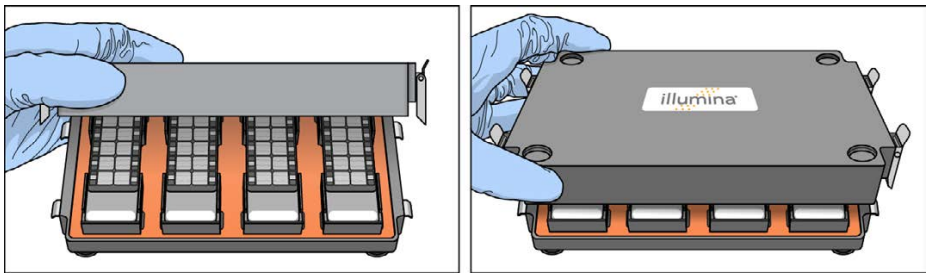
Figure 92 Placing Hyb Chamber Inserts into Hyb Chamber

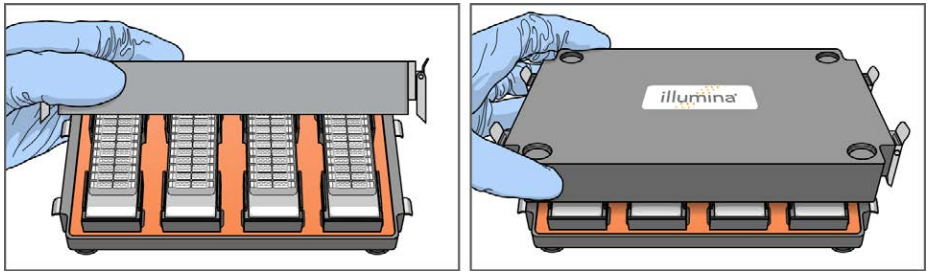


6 Make sure that Hyb Chamber inserts are seated properly.

7 Position the lid onto the Hyb Chamber by applying the backside of the lid first and then slowly bringing down the front end to avoid dislodging the Hyb Chamber inserts.

Figure 93 Seating Lid onto Hyb Chamber



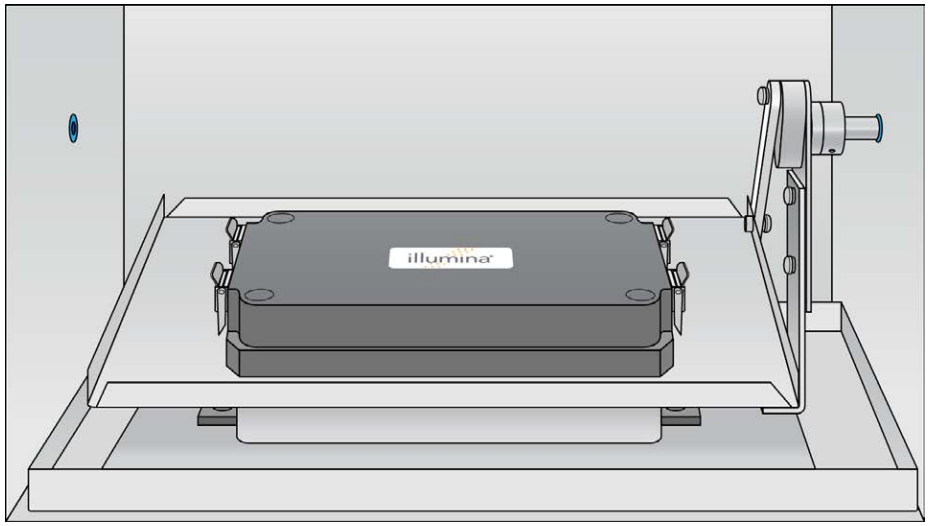


8 Close the clamps on both sides of the Hyb Chamber so that the lid is secure and even on the base (no gaps).
It is best to close them in a kitty-corner fashion, closing first the top left clamp, then the bottom right, then the top right followed by the bottom left.

NOTE
Keep the Hyb Chamber steady and level when moving it or transferring it to the Illumina Hybridization Oven.

9 Place the Hyb Chamber in the 48°C Illumina Hybridization Oven with the clamps on the left and right sides of the oven and the Illumina logo facing you.

Figure 94 Hyb Chamber Correctly Placed in Hyb Oven



NOTE

If you are stacking multiple Hyb Chambers in the Illumina Hybridization Oven, fit the feet of the top Hyb Chamber into the matching indents on the lid of the Hyb Chamber below it. The fitted feet and lid hold the Hyb Chambers in place while they are rocking. You can stack up to 3 Hyb Chambers per row for a maximum of 6 Hyb Chambers total in the Illumina Hybridization Oven.

Figure 95 Two Hyb Chambers Correctly Placed in Hyb Oven

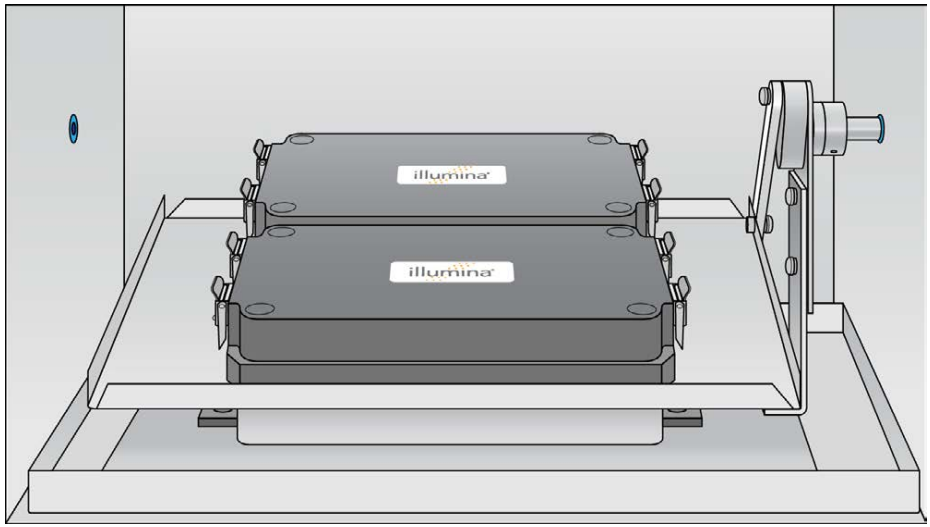
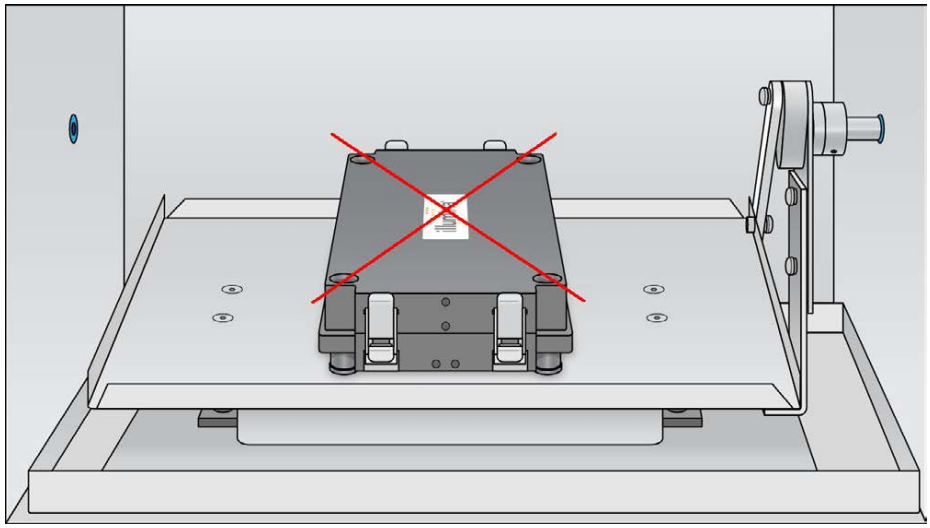


Figure 96 Incorrectly Placed Hyb Chamber



10 [Optional] Start the rocker, setting the speed to 5.

OVERNIGHT INCUBATION
Incubate at 48°C for at least 16 hours but no more than 24 hours.

11 Record the start and stop times on the lab tracking form.

12 Proceed to *Wash BeadChips (Post-Amp)* after the overnight incubation.

Resuspend XC4 Reagent for XStain BeadChip

Keep the XC4 in the bottle in which it was shipped until you are ready to use it. In preparation for the XStain protocol, follow these steps to resuspend the XC4 reagent.

1 Add 330 ml 100% EtOH to the XC4 bottle, for a final volume of 350 ml.
Each XC4 bottle has enough solution to process up to 24 BeadChips.

2 Shake the XC4 bottle vigorously to ensure complete resuspension. After it is resuspended, use XC4 at room temperature.
You can store it at 2°C to 8°C for 2 weeks if unused.

Wash the Robot Tip Alignment Guide

For optimal performance, wash and dry the Robot Tip Alignment Guides after every run.

1 Soak the tip guide inserts in a 1% aqueous Alconox solution (1 part Alconox to 99 parts water) using a 400 ml Pyrex beaker for 5 minutes.

NOTE
Do not use bleach or ethanol to clean the tip guide inserts.

2 After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the tip guides with DiH_2O at least 3 times to remove any residual detergent.

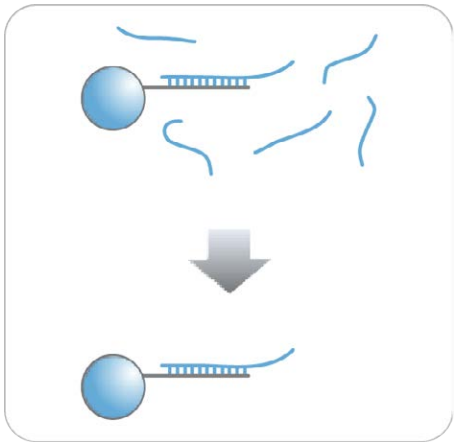
3 Dry the Robot Tip Alignment Guide using a Kimwipe or lint-free paper towels. Use a laboratory air gun to dry. Be sure to inspect the tip guide channels, including the top and bottom. Tip guides must be dry and free of any residual contaminates before next use.

Wash BeadChips (Post-Amp)

In this procedure, the BeadChips are prepared for the XStain process.

Remove the cover seals from the BeadChips and wash the BeadChips in 2 separate PB1 reagent washes. Then, assemble the BeadChips into flow-through chambers under the PB1 buffer.

Figure 97 Washing BeadChip



Estimated Time

- 20 minutes for 4 BeadChips
- 30 minutes for 8 BeadChips

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml for 1 alignment fixture 700 ml for 2 alignment fixtures 850 ml for 3 alignment fixtures	Room temperature	Illumina
Multisample BeadChip alignment fixture	1 (per 8 BeadChips)		Illumina
Te-Flow flow-through chambers, with black frames, spacers, glass back plates, and clamps	1 (per BeadChip)		Illumina
Wash dish	2 (up to 8 BeadChips)		Illumina
Wash rack	1 (up to 8 BeadChips)		Illumina

CAUTION

Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

WARNING

This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

1 Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 30 minutes before opening.

2 Have ready on the lab bench:
   a Two wash dishes:
      — Containing 200 ml PB1, and labeled as such
   b Multi-Sample BeadChip Alignment Fixture
      — Using a graduated cylinder, fill with 150 ml PB1
   c Te-Flow flow-through chamber components:
      — Black frames
      — Spacers (separated for ease of handling)
      — Clean glass back plates as directed in the *Infinium Lab Setup and Procedures Guide*.
      — Clamps

3 On the lab tracking form, record:
   - Date/Time
   - Operator
   - PB1 bottle barcode
   - Robot

NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Steps to Wash BeadChips

1 Attach the wire handle to the rack and submerge the wash rack in the wash dish containing 200 ml PB1.

Figure 98 Wash Rack in Wash Dish Containing PB1

2 Remove the Hyb Chamber inserts from the Hyb Chambers.

3 Remove each BeadChip from the Hyb Chamber insert.

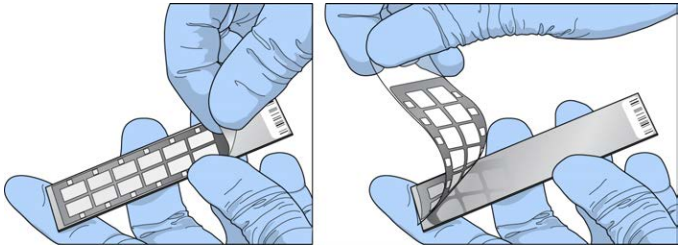
4 Remove the cover seal from each BeadChip.

NOTE
To make sure that no solution splatters on you, Illumina recommends removing the cover seal over an absorbent cloth or paper towels, preferably in a hood.

a Using powder-free gloved hands, hold the BeadChip securely and by the edges in one hand. Avoid contact with the sample inlets. Make sure that the barcode is facing up and closest to you, and that the top side of the BeadChip is angled slightly away from you.

b Remove the entire seal in a single, continuous motion. Start with a corner on the barcode end and pull with a continuous upward motion away from you and towards the opposite corner on the top side of the BeadChip.

Figure 99 Removing the Cover Seal



c Discard the cover seal.

CAUTION
Do not touch the arrays!

5 Immediately and carefully slide each BeadChip into the wash rack, making sure that the BeadChip is submerged in the PB1.

Figure 100 Submerging BeadChips in Wash Dish Containing PB1

6 Repeat steps 4 through 5 until all BeadChips (a maximum of 8) are transferred to the submerged wash rack.

7 After all BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

8 Move the wash rack to the other wash dish containing clean PB1. Make sure the BeadChips are submerged.

9 Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

10 When you remove the BeadChips from the wash rack, inspect them for remaining residue.

NOTE
Residue that can adversely affect results is sometimes left on BeadChips after seals are removed. If there is residue left on the BeadChips after the second PB1 wash, use a 200 µl pipette tip for each BeadChip and slowly and carefully scrape off the residues outward (away) from the bead-sections under PB1. Use a new pipette tip for each BeadChip. Then, continue with the protocol.

11 For each additional set of 8 BeadChips:

a Assemble the flow-through chambers for the first 8 BeadChips, as described in *Assemble Flow-Through Chambers* on page 58.

b Repeat the wash steps in this section to wash the next set of 8 BeadChips.

Assemble Flow-Through Chambers

1 If you have not done so, fill the BeadChip alignment fixture with 150 ml PB1.
If you plan to process more than 4 BeadChips, this 150 ml of PB1 can be reused for an additional set of 4 BeadChips. Use 150 ml of fresh PB1 for every additional set of 8 BeadChips.

2 For each BeadChip to be processed, place a black frame into the BeadChip alignment fixture prefilled with PB1.

Figure 101 Placing Black Frames into BeadChip Alignment Fixture

3 Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the alignment fixture.

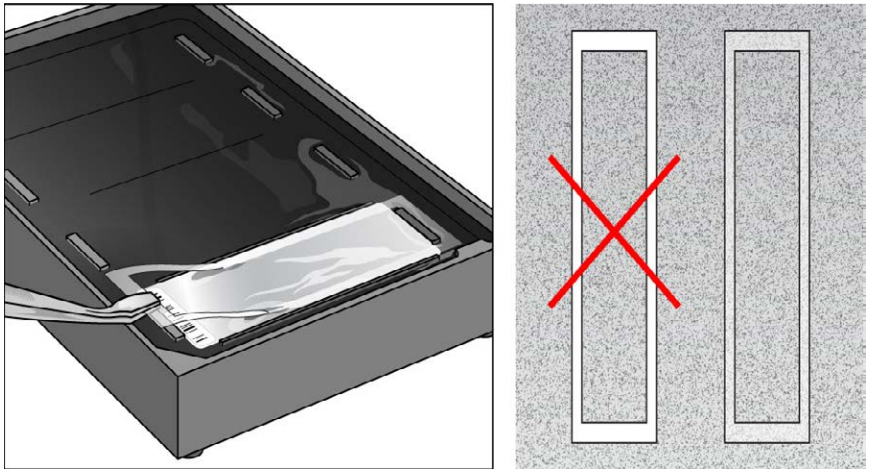
NOTE
Inspect the surface of each BeadChip for residue left by the seal. Use a pipette tip to remove any residue under buffer and be careful not to scratch the bead area.

Figure 102 Placing BeadChip into Black Frame on Alignment Fixture

4 Place a clear spacer onto the top of each BeadChip. Use the alignment fixture grooves to guide the spacers into proper position.

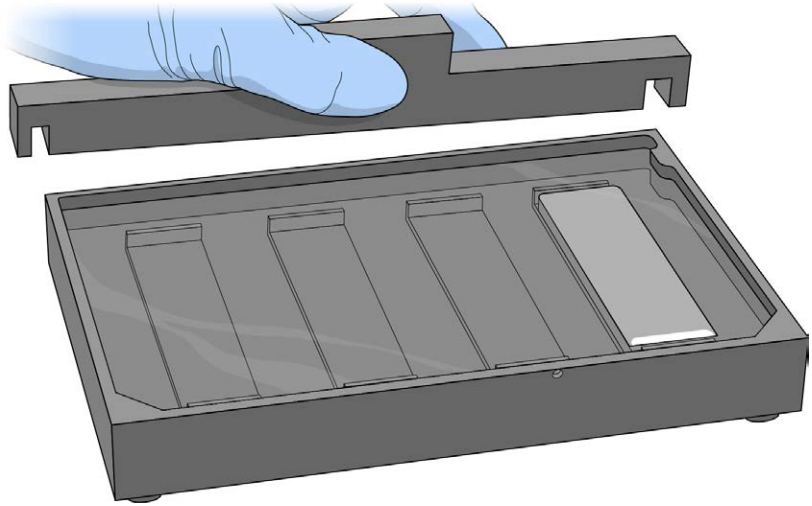
NOTE
Be sure to use the clear plastic spacers, not the white ones.

Figure 103 Placing Clear Plastic Spacer onto BeadChip



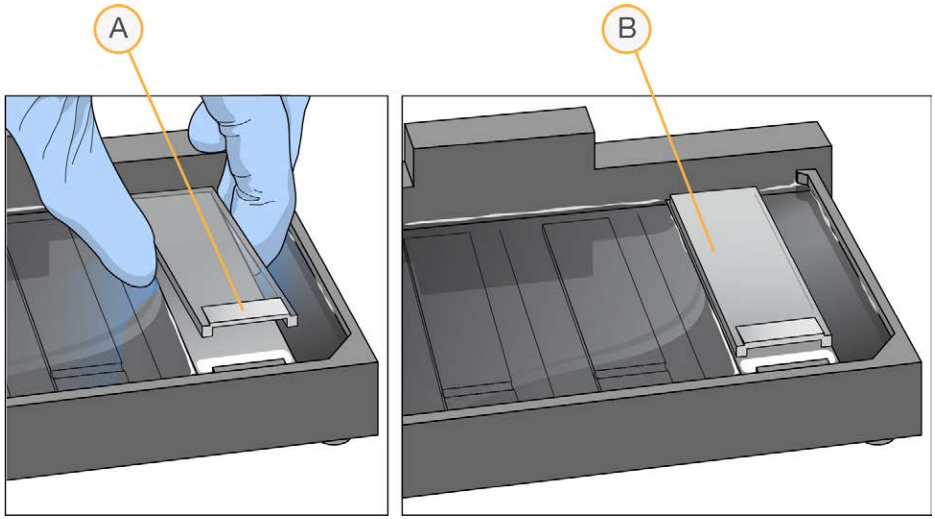
5 Place the alignment bar onto the alignment fixture.
The groove in the alignment bar fits over the tab on the alignment fixture.

Figure 104 Placing Alignment Bar onto Alignment Fixture



6 Place a clean glass back plate on top of the clear spacer covering each BeadChip. The plate reservoir is at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface.

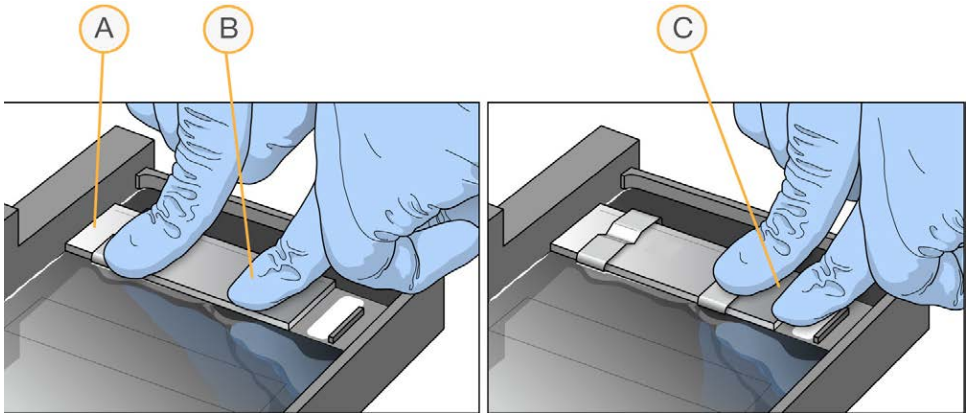
Figure 105 Placing Glass Back Plate onto BeadChip



A Reservoir at Barcode End of Glass Back Plate
B Glass Back Plate in Position

7 Attach the metal clamps to the flow-through chambers as follows:
   a Gently push the glass back plate against the alignment bar with one finger.
   b Place the first metal clamp around the flow-through chamber so that the clamp is approximately 5 mm from the top edge.
   c Place the second metal clamp around the flow-through chamber at the barcode end, approximately 5 mm from the reagent reservoir.

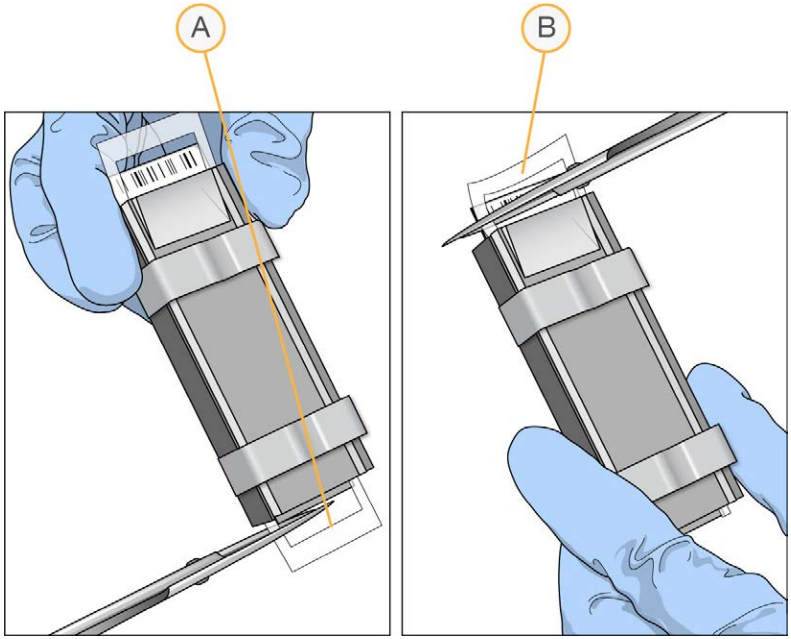
Figure 106 Securing Flow-Through Chamber Assembly with Metal Clamps



A One Stripe Shows Between First Clamp and Alignment Bar
B Glass Back Plate Pressed Against Alignment Bar
C No Stripes Show Between Second Clamp and Barcode

8 Using scissors, trim the ends of the clear plastic spacers from the flow-through chamber assembly. Slip scissors up over the barcode to trim the other end.

Figure 107 Trimming Spacer Ends from Flow-Through Chamber Assembly



A Trim Spacer at Non-Barcode End of Flow-Through Chamber
B Trim Spacer at Barcode End of Flow-Through Chamber

9 ***Immediately*** wash the Hyb Chamber reservoirs with DiH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains in the Hyb Chamber reservoir.

CAUTION
It is important to wash the Hyb Chamber reservoirs immediately and thoroughly to make sure that no traces of PB2 remain in the wells.

10 Discard unused reagents in accordance with facility standards.

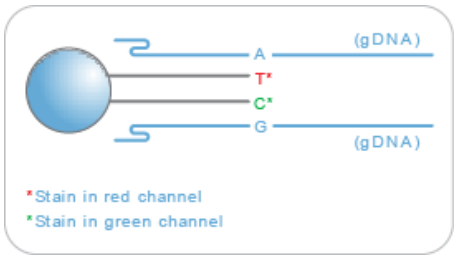
11 Proceed to *Extend and Stain (XStain) BeadChip (Post-Amp)*.

CAUTION
Place all assembled flow-through chambers on the lab bench in a horizontal position while you perform the preparation steps for the XStain BeadChip. Do not place the flow-through chambers in the chamber rack until the preparation is complete.

Extend and Stain (XStain) BeadChip

Following hybridization, RA1 reagent is used to wash away unhybridized and non-specifically hybridized DNA sample. XC1 and XC2 are added to condition the BeadChip surface for the extension reaction. TEM reagents are dispensed into the Flow-Through Chambers to perform single-base extension of primers hybridized to DNA on the BeadChip. This reaction incorporates labeled nucleotides into the extended primers. 95% formamide/1 mM EDTA is added to remove the hybridized DNA. After neutralization using the XC3 reagent, the labeled extended primers undergo a multi-layer staining process on the Chamber Rack. Next, the Flow-Through Chambers are disassembled. The BeadChips are washed in the PB1 reagent, and then coated with XC4 reagent and dried.

Figure 108 Extending and Staining BeadChip



Estimated Time

Robot time:

- 2 hours and 10 minutes for 8 BeadChips
- 2 hours and 25 minutes for 16 BeadChips
- 2 hours and 40 minutes for 24 BeadChips

Dry time: 55 minutes

Consumables

Item	Quantity	Storage	Supplied By
RA1	10 ml for 1-8 BeadChips 20 ml for 9-16 BeadChips 30 ml for 17-24 BeadChips	-25°C to -15°C	Illumina
XC1	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
XC2	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
TEM	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
XC3	50 ml for 1-8 BeadChips 100 ml for 9-16 BeadChips 150 ml for 17-24 BeadChips	Room temperature	Illumina
STM (Make sure that all STM tubes indicate the same stain temperature on the label)	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
ATM	1 tube (per 4 BeadChips)	-25°C to -15°C	Illumina
PB1	310 ml for 1-8 BeadChips 285 ml for 9-24 BeadChips	Room temperature	Illumina

Item	Quantity	Storage	Supplied By
XC4	310 ml for 1-8 BeadChips 285 ml for 9-24 BeadChips	Room temperature	Illumina
Alconox Powder Detergent	as needed	Room temperature	User
EtOH	as needed	Room temperature	User
95% formamide/1 mM EDTA	15 ml for 1-8 BeadChips 17 ml for 9-16 BeadChips 25 ml for 17-24 BeadChips	-25°C to -15°C	User

CAUTION

Pour out only the recommended reagent volume needed for the suggested number of beachships listed in the consumables table of each section. Some of the reagents are used later in the ptotocol.

NOTE

It is important to use fresh RA1 for each protocol step in the assay where it is required. RA1 that has been stored properly and has not been dispensed for use in either the XStain or Resuspension step is considered fresh RA1. After RA1 has been poured out into a reservoir and exposed to room temperature air for extended periods of time, it is no longer fresh.

WARNING

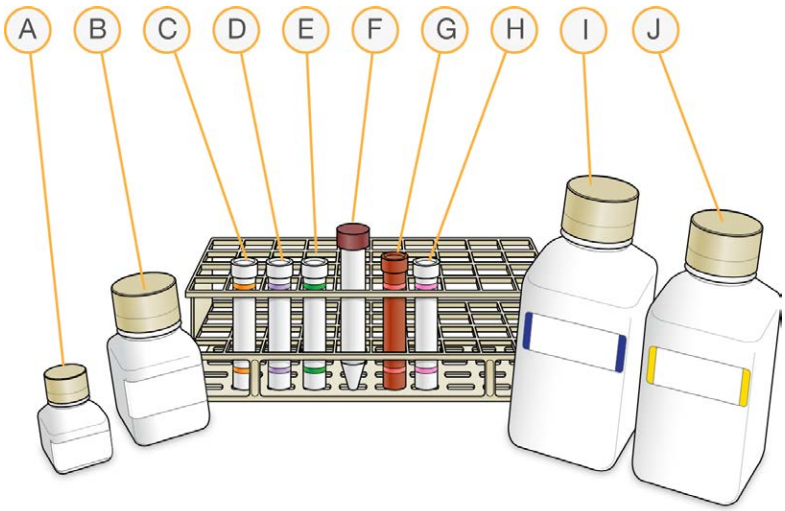
This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

1 RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that may be present.

2 Place all reagent tubes in a rack in the order in which they will be used. If frozen, allow them to thaw to room temperature, and then gently invert the reagent tubes at least 10 times to mix contents.

Figure 109 XStain BeadChip Reagent Tubes and Bottles



A RA1
B XC3
C XC1
D XC2
E TEM
F 95% Formamide / 1mM EDTA
G STM
H ATM
I PB1
J XC4

3 On the lab tracking form, record:
- Date/Time

- Operator
- Robot
- RA1 barcode
- XC3 barcode
- XC1 barcodes
- XC2 barcodes
- TEM barcodes
- STM barcodes
- ATM barcodes
- PB1 barcode
- XC4 barcodes

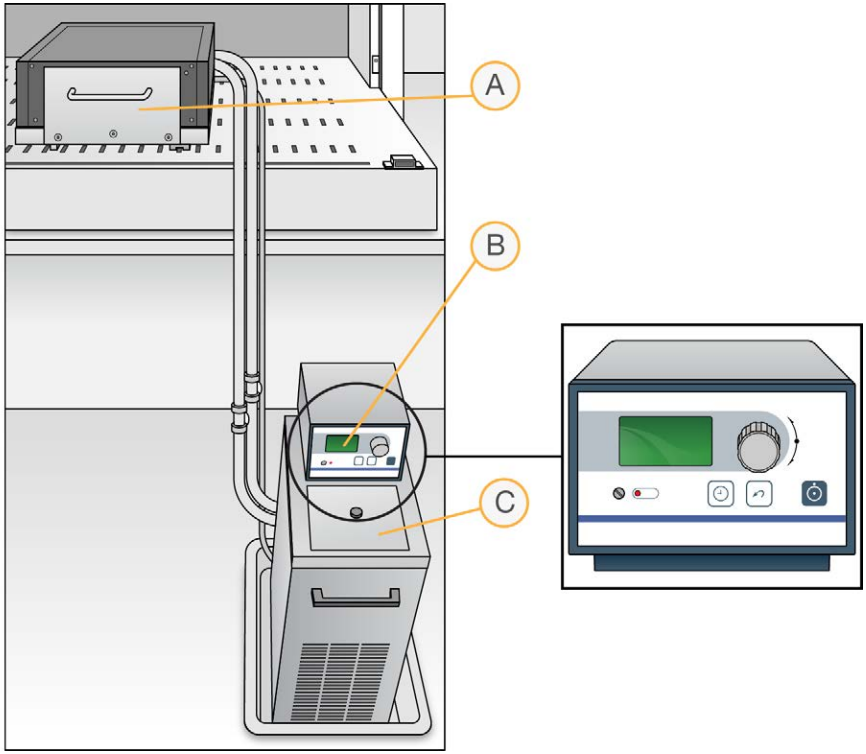
NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Set Up Chamber Rack

1. Make sure that the water circulator reservoir is filled with water to the appropriate level. See the *VWR Operator Manual*, VWR part # 110-229.
2. Turn on the water circulator and set it to a temperature that brings the chamber rack to 44°C at equilibrium.
   This temperature can vary depending on facility ambient conditions.

Figure 110 Water Circulator Connected to Chamber Rack



A Chamber Rack
B Water Circulator with Programmable Temperature Controls
C Reservoir Cover

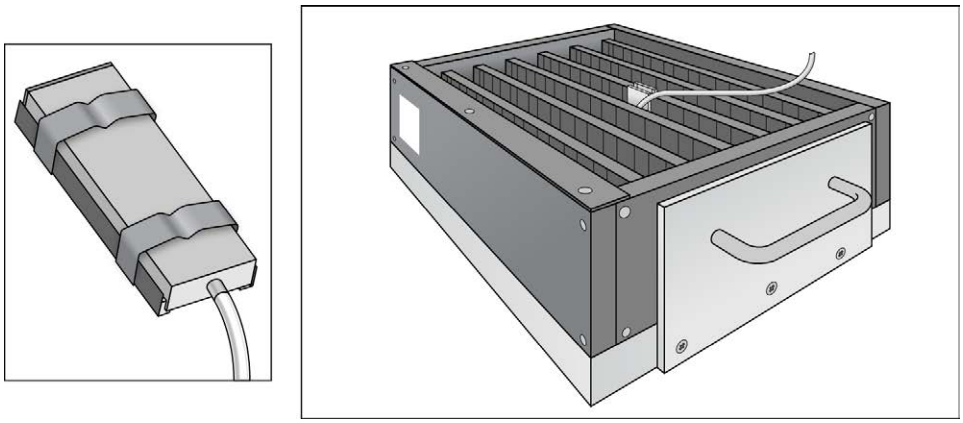
3 Confirm the temperature using the temperature probe for the chamber rack. The temperature displayed on the water circulator LCD screen might differ from the actual temperature on the chamber rack.

4 Make sure that you remove bubbles trapped in the chamber rack ***each time*** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.

5 Use the Illumina Temperature Probe in several locations to make sure that the chamber rack is at 44°C. Make sure that all locations are at 44°C ± 0.5°C.

NOTE
Do not leave the temperature probe in the first 3 rows of the chamber rack. Reserve this space for BeadChips.

Figure 111 Illumina Temperature Probe and Temperature Probe in Chamber Rack



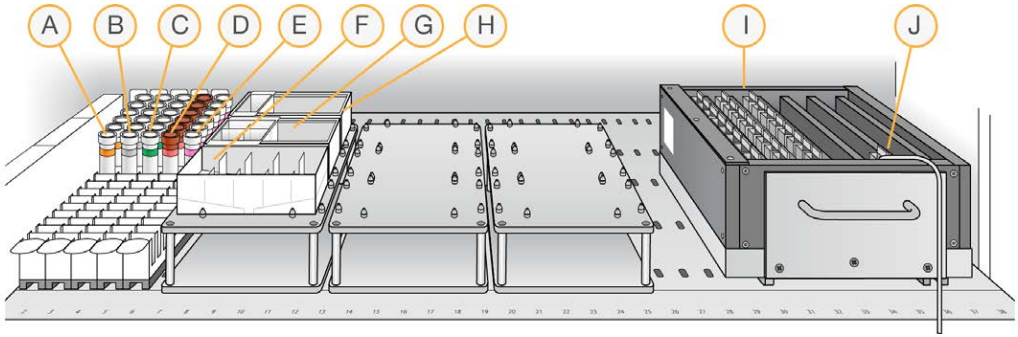
6 For accurate temperature measurement, make sure that the Illumina Temperature Probe is touching the base of the chamber rack.

Prepare Robot

For instructions on preparing the robot for use in a protocol, see the *Infinium Assay Lab Setup and Procedures Guide*

Refer to the following figure throughout this protocol.

Figure 112 Tecan 8-Tip Robot (XStain BeadChip Setup)



A XC1
B XC2
C TEM
D STM
E ATM
F XC3 in Full Reservoir
G RA1 in Half Reservoir
H 95% Formamide / 1 mM EDTA in Quarter Reservoir
I 24 BeadChips in Chamber Rack
J Temperature Probe

Single-Base Extension and Stain

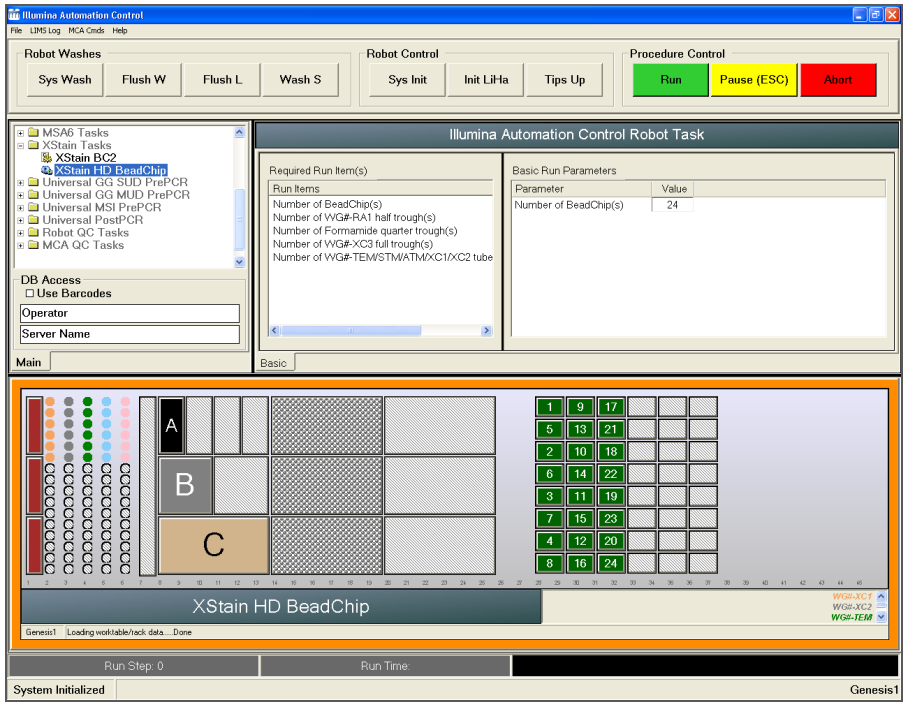
CAUTION
The remaining steps must be performed without interruption.

1 Slide the chamber rack into column 36 on the robot bed. Make sure that it is seated properly.

2 At the robot PC, select **XStain Tasks | XStain HD BeadChip**.

3 In the Basic Run Parameters pane, enter the number of BeadChips.
You can process up to 24 BeadChips in the XStain BeadChip process.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed. All barcodes must face to the left.

Figure 113 XStain HD BeadChip Screen



4 If you plan on imaging the BeadChip immediately after the staining process, turn on the iScan or HiScan now to allow the lasers to stabilize.

5 Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add 95% formamide/1 mM EDTA as follows:

- 15 ml to process 8 BeadChips
- 17 ml to process 16 BeadChips
- 25 ml to process 24 BeadChips

6 Place a half reservoir in the reservoir frame, according to the robot bed map, and add RA1 in the following volumes:

- 10 ml to process 8 BeadChips
- 20 ml to process 16 BeadChips
- 30 ml to process 24 BeadChips

7 Place a full reservoir in the reservoir frame, according to the robot bed map, and add XC3 in the following volumes:
- 50 ml to process 8 BeadChips
- 100 ml to process 16 BeadChips
- 150 ml to process 24 BeadChips

8 Place each reagent tube (XC1, XC2, TEM, STM, ATM) in the robot tube rack according to the bed map, and remove their caps.

9 Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start Robot

1 At the robot PC, click **Run**.

2 When prompted, enter the stain temperature indicated on the STM tube.

3 When the prompt appears, wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.

4 When the temperature probe registers 44°C, click **OK**.

5 When prompted, load the BeadChips and click **OK**.

6 Place each assembled flow-through chamber in the first row of the chamber rack. Refer to the robot bed map for the correct layout.

7 Make sure that each flow-through chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.

8 Record the chamber rack position associated with each BeadChip on the lab tracking form.

9 Click **OK**. A series of reactions begins, each with a wait time. Message boxes on the robot PC tell you which reaction is occurring and how long the wait time is.

Table 12 List of Reactions

#	Reagent	Wait Time
1	RA1	3 minutes
2	XC1	10 minutes
3	XC2	10 minutes
4	TEM	15 minutes
5	Formamide/EDTA	7 minutes
6	XC3	2 minutes
7	STM	10 minutes
8	XC3	7 minutes
9	ATM	10 minutes
10	XC3	7 minutes
11	STM	10 minutes
12	XC3	7 minutes
13	ATM	10 minutes
14	XC3	7 minutes
15	STM	10 minutes
16	XC3	7 minutes

10 When prompted, remove the BeadChips from the chamber rack immediately and place them horizontally on the lab bench at room temperature. Click **OK** in the message box.

11 The robot PC sounds an alert and opens a message when the process is complete. Click **OK** to finish the process.

Wash and Coat 8 BeadChips

Before starting the Wash and Coat process, read these important notes:

- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates before use.
- In preparation for XC4 BeadChip coating, wash the tube racks and wash dishes thoroughly before and after use. Rinse with DI water. Immediately following wash, place racks and wash dishes upside down on a wash rack to dry.
- Place Kimwipes in 3 layers on the lab bench. Place a tube rack on top of these Kimwipe layers. Do not place on absorbent lab pads. You will place the staining rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

Equipment Needed

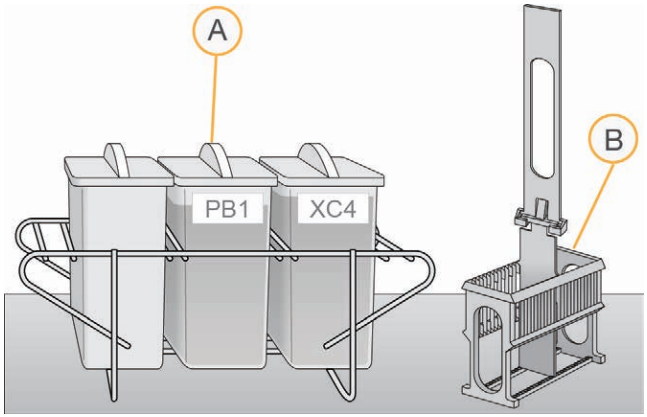
Place the following items on the bench:

- 1 staining rack
- 1 vacuum desiccator
- 1 tube rack
- Self-locking tweezers
- Large Kimwipes
- Vacuum hose

Steps

1. Set up 2 top-loading wash dishes, labeled "PB1" and "XC4".
2. To indicate the fill volume before filling wash dishes with PB1 and XC4, pour 310 ml water into the wash dishes and mark the water level on the side. Empty the water from the wash dish. Marking the level enables you to pour reagent directly from the PB1 and XC4 bottles into the wash dishes, minimizing contaminant transfer from labware to wash dishes.

Figure 114 PB1 and XC4 Wash Dishes with Staining Rack

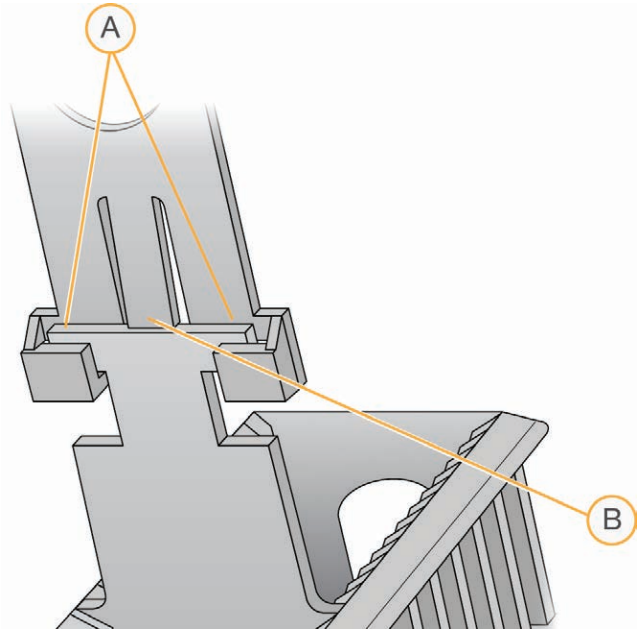


A Wash Dishes
B Staining Rack

3 Pour 310 ml PB1 into the wash dish labeled "PB1."

4 Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing towards*** you. This orientation makes it easier and safer to remove the BeadChips. Let the staining rack sit in the wash dish. You will use it to carry the BeadChips after disassembling the flow-through chambers.

Figure 115 Staining Rack Locking Arms and Tab



A Locking Arms
B Tab

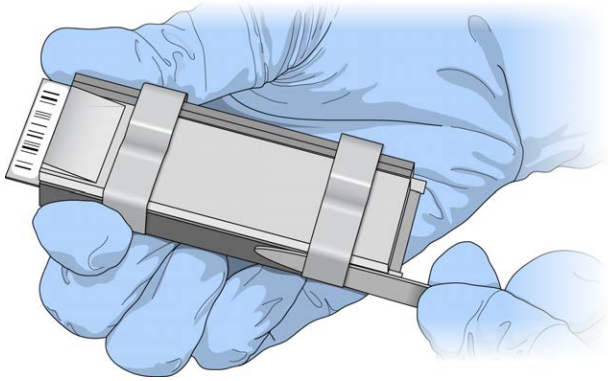
CAUTION
If the staining rack handle is not correctly oriented, the BeadChips can be damaged when you remove the staining rack handle before removing the BeadChips.

5 One at a time, disassemble each flow-through chamber:

a Use the dismantling tool to remove the 2 metal clamps.

CAUTION
It is important to use the dismantling tool to avoid chipping the glass back plates.

Figure 116 Removing the Metal Clamps from Flow-Through Chamber



b Remove the glass back plate.

c Set the glass back plate aside. When you finish the XStain HD BeadChip protocol, clean the glass back plates as described in the *Infinium Lab Setup and Procedures Guide*.

d Remove the spacer. To avoid damaging the stripes on the BeadChip, pull the spacer out so that the long sides slide along the sides of the BeadChip.

e Remove the BeadChip.

CAUTION
Do not touch the face of the BeadChips. Handle them by the barcode end or by the edges.

6 Place the BeadChips in the staining rack while it is submerged in PB1. Put 4 BeadChips above the staining rack handle and 4 below the handle. Make sure that the BeadChip barcodes ***face away*** from you and that the locking arms on the handle ***face towards*** you.

If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting each BeadChip.

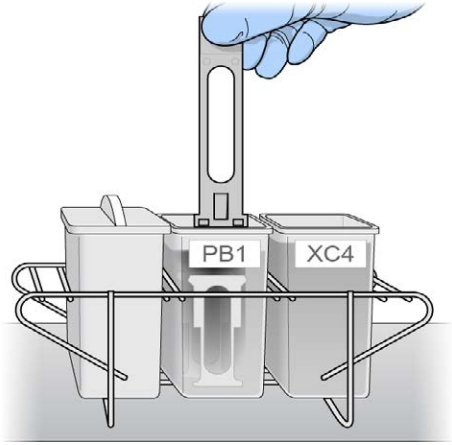
7 Make sure that the BeadChips are submerged.

CAUTION
Do not allow the BeadChips to dry. Submerge each BeadChip in the wash dish as soon as possible.

8 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

Figure 117 Washing BeadChips in PB1



9 Allow the BeadChips to soak for an additional 5 minutes.

CAUTION
Do not leave the BeadChips in the PB1 for more than 30 minutes.

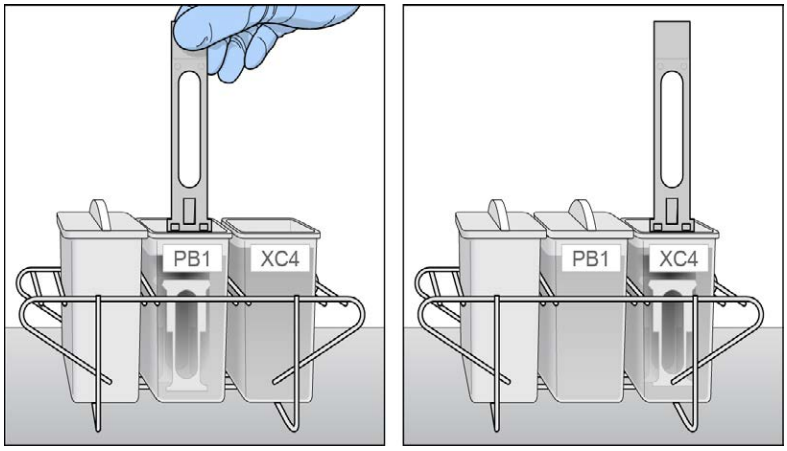
10 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until dissolved.

11 Pour 310 ml XC4 into the dish labeled "XC4," and cover the dish to prevent any lint or dust from falling into the solution.

CAUTION
Do not let the XC4 sit for longer than 10 minutes.

12 Remove the staining rack from the PB1 dish and place it directly into the wash dish containing XC4. For proper handling and coating, The barcode labels on the BeadChips must ***face away*** from you; the locking arms on the handle must ***face towards*** you.

Figure 118 Moving BeadChips from PB1 to XC4



13 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

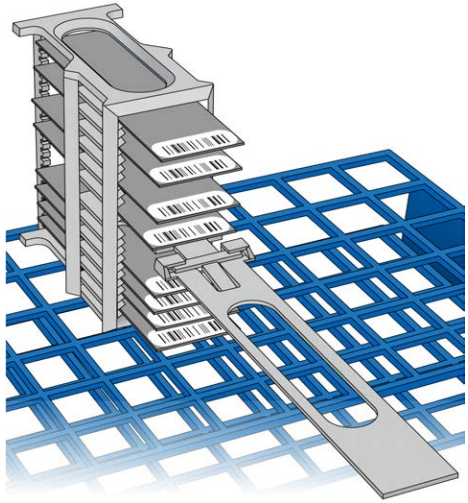
14 Allow the BeadChips to soak for an additional 5 minutes.

CAUTION
Use XC4 only one time. To process subsequent BeadChips, use a new, clean wash dish with fresh XC4.

15 Prepare 1 additional tube rack per 8 BeadChips (Illumina-provided from VWR catalog # 60916-748) that fits the internal dimensions of vacuum desiccator.

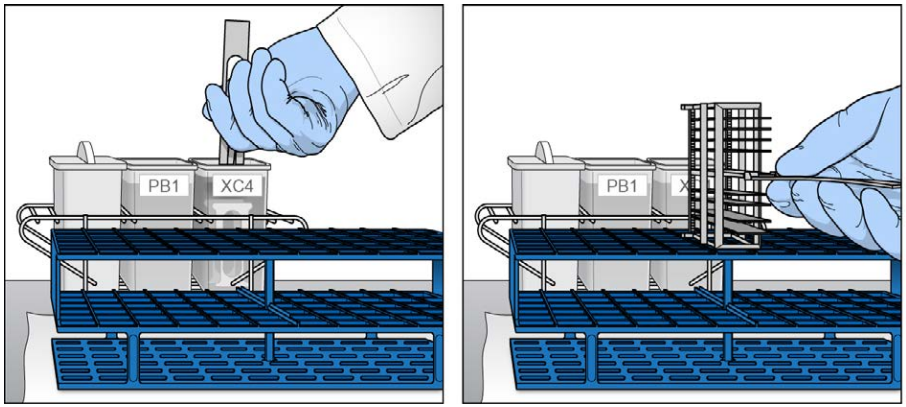
16 Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes ***face up***, and the locking arms and tabs ***face down***.

Figure 119 Staining Rack in Correct Orientation



To ensure uniform coating, place the staining rack on the center of the tube rack, avoiding the raised edges.

Figure 120 Moving the Staining Rack from XC4 to Tube Rack



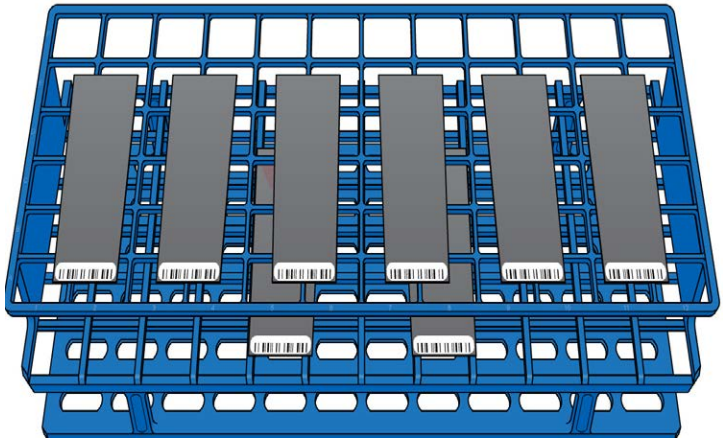
17 For each of the top 4 BeadChips, working top to bottom:

a Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

NOTE
The XC4 coat is slippery and makes the BeadChips difficult to hold. The self-locking tweezers grip the BeadChip firmly and help prevent damage.

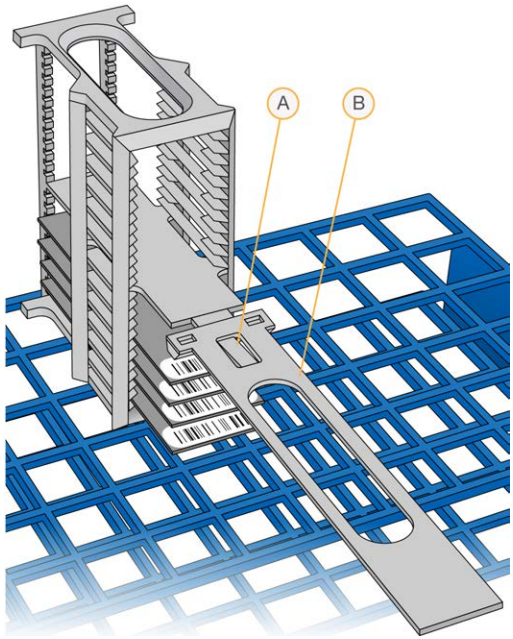
b Place each BeadChip on a tube rack with the barcode ***facing up and towards*** you.

Figure 121 BeadChips on Tube Rack



18 Holding the top of the staining rack in position, gently remove the staining rack handle by grasping the handle between the thumb and forefinger. Push up the tab with your thumb and push the handle away from you (unlocking the handle), then pull up the handle and remove.

Figure 122 Removing Staining Rack Handle



A Tab
B Handle

19 Remove the remaining BeadChips to the tube rack, with 6 BeadChips on top of the rack and 2 BeadChips on the bottom. Make sure that the barcode ends are towards you, and the BeadChips are completely horizontal.

CAUTION
To prevent wicking and uneven drying, do not allow the BeadChips to rest on the edge of the tube rack or to touch each other while drying.

20 Place the tube rack in the vacuum desiccator. Each desiccator can hold 1 tube rack (8 BeadChips).

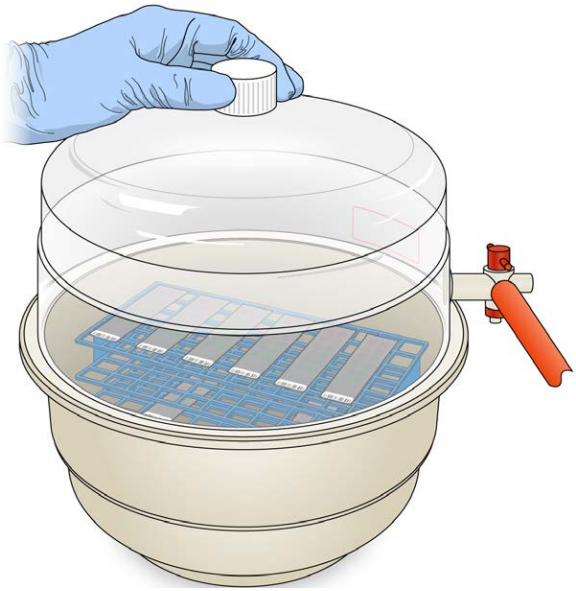
CAUTION
Make sure that the vacuum valve is seated tightly and securely.

21 Remove the red plug from the three-way valve before applying vacuum pressure.

22 Start the vacuum, using at least 675 mm Hg (0.9 bar).

23 To make sure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.

Figure 123 Testing Vacuum Seal



24 Dry under vacuum for 50–55 minutes.
Drying times can vary according to room temperature and humidity.

25 Release the vacuum by turning the handle very slowly.

WARNING
Make sure that air enters the desiccator very slowly to avoid disturbing the contents. Improper use of the vacuum desiccator can result in damage to the BeadChips, especially if you remove the valve plug while a vacuum is applied. For detailed vacuum desiccator instructions, see the documentation included with the desiccator.

26 Store the desiccator with the red valve plug in the 3-way valve of the desiccator to stop accumulation of dust and lint within the valve port.

27 Touch the borders of the BeadChips (**do not touch the stripes**) to make sure that the etched, barcoded sides are dry to the touch.

28 If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom 2 BeadChips are most likely to have some excess.
   a Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
   b Wipe along the underside of the BeadChip 5 or 6 times, until the surface is clean and smooth.

CAUTION
Do ***not*** touch the stripes with the wipe or allow EtOH to drip onto the stripes.

29 Clean the glass back plates. For instructions, see the *Infinium Lab Setup and Procedures Guide*.

30 Discard unused reagents in accordance with facility standards.

31 Do either of the following:
- Proceed to *Image BeadChip (Post-Amp)*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature. Be sure to image the BeadChips within 72 hours.

Wash and Coat 16–24 BeadChips

Follow either the 16–24 BeadChips Process (shown below) or the 8 BeadChips Process (see *Wash and Coat 8 BeadChips*).

Before starting the Wash and Coat process, please read these important notes:

- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates before use.
- In preparation for XC4 BeadChip coating, wash the tube racks and wash dishes thoroughly before and after use. Rinse with DI water. Immediately following wash, place racks and wash dishes upside down on a wash rack to dry.
- Place Kimwipes in three layers on the lab bench. Place a tube rack on top of these Kimwipe layers. Do not place on absorbent lab pads. You will place the staining rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

Equipment Needed

- 1 staining rack
- 3 vacuum desiccators (1 per 8 samples)
- 3 tube racks (1 per 8 samples)
- Self-locking tweezers
- Large Kimwipes
- Vacuum hose

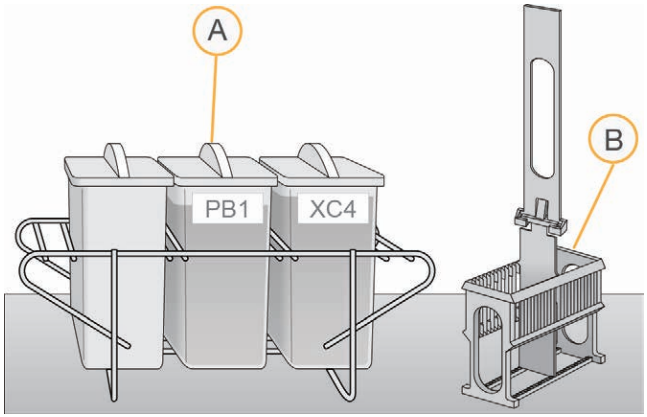
Steps

1 Dispense 285 ml PB1 into a wash dish, and then cover the dish.

2 Set up two top-loading wash dishes, labeled "PB1" and "XC4".

3 To indicate the fill volume before filling wash dishes with PB1 and XC4, pour 285 ml water into the wash dishes and mark the water level on the side. Empty the water from the wash dish. This enables you to pour reagent directly from the PB1 and XC4 bottles into the wash dishes, minimizing contaminant transfer from labware to wash dishes.

Figure 124 PB1 and XC4 Wash Dishes with Staining Rack

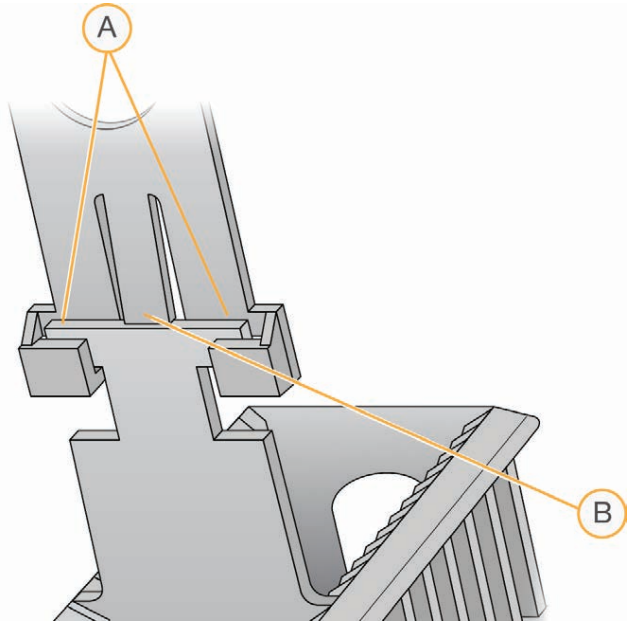


A Wash Dishes
B Staining Rack

4 Pour 285 ml PB1 into the wash dish labeled "PB1."

5 Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing you***. This orients the staining rack so that you can safely remove the BeadChips.

Figure 125 Staining Rack Locking Arms and Tabs



A Locking Arms
B Tab

CAUTION
If the staining rack handle is not correctly oriented, the BeadChips may be damaged when you remove the staining rack handle before removing the BeadChips.

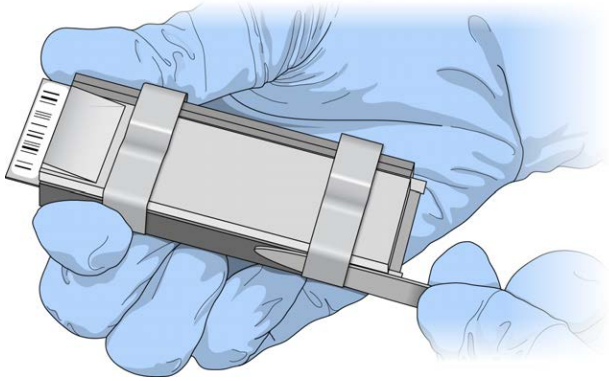
6 Let the staining rack sit in the wash dish. You will use it to carry the BeadChips after disassembling the Flow-Through Chambers.

7 One at a time, disassemble each Flow-Through Chamber:

a Using the dismantling tool, remove the two metal clamps.

CAUTION
It is important to use the dismantling tool to avoid chipping the glass back plates.

Figure 126 Removing Metal Clamps from Flow-Through Chamber



b Remove the glass back plate.

c Set the glass back plates aside. When you finish the XStain HD BeadChip protocol, clean the glass back plates as described in the *Infinium Assay Lab Setup and Procedures Guide*.

d Remove the spacer.

e Remove the BeadChip.

CAUTION
Do not touch the face of the BeadChips. Handle them by the barcode end or by the edges.

8 Place BeadChips in the staining rack while it is submerged in PB1.

- For 16 BeadChips, place 8 above the handle and 8 below.
- For 24 BeadChips, place 12 above the handle and 12 below.

The BeadChip barcodes should ***face away*** fromyou, while the locking arms and tab ***face towards*** you.

If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting the BeadChip.

9 Ensure that the BeadChips are completely submerged.

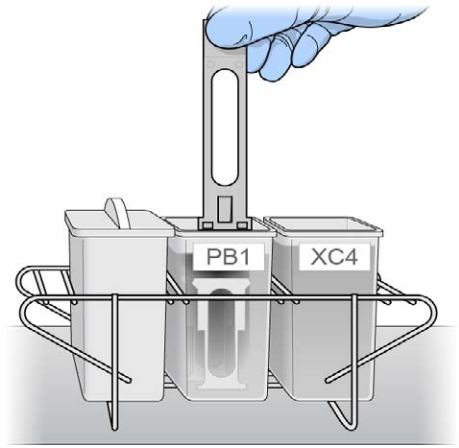
CAUTION
Do not allow the BeadChips to dry. Submerge each BeadChip in the wash dish as soon as possible.

10 Move the staining rack up and down 10 times, breaking the surface of the PB1.

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

11 Allow the BeadChips to soak for an additional 5 minutes.

Figure 127 Washing BeadChips in PB1



NOTE
Do not leave the BeadChips submerged in PB1 for longer than 30 minutes.

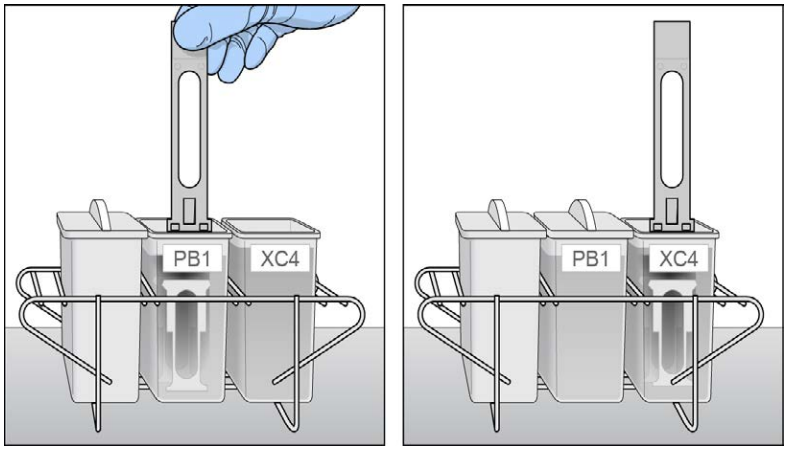
12 Pour 285 ml XC4 into the dish labeled "XC4," and cover the dish to prevent any lint or dust from falling into the solution. Place the bottle with excess XC4 in a readily available location for topping off the 'XC4' wash dish during the coating procedure.

NOTE
Use the XC4 within 10 minutes after filling the wash dish.

13 Remove the staining rack from the dish containing PB1 and place it directly into the wash dish containing XC4. The barcode labels on the BeadChips must ***face away*** from you, while the locking arms on the handle ***face towards*** you, for proper handling and coating.

14 Move the staining rack up and down 10 times, breaking the surface of the XC4.

Figure 128 Moving BeadChips from PB1 to XC4



NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

15 Allow the BeadChips to soak for an additional 5 minutes.

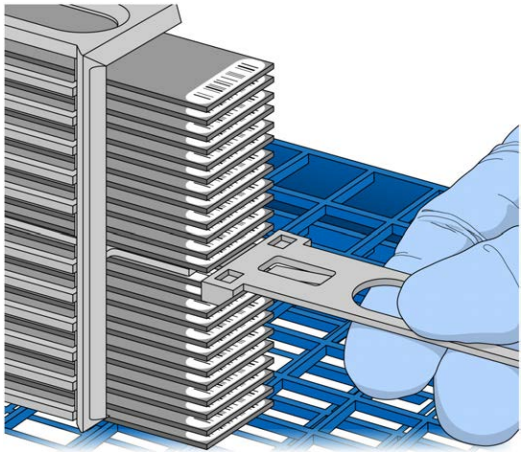
CAUTION
Use XC4 only once. To process subsequent BeadChips, use a new, clean wash dish with fresh XC4.

16 Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.

17 Prepare one additional tube rack per 8 BeadChips (Illumina-provided from VWR catalog # 60916-748) that fits the internal dimensions of the vacuum desiccator.

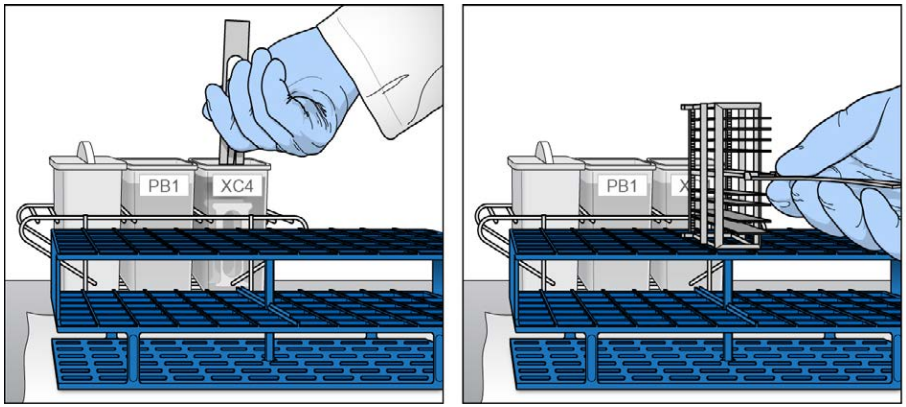
18 Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes face ***up*** and the locking arms and tab face ***down***.

Figure 129 Staining Rack in Correct Orientation



19 To ensure uniform coating, place the staining rack on the center of the tube rack, avoiding the raised edges.

Figure 130 Moving the Staining Rack from XC4 to Tube Rack



20 For the **top eight** BeadChips, working top to bottom:

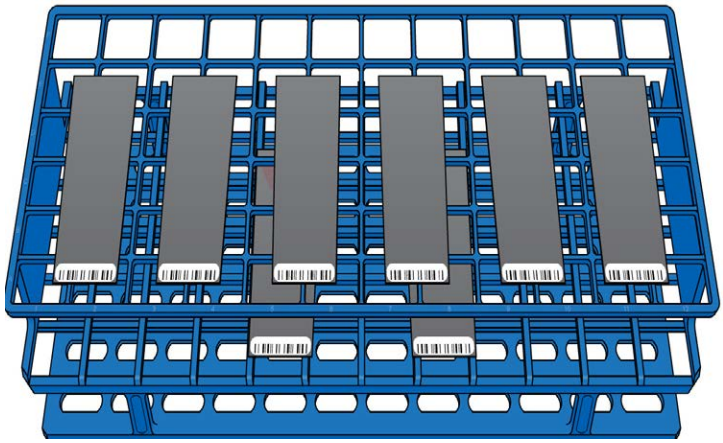
a Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers. Remove the rack handle if it facilitates removal of the BeadChips.

NOTE
The XC4 coat is slippery and makes the BeadChips difficult to hold. The self-locking tweezers grip the BeadChip firmly and help prevent damage.

b Put the eight BeadChips on the tube rack as shown in Figure 131, with six BeadChips on top of the rack and two BeadChips on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.

CAUTION
To prevent wicking and uneven drying, do not allow the BeadChips to rest on the edge of the tube rack or to touch each other while drying.

Figure 131 BeadChips on Tube Rack



If you are processing 16 BeadChips, 8 remain below the staining rack handle. If you are processing 24 BeadChips, 4 remain above the staining rack handle and 12 below.

21 Return the staining rack to the XC4 wash dish and top off wash dish until BeadChips are completely covered with remaining XC4 reagent.

22 Soak the BeadChips for 10 seconds.

23 Dry the **first 8** BeadChips:

a Place the tube rack with the first 8 BeadChips into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.
b Start the vacuum, using at least 675 mm Hg (0.9 bar).
c To ensure that the dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.
d Dry under vacuum for 50–55 minutes.

Drying times may vary according to room temperature and humidity.

24 Remove the staining rack with the remaining BeadChips in one rapid motion from the XC4 wash dish and place it directly on the tube rack. Ensure that the BeadChips are horizontal with the barcodes facing up.

25 If you are processing 24 BeadChips, remove the 4 BeadChips that remain above the staining rack handle and place them on the tube rack.

26 (For both 16- and 24-BeadChip processes) Holding the top of the staining rack in position, grasp the handle between your thumb and forefinger. Push the tab up with your thumb and push the handle away from you to unlock it. Pull up the handle and remove.

27 Place BeadChips on the tube rack until there are six BeadChips on top of the rack and two BeadChips on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.
If you are processing 24 BeadChips, 8 remain in the staining rack.

28 If you are processing 24 BeadChips:
a Return the staining rack with the last 8 BeadChips to the XC4 wash dish and top off the wash dish until BeadChips are completely covered with remaining XC4 reagent.
b Soak the BeadChips for 10 seconds.

29 Dry the **second set of 8** BeadChips:
a Place the tube rack with the second set of 8 BeadChips into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.
b Start the vacuum, using at least 675 mm Hg (0.9 bar).
c To ensure that the des sic at or is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.
d Dry under vacuum for 50–55 minutes.

30 If you are processing 24 BeadChips:

a Remove staining rack with the remaining 8 BeadChips in one rapid motion from the 'XC4' wash dish and place it directly on tube rack. Ensure that the BeadChips are horizontal with the barcodes facing up.

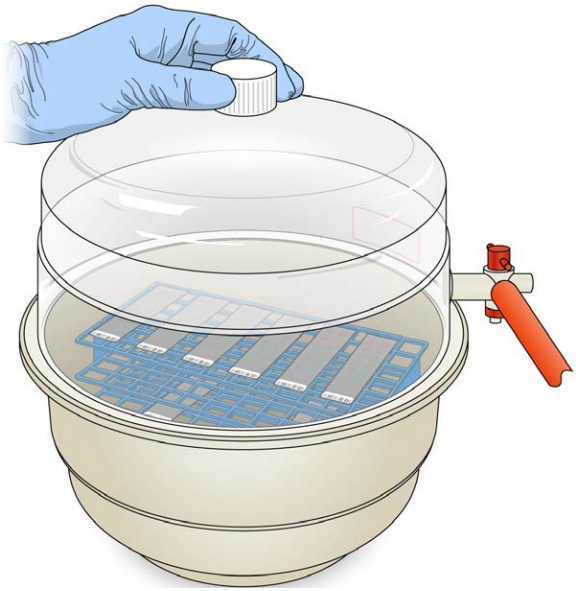
b Place BeadChips on the tube rack as shown in Figure 131 until there are six BeadChips on top of the rack and two BeadChips on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.

c Place the tube rack with the **third set of** 8 BeadChips into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.

d Start the vacuum, using at least 675 mm Hg (0.9 bar).

e To ensure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.

Figure 132 Testing Vacuum Seal



f Dry under vacuum for 50–55 minutes.

31 Release the vacuum by turning the handle very slowly.

WARNING

Air should enter the desiccator very slowly to avoid disturbing the contents. Improper use of the vacuum desiccator can result in damage to the BeadChips. This is especially true if you remove the valve plug while a vacuum is applied. For detailed vacuum desiccator instructions, see the documentation included with the desiccator.

32 Store the desiccator with the red valve plug in the desiccator's three-way valve to stop accumulation of dust and lint within the valve port. Remove the red plug from the three-way valve before applying vacuum pressure.

33 Touch the borders of the chips (**do not touch the stripes**) to ensure that the etched, barcoded side of the BeadChips are dry to the touch.

34 If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom two BeadChips are the most likely to have some excess.

a Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.

a Wrap a presaturated ProStat EtOH Wipe or Kimwipe soaked in EtOH around your index finger.

b Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

CAUTION

Do ***not*** touch the stripes with the wipe or allow EtOH to drip onto the stripes.

35 Clean the glass back plates. For instructions, see the *Infinium Assay Lab Setup and Procedures Guide*.

36 Clean the Hyb Chambers:

a Remove the rubber gaskets from the Hyb Chambers.

b Rinse all Hyb Chamber components with DI water.

c Thoroughly rinse the eight humidifying buffer reservoirs.

37 Discard unused reagents in accordance with facility standards.

38 Do one of the following:

- Proceed to Image BeadChip.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature. Image the BeadChips within 72 hours.

Image BeadChip

Follow the instructions in the *iScan System User Guide* or *HiScanSQ System User Guide* to scan your BeadChips.

Use the appropriate scan setting for your BeadChip, as outlined in the following table:

Table 13 Scan Settings for Infinium HD FFPE

BeadChip	Scan Setting Name
CytoSNP-FFPE	Infinium HD
HumanOmniExpress-FFPE	Infinium NXT

Illumina GenomeStudio

The Illumina GenomeStudio Genotyping Module, included with your Illumina Infinium Assay system, is an application for extracting genotyping data from intensity data files (*.idat files) collected from systems such as the Illumina HiScan System.

For feature descriptions and instructions on using the GenomeStudio platform to visualize and analyze genotyping data, see the *GenomeStudio Framework User Guide* and the *GenomeStudio Genotyping User Guide*.

System Controls

Infinium HD FFPE Assay System Controls 180

Infinium HD FFPE Assay System Controls

This appendix describes the controls used in the Infinium HD FFPE Assay. Standard Infinium controls apply to the Infinium HD FFPE Assay. The standard Infinium controls, including the bead type IDs used, their expected outcomes, and how to view them are documented in the Infinium Lab Setup and Procedures Guide. There is a restoration control specific to the Infinium HD FFPE Assay.

The Restore Control is included in the standard Infinium control panel to ensure that the Infinium HD FFPE Restore Kit is working properly and that degraded DNA is being restored efficiently. If you are working with fresh or fresh-frozen samples and have not used the Restore Kit to treat your samples, the restore control should show no activity.

The Restore Control is a short oligo sequence that has been "spiked" into the Infinium HD FFPE restore kit. A complementary sequence to this control has been included on Illumina's standard Infinium microarrays and is bound to a control bead. Due to the chemistry of the Infinium HD FFPE Restore Kit, the control oligo will be available to bind to its complement if the restore process is functioning properly. Detection of reduced intensity of the Restore Control may indicate that DNA restoration has not occurred or has been compromised.

Index

A

arrays 9
AutoLoader2 15

B

BeadChip
- alignment fixtures 124
- description 9
- imaging 8
- single-base extension 7
- washing 7
- Xstain, auto protocol 152

C

control oligos
- See controls 180

copy number variation 16
customer support 183

D

data analysis 11, 16, 85, 178
differential analysis 16, 85, 178
DNA
- amplification bias 4
- amplifying 4
- annealing 6
- captured and used as a template 7
- denaturing 4
- fragmenting 5
- hybridizing 6
- incubating 4, 6
- mapping samples to BeadChips 11
- neutralizing 4
- precipitating 5
- preparing for amplification 4
- preparing for staining, extension 7
- resuspending 6
- tracking samples 11
- washing away 7

documentation 183

E

equipment
- automated protocol
  - Illumina-supplied 90
  - user-supplied 90
- manual protocol
  - Illumina-supplied 20
  - user-supplied 20

experienced user cards 11

F

flourosphore 8
Flow-Through Chambers
- single-base extension 70
- stain 72

G

gene analysis 16, 85, 178
GenomeStudio 16, 85, 178
genotype calling for the sample 7

H

help, technical 183
HiScan 15-16, 85, 178
HiScan System 153

I

Infinium Assay
- overview 2

Infinium HD Assay
- controls
  - See controls 180

integrated informatics 16, 85, 178
intensity data files 16

iScan System 15, 153

L

lab setup and procedures 10

M

materials
    automated protocol
        Illumina-supplied 92
        user-supplied 20, 91
    manual protocol
        Illumina-supplied 22
multiplex 2

R

reagents
    manual protocol
        Illumina-supplied 22
Required Run Items 107, 153
robot
    BeadChip alignment fixtures 124
    bed map update 107
    dispensing sample 127
    preparing 102, 106, 113, 124, 152
    Required Run Items 107, 153
    resuspend setup 114
    scanning the barcode 127
    Tip Alignment Guide 119, 127
    tube rack 154
    XStain BeadChip setup 152
Robot Tip Alignment Guide 119, 133

S

sample sheet 11
SNPs
    number per sample 2
system controls
    See controls 180

T

Tecan Freedom Evo 14
Tecan GenePaint 14
Tecan Genesis 14
technical assistance 183
Tip Alignment Guide 119, 127

X

XStain BeadChips
    auto protocol 152
    single-base extension 70
    stain 72

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 14 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 15 Illumina Customer Support Telephone Numbers

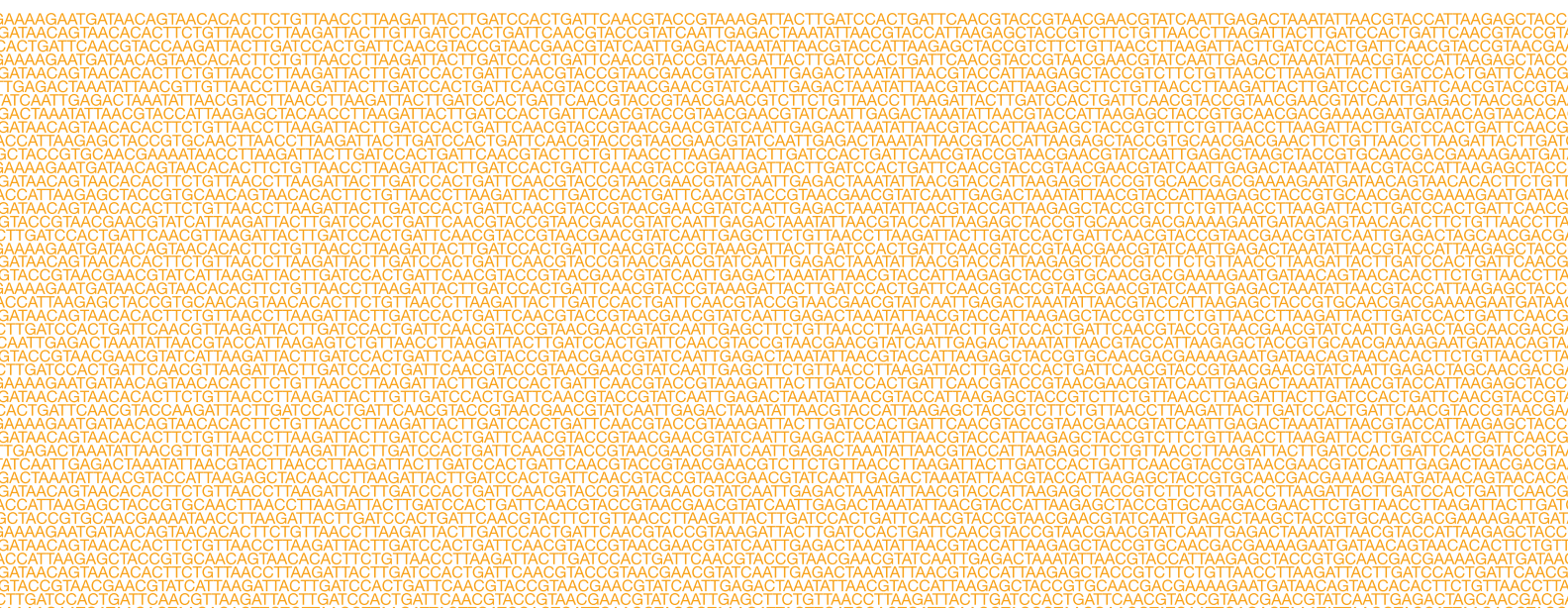
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America) techsupport@illumina.com
www.illumina.com