

Infinium® HD Assay Ultra Protocol Guide

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Revision History

Catalog # Part #, Revision Letter	Date
Catalog # WG-901-6102 Part # 11328087, Rev. A	December 2008
Catalog # WG-901-4007 Part # 11328087, Rev. B	November 2009

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Chapter 1

Overview

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Introduction

The Illumina® Infinium® HD Assay Ultra protocol revolutionizes DNA analysis by streamlining sample preparation and enabling unlimited multiplexing. Using Infinium I and Infinium II probe designs and dual color channel approach, the Infinium HD Assay Ultra protocol scales DNA analysis from 3,000 up to 300,000 SNPs and CNV markers per sample, dependent only on the number of features (bead types) on the array.

The Infinium HD Assay Ultra protocol 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 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.

Extension of the primer incorporates a biotin 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 Illumina Infinium HD Assay Ultra protocol offers:

- ▶ Sample throughput using 12x1 HD or 24x1 HD BeadChips
- ▶ Effectively unlimited multiplexing
- ▶ High call rate and accuracy
- ▶ Unlimited Genome-wide marker selection
- ▶ Single tube amplification—single chip—no PCR
- ▶ Minimal risk of carryover contamination
- ▶ Low DNA input of 200 ng (per sample)
- ▶ Walk-away automation using Tecan Genesis or Freedom EVO Robots and Tecan GenePaint system
- ▶ Illumina LIMS automation
- ▶ Compatibility with both Illumina iScan™ System and BeadArray™ Reader
- ▶ Multi-sample BeadChip format
- ▶ Unlimited genotype multiplexing, scaling with the number of BeadChip features

Audience and Purpose

This guide is for laboratory technicians running the Infinium HD Assay Ultra with Illumina multi-sample DNA Analysis BeadChips. The guide documents the laboratory protocols associated with the assay. Follow all of the protocols in the order shown.

Chapter 2, *Infinium HD Assay Ultra Manual Protocol*, explains how to run the assay manually in the lab.

Chapter 3, *Infinium HD Assay Ultra Automated Protocol*, explains how to automate the protocol with the aid of the Tecan eight-tip robot.

Important Note

Before following any of the procedures in this guide, read the *Infinium Assay Lab Setup and Procedures Guide*, which explains how to equip and run an Infinium HD Assay Ultra 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 DNA analysis genotyping chips provided by Illumina. All of the Infinium HD Assay Ultra Protocol guides assume that you have already set up the laboratory space and are familiar with the standard procedures and safety precautions.

The Infinium HD Assay Ultra

This section describes and illustrates the assay protocol. The assay requires only 200 ng of DNA (per sample) as input.

Quantitate DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples.

See *Quantitate DNA (Optional)* on page 19 for manual processing.
See *Quantitate DNA (Optional)* on page 109 for automated processing.

Amplify DNA

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

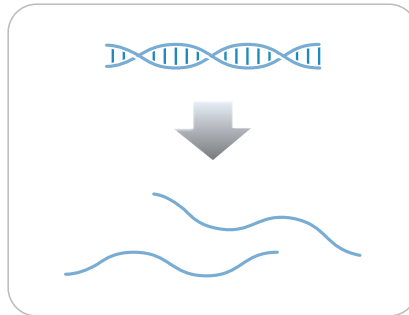


Figure 1 Denaturing and Neutralizing DNA

See *Make the MSA3 Plate* on page 31 for manual processing.
See *Make the MSA3 Plate* on page 119 for automated processing.

Incubate DNA

The denatured DNA is isothermally amplified in an overnight step. The whole-genome amplification uniformly increases the amount of the DNA sample by several thousandfold without introducing large amounts of amplification bias.

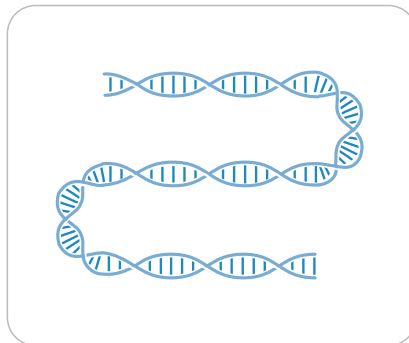


Figure 2 Incubating DNA to Amplify

See *Incubate the MSA3 Plate* on page 35 for manual processing.
See *Incubate the MSA3 Plate* on page 127 for automated processing.

Fragment DNA

The amplified product is fragmented by a controlled enzymatic process that does not require gel electrophoresis. The process uses end-point fragmentation to avoid overfragmenting the sample.

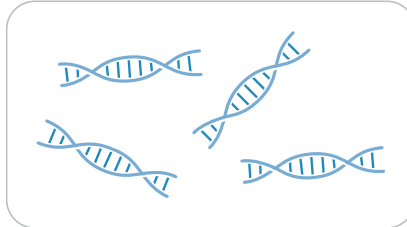


Figure 3 Fragmenting DNA

See *Fragment the MSA3 Plate* on page 36 for manual processing.
See *Fragment the MSA3 Plate* on page 130 for automated processing.

Precipitate DNA

After an isopropanol precipitation, the fragmented DNA is collected by centrifugation at 4°C.

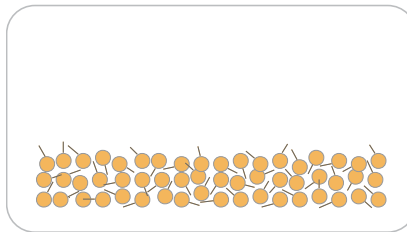


Figure 4 Precipitating DNA

See *Precipitate the MSA3 Plate* on page 38 for manual processing.
See *Precipitate the MSA3 Plate* on page 134 for automated processing.

Resuspend DNA

The precipitated DNA is resuspended in hybridization buffer.

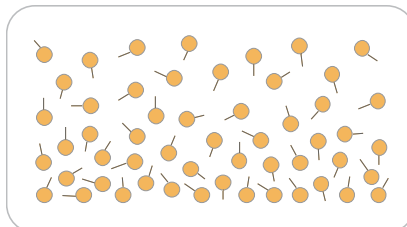


Figure 5 Resuspending DNA

See *Resuspend the MSA3 Plate* on page 42 for manual processing.
See *Resuspend the MSA3 Plate* on page 141 for automated processing.

Hybridize to BeadChip

The BeadChip is prepared for hybridization in a capillary flow-through chamber. Samples are applied to a BeadChip and divided 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 (covalently linked to one of up to 300,000 bead types) during hybridization.

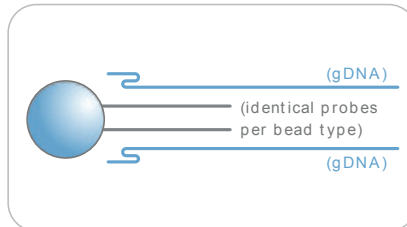


Figure 6 Hybridizing DNA to BeadChip

See *Hybridize Multi BeadChip* on page 44 for manual processing.
See *Hybridize Multi BeadChip* on page 145 for automated processing.

Wash BeadChip

Unhybridized and non-specifically hybridized DNA is washed away, and the BeadChip is prepared for staining and extension.

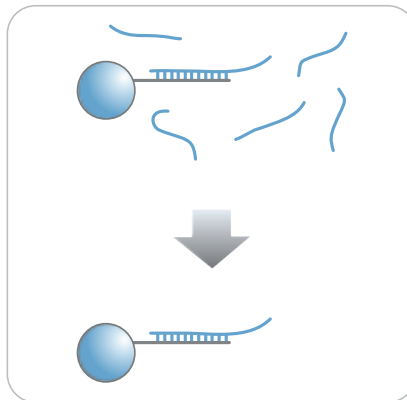


Figure 7 Washing BeadChip

See *Wash BeadChip* on page 57 for manual processing.
See *Wash BeadChip* on page 166 for automated processing.

Extend and Stain (XStain) BeadChip

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.

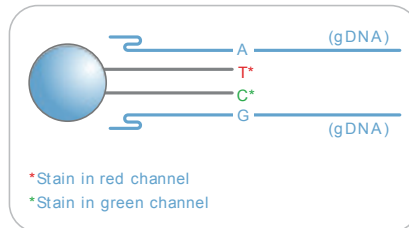


Figure 8 Extending and Staining BeadChip

See *Single-Base Extension and Stain HD BeadChip* on page 66 for manual processing.

See *Single-Base Extension and Stain HD BeadChip* on page 176 for automated processing.

Image BeadChip

The Illumina iScan or BeadArray Reader 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.

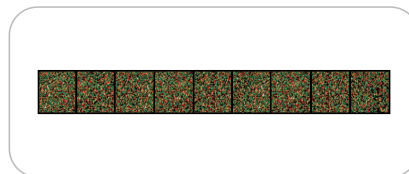


Figure 9 Imaging BeadChip

See *Image BeadChip on the iScan System* on page 81 or *Image BeadChip on the BeadArray Reader* on page 91 in the Manual protocol or *Image BeadChip on the iScan System* on page 204 or *Image BeadChip on the BeadArray Reader* on page 214 in the Automated protocol.

The Infinium HD BeadChip

Illumina Infinium HD BeadChips are sophisticated silicon-based array devices. The sample sections of the slide are separated by an IntelliHyb® seal so that you can run multiple samples simultaneously.

Each individual sample section may hold a minimum of 3,000 different oligonucleotide probe sequences. These are in turn 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.

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 of these documents are available for printing and reference at <http://www.illumina.com/documentation>.

Sample Sheet

To effectively track your samples and assay, Illumina recommends you create a Sample Sheet. The Sample Sheet will later be used by the GenomeStudio application for data analysis. For instructions on data analysis, see the *GenomeStudio Genotyping Module User Guide*.

Create your Sample Sheet according to the guidelines provided in Table 1.

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

Table 1 Sample Sheet Guidelines (Continued)

Column Heading	Description	Optional (O) or Required (R)
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
SentrixPosition_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. Used for exclusion in the Final Report Wizard.	O
Replicate	The Sample_ID of a replicate to this sample. Used in reproducibility error calculations.	O
Parent1	The Sample_ID of this sample's first parent.	O
Parent2	The Sample_ID of this sample's second parent.	O
NOTES	Your sample sheet header may contain whatever information you choose. Your sample sheet may contain any number of columns you choose. Your sample sheet must be in a comma-delimited (.csv) file format. Save the sample sheet under any name you wish; for example, the experiment name.	

Figure 10 provides an example of the Sample Sheet format. You can download an electronic, read-only Sample Sheet template file (*Sample Sheet Template.csv*) that you can copy and use from <http://www.illumina.com/documentation>.

	A	B	C	D	E	F	G	H	I	J	K
1	[Header]										
2	Investigator Name	BeadStudio 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_G	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											
17											
18											
19											

Figure 10 Sample Sheet Example

Tecan GenePaint

The Infinium HD Assay Ultra uses Tecan's GenePaint automated slide processor to process BeadChips. The GenePaint system employs a capillary gap flow-through chamber to enable reagent entrapment and exchange over the BeadChip's active surface. Washing, blocking, extension, and signal amplification are all performed by simple reagent additions 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.



WARNING

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

BeadArray Reader, iScan, AutoLoader, and AutoLoader2

BeadChips are imaged using either the Illumina iScan System or BeadArray Reader. Both 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 scanned area). The iScan System incorporates advanced optics and sensors to support much higher throughput than the BeadArray Reader, while providing equally high data quality.

The GenomeScan (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 individual bead pool map manifest file (*.bpm) to analyze the data from the assay.

Loading and unloading of BeadChips into the iScan System or BeadArray Reader can be automated with the optional AutoLoader2 or AutoLoader respectively. Both AutoLoaders support unattended processing by placing BeadChips carriers in the imaging system's tray, so that it can scan the BeadChips. Features include:

Table 2 AutoLoader and AutoLoader2 Features

	AutoLoader	AutoLoader2
Integrated with iScan Control Software		•
Integrated with BeadScan software	•	
Integrated with Illumina LIMS	•	•
Email alert system	•	•
Single-reader or dual-reader configuration	•	•

Table 2 *AutoLoader and AutoLoader2 Features*

	AutoLoader	AutoLoader2
Number of BeadChips supported per carrier	2	4
Number of carriers processed at a time	20	48

GenomeStudio Integrated Informatics Platform

GenomeStudio, Illumina's integrated data analysis software platform, provides a common environment for analyzing data obtained from microarray and sequencing technologies. Within this common environment, or framework, the GenomeStudio software modules allow you to perform application-specific analyses. The 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 iScan System or BeadArray Reader. For information on the latest software offerings, including those for applications like cytogenetics, please 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)

The GenomeStudio Genotyping Module can be fully integrated with the Illumina LIMS server.

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 Module User Guide*.

Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 3 *Illumina Customer Support Contacts*

Contact	Number
Toll-free Customer Hotline	1-800-809-ILMN (1-800-809-4566)
International Customer Hotline	1-858-202-ILMN (1-858-202-4566)
Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at <http://www.illumina.com/msds>.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website. Go to <http://www.illumina.com/documentation>. When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF.

If you do not already have an iCom account, then click New User on the iCom login screen and fill in your contact information. Indicate whether you wish to receive the iCommunity newsletter (a quarterly newsletter with articles about, by, and for the Illumina Community), illumiNOTES (a monthly newsletter that provides important product updates), and announcements about upcoming user meetings. After you submit your registration information, an Illumina representative will create your account and email login instructions to you.

Infinium HD Assay Ultra Manual Protocol

Topics

16	Introduction
16	Infinium HD Assay Ultra Manual Workflow
18	Equipment, Materials, and Reagents
19	Quantitate DNA (Optional)
31	Make the MSA3 Plate
35	Incubate the MSA3 Plate
36	Fragment the MSA3 Plate
38	Precipitate the MSA3 Plate
42	Resuspend the MSA3 Plate
44	Hybridize Multi BeadChip
57	Wash BeadChip
66	Single-Base Extension and Stain HD BeadChip
81	Image BeadChip on the iScan System
91	Image BeadChip on the BeadArray Reader
101	GenomeStudio Integrated Informatics Platform

Introduction

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

Infinium HD Assay Ultra Manual Workflow

Figure 11 graphically represents the Illumina Infinium HD Assay Ultra manual workflow for use with the 12x1 HD BeadChip or the 24x1 HD BeadChip. These protocols describe the procedure for preparing 96 DNA samples using either BeadChip.

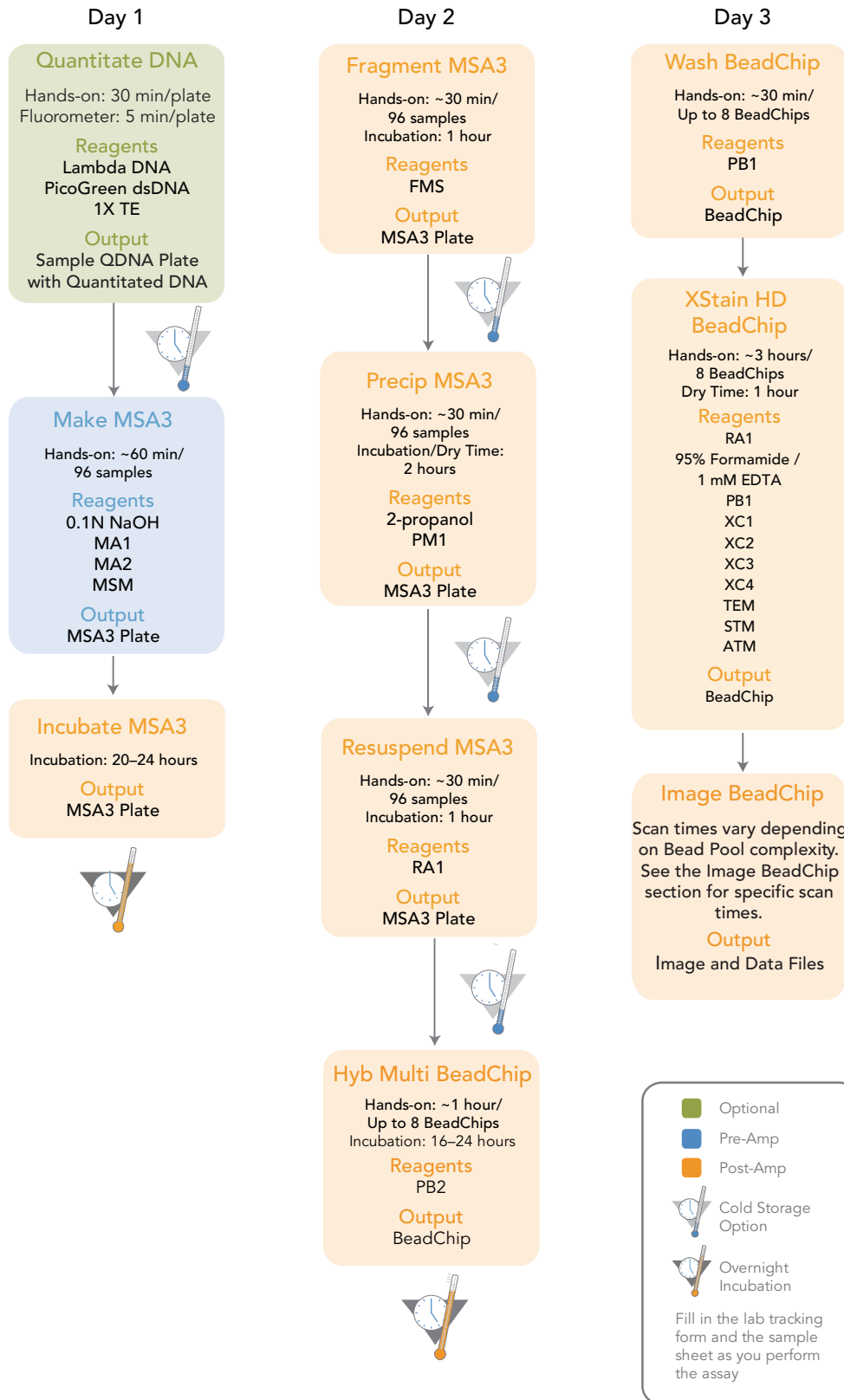


Figure 11 Infinium HD Assay Ultra Manual Workflow

Equipment, Materials, and Reagents

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

Equipment Illumina-Supplied

Table 4 *Illumina-Supplied Equipment*

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

User-Supplied

Table 5 *User-Supplied Equipment*

Item	Catalog #
Forceps	VWR Catalog # 25601-008
Auto-desiccator cabinet (Optional—allows you to hold prepared BeadChips for up to three days before scanning)	VWR Catalog # 74950-342

Materials Illumina-Supplied

- ▶ MSA3 barcode labels
- ▶ WG#-DNA barcode labels

Reagents Illumina-Supplied

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
MA2 —Multi-Sample Amplification 2 Mix	11203401
MSM —Multi-Sample Amplification Master Mix	11203410
PB1 —Reagent used to prepare BeadChips for hybridization	11191922
PB2 —Humidifying buffer used during hybridization	11191130

Table 6 Illumina-Supplied Reagents

Item	Part #
PM1 —Precipitation solution	11203436
RA1 —Resuspension, hybridization, and wash solution	11222442
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 (240 ml)—XStain BeadChip solution 3	11208421
XC4 —XStain BeadChip solution 4	11208430

Quantitate DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples. You can quantitate up to six plates, each containing up to 96 samples. If you already know the concentration you can proceed to *Make the MSA3 Plate* on page 31.

Illumina recommends the Molecular Probes PicoGreen assay to quantitate dsDNA samples. The PicoGreen assay can quantitate small DNA volumes, and measures DNA directly. Other techniques may pick up contamination such as RNA and proteins. Illumina recommends using a spectrofluorometer because fluorometry provides DNA-specific quantification. Spectrophotometry might also measure RNA and yield values that are too high.

Estimated Time

Hands-on time: ~20 minutes per plate, plus 10 minutes to prepare the PicoGreen

Spectrofluorometer read time: ~5 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	2° to 8°C	User
1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA (TE))	See Instructions	Room temperature	User
Lambda DNA	See Instructions	2° to 8°C	User
96-well 0.65 ml microtiter plate	1 per 96 samples		User

Item	Quantity	Storage	Supplied By
Fluotrac 200 96-well flat-bottom plate	1 per Std DNA plate 1 per Sample DNA plate		User

**NOTE**

PicoGreen is susceptible to differential contaminants. False positives may occur for whole-genome amplification. Therefore, it is important to quantitate the input into the whole-genome amplification reaction.

Preparation

- ▶ Thaw PicoGreen to room temperature for 60 minutes in a light-impermeable container.
- ▶ Hand-label the microtiter plate "Standard DNA."
- ▶ Hand-label one of the Fluotrac plates "Standard QNT."
- ▶ Hand-label the other Fluotrac plate "Sample QDNA." This plate will contain the quantitated DNA.
- ▶ In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Steps

In this section, you will perform the following steps:

- ▶ Make a Standard DNA plate with serial dilutions of stock Lambda DNA.
- ▶ Dilute PicoGreen with 1X TE.
- ▶ Create a Standard QDNA Fluotrac plate containing serial dilutions of DNA plus diluted PicoGreen.
- ▶ Create a Sample QDNA plate by adding diluted PicoGreen to the sample DNA you plan to assay.

Make Standard DNA Plate

In this process, you create a Standard DNA plate with serial dilutions of stock Lambda DNA in the wells of column 1 (Figure 12).

1. Add stock Lambda DNA to well A1 in the plate labelled "Standard DNA" and dilute it to 75 ng/μl in a final volume of 233.3 μl. Pipette up and down several times.
 - a. Use the following formula to calculate the amount of stock Lambda DNA to add to A1:

$$\frac{(233.3 \mu\text{l}) \times (75 \text{ ng}/\mu\text{l})}{(\text{stock Lambda DNA concentration})} = \mu\text{l of stock Lambda DNA to add to A1}$$

- b. Dilute the stock DNA in well A1 using the following formula:

$$\mu\text{l of 1X TE to add to A1} = 233.3 \mu\text{l} - \mu\text{l of stock Lambda DNA in well A1}$$

2. Add 66.7 μl 1X TE to well B1.
3. Add 100 μl 1X TE to wells C, D, E, F, G, and H of column 1.

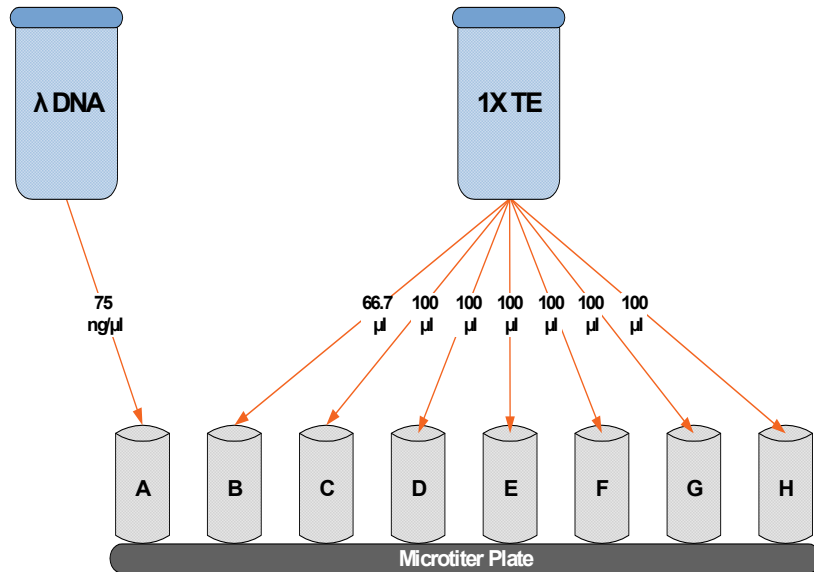


Figure 12 Dilution of Stock Lambda DNA Standard

4. Transfer 133.3 μl of Lambda DNA from well A1 into well B1. Pipette up and down several times.
5. Change tips. Transfer 100 μl from well B1 into well C1. Pipette up and down several times.
6. Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 ng/ μl Lambda DNA.

Table 7 Concentrations of Lambda DNA

Row-Column	Concentration (ng/ μl)	Final Volume in Well (μl)
A1	75	100
B1	50	100
C1	25	100
D1	12.5	100
E1	6.25	100
F1	3.125	100
G1	1.5262	200
H1	0	100

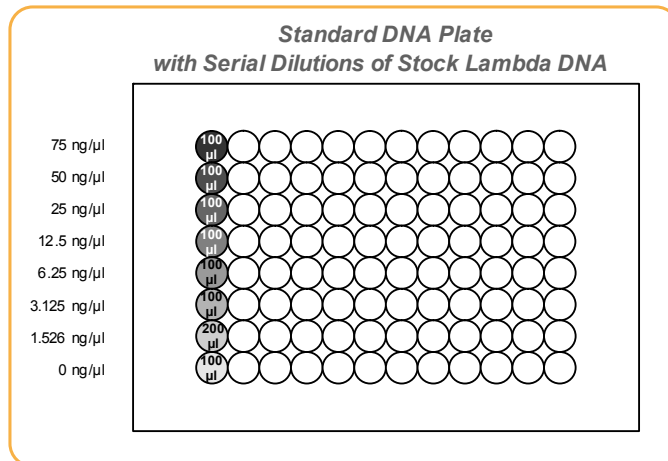


Figure 13 Serial Dilutions of Lambda DNA

7. Cover the Standard DNA plate with an adhesive seal.
8. Proceed to *Dilute PicoGreen*.

Dilute PicoGreen

The diluted PicoGreen will be added to both the Standard QDNA and Sample QDNA plates, to make the DNA fluoresce when read with the spectrofluorometer.



CAUTION

PicoGreen reagent degrades quickly in the presence of light. Do not use glass containers for PicoGreen reagent.

1. Prepare a 1:200 dilution of PicoGreen into 1X TE, using a sealed 100 ml or 250 ml Nalgene bottle wrapped in aluminum foil.
Refer to Table 8 to identify the volumes needed to produce diluted reagent for multiple 96-well QDNA plates. For fewer than 96 DNA samples, scale down the volumes.

Table 8 Volumes for PicoGreen Reagents

# QDNA Plates	PicoGreen Volume (μl)	1X TE Volume (ml)
1	115	23
2	215	43
3	315	63

2. Cap the foil-wrapped bottle and vortex to mix.

Create Standard QDNA Plate with Diluted PicoGreen

In this process you transfer the serial dilutions from the Standard DNA plate into the Standard QDNA Fluotrac plate and add diluted PicoGreen.

1. Pour the PicoGreen/1X TE dilution into a clean reagent reservoir.
2. Using a multichannel pipette, transfer 195 μl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled "Standard QDNA" (Figure 14).
3. Add 2 μl of each stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA Fluotrac plate.

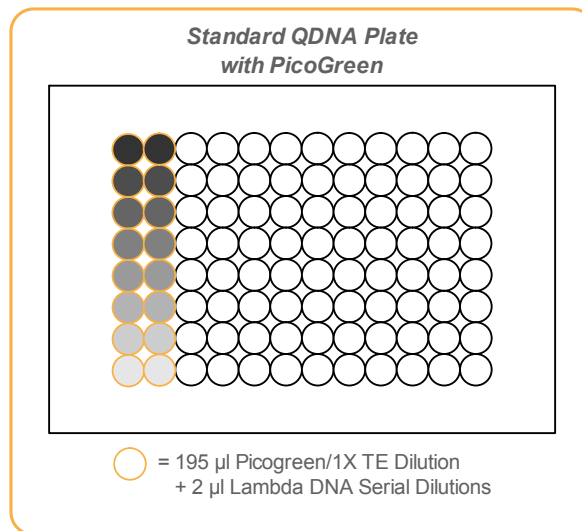


Figure 14 Standard QDNA Plate with PicoGreen

4. Immediately cover the plate with an adhesive aluminum seal.
5. Proceed to *Prepare Sample QDNA Plate with PicoGreen and DNA*.

Prepare Sample QDNA Plate with PicoGreen and DNA

In this process, you create a new Sample QDNA plate that contains DNA sample and PicoGreen.

1. Using a multichannel pipette, transfer 195 μl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled "Sample QDNA" (Figure 15).
2. Add 2 μl of DNA sample to all 96 wells of the Sample QDNA plate. Only the first two columns will also contain PicoGreen/1X TE dilution.

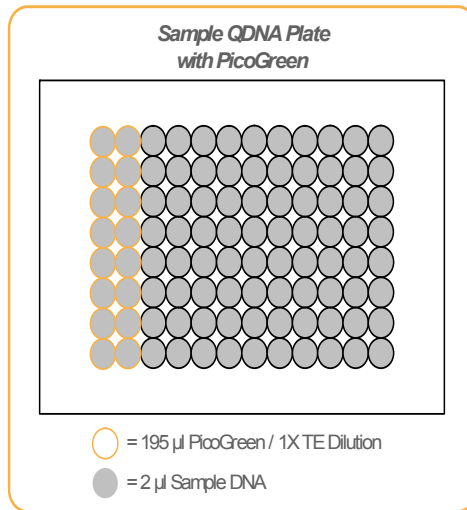


Figure 15 Sample QDNA Plate with PicoGreen



NOTE

For fewer than 96 DNA samples, add the diluted PicoGreen reagent into the number of wells needed.

3. Immediately cover the plate with an adhesive aluminum seal.
4. Proceed to *Read QDNA Plate With LIMS* if you are running Illumina LIMS (Laboratory Information Management System) or *Read QDNA Plate Without LIMS* and you are not running Illumina LIMS.

Read QDNA Plate With LIMS

In this process, you use the Gemini XS or XPS Spectrofluorometer to read the Standard QDNA and Sample QDNA plates. The spectrofluorometer creates a standard curve from the known concentrations in the Standard QDNA plate, which you use to determine the concentration of DNA in the Sample QDNA plates. This information is saved with the other project data in Illumina LIMS. For the best genotyping performance, Illumina recommends a minimum concentration of 50 ng/µl.

For information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

1. Turn on the spectrofluorometer.
2. Open the GTS Fluorometry Analysis software.

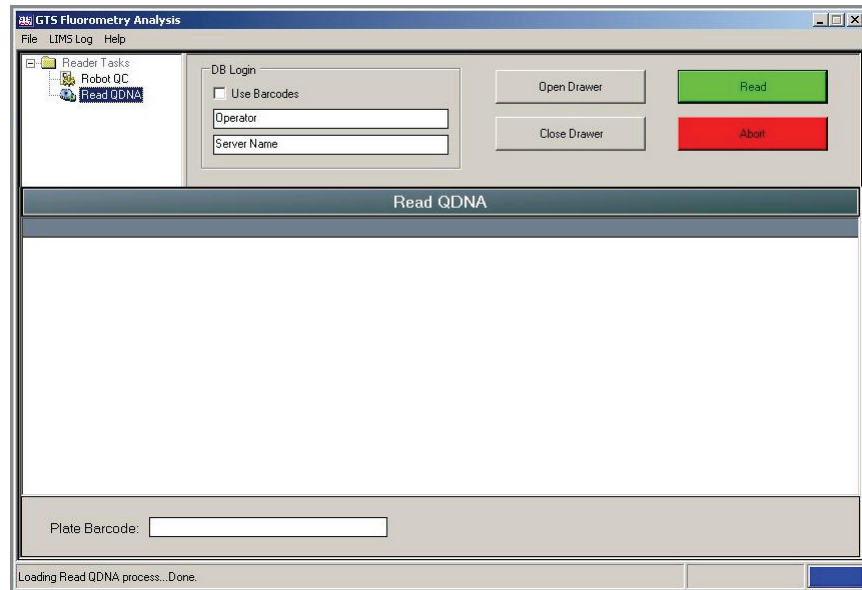


Figure 16 GTS Fluorometry Analysis Opening Screen

3. Select **Reader Tasks | Read QDNA**.
4. Select the **Use Barcodes** check box.
5. Click **Read**.
6. When prompted, log in to the Illumina LIMS database.
7. When asked if you want to read a new Standard QNT plate, click **Yes**. Load the Standard QNT plate in the open drawer of the fluorometer.
8. Review the data from the Standard QNT plate. Either accept it and go on to the next step, or reject it which will stop the Read Quant process.
9. Remove the Standard QNT plate from the spectrofluorometer tray.
10. When prompted, enter the number of Sample QNT plates you want to read (1, 2, or 3). Do not include the Standard QNT plate in this number. Click **OK**.

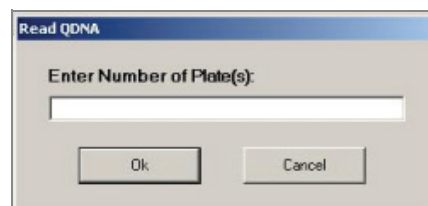


Figure 17 Number of QDNA Plates Dialog Box

11. When prompted, hand-scan or enter the Sample QNT plate barcode. Click **OK**.

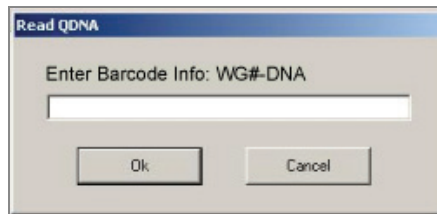


Figure 18 Plate Barcode Dialog Box

12. When prompted, remove the plate seal from the Sample QNT plate and load it into the spectrofluorometer tray, with well A1 at the upper left corner. Click **OK**.

The spectrofluorometer reads the Sample QNT plate.

13. When prompted, click **Yes** to review the raw Sample QNT plate data.

The GTS Fluorometry Analysis screen fills in with information about the fluorescence in the wells.

Read QDNA												
Index	01	02	03	04	05	06	07	08	09	10	11	12
A	901.187	574.264	2229.355	2743.684	1653.467	1240.680	2516.841	1806.896	957.319	1849.673	2062.137	1073.844
B	2511.246	1408.539	2197.872	2961.366	1817.009	1755.341	1478.821	2164.615	1279.077	544.411	1015.841	652.956
C	1354.520	586.959	618.364	1680.938	879.031	1186.237	251.373	1547.593	758.155	864.308	1459.477	975.849
D	2382.074	2444.011	1618.223	74.983	2172.284	2118.486	3367.836	1771.613	1255.763	2036.969	1436.691	342.354
E	2085.587	1933.228	2348.986	1112.462	1835.320	2230.770	2192.462	1427.822	2056.528	2417.382	1900.707	1438.428
F	1028.495	2637.335	1827.523	2219.094	2520.103	2360.154	907.099	1993.719	1426.916	1284.198	1066.274	791.380
G	1357.837	1753.874	1602.840	1527.622	1888.769	1178.043	1657.656	1804.497	1270.985	1495.533	1278.908	1461.335
H	1399.478	2255.065	1281.426	774.938	676.704	571.730	769.209	685.294	1086.497	1129.055	1142.966	1119.405

Figure 19 Fluorescence Data

Microsoft Excel opens automatically at the same time and displays the quant data for the QDNA plate. There are three tabs in the file:

- **SQDNA_STD**—Plots the RF values against the concentration (ng/μl).
- **QDNA**—Plots the concentration (ng/μl) for each well.
- **Data**—Compares the data from the Standard QDNA plate to the QDNA plate you just read.

The Infinium Fluorometer Analysis software prompts you to indicate whether you wish to use the QNT data shown in the Excel file.

14. Do one of the following:
 - Click **Yes** to send the data to Illumina LIMS. In Illumina LIMS, the QNT plate moves into the Make MSA3 queue.
 - Click **No** to delete the quant data. You can read the quant data again for the same plate.

15. If you entered more than one QNT plate to read, repeat steps 11 and 14 for each additional plate.
16. Discard the QNT plates and reagents in accordance with facility requirements.
17. Do one of the following:
 - Proceed to *Make the MSA3 Plate* on page 31.
 - Store the quantitated DNA at 2° to 8°C for up to one month.

Read QDNA Plate Without LIMS

In this process, you use the Gemini XS or XPS Spectrofluorometer to read the Standard QDNA and Sample QDNA plates. The spectrofluorometer creates a standard curve from the known concentrations in the Standard QDNA plate, which you use to determine the concentration of DNA in the Sample QDNA plates. For the best genotyping performance, Illumina recommends a minimum concentration of 50 ng/μl.



NOTE

The SoftMax Pro screens and menu options may vary from those depicted, depending up on the software version that you are running.

1. Turn on the spectrofluorometer. At the PC, open the SoftMax Pro program.
2. Load the Illumina QDNA.ppr file from the installation CD that came with your system.
3. Select **Protocols | GTS_QDNA** (Figure 20).

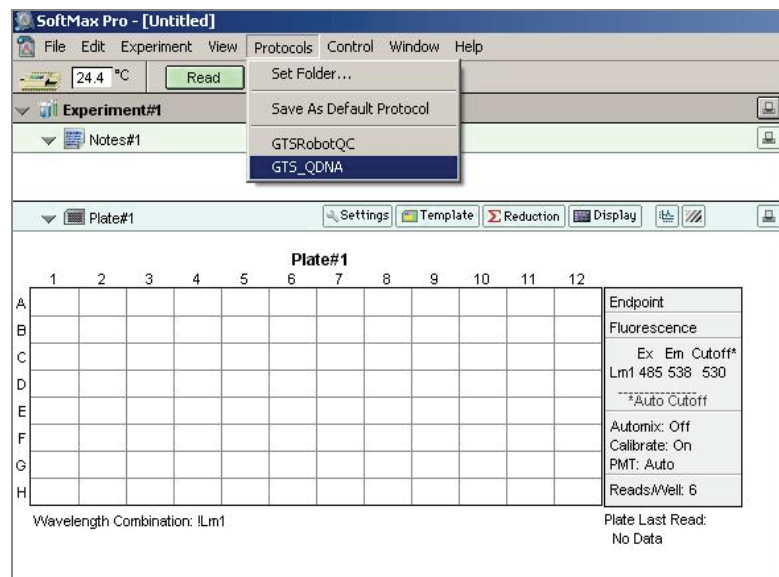


Figure 20 Load the Illumina QDNA Protocol in SoftMax Pro

4. Place the Standard QDNA Fluotrac Plate into the spectrofluorometer loading rack with well A1 in the upper left corner.

- Click the blue arrow next to **Illumina QDNA | SQDNA_STD** (Figure 21).

The screenshot shows the SoftMax Pro software interface. The main window is titled "SoftMax Pro - [Untitled 1]". The menu bar includes File, Edit, Experiment, View, Graph, Protocols, Control, Window, and Help. The temperature is set to 24.4 °C, and there is a "Read" button. The interface is divided into several sections:

- Illumina QDNA:** Contains a tree view with "SQDNA" and "SQDNA_STD". A blue arrow points to the "SQDNA_STD" item.
- SQDNA_STD (µg/ml):** A table with columns: SQDNA_START, Concentration, Wells, Values, MeanValue, Std.Dev., and CV%. The table lists 16 rows of standards from La01 to La08b.
- Standard Curve:** A section with a "Fit" dropdown set to "Linear".
- QDNA:** A grid for the QDNA plate with columns 1-12 and rows A-H. To the right of the grid is a panel with parameters: Endpoint, Fluorescence (Ex: 485, Em: 530, Cutoff*: 530), Auto Cutoff, Automix: Off, Calibrate: On, PMT: Low, Reads/Well: 6, Wavelength Combination: !Lm1, and Plate Last Read: No Data.

Figure 21 Select the SQDNA Standard Screen

- Click **Read** in the SoftMax Pro interface (Figure 22) to begin reading the Standard QDNA Plate.

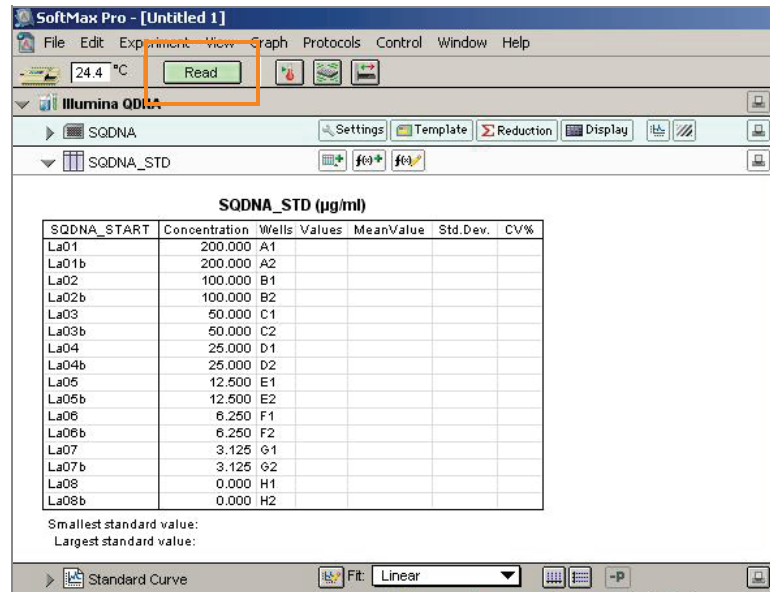


Figure 22 Read the Standard QDNA Plate

7. When the software finishes reading the data, remove the plate from the drawer.
8. Click the blue arrow next to **Standard Curve** to view the standard curve graph (Figure 23).

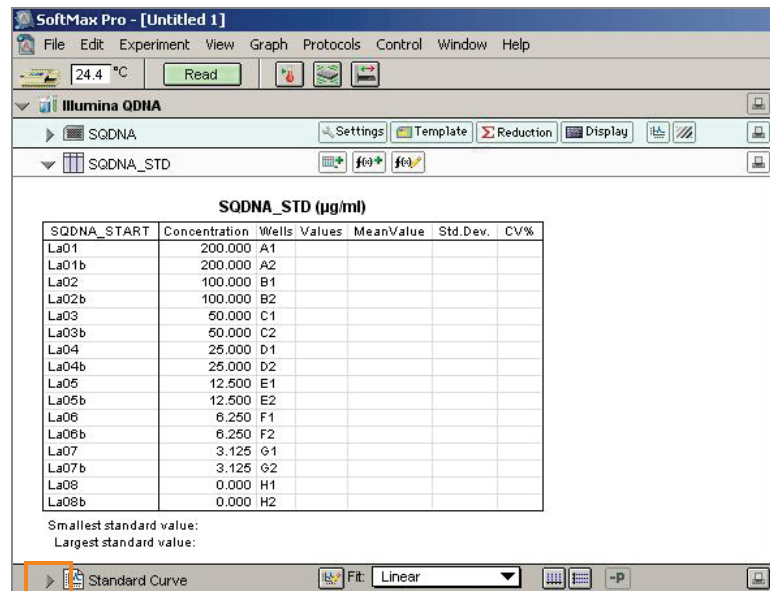


Figure 23 View Standard Curve

9. If the standard curve is acceptable, continue with the sample plate. Otherwise, click **Standard Curve** again.
10. Place the first Sample QDNA plate in the spectrofluorometer with well A1 in the upper left corner.
11. Click the blue arrow next to **SQDNA** and click **Read** (Figure 24).

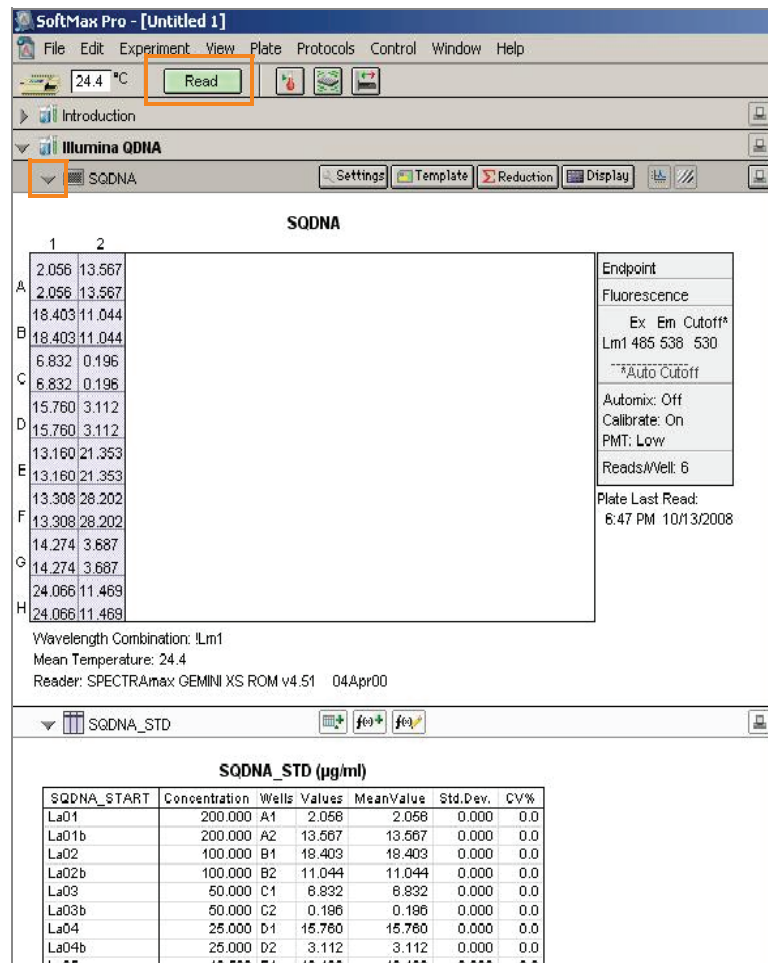


Figure 24 Read the Sample QDNA Plate

12. When the software finishes reading the plate, remove the plate from the drawer.
13. Repeat steps 10 through 12 to quantitate all Sample QDNA plates.
14. Once all plates have been read, click **File | Save** to save the output data file (*.pda).
15. When you have saved the *.pda file, click **File | Import/Export | Export** and export the file as a *.txt file. You can open the *.txt file in Microsoft Excel for data analysis.
16. Do one of the following:
 - Proceed to *Make the MSA3 Plate* on page 31.
 - Store the quantitated DNA at 2° to 8°C for up to one month.

Make the MSA3 Plate

This process creates a MSA3 plate for DNA amplification. MA1 is first added to the MSA3 plate, followed by the DNA samples. Next, the 0.1N NaOH is added to denature the DNA samples. The MA2 reagent neutralizes the sample. Lastly, MSM (Multi-Sample Amplification Master Mix) is added to the plate.

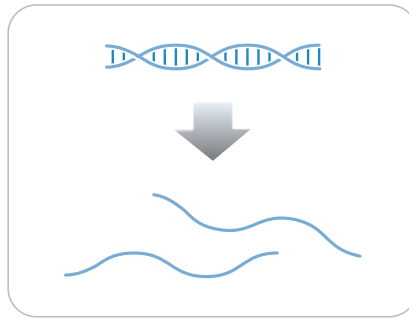


Figure 25 Denaturing and Neutralizing DNA

Estimated Time

Hands-on time:

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

Incubation time: 20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MA1	1 tube (per 96 samples)	-15° to -25°C	Illumina
MA2	1 tube (per 96 samples)	-15° to -25°C	Illumina
MSM	1 tube (per 96 samples)	-15° to -25°C	Illumina
0.1N NaOH	15 ml (per 96 samples)	2° to 8°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate		User
WG#-DNA plate with 96 DNA samples (50 ng/μl)	1 plate	-15° to -25°C	User



CAUTION

Only pour out 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.



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

Preparation

- ▶ In preparation for the Incubate MSA3 process (page 35), preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- ▶ Apply MSA3 barcode label to a new MIDI plate.
- ▶ Thaw MA1, MA2, and MSM tubes to room temperature. Pulse centrifuge to 280 xg.
- ▶ Thaw DNA samples to room temperature.
- ▶ In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well. You may also fill in the optional columns in the Sample Sheet: Gender, Sample_Group, Replicate, Parent1, and Parent2.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - WG#-DNA plate barcode
 - MSA3 plate barcode(s)
 - MA1 tube barcode(s)
 - MA2 tube barcode(s)
 - MSM tube barcode(s)



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

- Steps**
- If you do not already have a WG#-DNA plate, create one by adding DNA, normalized to 50 ng/ μ l, into either a:
 - MIDI plate: 20 μ l to each WG#-DNA plate well
 - TCY plate: 10 μ l to each WG#-DNA plate well
 Apply a barcode label to the new WG#-DNA plate.
 - Dispense 20 μ l MA1 into the MSA3 plate wells.
 - Transfer 4 μ l DNA sample, from the WG#-DNA plate to the corresponding wells in the MSA3 plate (Figures 26).

**CAUTION**

To ensure optimal performance, exchange tips between DNA samples and use aerosol filter tips when pipetting DNA.

- On the lab tracking form, record the original DNA sample ID for each well in the MSA3 plate.
- Dispense 4 μ l 0.1N NaOH into each well of the MSA3 plate that contains MA1 and sample.

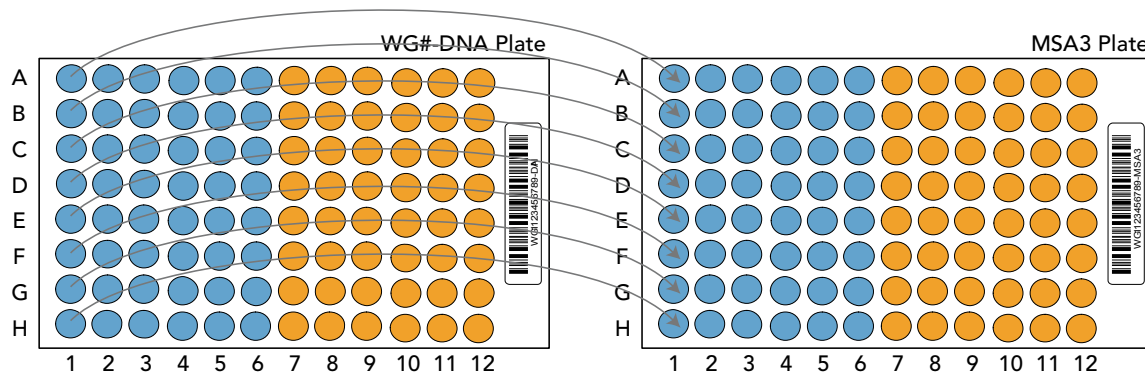


Figure 26 Distributing Sample to Wells

- Seal the MSA3 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, ensure that all 96 caps are securely seated in the wells.

- Vortex the plate at 1600 rpm for 1 minute.
- Centrifuge to 280 xg for 1 minute.
- Incubate for 10 minutes at room temperature.
- Carefully remove the cap mat.
- Dispense 34 μ l MA2 into each well of the MSA3 plate containing sample.



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

12. Dispense 38 μ l MSM into each well of the MSA3 plate containing sample.
13. Reseal the MSA3 plate with the cap mat.
14. Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.
15. Pulse centrifuge to 280 xg for 1 minute.
16. Discard unused reagents in accordance with facility standards.
17. Proceed immediately to *Incubate the MSA3 Plate* on page 35.

Incubate the MSA3 Plate

This process incubates the MSA3 plate for 20–24 hours at 37°C in the Illumina Hybridization Oven. It generates a sufficient quantity of each individual DNA sample to be used once in the Infinium HD Assay Ultra.

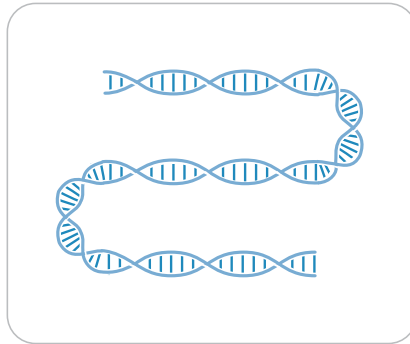


Figure 27 Incubating DNA to Amplify

Estimated Time Incubation time: 20–24 hours

Incubate MSA3

1. Incubate MSA3 plate in the Illumina Hybridization Oven for at least 20 but no more than 24 hours at 37°C.
2. On the lab tracking form, record the start and stop times.



NOTE

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

3. Proceed to *Fragment the MSA3 Plate* on page 36.

Fragment the MSA3 Plate

This process enzymatically fragments the amplified DNA samples. An end-point fragmentation is used to prevent over-fragmentation.

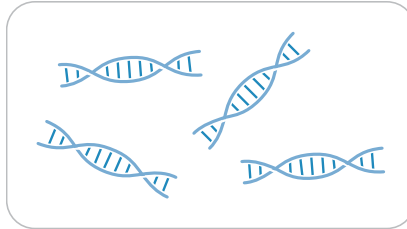


Figure 28 Fragmenting DNA

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)	-15° to -25°C	Illumina



Only pour out 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.



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

Preparation

- ▶ Remove the MSA3 plate from the Illumina Hybridization Oven.
- ▶ Remove the cap map.
- ▶ Preheat the heat block with the MIDI plate insert to 37°C.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - FMS 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. This form can be filled out and saved online, or printed and filled in by hand. Go to <http://www.illumina.com/documentation> to download the lab tracking form.

Steps

1. Centrifuge the plate to 50 xg for 1 minute.
2. 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. When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.
3. Add 25 μ l FMS to each well containing sample.
4. Seal the MSA3 plate with the 96-well cap mat.
5. Vortex the plate at 1600 rpm for 1 minute.
6. Centrifuge the plate to 50 xg for 1 minute at 22°C.
7. Place the sealed plate on the 37°C heat block for 1 hour.
8. On the lab tracking form, record the start and stop times.
9. Discard unused reagents in accordance with facility standards.
10. Do one of the following:
 - Proceed to *Precipitate the MSA3 Plate* on page 38. Leave the plate in the 37°C heat block until setup is complete.
 - Store the sealed MSA3 plate at -15° to -25°C if you do not plan to proceed to the next step immediately.



This is a good stopping point in the process.

Precipitate the MSA3 Plate

Add PM1 and 2-propanol to the MSA3 plate to precipitate the DNA samples.

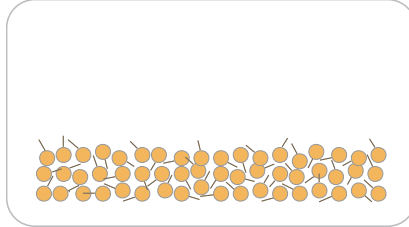


Figure 29 Precipitating DNA

Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	1 tube (per 96 samples)	2° to 8°C	Illumina
100% 2-propanol	30 ml (per 96 samples)	Room temperature	User



CAUTION

Only pour out 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

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

Preparation

- ▶ Preheat heat block to 37°C.
- ▶ If frozen, thaw MSA3 plate to room temperature. Pulse centrifuge to 50 xg.
- ▶ Thaw PM1 to room temperature. Centrifuge to 280 xg for 1 minute.
- ▶ Remove the 96-well cap mat.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - PM1 tube barcode(s)
 - 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. This form can be filled out and saved online, or printed and filled in by hand. Go to <http://www.illumina.com/documentation> to download the lab tracking form.

Steps

1. Add 50 μ l PM1 to each MSA3 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. Centrifuge to 50 xg at 22°C for 1 minute.

**NOTE**

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

6. Carefully remove the cap mat and discard it.
7. Add 155 μ l 100% 2-propanol to each well containing sample.
8. Carefully seal the MSA3 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 at least 10 times to mix contents thoroughly.
10. Incubate at 4°C for 30 minutes.
11. Place the sealed MSA3 plate in the centrifuge opposite another plate of equal weight (Figure 30).

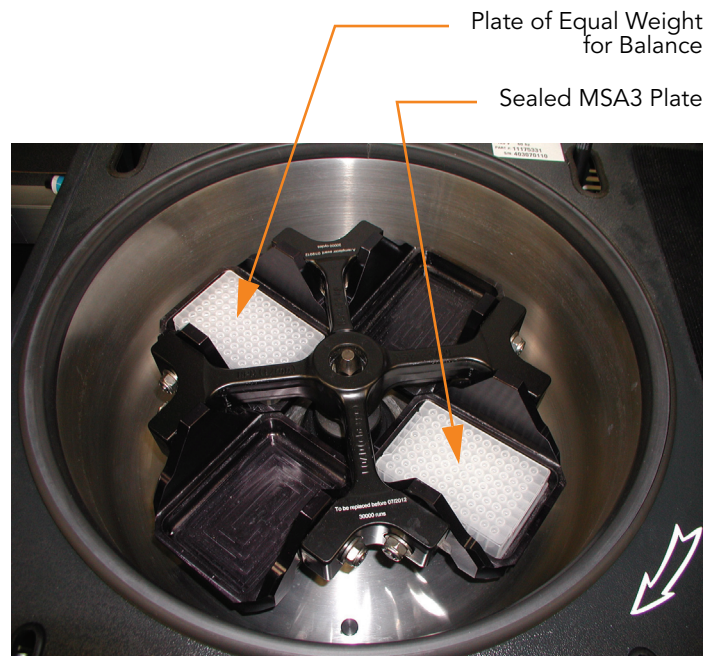


Figure 30 Balancing MSA3 Plate in Centrifuge

12. Centrifuge to 3,000 xg at 4°C for 20 minutes. Immediately remove the MSA3 plate from the centrifuge.
Perform the next step immediately, so that the blue pellet stays in place. If any delay occurs, repeat step 12 before proceeding.
13. Remove the cap mat and discard it.
14. Decant supernatant by quickly inverting the MSA3 plate and smacking it down onto an absorbent pad.
15. Tap firmly several times for 1 minute or until all wells are devoid of liquid. Do not allow supernatant to pour into other wells.



CAUTION

Keep 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 (Figure 31).
At this point, blue pellets should be present at the bottoms of the wells.



Figure 31 Uncovered MSA3 Plate Inverted for Air Drying



CAUTION

Do not over-dry the pellet. Pellets that are over-dried will be difficult to resuspend. Poorly resuspended samples will lead to poor genotyping results.

17. On the lab tracking form, enter the start and stop times.
18. Discard unused reagents in accordance with facility standards.
19. Do one of the following:
 - Proceed to *Resuspend the MSA3 Plate*.
 - Seal the MSA3 plate with a cap mat and store it at -15° to -25°C if you do not plan to proceed to the next step immediately.



This is a good stopping point in the process.

Resuspend the MSA3 Plate

Add RA1 to the MSA3 plate to resuspend the precipitated DNA samples.

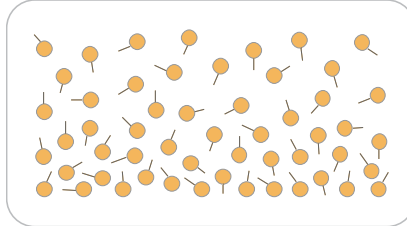


Figure 32 Resuspending DNA

Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation time: 1 hour

Consumables

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



NOTE

Only pour out 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 HD BeadChip** step.



WARNING

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the MSDS for this kit, which is available at <http://www.illumina.com/msds>.

Preparation

- ▶ RA1 is shipped frozen. Gradually warm the reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- ▶ If you stored the MSA3 plate at -15° to -25°C, thaw it to room temperature. Remove the cap mat and discard it.
- ▶ Preheat the Illumina Hybridization Oven to 48°C.
- ▶ Turn on the heat sealer to preheat. Allow 20 minutes.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - RA1 bottle barcode(s)



NOTE

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

Steps

1. Add 23 μ l RA1 to each well of the MSA3 plate containing a DNA pellet. Reserve any leftover reagent for XStain HD BeadChip.
2. Apply a foil seal to MSA3 plate by firmly holding the heat-sealer sealing block down for 5 seconds.
3. Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
4. Vortex the plate at 1800 rpm for 1 minute.
5. Pulse centrifuge to 280 xg.

If you stored the pellets at -15° to -25°C for extended periods of time after the Precip MSA3 process, you may need to repeat steps 3 to 5 until the pellets are completely resuspended.

6. Do one of the following:
 - Proceed to *Hybridize Multi BeadChip* on page 44. If you plan to do so immediately, it is safe to leave the MSA3 plate at room temperature.
 - If you do not plan to proceed to the next step immediately, store the sealed MSA3 plate at -15° to -25°C (-80°C if storing for more than 24 hours). Store RA1 at -15° to -25°C.



This is a good stopping point in the process.

Hybridize Multi BeadChip

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.

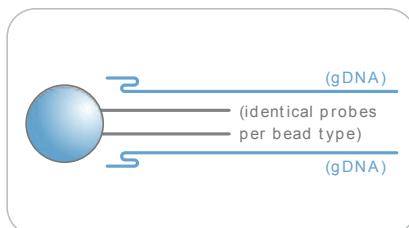


Figure 33 Hybridizing DNA to BeadChip

Estimated Time

Hands-on time:

- 24x1 HD BeadChips: ~1 hour for 4 BeadChips (96 samples)
- 12x1 HD BeadChips: ~1 hour for 8 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables 24x1 HD BeadChip

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	1 tube	Room temperature	Illumina
BeadChips	4		Illumina
Hyb Chambers	1		Illumina
Hyb Chamber gaskets	1		Illumina
Hyb Chamber inserts	4		Illumina

Consumables 12x1 HD BeadChip

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

Item	Quantity (per 96 Samples)	Storage	Supplied By
Hyb Chamber inserts	8		Illumina

**CAUTION**

Only pour out the recommended reagent volume needed for the suggested number of BeadChips listed in the Consumables table of each section. Some reagents are used later in the protocol.

**NOTE**

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

Preparation

- ▶ Preheat the heat block to 95°C.
- ▶ Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.
- ▶ Prepare the Illumina Hybridization Oven as follows:
 - a. Preheat the oven to 48°C:
 - Press the "F" button once 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.
- ▶ Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - PB2 tube barcode

**NOTE**

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

- Steps** This protocol includes the following steps:
- ▶ Assemble the Hyb Chambers on page 46
 - ▶ Hybridize Sample to Multi BeadChip on page 49
 - ▶ Load BeadChip on page 49
 - ▶ Set up Multi BeadChip for Hyb on page 53

Assemble the Hyb Chambers

1. Place the following items on the bench top per 96 samples (Figure 34):
 - For the 24x1 HD BeadChip:
 - BeadChip Hyb Chamber (1)
 - Hyb Chamber Gasket (1)
 - Hyb Chamber inserts (4)
 - For the 12x1 HD BeadChip:
 - BeadChip Hyb Chambers (2)
 - Hyb Chamber Gaskets (2)
 - Hyb Chamber inserts (8)

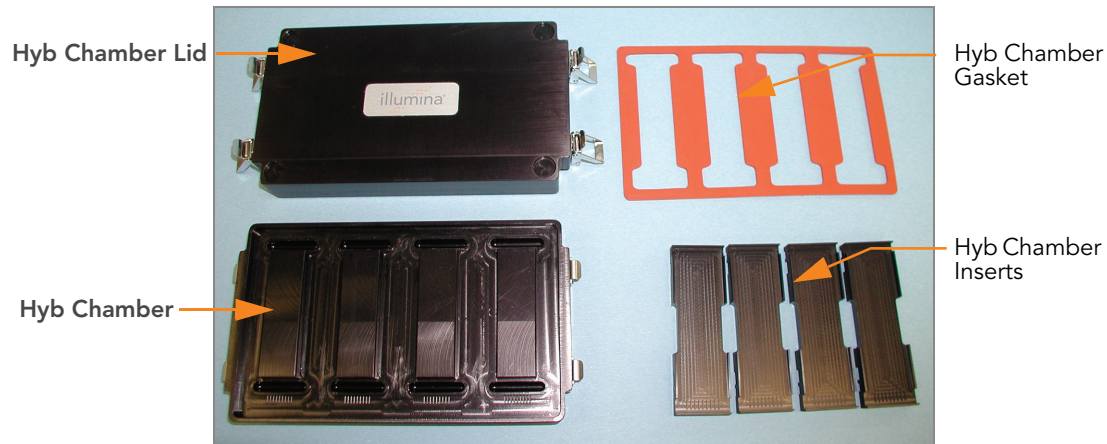


Figure 34 BeadChip Hyb Chamber Components

2. Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers as shown.
 - a. Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber (Figure 35).

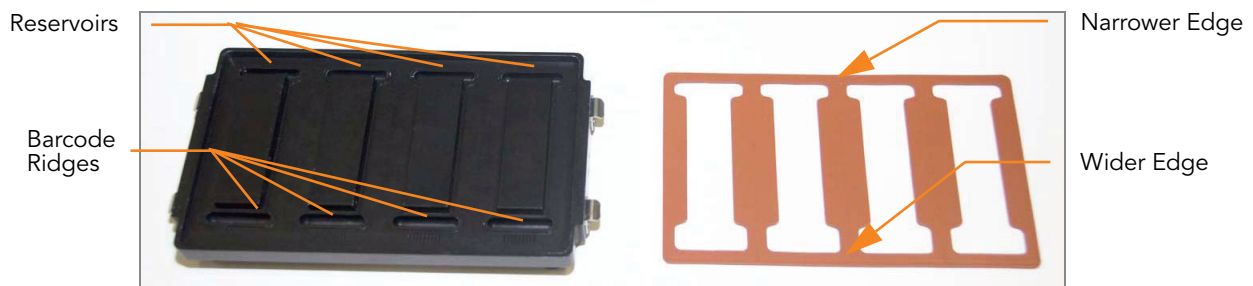


Figure 35 Hyb Chamber and Gasket

- b. Lay the gasket into the Hyb Chamber (Figure 36), and then press it down all around.



Figure 36 Placing Gasket into Hyb Chamber

- c. Make sure the Hyb Chamber gaskets are properly seated (Figure 37).



Figure 37 Hyb Chamber with Gasket in Place

3. Dispense 400 μ L PB2 into the humidifying buffer reservoirs in the Hyb Chambers (Figure 38).



Figure 38 Dispensing PB2 into Hyb Chamber Reservoir

4. Close and lock the BeadChip Hyb Chamber lid (Figure 39).
 - a. Seat the lid securely on the bottom plate.
 - b. Snap two clamps shut, diagonally across from each other.
 - c. Snap the other two clamps.



Figure 39 Sealing the Hyb Chamber

5. Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample.
6. Remove the BeadChips from 2°–8°C storage but do not unpackage.

Hybridize Sample to Multi BeadChip

1. Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.

**CAUTION**

Do not unpackage the BeadChips until you are ready to begin hybridization.

2. After the 20-minute incubation, remove the MSA3 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.
3. After the 30-minute cool down, pulse centrifuge the MSA3 plate to 280 xg. Remove the foil seal.

Load BeadChip

1. Just before loading DNA samples, remove all BeadChips from their packages.

**CAUTION**

When handling the BeadChip, 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 insert (Figure 40).

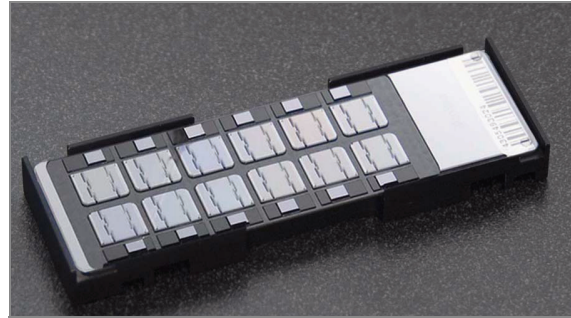
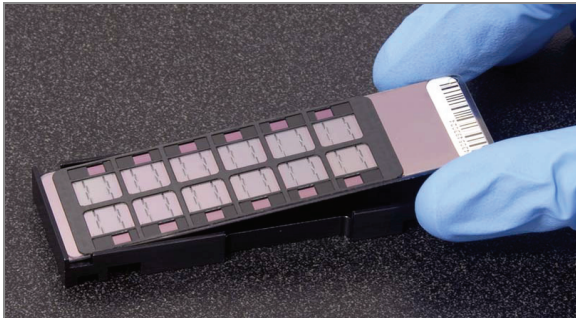


Figure 40 Placing BeadChips into Hyb Chamber Inserts

For the 24x1 HD BeadChip:

1. Using a multi-channel precision pipette, dispense 12 μ l of each DNA sample onto the appropriate BeadChip section, according to the chart

on the lab tracking form (repeated in Figure 44) and the illustrations below:

**CAUTION**

Load samples by directly placing pipette tip to the array surface and hold the pipette straight up above the array surface. Do not hold the pipette at an angle while you are loading sample. Proceed immediately to the next step as soon as all arrays have received sample.

2. Load samples A1–F1 from the MSA3 plate into the left side BeadChip (Figure 43) inlet ports A1–F1 (every other inlet port on the left side of the BeadChip).

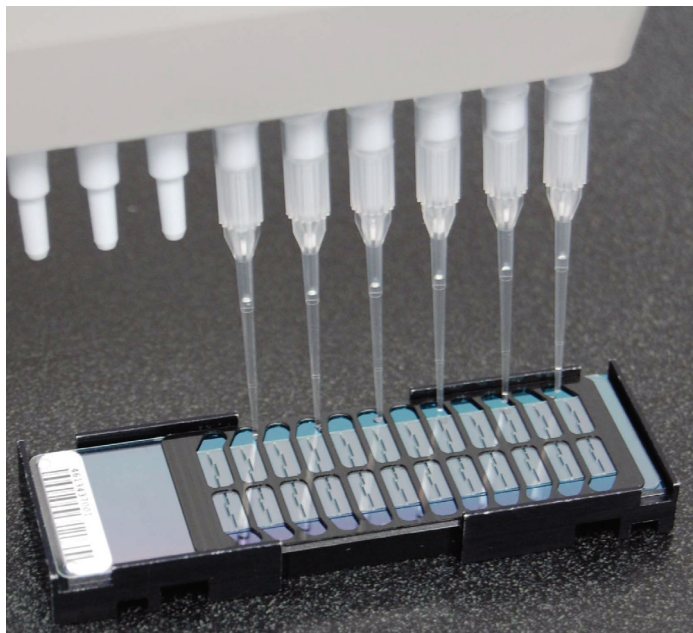


Figure 41 Loading Samples (24x1 HD BeadChip)

3. Load samples in G1 and H1 from the MSA3 plate into the left side BeadChip inlet ports G1 and H1.
4. Load samples A2–D2 from the MSA3 plate into the left side BeadChip inlet ports A2–D2.
5. Load samples E2–H2 from the MSA3 plate into the right side BeadChip inlet ports E2–H2.
6. Load samples A3 and B3 from the MSA3 plate into the right side BeadChip inlet ports A3 and B3.
7. Load samples C3–H3 from the MSA3 plate into the right side BeadChip inlet ports C3–H3 (every other inlet port on the right side of the BeadChip).
8. Continue loading samples by following the color-coded sections (Figure 44) shown in the following graphic for any additional BeadChips and until all samples are loaded.

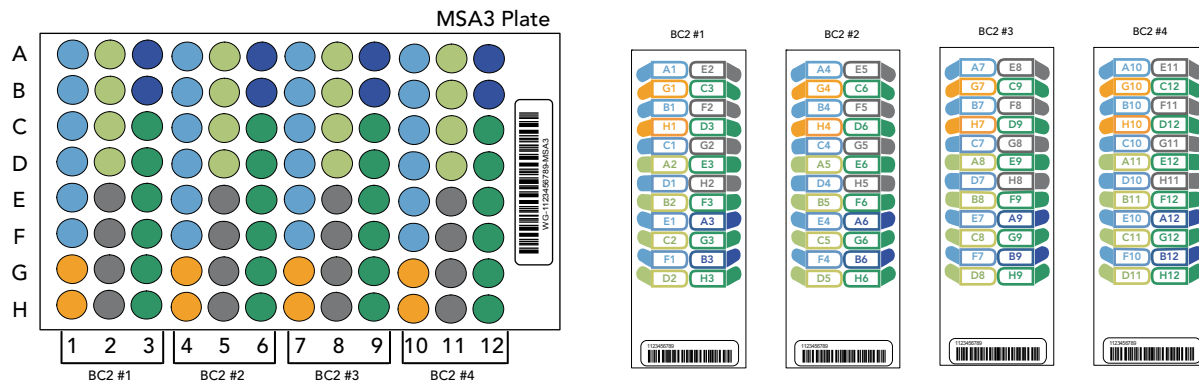


Figure 42 Distributing Sample from MSA3 Plate to BeadChip(s) (24x1 HD BeadChip)

9. On the lab tracking form, record the BeadChip barcode for each group of samples.
10. Visually inspect all sections of the BeadChips. Ensure the DNA sample entirely covers each bead stripe. Record any sections that are not completely covered.
11. To ensure optimal performance, after you have visually inspected all sections of the BeadChips, place the Hyb Chamber inserts inside the Hyb Chamber immediately as described in the next section, *Set up Multi BeadChip for Hyb*

For the 12x1 HD BeadChip:

1. Using a multi-channel precision pipette, dispense 15 μ l of each DNA sample onto the appropriate BeadChip section, according to the chart on the lab tracking form (repeated in Figure 44) and the following illustrations:



CAUTION

Load samples by directly placing pipette tip to the array surface and hold the pipette straight up above the array surface. Do not hold the pipette at an angle while you are loading sample. Proceed immediately to the next step as soon as all arrays have received sample.

2. Load samples A1–F1 from the MSA3 plate into the left side of the BeadChip (Figure 43).

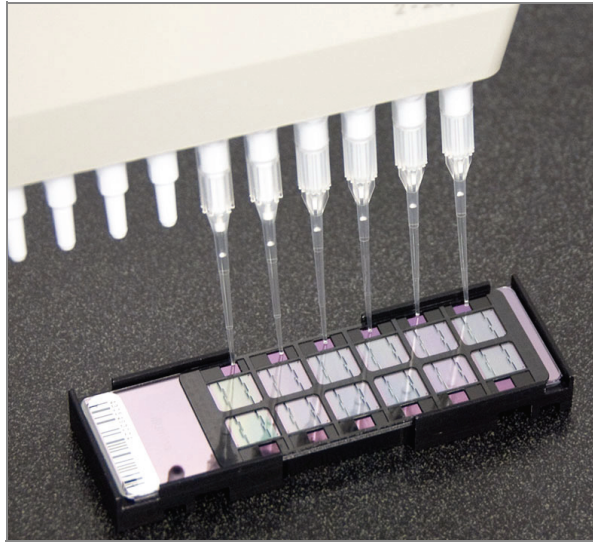


Figure 43 Loading Samples (12x1 HD BeadChip)

3. Load samples in G1 and H1 from the MSA3 plate into the top two inlets of the right side of the BeadChip.
4. Load samples A2–D2 into the remaining four inlets on the right side of the BeadChip.
5. Continue loading samples by following the color-coded sections shown in Figure 44 until all samples are loaded.

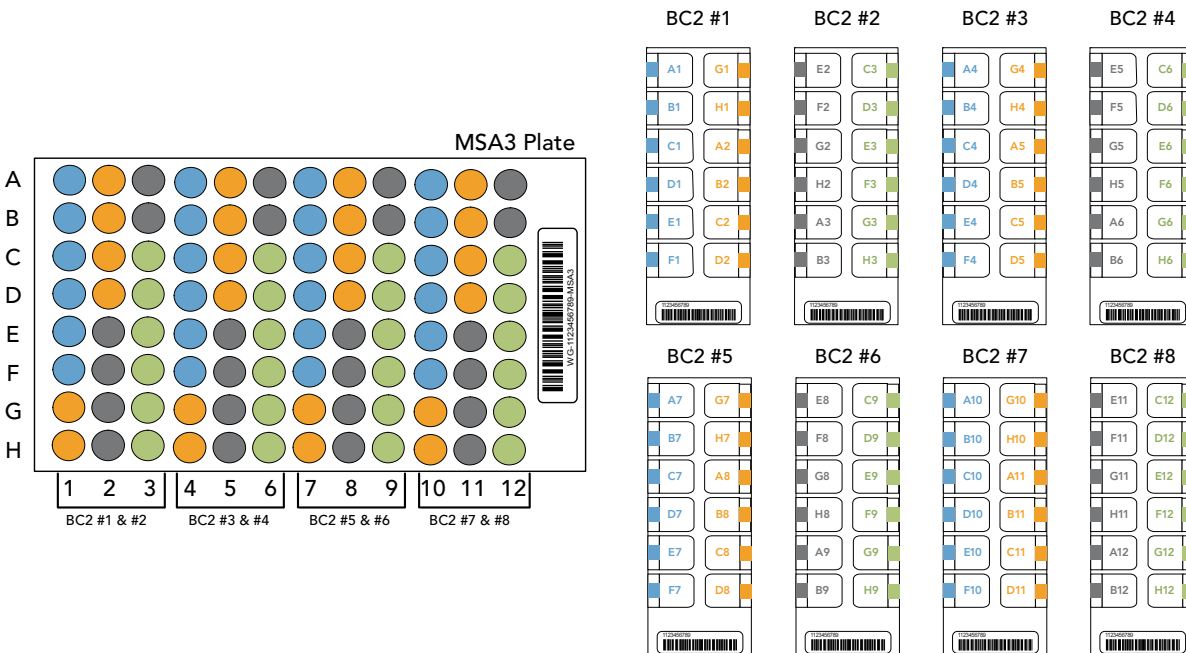


Figure 44 Distributing Sample from MSA3 Plate to BeadChip(s) (12x1 HD BeadChip)

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

7. Visually inspect all sections of the BeadChips. Ensure the DNA sample entirely covers each bead stripe. Record any sections that are not completely covered.
8. To ensure optimal performance, after you have visually inspected all sections of the BeadChips, place the Hyb Chamber inserts inside the Hyb Chamber immediately as described in the next section, *Set up Multi BeadChip for Hyb*.

Set up Multi BeadChip for Hyb

1. Ensure the Illumina Hybridization Oven is preheated to 48°C.
2. Remove the Hyb Chamber lid and load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chamber. Position the barcode end over the ridges indicated on the Hyb Chamber (Figure 45).



Figure 45 Placing Hyb Chamber Inserts into the Hyb Chamber



For optimal performance, keep the BeadChips steady and level when lifting or moving. Avoid shaking and keep parallel to the lab bench at all times. Avoid contacting the sample inlets when handling the BeadChips.

3. 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 46).
4. Close the clamps on both sides of the Hyb Chamber.



Figure 46 Seating Lid onto Hyb Chamber

**NOTE**

For optimal performance, keep the Hyb Chamber steady and level when lifting or moving. Avoid shaking the Hyb Chamber, and keep the Hyb Chamber parallel to the lab bench while you transfer it to the Illumina Hybridization Oven.

5. Place the Hyb Chamber into the 48°C Illumina Hybridization Oven so that the clamps of the Hyb Chamber face the left and right sides of the oven. The Illumina logo on top of the Hyb Chamber should be facing you.

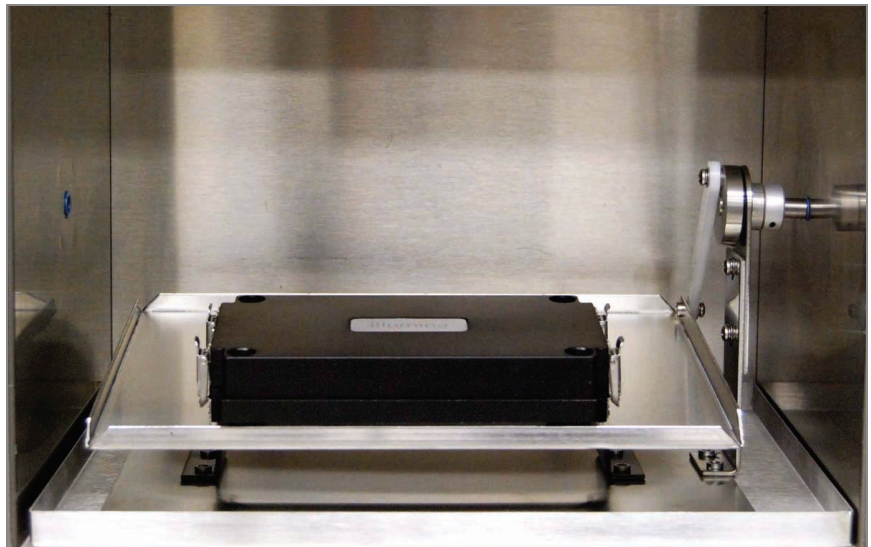


Figure 47 Hyb Chamber Correctly Placed in Hyb Oven

**NOTE**

If you are stacking multiple Hyb Chambers in the Illumina Hybridization Oven, make sure the feet of the top Hyb Chamber fit into the matching indents on top of the next Hyb Chamber. This will hold the Hyb Chambers in place while they are rocking. You can stack up to 3 Hyb Chambers per row for a maximum total of 6 Hyb Chambers in a Hyb Oven.

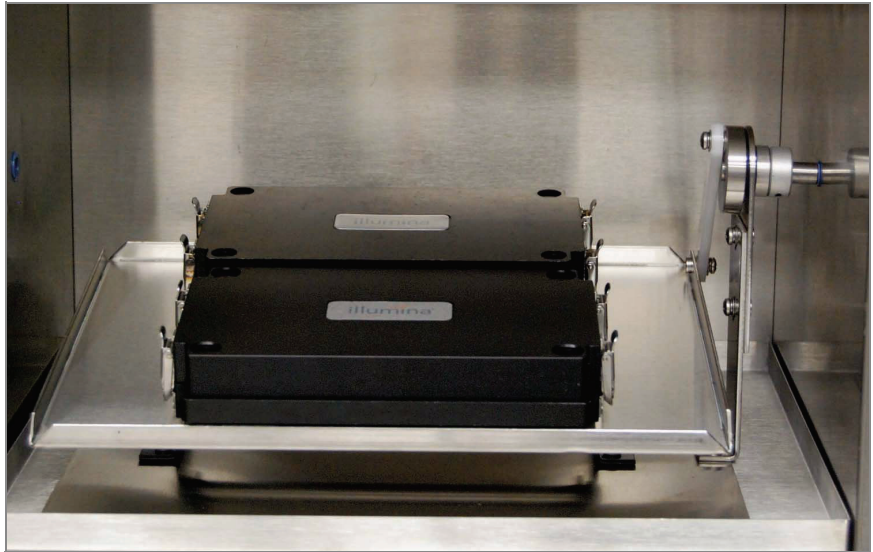


Figure 48 Two Hyb Chambers Correctly Placed in Hyb Oven



Figure 49 Incorrectly Placed Hyb Chamber

6. Incubate at 48°C for at least 16 hours but no more than 24 hours.
7. Discard the MSA3 plate.
8. On the lab tracking form, enter the start and stop times.

Resuspend XC4 Reagent for XStain HD BeadChip

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

1. Add 330 ml 100% EtOH to the XC4 bottle. The final volume will be 350 ml.
Each XC4 bottle (350 ml) has enough solution to process up to 24 BeadChips.
2. Shake vigorously for 15 seconds.
3. Leave the bottle upright on the lab bench overnight.



If the XC4 was not left to resuspend overnight, you can still proceed with the assay. Add the EtOH and put the XC4 on its side on a rocker to resuspend. Leave it there until the BeadChips are ready for coating.

Shake again to ensure that the pellet is completely resuspended. If any coating is visible, vortex at 1625 rpm until it is in complete suspension. Once resuspended with 330 ml 100% EtOH, use XC4 at room temperature.

4. After the overnight incubation, proceed to *Wash BeadChip* on page 57.

Wash BeadChip

This process prepares the BeadChips for the XStain HD BeadChip process. First, remove the IntelliHyb Seals from the BeadChips and wash the BeadChips in two separate PB1 reagent washes. Next, assemble the BeadChips into the Flow-Through Chambers under PB1 buffer.

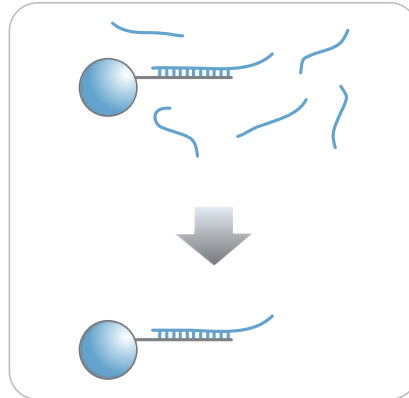


Figure 50 Washing BeadChip

Estimated Time

Hands-on time: ~30 minutes for 8 BeadChips

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml (up to 8 BeadChips)	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1 (per 4 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



This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the MSDS for this kit, which is available at <http://www.illumina.com/msds>.



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

Preparation

- ▶ Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 25 minutes prior to opening.
- ▶ Have ready on the lab bench:
 - Two wash dishes each containing 200 ml PB1, and labeled as such
 - Multi-Sample BeadChip Alignment Fixture
 - Using a graduated cylinder, fill with 150 ml PB1
 - Te-Flow Flow-Through Chamber components:
 - Black frames
 - Spacers (separated for ease of handling)
 - Clean glass back plates
 - Clamps
- ▶ Clean the glass back plates as directed in the *Infinium Assay Lab Setup and Procedures Guide*.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - PB1 bottle barcode



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

Steps Wash BeadChip

1. Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1 (Figure 51).

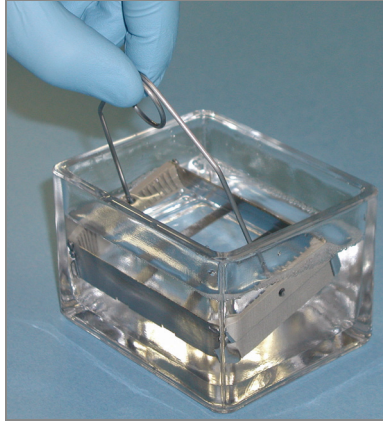


Figure 51 Wash Rack in Wash Dish Containing PB1

2. Remove the Hyb Chamber inserts from the Hyb Chambers.
3. Remove BeadChips from the Hyb Chamber inserts one at a time.
4. Remove the IntelliHyb Seal from each BeadChip (Figure 52):



CAUTION

To ensure no solution splatters on you, Illumina recommends removing the coverseal over an absorbent cloth or paper towels, preferably in a hood.

- a. Using powder-free gloved hands, hold the BeadChip in one hand with your thumb and forefinger on the long edges of the BeadChip. The BeadChip may also be held with the thumb and forefinger on the short edges of the BeadChip. In either case avoid contact with the sample inlets. The barcode should be facing up and be closest to you, and the top side of the BeadChip should be angled slightly away from you.
- b. Remove the entire seal in a single, slow, consistent motion by pulling it off in a diagonal direction. 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. Do not stop and start the pulling action. Do not touch the exposed active areas.
- c. Discard the seal.

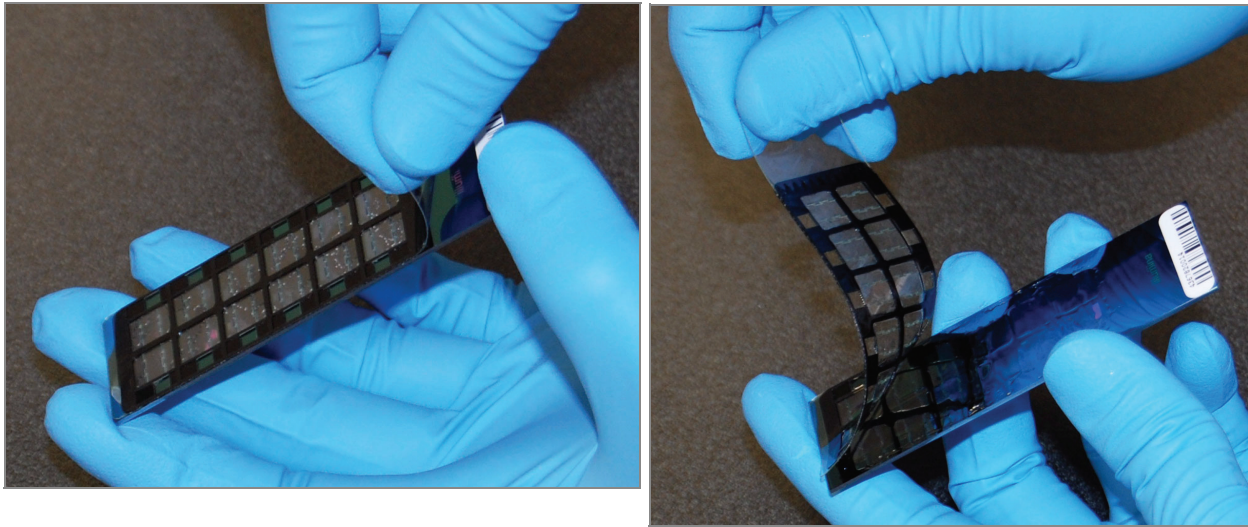


Figure 52 Removing the IntelliHyb Seal



CAUTION

Do not touch the arrays!

5. Immediately and carefully slide each BeadChip into the wash rack one at a time, making sure that the BeadChip is completely submerged in the PB1 (Figure 53).

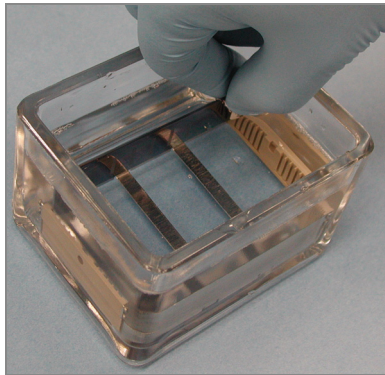


Figure 53 Placing BeadChips in Wash Dish Containing PB1

6. Repeat steps 4 through 5 for each BeadChip. The wash rack holds a max of 8 BeadChips.
7. Once all the 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. Once all BeadChips have been individually washed in PB1, remove the wash rack from the wash dish and place it immediately into the other wash dish containing PB1. Make sure that the BeadChips are completely submerged.

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

Assemble Flow-Through Chamber



NOTE

The 150 ml of PB1 used in the Flow-Through Chamber assembly can be used for up to eight BeadChips. You must use 150 ml of new PB1 for every additional set of eight BeadChips.

1. If you have not done so yet, fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PB1.
2. For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture (Figure 54).

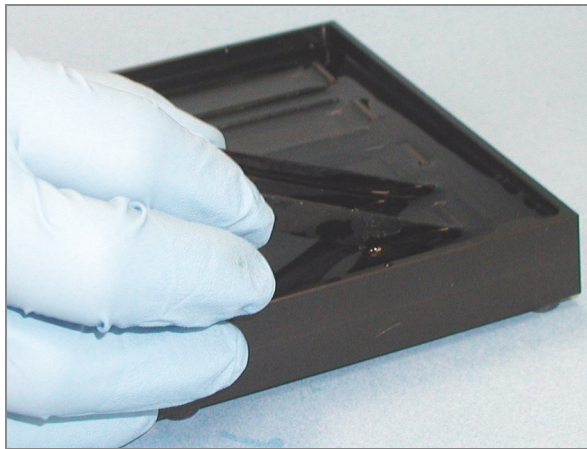


Figure 54 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

3. Place each BeadChip into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture. Each BeadChip should be fully immersed in PB1 (Figure 55).

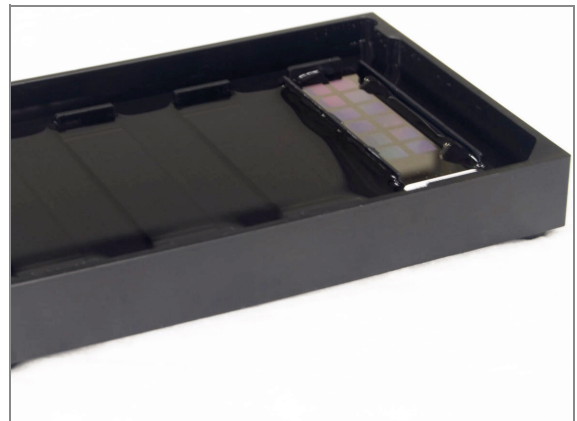
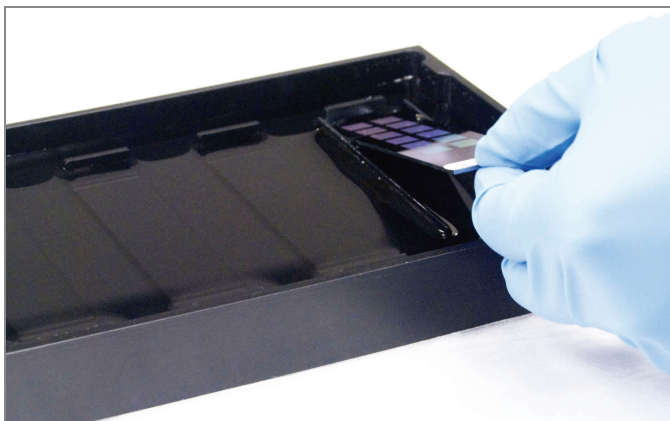


Figure 55 Placing BeadChip into Black Frame on Alignment Fixture

4. Place a clear spacer onto the top of each BeadChip (Figure 56). 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.

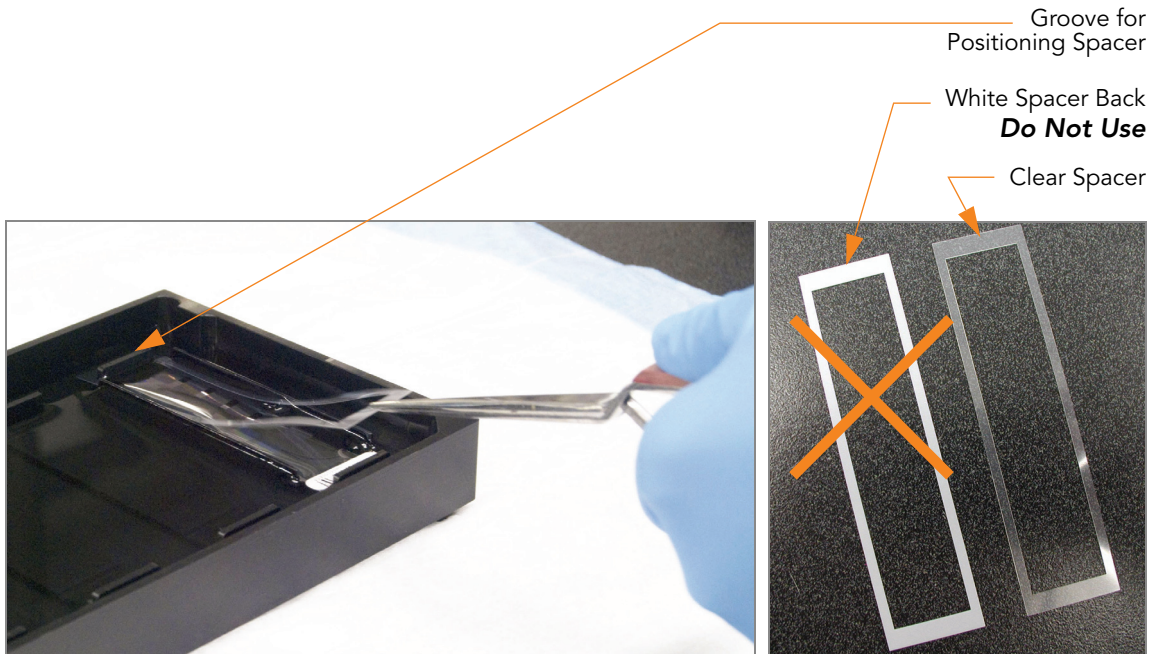


Figure 56 Placing Clear Plastic Spacer onto BeadChip

5. Place the Alignment Bar onto the Alignment Fixture (Figure 57). The groove in the Alignment bar should fit over the metal tab on the Alignment Fixture.

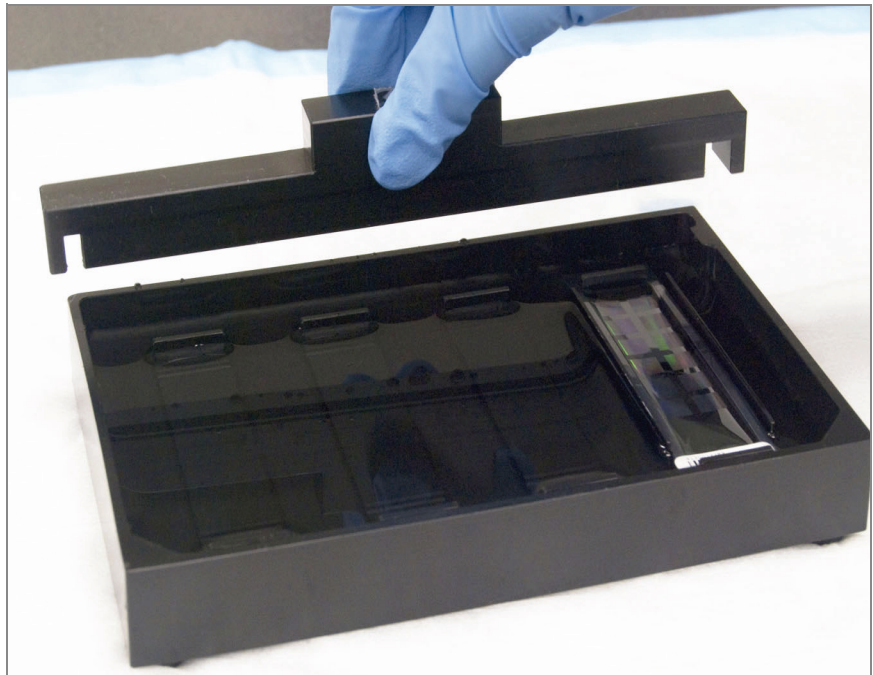


Figure 57 Placing Alignment Bar onto Alignment Fixture

6. Use a Whoosh duster or laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.
7. Place a clean glass back plate on top of the clear spacer covering each BeadChip. The plate reservoir should be at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface (Figure 58).

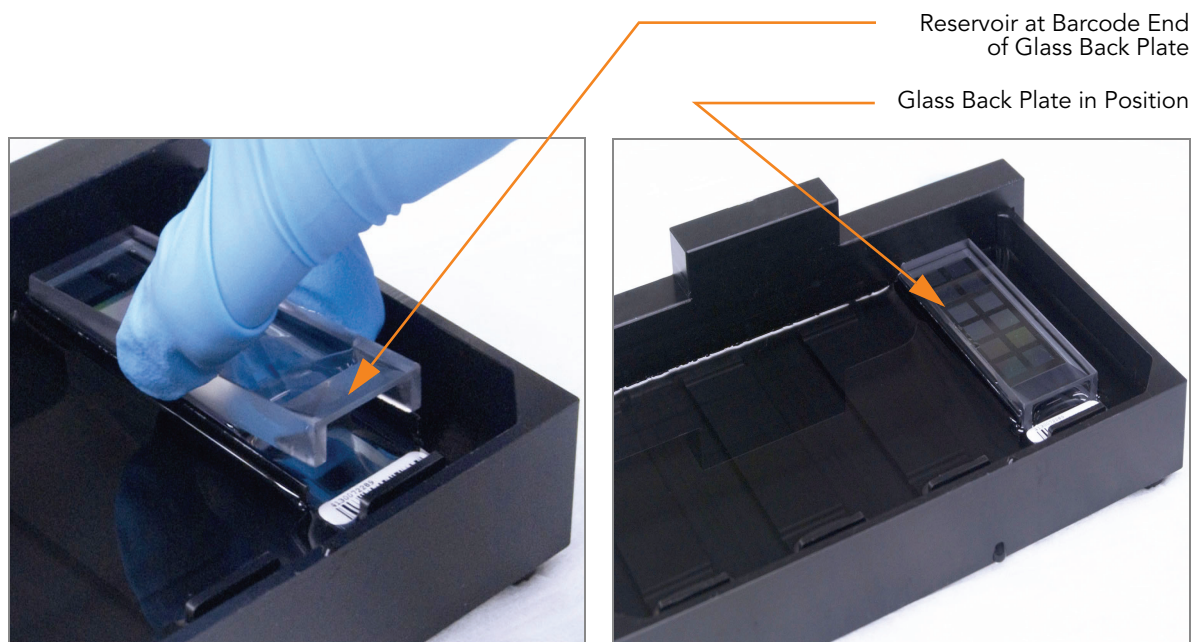


Figure 58 Placing Glass Back Plate onto BeadChip

8. Attach the metal clamps onto each Flow-Through Chamber as follows (Figure 59):
 - a. Gently push the glass back plate up against the Alignment Bar with one finger.
 - b. Place the first metal clamp around the Flow-Through Chamber so that the clamp is about 5 millimeters from the top edge.
 - c. Place the second metal clamp around the Flow-Through Chamber at the barcode end, about 5 millimeters from the reagent reservoir.

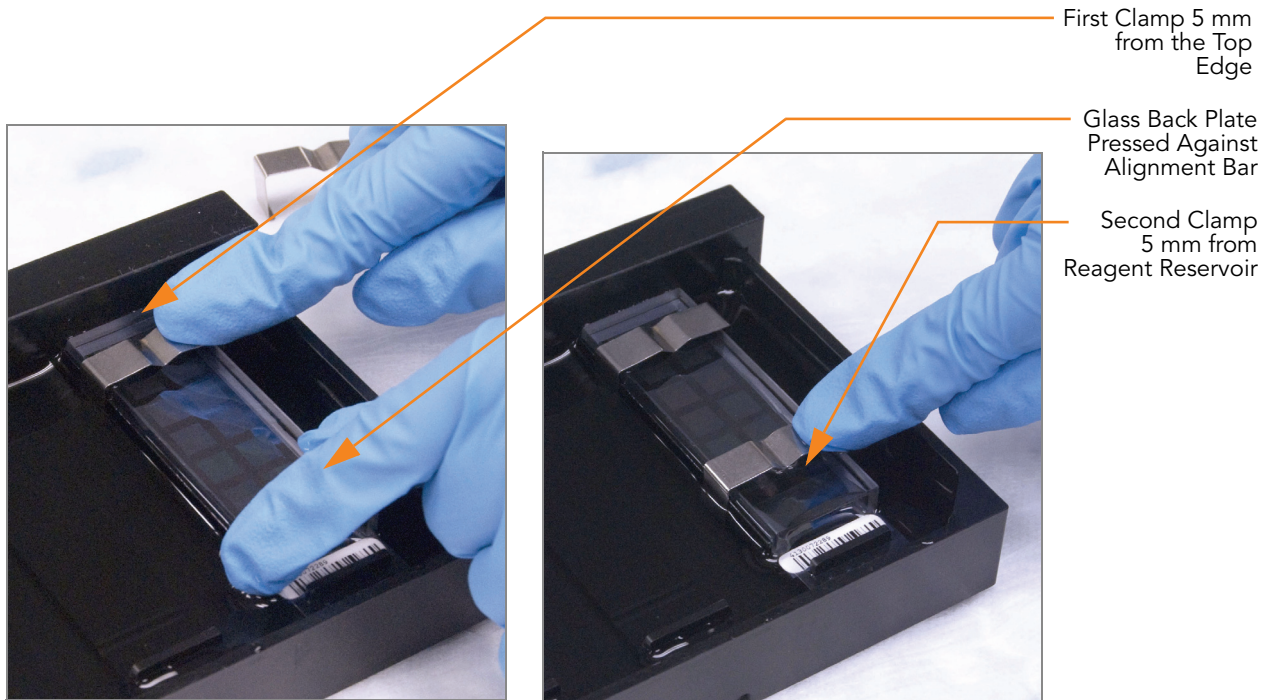


Figure 59 Securing Flow-Through Chamber Assembly with Metal Clamps

9. With scissors, trim the spacer at the non-barcode end of the assembly (Figure 60). Slip the scissors up over the barcode to trim the other end.

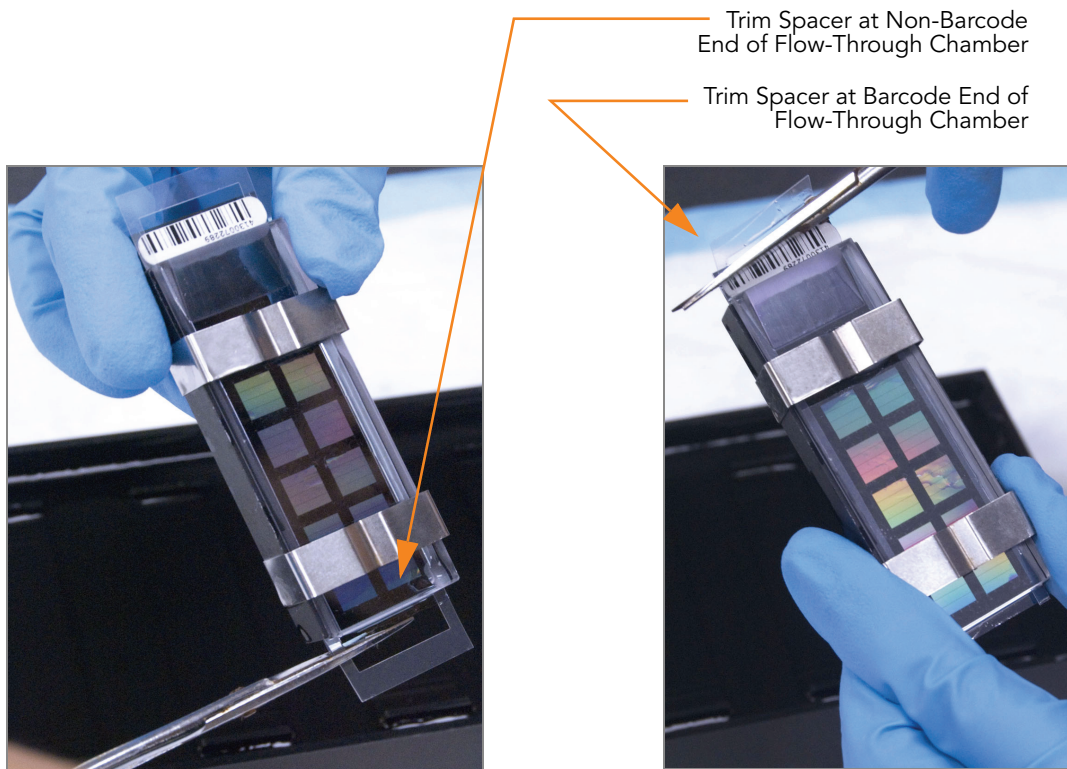


Figure 60 Trimming Spacer Ends from Flow-Through Chamber Assembly

10. Immediately wash the Hyb Chamber reservoirs with dH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains.



CAUTION

It is important to wash the reservoirs immediately and thoroughly to ensure that no traces of PB2 remain in the wells, as these could affect the results of future assays.

11. Discard unused reagents in accordance with facility standards.
12. Proceed to *Single-Base Extension and Stain HD BeadChip* on page 66.



CAUTION

Place all assembled Flow-Through Chambers on the lab bench in a horizontal position while you perform the preparation steps for XStain HD BeadChip. Do not place the Flow-Through Chambers in the Chamber Rack until the preparation is complete.

Single-Base Extension and Stain HD 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.

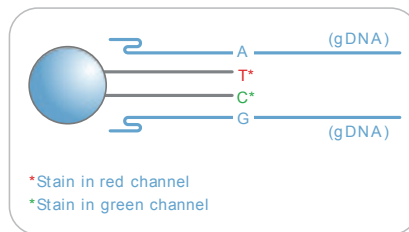


Figure 61 Extending and Staining BeadChip

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	-15° to -25°C	Illumina
XC1	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
XC2	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
TEM	1 tube (per 4 BeadChips)	-15° to -25°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)	-15° to -25°C	Illumina
ATM	1 tube (per 4 BeadChips)	-15° to -25°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	-15° to -25°C	User

**WARNING**

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the MSDS for this kit, which is available at <http://www.illumina.com/msds>.

Preparation

- ▶ Place all reagent tubes in a rack in the order in which they will be used (Figure 62). If frozen, allow them to thaw to room temperature and centrifuge to 3000 xg for 3 minutes.
- ▶ RA1 is shipped and stored at -15° to -25°C. Gradually warm the reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- ▶ Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

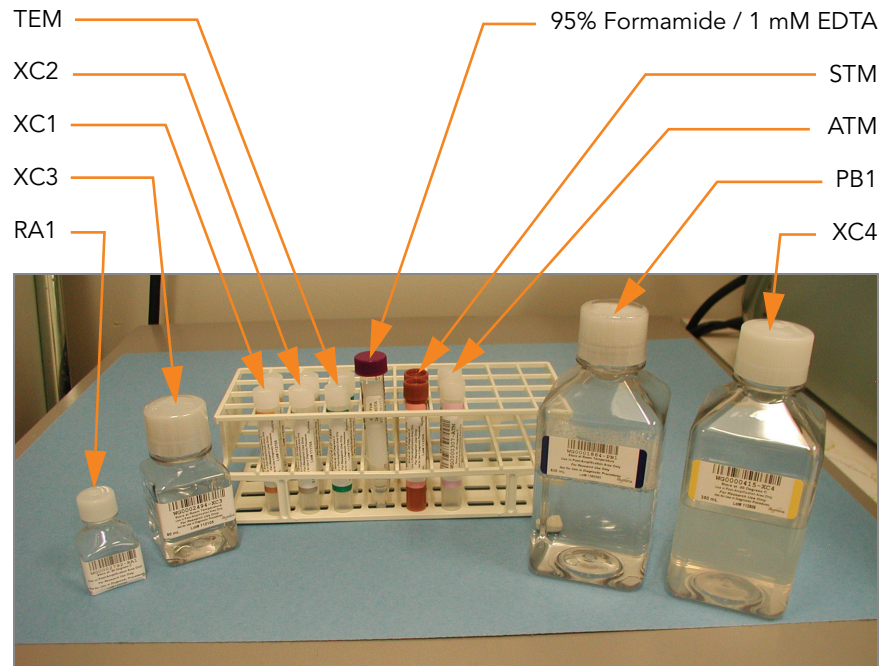


Figure 62 XStain HD BeadChip Reagent Tubes and Bottles

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



CAUTION

Only pour out the recommended reagent volume needed for the suggested number of BeadChips listed in the Consumables table of each section. Some reagents are used later in the protocol.

- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - RA1 barcode
 - XC3 barcode
 - XC1 barcode(s)
 - XC2 barcode(s)
 - TEM barcode(s)
 - STM barcode(s)
 - ATM barcode(s)
 - PB1 barcode
 - XC4 barcode(s)



NOTE

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

Set Up the Chamber Rack

1. Ensure the water circulator reservoir is filled with water to the appropriate level. See the *VWR Operator's Manual*.
2. Turn on the water circulator and set it to a temperature that brings the Chamber Rack to 44°C at equilibrium (Figure 63).

This temperature may vary depending on facility ambient conditions.

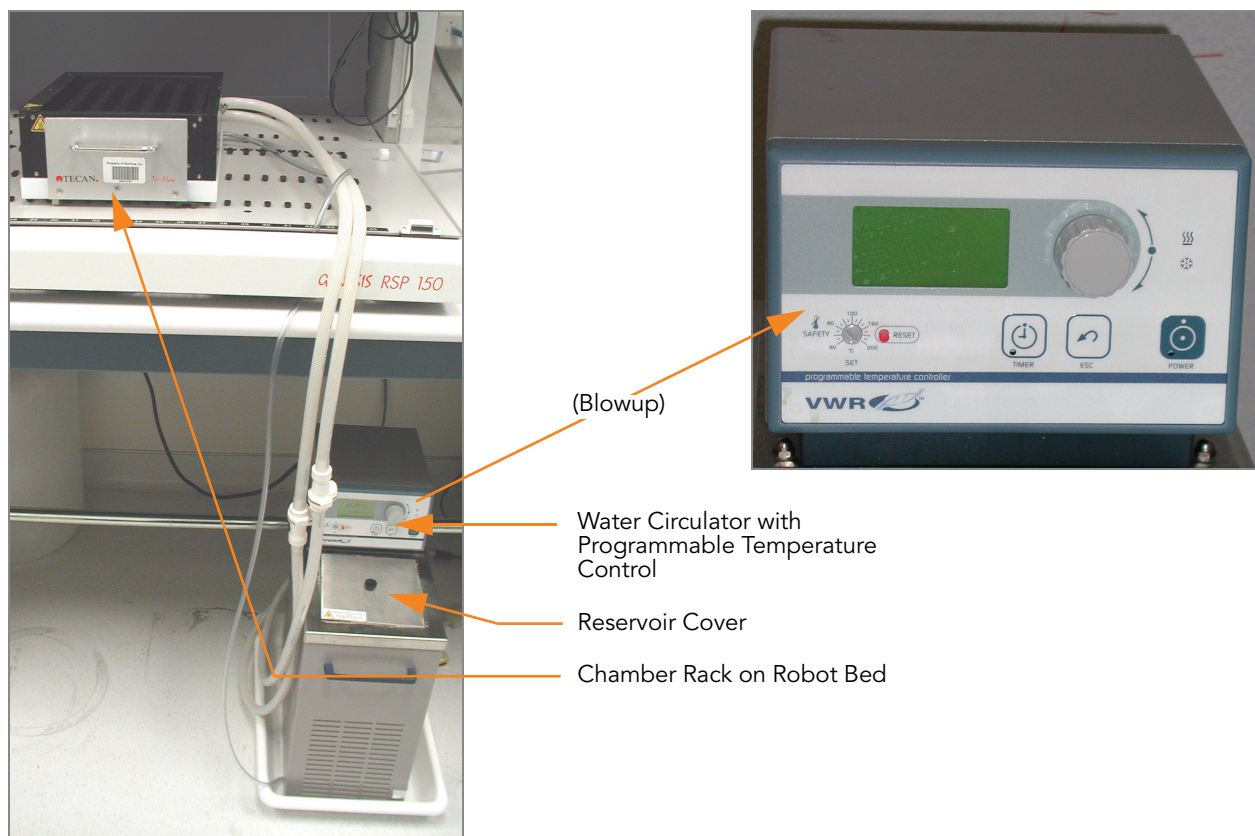


Figure 63 Water Circulator Connected to Chamber Rack

3. The temperature displayed on the water circulator LCD screen may differ from the actual temperature in the Chamber Rack. Confirm the actual Chamber Rack temperature using the temperature probe.
4. You must 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.

- Use the Illumina temperature probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 64).

**NOTE**

Do not leave the temperature probe in the first three rows of the Chamber Rack. Reserve this space for BeadChips.

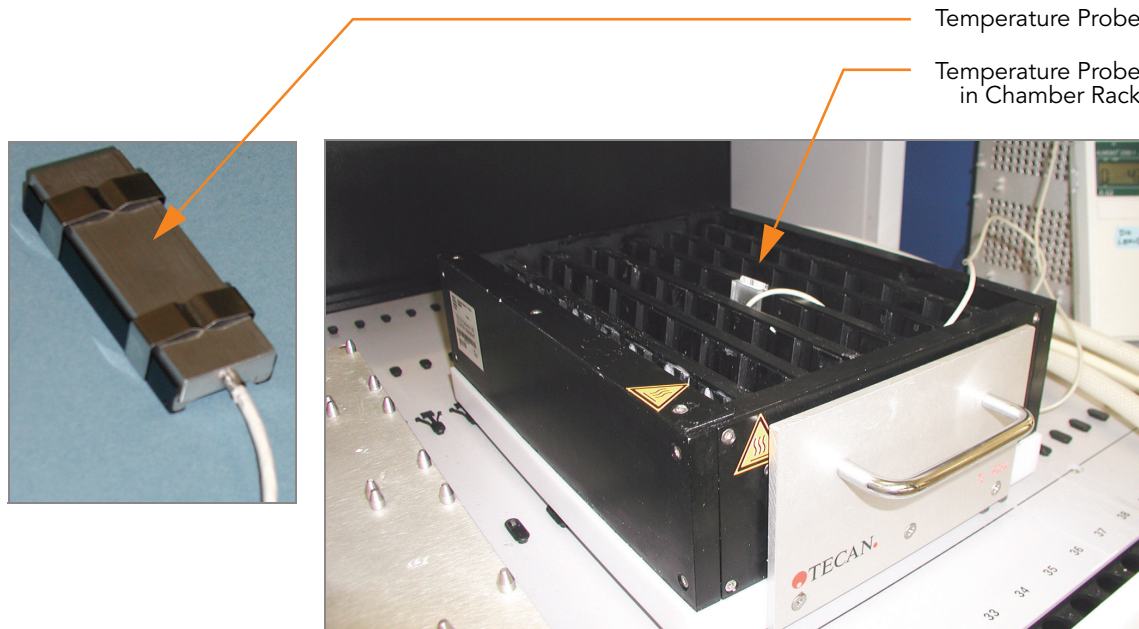


Figure 64 Illumina Temperature Probe in Chamber Rack

- For accurate temperature measurement, ensure the temperature probe is touching the floor of the Chamber Rack.
The remaining steps in this protocol must be performed without interruption.

Steps

This protocol includes the following steps:

- ▶ *Single-Base Extension* on page 70
- ▶ *Stain BeadChip* on page 72
- ▶ *Wash and Coat 8 BeadChips* on page 72

**CAUTION**

The remaining steps must be performed without interruption.

Single-Base Extension

- 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 num-

bers of BeadChips, fill all positions in the first row, then the second and third.

2. Ensure 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.

**CAUTION**

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

4. Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.
5. Into the reservoir of each Flow-Through Chamber, dispense:
 - a. 150 μ l RA1 (Figure 65). Incubate for 30 seconds. Repeat 5 times.



Pipette tip must not contact BeadChip surface

Figure 65 Dispensing RA1 into Each Flow-Through Chamber

- b. 450 μ l XC1. Incubate for 10 minutes.
 - c. 450 μ l XC2. Incubate for 10 minutes.
 - d. 200 μ l TEM. Incubate for 15 minutes.
 - e. 450 μ l 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat once.
 - f. Incubate 5 minutes.
 - g. Begin ramping the Chamber Rack temperature to the temperature indicated on the STM tube, or to 37°C if none is shown.
 - h. 450 μ l XC3. Incubate for 1 minute. Repeat once.
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 Illumina BeadArray Reader or iScan now to allow the lasers to stabilize.
2. Into the reservoir of each Flow-Through Chamber, dispense:
 - a. 250 μ l STM and incubate for 10 minutes.
 - b. 450 μ l XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - c. 250 μ l ATM and incubate for 10 minutes.
 - d. 450 μ l XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - e. 250 μ l STM and incubate for 10 minutes.
 - f. 450 μ l XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - g. 250 μ l ATM and incubate for 10 minutes.
 - h. 450 μ l XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - i. 250 μ l STM and incubate for 10 minutes.
 - j. 450 μ l XC3 and incubate for 1 minute. Repeat once, and then 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, 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.
1. Place the following items on the bench per 8 BeadChips:
 - 1 staining rack
 - 1 vacuum desiccator
 - 1 tube rack
 - Self-locking tweezers

- Large Kimwipes
 - Vacuum hose
2. Set up two top-loading wash dishes, labeled "PB1" and "XC4" (Figure 66).
 3. 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 and make sure the wash dish is dry. 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.

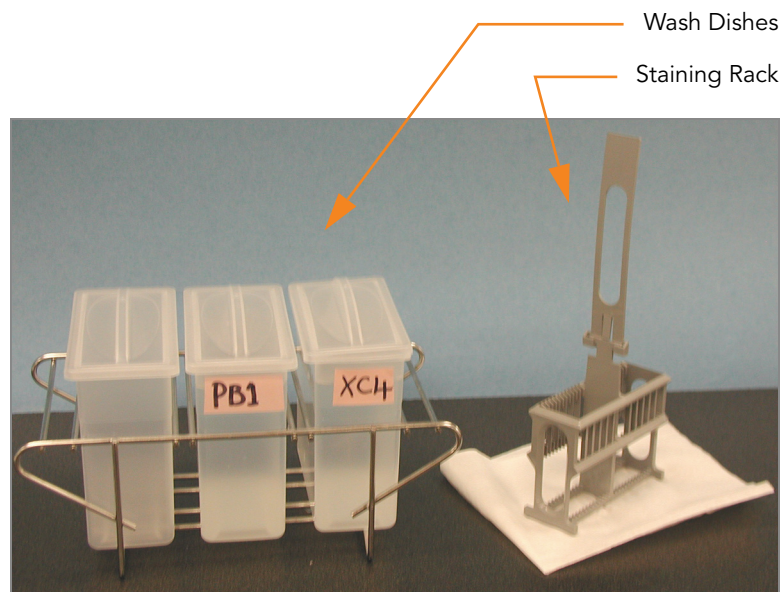


Figure 66 PB1 and XC4 Wash Dishes with Staining Rack

4. Pour 310 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 **towards** you (Figure 67). This orients the staining rack so that you can safely remove the BeadChips.

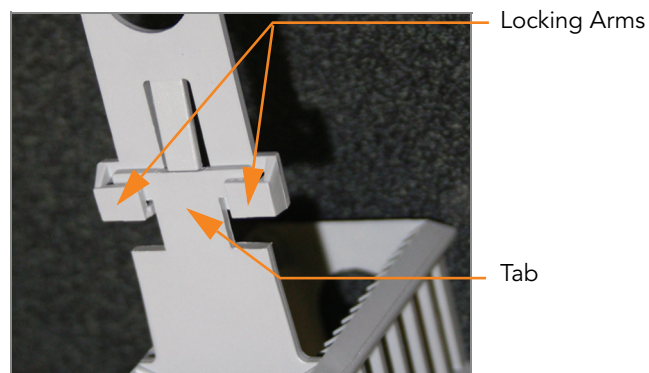


Figure 67 Staining Rack Locking Arms and Tab

**CAUTION**

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

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

6. One at a time, disassemble each Flow-Through Chamber:
 - a. Use the dismantling tool to remove the two metal clamps (Figure 68).

**CAUTION**

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

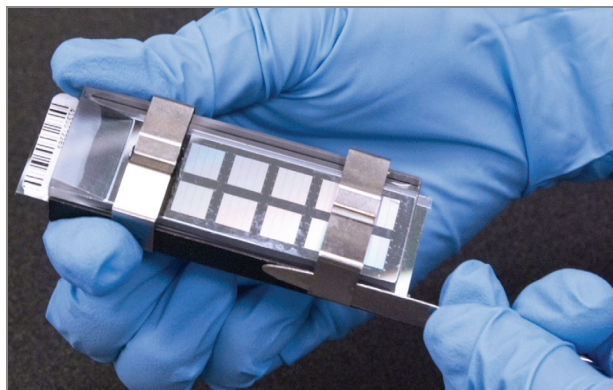


Figure 68 Removing Metal Clamps from Flow-Through Chamber

- b. Remove the glass back plate and set it 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*.
- c. 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.
- d. Remove the BeadChip.

**CAUTION**

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

7. Place the BeadChips in the staining rack while it is submerged in PB1. Put four BeadChips above the staining rack handle and four below. The BeadChip barcodes should face **away** from you, while 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.

8. 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.

9. Move the staining rack up and down 10 times, breaking the surface of the PB1 (Figure 69).

**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.

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

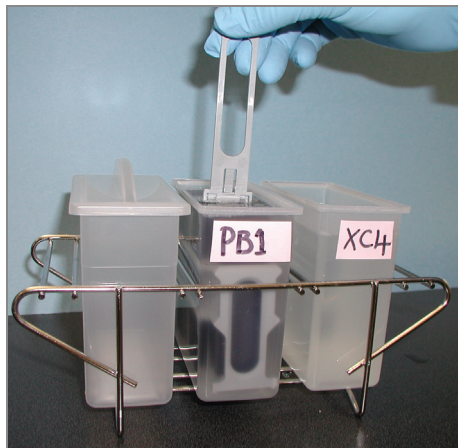


Figure 69 Washing BeadChips in PB1

**NOTE**

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

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.

**NOTE**

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

12. Remove the staining rack from the PB1 dish and place it directly into the wash dish containing XC4 (Figure 70). 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.

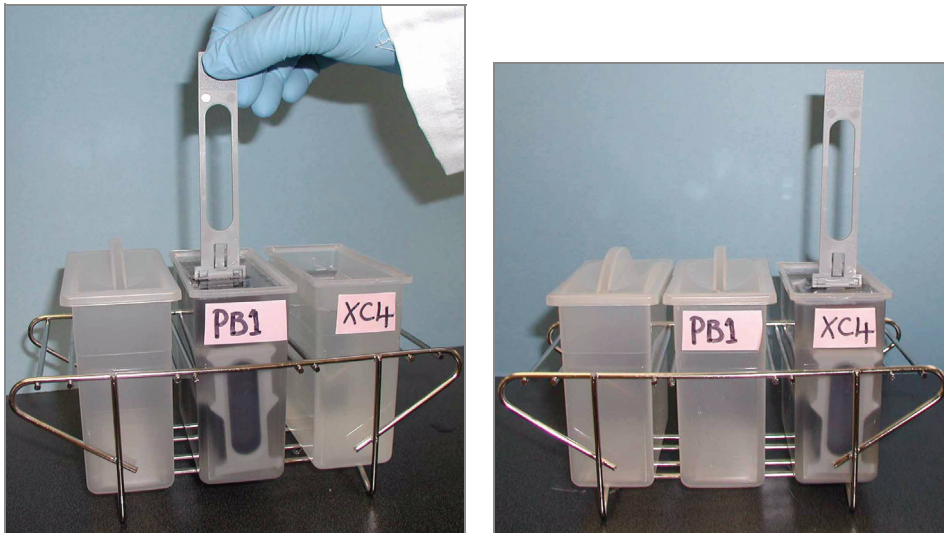


Figure 70 Moving BeadChips from PB1 to XC4

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

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



CAUTION

Up to 24 BeadChips can be processed per bottle of XC4. To process more than 24 BeadChips, use a new, clean wash dish with fresh XC4.

15. Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.
16. Prepare one additional tube rack per 8 BeadChips that fits the internal dimensions of the vacuum desiccator.
17. 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 71).



Figure 71 Staining Rack in Correct Orientation

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

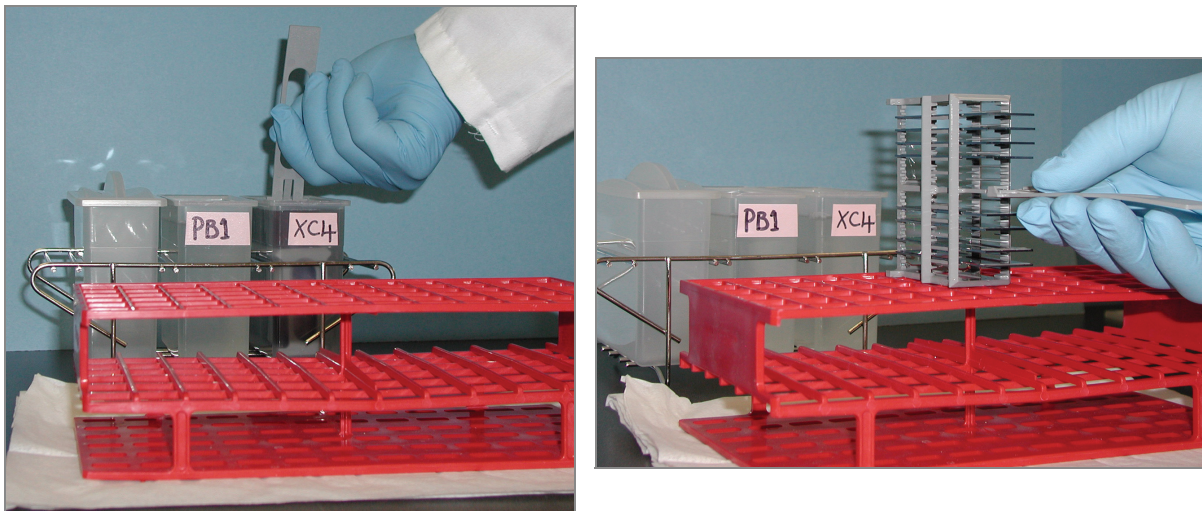


Figure 72 Moving the Staining Rack from XC4 to Tube Rack

19. For the top four 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 74).
20. 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 the tab up with your thumb and push the handle away from you (unlocking the handle), then pull up the handle and remove (Figure 73).

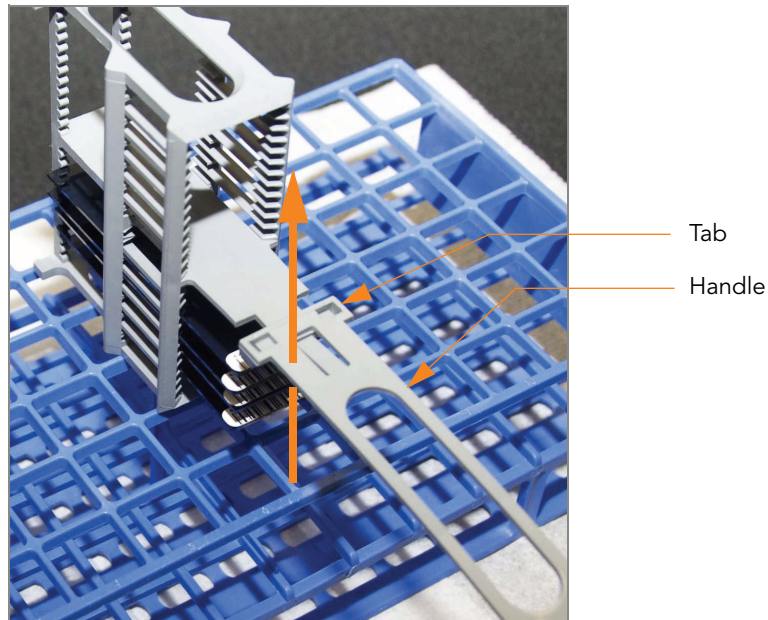


Figure 73 Removing Staining Rack Handle

21. Remove the remaining BeadChips to the tube rack as described in step 19, with six BeadChips on top of the rack and two BeadChips on the bottom (Figure 74). The barcode ends should be towards you, and the BeadChips should be completely horizontal.

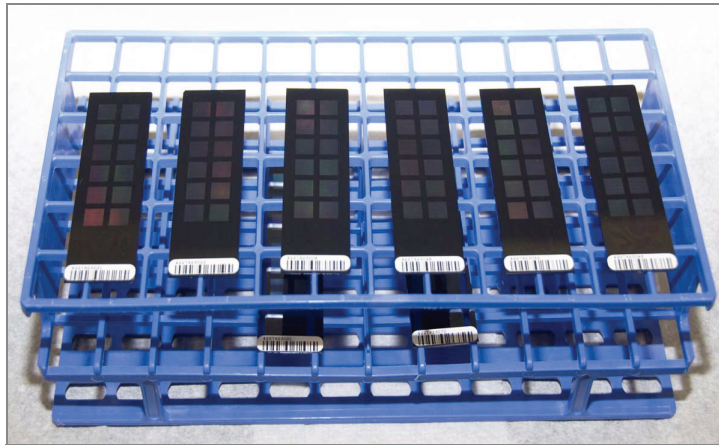


Figure 74 BeadChips on Tube Rack



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.

22. Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack (eight BeadChips).

**CAUTION**

Ensure the vacuum valve is seated tightly and securely.

23. Remove the red plug from the three-way valve before applying vacuum pressure.
24. Start the vacuum, using at least 508 mm Hg (0.68 bar).
25. To ensure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator (Figure 75). It should not lift off the desiccator base.

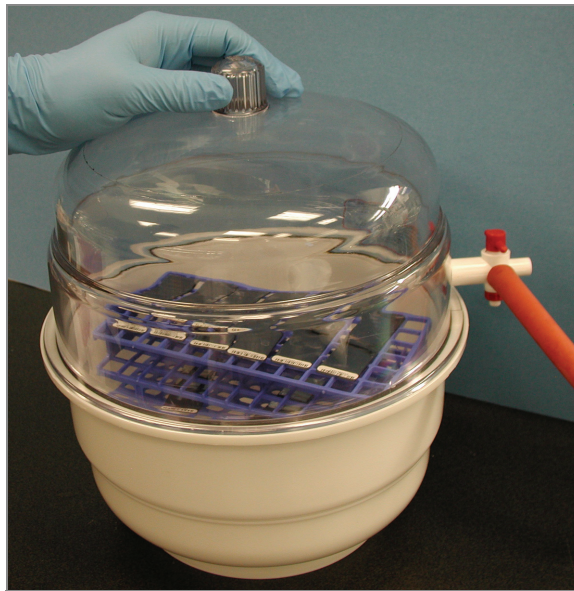


Figure 75 Testing Vacuum Seal

26. Dry under vacuum for 50–55 minutes.
Drying times may vary according to room temperature and humidity.
27. 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.

28. 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.
29. 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.

30. If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom two BeadChips are most likely to have some excess.
- Wrap a pre-saturated Prostat EtOH Wipe around your index finger.
 - Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
 - Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

**CAUTION**

Do not touch the stripes.

31. Clean the glass back plates. For instructions, see the *Infinium Assay Lab Setup and Procedures Guide*.
32. Clean the Hyb Chambers:
- Remove the rubber gaskets from the Hyb Chambers.
 - Rinse all Hyb Chamber components with DI water.
 - Thoroughly rinse the eight humidifying buffer reservoirs.
33. Discard unused reagents in accordance with facility standards.
34. Do one of the following:
- Proceed to *Image BeadChip on the iScan System* on page 81 or *Image BeadChip on the BeadArray Reader* on page 91.
 - 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 on the iScan System

The iScan Reader uses a laser to excite the fluor of the single-base extension product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

Scanning:

Bead Types	24x1 HD Format			12x1 HD Format	
	3K–20K	20K–40K	40K–60.8K	60.8K–100K	100K–200K
Stripes per Sample Section	1	2	3	2	4
Scan time per BeadChip	7 min	13 min	24 min	20 min	35 min



NOTE

Scan times may be shorter depending on the complexity of the Bead Pool loaded onto the BeadChip.

Preparation

- ▶ On the lab tracking form, record the following for each BeadChip:
 - Scanner ID
 - Scan date



NOTE

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

- ▶ For more information about the iScan System, iScan Control Software, or AutoLoader2, see the *iScan and AutoLoader2 System Guide*.

Steps Overview

The iScan Control Software leads you through the BeadChip scanning process, which is as follows:

1. Turn on the iScan Reader, boot up the iScan PC, and start the iScan Control Software application.
2. Let the iScan Reader warm up for at least 5 minutes before beginning a scan. It is fine to use the iScan Control Software during this time.



Turn on the iScan Reader before launching the iScan Control Software. If the software is launched when the instrument is turned off, an error message will alert you that the hardware is missing.

3. Load the BeadChips to be scanned, and copy their decode data into the Input Path.
4. Check the scan settings and input/output paths, making modifications if necessary.
5. If you wish, remove BeadChip sections or entire BeadChips from the scan.
6. Start the scan and monitor its progress.
7. Review the scan metrics.

Starting Up the iScan System

1. For each BeadChip, download the decode content from iCom or copy the contents of the DVD provided with the BeadChip (if purchased) into the Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).

If there is no decode folder, follow the instructions in *Setting Up Input and Output Paths* on page 88.

2. Double-click the iScan Control Software icon  on the desktop.

The Welcome window appears (Figure 76). The iScan Control Software automatically connects to the iScan Reader and initializes it. When the reader is initialized, the red dot in the status bar turns green, and the status changes to Initialized.

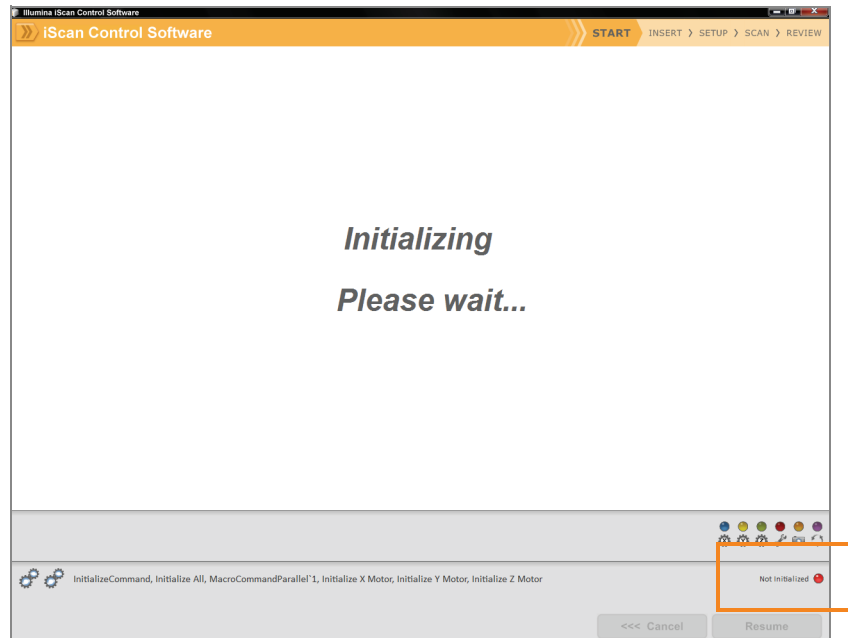


Figure 76 Initialize iScan Reader

3. Set the **LIMS** dropdown list to **None** and enter your Windows user name (Figure 77).

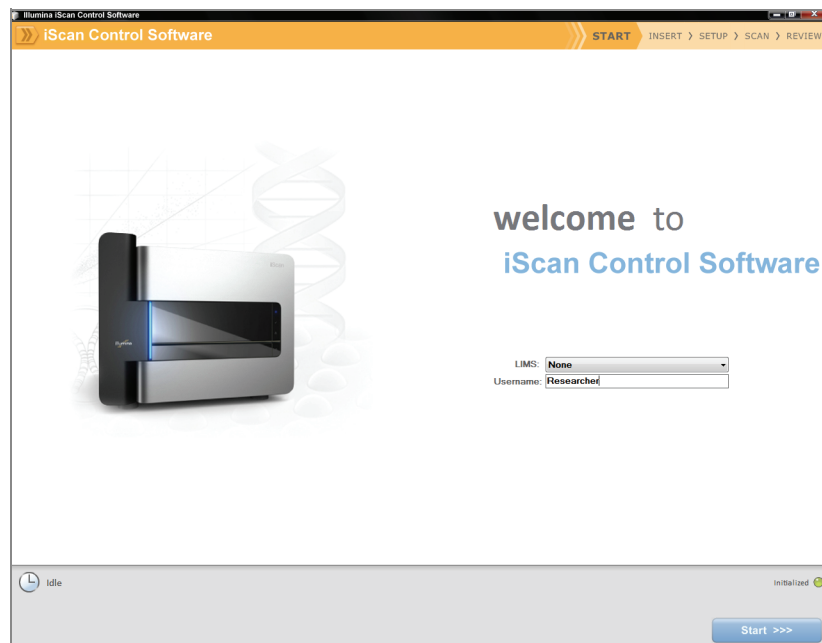


Figure 77 Welcome Window

4. Click **Start**.
The iScan Reader tray opens.

Loading BeadChips and Starting the Scan

1. Load the BeadChips into their carrier and place the carrier into the iScan Reader tray. Click **Next**.

The tray closes and the iScan Reader begins scanning the barcodes (Figure 78).



NOTE

Infinium HD assay screen shots are used as an example for the steps that follow. You may be required to choose different imaging options where appropriate or when prompted.

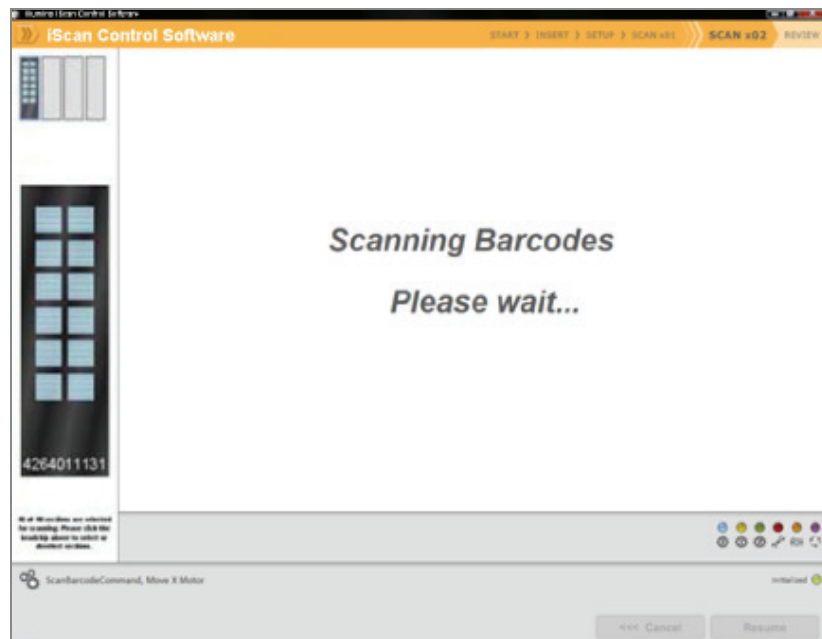


Figure 78 Scan BeadChip Barcodes

When the iScan Reader has read all of the barcodes, the Setup window displays the barcode, description, and scan setting for each BeadChip in the position corresponding to its location in the tray. You can click any barcode to view an image of the corresponding BeadChip.

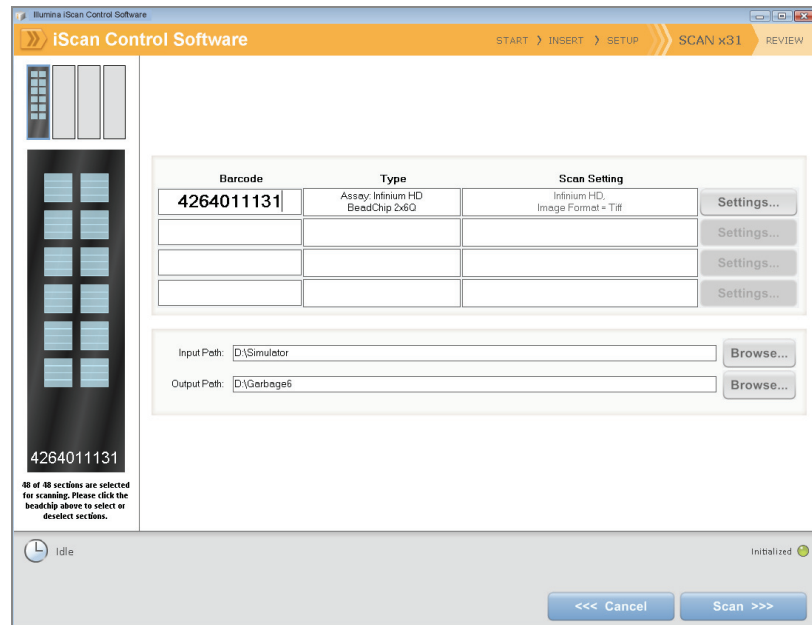


Figure 79 Scanned Infinium BeadChip Barcodes

- If the Scan Setting field beside each BeadChip does not contain "Infinium HD" (12x1 HD BeadChip) or "Infinium HD-HT" (24x1 HD BeadChip), click **Settings**.



NOTE

Ensure that the appropriate scan setting is used. An incorrect scan setting may result in the misregistration of images.

The Scan Settings File window appears.

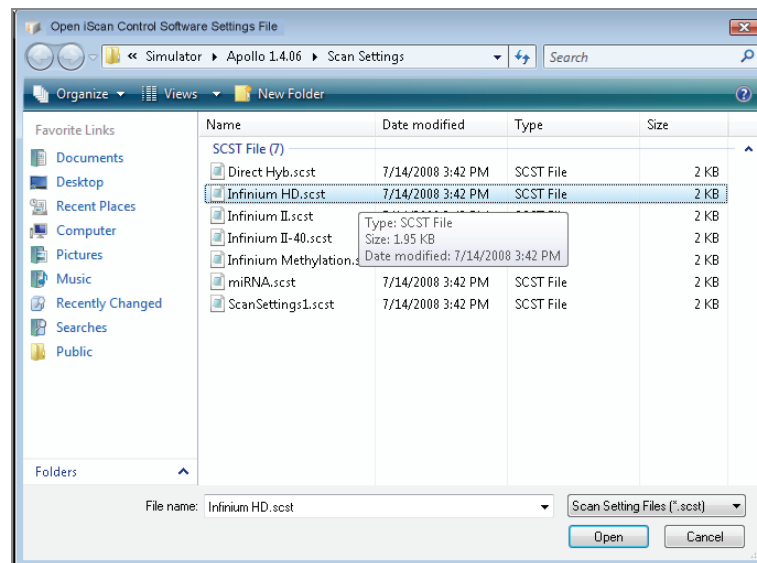


Figure 80 iScan Control Software Scan Settings File Window

3. Select **Infinium HD.scst** for the 12x1 HD BeadChip or **Infinium HD-HT.scst** for the 24x1 HD BeadChip and click **Open**.
4. If you want to change the image format (*.jpg or *.tif), click the Menu button and select **Tools | Options**.

The Options dialog box appears.

- **JPEG files** let you review the image of the scanned array sections, but you cannot extract bead intensity data.
- **TIFF files** let you review the scanned images and extract bead intensity data. The file size is much larger than *.jpg.

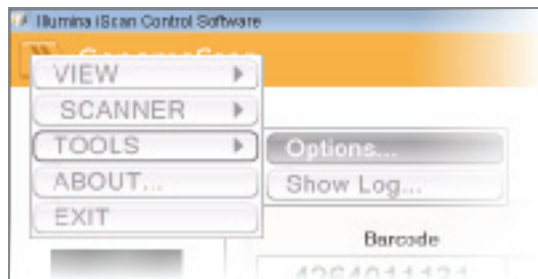


Figure 81 iScan Control Software Tools | Options

5. Click the Scan Settings tab.

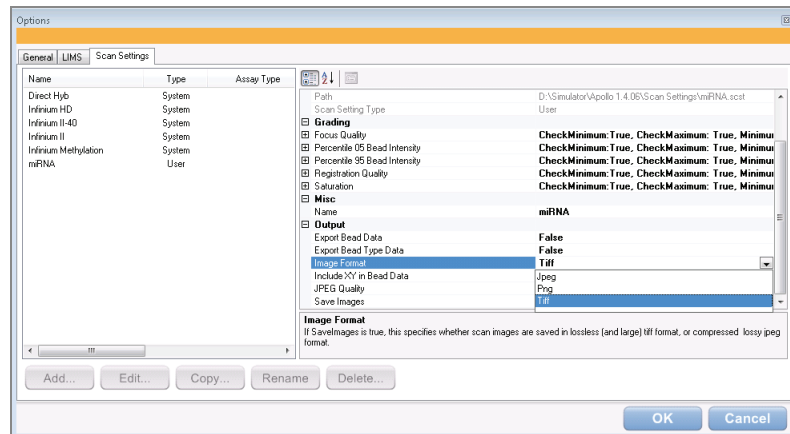
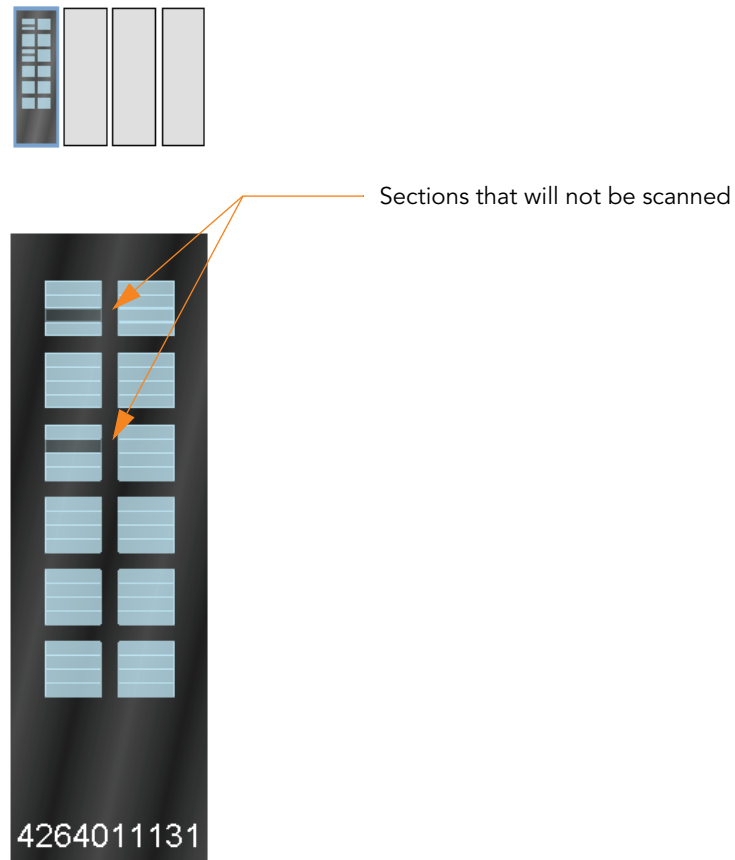


Figure 82 Options Dialog Box, Scan Settings Tab

6. Select **Infinium HD** or **Infinium HD-HT** in the left pane.
The scan settings appear in the right pane.
7. Click the down arrow beside **Image Format**, and select **Tiff**. Click **OK**.
Make sure that the input and output paths are correct.
For more information about the scan settings, see the *iScan and AutoLoader2 System Guide*.
8. If you do not want to scan certain sections of a BeadChip, click the barcode to display an image of the corresponding BeadChip in the Setup window. Click any BeadChip section to remove it from the scan (Figure 83). The section will no longer be highlighted blue.



46 of 48 sections are selected for scanning. Please click the beadchip above to select or deselect sections.

Figure 83 Deselect BeadChip Sections

9. If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.
10. To begin scanning the BeadChips, click **Scan**.
As the scan progresses, status icons and messages are displayed in the bottom left corner of window. For more information about what happens during the scan, see *During the Scan* on page 89.
11. At the end of the scan, a Review window appears (Figure 84). The Scan Metrics table at the top shows the intensity values, registration, and focus metrics for each stripe on the BeadChip.

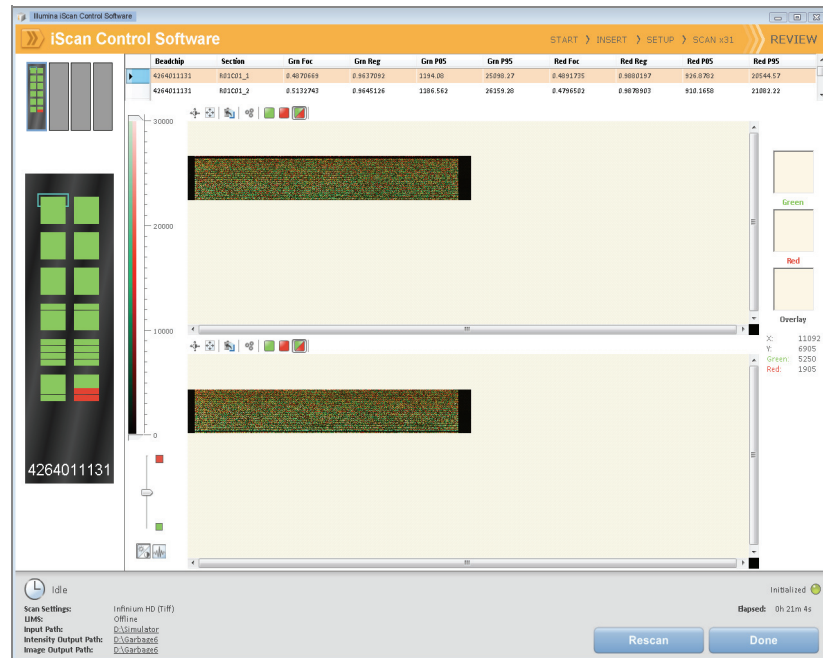


Figure 84 Review Window with Failed Stripes

You can also review scan metrics for any BeadChip in the Output Path folder. Scan metrics are in a document titled Barcode_qc.txt, where “Barcode” represents the barcode number for a single BeadChip.

The focus metric ranges between 0 and 1. High focus scores indicate a sharp, well defined image, leading to high bead intensity values.

12. If any stripes fail to scan successfully they are highlighted in red on the screen. The window contains a Rescan button. Click **Rescan** to automatically rescan all failed areas on the BeadChips in the carrier.
13. When you finish reviewing the data, click **Done** to return to the Start window.

If you click **Done** on the Review window without rescanning failed sections, the *.idat files will be saved for those sections but not accessible. The entire sample section will have to be rescanned to generate *.idat files.

When you return to the Start window, images from the scan are no longer available to be viewed in the iScan Control Software. Use another program such as Illumina’s GenomeStudio to view images from the scan.

Setting Up Input and Output Paths

This step should only occur once, when you install the iScan Control Software on the iScan PC. After that, all scans use these paths.

1. Create a folder on the iScan PC D: drive to contain the decode (*.dmap) and Sentrix descriptor (*.sdf) files that were downloaded from iCom or

came on each BeadChip DVD (for example, D:\Decode). The iScan Control Software refers to this folder as the Input Path.

2. Create another folder on the iScan PC D: drive where you want the iScan Control Software to store the image data from the scan (for example, D:\ImageData).

During the scan, the iScan Control Software automatically creates sub-folders named with each BeadChip's barcode number. The folder will be populated with image files (*.jpg or *.tif), scan metrics (*.txt), and intensity data files (*.idat) for each BeadChip. If the images are in *.tif format, the output path will also contain bead location files (*.locs).

During the Scan

Calibration

The iScan System begins with a calibration step, which may take several minutes to complete. The BeadChips are automatically tilted and aligned to ensure that they are in the optimal position for the scan.

- ▶ **Tilt**—The iScan Reader autofocus feature records the Z-position (height) of three corners of the BeadChip to determine its current tilt, and then adjusts the BeadChip until it is flat.
- ▶ **Align**—The iScan Reader identifies the X-Y position (lateral location) of the fiducials (focus points) on the BeadChip edges, and then adjusts the optics.

If there are defective or dirty sections at any of the three alignment corners, the software attempts to use alternate sections until satisfactory calibration is achieved. If no alternate sections are available, calibration fails and an error message is displayed.

Hard Drive Space

Before beginning a scan, the iScan Control Software checks the hard drive to ensure sufficient space is available. If sufficient disk space is not available, an error message is displayed, and the arrays will not be scanned.

Monitoring the Scan

After calibration, the iScan Reader begins scanning. You can view the progress of the scan in the Scan window (Figure 85).

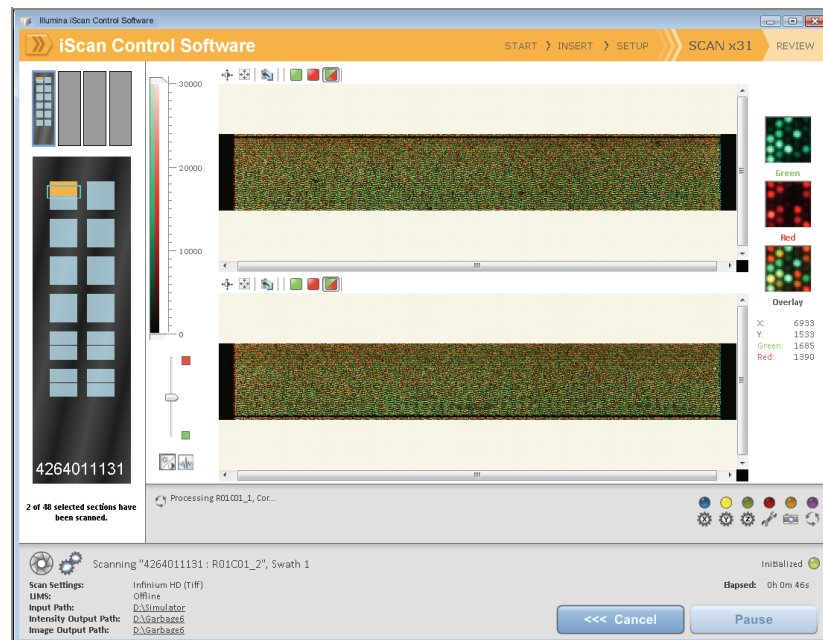


Figure 85 Monitor the Scan

Status and Controls

As each BeadChip section is scanned its status is indicated by a status color:

- ▶ **Light Blue**—Section has not yet been scanned.
- ▶ **Orange**—Section is in the process of being scanned or registered.
- ▶ **Green**—Scan and registration of section was successful.
- ▶ **Red**—Scan and registration of section failed.

While a scan is in progress, you can click the Pause or Cancel buttons to pause or stop the scan at any time. If you pause, the button changes to Resume. Click Resume to start scanning the next unscanned section.

Registration and Intensities

After images are scanned, they are registered and intensities are extracted for every bead type. Registration identifies beads by correlating their locations on the scanned image with information in the bead map (*.dmap) file. Registration and extraction are critical to obtaining results from your experiments.

Intensity extraction is the process by which intensity values are determined for every bead on the image. Statistics are generated for every bead type based on the intensities of the replicate beads for that type. Extracted information is saved in intensity data (*.idat) files. These files are saved on the iScan Reader hard drive or network under the Array ID (barcode identifier), in the Output Path folder. Intensity data (*.idat) files are only created for sections that have 100% of their stripes scanned successfully. These files are not created when scanning individual stripes within a sample section on a BeadChip.

Image BeadChip on the BeadArray Reader

The Illumina BeadArray Reader uses a laser to excite the fluor of the hybridized single-stranded product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

1–2 hours warm-up for the BeadArray Reader (first use of the day only)

Scanning:

	24x1 HD Format			12x1 HD Format	
Bead Types	3K–20K	20K–40K	40K–60.8K	60.8K–100K	100K–200K
Stripes per Sample Section	1	2	3	2	4
Scan time per BeadChip	58 min	115 min	171 min	96 min	180 min



NOTE

Scan times may be shorter depending on the complexity of the Bead Pool loaded onto the BeadChip.

Preparation

- ▶ If this is the first time the BeadArray Reader is being used today, follow the steps described in this section.
- ▶ On the lab tracking form, record the following for each BeadChip:
 - Scanner ID
 - Scan date



NOTE

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

Initializing the BeadArray Reader (Daily)

If this is the first time the scanner is being used today, follow these steps. Refer to Figure 86 throughout this protocol.

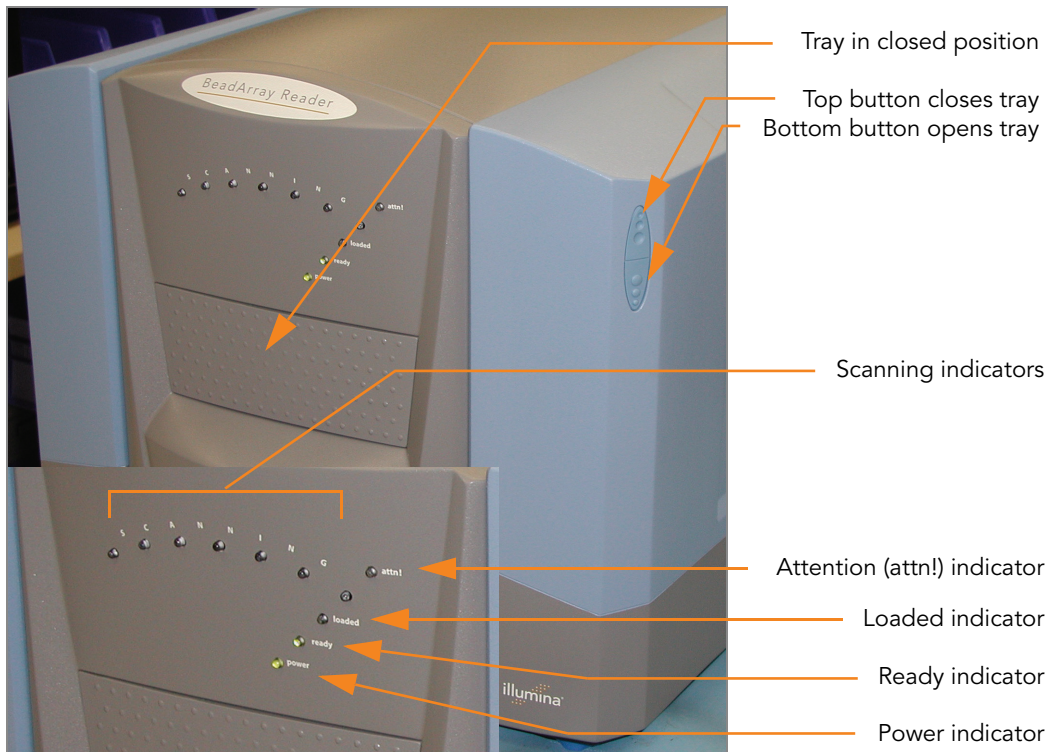


Figure 86 Illumina BeadArray Reader

Table 9 Illumina BeadArray Reader Indicators

Indicator	Description
Scanning (seven lights)	Flash blue in sequence when the BeadArray Reader is scanning.
Attention	Turns solid amber to indicate a hardware problem. Check the BeadArray Reader PC for an error message. You may need to cycle the power on the BeadArray Reader.
Loaded	Not currently used.
Ready	Flashes green during startup. Turns solid green when the BeadArray Reader is ready for use and when it is busy.
Power	Turns solid green when the BeadArray Reader is on.

1. Locate the power switch on the lower-left side of the BeadArray Reader back panel and turn it to the ON position.
2. Wait for the ready indicator to stop flashing.
3. Open the **BeadScan** software.
4. Log in and click **Scan** (Figure 87).

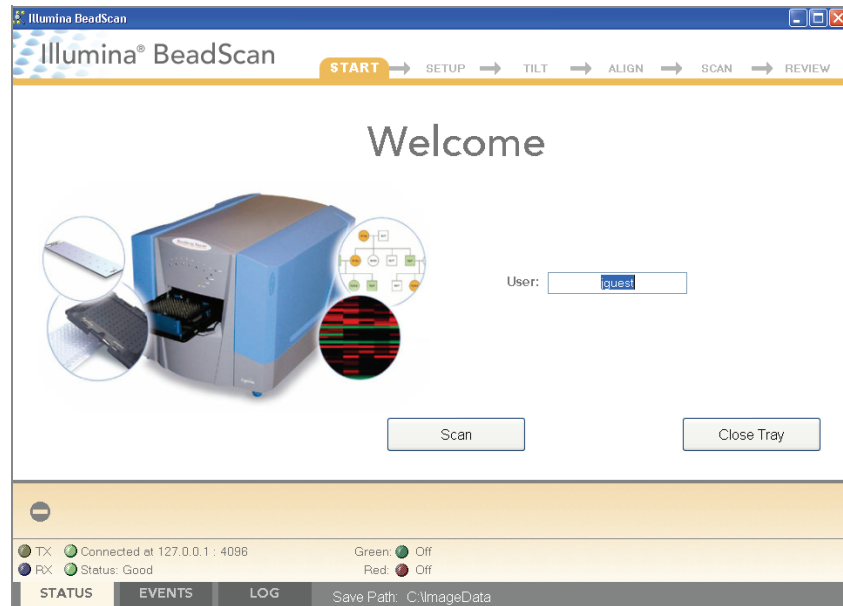


Figure 87 BeadScan Welcome Screen

Imaging BeadChip

When the BeadArray Reader is initialized, follow these steps to perform the scanning process.

1. From the **Docking Fixture** listbox, select BeadChip (Figure 88).

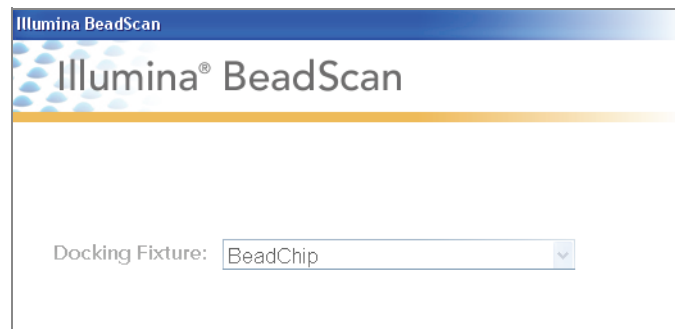


Figure 88 Select BeadChip Docking Fixture

2. Check the Data Repository path and the Decode Map path in the Settings area.
 - The **Data Repository path** indicates where the BeadArray Reader stores the images created during the scan. The default path is C:\ImageData.
 - The **Decode Map path** points to the location where you will copy the files that were downloaded from iCom or copied from each DVD provided with the BeadChip (if purchased). The default path is C:\DecodeData.
3. If either path is not correct:
 - a. Click **Edit** to open the Options dialog box.

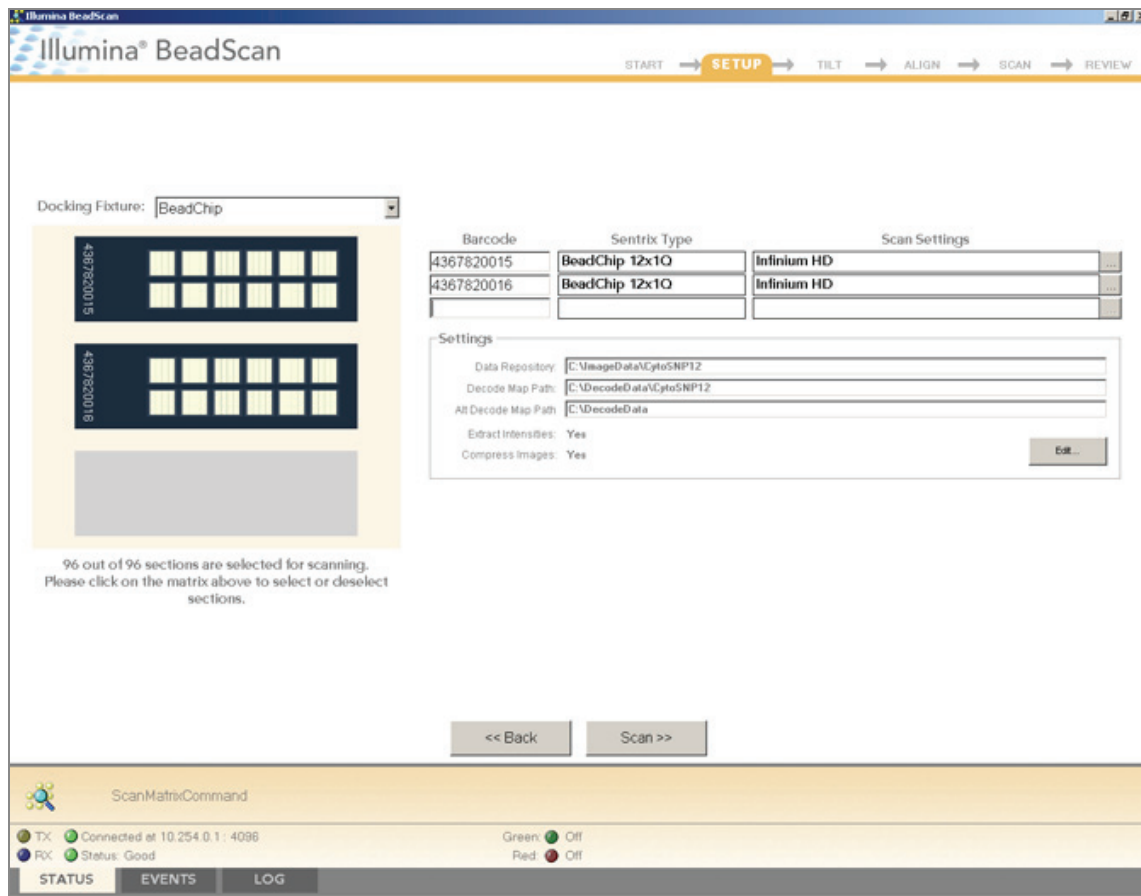


Figure 90 Scan Barcode Numbers



NOTE

BeadChip layout and color channel employed may differ from product to product.

- c. If either the **Sentrix Type** or **Scan Settings** are not correct, click **Browse (...)** to open the Select Scan Settings dialog box (Figure 91).

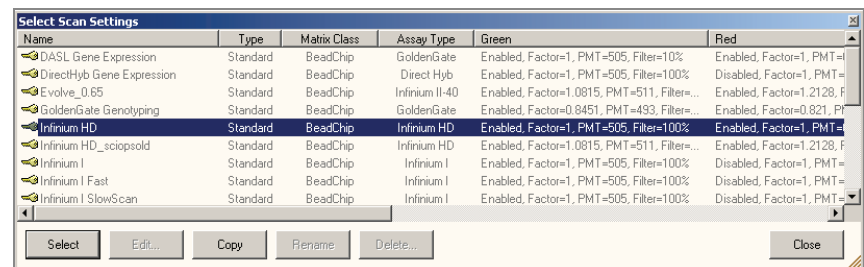


Figure 91 Select Scan Settings Dialog Box

- d. Select **Infinium HD.scst** for the 12x1 HD BeadChip or **Infinium HD-HT.scst** for the 24x1 HD BeadChip and click **Select**.

**NOTE**

Ensure that the appropriate scan setting is used. An incorrect scan setting may result in the misregistration of images.

6. Make sure that the BeadChips are properly seated in the BeadArray Reader tray (Figure 92).



Figure 92 Place BeadChips into Illumina BeadArray Reader Tray

7. Click **Scan**.

Scanning Process

BeadScan begins the BeadArray Reader Tilt and Align processes (Figure 94):

- **Tilt**—The BeadArray Reader Autofocus feature records the Z-position (height) of three corners of the BeadChip to determine its current tilt, and then adjusts the BeadChip until it is flat.

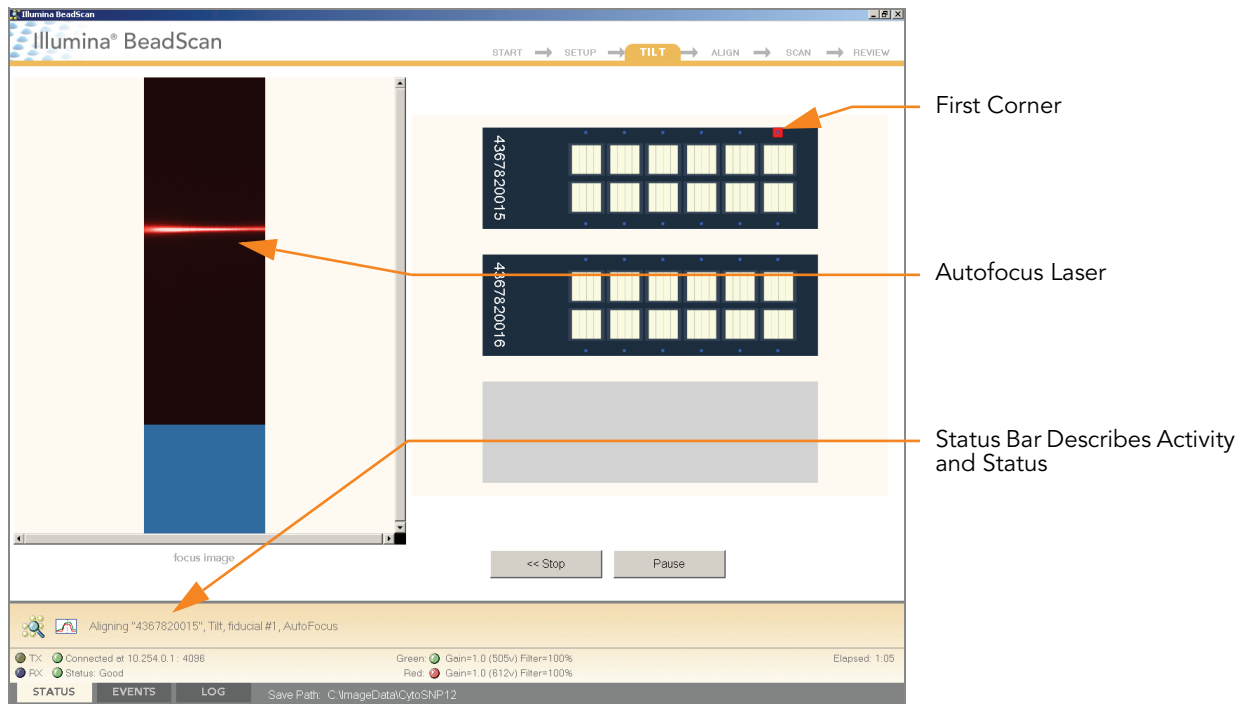


Figure 93 BeadArray Reader Tile Process



NOTE

The BeadChip image on the screen will be specific to the type of BeadChip being scanned.

- **Align**—The BeadArray Reader identifies the X-Y position (lateral location) of the fiducials (focus points) on the BeadChip edges, and then adjusts the optics.

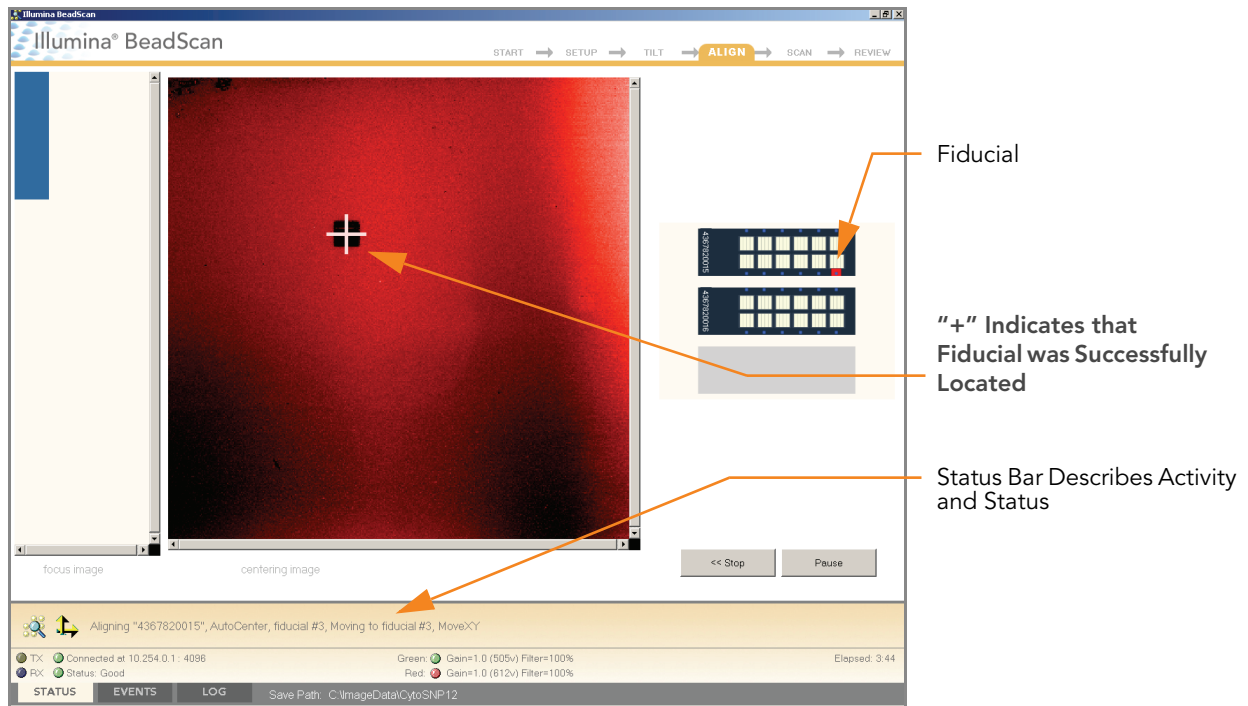


Figure 94 BeadArray Reader Tilt & Align Processes

Once the Tilt and Align processes are complete, the Scan process begins (Figure 95). Hover over any of the red or green dots in the closeup image to see the relative intensity and the XY position.¹

1. The 0/0 position is at the upper left corner of the BeadChip, with X increasing rightwards and Y increasing downwards.

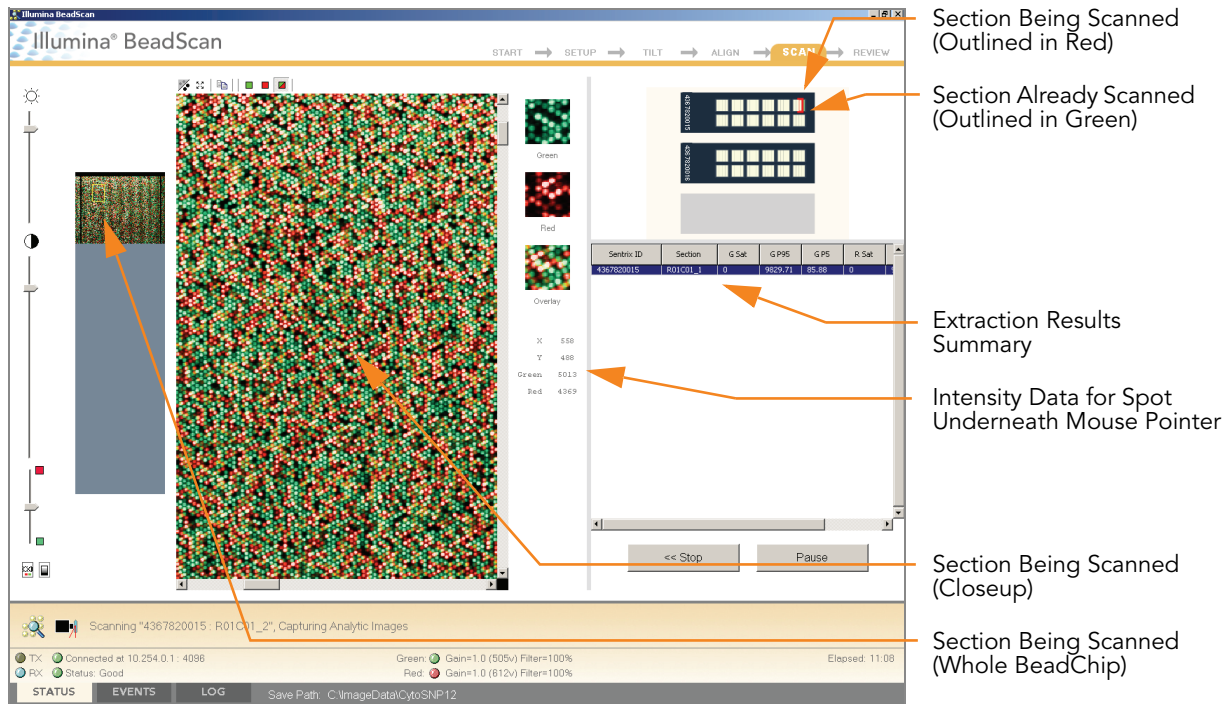


Figure 95 BeadArray Reader Scan Process

As the BeadArray Reader scans, the front panel blue Scanning indicator lights flash in sequence.

When the BeadArray Reader finishes scanning, a green message screen appears if the scan was successful, or a red message if it completed with any warnings. These screens are designed to be visible from across the lab.

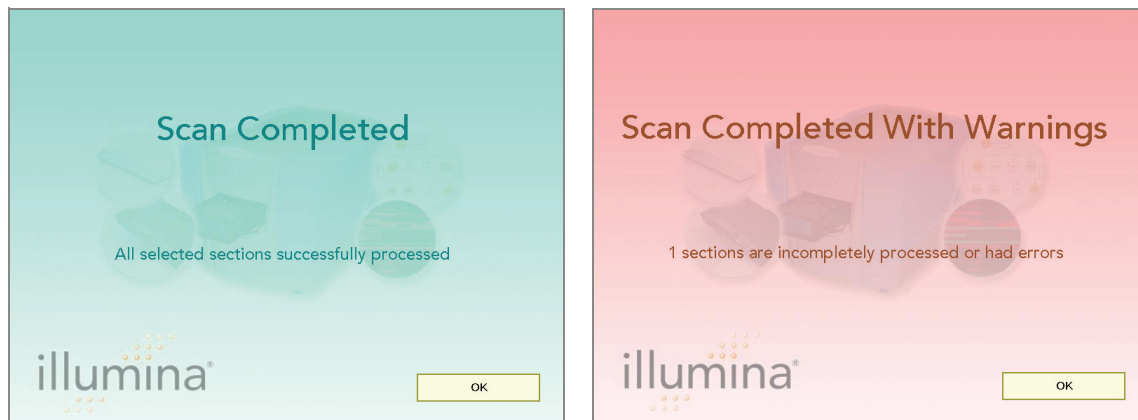


Figure 96 Scan Completed and Scan Completed with Warnings Screens

If Scan is Successful

1. Click **OK** on the Scan Completed message to view the next screen.
2. Click **Done** in the Review pane.

3. When the application returns to the Welcome screen, click **Open Tray**. The BeadArray Reader tray, loaded with the scanned BeadChips, will eject.
4. Remove the BeadChips from the tray.
5. Do one of the following:
 - ▶ If you have more BeadChips to scan, repeat the scanning process.
 - ▶ If this is the last use of the day:
 - a. Wipe the BeadArray Reader tray with a lint-free, absorbent towel. Pay particular attention to the tray edges where reagent may have wicked out.
 - b. Close the tray.
 - c. Turn the power switch at the back of the scanner to the **OFF** position.
 - d. Shut down the BeadArray Reader BeadScan software. To exit, right-click near the Illumina logo and click **Exit** (Figure 97).

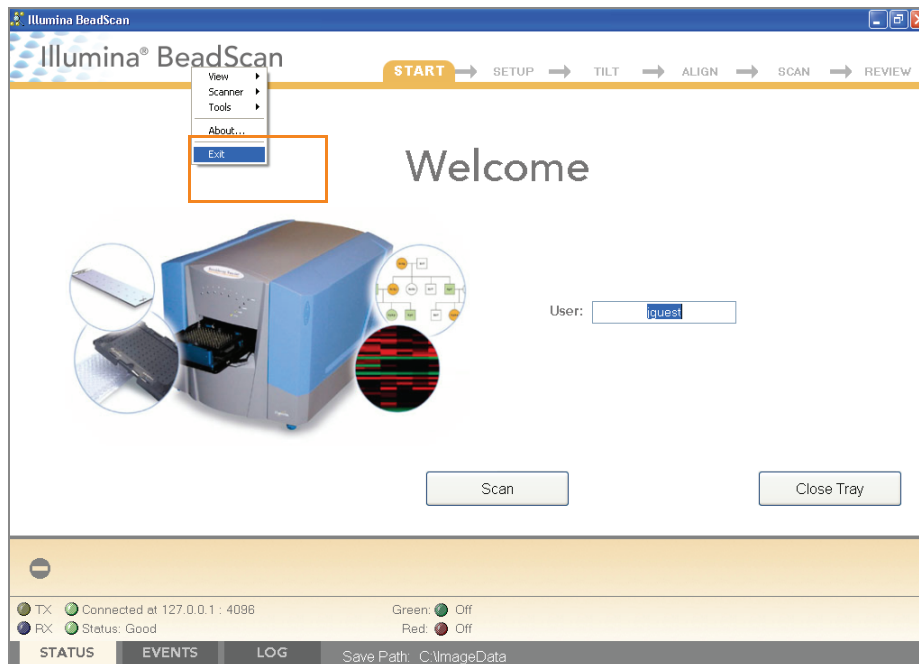


Figure 97 Exit the BeadArray Reader Software

If Scan is not Successful

Re-scan the array. For more information, refer to the *Illumina BeadArray Reader User Guide*.

If the scanner was unable to locate the alignment fiducials (focus points), you may need to clean the edges of the BeadChip before re-scanning.

Clean BeadChip Edges

1. To clean the BeadChips, wrap a Prostat EtOH presaturated wipe around your index finger. Place the wipe against the edge of BeadChip, no further than 2 mm from the edge of the slide (Figure 98).



CAUTION

The wipe should not pass over the etched stripes on the BeadChip.

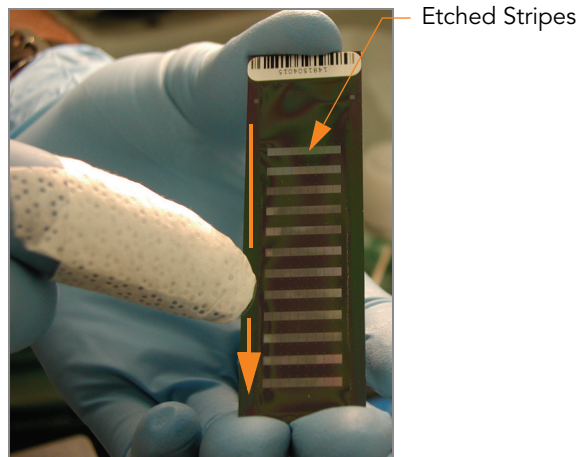


Figure 98 Wiping Edges of BeadChip

2. Wipe along the edge of each BeadChip five or six times (Figure 98), until the surface is clean and smooth and no streaks are visible.
To completely remove the coating along the edges and back of BeadChip, you may need to use a different part of the ProStat EtOH wipe.



CAUTION

Any remaining visible streaks may lead to alignment errors.

GenomeStudio Integrated Informatics Platform

The 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 iScan System or BeadArray Reader.

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 Module User Guide*.

Chapter 3

Infinium HD Assay Ultra Automated Protocol

Topics

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224	GenomeStudio Integrated Informatics Platform

Introduction

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

Some of the tasks in this chapter make reference to Illumina LIMS (Laboratory Information Management System). If you are not running Illumina LIMS, disregard those instructions. For information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

Infinium HD Assay Ultra Automated Workflow

Figure 99 graphically represents the Illumina Infinium HD Assay Ultra automated workflow for use with the 12x1 HD BeadChip or the 24x1 HD BeadChip. These protocols describe the procedure for preparing 96 DNA samples using either BeadChip.

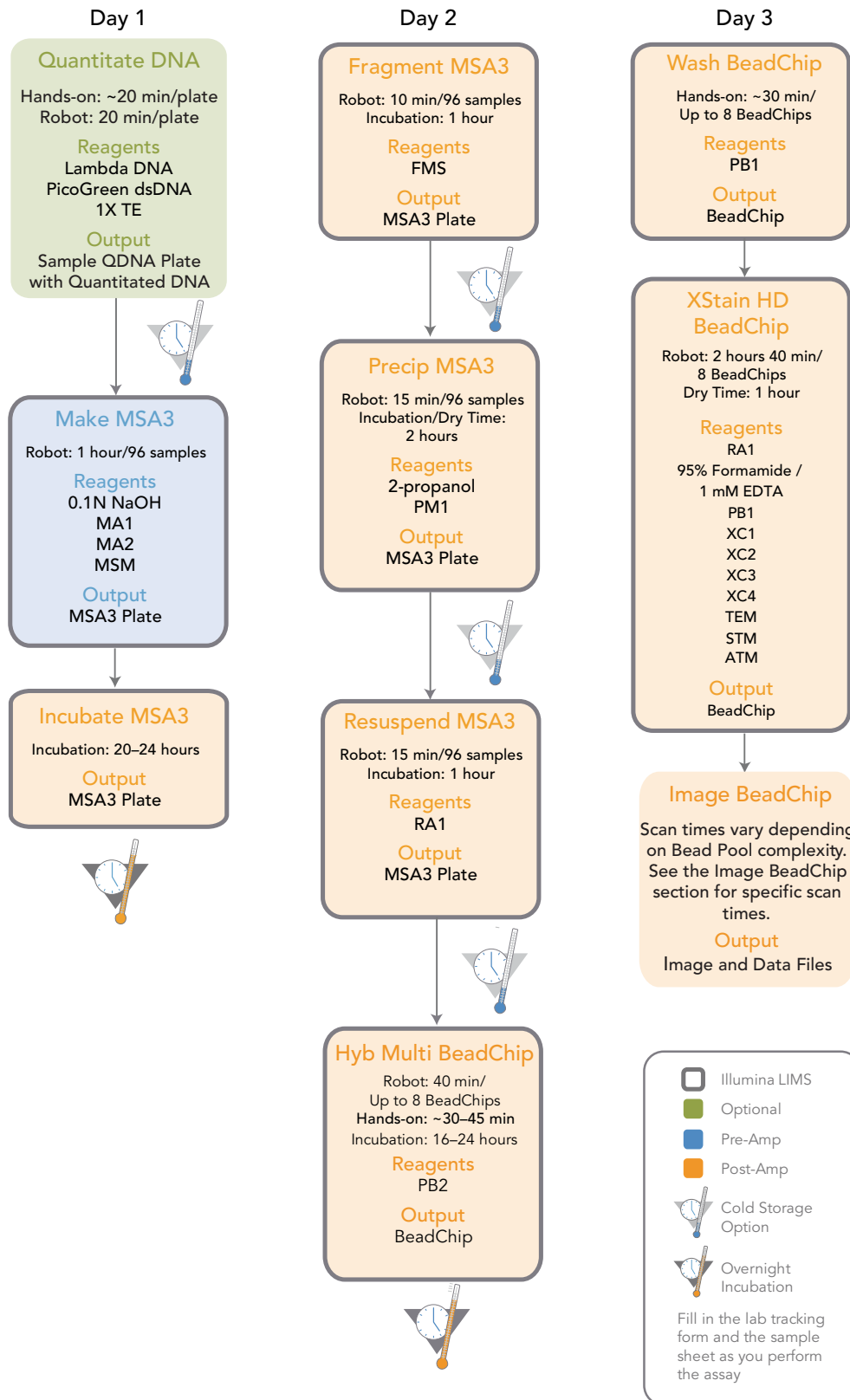


Figure 99 Infinium HD Assay Ultra Automated Workflow

Equipment, Materials, and Reagents

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

Equipment User-Supplied

Table 10 User-Supplied Equipment

Item	Source
Vacuum desiccator (one per 8 BeadChips processed simultaneously)	VWR Catalog # 24988-197
Vacuum tubing	VWR Catalog # 62995-335
2 Tecan eight-tip robots (one for pre- and one for post-amplification processes)	Non-LIMS customers <ul style="list-style-type: none"> • SC-30-401 (110V) – North America and Japan • SC-30-402 (220V) – EU and Asia Pacific (Except Japan) Illumina LIMS customers <ul style="list-style-type: none"> • SC-30-403 (110V) – North America and Japan • SC-30-404 (220V) – EU and Asia Pacific (Except Japan)
Forceps	VWR International, Catalog # 25601-008
Auto-desiccator cabinet (Optional—allows scanning of BeadChips up to three days after processing)	VWR International, Catalog # 74950-342

Illumina-Supplied

Table 11 Illumina-Supplied Materials

Item	Catalog or Part #
Multi-Sample BeadChip Alignment Fixture	Part # 218528
Robot BeadChip Alignment Fixture (4)	Part # 222691
Robot Tip Alignment Guide inserts Guide-B (two piece)	Part # 11294913
Robot Tip Alignment Guide base Guide-B (two piece)	Part # 11292731
Robot Tip Alignment Guide-B (one piece)	Part # 11323260
Robot Tip Alignment Guide-D (one piece)	Part # 219213

Materials User-Supplied

Table 12 *User-Supplied Materials*

Item	Source
96-well, black, flat-bottom Fluotrac 200 plates	Greiner, catalog # 655076
Foil adhesive seals (Microseal "F")	MJ Research, Catalog # MSF-1001
Aluminum foil	
Reservoir, full, 150 ml	Beckman Coulter, catalog # 372784
Reservoir, half, 75 ml	Beckman Coulter, catalog # 372786
Reservoir, quarter, 40 ml	Beckman Coulter, catalog # 372790
Reservoir frame	Beckman Coulter, catalog # 372795
Tube racks for vacuum desiccator (one for every 8 BeadChips to be processed simultaneously; must fit internal dimensions of vacuum desiccator)	VWR catalog # 66023-526
Vacuum source (greater than 508 mm Hg (0.68 bar)	
(Recommended) Vacuum gauge for vacuum desiccator	

Illumina-Supplied

- ▶ MSA3 barcode labels
- ▶ WG#-DNA barcode labels
- ▶ QNT barcode labels

Reagents Illumina-Supplied

Table 13 *Illumina-Supplied Reagents*

Item	Part #
ATM —Anti-Stain Two-Color Master Mix	11208317
FMS —Fragmentation solution	11203428
MA1 —Multi-Sample Amplification 1 Mix	11202880
MA2 —Multi-Sample Amplification 2 Mix	11203401
MSM —Multi-Sample Amplification Master Mix	11203410
PB1 —Reagent used to prepare BeadChips for hybridization	11191922
PB2 —Humidifying buffer used during hybridization	11191130

Table 13 *Illumina-Supplied Reagents*

Item	Part #
PM1 —Precipitation solution	11203436
RA1 —Resuspension, hybridization, and wash solution	11222442
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 (240 ml)—XStain BeadChip solution 3	11208421
XC4 —XStain BeadChip solution 4	11208430

Quantitate DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples. You can quantitate up to three plates, each containing up to 96 samples. If you already know the concentration and you are not running Illumina LIMS (Laboratory Information Management System), proceed to *Make the MSA3 Plate* on page 119.

If you are running Illumina LIMS, for information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

Illumina recommends the Molecular Probes PicoGreen assay to quantitate dsDNA samples. The PicoGreen assay can quantitate small DNA volumes, and measures DNA directly. Other techniques may pick up contamination such as RNA and proteins. Illumina recommends using a spectrofluorometer because fluorometry provides DNA-specific quantification. Spectrophotometry might also measure RNA and yield values that are too high.

Estimated Time Hands-on time: ~20 minutes per plate
Robot: 20 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	-15° to -25°C	User
1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA (TE))	See Instructions	Room temperature	User
Lambda DNA	See Instructions	2° to 8°C	User
96-well 0.65 ml microtiter plate	1 per 96 samples		User
Fluotrac 200 96-well flat-bottom plate	1 per Std DNA plate 1 per Sample DNA plate		User
WG#-DNA plate with DNA samples	8, 16, 24, 32, 48, or 96 DNA samples in 1 to 3 plates	2° to 8°C	User



NOTE

PicoGreen is susceptible to differential contaminants. False positives may occur for whole-genome amplification. Therefore, it is important to quantitate the input into the whole-genome amplification reaction.

- Preparation**
- ▶ Thaw PicoGreen to room temperature in a light-impermeable container.
 - ▶ Follow the instructions for preparing the robot before each use in the *Infinium Assay Lab Setup and Procedures Guide*.
 - ▶ Prepare the robot for use.

- ▶ Thaw the sample DNA plates to room temperature.
- ▶ Apply a QDNA barcode label to a new Fluotrac plate for each WG#-DNA plate to be quantified.
- ▶ Hand-label the microtiter plate "Standard DNA."
- ▶ Hand-label one of the Fluotrac plates "Standard QNT."
- ▶ In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Steps

In this section, you will perform the following steps:

- ▶ Make a Standard DNA plate with serial dilutions of stock Lambda DNA.
- ▶ Dilute PicoGreen with 1X TE.
- ▶ Create a Standard QNT Fluotrac plate containing serial dilutions of DNA plus diluted PicoGreen.
- ▶ Create a QNT plate by adding diluted PicoGreen to the sample DNA you plan to assay.

Make Standard DNA Plate

In this process, you create a Standard DNA plate with serial dilutions of stock Lambda DNA in the wells of column 1 (Figure 100).

1. Add stock Lambda DNA to well A1 in the plate labelled "Standard DNA" and dilute it to 75 ng/μl in a final volume of 233.3 μl. Pipette up and down several times.
 - a. Use the following formula to calculate the amount of stock Lambda DNA to add to A1:

$$\frac{(233.3 \mu\text{l}) \times (75 \text{ ng}/\mu\text{l})}{(\text{stock Lambda DNA concentration})} = \mu\text{l of stock Lambda DNA to add to A1}$$

- b. Dilute the stock DNA in well A1 using the following formula:

$$\mu\text{l of 1X TE to add to A1} = 233.3 \mu\text{l} - \mu\text{l of stock Lambda DNA in well A1}$$

2. Add 66.7 μl 1X TE to well B1.
3. Add 100 μl 1X TE to wells C, D, E, F, G, and H of column 1.

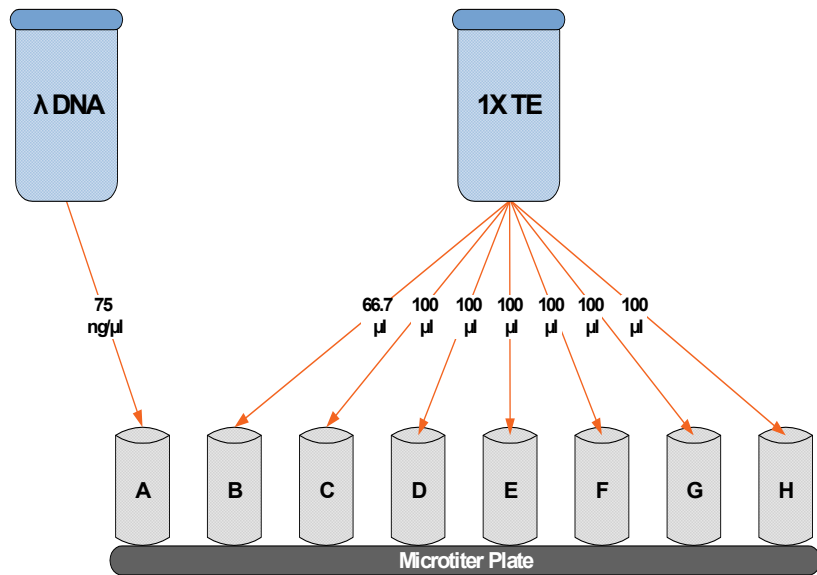


Figure 100 Dilution of Stock Lambda DNA Standard

4. Transfer 133.3 μl of Lambda DNA from well A1 into well B1. Pipette up and down several times.
5. Change tips. Transfer 100 μl from well B1 into well C1. Pipette up and down several times.
6. Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 $\text{ng}/\mu\text{l}$ Lambda DNA.

Table 14 Concentrations of Lambda DNA

Row-Column	Concentration ($\text{ng}/\mu\text{l}$)	Final Volume in Well (μl)
A1	75	100
B1	50	100
C1	25	100
D1	12.5	100
E1	6.25	100
F1	3.125	100
G1	1.5262	200
H1	0	100

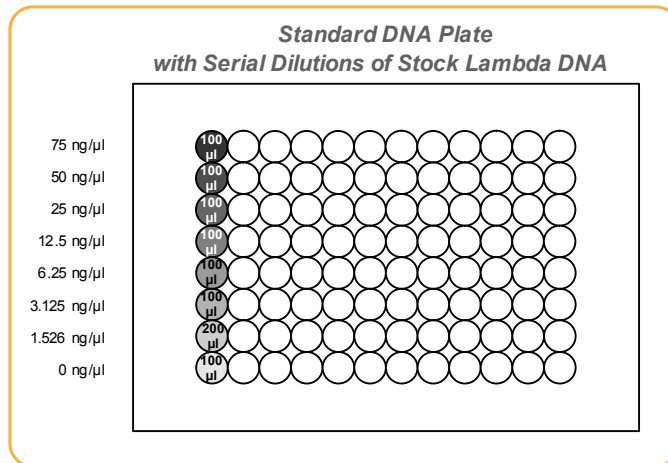


Figure 101 Serial Dilutions of Lambda DNA

7. Cover the Standard DNA plate with cap mat.
8. Proceed to *Dilute PicoGreen*.

Dilute PicoGreen

Diluted PicoGreen will be added to both the Standard QNT and QNT plates, intercalating into available dsDNA and fluorescing upon excitation in a spectrofluorometer.



CAUTION

PicoGreen reagent degrades quickly in the presence of light. Also, do not use glass containers for PicoGreen reagent, because it adheres to glass, thereby lowering its effective concentration in solution and effecting the upper response range accuracy.

1. Prepare a 1:200 dilution of PicoGreen into 1X TE, using a sealed 100 ml or 250 ml Nalgene bottle wrapped in aluminum foil.
Refer to Table 15 to identify the volumes needed to produce diluted reagent for up to three 96-well QNT plates. For fewer than 96 DNA samples, scale down the volumes.

Table 15 Volumes for PicoGreen Reagents

# QNT Plates	PicoGreen Volume (μl)	1X TE Volume (ml)
1	125	25
2	230	45
3	325	65

2. Cap the foil-wrapped bottle and vortex to mix.

Create Standard QNT Standard and Sample Plates

In this process, PicoGreen is distributed to Standard QNT and Sample QNT Fluotrac plates and mixed with aliquots of DNA from the respective DNA plates.



WARNING

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

1. At the robot PC, select **GTS Pre-PCR Tasks | DNA Prep | Make QDNA**.
2. In the DNA Plate Selection dialog box, select the plate type of the Standard DNA and Sample DNA plates. They should all be MIDI plates, TCY plates or ABGN plates. Roll the mouse pointer over each picture to see a description of the plate.

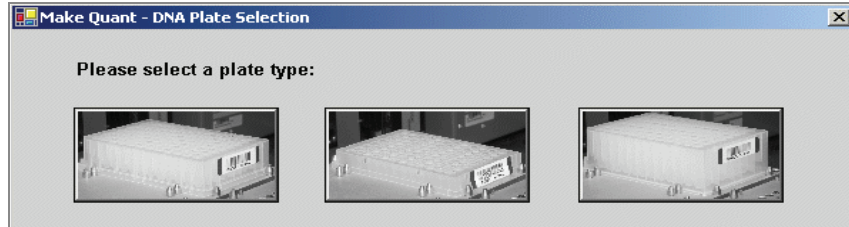


Figure 102 DNA Plate Selection Dialog Box

3. In the Basic Run Parameters pane, enter the **Number of DNA/QNT plates** (1, 2, or 3 pairs) and the **Number of DNA 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.

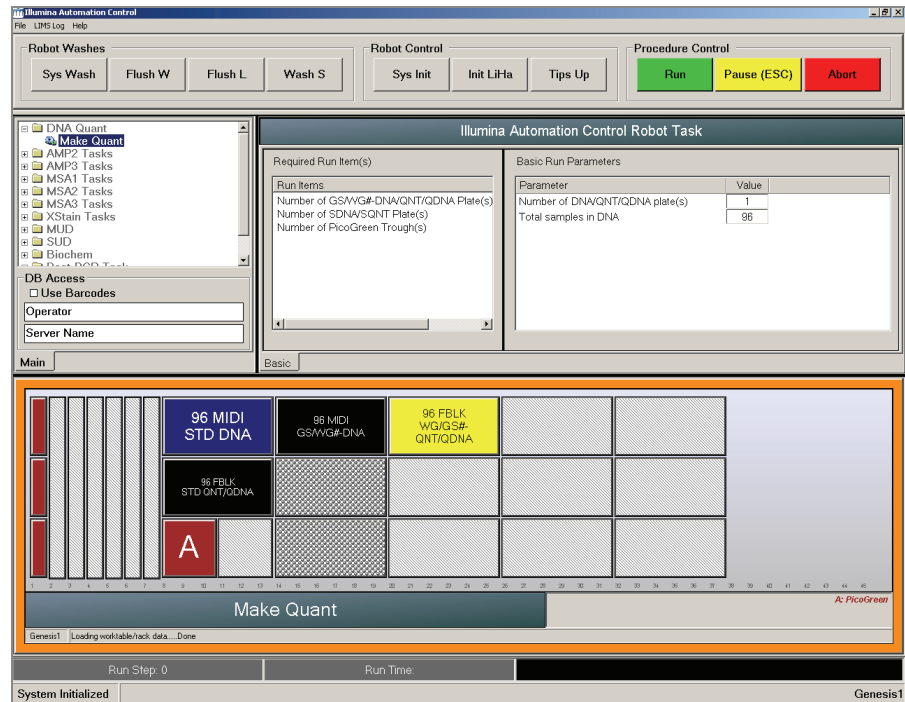


Figure 103 Make Quant Screen

4. Vortex each plate for 1 minute.
5. Centrifuge the WG#-DNA Sample plate to 280 xg for 1 minute.
6. Vortex the Standard DNA plate at 1450 rpm for 1 minute.
7. Centrifuge the Standard DNA plate to 280 xg for 1 minute.
8. Place the WG#-DNA Sample, Standard DNA, Standard QNT, and QNT Sample plates on the robot bed according to the robot bed map. Place well A1 at the top-left corner of its robot bed carrier. Remove any plate seals.
9. Pour the PicoGreen dilution into half reservoir A and place it on the robot bed.
10. Make sure that all items are placed properly on the robot bed, that all seals have been removed, and that all the barcodes face to the right.
11. On the lab tracking form, record the position of the plates on the robot bed.
12. If you are not using LIMS, clear the **Use Barcodes** checkbox.
13. Click **Run**. Observe the beginning of the robot run to ensure there are no problems.
The robot transfers 195 μ l of diluted PicoGreen to all Fluotrac plates, then transfers 2 μ l aliquots of DNA from Standard DNA plate to Standard Quant plate and from WG#-DNA plate to sample QNT plates.
The robot PC sounds an alert and displays a message when the process is complete.
14. Click **OK** in the message box.

15. On the lab tracking form, record:
 - Date/Time
 - Operator
 - Robot
 - The QNT barcode that corresponds to each WG#-DNA barcode
 - The Standard QNT plate that corresponds to each Standard DNA plate
16. After the robot finishes, immediately seal all plates:
 - a. Place foil adhesive seals over Sample QNT and Standard QNT plates.
 - b. Place cap mats on WG#-DNA Sample and Standard DNA plates.
17. Discard unused reagents in accordance with facility requirements.
18. Store the WG#-DNA and Standard DNA plates at 2° to 8°C or -15° to -25°C.
19. Centrifuge the Sample QNT Plate and Standard QNT plates to 280 xg for 1 minute.
20. Proceed to *Read QNT Plate*.

Read QNT Plate

In this process, you use the Gemini XS or XPS Spectrofluorometer along with the Infinium Fluorometry Analysis software to read the Standard QNT and Sample QNT plates. You use the software to create a standard curve based on the quantities of Standard DNA with PicoGreen. Then you read the Sample QNT plates to compare their data against the standard curve to obtain the concentration of sample DNA. For the best genotyping performance, Illumina recommends a minimum concentration of 50 ng/ μ l.

1. Turn on the spectrofluorometer. At the PC, open the Infinium Fluorometry Analysis program.

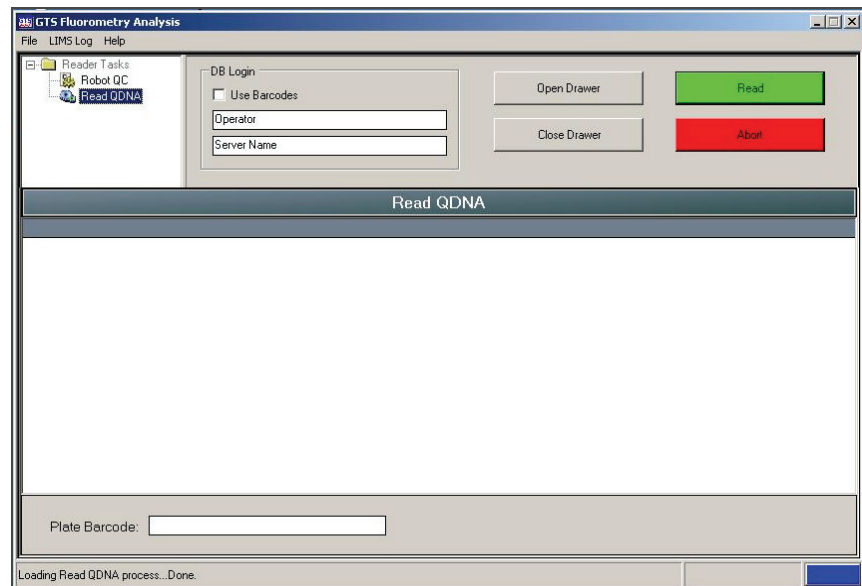


Figure 104 GTS Fluorometry Analysis Main Screen

2. Select **Reader Tasks | Read QDNA**.
3. If you are not using LIMS, clear the **Use Barcodes** checkbox.
4. Click **Read**.
5. When prompted, log in to the Illumina LIMS database.
6. When asked if you want to read a new Standard QNT plate, click **Yes**. Remove the plate seal and load the Standard QNT plate in the open fluorometer tray. Click **OK**. The spectrofluorometer reads the plate data.
7. Review the data from the Standard QNT plate. Either accept it and go on to the next step, or reject it which will stop the Read Quant process.
8. Remove the Standard QNT plate from the spectrofluorometer tray.
9. When prompted, enter the number of Sample QNT plates you want to read (1, 2, or 3). Do not include the Standard QNT plate in this number. Click **OK**.

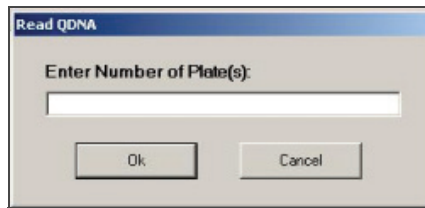


Figure 105 Number of Sample QDNA Plates

10. When prompted, hand-scan or enter the Sample QNT plate barcode. Click **OK**.

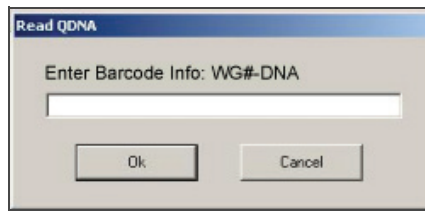


Figure 106 Plate Barcode Dialog Box

11. When prompted, remove the plate seal from the Sample QNT plate and load it into the spectrofluorometer tray, with well A1 at the upper left corner. Click **OK**.

The spectrofluorometer reads the Sample QNT plate.

12. When prompted, click **Yes** to review the raw Sample QNT plate data. The Infinium Fluorometry Analysis screen fills in with information about the fluorescence in the wells.

Read QDNA												
Index	01	02	03	04	05	06	07	08	09	10	11	12
A	901.187	574.264	2229.355	2743.684	1653.467	1240.680	2516.841	1806.896	957.319	1849.673	2062.137	1073.844
B	2511.246	1408.539	2197.872	2961.366	1817.009	1755.341	1478.821	2164.615	1279.077	544.411	1015.841	652.956
C	1354.520	586.959	618.364	1680.938	879.031	1186.237	251.373	1547.593	758.155	864.308	1459.477	975.849
D	2382.074	2444.011	1618.223	74.983	2172.284	2118.486	3367.836	1771.613	1255.763	2036.969	1436.691	342.354
E	2085.587	1933.228	2348.986	1112.462	1835.320	2230.770	2192.462	1427.822	2056.528	2417.382	1900.707	1438.428
F	1028.495	2637.335	1827.523	2219.094	2520.103	2360.154	907.099	1993.719	1426.916	1284.198	1066.274	791.380
G	1357.837	1753.874	1602.840	1527.622	1888.769	1178.043	1657.656	1804.497	1270.985	1495.533	1278.908	1461.335
H	1399.478	2255.065	1281.426	774.938	676.704	571.730	769.209	685.294	1086.497	1129.055	1142.966	1119.405

Figure 107 Sample QDNA Data

Microsoft Excel opens automatically at the same time and displays the quantitation data for the Sample QNT plate. There are three tabs in the file:

- **SQDNA_STD**—Generates the standard curve by plotting the Relative Fluorescence (RF) values measured in the Standard QNT plate against assumed concentrations in the Standard DNA Plate.
- **QDNA**—Plots the concentration (ng/ μ l) of each well of the Sample QDNA Plate as derived from the standard curve.
- **Data**—A readout of the raw data values for the Standard QNT plate and the Sample QNT Plate.

The GTS Fluorometry Analysis software prompts you to indicate whether you wish to save the QNT data shown in the Excel file.

13. Do one of the following:
 - Click **Yes** to send the data to Illumina LIMS. In Illumina LIMS, the QNT plate moves into the *Make the MSA3 Plate* queue.
 - Click **No** to delete the quant data. You can read the quant data again for the same plate.
14. If you entered more than one Sample QNT plate to read, repeat steps 10 to 13 for each additional plate.
15. Discard the QNT plates and reagents in accordance with facility requirements.
16. Do one of the following:
 - Proceed to *Make the MSA3 Plate* on page 119.
 - Store the Sample DNA plate at 2° to 8°C for up to one month.

Make the MSA3 Plate

This process creates a MSA3 plate for DNA amplification. MA1 is first added to the MSA3 plate, followed by the DNA samples. 0.1N NaOH is added to denature the DNA samples. The MA2 reagent neutralizes the sample. MSM (Multi-Sample Amplification Master Mix) is then added to the DNA samples.

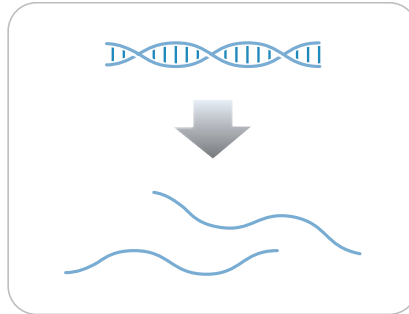


Figure 108 Denaturing and Neutralizing DNA

Estimated Time

Robot time:

- 30 minutes for 48 samples
- 1 hour for 96 samples

Consumables

Item	Quantity	Storage	Supplied By
MA1	1 tube (per 96 samples)	-15° to -25°C	Illumina
MA2	1 tube (per 96 samples)	-15° to -25°C	Illumina
MSM	1 tube (per 96 samples)	-15° to -25°C	Illumina
0.1N NaOH	15 ml (per 96 samples)	2° to 8°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate		User
WG#-DNA plate with 48 or 96 DNA samples (50 ng/μl)	1 plate	-15° to -25°C	User



CAUTION

Only pour out 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.



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

Preparation

- ▶ In preparation for the Incubate MSA3 process (page 127), preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- ▶ On the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.
- ▶ Apply an MSA3 barcode label to a new MIDI.
- ▶ Thaw DNA samples to room temperature.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - Robot
 - Batch number
 - Number of samples (48 or 96)
 - WG#-DNA plate barcode(s)
 - MSA3 plate barcode(s)
 - MA1 tube barcode(s)
 - MA2 tube barcode(s)
 - MSM tube barcode(s)



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

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 Figure 109 throughout this protocol. Note that all of the barcodes face to the right.

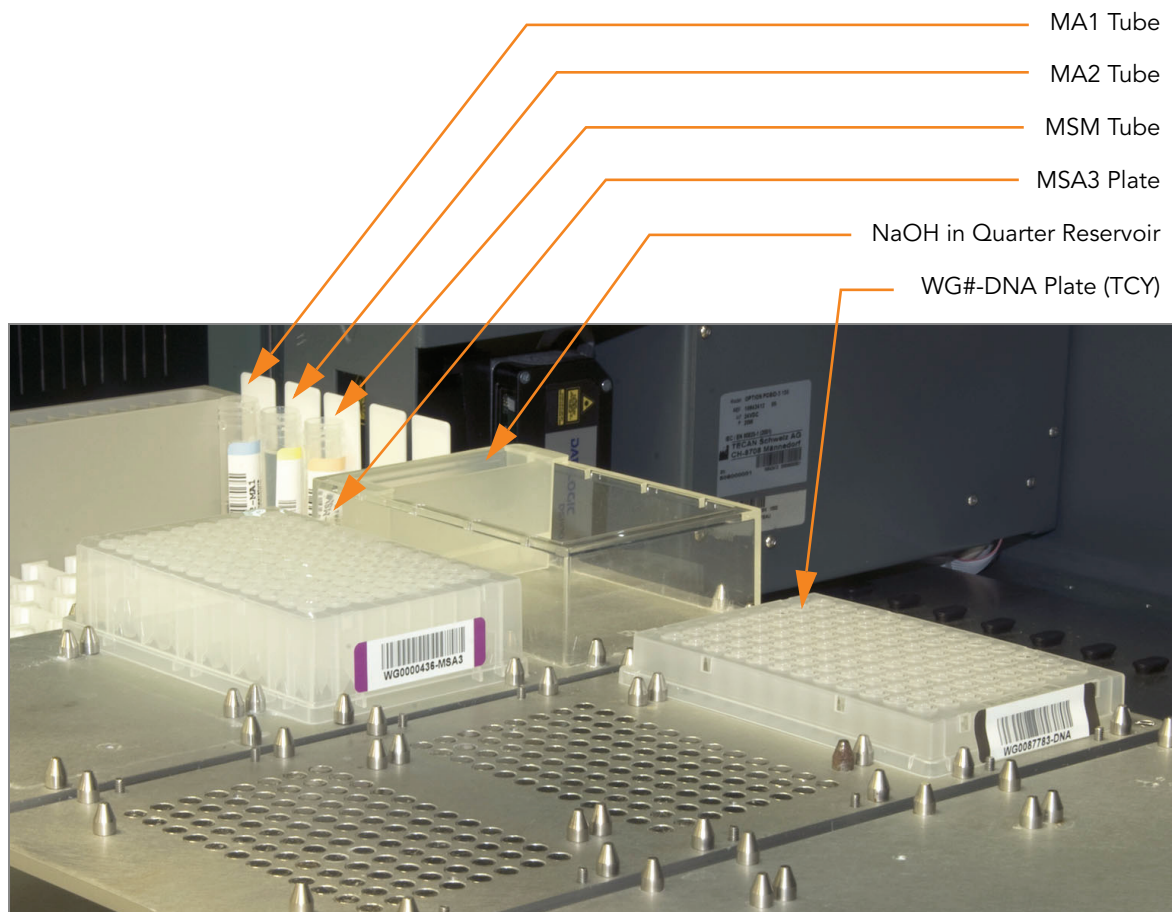


Figure 109 Setting up the Tecan Eight-Tip Robot for Make MSA3

Steps Set up the Robot

1. If you do not already have a WG#-DNA plate, create one by adding DNA into either a:
 - MIDI plate: 20 μ l to each WG#-DNA plate well
 - TCY plate: 10 μ l to each WG#-DNA plate wellApply a barcode label to the new WG#-DNA plate.
2. At the robot PC, select **MSA3 Tasks | Make MSA3**.
3. In the DNA Plate Selection dialog box (Figure 110), click on the plate type you wish to use. Roll the mouse pointer over each picture to see a description of the plate.

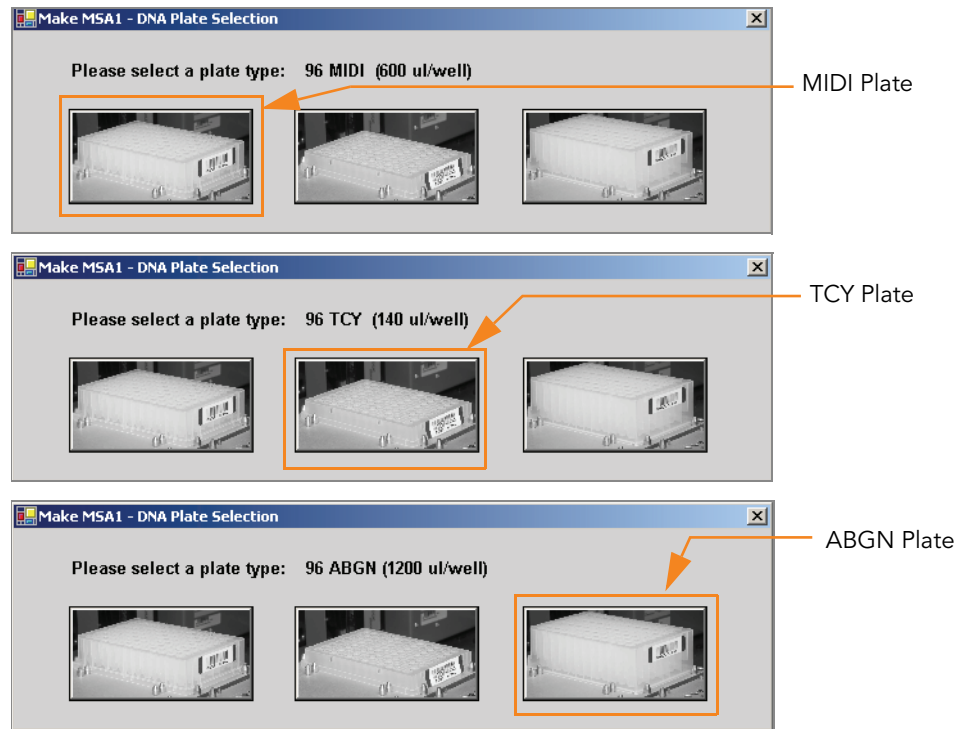


Figure 110 Selecting the DNA Plate Type



NOTE

Do not mix plate types on the robot bed.

4. (Non-Illumina LIMS only) 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. This value must match the number of DNAs in the plate and the number of DNAs identified in the DNA manifest.



NOTE

(Non-Illumina LIMS only) If you are running 48 or less samples enter 48 in the Basic Run Parameters. Enter 96 if you are running 49–96 samples.



NOTE

If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of samples.

You can process up to 96 DNA samples per robot run.

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.

**NOTE**

If you are using Illumina LIMS, then you must click **Run** and select batches before the robot bed map will display the correct layout for the WG#-DNA plates.

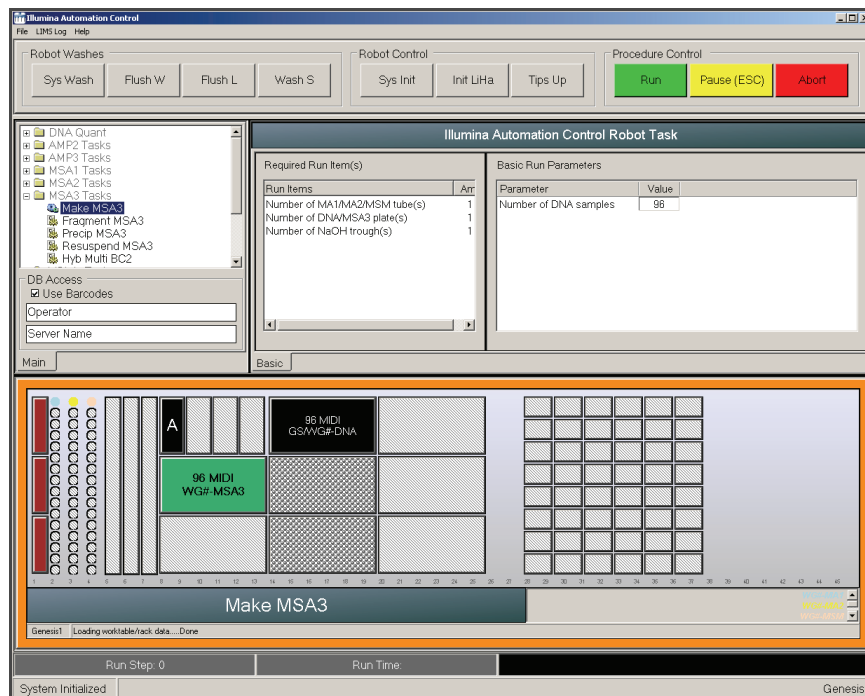


Figure 111 Make MSA3 Screen

5. Remove caps from MA1, MA2, and MSM tubes, then place the tubes in the robot tube rack according to the bed map.
6. Add 15 ml NaOH to the quarter reservoir, then place the reservoir on the robot bed according to the bed map (Figure 111).
7. Place the WG#-DNA and MSA3 plates on the robot bed according to the bed map (Figure 111).
8. In 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. (Non-Illumina LIMS only) At the robot PC, click **Run**. Observe the robot start to run to ensure that there are no problems.
11. (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - a. Log in when prompted.
 - After the robot initializes, the Make MSA3 screen appears after a moment.

The screenshot shows a dialog box titled "Make MSA3". It is divided into several sections. On the left, there is a "Project Name" field containing the text "Ovine_iSelect_Fall08". Below this are search fields: "Search By:" with a dropdown arrow and "Search For:" with a text input. There are "Search" and "Reset" buttons below these fields. On the right side, there are three stacked fields: "Sample Batch ID:" (empty), "Plate(s):" (empty), and "Selected Batches:" (empty). A "Remove" button is located below the "Selected Batches" field. At the bottom of the dialog, there are "OK" and "Cancel" buttons.

Figure 112 Selecting Project or Batch for Make MSA3

- b. Do one of the following:
- Select your current project. The available batches appear in the Sample Batch ID pane. Select a batch to see the associated DNA plate appear in the DNA Plate(s) pane.

The screenshot shows a dialog box titled "Make MSA3 - KIT-OVINESNP50". It is divided into several sections. On the left, there is a "Project Name" field containing the text "Ovine_iSelect_Fall08". Below this are search fields: "Search By:" with a dropdown arrow and "Search For:" with a text input. There are "Search" and "Reset" buttons below these fields. On the right side, there are three stacked fields: "Sample Batch ID:" containing "5893", "Plate(s):" containing "\WG0087490-DNA", and "Selected Batches:" containing "5893". A "Remove" button is located below the "Selected Batches" field. At the bottom of the dialog, there are "OK" and "Cancel" buttons.

Figure 113 Make MSA3 Screen with Project and Batch Selected

- Use the **Search** box to search for a specific Batch ID or DNA Plate.
- c. Select the batch you want to run and click **OK**.
- d. Click **OK** to confirm the required DNAs.

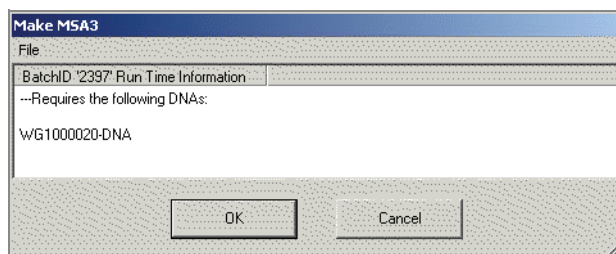


Figure 114 Confirm DNAs

- e. When prompted, enter the barcode of each WG#-DNA plate. The robot bed map is updated with the WG#-DNA plate locations.
 - f. Place the WG#-DNA plate(s) on the robot bed according to the bed map and click **OK**. The robot begins running when the plates are in place.
12. Observe the robot run to ensure there are no problems.
 13. After the robot adds the 0.1N NaOH to the DNA in the MSA3 plate, follow the instructions at the prompt (Figure 115).

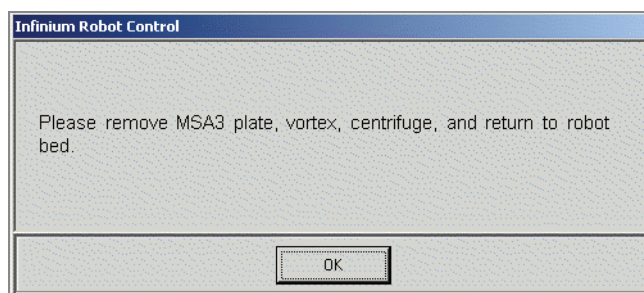


Figure 115 Vortex & Centrifuge Prompt

14. Seal the plate with a cap mat.
15. Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.
16. Centrifuge to 280 xg for 1 minute at 22°C.
17. 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. When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

18. Place the MSA3 plate back on the robot bed in its original position and click **OK**.
The Wait for reaction time message appears. The wait time for this reaction is 10 minutes.
The robot PC sounds an alert and displays a message when the process is complete.
19. After the Make MSA3 process is complete click **OK** in the message box. Remove the MSA3 plate from the robot bed and seal with the 96-well cap mat.

20. Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.
21. Centrifuge to 280 xg for 1 minute.
22. Discard unused reagents in accordance with facility standards.
23. Proceed immediately to *Incubate the MSA3 Plate* on page 127.

Incubate the MSA3 Plate

This process incubates the MSA3 plate for at least 20 but no more than 24 hours at 37°C in the Illumina Hybridization Oven. It generates a sufficient quantity of each individual DNA sample to be used once in the Infinium HD Assay Ultra.

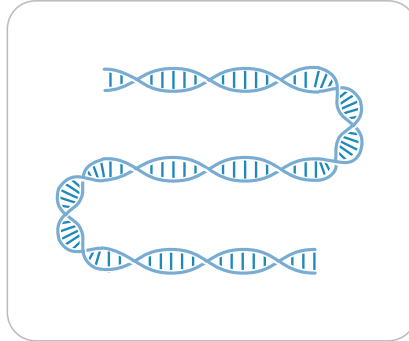


Figure 116 Incubating DNA to Amplify

Estimated Time Incubation time: 20–24 hours

Preparation **Verify MSA3 for Incubation (LIMS only)**

1. In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Incubate MSA3**.
2. Scan the barcode of the MSA3 plate and click **Verify**.

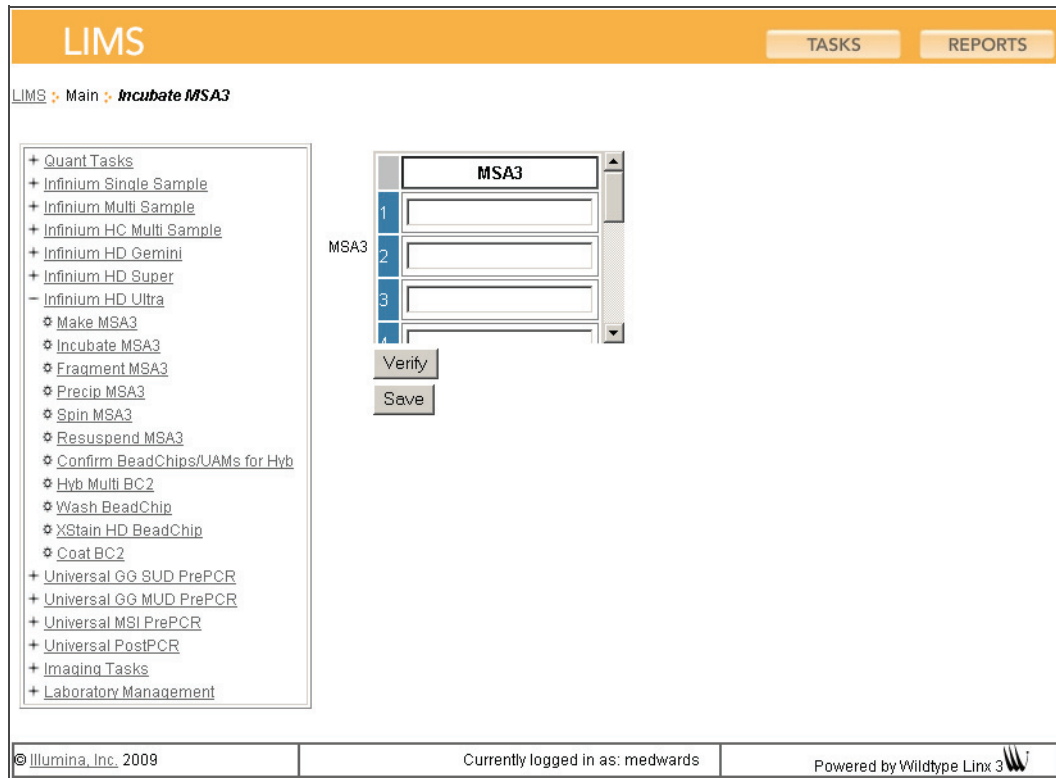


Figure 117 (Illumina LIMS) Verifying MSA3 for Incubation

3. If the MSA3 plate is queued for incubation, a blue confirmation message appears at the top of the window. Proceed to *Incubate MSA3*.
4. If the MSA3 plate is not queued for incubation, a red error message appears at the top of the window. Do **not** proceed with incubation. Instead, follow these steps to troubleshoot the problem:
 - a. Click the Reports tab in the upper-right corner.
 - b. In the left sidebar, click **Tracking | Get Queue Status**.
 - c. Scan the plate barcode and click **Go**.
 - d. Note what step the plate is queued for, and proceed with that step.

For information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

Steps Incubate MSA3

1. Incubate the MSA3 plate in the Illumina Hybridization Oven for at least 20 but no more than 24 hours at 37°C.
2. On the lab tracking form, record the start and stop times.



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

3. If you are using Illumina LIMS:
 - a. In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Incubate MSA3**.
 - b. Scan the barcode of the MSA3 plate and click **Save**. Illumina LIMS records the data and queues the plate for the next step.
4. Proceed to *Fragment the MSA3 Plate*.

Fragment the MSA3 Plate

This process enzymatically fragments the amplified DNA samples. An end-point fragmentation is used to prevent over-fragmentation.

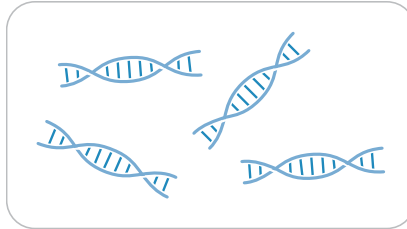


Figure 118 Fragmenting DNA

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	1 tube (per 96 samples)	-15° to -25°C	Illumina



CAUTION

Only pour out 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

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

Preparation

- ▶ Preheat the heat block with the MIDI plate insert to 37°C.
- ▶ Remove the MSA3 plate from the Illumina Hybridization Oven.
- ▶ Remove the cap mat.
- ▶ If you plan to Resuspend MSA3 today then remove the RA1 from the freezer to thaw.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator

- Robot
- FMS 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. This form can be filled out and saved online, or printed and filled in by hand. Go to <http://www.illumina.com/documentation> to download the lab tracking form.

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 Figure 119 throughout this protocol.

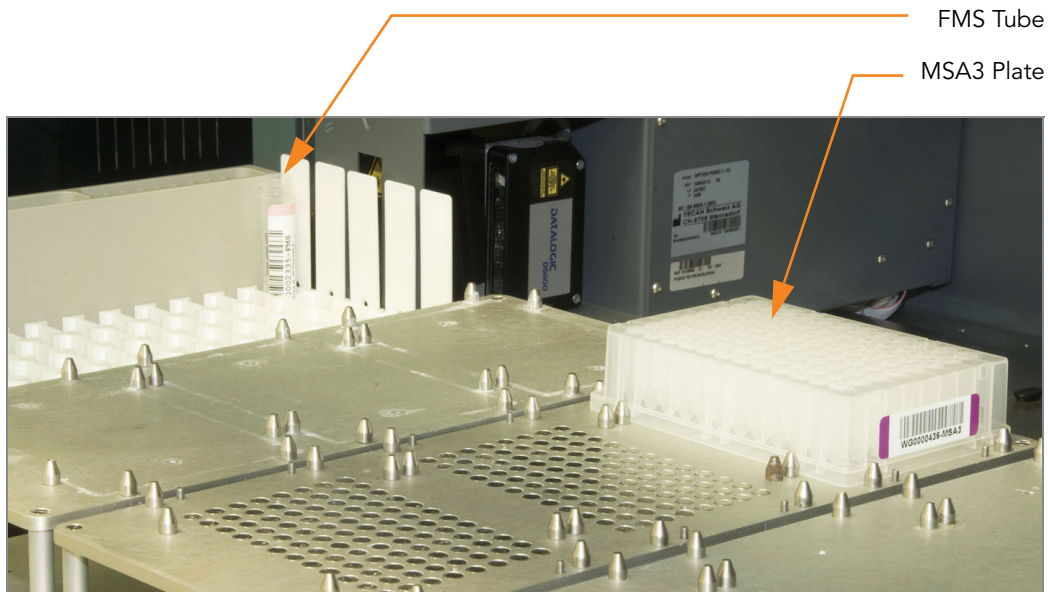


Figure 119 Tecan Eight-Tip Robot (Fragment MSA3 Setup)

Steps Set Up the Robot

1. Centrifuge the MSA3 plate to 50 xg for 1 minute.
2. Remove the cap mat.

**NOTE**

When you remove a cap mat, set it aside, upside down, in a safe location for use later in the protocol. When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

3. At the robot PC, select **MSA3 Tasks | Fragment MSA3**.

- (Non-Illumina LIMS only) In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect the number of samples being processed.

**NOTE**

If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of 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.

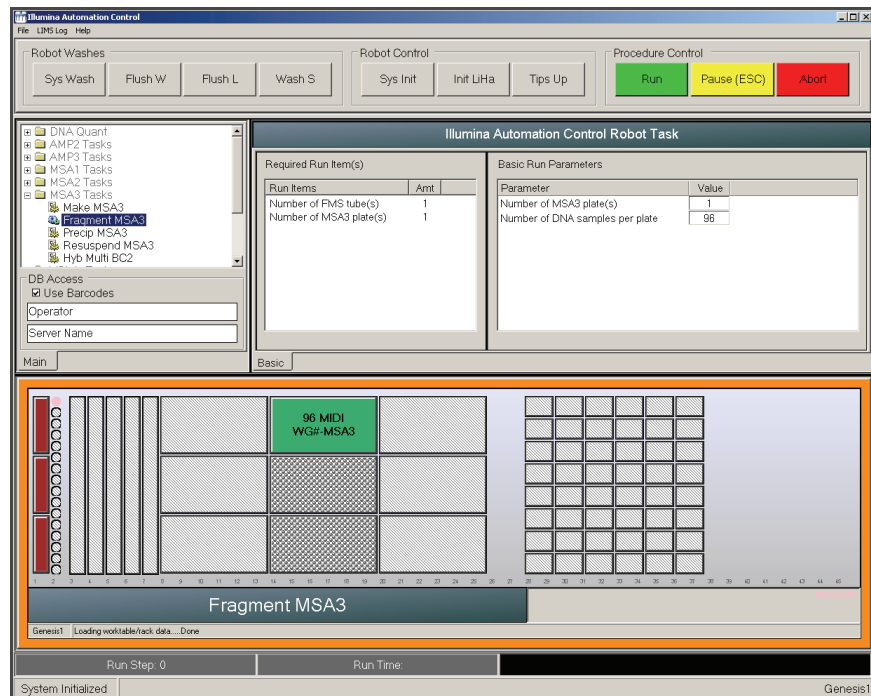


Figure 120 Fragment MSA3 Screen

- Place the MSA3 plate on the robot bed according to the bed map (Figure 120). Remove the plate seal. If you have not already done so, remove the cap mat from the plate.
- Place the FMS tube in the robot tube rack according to the bed map. Remove the cap.
- In the lab tracking form, record the plate positions on the robot bed.

Start the Robot

- (Non-Illumina LIMS) At the robot PC, click **Run**.
- (Illumina LIMS only) Make sure the Use Barcodes check box is selected and click **Run**.
 - Log in if prompted.
 - Observe the robot start to run to ensure that there are no problems.

The robot PC sounds an alert and displays a message when the process is complete.

3. Click **OK** in the message box. Remove the MSA3 plate from the robot bed and seal with the 96-well cap mat.
4. Vortex at 1600 rpm for 1 minute.
5. Centrifuge to 50 xg for 1 minute at 22°C.
6. Place the sealed plate on the 37°C heat block for 1 hour.
7. On the lab tracking form, record the start and stop times.
8. Discard unused reagents in accordance with facility standards.
9. Do one of the following:
 - Proceed to *Precipitate the MSA3 Plate* on page 134. Leave plate in 37°C heat block until you have completed the preparatory steps.
 - Store the sealed MSA3 plate at -15° to -25°C if you do not plan to proceed to the next step immediately.



This is a good stopping point in the process.

Precipitate the MSA3 Plate

PM1 and 2-propanol are added to the MSA3 plate to precipitate the DNA samples.

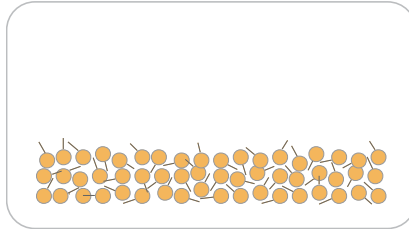


Figure 121 Precipitating DNA

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	1 tube (per 96 samples)	2° to 8°C	Illumina
100% 2-propanol	30 ml (per 96 samples)	Room temperature	User



CAUTION

Only pour out 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

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

Preparation

- ▶ Preheat the heat block to 37°C.
- ▶ If frozen, thaw MSA3 plate to room temperature. Pulse centrifuge to 50 xg.
- ▶ Thaw PM1 to room temperature. Centrifuge to 280 xg for 1 minute.
- ▶ On the lab tracking form, record:
 - Date/Time

- Operator
- Robot
- PM1 tube barcode(s)
- 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. This form can be filled out and saved online, or printed and filled in by hand. Go to <http://www.illumina.com/documentation> to download the lab tracking form.

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 Figure 122 throughout this protocol. Note that all of the barcodes face to the right.

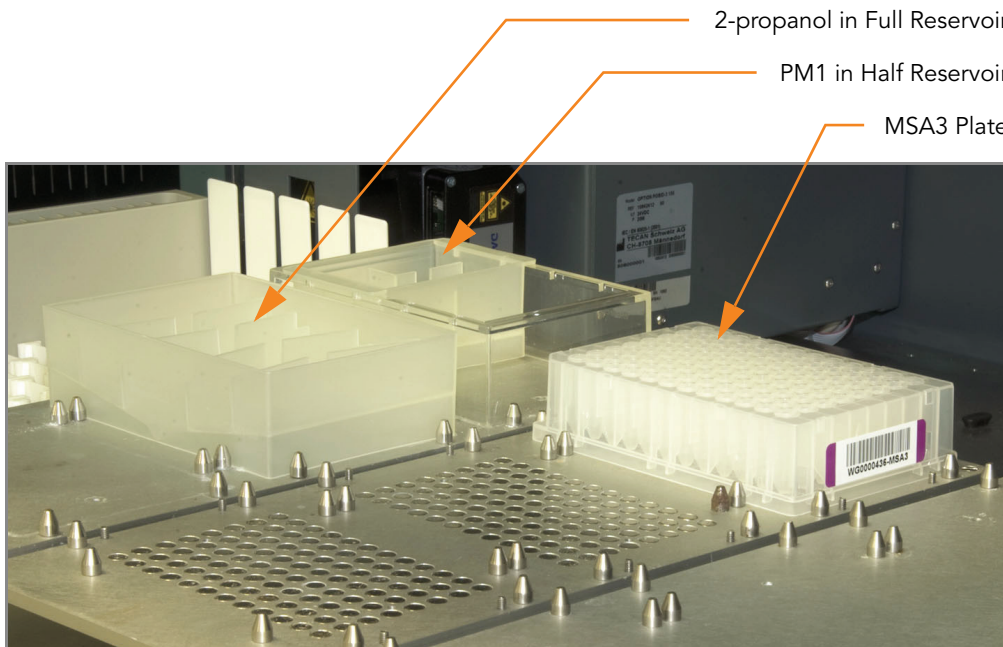


Figure 122 Tecan Eight-Tip Robot (Precip MSA3 Setup)

Verify MSA3 for Centrifugation (LIMS only)

1. In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Spin MSA3**.
2. Scan the barcode of the MSA3 plate and click **Verify** and then **Save**. You can scan up to four plates.

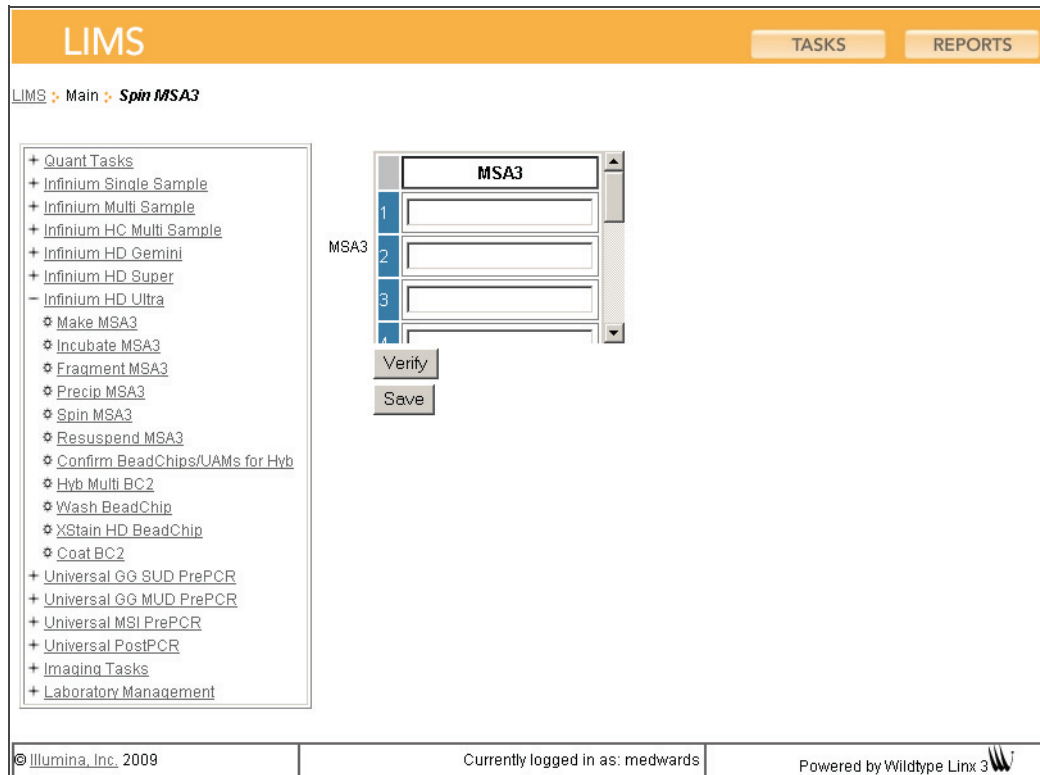


Figure 123 (Illumina LIMS) Verifying MSA3 for Centrifugation

3. If the MSA3 plate is queued for centrifugation, a blue confirmation message appears at the top of the window. Proceed to *Set Up the Robot* on page 136.
4. If the MSA3 plate is not queued for centrifugation, a red error message appears at the top of the window. Do **not** proceed with centrifugation. Instead, follow these steps to troubleshoot the problem:
 - a. Click the Reports tab in the upper-right corner.
 - b. In the left sidebar, click **Tracking | Get Queue Status**.
 - c. Scan the plate barcode and click **Go**.
 - d. Note what step the plate is queued for, and proceed with that step.

For information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

Steps Set Up the Robot

1. At the robot PC, select **MSA3 Tasks | Precip MSA3**.
2. (Non-Illumina LIMS only) Make sure the Use Barcodes check box is cleared. In the Basic Run Parameters pane, change the value for **Number of MSA3 plate(s)** and **Number of DNA samples per plate** to reflect the number of DNA samples being processed.

**NOTE**

If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of 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.

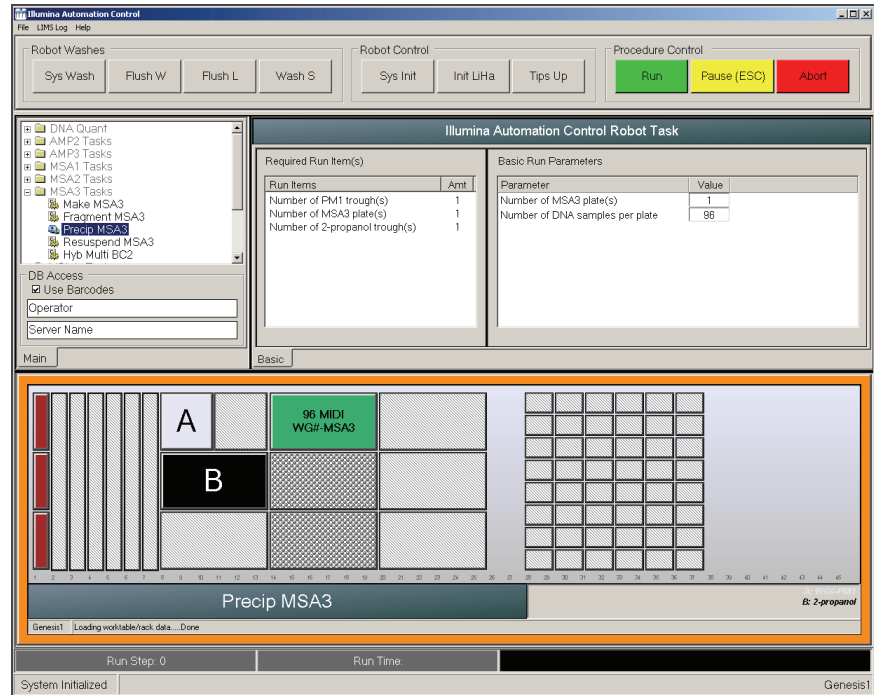


Figure 124 Precip MSA3 Screen

3. Centrifuge the sealed MSA3 plate to 50 xg for 1 minute at 22°C.
4. Remove the cap mat and place the MSA3 plate on the robot bed according to the bed map (Figure 124).
5. Place a half reservoir in the reservoir frame, according to the robot bed map, and add PM1 as follows:
 - For 96 samples, dispense 1 PM1 tube
6. Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:
 - For 96 samples, dispense 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. (Non-Illumina LIMS) At the robot PC, click **Run**.
2. (Illumina LIMS only) At the robot PC, make sure the Use Barcodes check box is selected and click **Run**.
 - a. Log in if prompted.
 - b. Observe the robot start to run to ensure that there are no problems.
3. When prompted, remove the MSA3 plate from the robot bed. Do not click **OK** in the message box yet.
4. Seal the MSA3 plate with the same cap mat removed earlier.
5. Vortex the sealed plate at 1600 rpm for 1 minute.
6. Incubate at 37°C for 5 minutes.
7. Centrifuge to 50 xg at room temperature for 1 minute.
8. Remove the cap mat and place the MSA3 plate back on the robot bed according to the bed map (Figure 124).
9. Click **OK** in the **Please Remove MSA3 Plate** message box.
10. In preparation for the 4°C spin, set centrifuge to 4°C.
The robot PC sounds an alert and displays a message when the process is complete.
11. Click **OK** in the message box. Remove the MSA3 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.
12. Invert the plate at least 10 times to mix contents thoroughly.
13. Incubate at 4°C for 30 minutes.
14. Place the sealed MSA3 plate in the centrifuge opposite another plate of equal weight (Figure 125).

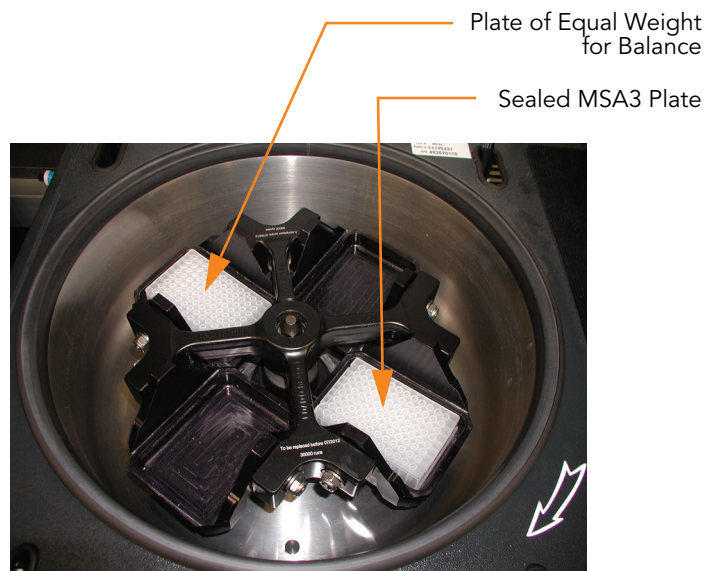


Figure 125 Balancing MSA3 Plate in Centrifuge

15. Centrifuge to 3000 xg at 4°C for 20 minutes.
16. Immediately remove the MSA3 plate from the centrifuge. Remove the cap mat and discard it.
Perform the next step immediately to avoid dislodging the blue pellet. If any delay occurs, repeat steps 15 through 16 before proceeding.
17. Over an absorbent pad appropriate for 2-propanol disposal, decant the supernatant by quickly inverting the MSA3 plate and smacking it down.
18. Tap the plate firmly on the pad several times over a period of 1 minute or until all wells are completely devoid of liquid.

**CAUTION**

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

19. Leave the uncovered plate inverted on the tube rack for 1 hour at room temperature to air dry the pellet (Figure 126).
At this point, blue pellets should be present at the bottoms of the wells.



Figure 126 Uncovered MSA3 Plate Inverted for Air Drying

**CAUTION**

Do not over-dry the pellet. Pellets that are over-dried will be difficult to resuspend. Poorly resuspended samples will lead to poor genotyping results.

20. On the lab tracking form record the start and stop times.
21. Discard unused reagents in accordance with facility standards.
22. Do one of the following:
 - Proceed to *Resuspend the MSA3 Plate* on page 141.

- If you do not plan to proceed to the next step immediately, seal the MSA3 plate with a new cap mat and store at -15° to -25°C .



This is a good stopping point in the process.

Resuspend the MSA3 Plate

RA1 is added to the MSA3 plate to resuspend the precipitated DNA samples.

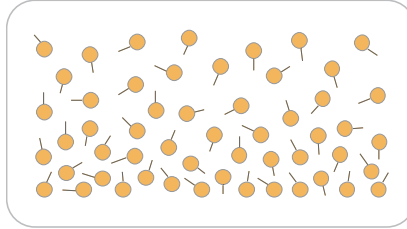


Figure 127 Resuspending DNA

Estimated Time

Robot time: 15 minutes for 96 samples

Incubation time: 1 hour

Consumables

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



NOTE

Only pour out 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 HD BeadChip** step.



WARNING

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the MSDS for this kit, which is available at <http://www.illumina.com/msds>.

Preparation

- ▶ RA1 is shipped frozen. Gradually warm the reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- ▶ If you stored the MSA3 plate at -15° to -25°C, thaw it to room temperature. Remove the cap mat and discard it.
- ▶ Preheat the Illumina Hybridization Oven to 48°C.
- ▶ Preheat the heat sealer. Allow 20 minutes.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator

- Robot
- RA1 bottle barcode(s)



NOTE

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

Prepare the Robot

For instructions on preparing the robot for use in a protocol and ensuring that the Chamber Rack is properly installed on the post-amplification robot bed, see the *Infinium Assay Lab Setup and Procedures Guide*.

Refer to Figure 128 throughout this protocol. Note that all of the barcodes face to the right.

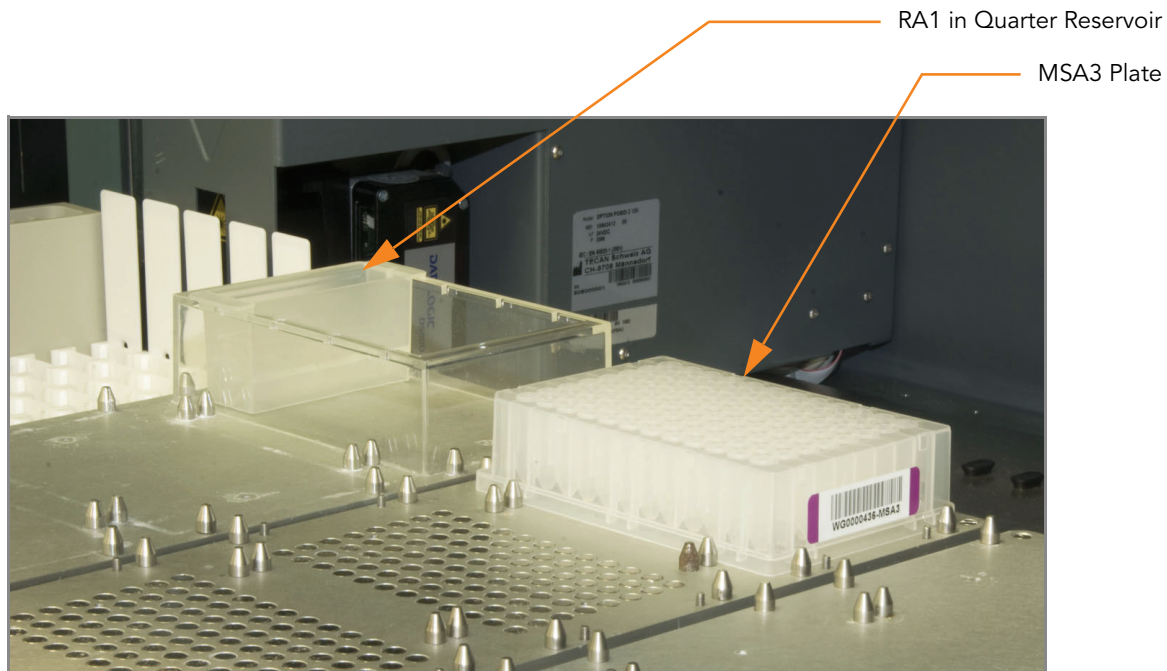


Figure 128 Tecan Eight-Tip Robot (Resuspend MSA3 Setup)

Steps Set Up the Robot

1. At the robot PC, select **MSA3 Tasks | Resuspend MSA3**.
2. (Non-Illumina LIMS only) Make sure the Use Barcodes check box is cleared. In the Basic Run Parameters pane, change the value for **Number of MSA3 plate(s)** and **Number of DNA samples per plate** to reflect the number of DNAs being processed.

**NOTE**

If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of 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.

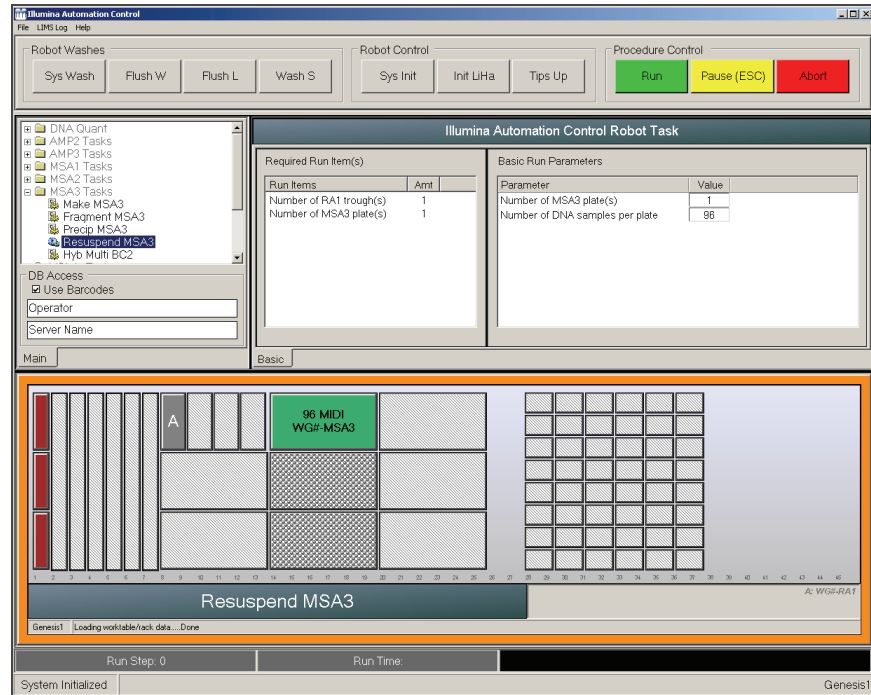


Figure 129 Resuspend MSA3 Screen

3. Place the MSA3 plate on the robot bed according to the bed map (Figure 129).
4. Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add RA1 as follows:
 - 7 ml for 96 samples
5. In the lab tracking form, record the plate positions on the robot bed.
6. 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. (Non-Illumina LIMS) At the robot PC, click **Run**.
2. (Illumina LIMS only) At the robot PC, make sure the Use Barcodes check box is selected and click **Run**.
 - a. Log in if prompted.

Hybridize Multi BeadChip

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

Once the DNA samples are loaded into the Hyb Chambers, incubate the chambers for 16–24 hours at 48°C in the Illumina Hybridization Oven. Hybridization occurs during the incubation period. Each sample will be hybridized to an individual section of the BeadChip.

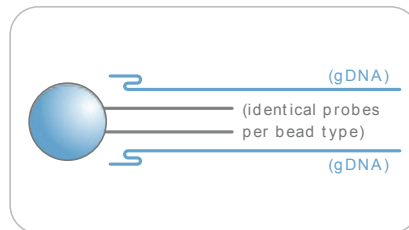


Figure 130 Hybridizing DNA to BeadChip

Estimated Time

Robot time:

- 24x1 HD BeadChip: ~40 minutes for 4 BeadChips (96 samples)
- 12x1 HD BeadChip: ~40 minutes for 8 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables 24x1 HD BeadChip

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	1 tube	Room temperature	Illumina
BeadChips	4		Illumina
Hyb Chambers	1		Illumina
Hyb Chamber gaskets	1		Illumina
Hyb Chamber inserts	4		Illumina
Robot BeadChip Alignment Fixtures	2		Illumina
Robot Tip Alignment Guide-D (One-piece guide)	2		Illumina
1% aqueous Alconox solution	As needed		User

Consumables 12x1 HD BeadChip

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	2 tubes	Room temperature	Illumina
BeadChips	8		Illumina
Hyb Chambers	2		Illumina
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 inserts (for multi-piece guide)	8		Illumina
Robot Tip Alignment Guide-B frames (for multi-piece guide)	4		Illumina
1% aqueous Alconox solution	As needed		User



CAUTION

Only pour out the recommended reagent volume needed for the suggested number of BeadChips listed in the Consumables table of each section. Some reagents are used later in the protocol.



NOTE

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

Preparation

- ▶ Preheat the heat block to 95°C.
- ▶ Preheat the Illumina Hybridization Oven to 48°C.
- ▶ Prepare the Illumina Hybridization Oven as follows:
 - a. Preheat the oven to 48°C:
 - Press the "F" button once 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.
- ▶ Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
 - ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - Robot
 - PB2 tube barcode



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

Prepare Robot Tip Alignment Guide(s)

There are two versions of the Guide-B Robot Tip Alignment Guide (both used with the 12x1 HD BeadChip only). There is a multi-piece that includes two plastic inserts and a metal frame per set and a plastic one-piece. The Guide-D Robot Tip Alignment Guide is used with the 24x1 HD BeadChip only.

1. Make sure you have the correct Robot Tip Alignment Guide for the Infinium HD Ultra Assay. The barcode should say "Guide B" (if using 12x1 HD BeadChips) or "Guide D" (if using 24x1 HD BeadChips) on it.



Figure 131 Multi-Piece Guide-B Robot Tip Alignment Guide Insert



Figure 132 Multi-Piece Guide-B Robot Tip Alignment Guide with Inserts

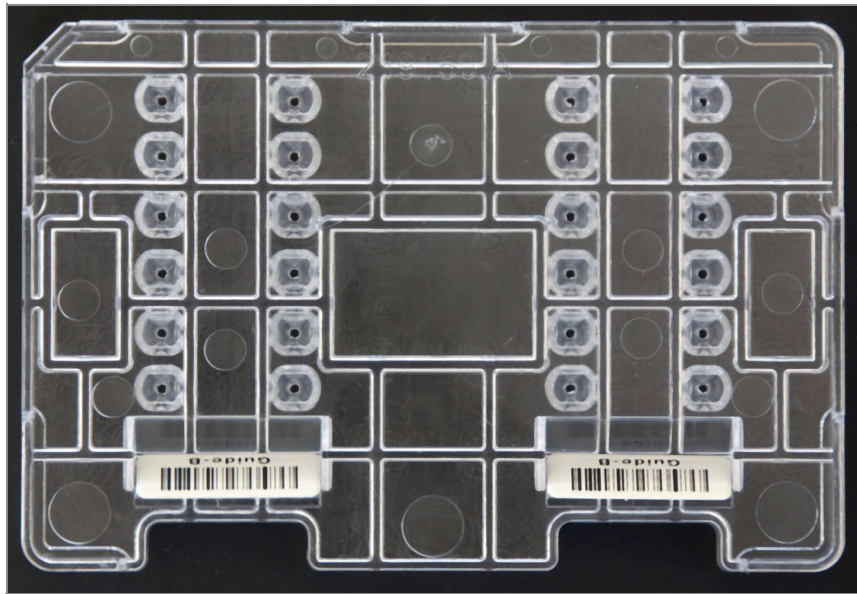


Figure 133 One-Piece Guide-B Robot Tip Alignment Guide

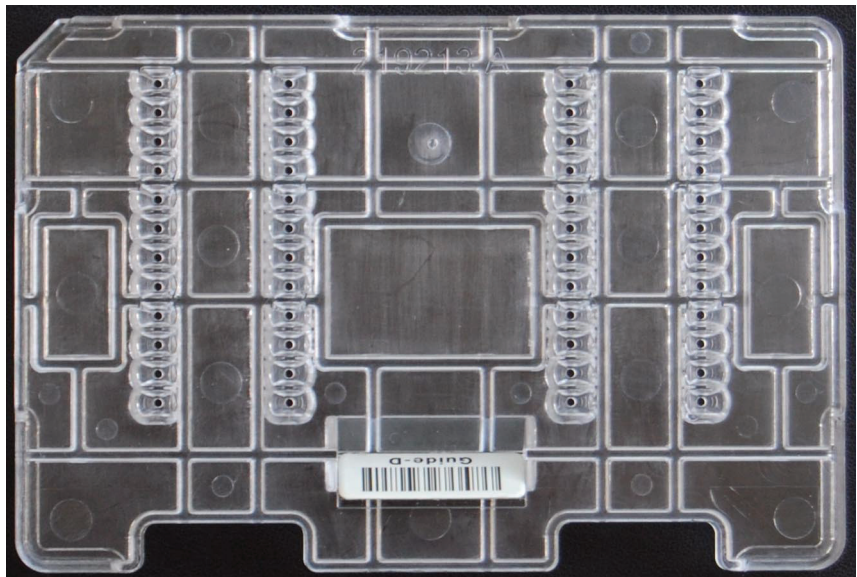


Figure 134 One-Piece Guide-D Robot Tip Alignment Guide

2. Wash and dry the multi-piece Robot Tip Alignment Guide inserts or the entire one-piece Robot Tip Alignment Guide. See *Wash Robot Tip Alignment Guide* on page 165 for washing instructions.

The remaining steps apply to the multi-piece Robot Tip Alignment Guide only.

3. Hold the insert with the barcode side closest to you and the top side of the Robot Tip Alignment Guide frame facing you.
4. Orient the Robot Tip Alignment Guide frame so that the legs face down and the angled corners are closest to you.



Figure 135 Loading the Insert in Robot Tip Alignment Guide Frame

5. Slide the top of the insert (non-barcode end) into the top of the frame at an angle.



Figure 136 Loading Robot Tip Alignment Guide Insert at an Angle

6. Using your thumb, snap in the insert at the barcode end. The angled cutout on the back of the insert should fit into the angled corner of the frame.



Figure 137 Snapping in the Insert

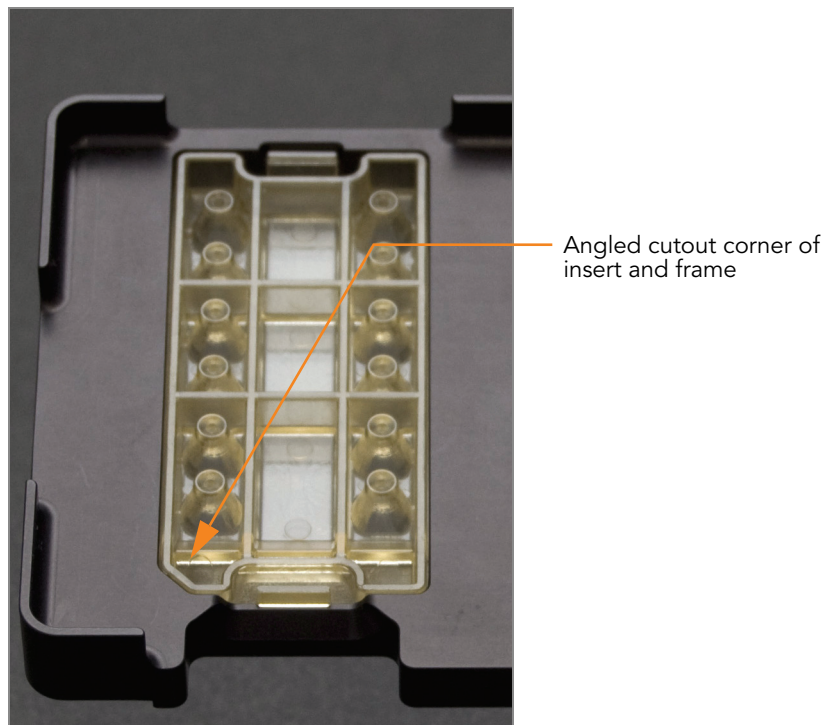


Figure 138 Matching Angled Corner of Insert and Frame

7. Repeat steps 3–6 for each insert.
8. Place the assembled Robot Tip Alignment Guide(s) on the lab bench until it is time to place them on the robot bed.

Assemble the Hyb Chambers



NOTE

Perform the Hyb Chamber assembly near the robot to minimize the distance over which you need to transfer the BeadChips. Keep the Hyb Chamber steady and level when lifting and moving. Avoid shaking and keep the Hyb Chamber parallel to the lab bench at all times.

1. Place the following items on the bench top per 96 samples (Figure 139):
 - For the 24x1 HD BeadChip:
 - BeadChip Hyb Chamber (1)
 - Hyb Chamber Gasket (1)
 - Robot BeadChip Alignment Fixtures (2)
 - Hyb Chamber inserts (4)
 - For the 12x1 HD BeadChip:
 - BeadChip Hyb Chambers (2)
 - Hyb Chamber Gaskets (2)
 - Robot BeadChip Alignment Fixtures (4)
 - Hyb Chamber inserts (8)

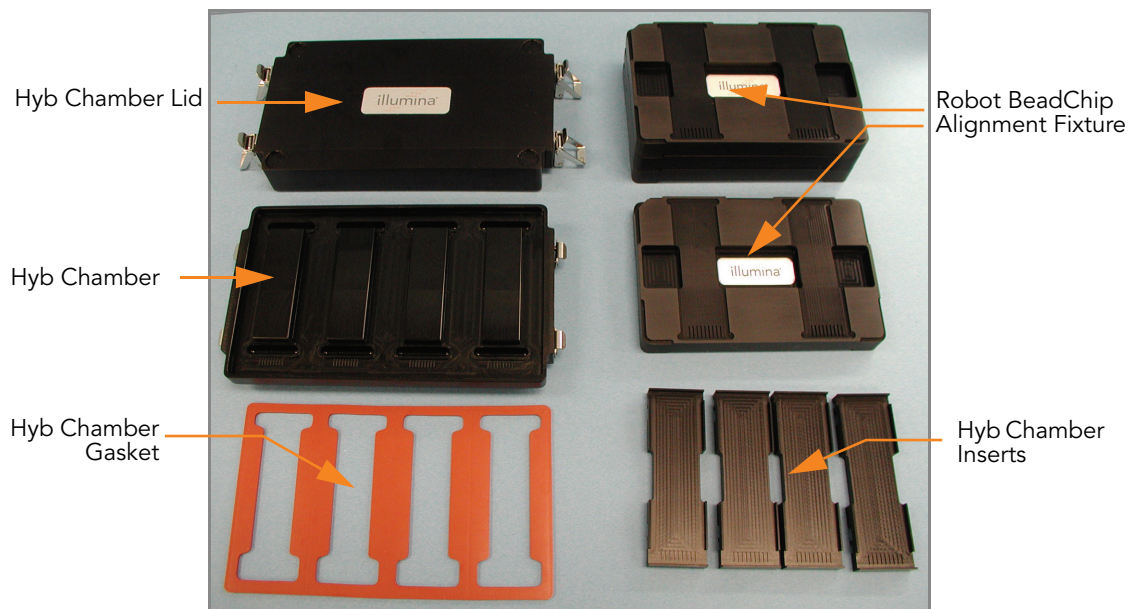


Figure 139 BeadChip Hyb Chamber Components

2. Place the gaskets into the BeadChip Hyb Chambers.
 - a. Match the wider edge of the gasket to the barcode-ridge side of the Hyb Chamber (Figure 140).

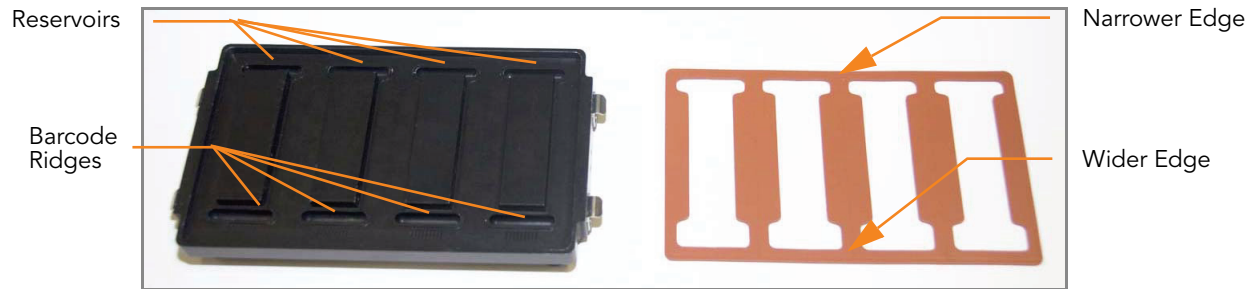


Figure 140 Hyb Chamber and Gasket

- b. Lay the gasket into the Hyb Chamber (Figure 141), and then press it down all around.

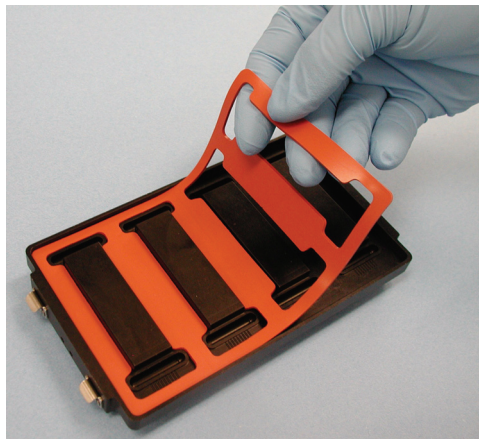


Figure 141 Placing Gasket into Hyb Chamber

- c. Make sure the gaskets are properly seated (Figure 142).



Figure 142 Hyb Chamber with Gasket in Place

3. Dispense 400 μ l PB2 into the eight humidifying buffer reservoirs in the Hyb Chambers (Figure 143).



Figure 143 Dispensing PB2 into Hyb Chamber Reservoirs

4. Close and lock the BeadChip Hyb Chamber lid (Figure 144).
 - a. Seat the lid securely on the bottom plate.
 - b. Snap two clamps shut, diagonally across from each other.
 - c. Snap the other two clamps.

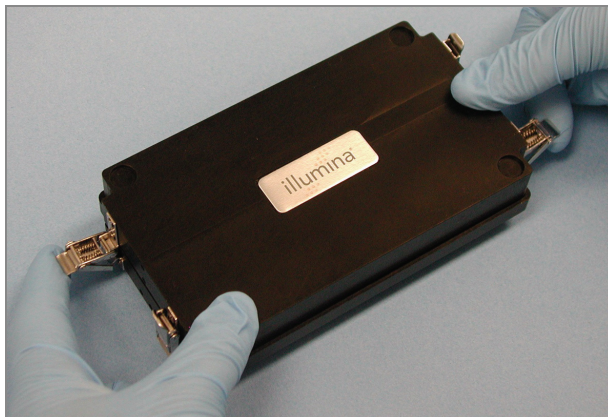


Figure 144 Sealing the Hyb Chamber

5. Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample (Figure 144).
6. Remove the BeadChips from 2°–8°C storage but do not unpackage.

Prepare the Robot

For instructions on preparing the robot for use in a protocol and ensuring that the Chamber Rack is properly installed on the post-amplification robot bed, see the *Infinium Assay Lab Setup and Procedures Guide*.



NOTE

Robot QC procedures should be performed on a regular basis before running samples. See the **Using the Robot** chapter of the *Infinium Assay Lab Setup and Procedures Guide* for Robot QC instructions.

Refer to Figure 145 throughout this protocol. Note that all of the plate barcodes face to the right.

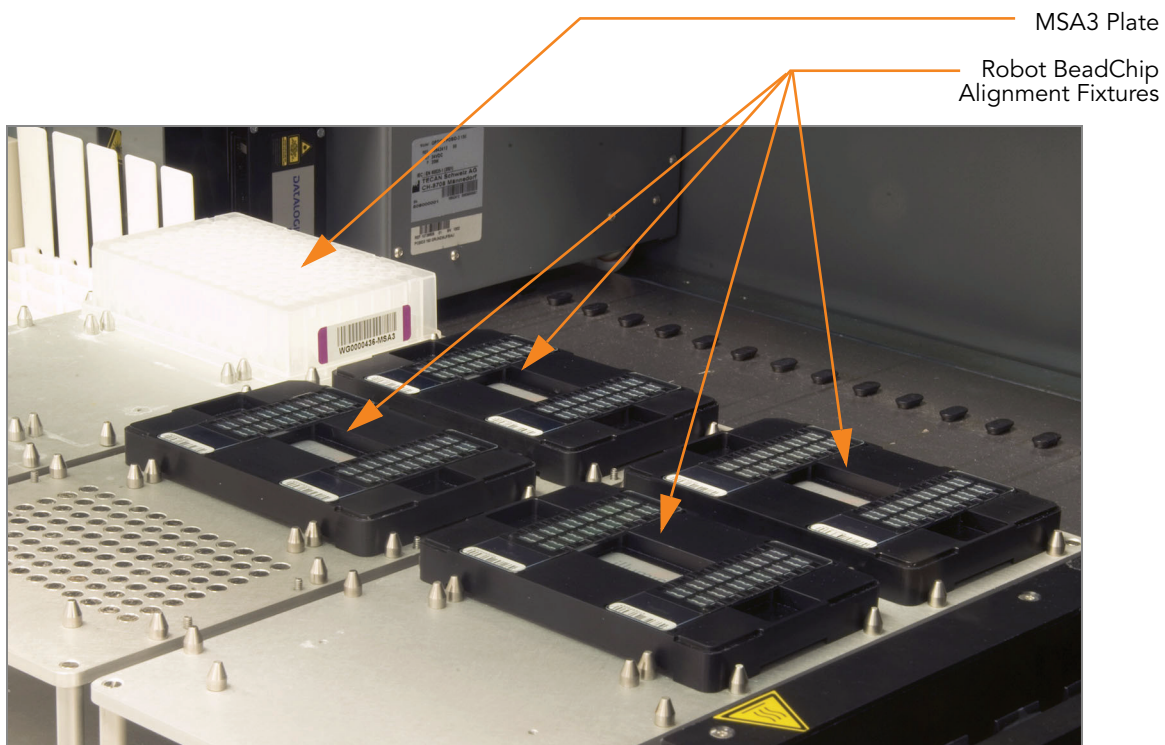


Figure 145 Placing Alignment Fixtures and MSA3 Plate onto Robot Bed

Verify MSA3 and BeadChips for Hyb (LIMS only)

1. In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Confirm BeadChips for Hyb**.
2. Scan the barcode of the MSA3 plate.
3. Scan the barcodes of all the BeadChips you plan to hybridize with the plate. You can scan up to 8 BeadChips.



NOTE

Only scan BeadChips that have been accessioned into the system. The BeadChip type must match the type associated with this batch in Illumina LIMS.

4. Click **Verify**.

The screenshot shows the LIMS interface for the task "Confirm BeadChips/UAMs for Hyb". On the left is a sidebar with a tree view of tasks, including "Quant Tasks", "Infinium Single Sample", "Infinium Multi Sample", "Infinium HC Multi Sample", "Infinium HD Gemini", "Infinium HD Super", "Infinium HD Ultra", "Make MSA3", "Incubate MSA3", "Fragment MSA3", "Precip MSA3", "Spin MSA3", "Resuspend MSA3", "Confirm BeadChips/UAMs for Hyb", "Hyb Multi BC2", "Wash BeadChip", "XStain HD BeadChip", "Coat BC2", "Universal GG SUD PrePCR", "Universal GG MUD PrePCR", "Universal MSI PrePCR", "Universal PostPCR", "Imaging Tasks", and "Laboratory Management". The main area contains a "Plate" input field, a "Get Plate Information" button, and a list of 9 "Items" for verification. Below the items list are "Verify" and "Reset" buttons. The footer shows "© Illumina, Inc. 2009", "Currently logged in as: medwards", and "Powered by Wildtype Linx 3".

Figure 146 (Illumina LIMS) Verifying MSA3 and BeadChips for Hyb

5. If the MSA3 plate and BeadChips are queued for hybridization, a blue confirmation message appears at the top of the window. Proceed to *Load BeadChips* on page 157.
If the MSA3 plate is not queued for hybridization, if any of the BeadChips have not been accessioned into the system, or if any of the BeadChips are the wrong type, a red error message appears at the top of the window. The error message indicates the first incorrect barcode it finds. Do **not** proceed with hybridization.
6. If the plate is not queued for hybridization:
 - a. Click the Reports tab in the upper-right corner.
 - b. In the left sidebar, click **Tracking | Get Queue Status**.
 - c. Scan the plate barcode and click **Go**.
 - d. If the plate is queued for another step, proceed with that step.
7. If one of the BeadChips is not accessioned into the system, accession it and then repeat the verification step.
8. If one of the BeadChips is not the right type for this batch, accession one that is the right type and repeat the verification step.
9. When the verification is successful, proceed to *Load BeadChips* on page 157.

For information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

Steps Load BeadChips

Perform the BeadChip loading near the robot to minimize the distance you need to move the loaded Robot BeadChip Alignment Fixtures.



NOTE

Make sure the Robot Tip Alignment Guide inserts are washed and thoroughly dried before you begin the following steps. See *Wash Robot Tip Alignment Guide* at the end of the *Hybridize Multi BeadChip* section.

1. Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.



CAUTION

Do not unpackage BeadChips until you are ready to begin hybridization.

2. After the 20-minute incubation, remove the MSA3 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.
3. After the 30-minute cool down, pulse centrifuge the MSA3 plate to 280 xg. Remove the foil seal.
4. Remove all BeadChips from their packages.



CAUTION

When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.

5. Place two BeadChips into each Robot BeadChip Alignment Fixture with the barcode end aligned to the ridges on the fixture (Figure 147).

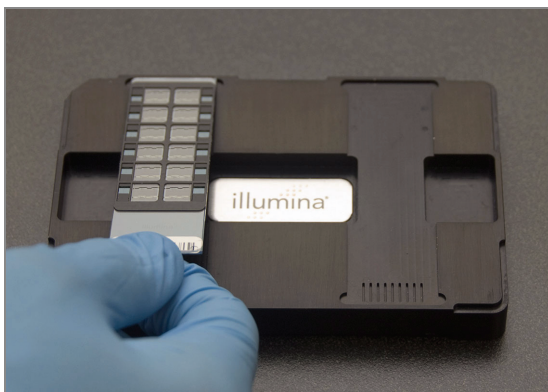


Figure 147 Placing BeadChips in Robot BeadChip Alignment Fixture

6. If you did not load the BeadChips near the robot, stack the Robot BeadChip Alignment Fixtures (Figure 148) and carry them to the robot.



Figure 148 Four Stacked Robot BeadChip Alignment Fixtures

7. At the robot PC, select **MSA3 Tasks | Hyb Multi BC2**.
8. Choose the appropriate BeadChip type from the dialog box.
9. (Non-Illumina LIMS only) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, change the value for the **Number of MSA3 plate(s)** and **Number of DNA samples per plate** to reflect the number of DNA samples being processed.



NOTE

If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of 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.

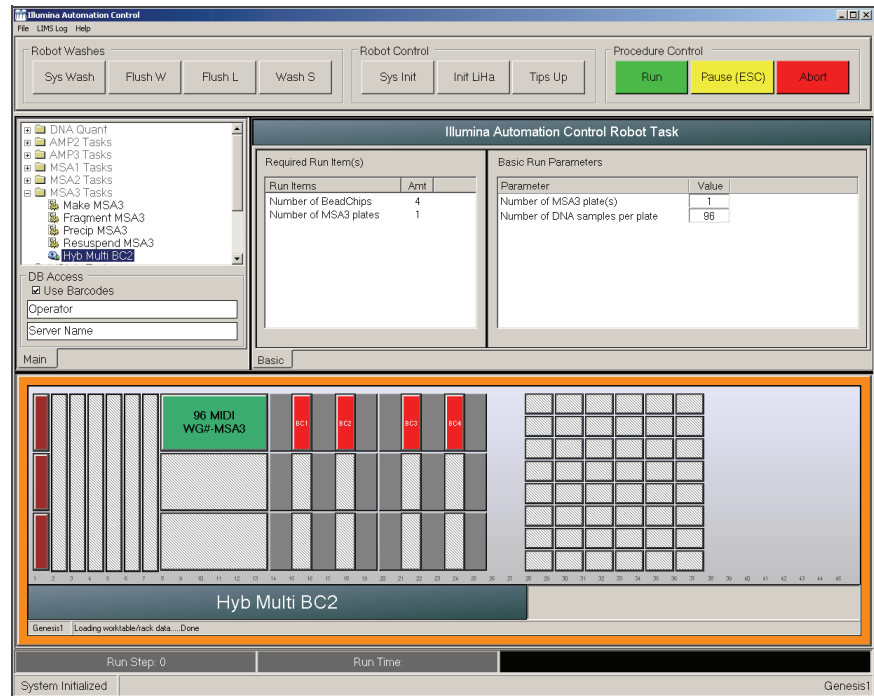


Figure 149 Hyb Multi BC2 Screen

10. Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map (Figure 149).
11. On the lab tracking form, record the plate position on the robot bed.
12. Remove the foil seal.
13. Place the MSA3 plate onto the robot bed according to the bed map.

Start the Robot

1. (Non-Illumina LIMS) At the robot PC, click **Run**.
2. (Illumina LIMS only) Make sure the Use Barcodes check box is selected and click **Run**.
 - a. Log in if prompted.
 - b. Observe the robot start to run to ensure that there are no problems.
3. (Illumina LIMS only) The robot scans the barcodes on the BeadChips to confirm the correct BeadChips are loaded. Once the correct BeadChips are confirmed, the robot pauses.
4. Place the Robot Tip Alignment Guide with the correct inserts loaded on top of the Robot BeadChip Alignment Fixture (Figure 150 or Figure 151 depending on which Tip Guide version you are using). The Guide-B (12x1 HD BeadChip) or Guide-D (24x1 HD BeadChip) barcode should be 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. See *Prepare Robot Tip Alignment Guide(s)* on page 147 for the correct multi-piece Robot Tip Alignment Guide preparation instructions.

- At the robot PC, click **OK** to confirm you have placed the correct Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture.



Figure 150 Full Set of Robot Tip Alignment Guides (Two Piece) on Robot Bed

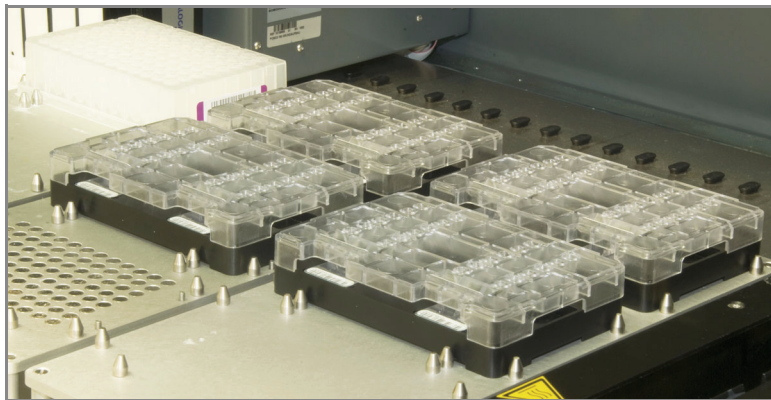


Figure 151 Full Set of Robot Tip Alignment Guides (One Piece) on Robot Bed

- (Illumina LIMS only) The robot scans the barcode on the Robot Tip Alignment Guide to confirm the correct tip guide is being used.

**NOTE**

In the rare occasion the robot scanner fails to read a barcode, use the hand scanner attached to the PC to manually scan the barcode.

The robot PC sounds an alert and displays a message when the process is complete.

- Click **OK** in the message box.
- Carefully remove each Robot Tip Alignment Guide from the robot bed and set it to the side. Next, carefully remove the Robot BeadChip Alignment Fixtures from the robot bed and visually inspect all sections of the BeadChips. Ensure DNA sample covers all of the sections of each bead stripe. Record any sections that are not completely covered.

**NOTE**

For optimal performance, the Robot Tip Alignment Guide should be washed and dried after every run. See *Wash Robot Tip Alignment Guide* at the end of this section for wash instructions.

9. Discard the MSA3 plate if you have not done so.
10. To ensure optimal performance, immediately after the robot has finished delivering samples to all BeadChips, remove each Robot BeadChip Alignment Fixture from the robot bed and visually inspect all sections of the BeadChips. Next, place the Hyb Chamber inserts inside the Hyb Chamber as described in the next section, *Set Up Multi BeadChip for Hyb*.

Set Up Multi BeadChip for Hyb

1. Ensure the Illumina Hybridization Oven is set to 48°C.

**CAUTION**

When holding the BeadChips, avoid contacting the beadstripe area and sample inlets.

2. Remove the Hyb Chamber lid and place the Hyb Chamber inserts inside the Hyb Chamber as shown (Figure 152). Position the barcode over the ridges indicated on the Hyb Chamber.

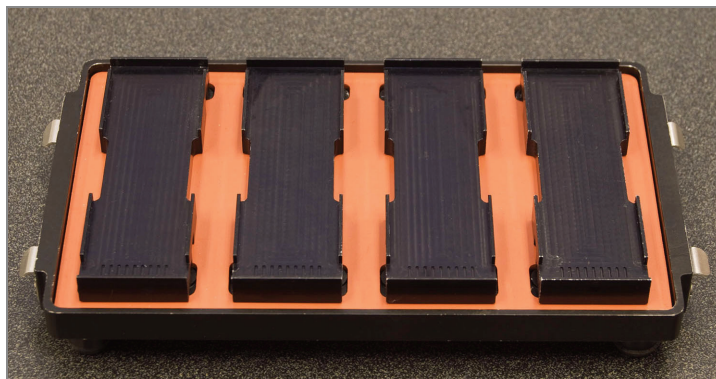


Figure 152 Hyb Chamber Inserts in Hyb Chamber

3. Carefully remove each BeadChip from the Robot BeadChip Alignment Fixtures when the robot finishes. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the insert (Figure 153).

**CAUTION**

For optimal performance, keep the BeadChips steady and level when lifting or moving. Avoid shaking and keep parallel to the lab bench at all times. Avoid contacting the sample inlets when handling the BeadChips.

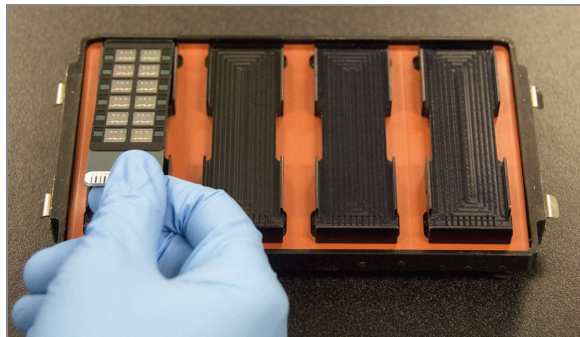


Figure 153 Placing BeadChips in the Hyb Chamber

4. Position the lid onto the Hyb Chamber by applying the back side of the lid first and then slowly bringing down the front end to avoid dislodging the Hyb Chamber inserts (Figure 154).



Figure 154 Seating Lid onto Hyb Chamber

5. Secure the lid by closing down the clamps on both sides of the Hyb Chamber.



NOTE

For optimal performance, keep the Hyb Chamber steady and level when lifting or moving. Avoid shaking the Hyb Chamber, and keep the Hyb Chamber parallel to the lab bench while you transfer it to the Illumina Hybridization Oven.

6. Place the Hyb Chamber in the 48°C Illumina Hybridization Oven so that the clamps of the Hyb Chamber face the left and right sides of the oven. The Illumina logo on top of the Hyb Chamber should be facing you.

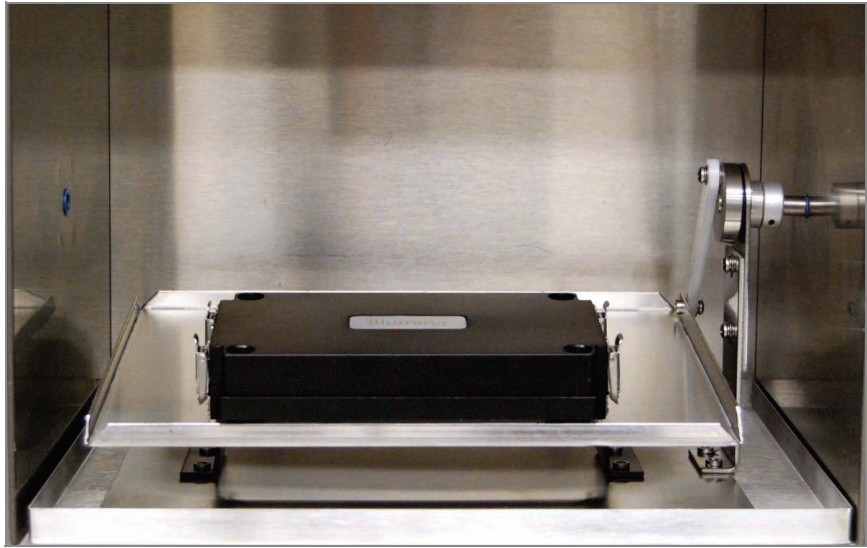


Figure 155 Hyb Chamber Correctly Placed in Hyb Oven



NOTE

If you are stacking multiple Hyb Chambers in the Illumina Hybridization Oven, make sure the feet of the top Hyb Chamber fit into the matching indents on top of the next Hyb Chamber. This will hold the Hyb Chambers in place while they are rocking. You can stack up to 3 Hyb Chambers per row for a maximum total of 6 Hyb Chambers in a Hyb Oven.

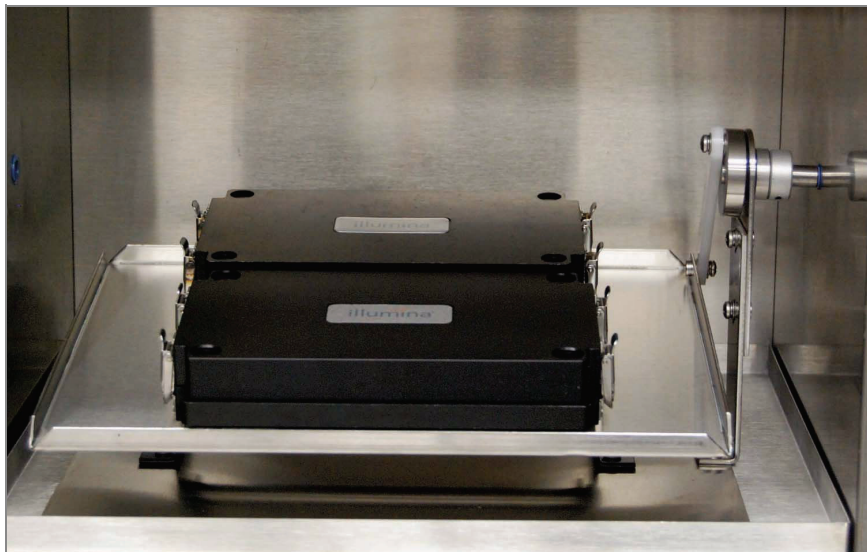


Figure 156 Two Hyb Chambers Correctly Placed in Hyb Oven



Figure 157 Incorrectly Placed Hyb Chamber

7. Incubate at 48°C for at least 16 hours but no more than 24 hours.
8. On the lab tracking form, record the start and stop times.
9. After the overnight hybridization, proceed to *Wash BeadChip* on page 166.

Resuspend XC4 Reagent for XStain HD BeadChip

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

1. Add 330 ml 100% EtOH to the XC4 bottle. The final volume will be 350 ml.
Each XC4 bottle (350 ml) has enough solution to process up to 24 BeadChips.
2. Shake vigorously for 15 seconds.
3. Leave the bottle upright on the lab bench overnight.



NOTE

If the XC4 was not left to resuspend overnight, you can still proceed with the assay. Add the EtOH and put the XC4 on its side on a rocker to resuspend. Leave it there until the BeadChips are ready for coating.

4. Shake again to ensure that the pellet is completely resuspended. If any coating is visible, vortex at 1625 rpm until it is in complete suspension. Once resuspended with 330 ml 100% EtOH, use XC4 at room temperature.

Wash Robot Tip Alignment Guide

For optimal performance, the Robot Tip Alignment Guides should be washed and dried after every run.

1. This first step applies to the multi-piece Robot Tip Alignment Guide only. Hold the tip guide upside down with the barcode side facing down. Remove tip guide inserts from the underside of the tip guide frame by using thumb pressure on the beveled, barcode end and pushing the insert down and out. The metal frame does not need to be washed.

The remaining steps apply to both the multi-piece (once the plastic inserts are removed) and the one-piece Robot Tip Alignment Guides.

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



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

3. After the 5 minute soak in the 1% Alconox solution, thoroughly rinse with DiH_2O at least three times to remove any residual detergent. Make sure the DiH_2O runs through all the tip guide channels.
4. Dry the inserts or one-piece Robot Tip Alignment Guide, especially the tip guide channels, 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 guide inserts and frames should be completely dry and free of any residual contaminants before next use.

Wash BeadChip

In this process, the BeadChips are prepared for the XStain HD BeadChip process. First, remove the IntelliHyb seals from the BeadChips and wash the BeadChips in two separate PB1 reagent washes. BeadChips are then assembled into Flow-Through Chambers under the PB1 buffer.

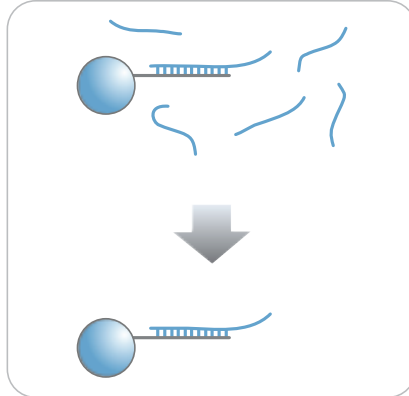


Figure 158 Washing BeadChip

Estimated Time

- ~30 minutes for 8 BeadChips

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml (up to 8 BeadChips)	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1 (per 4 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

Only pour out the recommended reagent volume needed for the suggested number of BeadChips listed in the Consumables table of each section. Some reagents are used later in the protocol.



This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the MSDS for this kit, which is available at <http://www.illumina.com/msds>.



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

Preparation

- ▶ Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 25 minutes prior to opening.
- ▶ Have ready on the lab bench:
 - Two wash dishes each containing 200 ml PB1, and labeled as such
 - Multi-Sample BeadChip Alignment Fixture
 - Using a graduated cylinder, fill with 150 ml PB1
 - Te-Flow Flow-Through Chamber components:
 - Black frames
 - Spacers (separated for ease of handling)
 - Clean glass back plates
 - Clamps
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - Robot
 - PB1 bottle barcode



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

Verify Reagents and BeadChips for Washing (LIMS only)

1. In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Wash BeadChip**.
2. Scan the barcode(s) of the PB1.

3. Scan the BeadChip barcodes.
4. Click **Verify**.

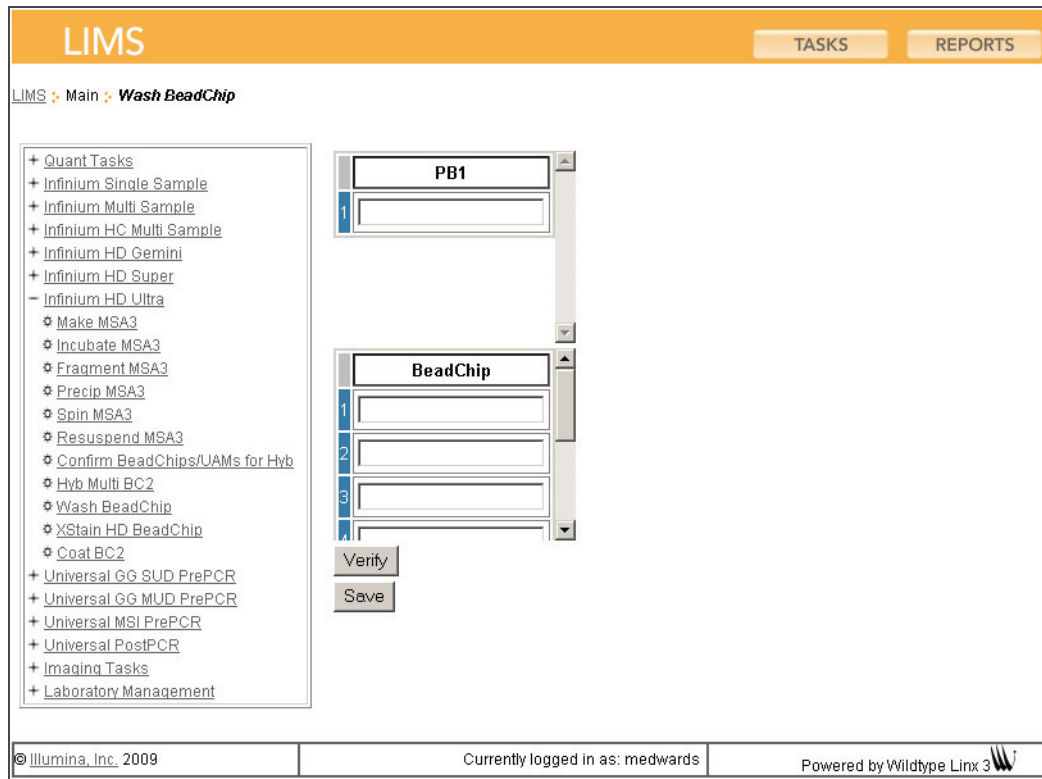


Figure 159 (Illumina LIMS) Verify Reagents and BeadChips for Washing

5. If the reagents are correct and the BeadChips are queued for washing, a blue confirmation message appears at the top of the window. Proceed to *Wash BeadChips*.
6. If any of the reagents are invalid, check the reagent type before re-scanning. The reagent name (e.g., PB1) appears at the end of the barcode. Make sure to scan the correct reagent into each box.
7. If any of the BeadChips are not queued for washing, a red error message appears at the top of the window. The error message indicates the first incorrect barcode it finds. Do **not** proceed with washing. Instead, follow these steps to troubleshoot the problem:
 - a. Click the Reports tab in the upper-right corner.
 - b. In the left sidebar, click **Tracking | Get Queue Status**.
 - c. Scan the BeadChip barcode that appeared in the error message and click **Go**.
 - d. Note what step the BeadChip is queued for, and proceed with that step.

Steps Wash BeadChips

1. Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1 (Figure 160).

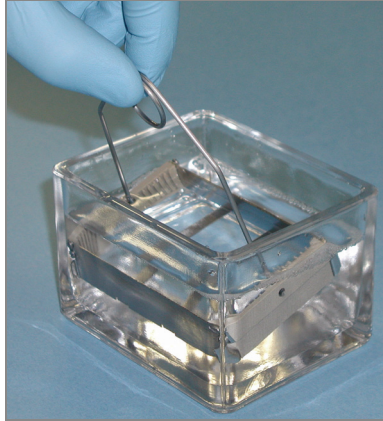


Figure 160 Wash Rack in Wash Dish Containing PB1

2. Remove the Hyb Chamber inserts from the Hyb Chambers.
3. Remove BeadChips from the Hyb Chamber inserts one at a time.
4. Remove the IntelliHyb seal from each BeadChip as follows (Figure 161):
 - a. Using powder-free gloved hands, hold the BeadChip in one hand with your thumb and forefinger on the long edges of the BeadChip. The BeadChip may also be held with the thumb and forefinger on the short edges of the BeadChip. In either case avoid contact with the sample inlets. The barcode should be facing up and be closest to you, and the top side of the BeadChip should be angled slightly away from you.
 - b. Remove the entire seal in a single, slow, consistent motion by pulling it off in a diagonal direction. 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. Do not stop and start the pulling action. Do not touch the exposed active areas.
 - c. Discard the seal.



NOTE

To ensure no solution splatters on you, be sure to pull the seal down and away from yourself. Illumina recommends removing the seal over an absorbent cloth or paper towels, preferably under a hood.

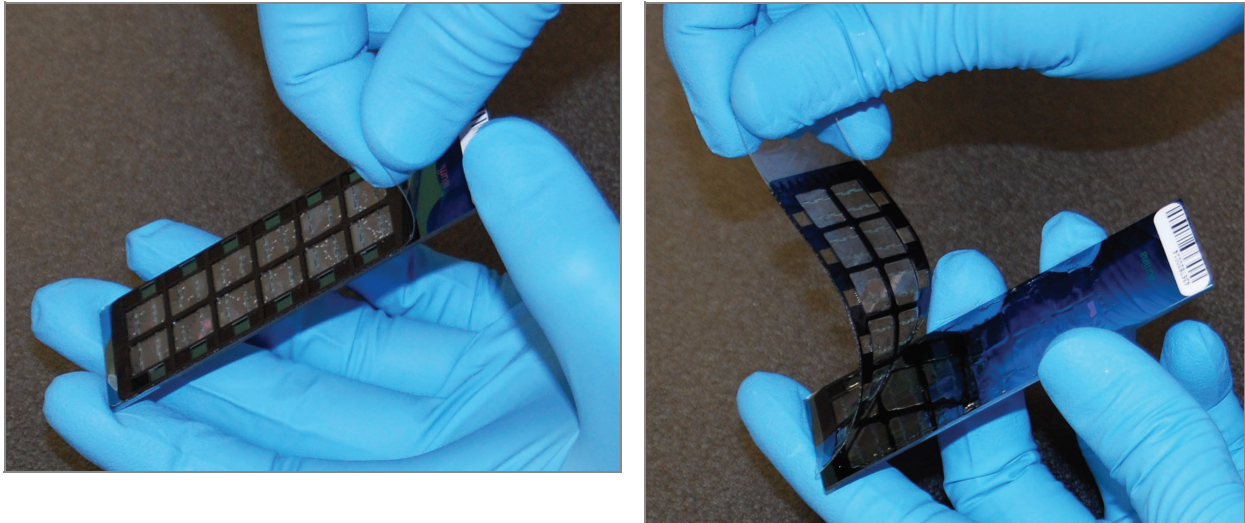


Figure 161 Removing the IntelliHyb Seal



CAUTION

Do not touch the arrays!

5. Immediately and carefully slide each BeadChip into the wash rack one at a time, making sure that the BeadChip is completely submerged in the PB1 (Figure 162).

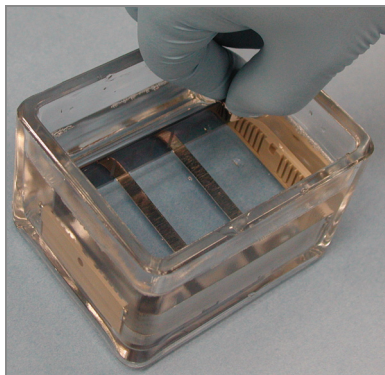


Figure 162 Placing BeadChips in Wash Dish Containing PB1

6. Repeat steps 5 and 6 for each BeadChip to be processed. The wash rack holds up to 8 BeadChips.
7. Once 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 PB1. Make sure the BeadChips are completely submerged.
9. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

Assemble Flow-Through Chambers



NOTE

The 150 ml of PB1 used in the Flow-Through Chamber assembly can be used for up to eight BeadChips. You must use 150 ml of new PB1 for every additional set of eight BeadChips.

1. If you have not done so yet, fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PB1.
2. For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture (Figure 163) pre-filled with PB1 (see *Preparation* on page 167).

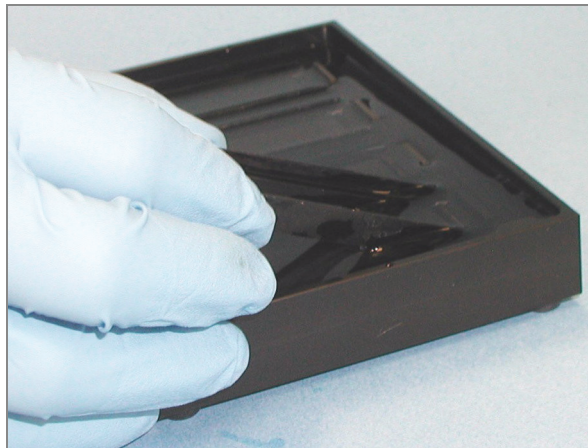


Figure 163 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

3. Place each BeadChip into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture. Each BeadChip should be fully immersed in PB1 (Figure 164).

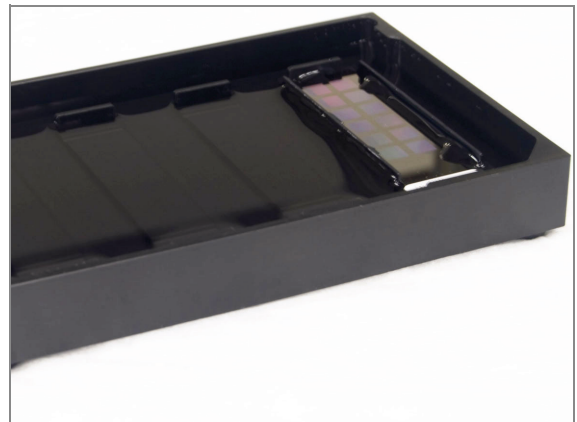
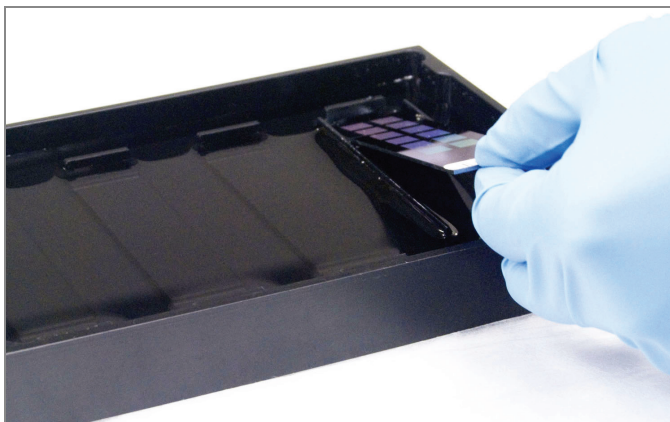


Figure 164 Placing BeadChip into Black Frame on Alignment Fixture

4. Place a clear spacer onto the top of each BeadChip to be processed (Figure 165). Use the Alignment Fixture grooves to guide the spacers into proper position.



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

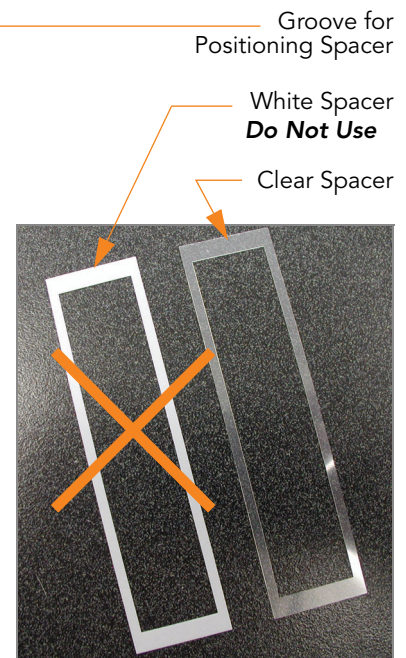
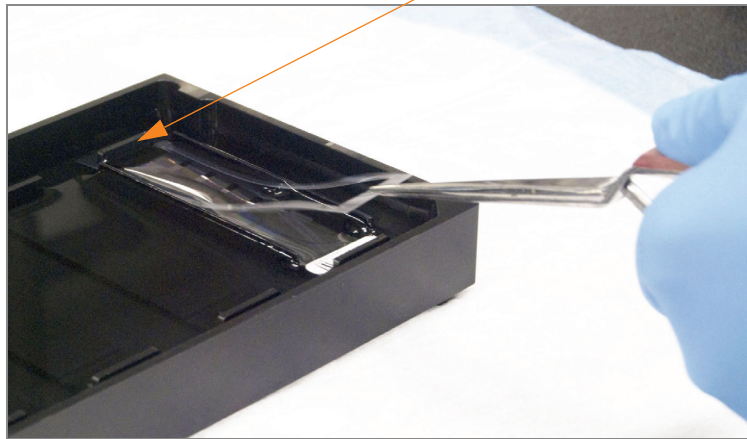


Figure 165 Placing Clear Plastic Spacer onto BeadChip

5. Place the Alignment Bar onto the Alignment Fixture (Figure 166).

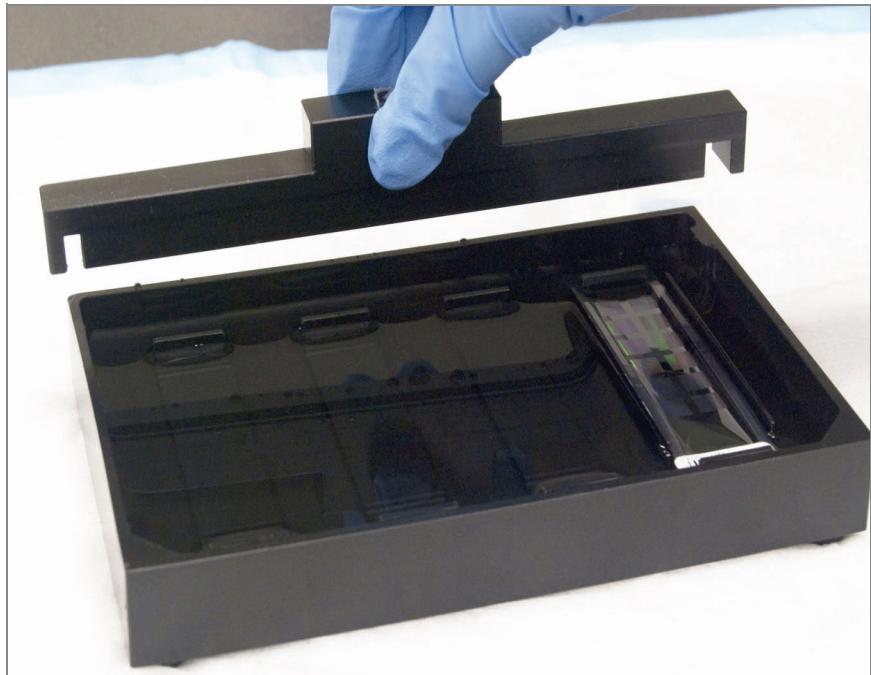


Figure 166 Placing Alignment Bar onto Alignment Fixture

6. Use a Whoosh duster or laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.
7. Place a clean glass back plate on top of the clear spacer covering each BeadChip. The plate reservoir should be at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface (Figure 167).

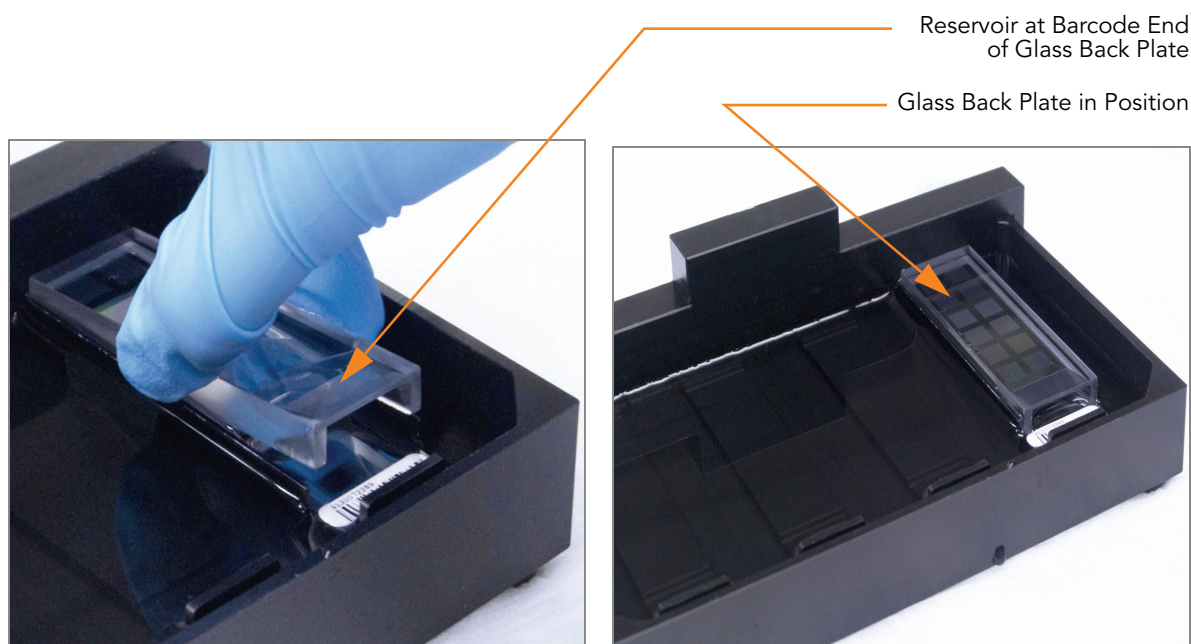


Figure 167 Placing Glass Back Plate onto BeadChip

8. Attach the metal clamps to the Flow-Through Chambers as follows (Figure 168):
 - a. Gently push the glass back plate up against the Alignment Bar with one finger.
 - b. Place the first metal clamp around the Flow-Through Chamber so that the clamp is about 5 mm from the top edge.
 - c. Place the second metal clamp around the Flow-Through Chamber at the barcode end, about 5 mm from the reagent reservoir.

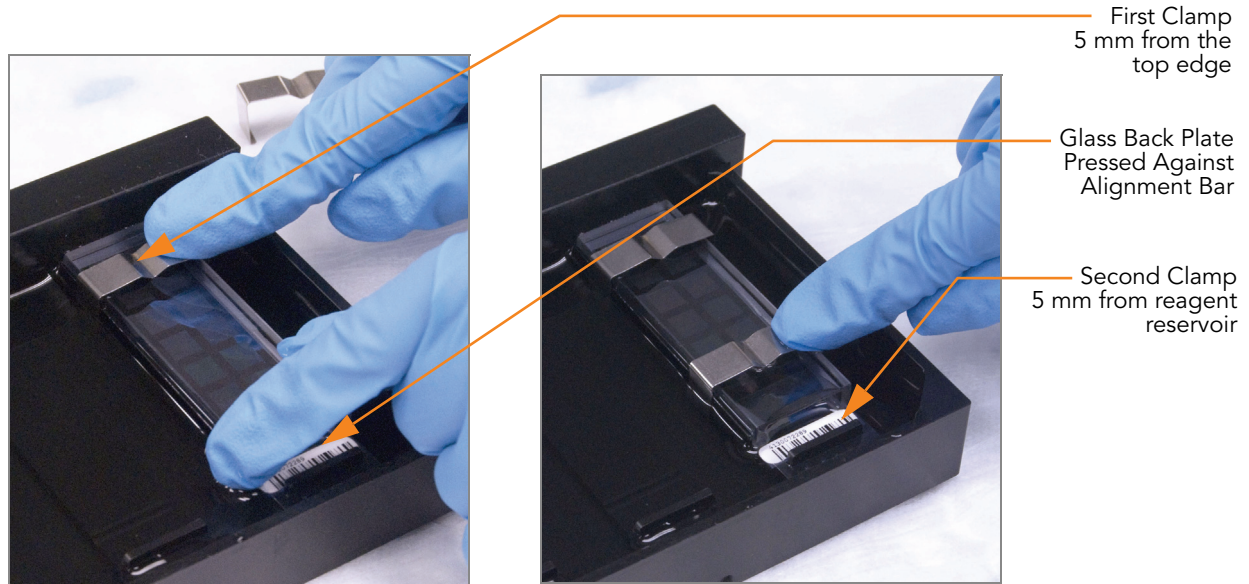


Figure 168 Securing Flow-Through Chamber Assembly with Metal Clamps

9. Using scissors, trim the ends of the clear plastic spacers from the Flow-Through Chamber assembly (Figure 169):
 - a. Trim spacer ends at the non-barcode end of the assembly.
 - b. On the barcode end of the assembly, slip scissors up over the barcode to trim spacer ends.

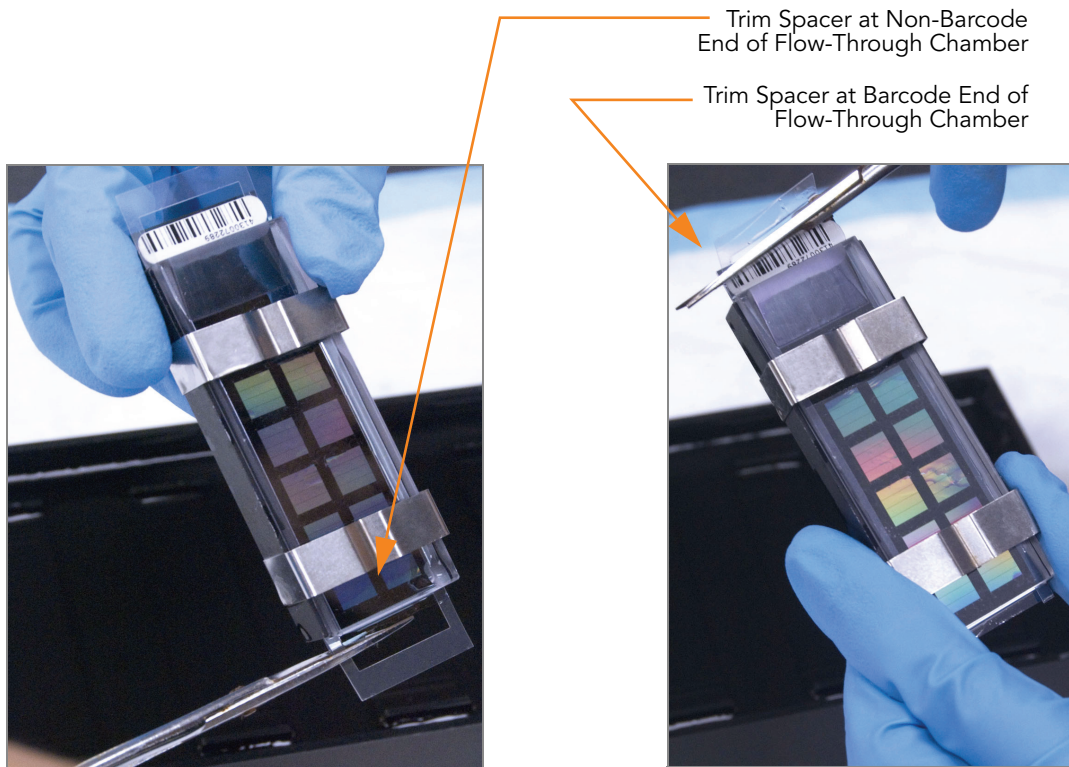


Figure 169 Trimming Spacer Ends from Flow-Through Chamber Assembly

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



CAUTION

It is important to wash the reservoirs immediately and thoroughly to ensure that no traces of PB2 remain in the wells.

11. Discard unused reagents in accordance with facility standards.
12. Proceed to *Single-Base Extension and Stain HD BeadChip* on page 176.



CAUTION

Place all assembled Flow-Through Chambers on the lab bench in a horizontal position while you perform the preparation steps for XStain HD BeadChip. Do not place the Flow-Through Chambers in the Chamber Rack until all necessary steps are completed.

Single-Base Extension and Stain HD 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.

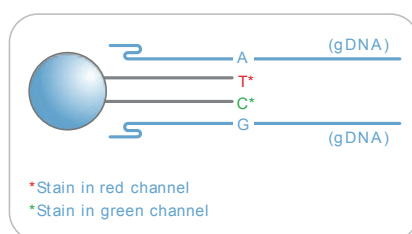


Figure 170 Extending and Staining BeadChip

Estimated Time

Robot time:

- 2 hours and 15 minutes for 4 BeadChips
- 2 hours and 40 minutes for 8 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	-15° to -25°C	Illumina
XC1	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
XC2	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
TEM	1 tube (per 4 BeadChips)	-15° to -25°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)	-15° to -25°C	Illumina
ATM	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina

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

**CAUTION**

Only pour out the recommended reagent volume needed for the suggested number of BeadChips listed in the Consumables table of each section. Some reagents are used later in the protocol.

**WARNING**

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the MSDS for this kit, which is available at <http://www.illumina.com/msds>.

Preparation

- ▶ Place all reagent tubes to be used in the assay in a tube rack. If frozen, thaw to room temperature and centrifuge to 3000xg for 3 minutes.
- ▶ RA1 is shipped frozen. Gradually warm the reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- ▶ On the lab tracking form, record:
 - Date/Time
 - Operator
 - Robot
 - RA1 barcode
 - XC1 barcode(s)
 - XC2 barcode(s)
 - XC3 barcode
 - TEM barcode(s)
 - STM barcode(s)
 - ATM barcode(s)
 - PB1 barcode

- XC4 barcode(s)

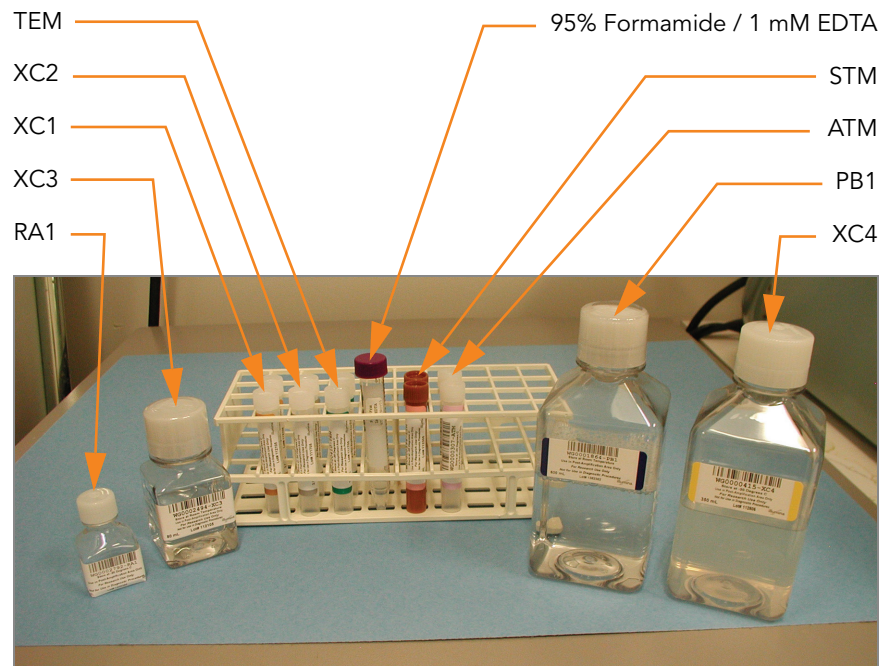


Figure 171 XStain HD BeadChip Reagent Tubes and Bottles



NOTE

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

Set Up the Chamber Rack

1. Ensure the water circulator reservoir is filled with water to the appropriate level. See the *VWR Operator's Manual*.
2. Turn on the water circulator and set it to a temperature that brings the Chamber Rack to 44°C at equilibrium (Figure 172).
This temperature may vary depending on facility ambient conditions.

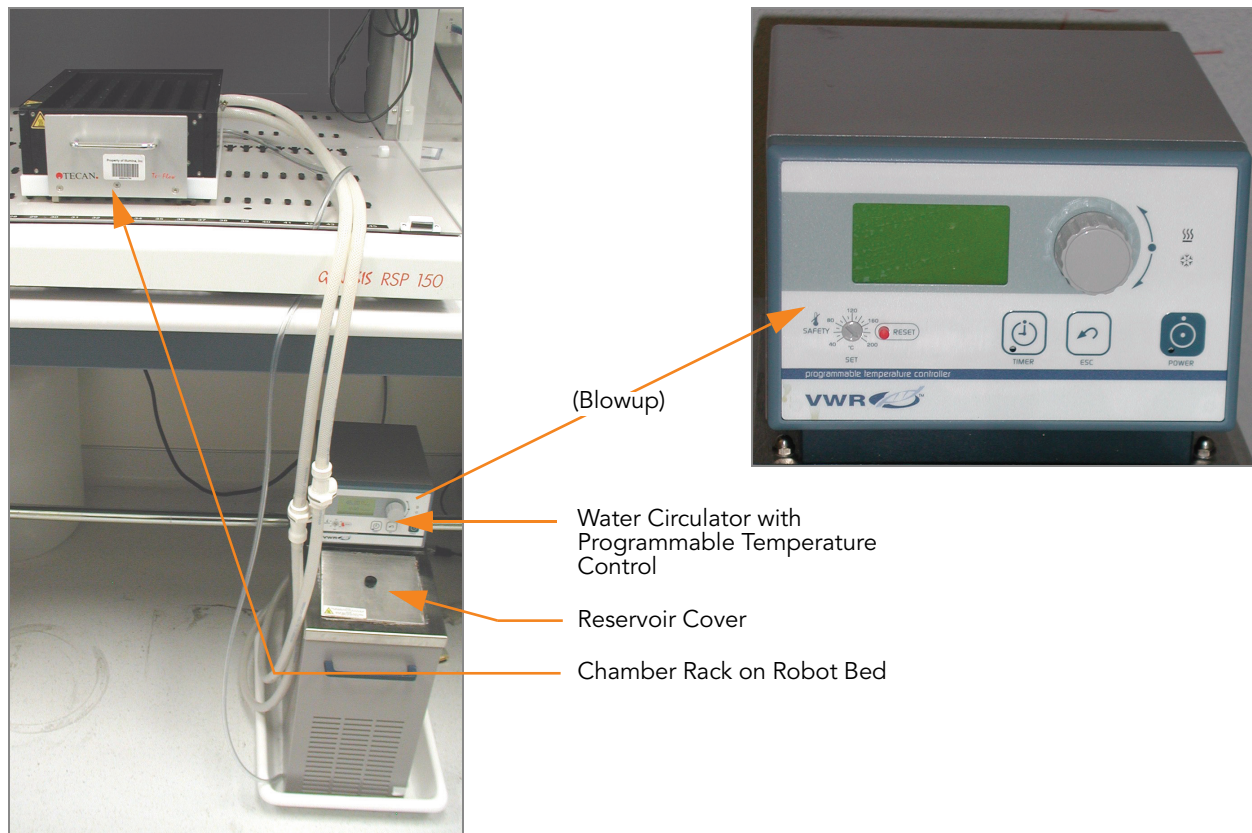


Figure 172 Water Circulator Connected to Chamber Rack

3. You must 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.
4. Use the Illumina Temperature Probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 173).
Do not leave the temperature probe in the first three rows of the Chamber Rack. Reserve this space for BeadChips.

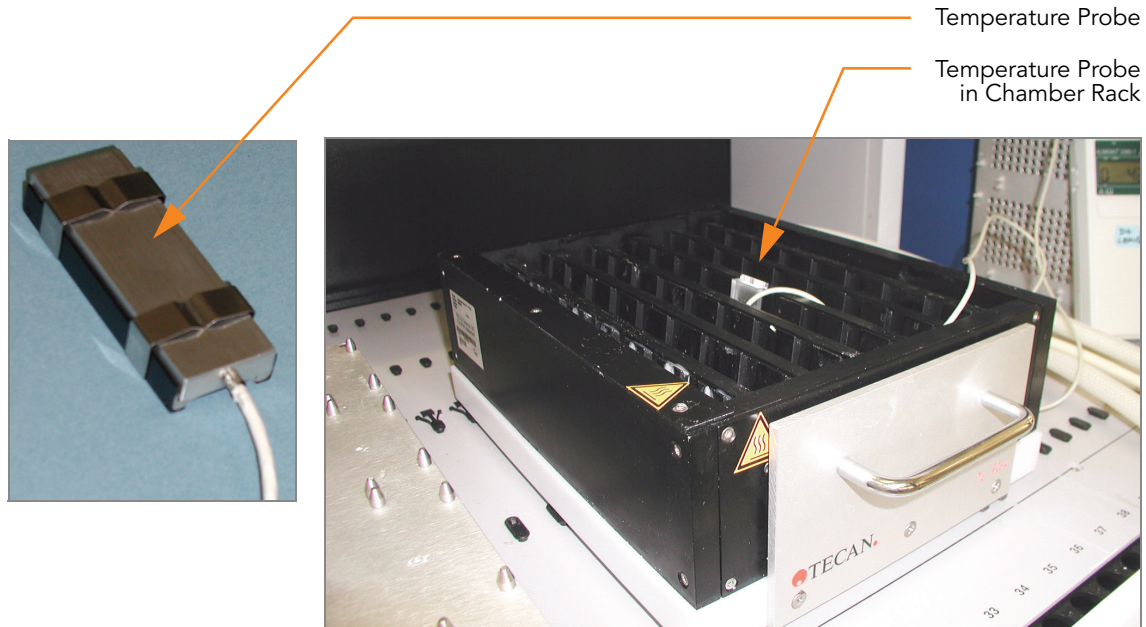


Figure 173 Illumina Temperature Probe in Chamber Rack

5. For accurate temperature measurement, ensure the Temperature Probe is touching the base of the Chamber Rack.
6. Confirm the Chamber Rack is seated in column 28 on the robot bed.
7. Slide the Chamber Rack back to ensure it is firmly seated.

Prepare the Robot

For instructions on preparing the robot for use in a protocol and ensuring that the Chamber Rack is properly installed on the post-amplification robot bed, see the *Infinium Assay Lab Setup and Procedures Guide*.

Refer to Figure 174 throughout this protocol.

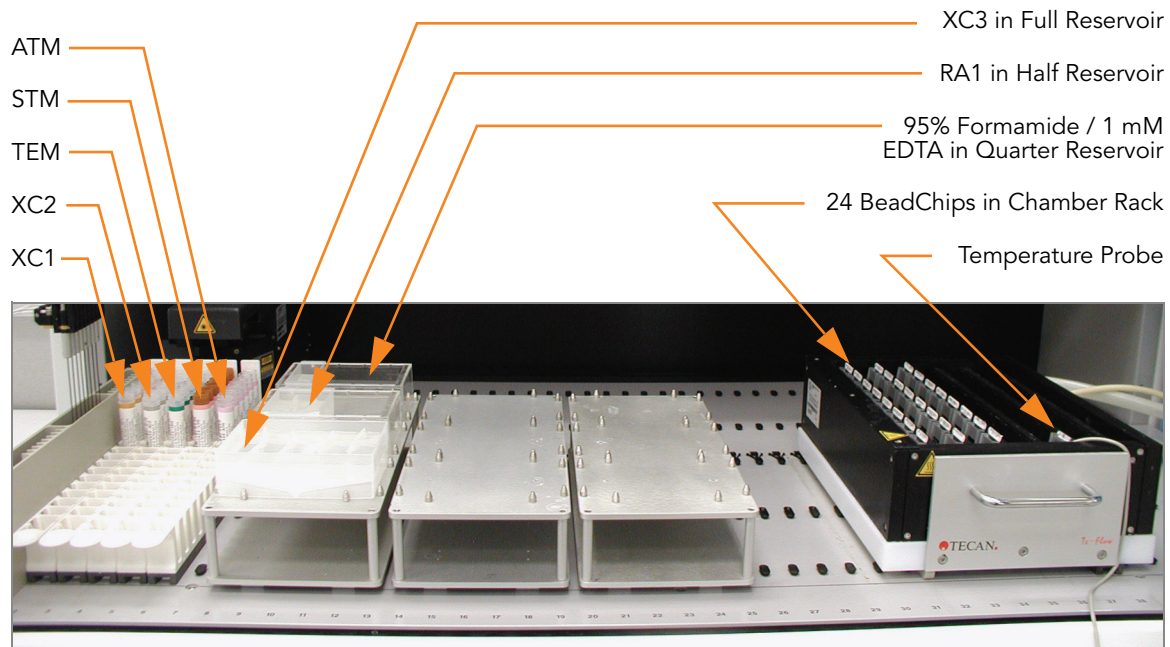


Figure 174 Tecan Eight-Tip Robot (XStain HD BeadChip Setup)

Steps



CAUTION

The remaining steps must be performed without interruption.

1. At the robot PC, select **XStain Tasks | Infinium II Chemistry | XStain HD BeadChip**.
2. In the Basic Run Parameters pane, enter the number of BeadChips.
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.

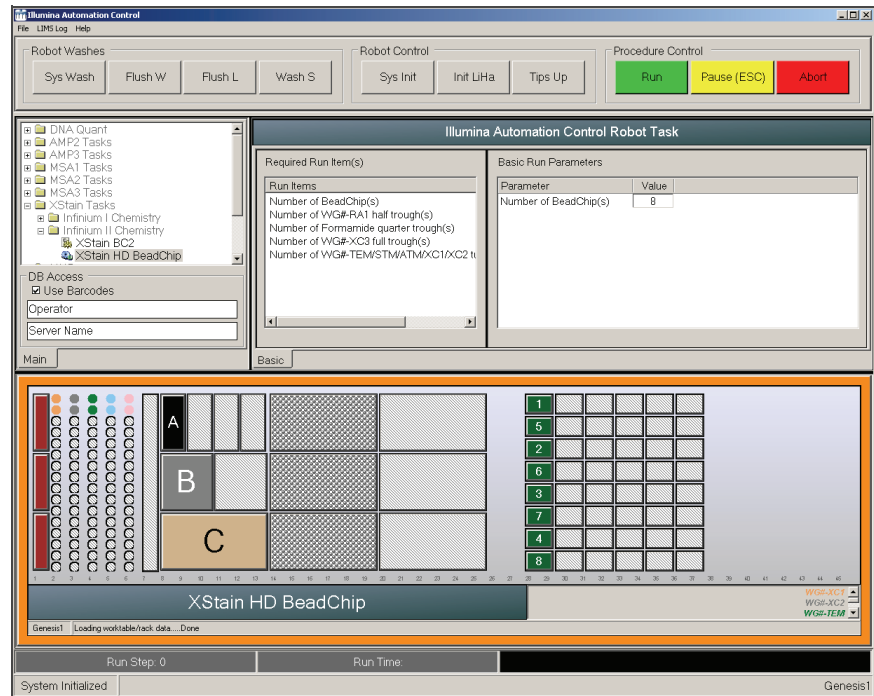


Figure 175 XStain HD BeadChip Screen

Single-Base Extension and Stain

- Place a quarter reservoir in the reservoir frame, according to the robot bed map (Figure 175), and add 95% formamide/1 mM EDTA as follows:
 - 15 ml to process 1–8 BeadChips
 - 17 ml to process 9–16 BeadChips
 - 25 ml to process 17–24 BeadChips
- Place a half reservoir in the reservoir frame, according to the robot bed map, and add RA1 as follows:
 - 10 ml to process 1–8 BeadChips
 - 20 ml to process 9–16 BeadChips
 - 30 ml to process 17–24 BeadChips
- Place a full reservoir in the reservoir frame, according to the robot bed map, and add XC3 as follows:
 - 50 ml to process 1–8 BeadChips
 - 100 ml to process 9–16 BeadChips
 - 150 ml to process 17–24 BeadChips
- Place each reagent tube (XC1, XC2, TEM, STM, ATM) in the robot tube rack according to the bed map, and remove their caps.
 - 1 tube to process 1–4 BeadChips
 - 2 tubes to process 5–8 BeadChips
- Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

6. 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:
 - a. If you are not running Illumina LIMS, clear the **Use Barcodes** check box.
 - b. If you are running Illumina LIMS, leave the check box selected.
 - c. Click **Run** to start the process.
 - d. Log in if prompted.
 - e. (Non-Illumina LIMS only) At the prompt (Figure 176), enter the staining temperature. The correct temperature is listed on the STM reagent label. If no temperature is listed, enter 37°C.

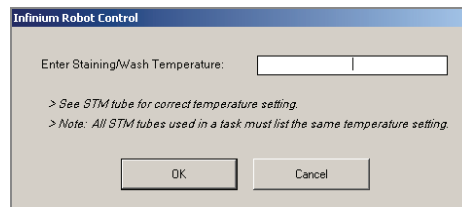


Figure 176 Entering XStain Temperature

- f. Observe the robot start to run to ensure that there are no problems.
2. When the prompt appears (Figure 177), wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.

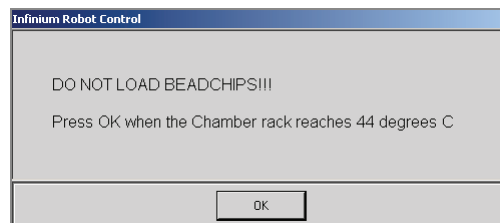


Figure 177 Adjusting Chamber Rack to 44°C Message

3. Once the temperature probe registers 44°C, click **OK** (Figure 177).
4. When prompted (Figure 178), load the BeadChips and click **OK**.

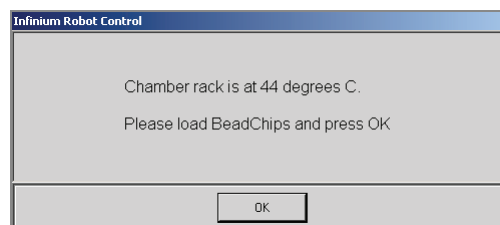


Figure 178 Load BeadChips Message

5. (Illumina LIMS only) At the prompt (Figure 176), enter the staining temperature. The correct temperature is listed on the STM reagent label. If no temperature is listed, enter 37°C.

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. Ensure each Flow-Through Chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.
8. On the lab tracking form record the chamber rack position associated with each BeadChip.
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. The total wait time is 1 hour and 25 minutes.

Table 16 *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.

The robot PC sounds an alert and displays a message when the process is complete.

Click **OK** to finish the process.

Verify Reagents and BeadChips for Coating (LIMS only)

1. In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Coat BC2**.
2. Scan the barcode(s) of the PB1.
3. Scan the barcode(s) of the XC4.
4. Scan the BeadChip barcodes.
5. Click **Verify** and then **Save**.

The screenshot shows the LIMS interface for the 'Coat BC2' task. On the left is a sidebar with a tree view of tasks, including 'Coat BC2'. The main area is titled 'Items in each column will be grouped together for save' and contains three columns of input fields. Each column has a 'PB1' field, an 'XC4' field, and a 'BeadChips' section with eight rows (1-8). At the bottom right are 'Verify' and 'Save' buttons. The footer shows '© Illumina, Inc. 2009', 'Currently logged in as: medwards', and 'Powered by Wildtype Linx 3'.

Figure 179 (Illumina LIMS) Verify Reagents and BeadChips for Coating

6. If the reagents are correct and the BeadChips are queued for coating, a blue confirmation message appears at the top of the window. Proceed to *Wash and Coat 8 BeadChips* on page 186.
7. If any of the reagents are invalid, check the reagent type before re-scanning. The reagent name (e.g., PB1) appears at the end of the barcode. Make sure to scan the correct reagent into each box.
8. If any of the BeadChips are not queued for coating, a red error message appears at the top of the window. The error message indicates the first incorrect barcode it finds. Do **not** proceed with coating. Instead, follow these steps to troubleshoot the problem:
 - a. Click the Reports tab in the upper-right corner.
 - b. In the left sidebar, click **Tracking | Get Queue Status**.
 - c. Scan the BeadChip barcode that appeared in the error message and click **Go**.
 - d. Note what step the BeadChip is queued for, and proceed with that step.

For information about how to use Illumina LIMS, see the *Illumina LIMS User Guide*.

Wash and Coat 8 BeadChips

Follow either the 8 BeadChips Process (*Wash and Coat 8 BeadChips* on page 186) or the 16–24 BeadChips Process (*Wash and Coat 16–24 BeadChips* on page 195). Follow these best practices to optimize the wash and coat process.

- ▶ 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 prior to use.
- ▶ In preparation for XC4 BeadChip coating, wash 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 three layers of Kimwipes on the lab bench. Place a tube rack on top of these Kimwipe layers (do not place on absorbent lab pads). The staining rack containing BeadChips will be placed on this tube rack after you remove it from the XC4 wash dish.
- ▶ Prepare an additional clean tube rack (Illumina-provided from VWR catalog # 60916-748, must fit internal dimensions of vacuum desiccator) for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

1. Place the following items on the bench per 8 BeadChips:
 - 1 staining rack
 - 1 vacuum desiccator
 - 1 tube rack
 - Self-locking tweezers
 - Large Kimwipes
 - Vacuum hose
2. Set up two top-loading wash dishes, labeled “PB1” and “XC4” (Figure 180).
3. 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 and make sure the wash dish is dry. 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.

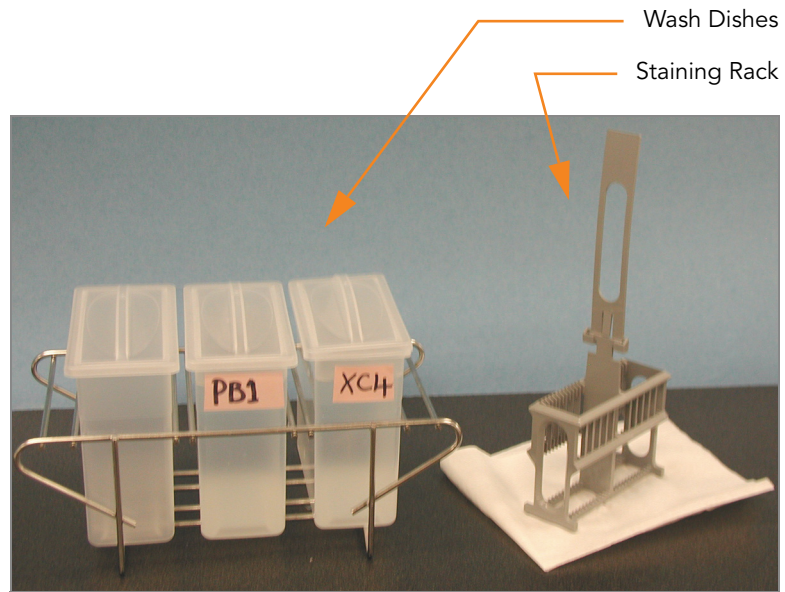


Figure 180 PB1 & XC4 Wash Dishes with BeadChip Rack

4. Dispense 310 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 **towards** you (Figure 181). This orients the staining rack so that you can safely remove the BeadChips.

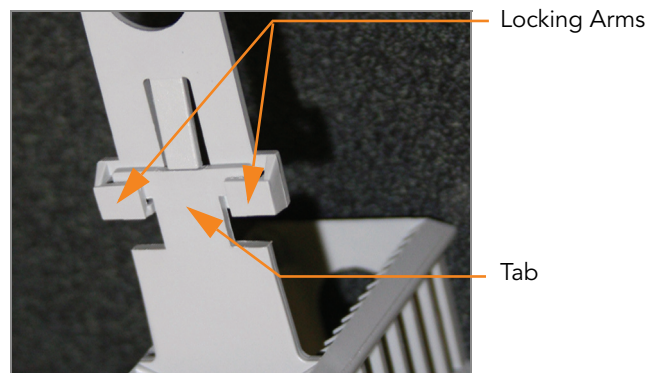


Figure 181 Staining Rack Locking Arms and Tabs



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.

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

6. One at a time, disassemble each Flow-Through Chamber:
 - a. Use the dismantling tool to remove the two metal clamps (Figure 182).

**CAUTION**

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

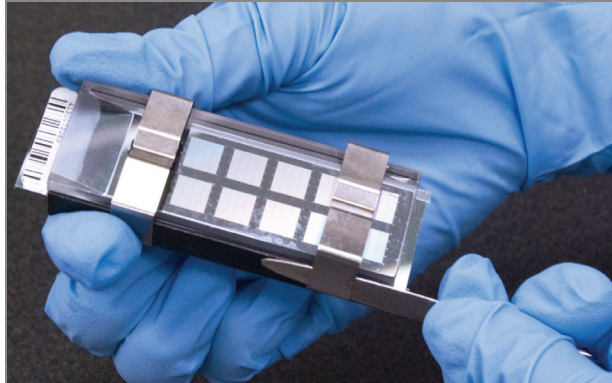


Figure 182 Removing the Metal Clamps from Flow-Through Chamber

- 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. 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.

7. Place BeadChips in the staining rack while it is submerged in PB1. Put four BeadChips above the staining rack handle and four below. The BeadChip barcodes should face **away** from you, while 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 the BeadChip.
8. 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.

9. Move the staining rack up and down 10 times, breaking the surface of the PB1 (Figure 183).

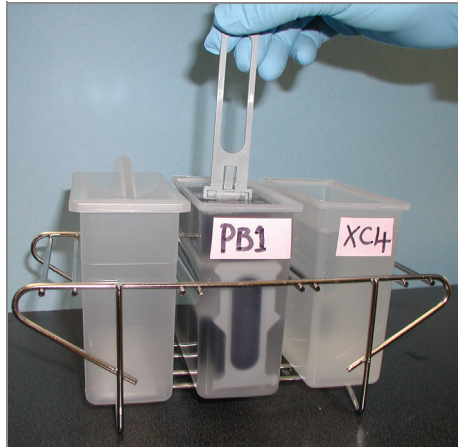


Figure 183 Washing BeadChips in PB1



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.

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



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

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



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

12. Remove the staining rack from the dish containing PB1 and place it directly into the wash dish containing XC4 (Figure 184). 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.

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

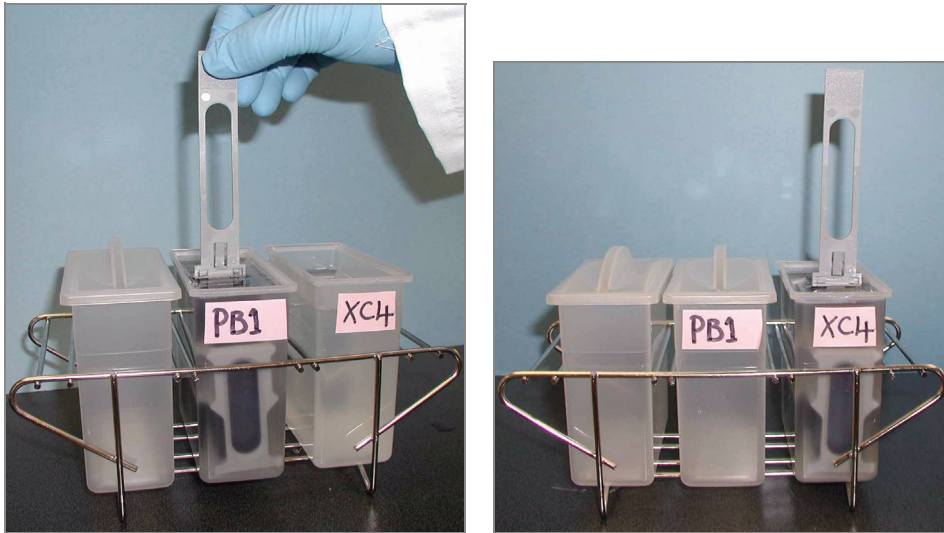


Figure 184 Moving BeadChips from PB1 to XC4



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.

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



CAUTION

Up to 24 BeadChips can be processed per bottle of XC4. To process more than 24 BeadChips, use a new, clean wash dish with fresh XC4.

15. Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.
16. Prepare one additional tube rack per 8 BeadChips that fits the internal dimensions of vacuum desiccator
17. Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes **face upwards** and the locking arms and tab **face down** (Figure 185).

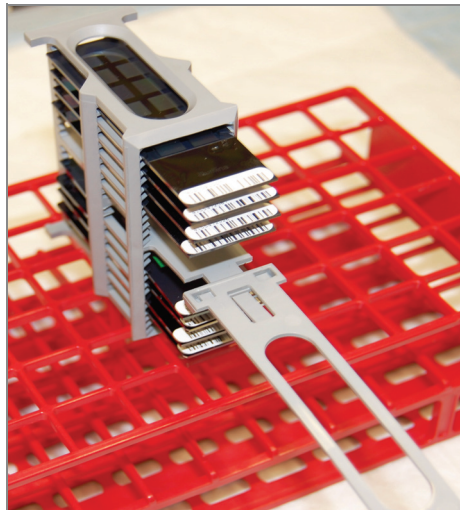


Figure 185 Staining Rack in Correct Orientation

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

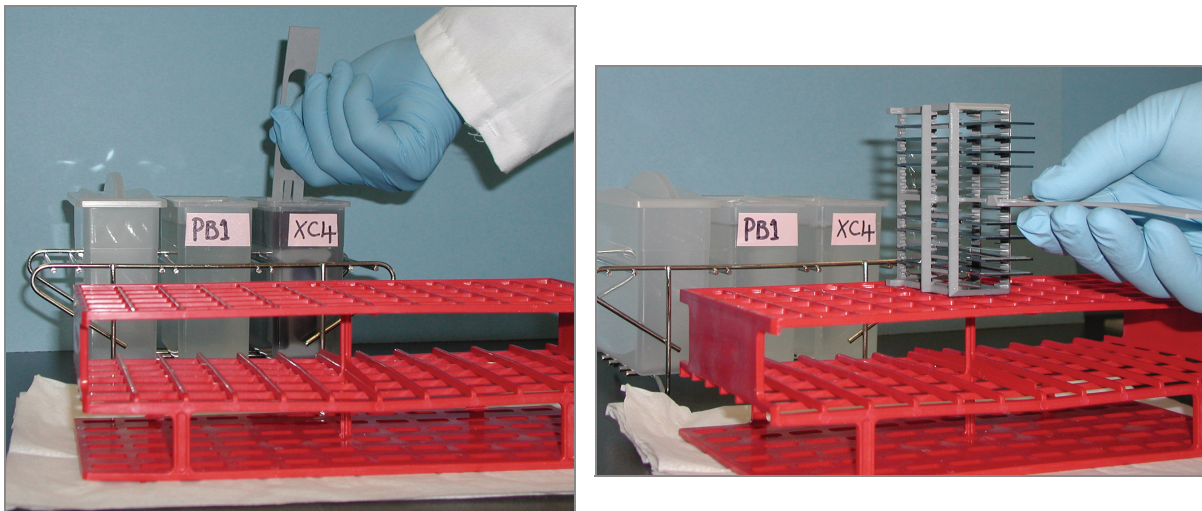


Figure 186 Moving BeadChip Carrier from XC4 to Tube Rack

19. For each of the top four 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.



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 the BeadChip horizontally on a tube rack with the barcode facing up and towards you (Figure 188).
20. 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 (Figure 187).

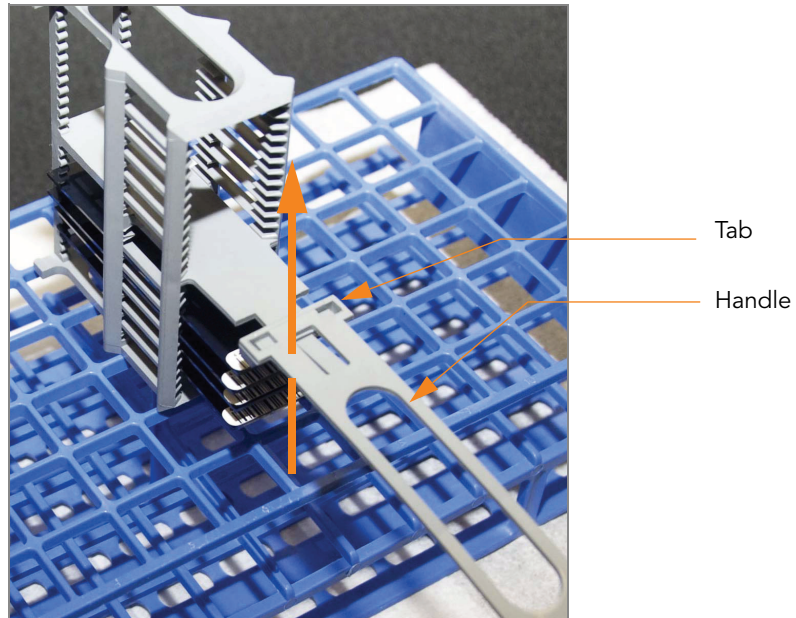


Figure 187 Removing Staining Rack Handle

21. Remove the remaining four BeadChips to the tube rack as described in step 19, so that six BeadChips are on top of the rack and two BeadChips are on the bottom (Figure 188). The barcode ends should be towards you, and the BeadChips should be completely horizontal.



Figure 188 BeadChips on Tube Rack



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.

22. Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack (eight BeadChips).

**CAUTION**

Ensure the vacuum valve is seated tightly and securely.

23. Remove the red plug from the three-way valve before applying vacuum pressure.
24. Start the vacuum, using at least 508 mm Hg (0.68 bar).
25. To ensure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator (Figures 189). It should not lift off the desiccator base.

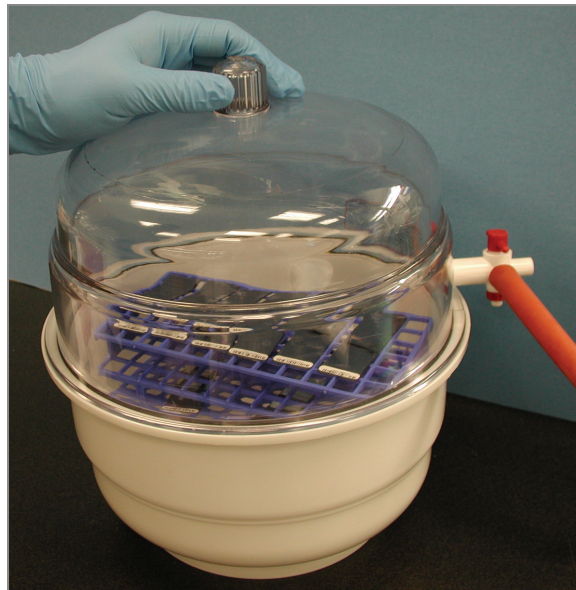


Figure 189 Testing Vacuum Seal

26. Dry under vacuum for 50–55 minutes.
Drying times may vary according to room temperature and humidity.
27. 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.

28. 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.
29. Touch the borders of the chips (**do not touch the stripes**) to ensure that the etched, bar-coded side of the BeadChips are dry to the touch.

30. 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.
- Wrap a pre-saturated Prostat EtOH Wipe around your index finger.
 - Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
 - Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

**CAUTION**

Do not touch the stripes.

31. Clean the glass back plates. For instructions, see the *Infinium Assay Lab Setup and Procedures Guide*.
32. Clean the Hyb Chambers:
- Remove the rubber gaskets from the Hyb Chambers.
 - Rinse all Hyb Chamber components with DI water.
 - Thoroughly rinse the eight humidifying buffer reservoirs.
33. Discard unused reagents in accordance with facility standards.
34. Do one of the following:
- Proceed to *Image BeadChip on the iScan System* on page 204 or *Image BeadChip on the BeadArray Reader* on page 214.
 - Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature. Image the BeadChips within 72 hours.

Wash and Coat 16–24 BeadChips

1. Place the following items on the bench:
 - 1 staining rack
 - 3 vacuum desiccators (1 per 8 BeadChips)
 - 3 tube racks (1 per 8 BeadChips)
 - Self-locking tweezers
 - Large Kimwipes
 - Vacuum hose
2. Set up two top-loading wash dishes, labeled "PB1" and "XC4" (Figure 190).
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.

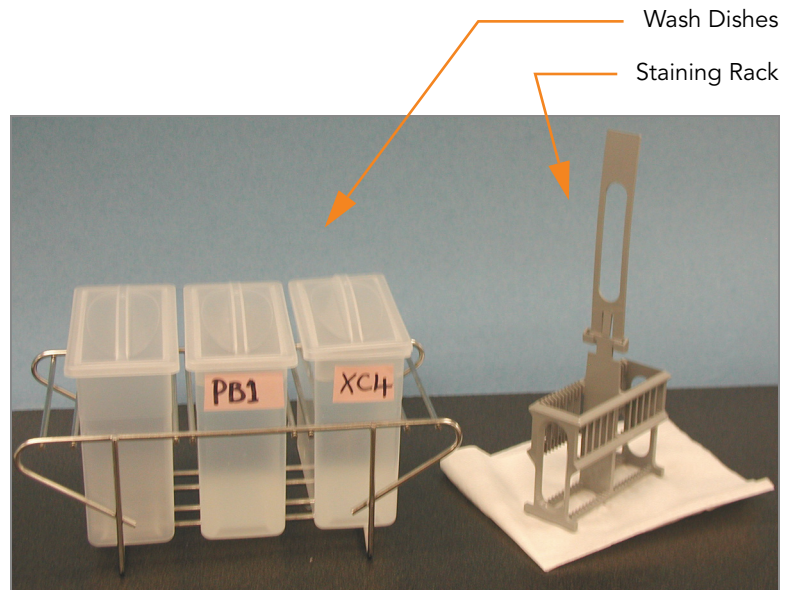


Figure 190 PB1 and XC4 Wash Dishes with Staining Rack

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 **towards** you (Figure 191). This orients the staining rack so that you can safely remove the BeadChips.

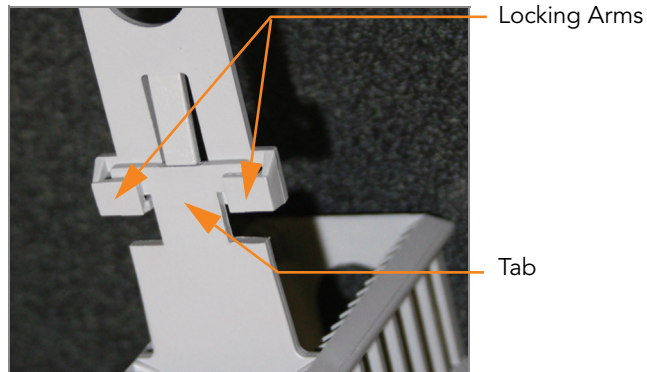


Figure 191 Staining Rack Locking Arms and Tabs



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 (Figure 192).



CAUTION

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

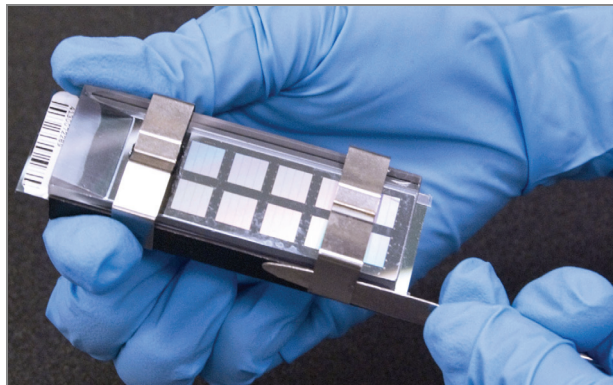


Figure 192 Removing Metal Clamps from Flow-Through Chamber

- 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** from you, 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 (Figure 193).

**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 193 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 (Figure 194). 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.

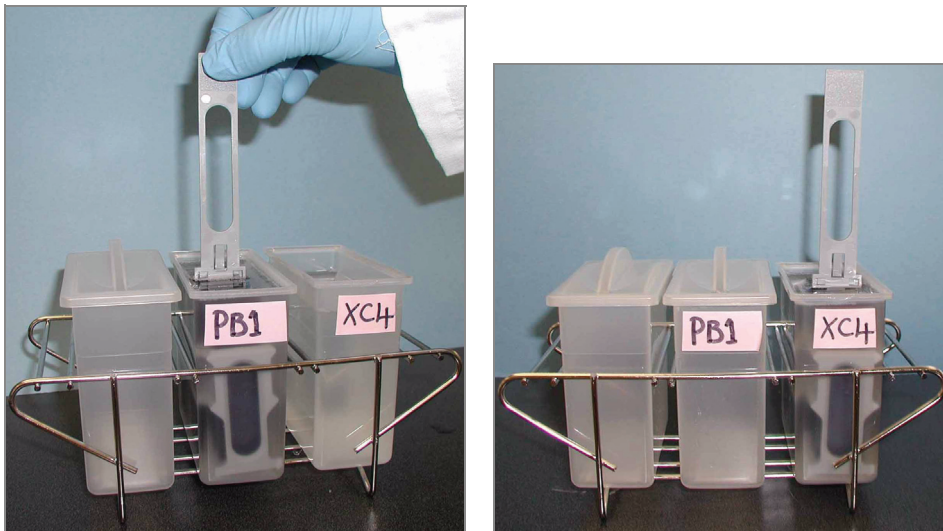


Figure 194 Moving BeadChips from PB1 to XC4



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**

Up to 24 BeadChips can be processed per bottle of XC4. To process more than 24 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 196).

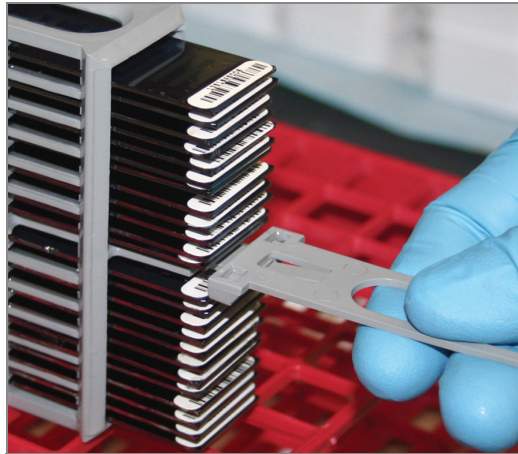


Figure 195 Staining Rack in Correct Orientation

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

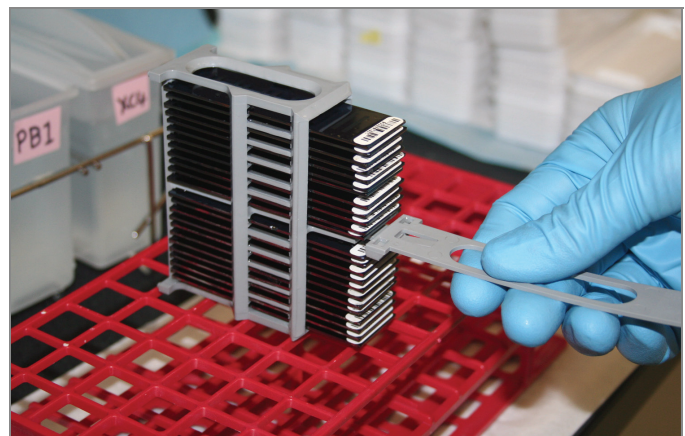
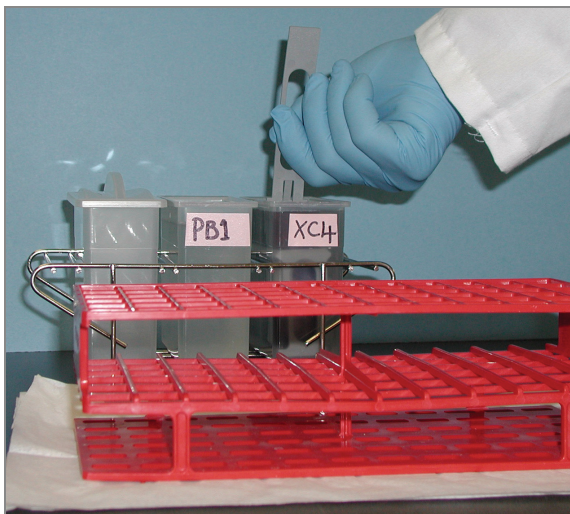


Figure 196 Moving the Staining Rack from XC4 to Tube Rack

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

- Put the eight BeadChips on the tube rack as shown in Figure 197, 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 197 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.

- Return the staining rack to the XC4 wash dish and top off the wash dish until BeadChips are completely covered with remaining XC4 reagent.
- Soak the BeadChips for 10 seconds.
- Dry the **first 8** BeadChips:
 - Place the tube rack with the first 8 BeadChips (Figure 197) into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.
 - Start the vacuum, using at least 508 mm Hg (0.68 bar).
 - To ensure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.
 - 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 (Figure 198).

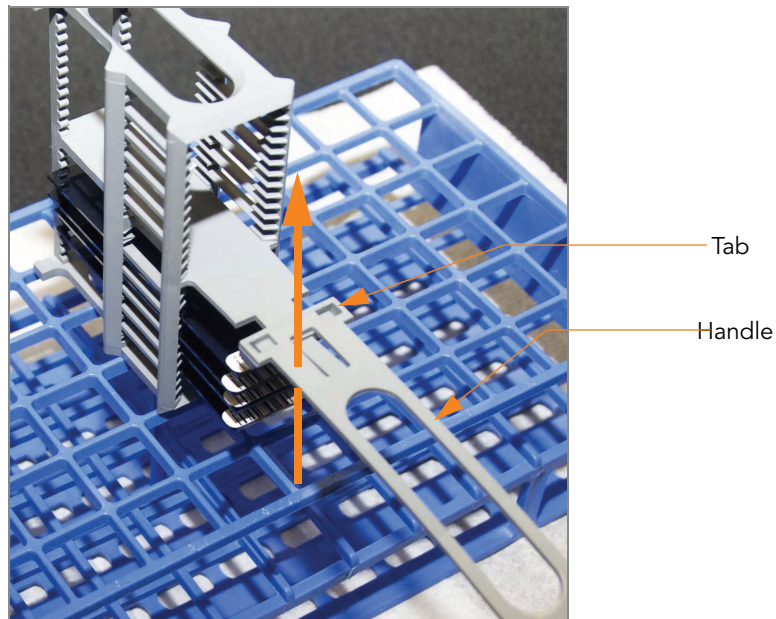


Figure 198 Removing Staining Rack Handle

27. Place BeadChips on the tube rack (Figure 197) 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 (Figure 197) into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.
 - b. Start the vacuum, using at least 508 mm Hg (0.68 bar).
 - c. To ensure that the desiccator 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 197 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.
 - a. Place the tube rack with the **third set of 8** BeadChips (Figure 197) into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.
 - b. Start the vacuum, using at least 508 mm Hg (0.68 bar).
 - c. 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 199 Testing Vacuum Seal

- d. 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, bar-coded 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. Wrap a pre-saturated Prostat
 - b. Wipe around your index finger.
 - c. Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
 - d. Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.



Do not touch 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 on the iScan System* on page 204 or *Image BeadChip on the BeadArray Reader* on page 214.
 - 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 on the iScan System

The iScan Reader uses a laser to excite the fluor of the single-base extension product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

Scanning:

Bead Types	24x1 HD Format			12x1 HD Format	
	3K–20K	20K–40K	40K–60.8K	60.8K–100K	100K–200K
Stripes per Sample Section	1	2	3	2	4
Scan time per BeadChip	7 min	13 min	24 min	20 min	35 min



NOTE

Scan times may be shorter depending on the complexity of the Bead Pool loaded onto the BeadChip.

Preparation

- ▶ On the lab tracking form, record the following for each BeadChip:
 - Scanner ID
 - Scan date



NOTE

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

- ▶ For more information about the iScan System, iScan Control Software, or AutoLoader2, see the *iScan and AutoLoader2 System Guide*.

Steps Overview

The iScan Control Software leads you through the BeadChip scanning process, which is as follows:

1. Turn on the iScan Reader, boot up the iScan PC, and start the iScan Control Software application.
2. Let the iScan Reader warm up for at least 5 minutes before beginning a scan. It is fine to use the iScan Control Software during this time.




Turn on the iScan Reader before launching the iScan Control Software. If the software is launched when the instrument is turned off, an error message will alert you that the hardware is missing.

3. Load the BeadChips to be scanned, and copy their decode data into the Input Path.
4. Check the scan settings and input/output paths, making modifications if necessary.
5. If you wish, remove BeadChip sections or entire BeadChips from the scan.
6. Start the scan and monitor its progress.
7. Review the scan metrics.

Starting Up the iScan System

1. For each BeadChip, download the decode content from iCom or copy the contents of the DVD provided with the BeadChip (if purchased) into the Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).

If there is no decode folder, follow the instructions in *Setting Up Input and Output Paths* on page 211.

2. Double-click the iScan Control Software icon  on the desktop.

The Welcome window appears (Figure 200). The iScan Control Software automatically connects to the iScan Reader and initializes it. When the reader is initialized, the red dot in the status bar turns green, and the status changes to Initialized.

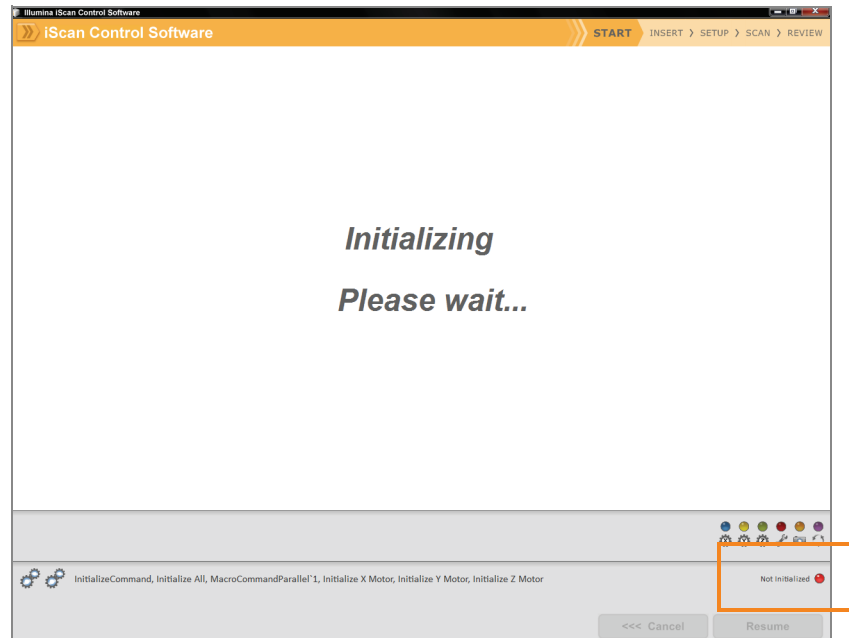


Figure 200 Initialize iScan Reader

3. (Non-Illumina LIMS) Set the **LIMS** dropdown list to **None** and enter your Windows user name (Figure 201).
4. (Illumina LIMS only) Select the **LIMS** server from the dropdown list and enter your LIMS user name and password.

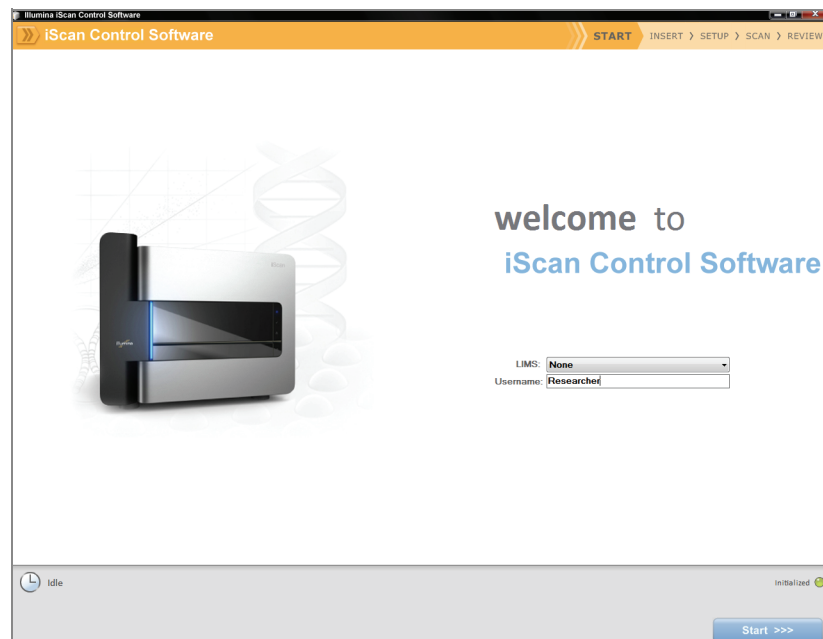


Figure 201 Welcome Window

5. Click **Start**.
The iScan Reader tray opens.

Loading BeadChips and Starting the Scan

1. Load the BeadChips into their carrier and place the carrier into the iScan Reader tray. Click **Next**.

The tray closes and the iScan Reader begins scanning the barcodes (Figure 202).



NOTE

Infinium HD assay screen shots are used as an example for the steps that follow. You may be required to choose different imaging options where appropriate or when prompted.

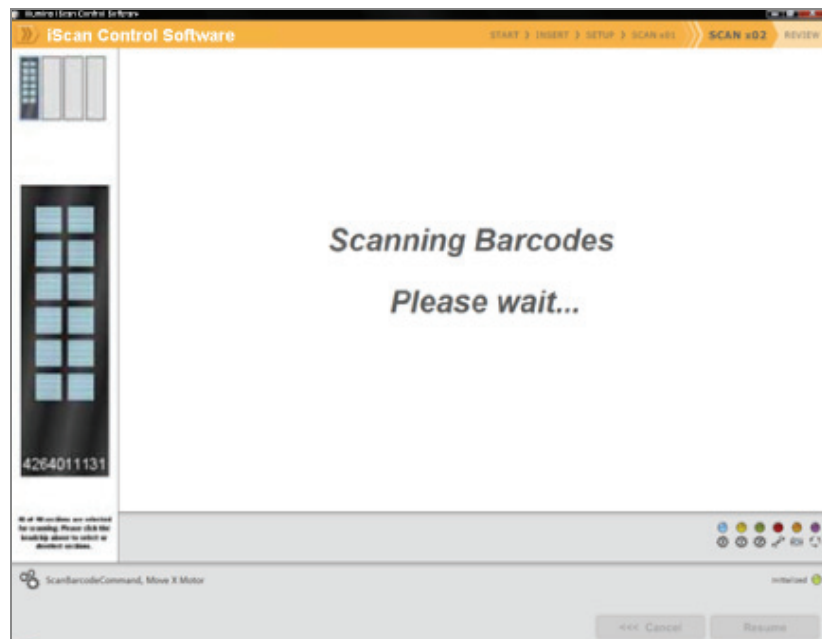


Figure 202 Scan BeadChip Barcodes

When the iScan Reader has read all of the barcodes, the Setup window displays the barcode, description, and scan setting for each BeadChip in the position corresponding to its location in the tray. You can click any barcode to view an image of the corresponding BeadChip.

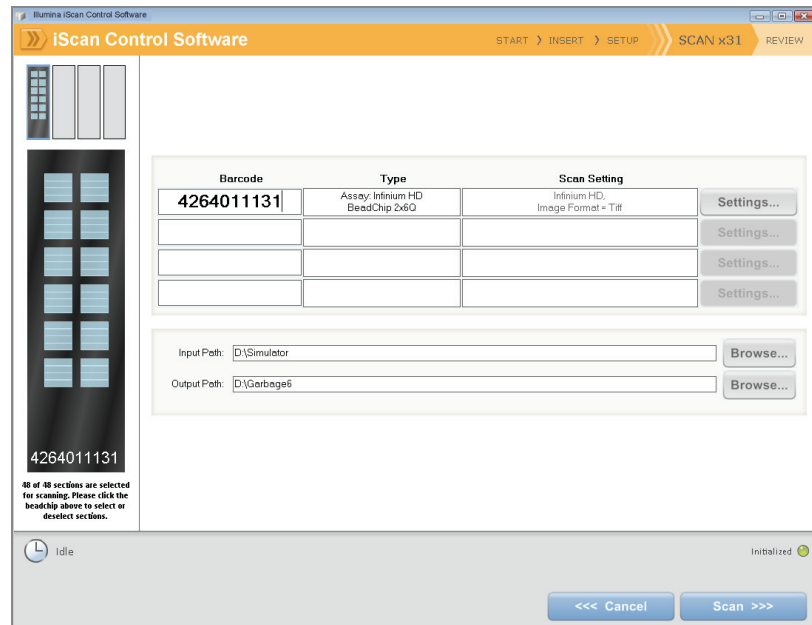


Figure 203 Scanned Infinium BeadChip Barcodes

- If the Scan Setting field beside each BeadChip does not contain "Infinium HD" (12x1 HD BeadChip) or "Infinium HD-HT" (24x1 HD BeadChip), click **Settings**.



NOTE

Ensure that the appropriate scan setting is used. An incorrect scan setting may result in the misregistration of images.

The Scan Settings File window appears.

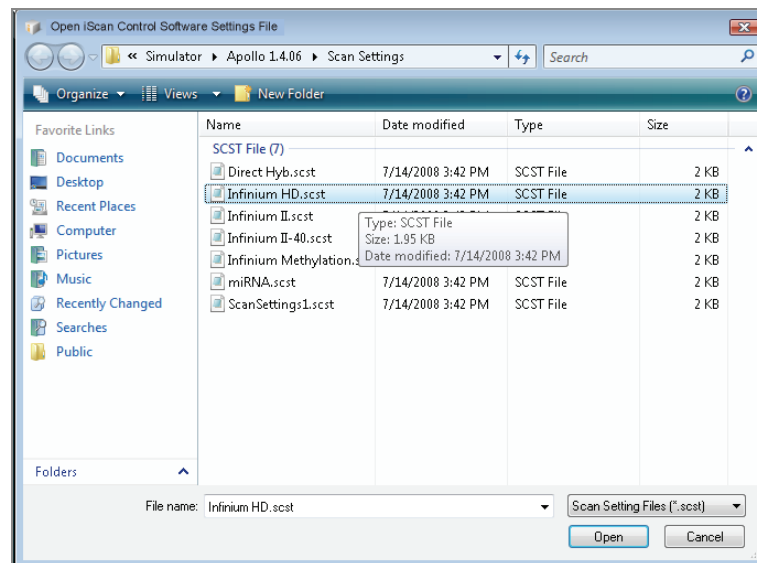


Figure 204 iScan Control Software Scan Settings File Window

3. Select **Infinium HD.scst** for the 12x1 HD or **Infinium HD-HT.scst** for the 24x1 HD BeadChip and click **Open**.
4. If you want to change the image format (*.jpg or *.tif), click the Menu button and select **Tools | Options**.

The Options dialog box appears.

- **JPEG files** let you review the image of the scanned array sections, but you cannot extract bead intensity data.
- **TIFF files** let you review the scanned images and extract bead intensity data. The file size is much larger than *.jpg.

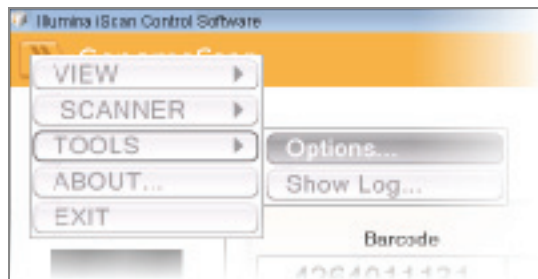


Figure 205 iScan Control Software Tools | Options

5. Click the Scan Settings tab.

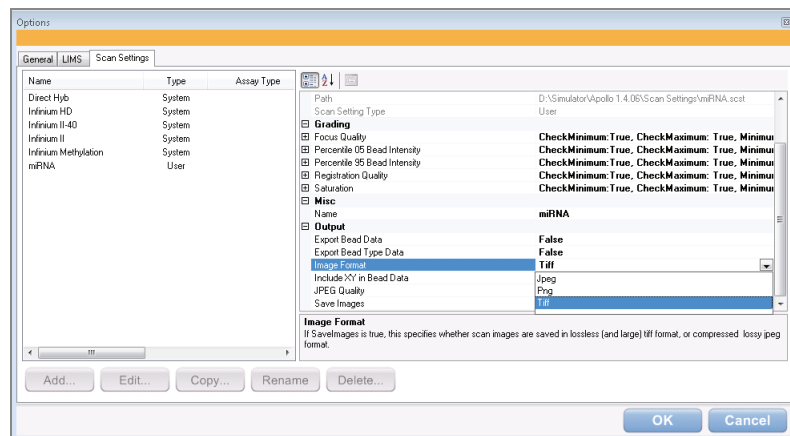
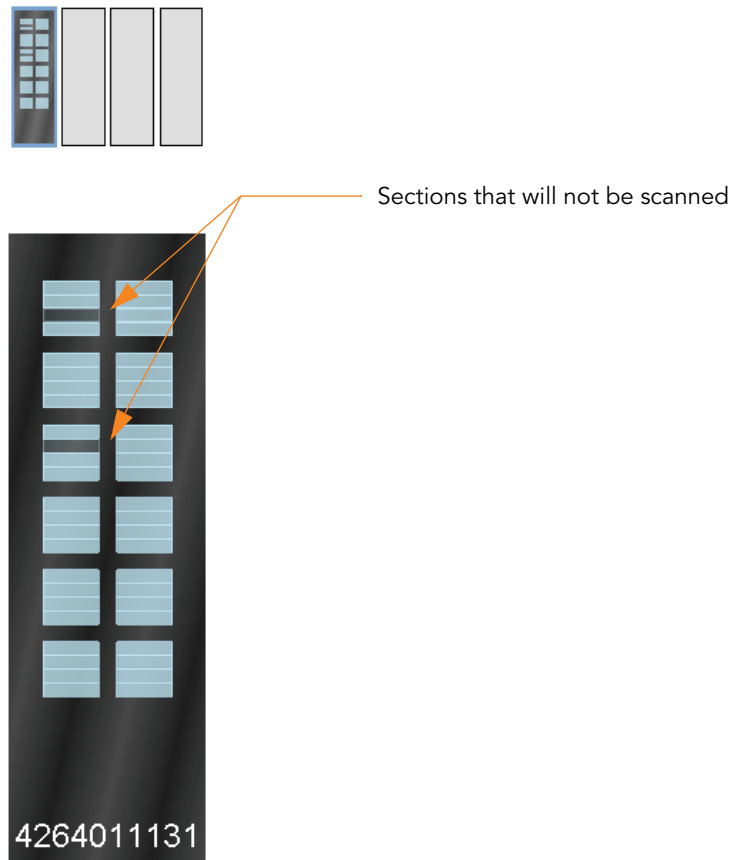


Figure 206 Options Dialog Box, Scan Settings Tab

6. Select **Infinium HD** or **Infinium HD-HT** in the left pane.
The scan settings appear in the right pane.
7. Click the down arrow beside **Image Format**, and select **Tiff**. Click **OK**.
Make sure that the input and output paths are correct.
For more information about the scan settings, see the *iScan and AutoLoader2 System Guide*.
8. (Non-Illumina LIMS only) If you do not want to scan certain sections of a BeadChip, click the barcode to display an image of the corresponding BeadChip in the Setup window. Click any BeadChip section to remove it from the scan (Figure 207). The section will no longer be highlighted blue.



46 of 48 sections are selected for scanning. Please click the beadchip above to select or deselect sections.

Figure 207 Deselect BeadChip Sections

9. If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.
10. To begin scanning the BeadChips, click **Scan**.
As the scan progresses, status icons and messages are displayed in the bottom left corner of window. For more information about what happens during the scan, see *During the Scan* on page 212.
11. At the end of the scan, a Review window appears (Figure 208). The Scan Metrics table at the top shows the intensity values, registration, and focus metrics for each stripe on the BeadChip.

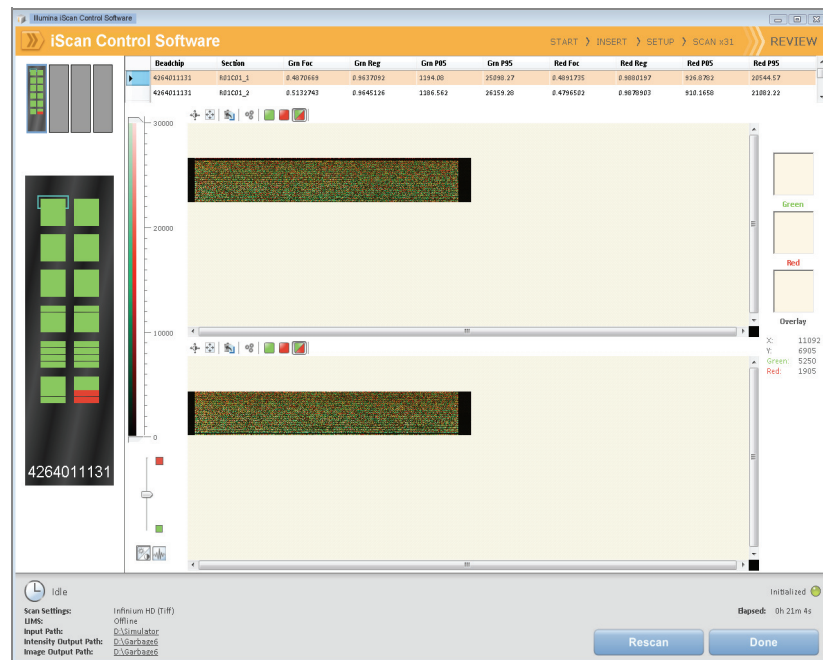


Figure 208 Review Window with Failed Stripes

You can also review scan metrics for any BeadChip in the Output Path folder. Scan metrics are in a document titled Barcode_qc.txt, where “Barcode” represents the barcode number for a single BeadChip.

The focus metric ranges between 0 and 1. High focus scores indicate a sharp, well defined image, leading to high bead intensity values.

12. If any stripes fail to scan successfully they are highlighted in red on the screen. The window contains a Rescan button. Click **Rescan** to automatically rescan all failed areas on the BeadChips in the carrier.
13. When you finish reviewing the data, click **Done** (Non-Illumina LIMS only) or **Submit to LIMS** (Illumina LIMS only) to return to the Start window. If you click **Done** on the Review window without rescanning failed sections, the *.idat files will be saved for those sections but not accessible. The entire sample section will have to be rescanned to generate *.idat files.

When you return to the Start window, images from the scan are no longer available to be viewed in the iScan Control Software. Use another program such as Illumina’s GenomeStudio to view images from the scan.

Setting Up Input and Output Paths

Input and Output paths can only be set in the iScan Control Software when not scanning in the LIMS environment. This step should only occur once, when you install the iScan Control Software on the iScan PC. After that, all scans use these paths.

1. Create a folder on the iScan PC D: drive to contain the decode (*.dmap) and Satrix descriptor (*.sdf) files that were downloaded from iCom or

came on each BeadChip DVD (for example, D:\Decode). The iScan Control Software refers to this folder as the Input Path.

2. Create another folder on the iScan PC D: drive where you want the iScan Control Software to store the image data from the scan (for example, D:\ImageData).

During the scan, the iScan Control Software automatically creates sub-folders named with each BeadChip's barcode number. The folder will be populated with image files (*.jpg or *.tif), scan metrics (*.txt), and intensity data files (*.idat) for each BeadChip. If the images are in *.tif format, the output path will also contain bead location files (*.locs).

During the Scan

Calibration

The iScan System begins with a calibration step, which may take several minutes to complete. The BeadChips are automatically tilted and aligned to ensure that they are in the optimal position for the scan.

- ▶ **Tilt**—The iScan Reader autofocus feature records the Z-position (height) of three corners of the BeadChip to determine its current tilt, and then adjusts the BeadChip until it is flat.
- ▶ **Align**—The iScan Reader identifies the X-Y position (lateral location) of the fiducials (focus points) on the BeadChip edges, and then adjusts the optics.

If there are defective or dirty sections at any of the three alignment corners, the software attempts to use alternate sections until satisfactory calibration is achieved. If no alternate sections are available, calibration fails and an error message is displayed.

Hard Drive Space

Before beginning a scan, the iScan Control Software checks the hard drive to ensure sufficient space is available. If sufficient disk space is not available, an error message is displayed, and the arrays will not be scanned.

Monitoring the Scan

After calibration, the iScan Reader begins scanning. You can view the progress of the scan in the Scan window (Figure 209).

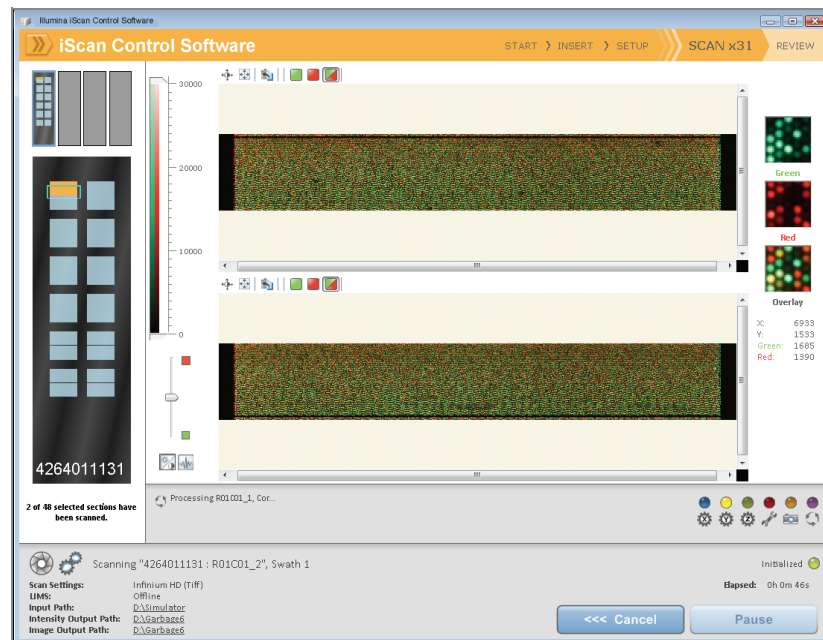


Figure 209 Monitor the Scan

Status and Controls

As each BeadChip section is scanned its status is indicated by a status color:

- ▶ **Light Blue**—Section has not yet been scanned.
- ▶ **Orange**—Section is in the process of being scanned or registered.
- ▶ **Green**—Scan and registration of section was successful.
- ▶ **Red**—Scan and registration of section failed.

While a scan is in progress, you can click the Pause or Cancel buttons to pause or stop the scan at any time. If you pause, the button changes to Resume. Click Resume to start scanning the next unscanned section.

Registration and Intensities

After images are scanned, they are registered and intensities are extracted for every bead type. Registration identifies beads by correlating their locations on the scanned image with information in the bead map (*.dmap) file. Registration and extraction are critical to obtaining results from your experiments.

Intensity extraction is the process by which intensity values are determined for every bead on the image. Statistics are generated for every bead type based on the intensities of the replicate beads for that type. Extracted information is saved in intensity data (*.idat) files. These files are saved on the iScan Reader hard drive or network under the Array ID (barcode identifier), in the Output Path folder. Intensity data (*.idat) files are only created for sections that have 100% of their stripes scanned successfully. These files are not created when scanning individual stripes within a sample section on a BeadChip.

Image BeadChip on the BeadArray Reader

The Illumina BeadArray Reader uses a laser to excite the fluor of the hybridized single-stranded product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

1–2 hours warm-up for the BeadArray Reader (first use of the day only)

Scanning:

	24x1 HD Format			12x1 HD Format	
Bead Types	3K–20K	20K–40K	40K–60.8K	60.8K–100K	100K–200K
Stripes per Sample Section	1	2	3	2	4
Scan time per BeadChip	58 min	115 min	171 min	96 min	180 min



NOTE

Scan times may be shorter depending on the complexity of the Bead Pool loaded onto the BeadChip.

Preparation

- ▶ If this is the first time the BeadArray Reader is being used today, follow the steps described in this section.
- ▶ On the lab tracking form, record the following for each BeadChip:
 - Scanner ID
 - Scan date



NOTE

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

Initializing the BeadArray Reader (Daily)

If this is the first time the scanner is being used today, follow these steps. Refer to Figure 210 throughout this protocol.

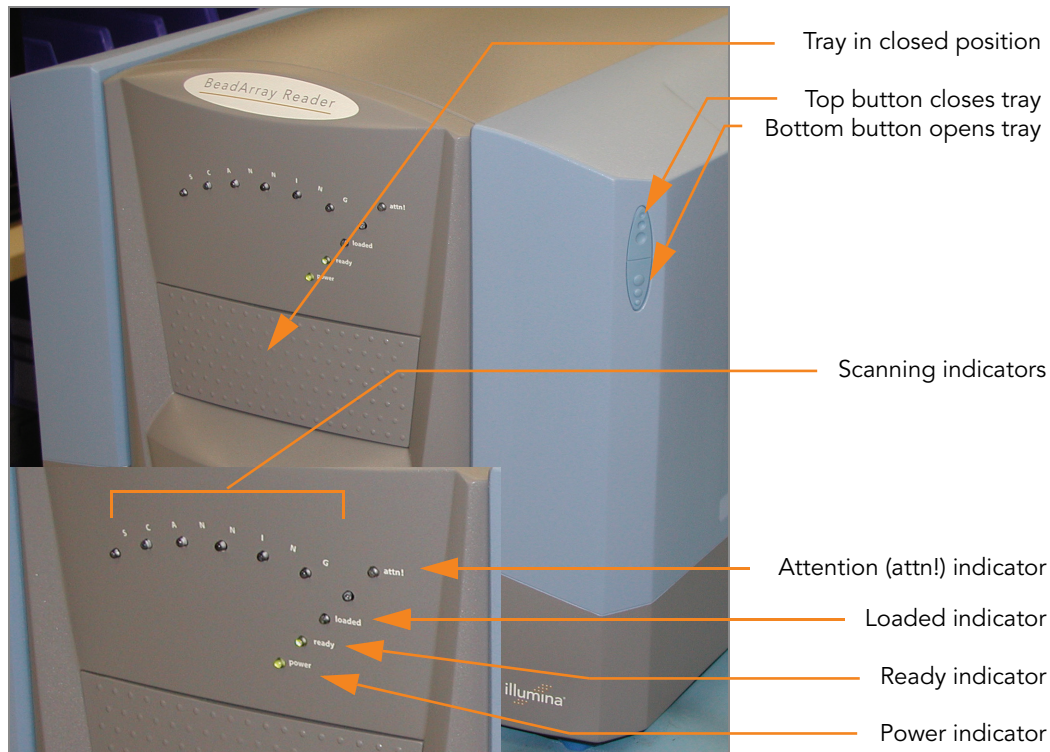


Figure 210 Illumina BeadArray Reader

Table 17 Illumina BeadArray Reader Indicators

Indicator	Description
Scanning (seven lights)	Flash blue in sequence when the BeadArray Reader is scanning.
Attention	Turns solid amber to indicate a hardware problem. Check the BeadArray Reader PC for an error message. You may need to cycle the power on the BeadArray Reader.
Loaded	Not currently used.
Ready	Flashes green during startup. Turns solid green when the BeadArray Reader is ready for use and when it is busy.
Power	Turns solid green when the BeadArray Reader is on.

1. Locate the power switch on the lower-left side of the BeadArray Reader back panel and turn it to the ON position.
2. Wait for the ready indicator to stop flashing.
3. Open the **BeadScan** software.
4. (Illumina LIMS only) Log in and click **Scan** (Figure 211).

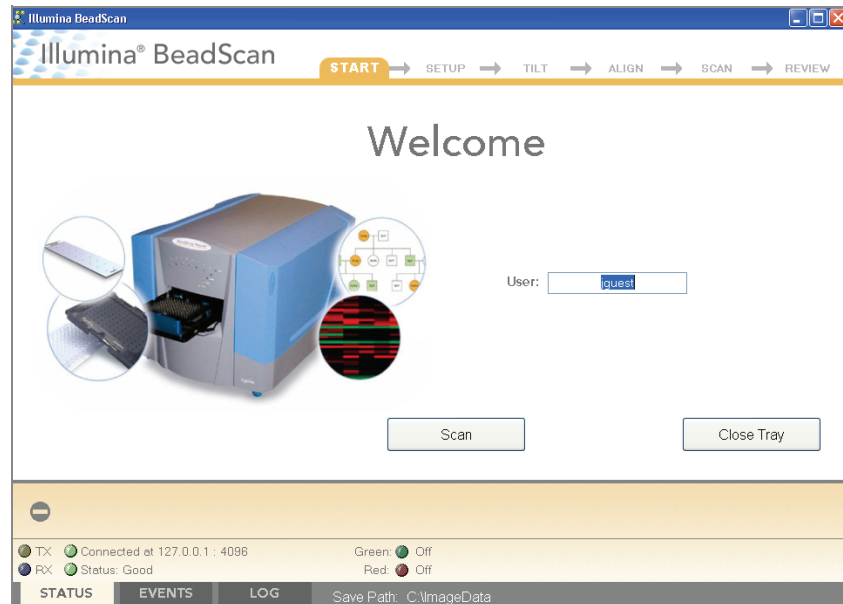


Figure 211 BeadScan Welcome Screen

Imaging BeadChip

When the BeadArray Reader is initialized, follow these steps to perform the scanning process.

1. From the **Docking Fixture** listbox, select BeadChip (Figure 212).

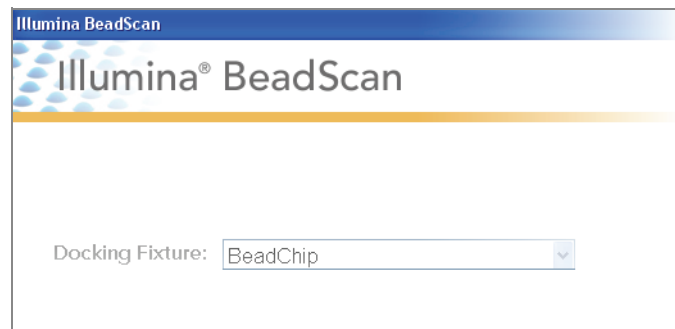


Figure 212 Select BeadChip Docking Fixture

2. Check the Data Repository path and the Decode Map path in the Settings area.
 - The **Data Repository path** indicates where the BeadArray Reader stores the images created during the scan. The default path is C:\ImageData.
 - The **Decode Map path** points to the location where you will copy the files that were downloaded from iCom or copied from each DVD provided with the BeadChip (if purchased). The default path is C:\DecodeData.
3. If either path is not correct:
 - a. Click **Edit** to open the Options dialog box.

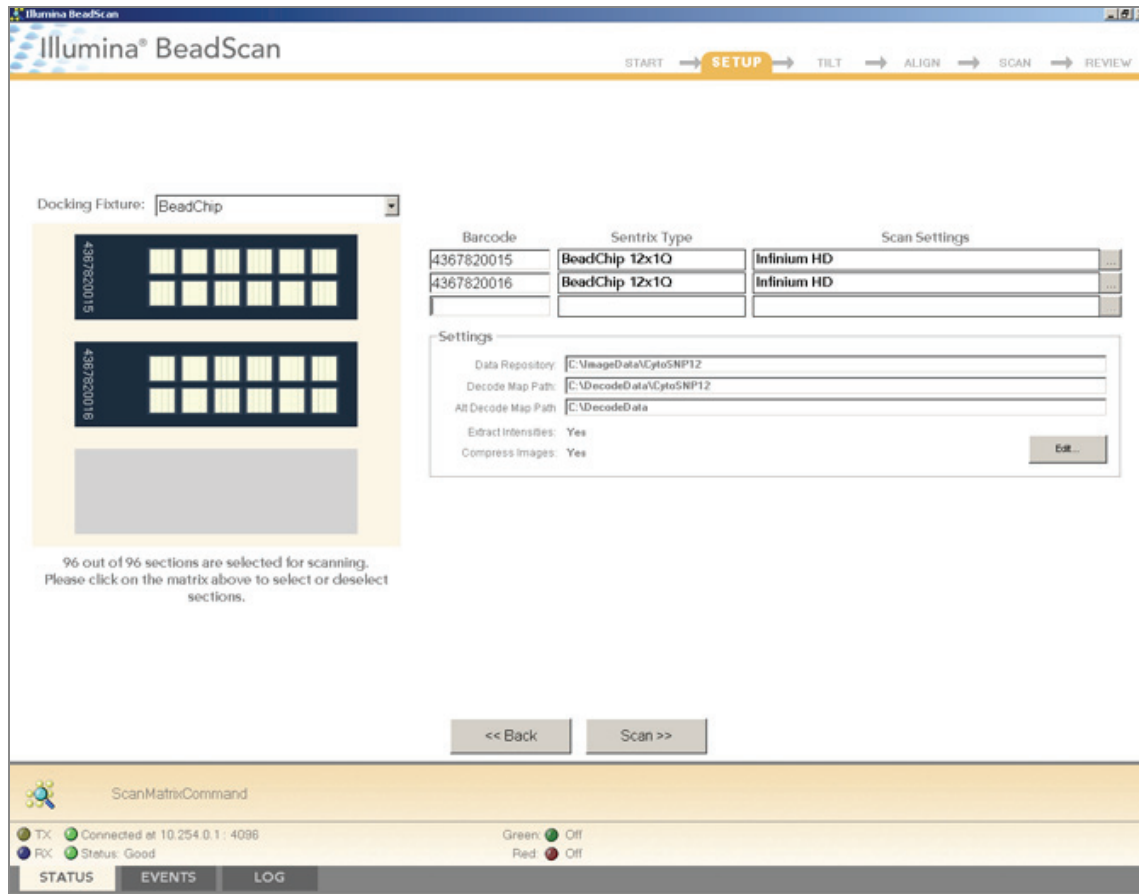


Figure 214 Scan Barcode Numbers

**NOTE**

BeadChip layout and color channel employed may differ from product to product.

- c. If either the **Sentrix Type** or **Scan Settings** are not correct, click **Browse (...)** to open the Select Scan Settings dialog box (Figure 215).

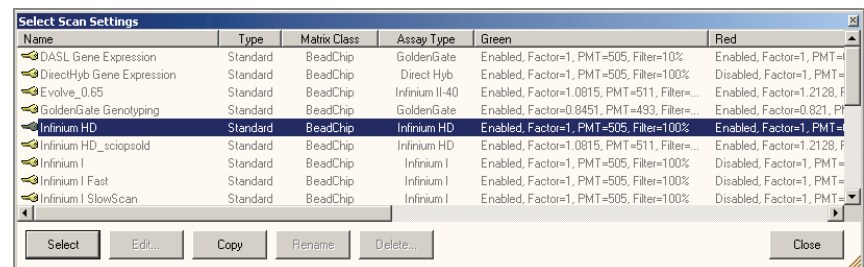


Figure 215 Select Scan Settings Dialog Box

- d. Select **Infinium HD.scst** for the 12x1 HD BeadChip or **Infinium HD-HT.scst** for the 24x1 HD BeadChip and click **Select**.

**NOTE**

Ensure that the appropriate scan setting is used. An incorrect scan setting may result in the misregistration of images.

6. Make sure that the BeadChips are properly seated in the BeadArray Reader tray (Figure 216).

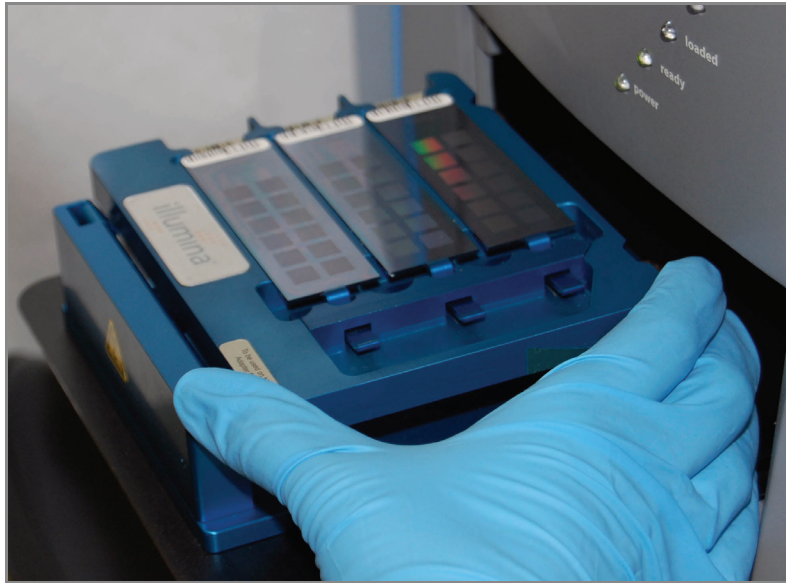


Figure 216 Place BeadChips into Illumina BeadArray Reader Tray

7. Click **Scan**.

Scanning Process

BeadScan begins the BeadArray Reader Tilt and Align processes (Figure 218):

- **Tilt**—The BeadArray Reader Autofocus feature records the Z-position (height) of three corners of the BeadChip to determine its current tilt, and then adjusts the BeadChip until it is flat.

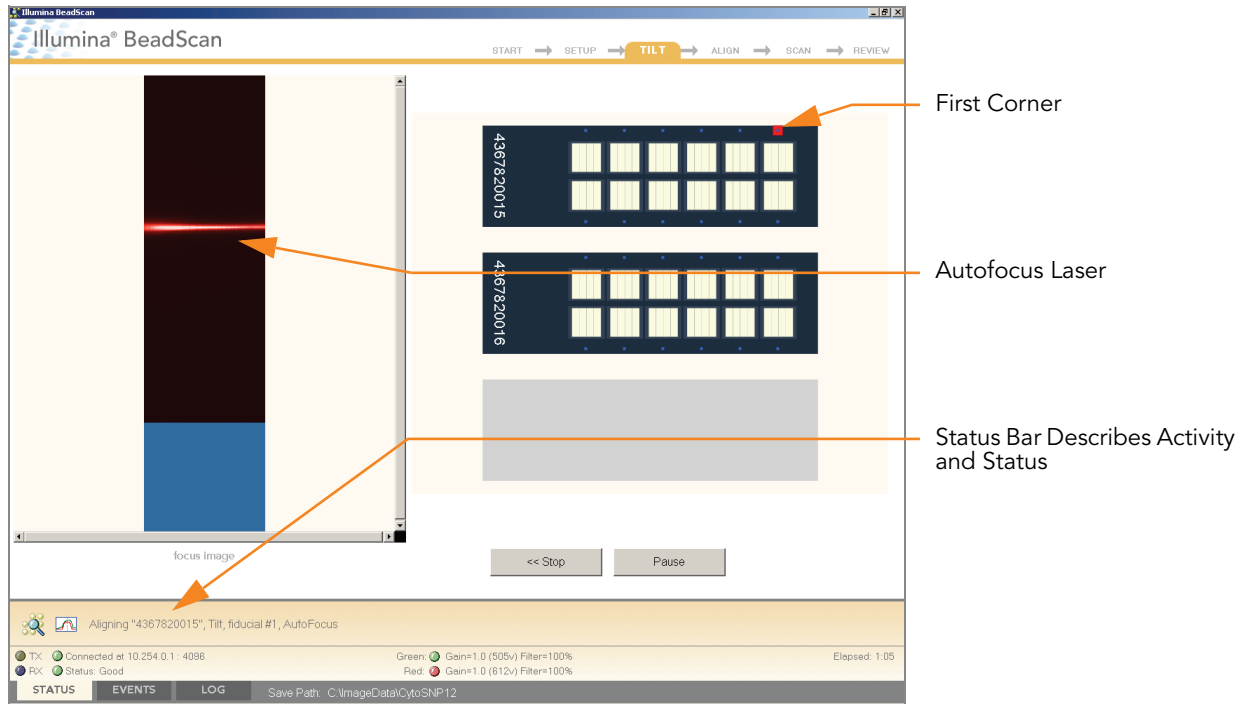


Figure 217 BeadArray Reader Tile Process



NOTE

The BeadChip image on the screen will be specific to the type of BeadChip being scanned.

- **Align**—The BeadArray Reader identifies the X-Y position (lateral location) of the fiducials (focus points) on the BeadChip edges, and then adjusts the optics.

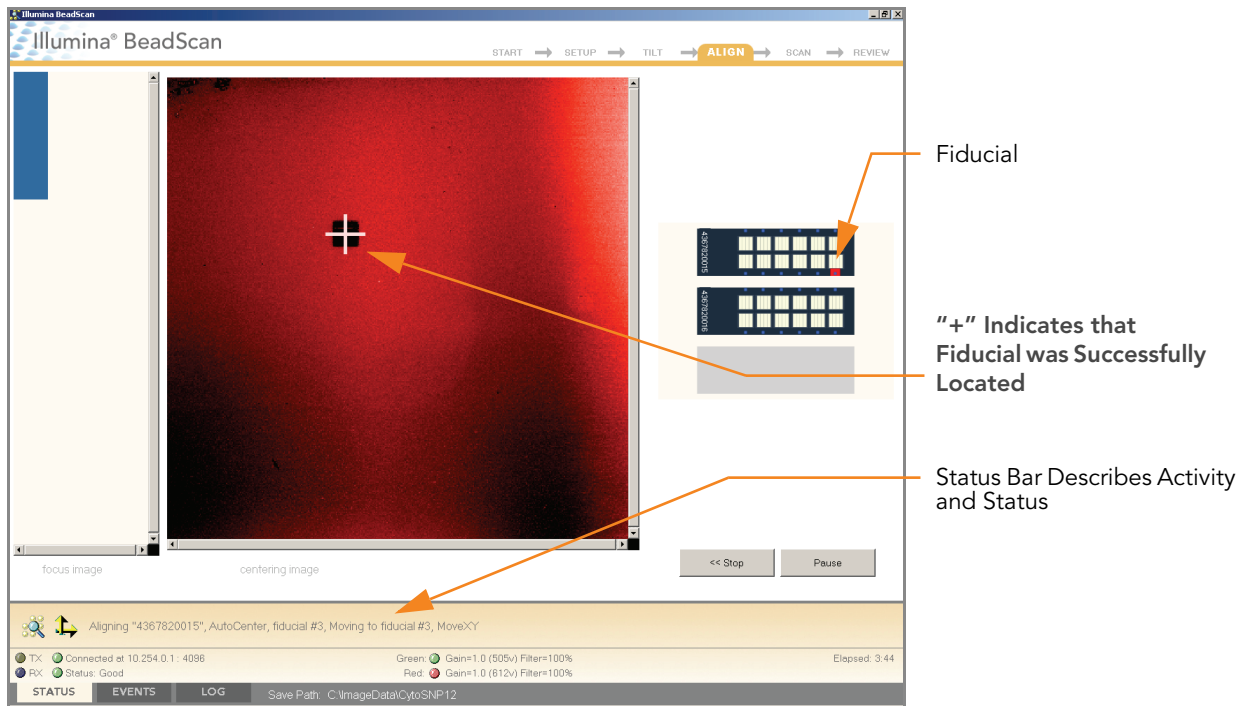


Figure 218 BeadArray Reader Tilt & Align Processes

Once the Tilt and Align processes are complete, the Scan process begins (Figure 219). Hover over any of the red or green dots in the closeup image to see the relative intensity and the XY position.¹

1. The 0/0 position is at the upper left corner of the BeadChip, with X increasing rightwards and Y increasing downwards.

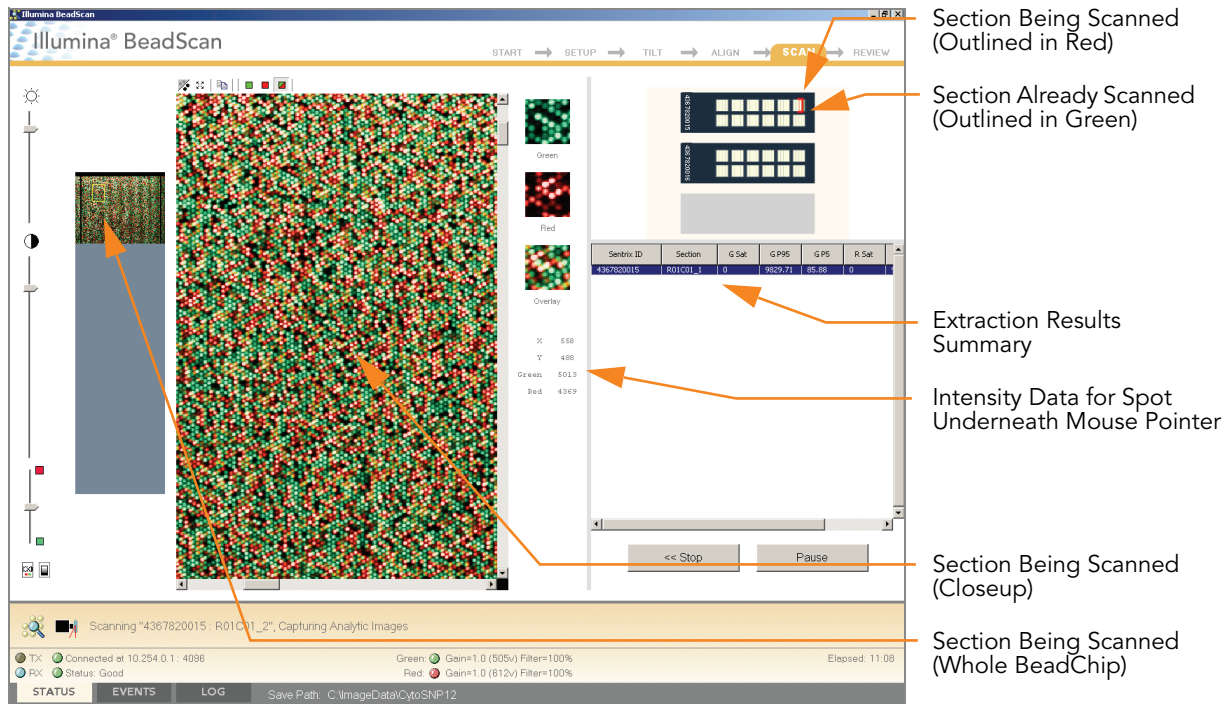


Figure 219 BeadArray Reader Scan Process

As the BeadArray Reader scans, the front panel blue Scanning indicator lights flash in sequence.

When the BeadArray Reader finishes scanning, a green message screen appears if the scan was successful, or a red message if it completed with any warnings. These screens are designed to be visible from across the lab.

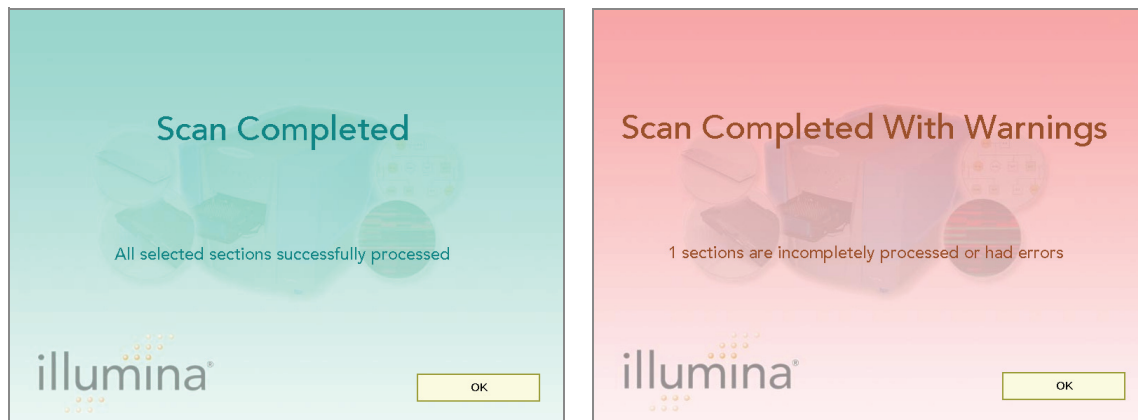


Figure 220 Scan Completed and Scan Completed with Warnings Screens

If Scan is Successful

1. Click **OK** on the Scan Completed message to view the next screen.
2. Click **Done** in the Review pane.

3. When the application returns to the Welcome screen, click **Open Tray**. The BeadArray Reader tray, loaded with the scanned BeadChips, will eject.
4. Remove the BeadChips from the tray.
5. Do one of the following:
 - ▶ If you have more BeadChips to scan, repeat the scanning process.
 - ▶ If this is the last use of the day:
 - a. Wipe the BeadArray Reader tray with a lint-free, absorbent towel. Pay particular attention to the tray edges where reagent may have wicked out.
 - b. Close the tray.
 - c. Turn the power switch at the back of the scanner to the **OFF** position.
 - d. Shut down the BeadArray Reader BeadScan software. To exit, right-click near the Illumina logo and click **Exit** (Figure 221).

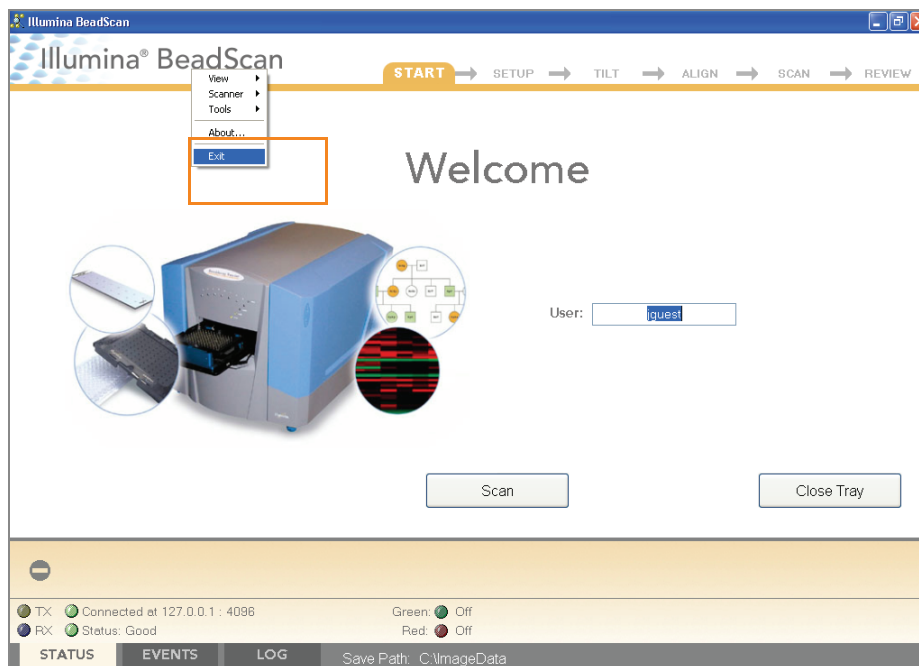


Figure 221 Exit the BeadArray Reader Software

If Scan is not Successful

Re-scan the array. For more information, refer to the *Illumina BeadArray Reader User Guide*.

If the scanner was unable to locate the alignment fiducials (focus points), you may need to clean the edges of the BeadChip before re-scanning.

Clean BeadChip Edges

1. To clean the BeadChips, wrap a Prostat EtOH presaturated wipe around your index finger. Place the wipe against the edge of BeadChip, no further than 2 mm from the edge of the slide (Figure 222).

**CAUTION**

The wipe should not pass over the etched stripes on the BeadChip.

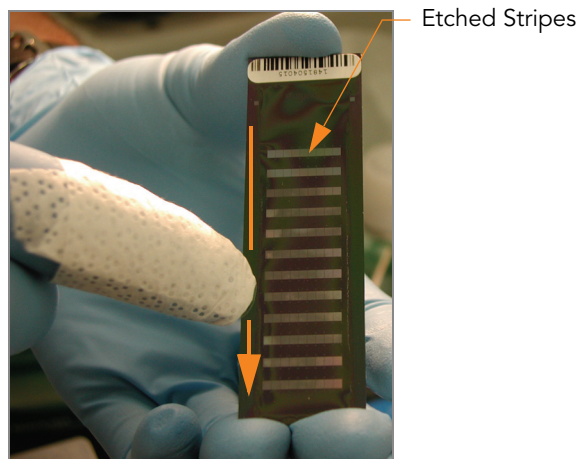


Figure 222 Wiping Edges of BeadChip

2. Wipe along the edge of each BeadChip five or six times (Figure 222), until the surface is clean and smooth and no streaks are visible.
To completely remove the coating along the edges and back of BeadChip, you may need to use a different part of the ProStat EtOH wipe.

**CAUTION**

Any remaining visible streaks may lead to alignment errors.

GenomeStudio Integrated Informatics Platform

The 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 iScan System or BeadArray Reader.

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 Module User Guide*.

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