Infinium® HD Assay
Gemini Protocol Guide

FOR RESEARCH ONLY

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illumina®

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Introduction

The Illumina® Infinium® HD Assay Gemini 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 Gemini protocol scales DNA analysis to more than a million SNPs and CNV markers per sample, dependent only on the number of features (bead types) on the array.

The Infinium HD Assay Gemini 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 is provided by a combination of sequence-specific hybridization capture and array-based, single-base primer extension. In the case of Infinium II probe design, the 3' end of the primer is positioned directly adjacent to the SNP locus, or the non-polymorphic assay locus in the case of non-polymorphic probes. In the case of 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 Gemini protocol offers:

- Effectively unlimited loci multiplexing
- High call rate and accuracy
- Genome-wide marker selection
- Minimal risk of carryover contamination
- Low DNA input of 400 ng
- Walk-away automation using Tecan Genesis or Freedom EVO Robots and Tecan GenePaint system
- Infinium LIMS automation
- Compatibility with both Illumina iScan™ System and BeadArray™ Reader
- Multi-sample BeadChip format

Audience and Purpose

This guide is for laboratory technicians running the Infinium HD Assay Gemini 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 Gemini Manual Protocol* explains how to run the assay manually in the lab.

Chapter 3, *Infinium HD Assay Gemini 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 Gemini 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 whole-genome genotyping chips provided by Illumina. All of the Infinium HD Assay Gemini 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 Gemini

This section describes and illustrates the assay protocol. The assay requires only 400 ng of DNA sample as input.

Amplify DNA

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

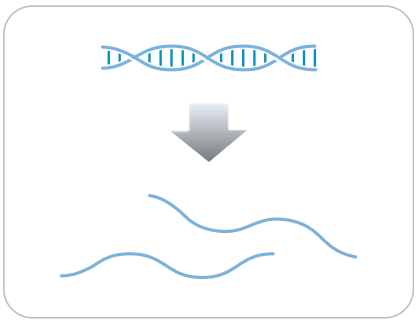


Figure 1 Denaturing and Neutralizing DNA

See *Make the AMP3 Plate* on page 27 for manual processing.
See *Make the AMP3 Plate* on page 106 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 thousand-fold without introducing large amounts of amplification bias.

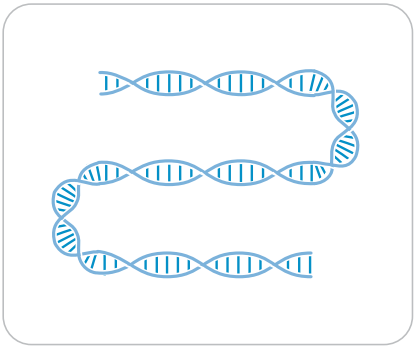


Figure 2 Incubating DNA to Amplify

See *Incubate the AMP3 Plate* on page 30 for manual processing.
See *Incubate the AMP3 Plate* on page 119 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.

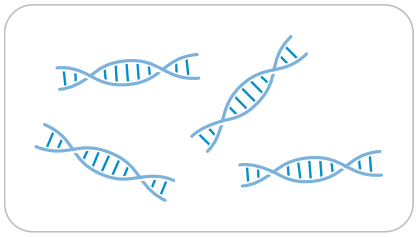


Figure 3 Fragmenting DNA

See *Fragment the AMP3 Plate* on page 31 for manual processing.
See *Fragment the AMP3 Plate* on page 121 for automated processing.

Precipitate DNA

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

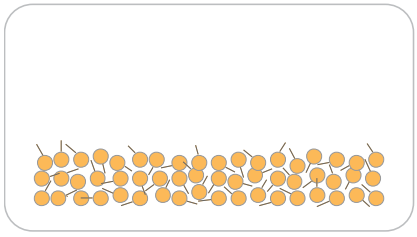


Figure 4 Precipitating DNA

See *Precipitate the AMP3 Plate* on page 34 for manual processing.
See *Precipitate the AMP3 Plate* on page 125 for automated processing.

Resuspend DNA

The precipitated DNA is resuspended in hybridization buffer.

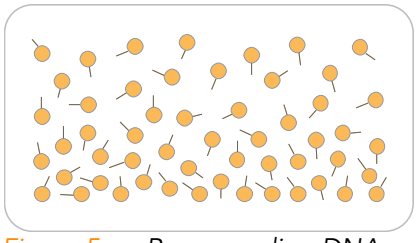


Figure 5 Resuspending DNA

See *Resuspend the AMP3 Plate* on page 37 for manual processing.
See *Resuspend the AMP3 Plate* on page 132 for automated processing.

Hybridize to BeadChip

The BeadChip is prepared for hybridization in a capillary flow-through chamber. Samples are applied to a Beadchip divided into halves 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 50mers (covalently linked to one of over 1,100,000 bead types) during hybridization.

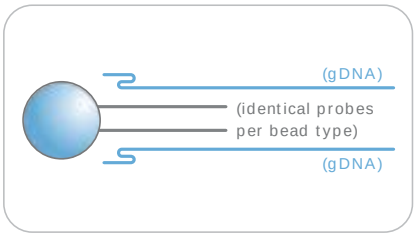


Figure 6 Hybridizing DNA to BeadChip

See *Hybridize Duo BeadChip* on page 39 for manual processing.
See *Hybridize Duo BeadChip* on page 136 for automated processing.

Wash BeadChip

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

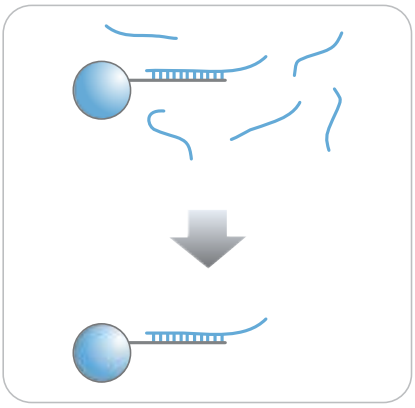


Figure 7 Washing BeadChip

See *Wash BeadChips* on page 50 for manual processing.
See *Wash BeadChips* on page 152 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.

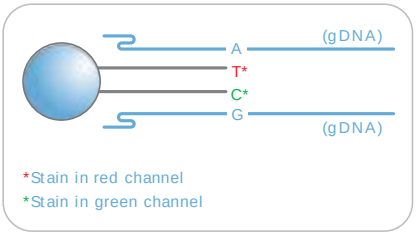


Figure 8 Extending and Staining BeadChip

See *Single-Base Extension and Stain HD BeadChip* on page 59 for manual processing.
See *Single-Base Extension and Stain HD BeadChip* on page 163 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 the chapter on imaging BeadChips in the *Infinium Assay Lab Setup and Procedures Guide*.

The HD BeadChip

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

Each individual array in the matrix may hold over 1,100,000 different bead types. Distinct oligonucleotide probe sequences are attached to each bead type. Mixed pools of beads are assembled into the microwells of the BeadChip substrate. Because the microwells outnumber the distinct probe sequences, 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 control of each array feature, allowing consistent production of high-quality, reproducible arrays.

Illumina Lab Protocols

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

Chapter 2, *Infinium HD Assay Gemini Manual Protocol* and Chapter 3, *Infinium HD Assay Gemini Automated Protocol*, show how to perform the assay protocol with clearly divided pre- and post-amplification processes.

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 on your Documentation CD for printing and reference.

Sample Sheet

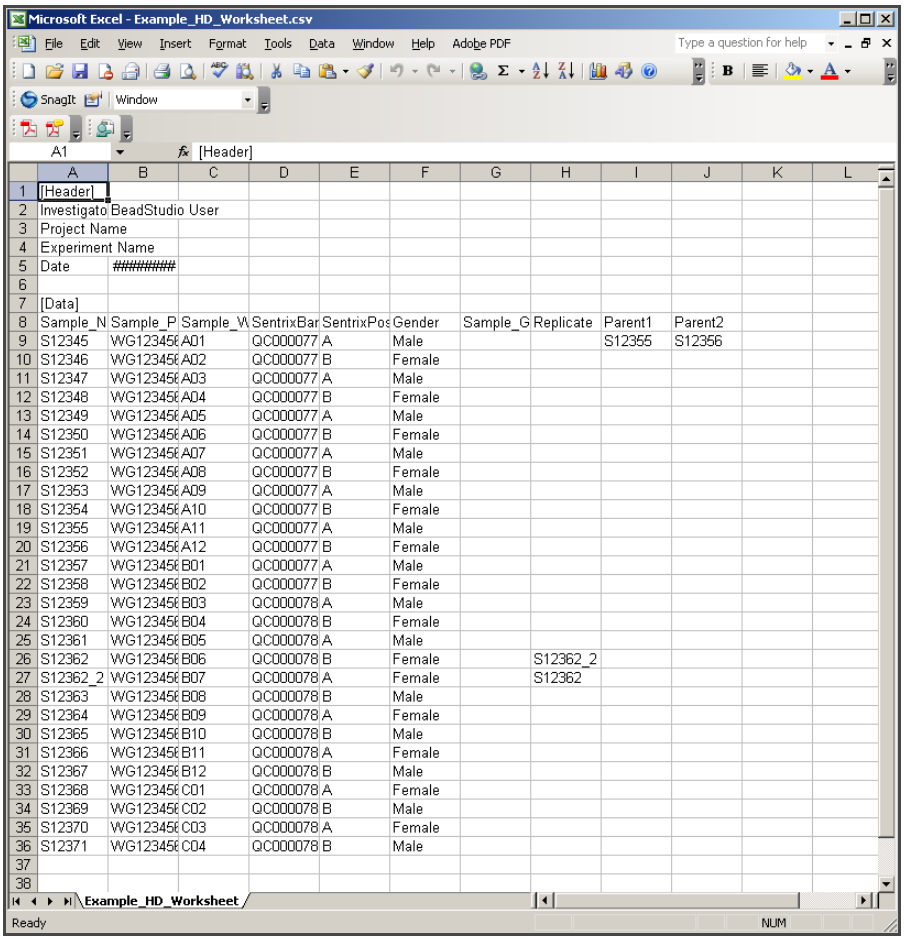
To effectively track your samples and assay, Illumina recommends you create a Sample Sheet. The Sample Sheet will later be used by the GenomeStudio™ application for data analysis. For instructions on data analysis, see the *GenomeStudio Genotyping Module User Guide*.

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_Name	Name of the sample. Used only for display in the table.	O
Sample_Plate	The barcode of the sample plate for this sample. Used only for display in the table.	O
Sample_Well	The sample plate well for this sample. Used only for display in the table.	O
SentrixBarcode_A	The barcode of the array product (BeadChip) to which this sample was hybridized (for Manifest A).	R
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. Your Documentation CD includes an electronic, read-only Sample Sheet template file (*Sample Sheet Template.csv*) that you can copy and use.



	A	B	C	D	E	F	G	H	I	J	K	L
1	[Header]											
2	Investigato	BeadStudio User										
3	Project Name											
4	Experiment Name											
5	Date	#########										
6												
7	[Data]											
8	Sample_N	Sample_P	Sample_W	SentrixBar	SentrixPos	Gender	Sample_G	Replicate	Parent1	Parent2		
9	S12345	WG12345(	A01	QC000077	A	Male			S12355	S12356		
10	S12346	WG12345(	A02	QC000077	B	Female						
11	S12347	WG12345(	A03	QC000077	A	Male						
12	S12348	WG12345(	A04	QC000077	B	Female						
13	S12349	WG12345(	A05	QC000077	A	Male						
14	S12350	WG12345(	A06	QC000077	B	Female						
15	S12351	WG12345(	A07	QC000077	A	Male						
16	S12352	WG12345(	A08	QC000077	B	Female						
17	S12353	WG12345(	A09	QC000077	A	Male						
18	S12354	WG12345(	A10	QC000077	B	Female						
19	S12355	WG12345(	A11	QC000077	A	Male						
20	S12356	WG12345(	A12	QC000077	B	Female						
21	S12357	WG12345(	B01	QC000077	A	Male						
22	S12358	WG12345(	B02	QC000077	B	Female						
23	S12359	WG12345(	B03	QC000078	A	Male						
24	S12360	WG12345(	B04	QC000078	B	Female						
25	S12361	WG12345(	B05	QC000078	A	Male						
26	S12362	WG12345(	B06	QC000078	B	Female		S12362_2				
27	S12362_2	WG12345(	B07	QC000078	A	Female		S12362				
28	S12363	WG12345(	B08	QC000078	B	Male						
29	S12364	WG12345(	B09	QC000078	A	Female						
30	S12365	WG12345(	B10	QC000078	B	Male						
31	S12366	WG12345(	B11	QC000078	A	Female						
32	S12367	WG12345(	B12	QC000078	B	Male						
33	S12368	WG12345(	C01	QC000078	A	Female						
34	S12369	WG12345(	C02	QC000078	B	Male						
35	S12370	WG12345(	C03	QC000078	A	Female						
36	S12371	WG12345(	C04	QC000078	B	Male						
37												
38												

Figure 10 Sample Sheet Example

Tecan GenePaint

The Infinium HD Assay Gemini 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, 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 array). 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) software 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 (*.bpm) or manifest file (*.bgx) to analyze the data from the assay.

Loading and unloading the iScan System can be automated with the optional AutoLoader2. AutoLoader2 is fully integrated with GenomeScan software and Infinium LIMS, and contains an email alert system. The AutoLoader2 places carriers with up to four BeadChips in the iScan Reader tray, so that the iScan Reader can scan the BeadChips. The AutoLoader2 supports unattended processing of up to 48 carriers at a time in a single-reader or dual-reader configuration.

For instructions on imaging the BeadChip, see the *Infinium Assay Lab Setup and Procedures Guide*. For instructions on using the iScan System, see the *iScan and AutoLoader2 System Guide*. For instructions on using the BeadArray Reader, see the *Illumina BeadArray Reader User Guide*.

GenomeStudio Integrated Informatics Platform

GenomeStudio, Illumina's new 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.

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 Infinium 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 2 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.

Chapter 2

Infinium HD Assay Gemini Manual Protocol

Topics

Introduction

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

Infinium HD Assay Gemini Manual Workflow

Figure 11 graphically represents the Illumina Infinium HD Assay Gemini manual workflow. These protocols describe the procedure for preparing sixteen DNA samples. To process 48 or 96 samples, scale up the protocols accordingly.

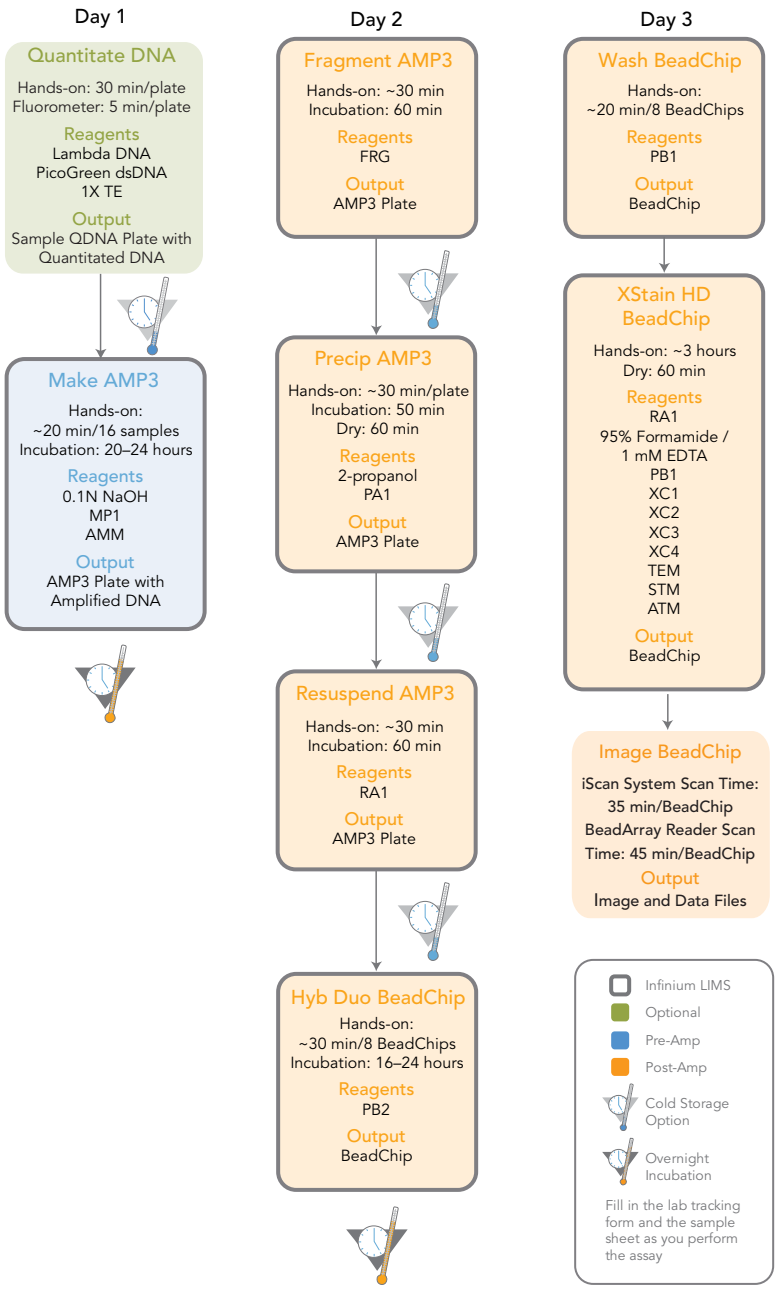


Figure 11 Infinium HD Assay Gemini Manual Workflow

Equipment, Materials, and Reagents

These items are specific to the two-sample, manual BeadChip assay. For a list of other equipment, materials, and reagents needed in an Infinium HD Assay Gemini lab, see the *Infinium Assay Lab Setup and Procedures Guide*.

Equipment Illumina-Supplied

Table 3 Illumina-Supplied Equipment

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

Materials User-Supplied

Table 4 User-Supplied Equipment

Item	Source
Vacuum desiccator (1 per 8 BeadChips processed simultaneously)	VWR Catalog # 24988-197
Vacuum tubing	VWR Catalog # 62995-335
Tube racks for vacuum desiccators (must fit internal dimensions of vacuum desiccator)	VWR Catalog # 60916-748
Vacuum source (greater than 508 mm Hg (0.68 bar))	
Vacuum gauge for vacuum desiccator (Recommended)	

Illumina-Supplied

- AMP3 barcode labels
- WG#-DNA barcode labels

Reagents Illumina-Supplied

Table 5 Illumina-Supplied Reagents

Item	Part #
MP1—Neutralization solution	11190751
AMM—Amplification Master Mix	11192044
FRG—Fragmentation solution	11190022
PA1—Precipitation solution	11190031

Table 5 Illumina-Supplied Reagents

Item	Part #
RA1—Resuspension, hybridization, and wash solution	11191914
PB1—Reagent used to prepare BeadChips for hybridization	11191922
PB2—Humidifying buffer used during hybridization	11191130
XC1—XStain BeadChip solution 1	11208288
XC2—XStain BeadChip solution 2	11208296
TEM—Two-Color Extension Master Mix	11208309
XC3 (80 ml)—XStain BeadChip solution 3	11208392
XC3 (240 ml)—XStain BeadChip solution 3	11208421
STM—Superior Two-Color Master Mix	11288046
ATM—Anti-Stain Two-Color Master Mix	11208317
XC4—XStain BeadChip solution 4	11208430

Quantitate DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples before bilsulfite conversion. You can quantitate up to six plates, each containing up to 96 samples. If you already know the concentration, proceed to *Make the AMP3 Plate on page 27*.

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	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

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 QDNA.”
- 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:

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

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

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

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.

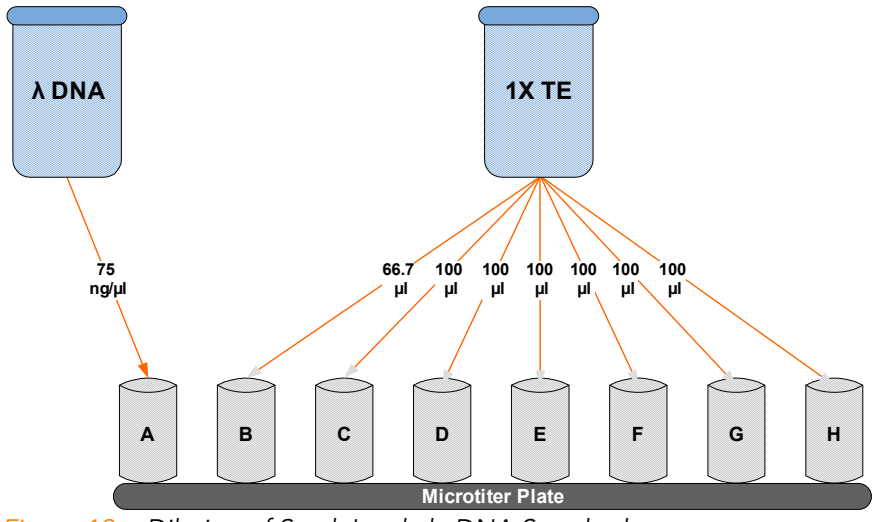


Figure 12 Dilution of Stock Lambda DNA Standard

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 6 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

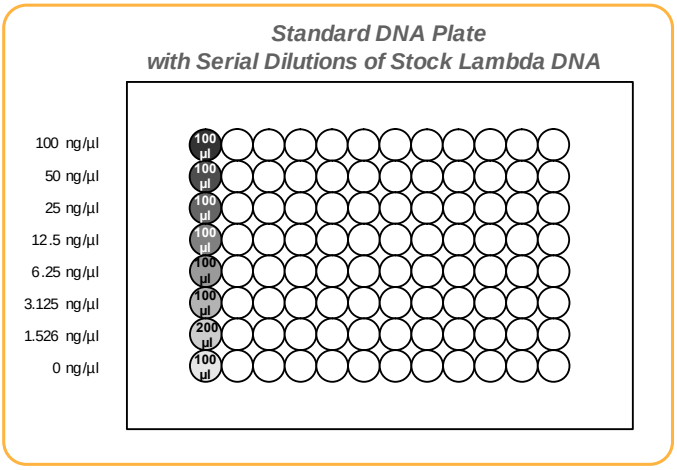


Figure 13 Serial Dilutions of Lambda DNA

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 7 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 7 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.

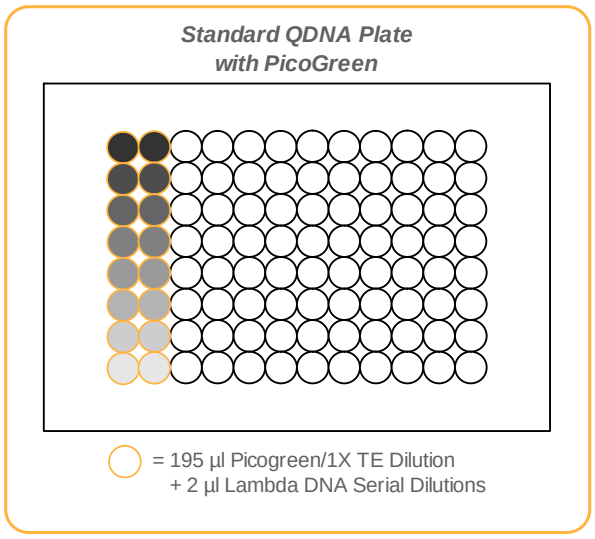


Figure 14 Standard QDNA Plate with PicoGreen

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.

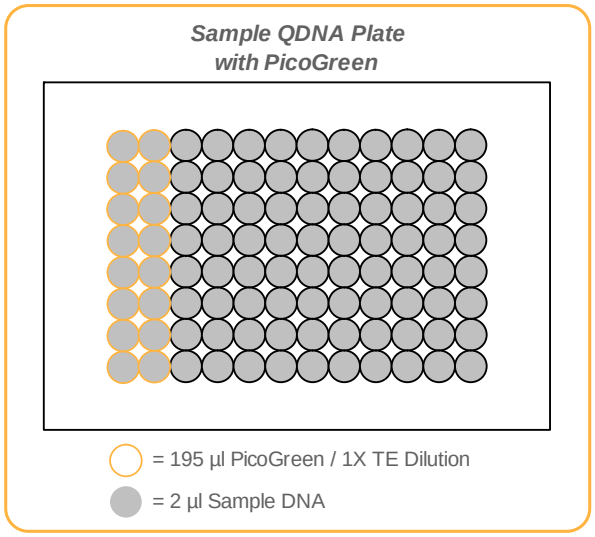


Figure 15 Sample QDNA Plate with PicoGreen

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*.

Read QDNA Plate

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.

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 **Assays | Nucleic Acids | Illumina QDNA** (Figure 16).

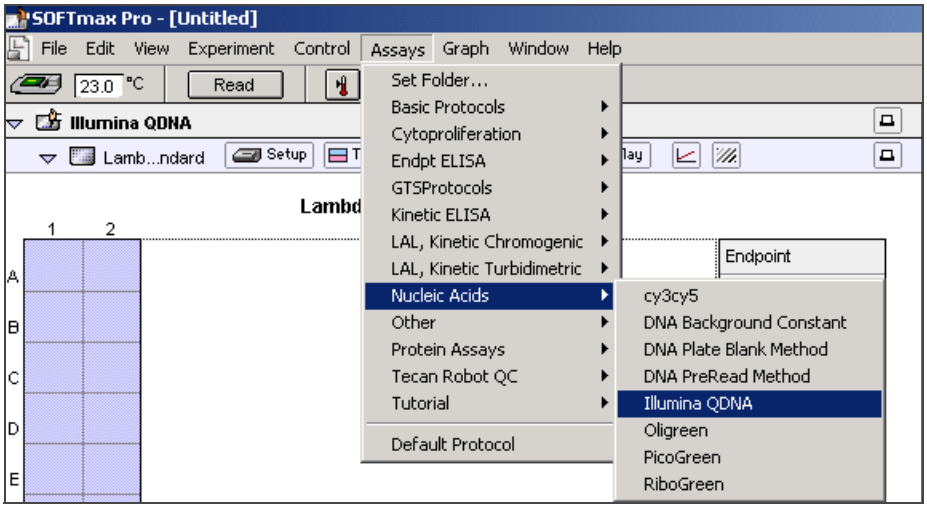


Figure 16 Load the Illumina QDNA Protocol in SoftMax Pro

4. Place the Standard QDNA Fluotrac Plate into the spectrofluorometer loading rack with well A1 in the upper left corner.
5. Click the blue arrow next to **Lambda Standard** (Figure 17).

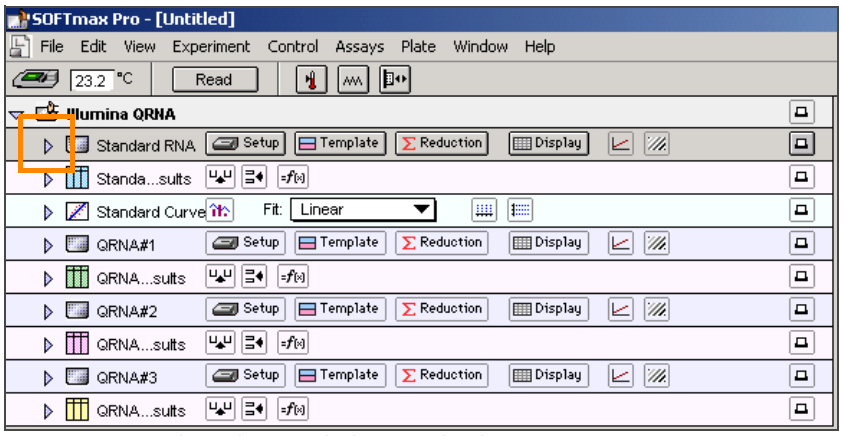


Figure 17 Select the Lambda Standard Screen

6. Click **Read** in the SoftMax Pro interface (Figure 18) to begin reading the Standard QDNA Plate.

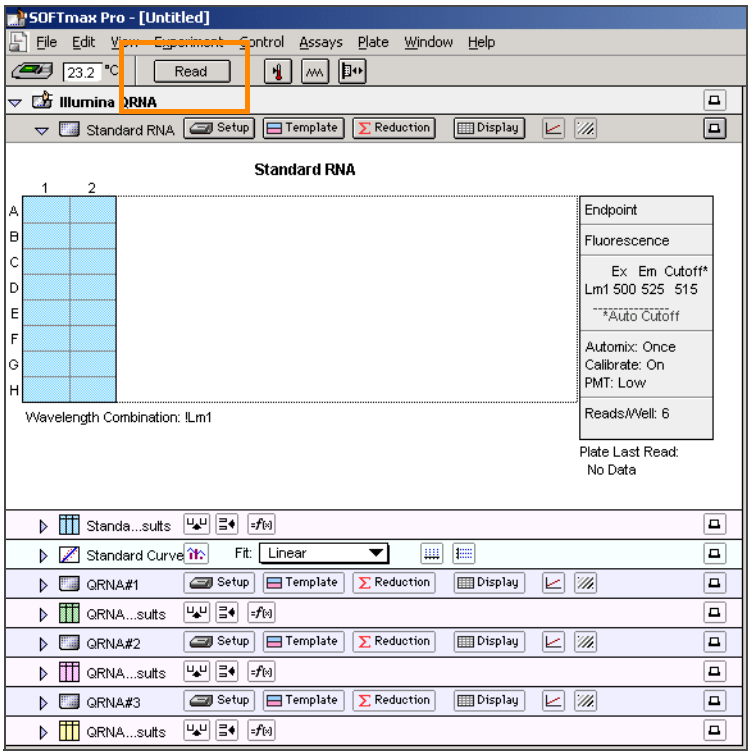


Figure 18 Read the Standard QDNA Plate

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 19).
9. If the standard curve is acceptable, continue with the sample plate. Otherwise, click **Standard Curve** again.

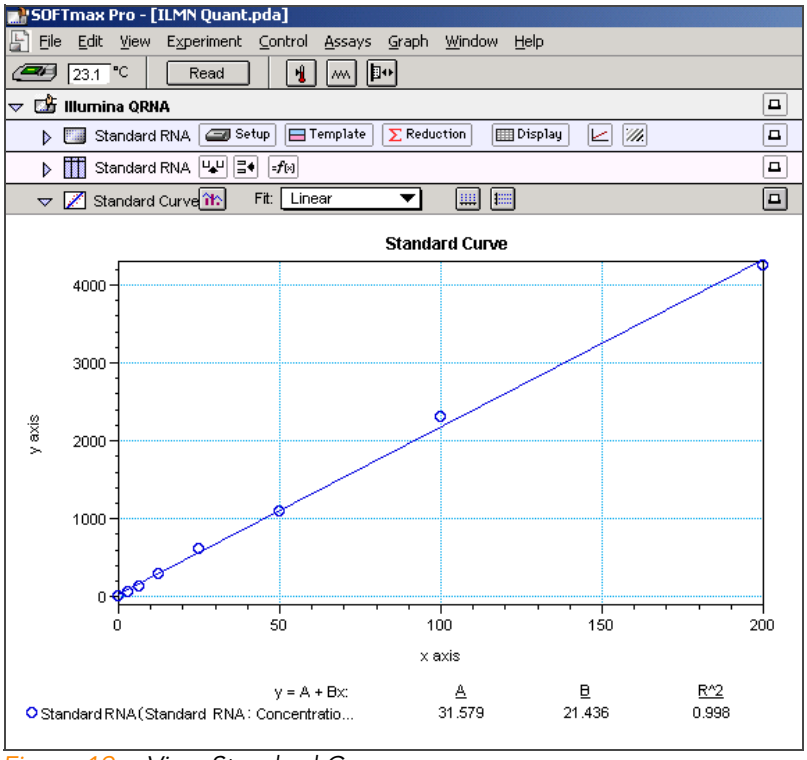


Figure 19 View Standard Curve

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 **QDNA#1** then click **Read** (Figure 20).

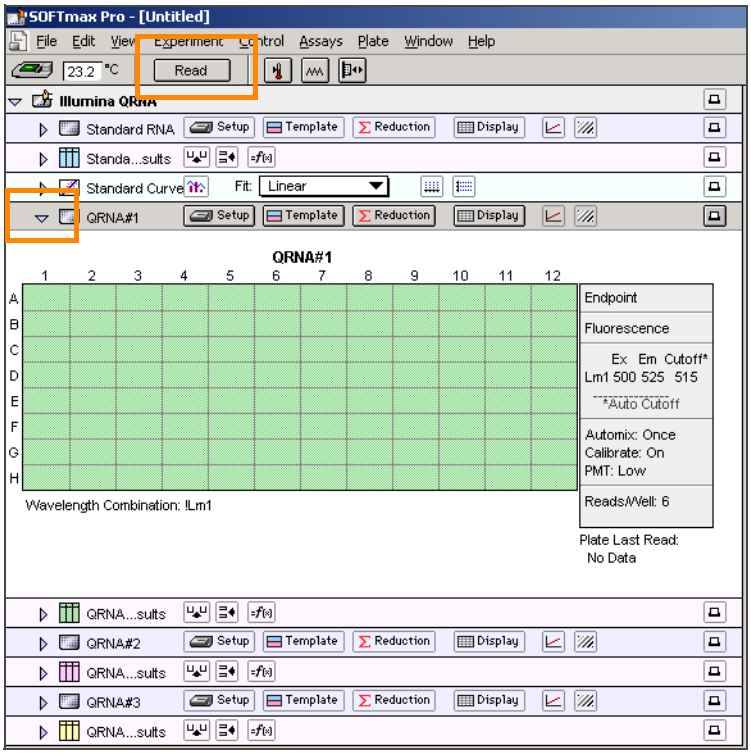


Figure 20 Read the Sample QDNA Plate

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 AMP3 Plate* on page 27.
    - Store the quantitated DNA at 2 to 8°C for up to one month.

Make the AMP3 Plate

This process creates a AMP3 plate for DNA amplification. The DNA sample is denatured with NaOH and then neutralized with MP1 reagent. The last reagent added is AMM (Amplification Master Mix).

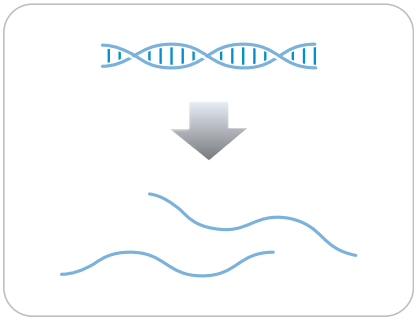


Figure 21 Denaturing and Neutralizing DNA

Estimated Time

Hands-on time: ~20 minutes per 16 samples

Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MP1	1 tube per 16 samples	-15 to -25°C	Illumina
AMM	1 tube per 16 samples	-15 to -25°C	Illumina
0.1N NaOH	15 ml for 16–48 samples	2 to 8°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate per 48 samples		User
WG#-DNA plate with up to 96 DNA samples (50 ng/µl)	1 plate for up to 48 samples	-15 to -25°C	User

Preparation

- Preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- Apply an AMP3 barcode label to a new MIDI plate.
- Thaw MP1 and AMM tubes to room temperature. Gently invert to mix, then pulse centrifuge to 280 xg.
- Thaw DNA samples to room temperature.
- Enter the Sample_Name (optional) and Sample_Plate for each Sample_Well in the sample sheet.
- On the lab tracking form, record:
  - Date/Time
  - Operator

- WG#-DNA plate barcode
- AMP3 plate barcode
- MP1 tube barcode(s)
- AMM tube barcode(s)

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

Steps

1. If you do not already have a WG#-DNA plate, create one by adding DNA, normalized to 50 ng/µl, into either a:
   - MIDI plate: 40 µl to each WG#-DNA plate well
   - TCY plate: 30 µl to each WG#-DNA plate well

   Apply a barcode label to the new WG#-DNA plate.
2. Vortex the WG#-DNA plate at 1600 rpm (actual vortex speed) for 1 minute.
3. Centrifuge to 280 xg for 1 minute.
4. Transfer 8 µl DNA sample, into each well in the following AMP3 plate columns:
   - Column 1 (8 samples)
   - Columns 1 and 3 (16 samples)
   - Columns 1, 3, 5, 7, 9, and 11 (48 samples)

To ensure optimal performance of sample and lab equipment, use aerosol filter tips when pipetting DNA.

5. Dispense 8 µl 0.1N NaOH into each well that contains DNA (Figure 22).

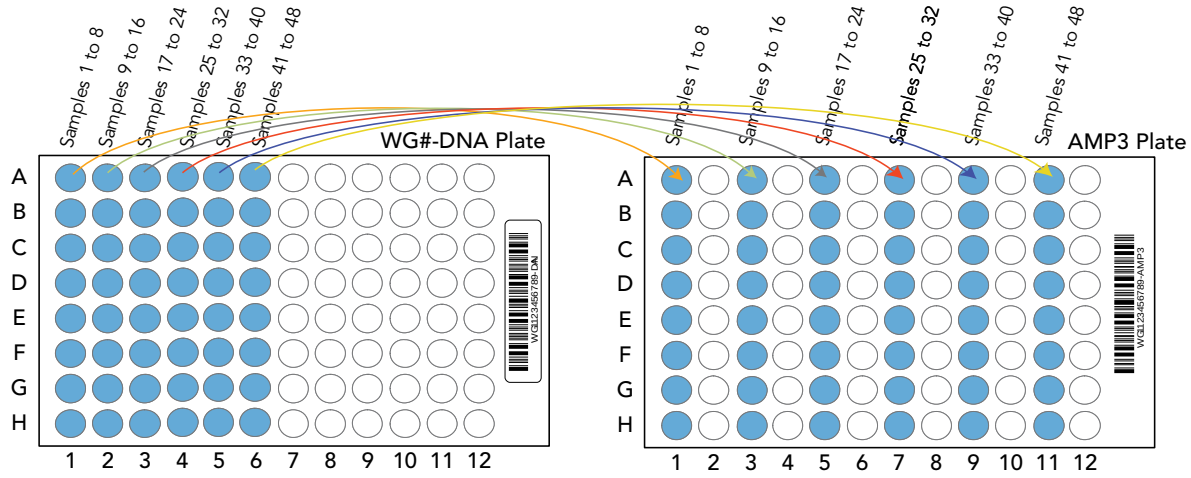


Figure 22 Distributing Sample to AMP3 Plate Wells

6. Incubate for 10 minutes at room temperature.
7. Record each DNA sample in the lab tracking form.
8. Dispense 135 µl MP1 into each well containing sample.
9. Dispense 150 µl AMM into each well containing sample.
10. Seal the plate with a cap mat.
11. Invert the sealed plate at least 10 times to mix contents.
12. Pulse centrifuge to 280 xg.
13. Discard unused reagents in accordance with facility standards.
14. Proceed immediately to *Incubate the AMP3 Plate* on page 30.

Incubate the AMP3 Plate

This process uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used twice in the Infinium HD Assay Gemini.

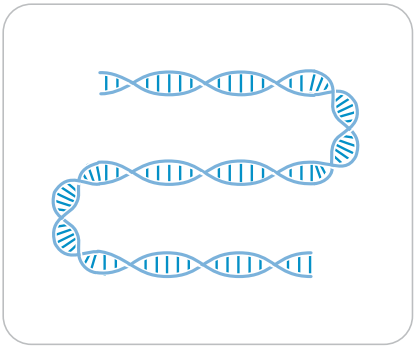


Figure 23 Incubating DNA to Amplify

Incubation Time

~20–24 hours.

Steps

1. Incubate the AMP3 plate in the Illumina Hybridization Oven for at least 20 and 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 provided on your documentation CD. This form can be filled out and saved online, or printed and filled in by hand.

3. Proceed to *Fragment the AMP3 Plate* on page 31.

Fragment the AMP3 Plate

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

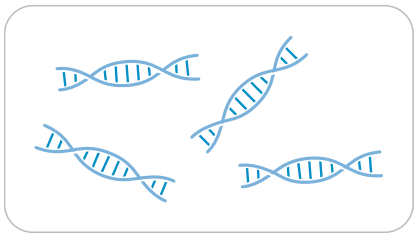


Figure 24 Fragmenting DNA

Estimated Time

Hands-on time: ~30 minutes per 48 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FRG	1 tube per 16 samples	-15 to -25°C	Illumina

Preparation

- Preheat the heat block with the MIDI plate insert to 37°C.
- Thaw the FRG tube to room temperature. Invert several times to mix contents. Pulse centrifuge to 280 xg for 1 minute.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - FRG tube barcode(s)
- Remove the AMP3 plate from the Illumina Hybridization Oven.

NOTE

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

Steps

1. Centrifuge the 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. Thoroughly pipette-mix all wells containing sample to evenly distribute precipitate.
4. Split the sample into 1 additional well, for a total of 2 wells per sample. Each well should contain 150 µl.

   For example, move 150 µl sample from A1 into A2.
   - Divide DNA sample in A1 into A2, A3, and A4.
   - Divide DNA sample in A5 into A6, A7, and A8.
   - Divide DNA sample in A9 into A10, A11, and A12.

   Follow this pattern for rows B–H, columns 1, 5, and 9 (see Figure 25).

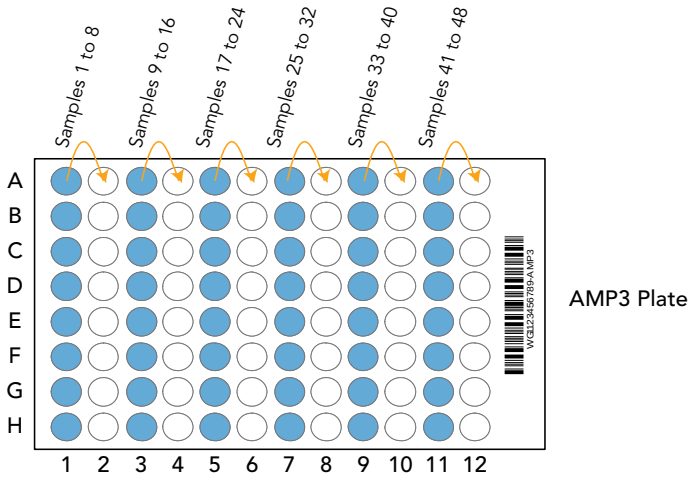


Figure 25 Distributing Sample in AMP3 Plate

5. Dispense 50 µl FRG to each well containing sample.
6. Seal the AMP3 plate with the cap mat.
7. Place the sealed plate on the vortexer and secure it with the provided Velcro straps (Figure 26).

Figure 26 Securing Plates to Vortexer Platform with Velcro Straps

8. Vortex the AMP3 plate at 1600 rpm for 1 minute.
9. Centrifuge the plate to 50 xg for 1 minute at 22°C.
10. Incubate the sealed plate on the 37°C heat block for 1 hour.
11. On the lab tracking form, record the start and stop times.
12. Discard unused reagents in accordance with facility standards.
13. Do one of the following:
    - Proceed to *Precipitate the AMP3 Plate* on page 34. Leave the plate in 37°C heat block until setup is complete.
    - Store the sealed AMP3 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 AMP3 Plate

Add PA1 and 2-propanol to the AMP3 plate to precipitate the DNA samples.

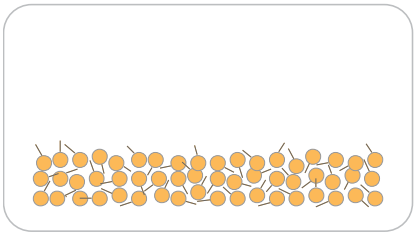


Figure 27 Precipitating DNA

Estimated Time

Hands-on time: ~30 minutes per 48 samples

Incubation time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PA1	1 tube per 16 samples	2 to 8°C	Illumina
100% 2-propanol	12 ml per 16 samples 40 ml per 48 samples	Room temperature	User

Preparation

- Do one of the following:
  - If you froze the AMP3 plate after fragmentation, thaw it to room temperature. Centrifuge to 280 xg for 1 minute.
  - If you proceeded immediately from Fragment AMP3, leave the plate in the 37°C heat block until setup is complete.
- Thaw PA1 to room temperature. Centrifuge to 280 xg for 1 minute.
- Preheat the heat block to 37°C, if it is not already.
- Turn on the heat sealer.
- In preparation for the 4°C spin, set the centrifuge to 4°C.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - PA1 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 provided on your documentation CD. This form can be filled out and saved online, or printed and filled in by hand.

Steps

1. Dispense 100 µl PA1 to each well containing sample.
2. Seal the plate with the cap mat.
3. Vortex the plate at 1600 rpm for 1 minute.
4. Centrifuge to 50 xg at 22°C for 1 minute.
5. Incubate at 37°C for 5 minutes.
6. Add 300 µl 100% 2-propanol to each well containing sample.
7. Seal the plate with a new, dry cap mat, taking care not to shake the plate in any way until the heat-seal is fully seated.
8. Invert at least 10 times to mix contents.
9. Incubate at 4°C for 30 minutes.
10. Place the sealed AMP3 plate in the centrifuge opposite another plate of equal weight (Figure 28).

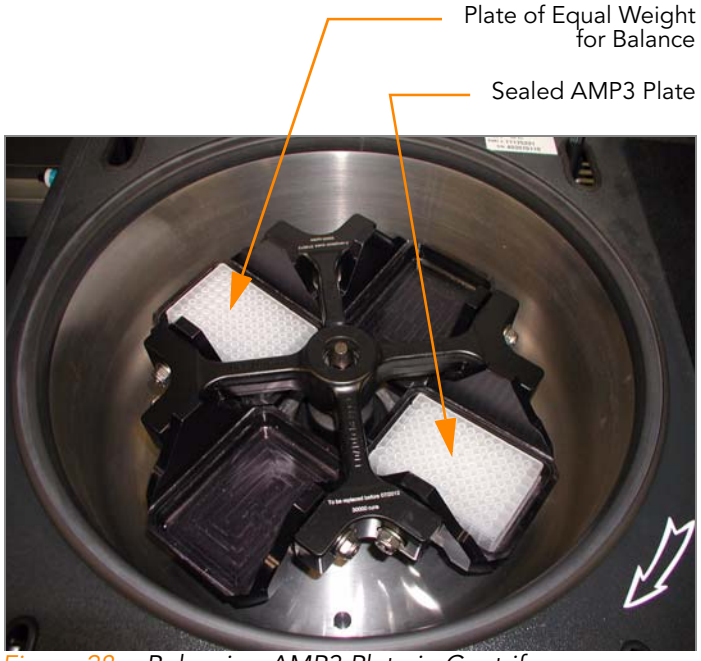


Figure 28 Balancing AMP3 Plate in Centrifuge

11. Centrifuge to 3000 xg at 4°C for 20 minutes. When the spin finishes, immediately remove the AMP3 plate from the centrifuge.

    Perform the next steps immediately, to avoid dislodging the blue pellet. If any delay occurs, repeat the 20-minute centrifugation before proceeding.
12. Remove the cap mat.
13. Decant the supernatant by quickly inverting the AMP3 plate and smacking it down onto an absorbent pad appropriate for 2-propanol disposal.
14. 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 plate inverted. To ensure optimal performance while decanting. Do not allow supernatant in wells to pour into other wells.

15. Place the inverted, uncovered plate on a tube rack for 1 hour at room temperature to air dry the pellet (Figure 29).

At this point, blue pellets should be present at the bottoms of the wells.

Figure 29 Uncovered AMP3 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.

16. On the lab tracking form, record the start and stop times.

17. Discard unused reagents in accordance with facility standards.

18. Do one of the following:

- Proceed to *Resuspend the AMP3 Plate* on page 37.
- Heat-seal the AMP3 plate and store it at -15 to -25°C for up to 24 hours or -80°C for long-term storage.

This is a good stopping point in the process.

Resuspend the AMP3 Plate

Add RA1 to the AMP3 plate to resuspend the precipitated DNA samples.

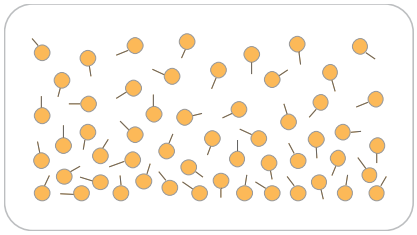


Figure 30 Resuspending DNA

Estimated Time

Hands-on time: ~30 minutes per 48 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	9 ml per 48 samples	-15 to -25°C	Illumina

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.

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.

Preparation

- RA1 is shipped frozen. Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- If you stored the AMP3 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)

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

Steps

1. Add 46 µl RA1 to each well of the AMP3 plate containing a DNA pellet.
2. Heat-seal the AMP3 plate with a foil seal.
3. Incubate it in the Illumina Hybridization Oven for 1 hour at 48°C.
4. On the lab tracking form, record the start and stop times.
5. Vortex the plate at 1800 rpm for 1 minute.
6. Pulse centrifuge to 280 xg.

   If you stored the pellets at -15 to -25°C for more than 72 hours after the Precip AMP3 process, you may need to repeat steps 3 to 6 until the pellets are completely resuspended.
7. Do one of the following:
   - Proceed to *Hybridize Duo BeadChip* on page 39. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
   - Store the sealed AMP3 plate and the RA1 at -15 to -25°C (-80°C if storing for more than 24 hours).

This is a good stopping point in the process.

Hybridize Duo BeadChip

In this process, you dispense the resuspended, denatured DNA samples onto BeadChips. First, the BeadChips are placed into Hyb Chamber inserts. Each slide is loaded with two DNA samples. Place the inserts into the Hyb Chambers. Incubate the Hyb Chambers in the Illumina Hybridization Oven for 16–24 hours at 48°C.

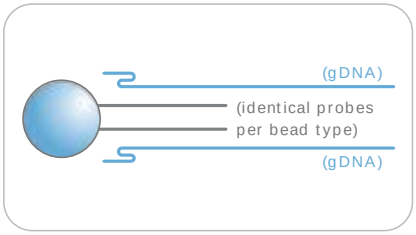


Figure 31 Hybridizing DNA to BeadChip

Estimated Time

Hands-on time:

- ~30 minutes for 8 BeadChips (16 samples)
- ~45 minutes for 16 BeadChips (32 samples)
- ~1 hour for 24 BeadChips (48 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 16 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

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.

- If you plan to perform the XStain process tomorrow, begin thawing the XC4 reagent. For instructions, see *Resuspend XC4 Reagent for XStain HD BeadChip* on page 49.
- 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.*
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - PB2 tube barcode(s)
- Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.

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

Steps

This protocol includes the following steps:

- *Assemble Hyb Chambers* on page 40
- *Hybridize Sample to Duo BeadChip* on page 43
- *Load BeadChip* on page 44
- *Set Up Duo BeadChip for Hyb* on page 46

Perform the Hyb Chamber assembly near the Hyb Oven to minimize the distance you need to move the BeadChip-loaded Hyb Chamber. Take care to 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.

Assemble Hyb Chambers

1. For every 4 BeadChips, place the following items on the bench top (Figure 32):
   - BeadChip Hyb Chambers (1)

- Hyb Chamber Gaskets (1)
- BeadChip Hyb Chamber Inserts (4)

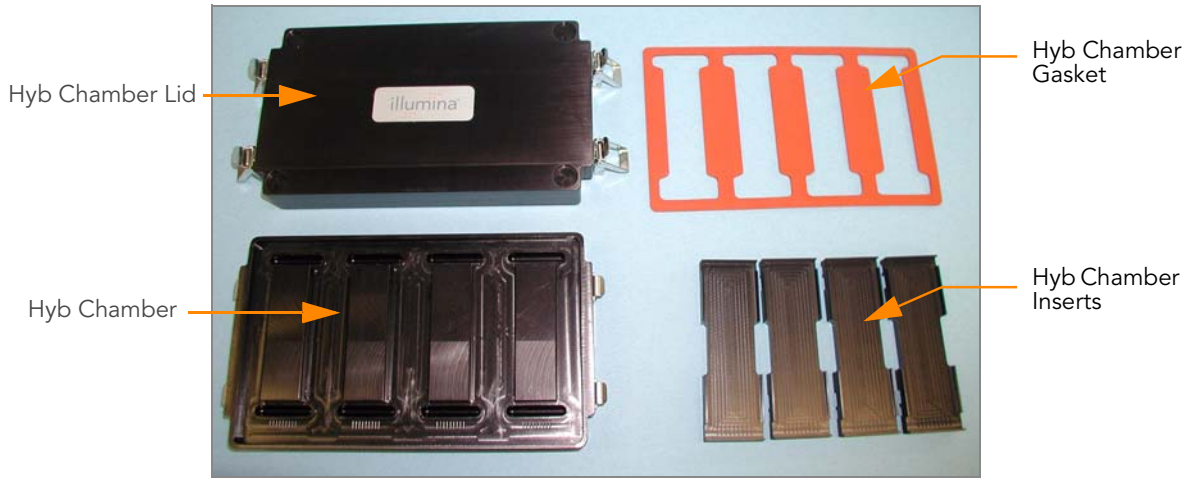


Figure 32 BeadChip Hyb Cartridge Components

2. Place the BeadChip Hyb Chamber gasket into the BeadChip Hyb Chambers:
   a. Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber (Figure 33).

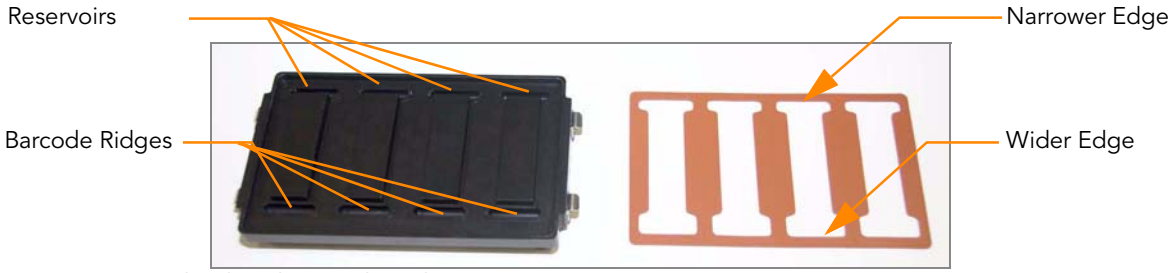


Figure 33 Hyb Chamber and Gasket

   b. Lay the gasket into the Hyb Chamber (Figure 34), and then press it down all around.

Figure 34 Placing Gasket into Hyb Chamber

c. Make sure the Hyb Chamber gasket is properly seated (Figure 35).

Figure 35 Hyb Chamber with Gasket in Place

3. Dispense 400 µl PB2 into each of the humidifying buffer reservoirs in the Hyb Chamber (Figure 36).

Figure 36 Dispensing PB2 into Hyb Chamber Reservoirs

4. Close and lock the BeadChip Hyb Chamber lid (Figure 37).
   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 37 Sealing the Hyb Chamber

5. Leave the closed Hyb Chamber on the bench at room temperature until the BeadChips are loaded with DNA sample.

Hybridize Sample to Duo BeadChip

In this step, you will denature and consolidate the DNA sample.

1. Place the resuspended AMP3 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. Remove the BeadChips from 2 to 8°C storage but do not unpackage.
3. After the 20-minute incubation, pulse centrifuge the AMP3 plate to 280 xg. Remove the foil seal.
4. Combine the two separate wells back into the original well. Refer to Figure 38:
   a. Combine contents of well A2 back into well A1.
   b. Combine contents of well A4 back into well A3.
   c. Combine contents of well A6 back into well A5.
   d. Combine contents of well A8 back into well A7.
   e. Combine contents of well A10 back into well A9.
   f. Combine contents of well A12 back into well A11.
   g. Repeat for rows B–H.

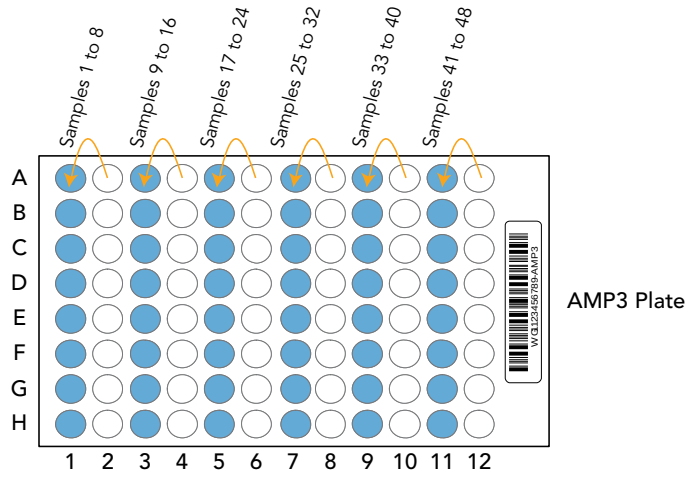


Figure 38 Consolidating Sample Back Into Original Sample Well

Load BeadChip

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

CAUTION Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Avoid contacting the beadstripe area and sample inlets.

2. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the Hyb Chamber insert (Figure 39). Make sure you place each BeadChip into the Hyb Chamber insert prior to loading the DNA sample.

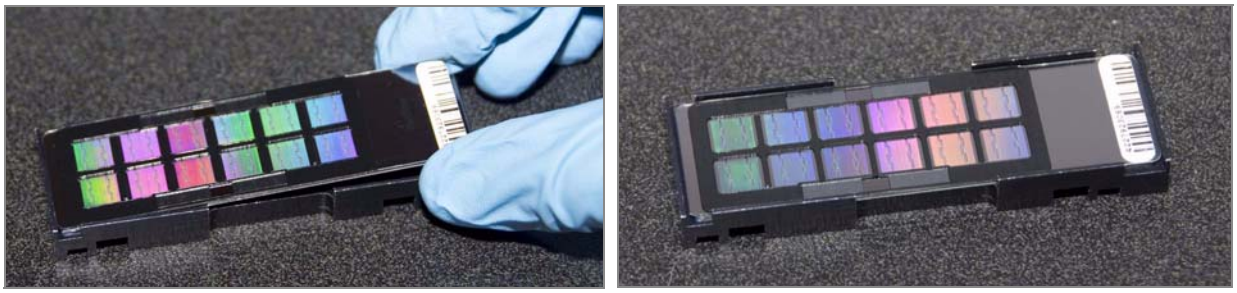


Figure 39 Placing BeadChips into Hyb Chamber Inserts

3. Dispense 84 µl of each DNA sample into the appropriate BeadChip inlet port according to the lab tracking form. See Figure 40 for a diagram and Figure 41 for a photo of the BeadChip.

CAUTION Load samples by directly placing pipette tips to the array surface. To avoid evaporation, proceed immediately to the next step as soon as all arrays have received sample.

a. Load samples in the A1 and B1 wells of the AMP3 plate into the first BeadChip.
b. Load samples in C1 and D1 into the second BeadChip.
c. Load samples in E1 and F1 into the third BeadChip.
d. Load samples in G1 and H1 into the fourth BeadChip.

Repeat the same pattern to transfer sample from column 3 to BeadChips 5–8, and from column 5 to BeadChips 9–12.

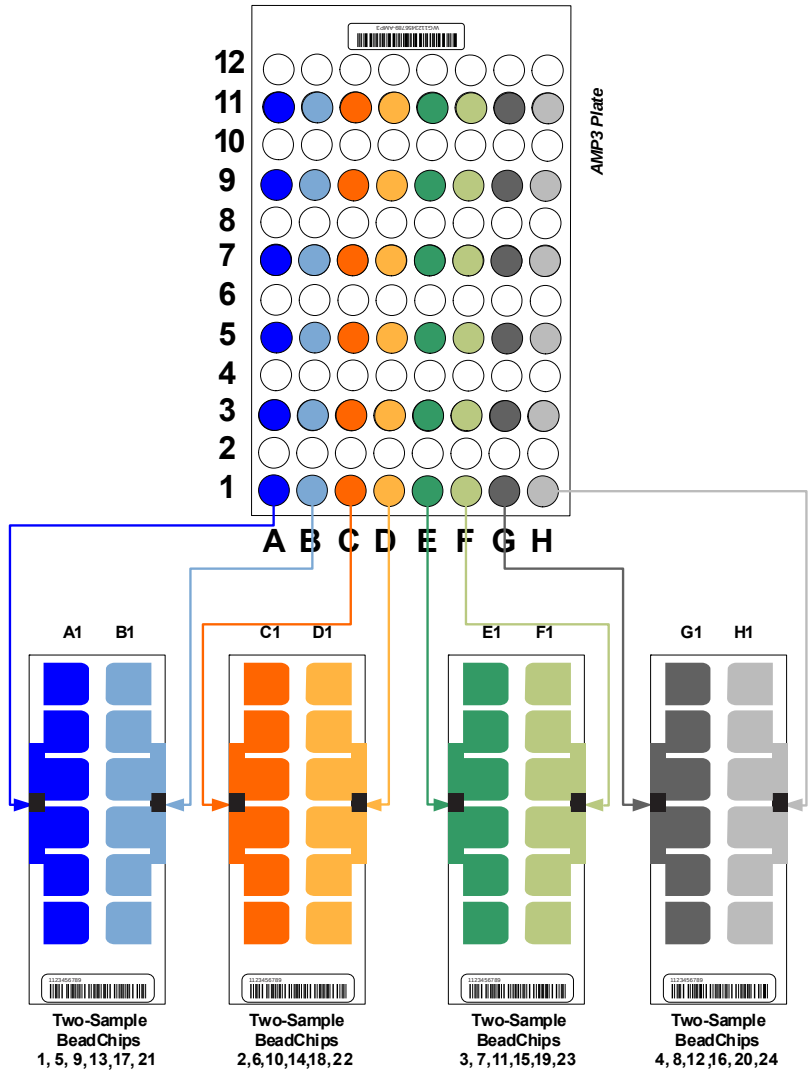


Figure 40 Distributing Sample to BeadChips (Pattern)

Figure 41 Dispensing Sample onto BeadChip (Photo)

4. On the lab tracking form, record the BeadChip barcodes associated with each sample well.
5. Visually inspect all sections of the BeadChips to ensure the DNA sample covers all of each bead stripe. Record any sections that are not completely covered.

Set Up Duo BeadChip for Hyb

CAUTION

For optimal performance, keep the Hyb Chamber inserts containing BeadChips steady and level when lifting or moving. Avoid shaking and keep parallel to the lab bench at all times. Avoid contacting the beadstripe area and sample inlets.

1. Load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chamber (Figure 42). Position the barcode end over the ridges indicated on the Hyb Chamber.

Figure 42 Placing Hyb Chamber Inserts into Hyb Chamber

2. Place the back side of the lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts (Figure 43).
3. Close the clamps on both sides of the Hyb Chamber.

Figure 43 Securing Hyb Chamber Lid

NOTE

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

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

Figure 44 Hyb Chamber Correctly Placed in Hyb Oven

5. If you are loading multiple Hyb Chambers, stack them on top of each other. You can stack up to 3 Hyb Chambers, for a total of 6 in the 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.

Figure 45 Two Hyb Chambers Correctly Placed in Hyb Oven

Figure 46 Incorrectly Placed Hyb Chamber

6. Start the rocker (optional).

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

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.

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. You can store it at 2 to 8°C overnight, but thaw it again before use.

5. Proceed to *Wash BeadChips* on page 50.

Wash BeadChips

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

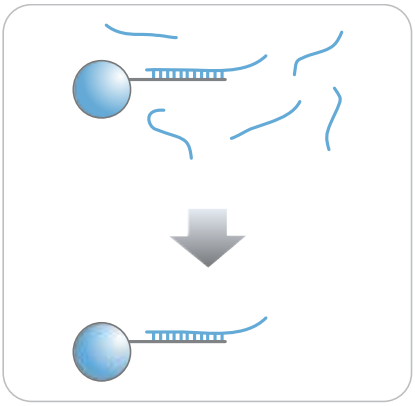


Figure 47 Washing BeadChip

Estimated Time

Hands-on time:

- 20 minutes per 8 BeadChips (16 samples)
- 30 minutes per 16 BeadChips (32 samples)
- 50 minutes per 24 BeadChips (48 samples)

Consumables

Item	Quantity (per 8 BeadChips)	Storage	Supplied By
PB1	550 ml	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1		Illumina
Te-Flow Flow-Through Chambers (with black frames, spacers, glass back plates, and clamps)	1 per BeadChip		Illumina
Wash Dish	8 BeadChips: 2 dishes 24 BeadChips: 6 dishes		Illumina
Wash Rack	8 BeadChips: 1 rack 24 BeadChips: 3 racks		Illumina

NOTE

Only pour out the recommended volume of PB1 needed for the suggested number of samples listed in the consumables table. Additional PB1 is used later in the XStain HD BeadChip step. One bottle of PB1 should be used per 8 BeadChips.

Preparation

- Fill 2 wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".
- Fill the BeadChip Alignment Fixture with 150 ml PB1.
- Separate the clear plastic spacers from the white backs.
- Clean the glass back plates according to the directions in the *Infinium Assay Lab Setup and Procedures Guide*.
- On the lab tracking form record:
  - Date/Time
  - Operator
  - Robot
  - PB1 bottle barcode

NOTE

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

Steps Wash BeadChip

1. Remove each Hyb Chamber from the Illumina Hybridization Oven.
2. Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1 (Figure 48).
3. Remove the Hyb Chamber inserts from the Hyb Chambers.
4. Remove BeadChips from the Hyb Chamber inserts one at a time.

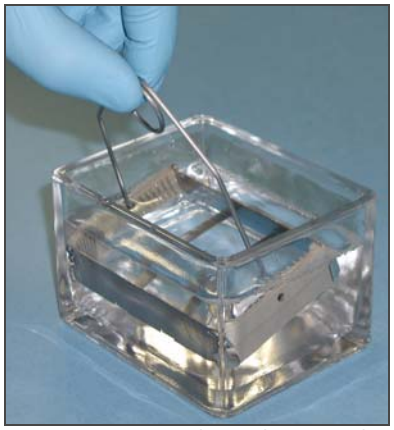


Figure 48 Wash Rack in Wash Dish Containing PB1

5. Remove the IntelliHyb seal from each BeadChip as follows:

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, rapid 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.

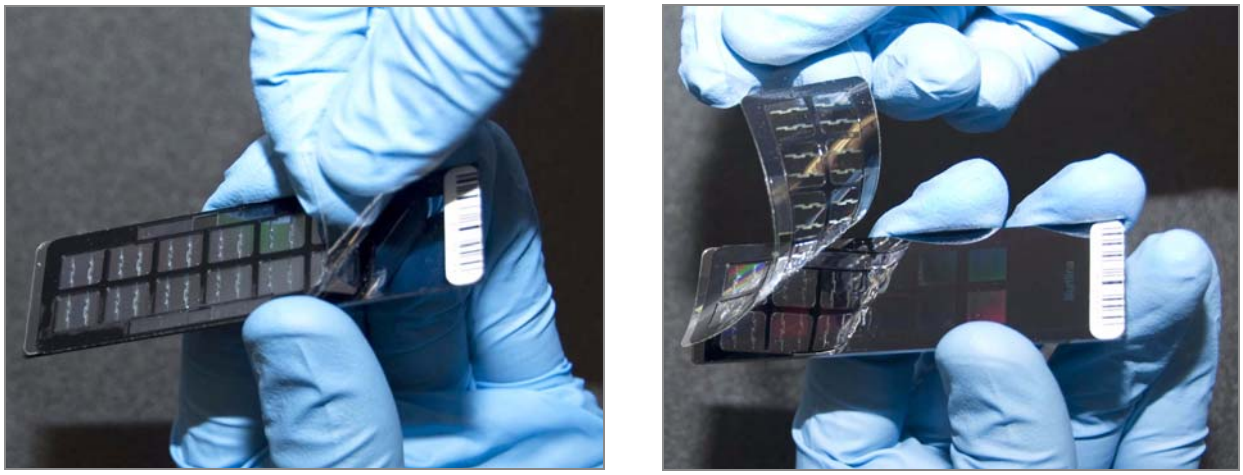


Figure 49 Removing the Coverseal

CAUTION

Do not touch the arrays!

6. 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 50).

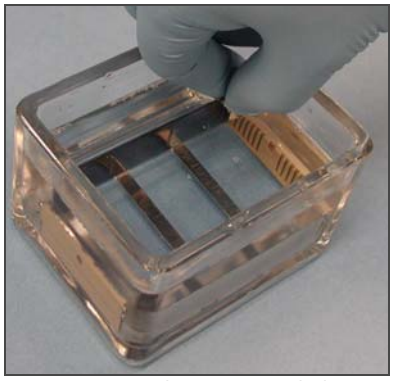


Figure 50 Placing BeadChips in Wash Dish Containing PB1

7. Repeat steps 5 and 6 until all BeadChips are transferred to the submerged wash rack. The wash rack holds up to 8 BeadChips.
8. 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.
9. Move the wash rack to the other wash dish containing PB1. Make sure the BeadChips are completely submerged.
10. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.
11. If you are processing more than 8 BeadChips:
    a. Complete the steps in the next section, *Assemble Flow-Through Chambers*, for the first eight BeadChips.
    b. Place the assembled Flow-Through Chambers of the first eight BeadChips on the lab bench in a horizontal position.

NOTE Keep them in a horizontal position on the lab bench until all assembled Flow-Through Chambers are ready to be loaded into the Do not place the Flow-Through Chambers in the Chamber Rack until all necessary steps are completed.

    c. Repeat steps 3 through 11 from this section for any additional BeadChips. Use new PB1 for each set of eight BeadChips.

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

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 for every additional set of eight BeadChips.

1. If you have not done so yet, fill the 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 51) pre-filled with PB1 (see *Preparation* on page 51).

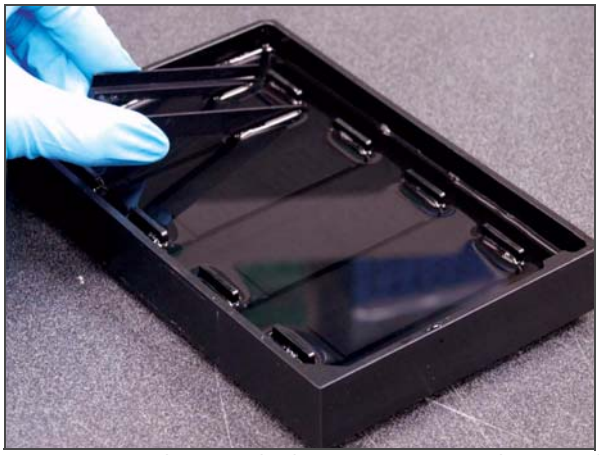


Figure 51 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

3. Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture (Figure 52). Each BeadChip should be fully immersed in PB1.

Figure 52 Placing BeadChip into Black Frame on Alignment Fixture

4. Place a clear spacer onto the top of each BeadChip to be processed (Figure 53). 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.

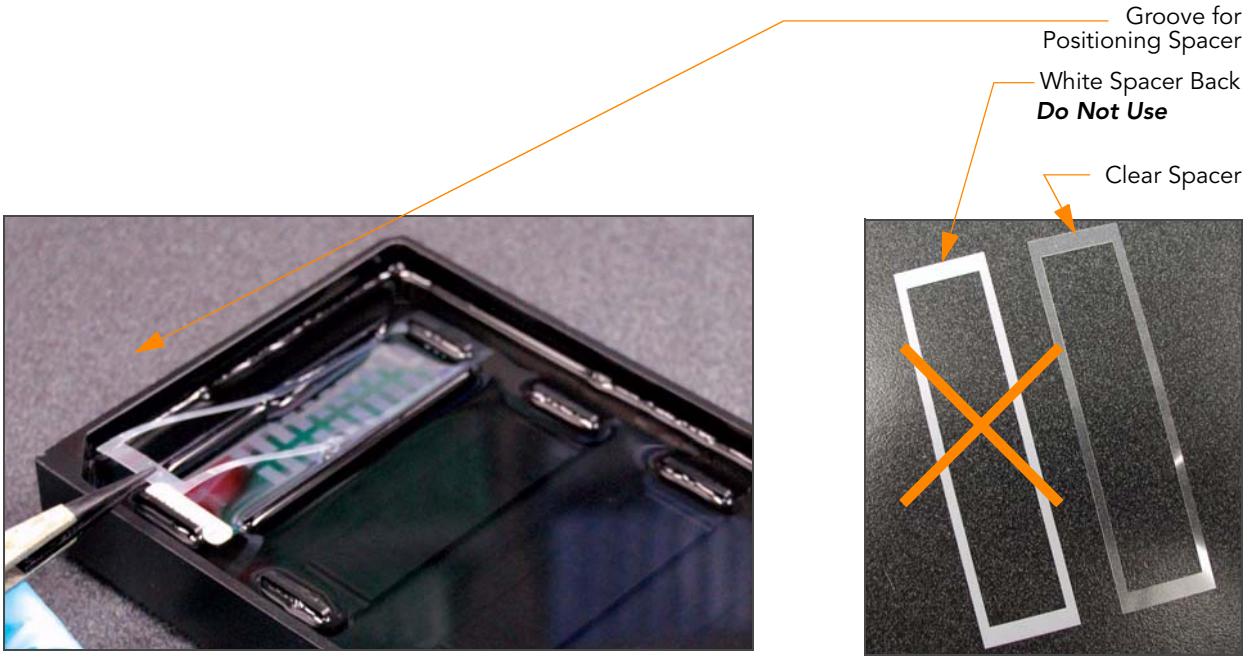


Figure 53 Placing Clear Plastic Spacer onto BeadChip

5. Place the Alignment Bar onto the Alignment Fixture (Figure 54).

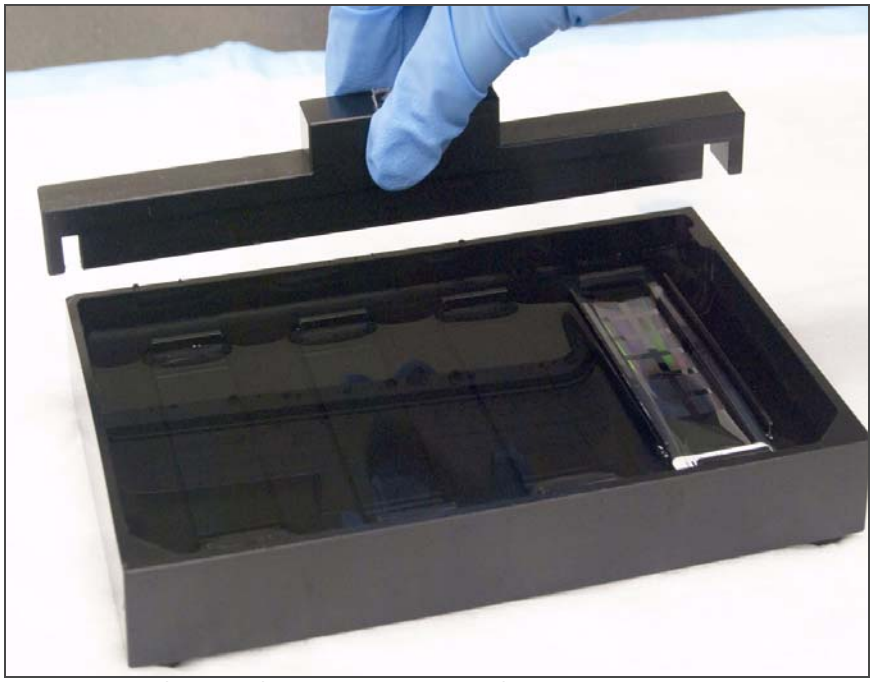


Figure 54 Placing Alignment Bar onto Alignment Fixture

6. Use a 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 55).

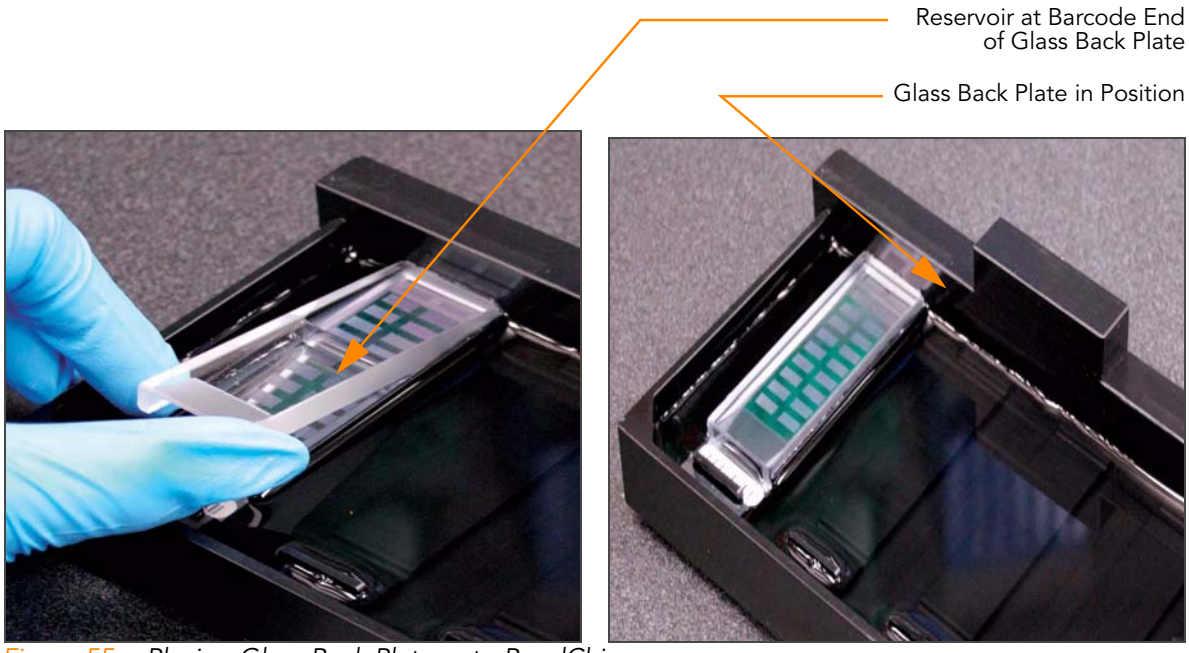


Figure 55 Placing Glass Back Plate onto BeadChip

8. Attach the metal clamps to the Flow-Through Chambers as follows (Figure 56):
   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.

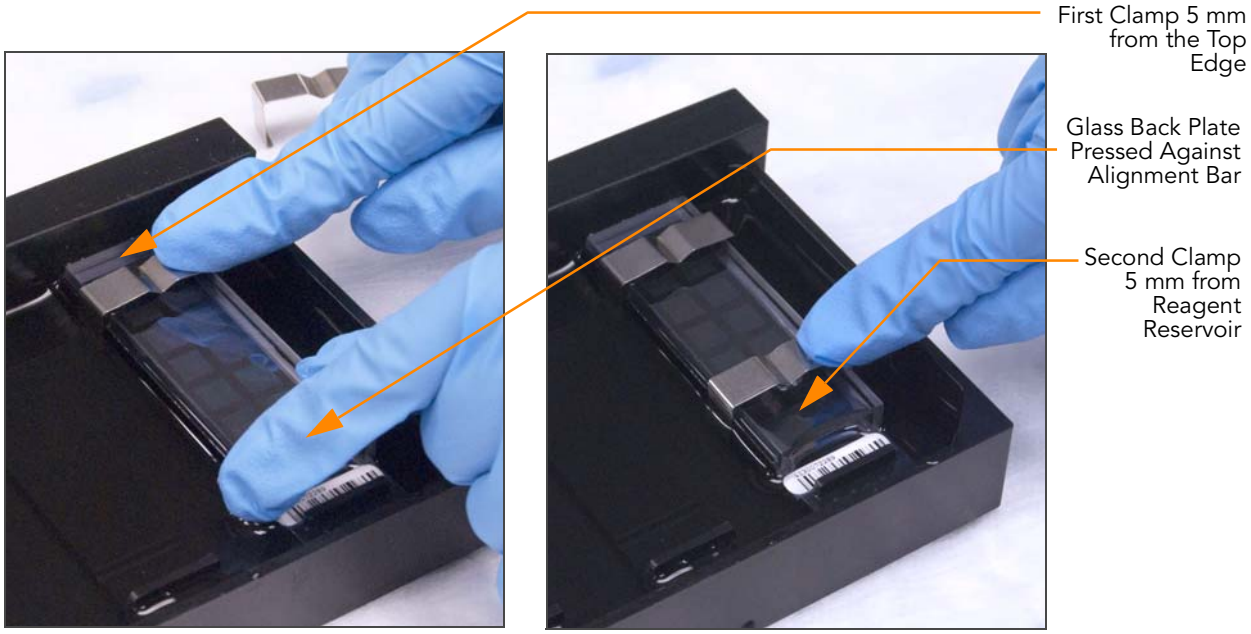


Figure 56 Securing Flow-Through Chamber Assembly with Metal Clamps

9. Using scissors, trim the ends of the clear plastic spacers from the Flow-Through Chamber assembly (Figure 57):

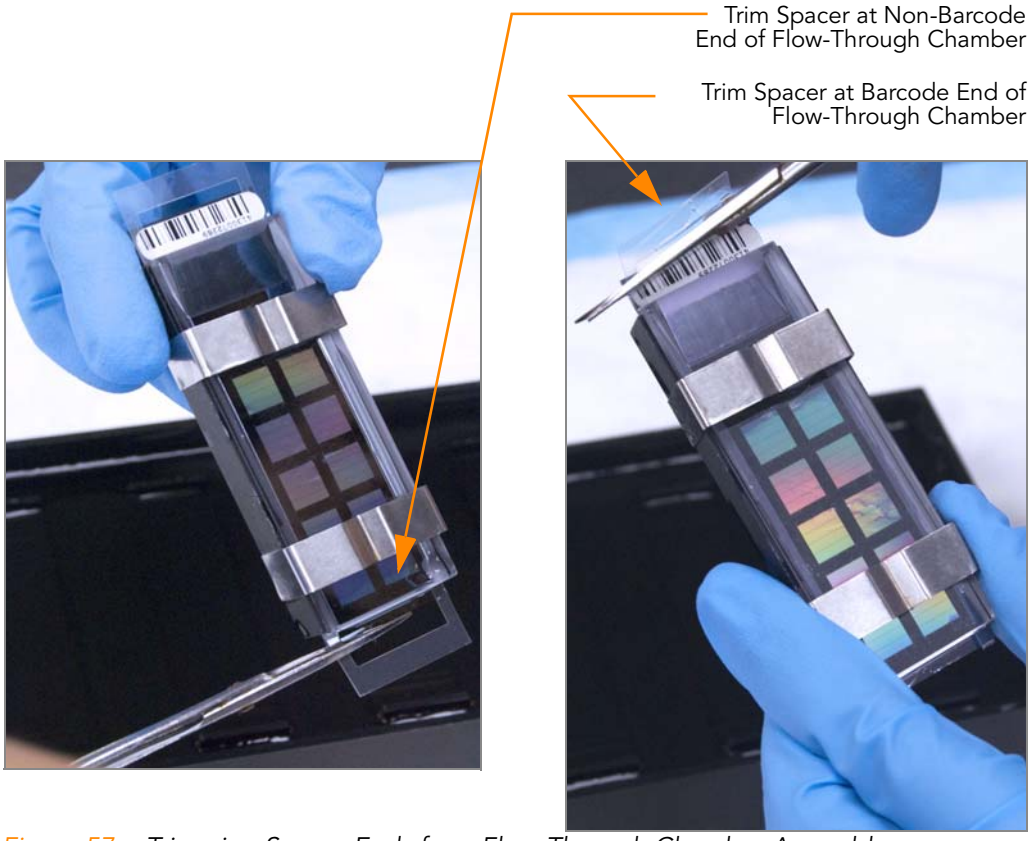


Figure 57 Trimming Spacer Ends from Flow-Through Chamber Assembly

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.

10. Immediately wash the Hyb Chamber reservoirs with dH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains.
11. Discard unused reagents in accordance with facility standards.
12. Proceed to *Single-Base Extension and Stain HD BeadChip* on page 59.

Single-Base Extension and Stain HD BeadChip

In this process, RA1 reagent is used to wash away unhybridized and non-specifically hybridized DNA sample. TEM reagent is dispensed into the Flow-Through Chambers to extend primers hybridized to DNA on the BeadChip. This reaction incorporates labeled nucleotides into the extended primers. 95% formamide/1 mM EDTA is then 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. Finally, the BeadChips are removed from the Flow-Through Chambers, washed in the PB1 reagent, coated with XC4, and dried.

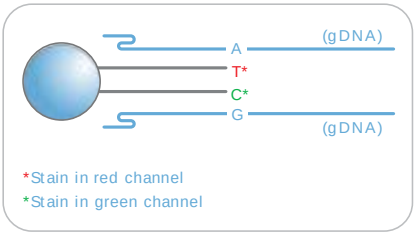


Figure 58 Extending and Staining BeadChip

Estimated Time

Hands-on time: ~3 hours for 8–24 BeadChips

Dry time: 55 minutes

Consumables

Item	Quantity	Storage	Supplied By
RA1	10 ml for 1–8 BeadChips 20 ml for 9–16 BeadChips 30 ml for 17–24 BeadChips	-15 to -25°C	Illumina
XC1	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
XC2	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
TEM	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
XC3	50 ml for 1–8 BeadChips 100 ml for 9–16 BeadChips 150 ml for 24 BeadChips	Room temperature	Illumina
STM (Make sure that all STM tubes indicate the same stain temperature on the label)	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina

Item	Quantity	Storage	Supplied By
ATM	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
PB1	310 ml for 1–8 BeadChips 285 ml for 9– 24 BeadChips	Room temperature	Illumina
XC4	310 ml for 1–8 BeadChips 285 ml for 9– 24 BeadChips	-15 to -25°C	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

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.

Preparation

- RA1 is shipped and stored at -15 to -25°C. Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- Place all reagent tubes in a rack in the order in which they will be used (Figure 59). If frozen, allow them to thaw to room temperature and centrifuge to 3000 xg for 3 minutes.

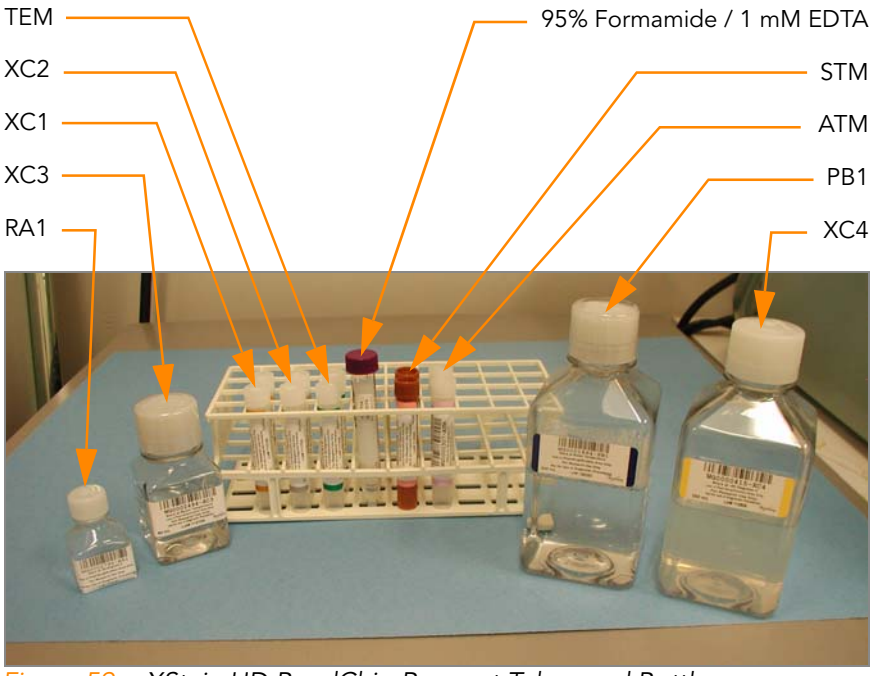


Figure 59 XStain HD BeadChip Reagent Tubes and Bottles

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

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, VWR part # 110-229.
2. Turn on the water circulator and set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.

   This temperature may vary depending on facility ambient conditions.

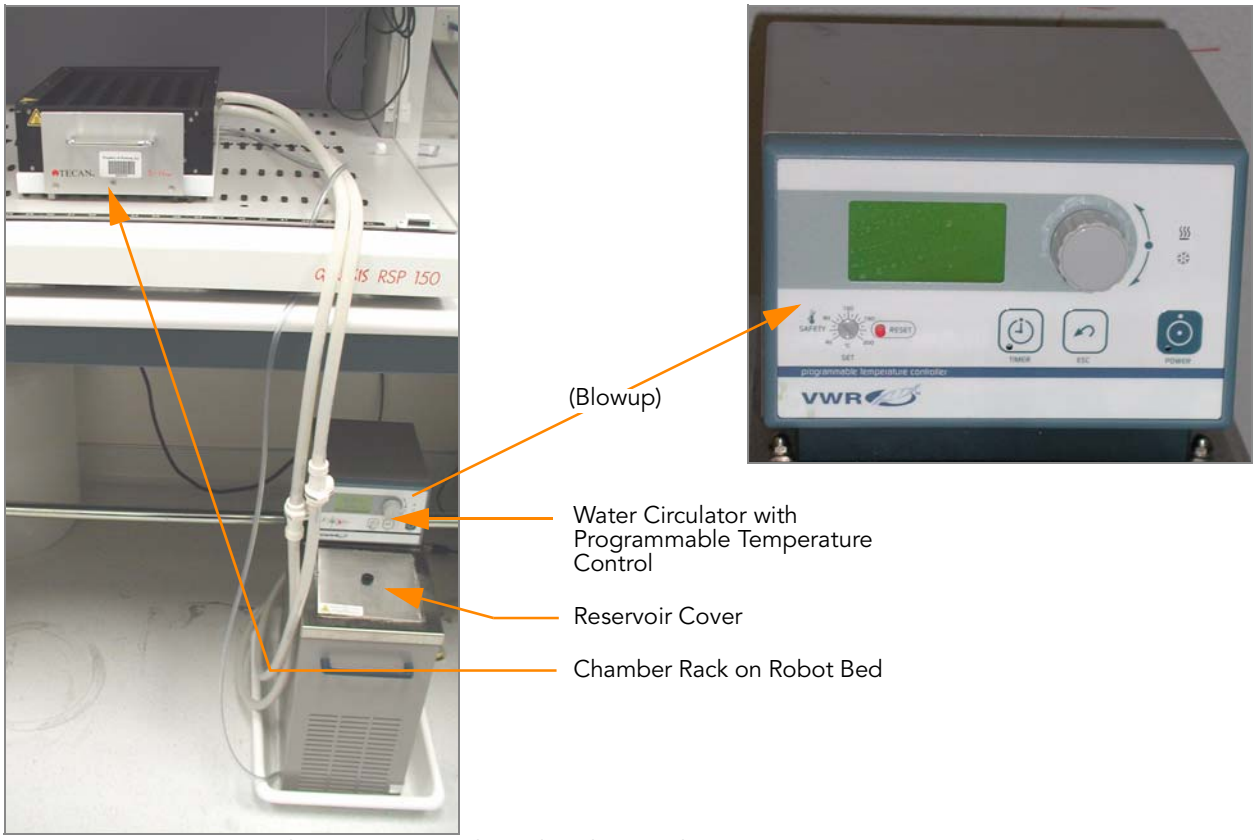


Figure 60 Water Circulator Connected to Chamber Rack

3. The temperature displayed on the water circulator LCD screen may differ from the actual temperature in the Chamber Rack. Confirm this using the temperature probe for the Chamber Rack.
4. Remove the bubbles trapped in the Chamber Rack. You must do this every time you run this process. Follow instructions in the Te-Flow (Tecan Flow-Through Module) Operating Manual, Tecan Doc ID 391584.
5. Use the Illumina temperature probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 61).

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

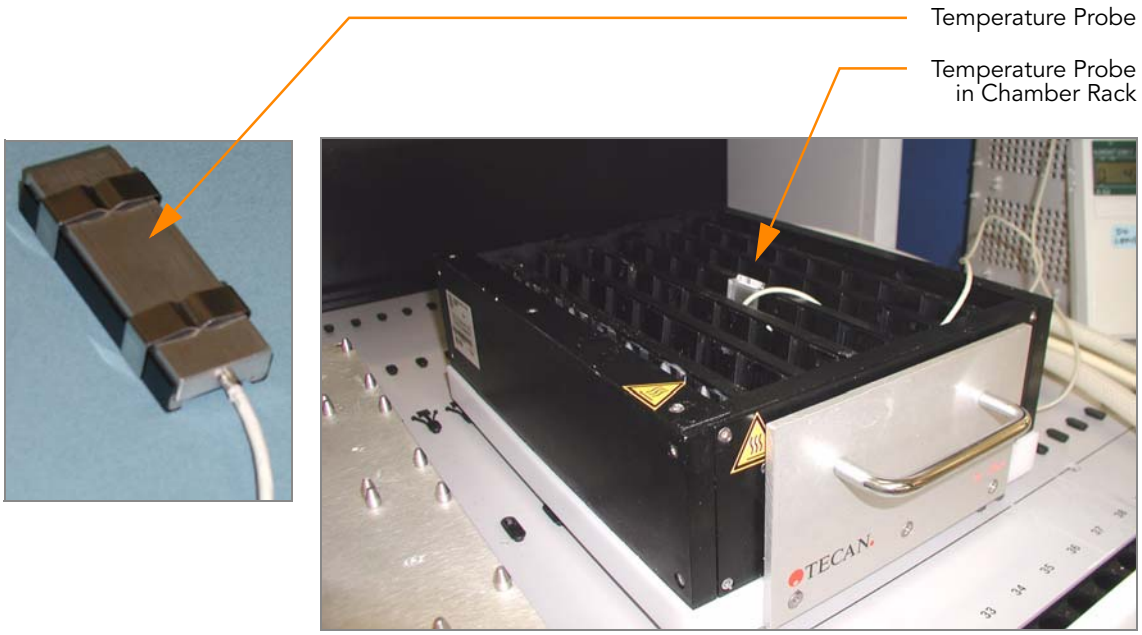


Figure 61 Illumina Temperature Probe in Chamber Rack

6. For accurate temperature measurement, ensure the temperature probe is touching the floor of the Chamber Rack.

Steps

This protocol includes the following steps:

- *Single-Base Extension* on page 63
- *Stain BeadChip* on page 64
- *Wash and Coat 8 BeadChips* on page 65

CAUTION The remaining steps must be performed without interruption.

Single-Base Extension

1. When the Chamber Rack reaches 44°C, quickly place each Flow-Through Chamber assembly into the Chamber Rack.

   For 4 BeadChips, place the Flow-Through Chambers in every other position, starting at 1, in the first row of the Chamber Rack. For larger numbers of BeadChips, fill all positions in the first row, then the second and third.

2. 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.
4. Into the reservoir of each Flow-Through Chamber, dispense:
   a. 150 µl RA1 (Figure 62). Incubate for 30 seconds. Repeat 5 times.

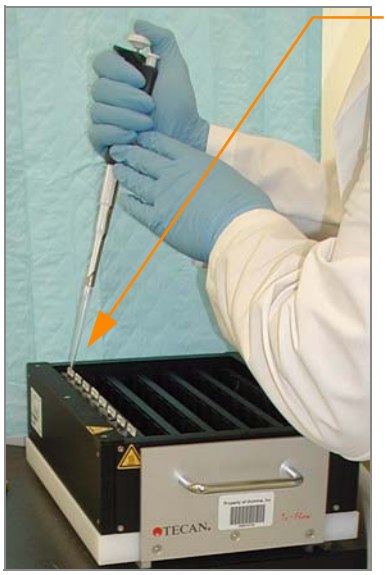


Figure 62 Dispensing RA1 into Each Flow-Through Chamber

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

b. 450 µl XC1. Incubate for 10 minutes.
c. 450 µl XC2. Incubate for 10 minutes.
d. 200 µl TEM. Incubate for 15 minutes.
e. 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat 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 µl XC3. Incubate for 1 minute. Repeat once.

5. 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 iScan or BeadArray Reader now to allow the lasers to stabilize.

2. Into the reservoir of each Flow-Through Chamber, dispense:
a. 250 µl STM and incubate for 10 minutes.
b. 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
c. 250 µl ATM and incubate for 10 minutes.
d. 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
e. 250 µl STM and incubate for 10 minutes.

f. 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
g. 250 µl ATM and incubate for 10 minutes.
h. 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
i. 250 µl STM and incubate for 10 minutes.
j. 450 µl 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 equipment on the lab bench:
   - 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 as shown (Figure 63).
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. 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.

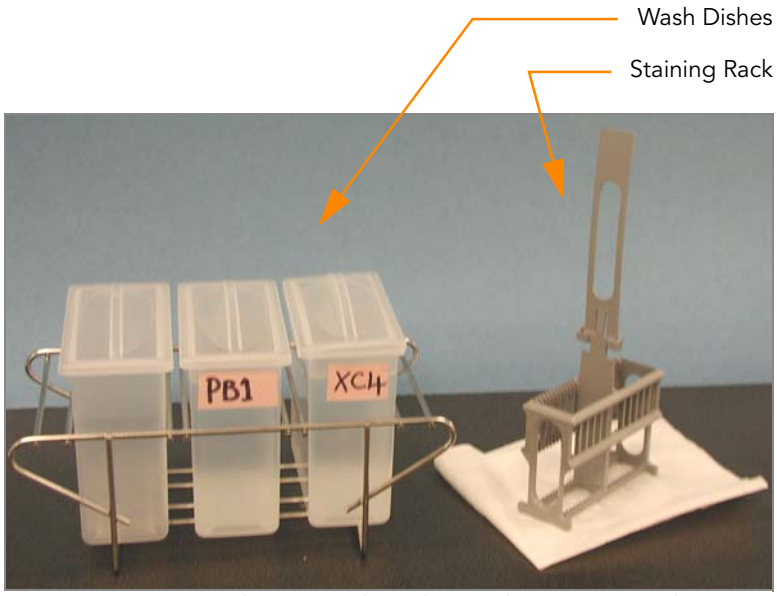


Figure 63 PB1 and XC4 Wash Dishes with Staining Rack

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 facing you. This orients the staining rack so that you can safely remove the BeadChips.

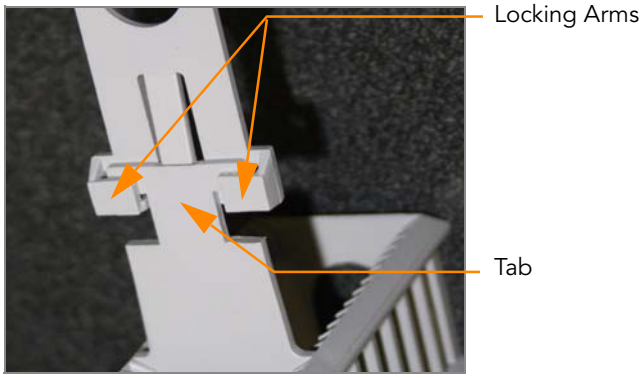


Figure 64 Staining Rack Locking Arms and Tab

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 65).

CAUTION

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

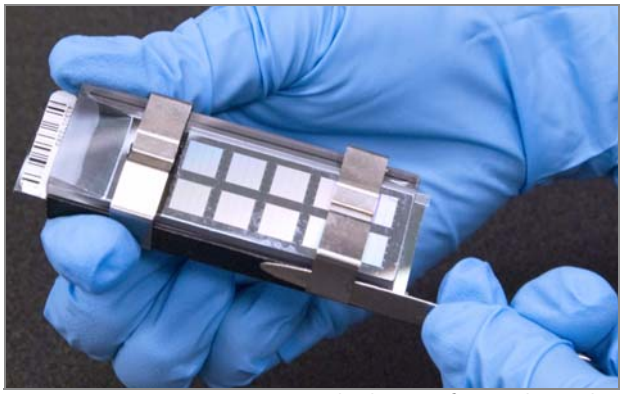


Figure 65 Removing Metal Clamps from Flow-Through Chamber

b. Remove the glass back plate and set it aside.
c. 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.

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 BeadChip barcodes are correctly positioned in the staining rack, with the labels facing ***away*** from you. This is essential for proper handling and coating.

CAUTION

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

9. Ensure that the BeadChips are completely submerged.

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

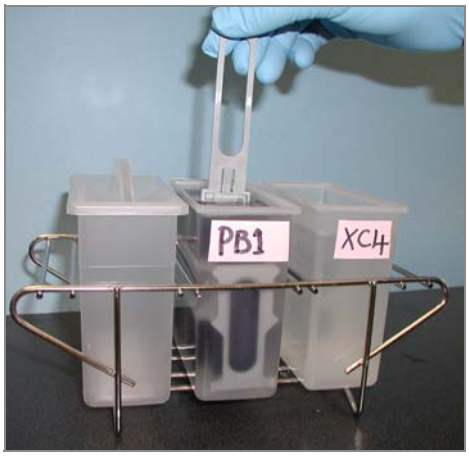


Figure 66 Washing BeadChips in PB1

NOTE

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

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

CAUTION

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

12. 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.

13. Remove the staining rack from the PB1 dish and place it directly into the wash dish containing XC4 (Figure 67). 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.

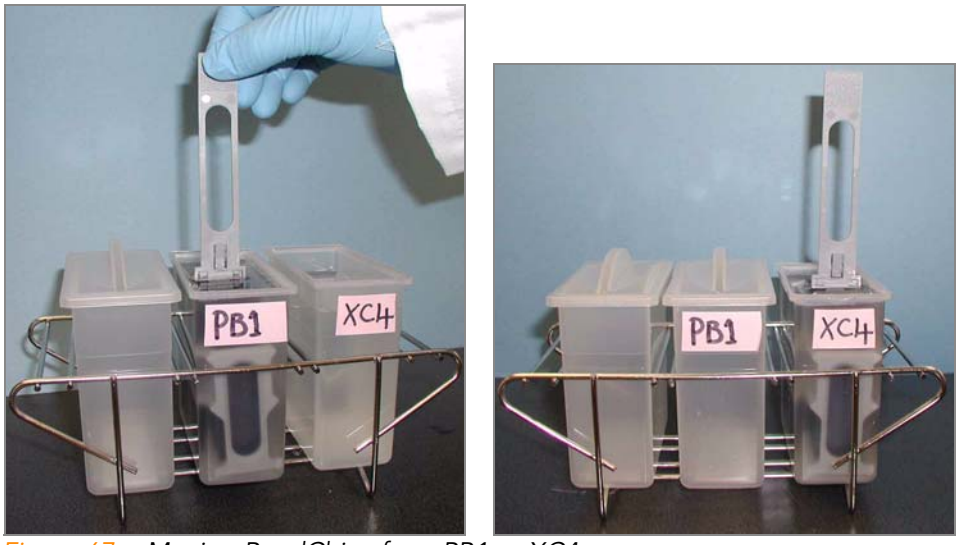


Figure 67 Moving BeadChips from PB1 to XC4

14. 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.

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

CAUTION

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

16. Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.
17. Prepare one additional tube rack per 8 BeadChips that fits the internal dimensions of the vacuum desiccator.
18. Remove the staining rack in one smooth, rapid motion and place it directly on prepared tube rack, making sure the barcodes face ***up*** and the locking arms and tab face ***down*** (Figure 68).

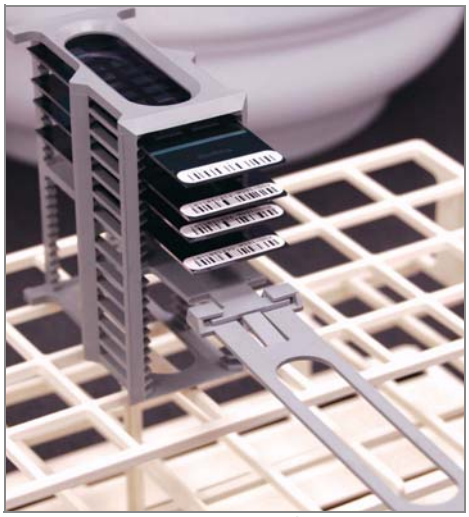


Figure 68 Staining Rack in Correct Orientation

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

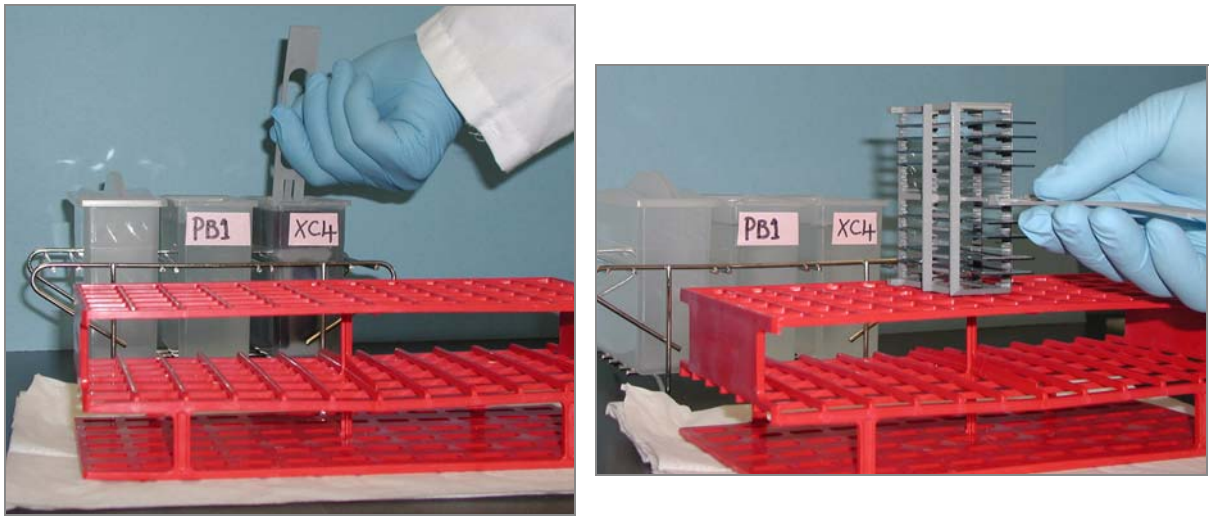


Figure 69 Moving the Staining Rack from XC4 to Tube Rack

20. 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 the BeadChip on a tube rack with the barcode facing up and towards you (Figure 71).

21. 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 70).

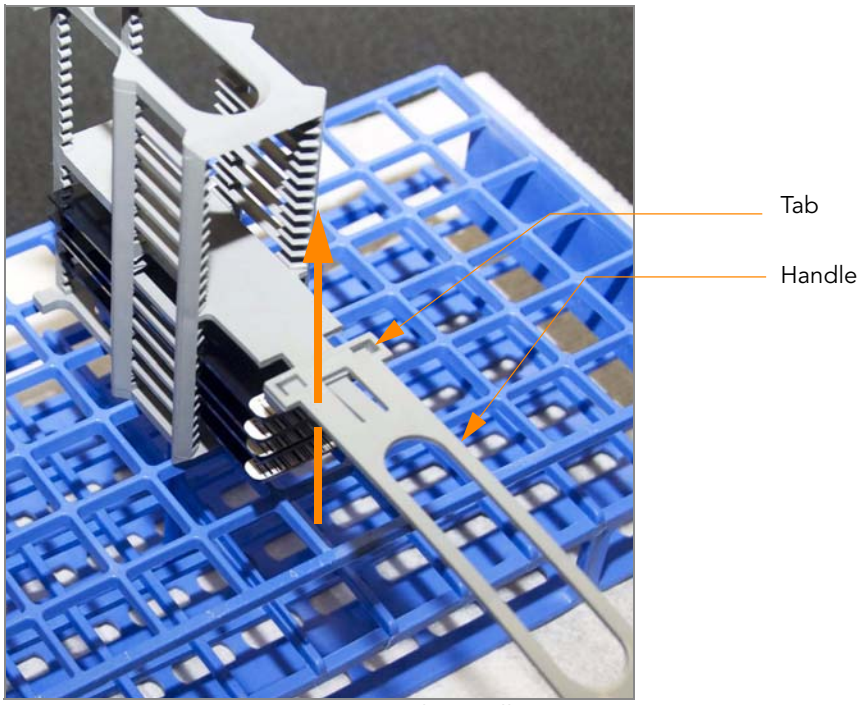


Figure 70 Removing Staining Rack Handle

22. Remove the remaining BeadChips to the tube rack as shown in Figure 71, 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.

Figure 71 Placing 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.

23. Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack (Eight BeadChips) (Figure 72).
24. Remove the red plug from the three-way valve before applying vacuum pressure.
25. Start the vacuum, using at least 508 mm Hg (0.68 bar).
26. To ensure that the dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.

Figure 72 Testing Vacuum Seal

27. Dry under vacuum for 50–55 minutes.

    Drying times may vary according to room temperature and humidity.
28. 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.

29. Store desiccator with the red valve plug in the desiccator's three-way valve to stop accumulation of dust and lint within the valve port.
30. 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.
31. 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 EtOH Wipe around your index finger.

b. Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
c. Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

Do not touch the stripes.

32. Clean the glass back plates. For instructions, see the *Infinium Assay Lab Setup and Procedures Guide*.
33. 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 humidifying buffer reservoirs.
34. Discard unused reagents in accordance with facility standards.
35. Do one of the following:
    - Proceed to *Image BeadChip on the iScan System* on page 74 or *Image BeadChip on the BeadArray Reader* on page 82.
    - 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: 35 minutes per BeadChip

Preparation

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

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

- For more information about the iScan System, GenomeScan software, or AutoLoader2, see the *iScan and AutoLoader2 System Guide*.

Steps

Overview

The GenomeScan 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 GenomeScan application.

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

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

Starting Up the iScan System

1. Turn on the iScan Reader and the attached PC.
2. Let the iScan Reader warm up for at least 5 minutes before beginning a scan. It is fine to use the GenomeScan software during this time.
3. For each BeadChip you plan to scan, copy the contents of the mini-CD provided with the BeadChip 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 79.
4. Double-click the GenomeScan icon on the desktop.

   The Welcome window appears (Figure 73). The GenomeScan 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.

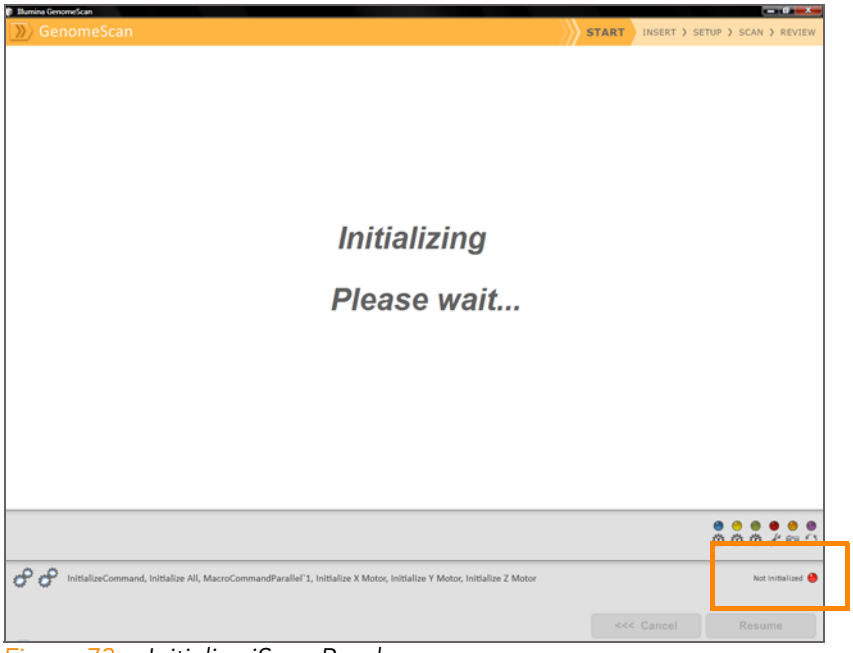


Figure 73 Initialize iScan Reader

5. Set the **LIMS** dropdown list to **None** and enter your Windows user name (Figure 74).

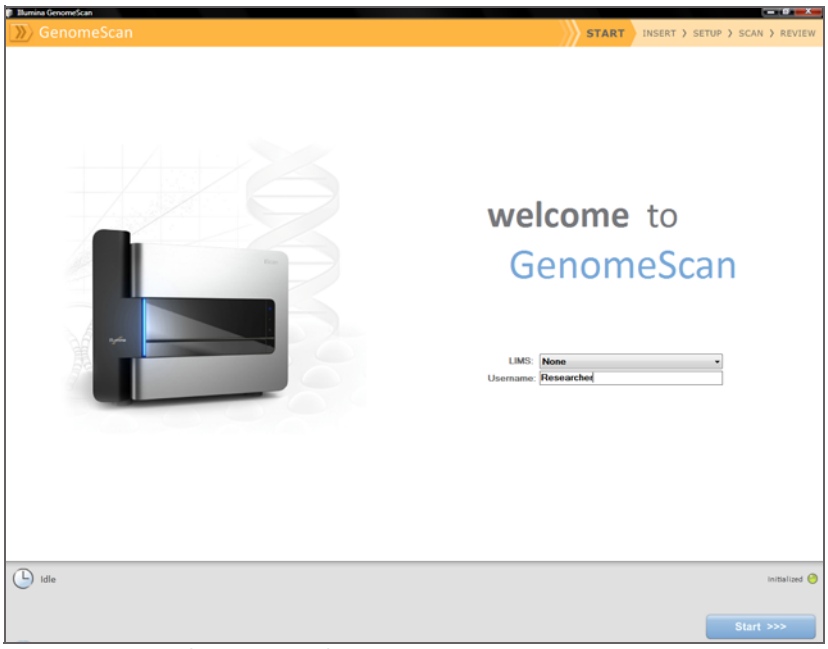


Figure 74 Welcome Window

6. 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 75).

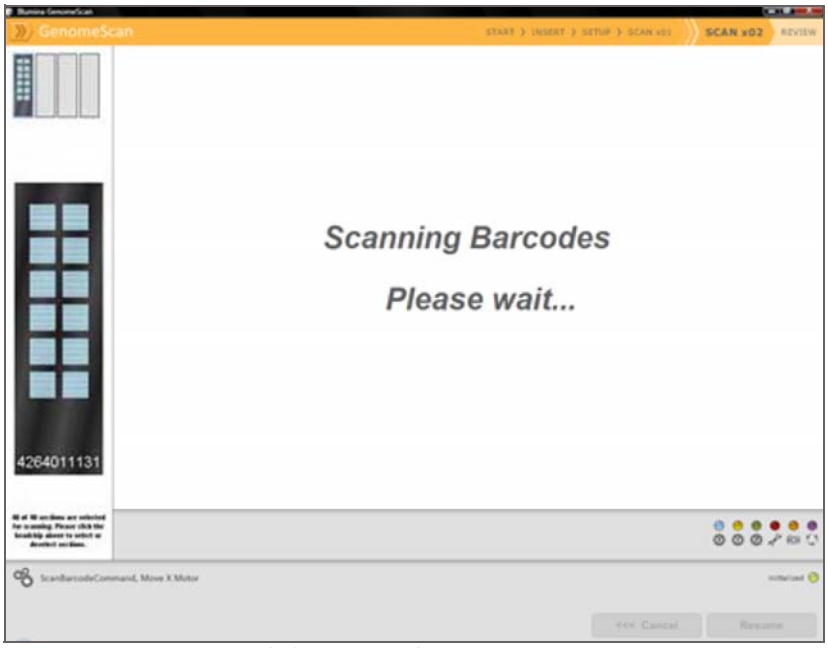


Figure 75 Scan BeadChip Barcodes

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.

For more information about the scan settings, see the *iScan and AutoLoader2 System Guide*.

2. 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 76). The section will no longer be highlighted blue.

Figure 76 Deselect BeadChip Sections

3. If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.
4. To begin scanning the BeadChips, click **Scan**.

   Scanning should take 8–10 minutes per BeadChip.

   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 80.
5. At the end of the scan, a Review window appears (Figure 77). The Scan Metrics table at the top shows the intensity values, registration, and focus metrics for each stripe on the BeadChip.

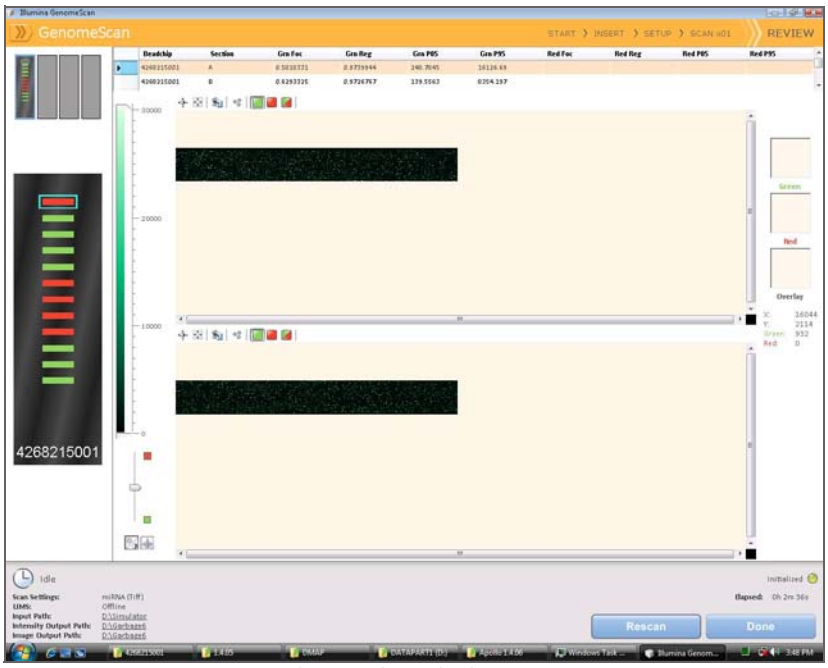


Figure 77 Review Window with Failed Stripes

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.

6. 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.

7. 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, no *.idat files will be saved for those sections. 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 GenomeScan software. Use another program such as Illumina's GenomeStudio software to view images from the scan.

Setting Up Input and Output Paths

This step should only occur once, when you install the GenomeScan 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 came on each BeadChip CD (for

example, D:\Decode). The GenomeScan software refers to this folder as the Input Path.

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

   During the scan, GenomeScan automatically creates subfolders 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 GenomeScan 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 78).

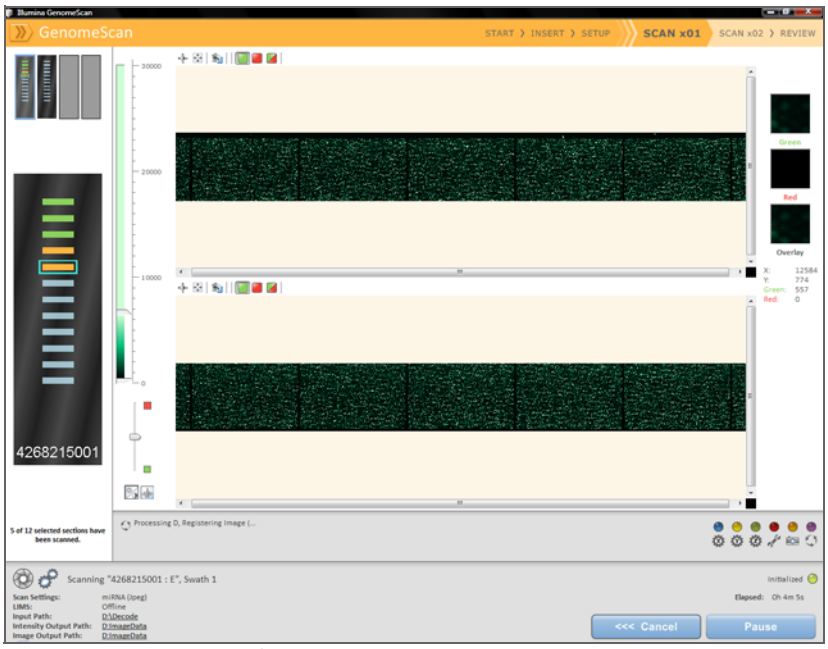


Figure 78 Monitor the Scan

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 register 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 warmup for the BeadArray Reader (first use of the day only)

45 minutes to scan each BeadChip using BeadScan 3.2 FastScan settings

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

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

Initializing the BeadArray Reader (Daily)

If this is the first time the scanner is being used today, follow these steps.

Refer to Figure 79 throughout this protocol.

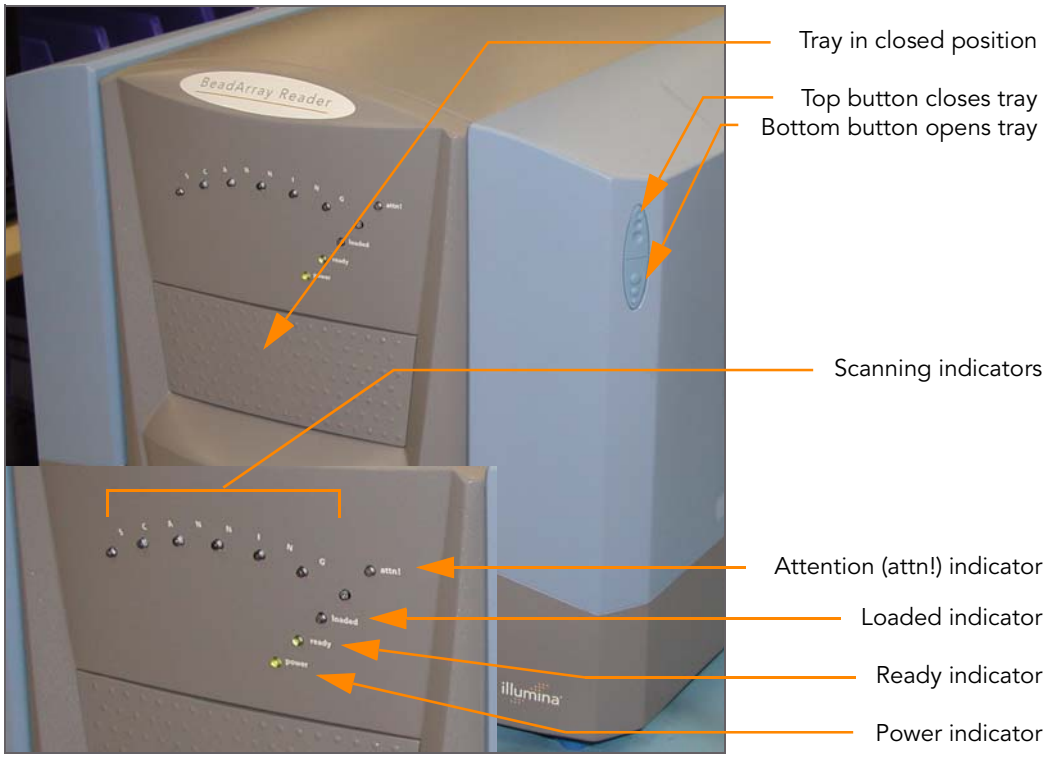


Figure 79 Illumina BeadArray Reader

Table 8 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 80).

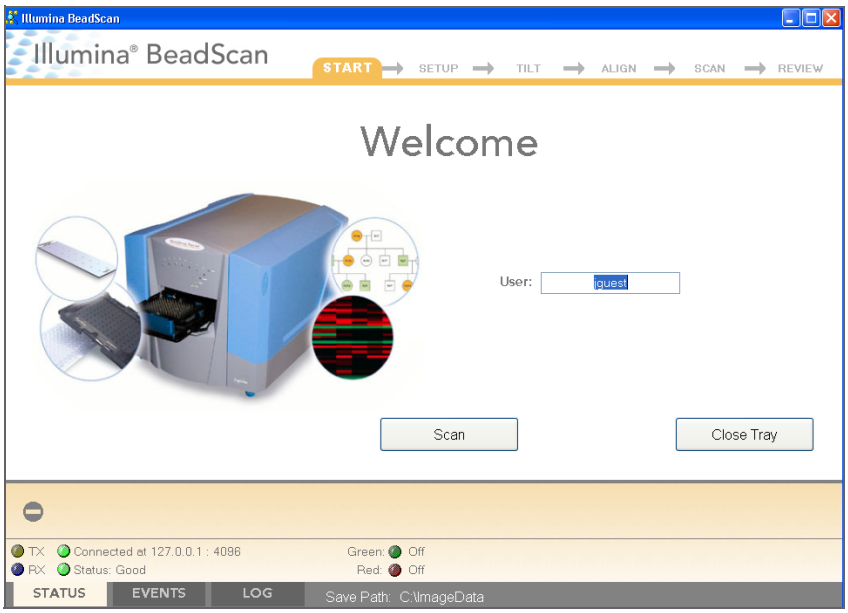


Figure 80 BeadScan Welcome Screen

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 81).

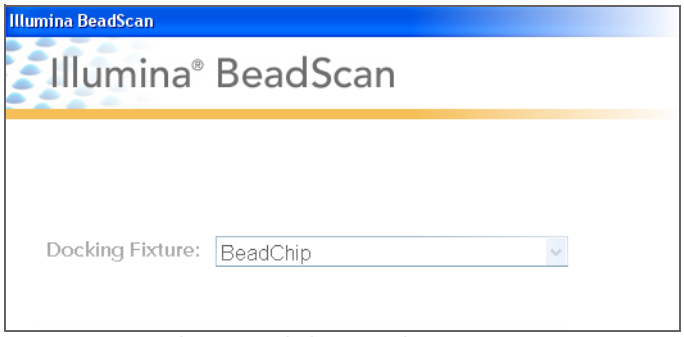


Figure 81 Select BeadChip Docking Fixture

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 from the BeadChip CD. The default path is C:\DecodeData.
3. If either path is not correct:
   a. Click **Edit** to open the Options dialog box.

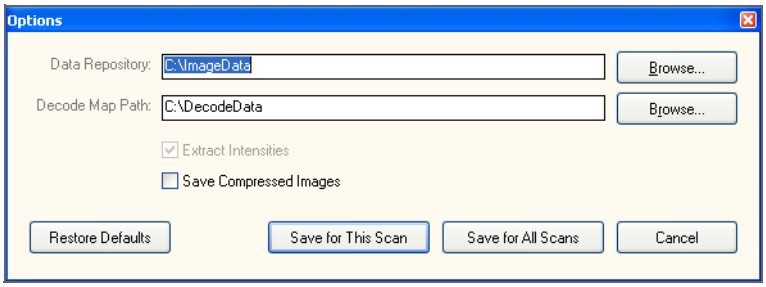


Figure 82 Enter Data Repository and Decode Data Paths

b. Click **Browse** to navigate to and select the Data Repository path and the Decode Map path.

c. Select or clear the **Save Compressed Images** check box. Compressed images use the JPG format. Uncompressed images use the TIFF format and may be 75 MB or more.

d. After changing settings, click either **Save for this Scan** or **Save for All Scans**.

4. Copy the decode map (*.dmap) files for each BeadChip from the BeadChip CD to the Decode Map path directory.

5. For each BeadChip:

a. Place the BeadChip into the BeadArray Reader tray.

b. Using the hand-held barcode scanner, scan the BeadChip barcode. The barcode appears on the screen in the position corresponding to the BeadChip position in the tray.

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

d. Select the appropriate scan method and click **Select**.

6. Make sure that the BeadChips are properly seated in the BeadArray Reader tray (Figure 83).

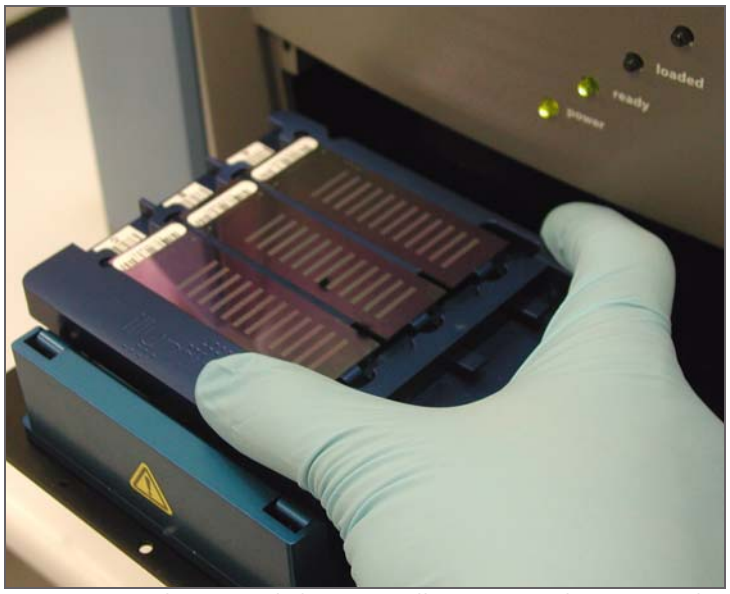


Figure 83 Place BeadChips into Illumina BeadArray Reader Tray

7. Click **Scan**.

Scanning Process

BeadScan begins the BeadArray Reader Tilt and Align processes (Figure 85):

- **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.

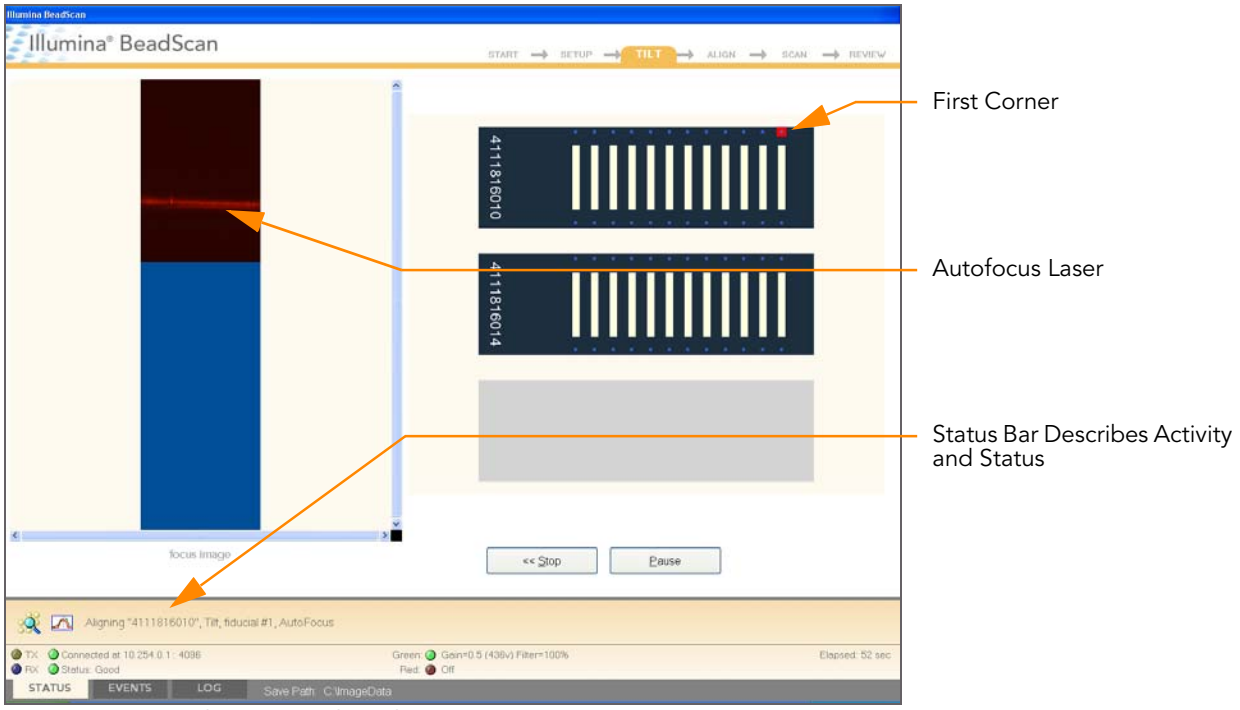


Figure 84 BeadArray Reader Tile Process

- **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.

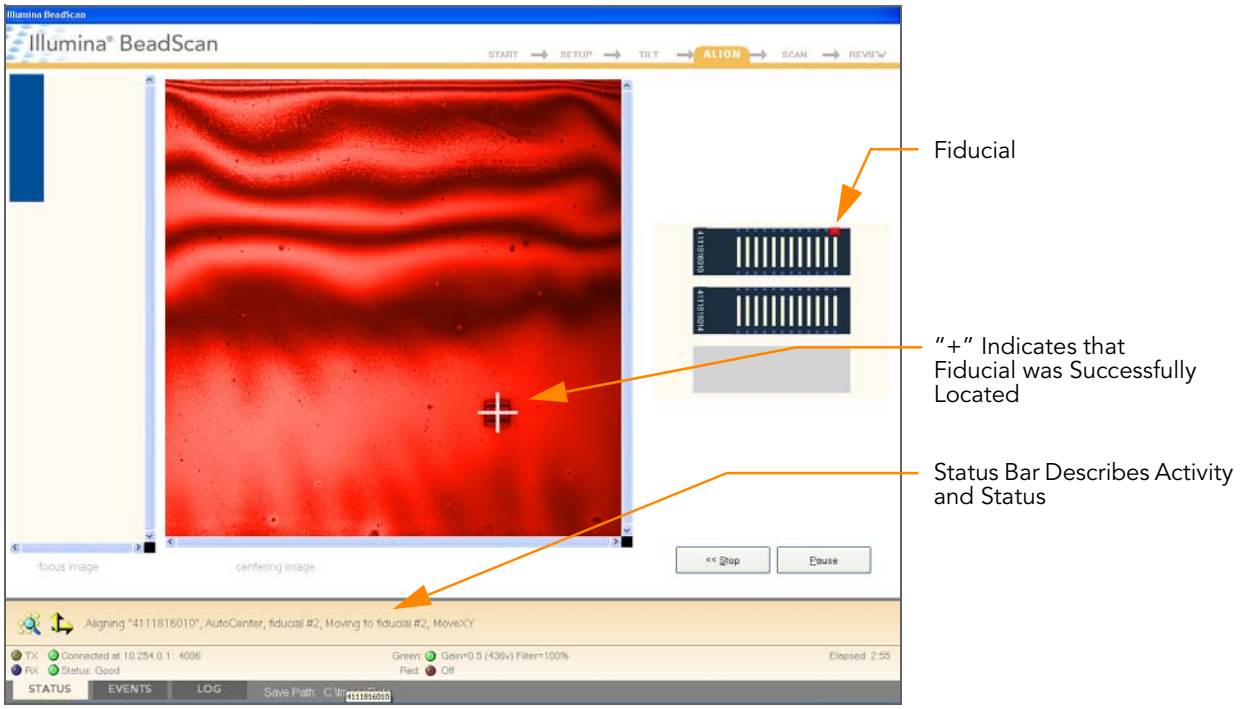


Figure 85 BeadArray Reader Tilt & Align Processes

Once the Tilt and Align processes are complete, the Scan process begins (Figure 86). Hover over any of the green dots in the closeup image to see the relative intensity and the XY position.[1] The red value should be at or close to zero, because this is a one-color assay.

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

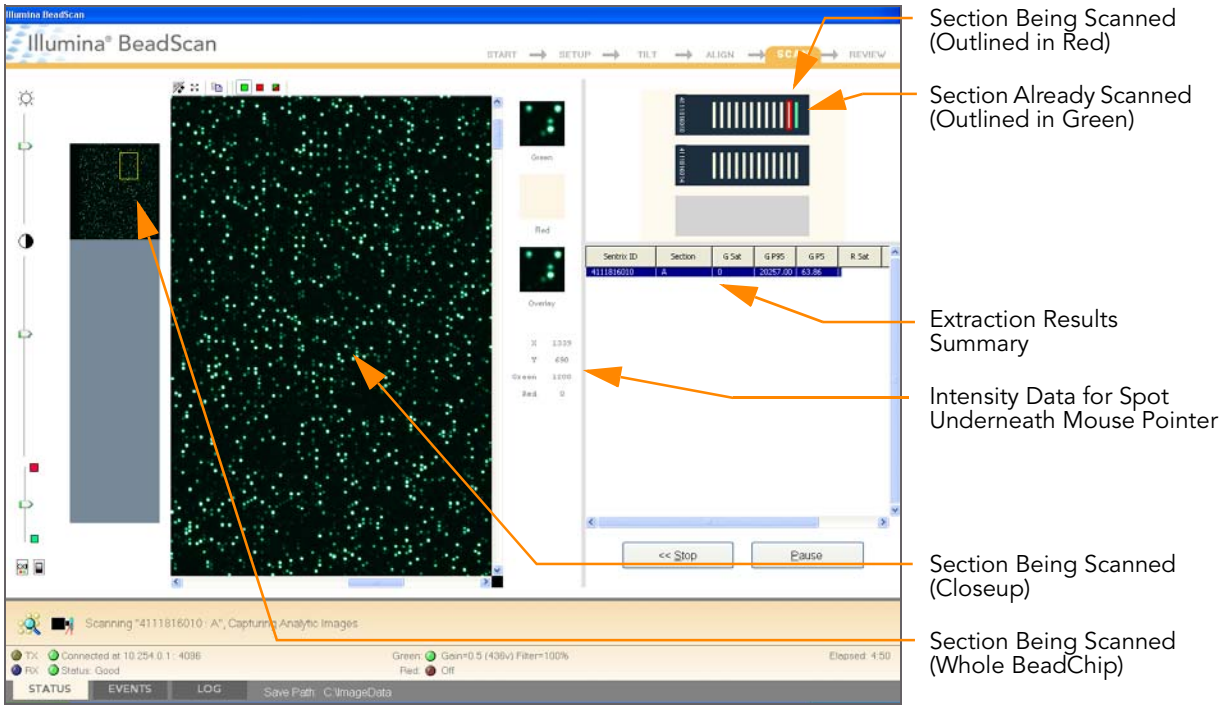


Figure 86 BeadArray Reader Scan Process

As the BeadArray Reader scans, the front panel blue Scanning indicator lights flash in sequence (see Figure 10 on page 25).

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.

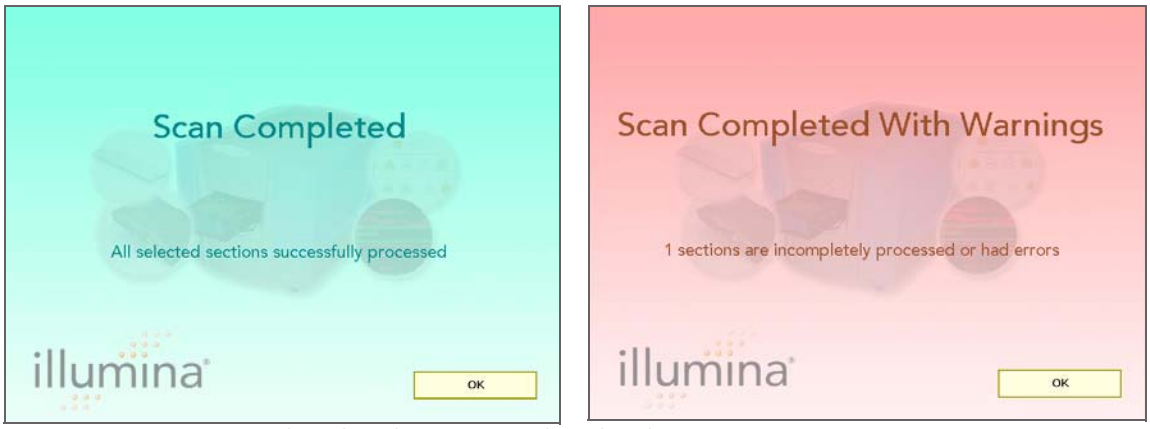


Figure 87 Scan Completed and Scan Completed with Warnings Screens

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 88).

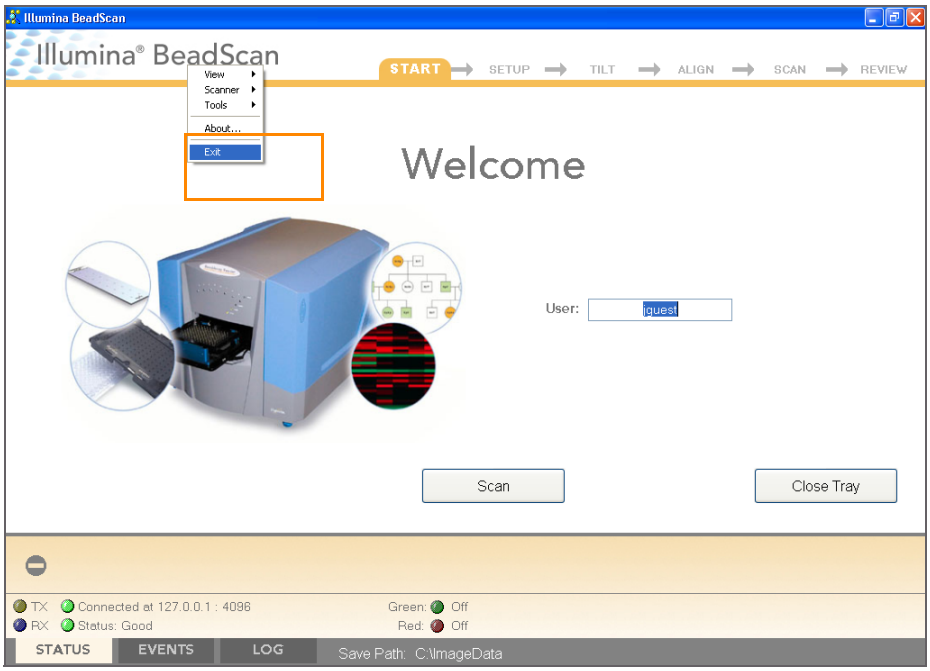


Figure 88 Exit the BeadArray Reader Software

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 89 inset).

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

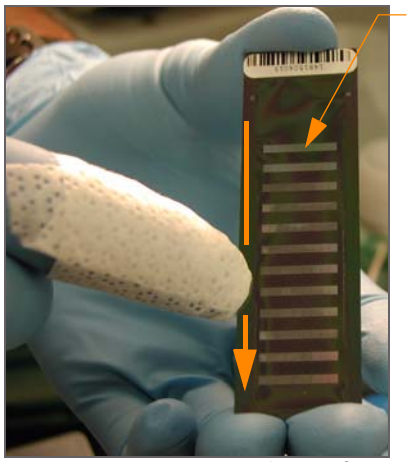


Figure 89 Wiping Edges of BeadChip

2. Wipe along the edge of each BeadChip five or six times (Figure 89), 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 Gemini Automated Protocol

Topics

Introduction

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

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

Infinium HD Assay Gemini Automated Workflow

Figure 90 graphically represents the Illumina Infinium HD Assay Gemini automated workflow, with or without Infinium LIMS. These protocols describe the procedure for preparing eight DNA samples. To process 48 or 96 samples, scale up the protocols accordingly.

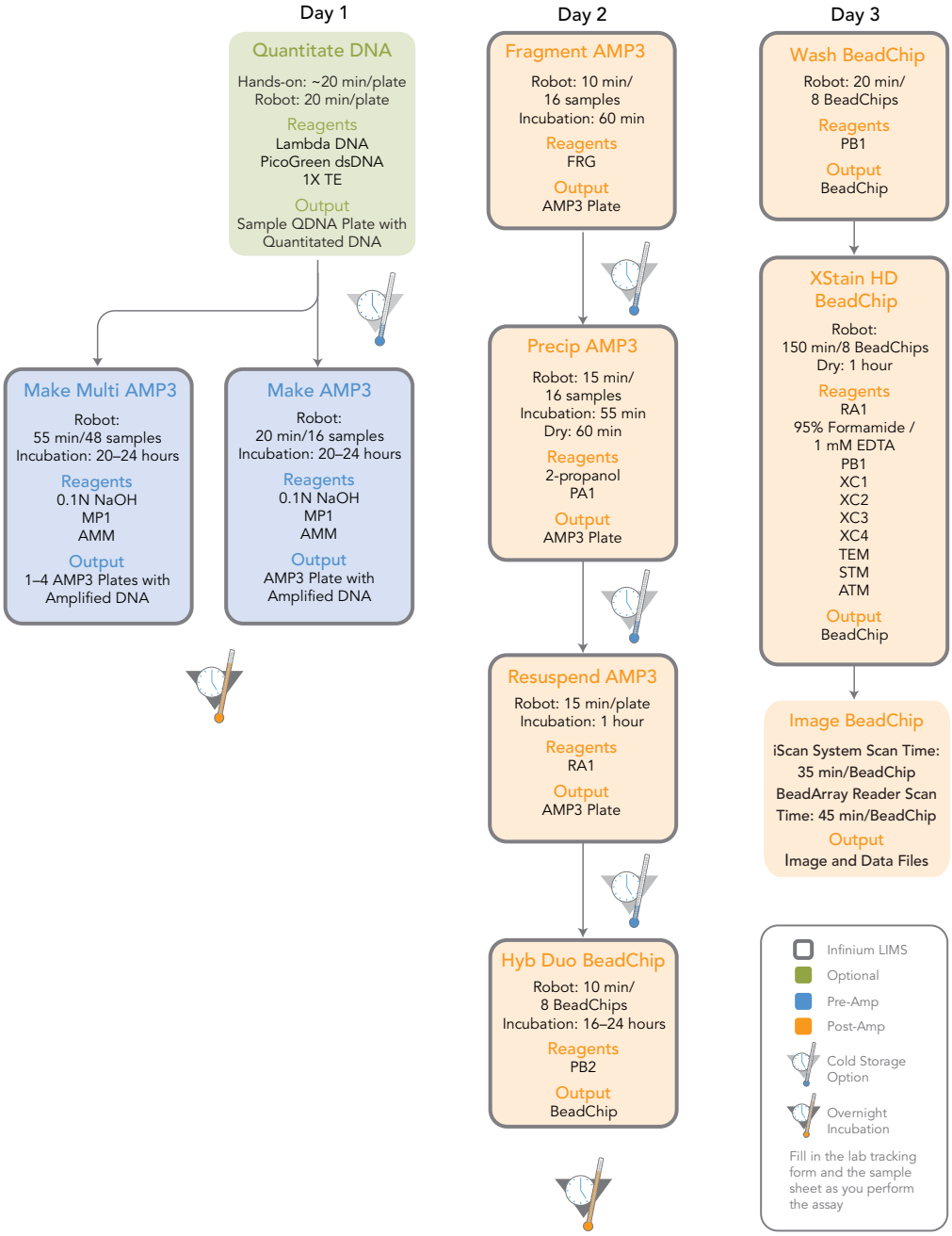


Figure 90 Infinium HD Assay Gemini Automated Workflow

Equipment, Materials, and Reagents

These items are specifically required for the Infinium HD Assay Gemini. 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 9 User-Supplied Equipment

Item	Source
Vacuum desiccator (1 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 • SC-30-401 (110V) - North America and Japan • SC-30-402 (220V) - EU and Asia Pacific (Except Japan) Infinium LIMS customers • 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 10 Illumina-Supplied Materials

Item	Catalog or Part #
Multi-Sample BeadChip Alignment Fixture	Part # 218528
Robot BeadChip Alignment Fixture (6)	Part # 222691
Robot Tip Alignment Guide	Part # 11310856

Materials User-Supplied

Table 11 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 (1 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

- AMP3 barcode labels
- WG#-DNA barcode labels
- QNT barcode labels

Reagents Illumina-Supplied

Table 12 Illumina-Supplied Reagents

Item	Part #
MP1—Neutralization solution	11190751
AMM—Amplification Master Mix	11192044
FRG—Fragmentation solution	11190022
PA1—Precipitation solution	11190031
RA1—Resuspension, hybridization, and wash solution	11191914
PB1—Reagent used to prepare BeadChips for hybridization	11191922
PB2—Humidifying buffer used during hybridization	11191130

Table 12 Illumina-Supplied Reagents

Item	Part #
XC1—XStain BeadChip solution 1	11208288
XC2—XStain BeadChip solution 2	11208296
TEM—Two-Color Extension Master Mix	11208309
XC3 (80 ml)—XStain BeadChip solution 3	11208392
XC3 (240 ml)—XStain BeadChip solution 3	11208421
STM—Superior Two-Color Master Mix	11288046
ATM—Anti-Stain Two-Color Master Mix	11208317
XC4—XStain BeadChip solution 4	11208430

Quantitate DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples before bisulfite conversion. You can quantitate up to three plates, each containing up to 96 samples. If you already know the concentration and your are not running Infinium LIMS (Laboratory Information Management System), Proceed to *Make the AMP3 Plate* on page 106 or *Make Multiple AMP3 Plates on page 113.*

If you are running Infinium LIMS, for information about how to use Infinium LIMS, see the *Infinium 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

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*.

- Thaw the sample DNA plates to room temperature.
- Apply a QNT 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 91).

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:

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

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

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

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.

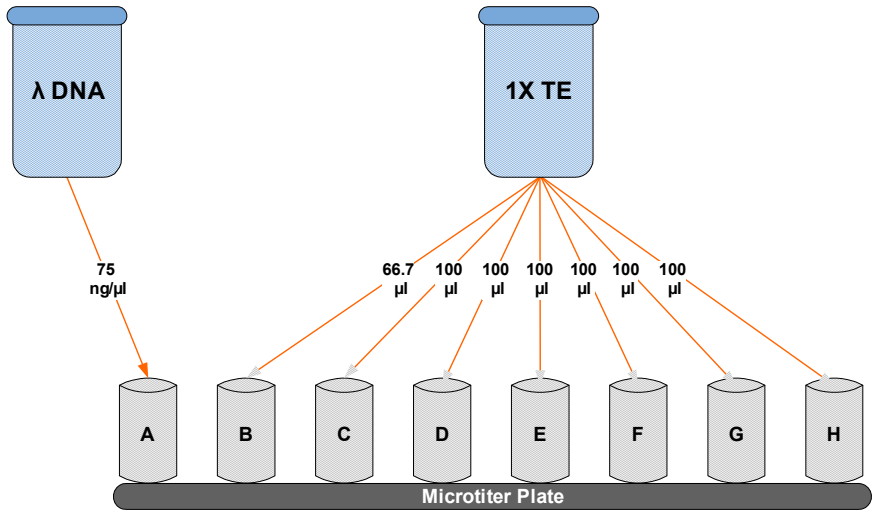


Figure 91 Dilution of Stock Lambda DNA Standard

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 13 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

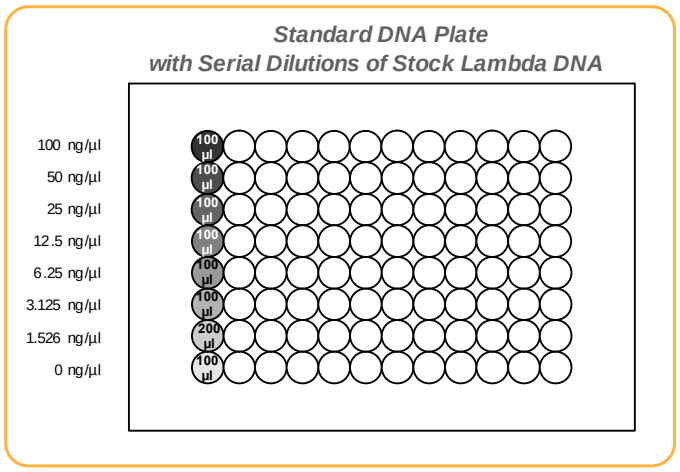


Figure 92 Serial Dilutions of Lambda DNA

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 14 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 14 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 **DNA Quant | Make Quant**.
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 93 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.

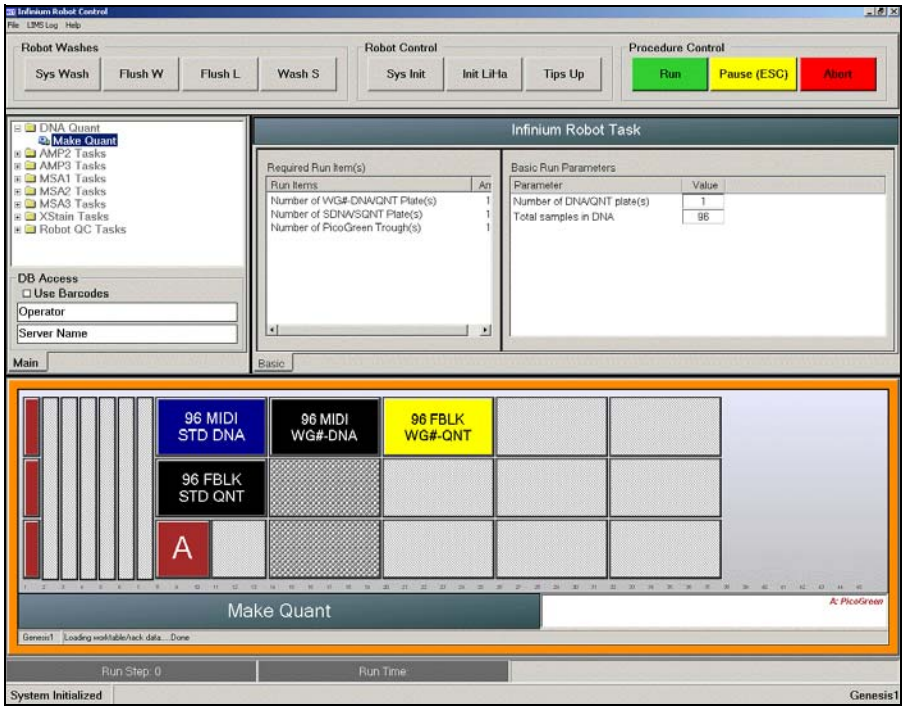


Figure 94 Make Quant Screen

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 µl of diluted PicoGreen to all Fluotrac plates, then transfers 2 µl 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/µl.

1. Turn on the spectrofluorometer. At the PC, open the Infinium Fluorometry Analysis program.
2. Select **Reader Tasks | Read Quant**.

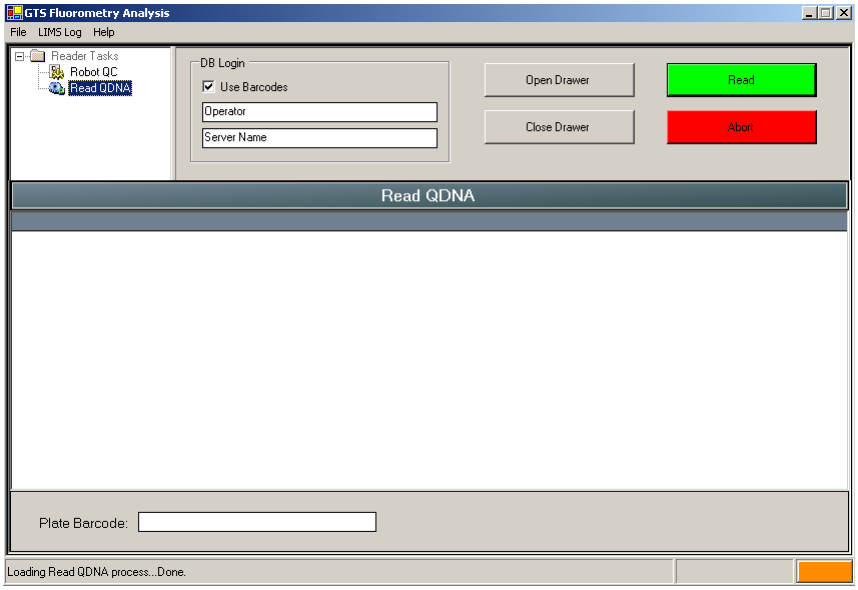


Figure 95 Infinium Fluorometry Analysis Main Screen

3. If you are not using LIMS, clear the **Use Barcodes** checkbox.
4. Click **Read**.
5. 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**.

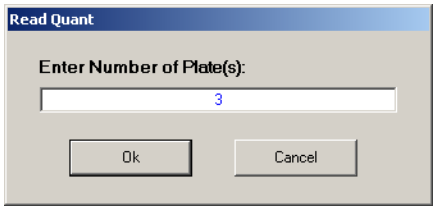


Figure 96 Number of Sample QNT Plates

6. Remove the seal from the Standard QNT plate, place the plate in the spectrofluorometer tray, and 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, hand-scan the Sample QNT plate barcode. Click **OK**.
10. 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.
11. When prompted, click **Yes** to review the raw Sample QNT plate 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:

- **SQNT_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.
- **QNT**—Plots the concentration (ng/µl) of each well of the Sample QNT 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.

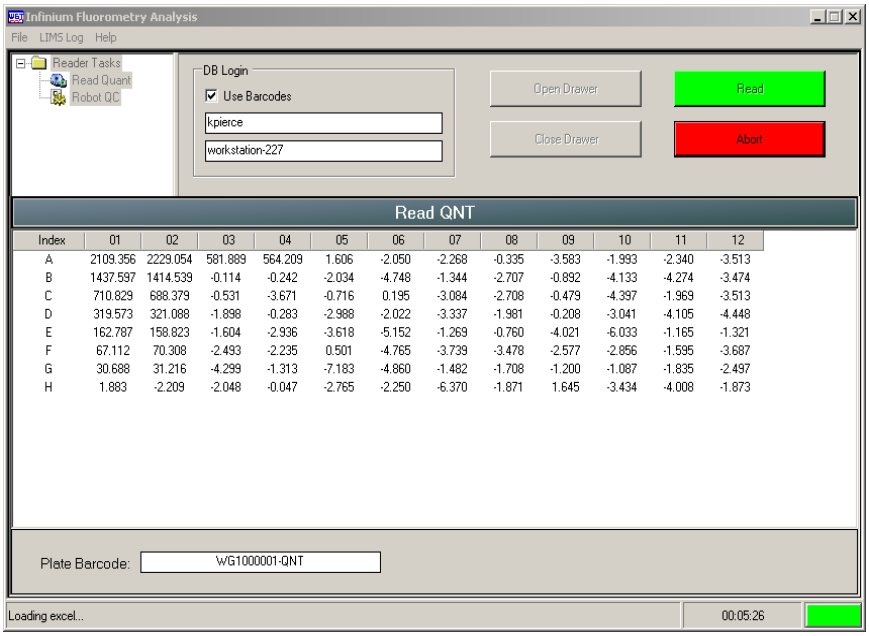


Figure 97 Sample QNT Data

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

12. If you entered more than one Sample QNT plate to read, repeat steps 9 to 11 for each additional plate.
13. Discard the QNT plates and reagents in accordance with facility requirements.
14. Do one of the following:
    - Proceed to *Make the AMP3 Plate* on page 106 or *Make Multiple AMP3 Plates on page 113*.
    - Store the Sample DNA plate at 2 to 8°C for up to one month.

Make the AMP3 Plate

This process creates an AMP3 plate for DNA amplification. DNA samples are added to designated well locations, and then 0.1N NaOH is added, denaturing the DNA. Neutralization occurs when the MP1 reagent is added. Finally, the robot adds AMM (Amplification Master Mix) to the DNA samples.

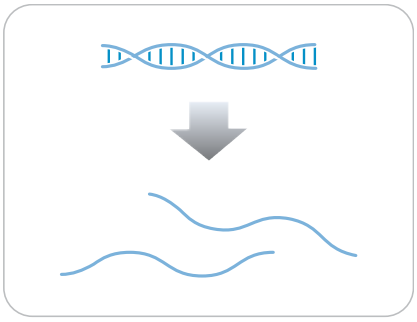


Figure 98 Denaturing and Neutralizing DNA

Estimated Time

Robot time:

- 20 minutes per 16 samples
- 30 minutes per 32 samples
- 55 minutes for 48 samples

Consumables

Item	Quantity	Storage	Supplied By
0.1N NaOH	15 ml per 16–48 samples	2 to 8°C	User
WG#-DNA plate with up to 96 DNA samples (50 ng/µl)	1 plate	-15 to -25°C	User
MP1	1 tube per 16 samples	-15 to -25°C	Illumina
AMM	1 tube per 16 samples	-15 to -25°C	Illumina
96-well 0.8 ml microtiter plate (MIDI)	1 plate for up to 48 samples		User

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

- In preparation for Incubate AMP3, preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- Thaw DNA samples to room temperature.
- Apply an AMP3 barcode to a new MIDI plate.
- In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - WG#-DNA plate barcode
  - AMP3 plate barcode
  - MP1 tube barcode(s)
  - AMM tube barcode(s)

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

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 99 throughout this protocol. Note that the barcodes all face to the right.

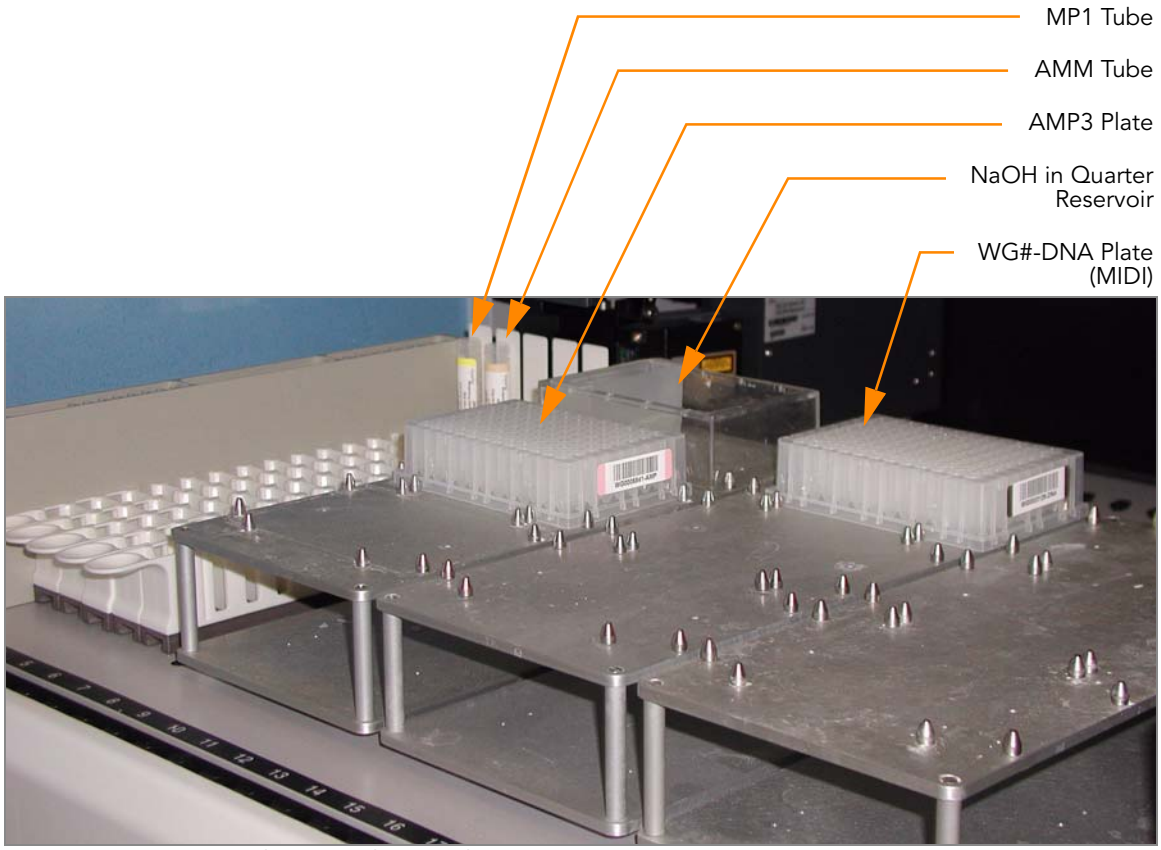


Figure 99 Tecan Eight-Tip Robot (Make AMP3 Setup)

Steps

1. If you do not already have a WG#-DNA plate, dispense DNA into either a:
   - MIDI plate: 40 µl to each WG#-DNA plate well
   - TCY plate: 30 µl to each WG#-DNA plate well

   Apply a barcode label to the new WG#-DNA plate.
2. At the robot PC, select **AMP3 Tasks | Make AMP3**.

   If you want to process multiple AMP3 plates at once, see *Make Multiple AMP3 Plates on page 113*.
3. Select the WG#-DNA plate type (MIDI or TCY).

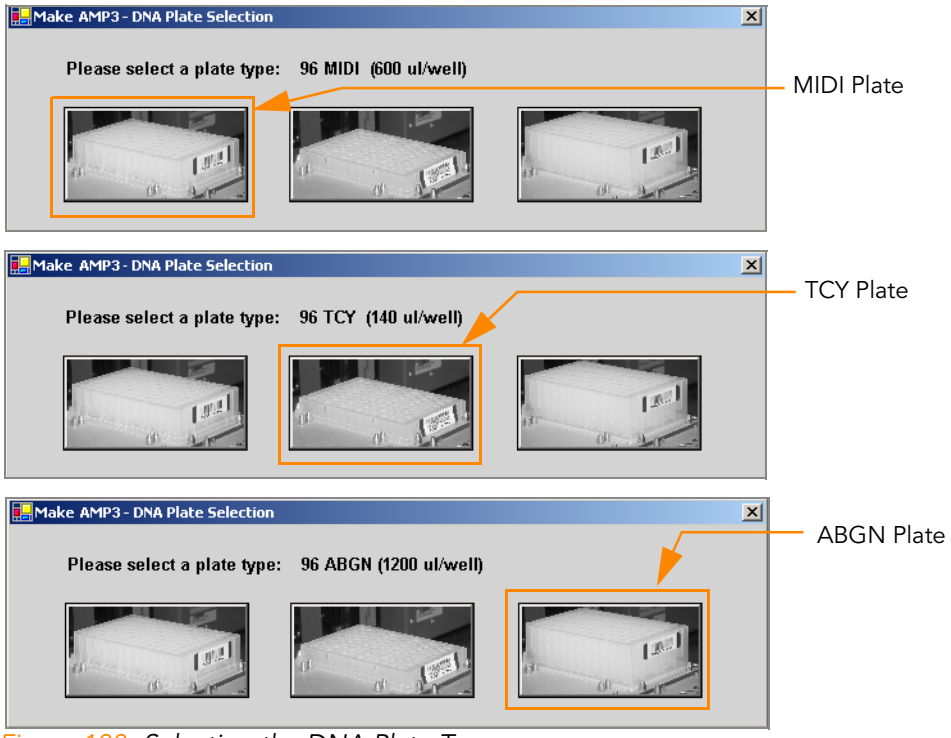


Figure 100 Selecting the DNA Plate Type

NOTE

Do not mix plate types on the robot bed.

4. (Non-Infinium LIMS only) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of DNA samples** (16, 32, or 48) 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

If you are using Infinium 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.

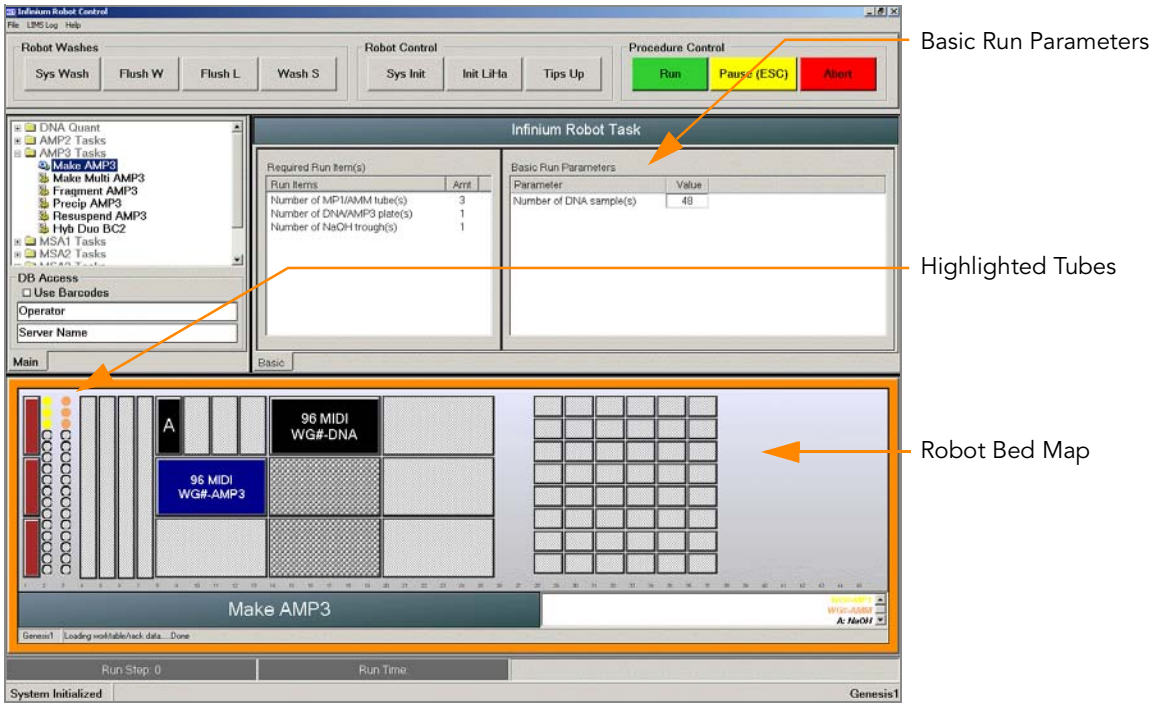


Figure 101 Make AMP3 Basic Run Parameters

5. Remove the caps. Place the MP1 and AMM tubes in the robot tube rack according to the robot bed map (Figure 101).
6. Place a quarter reservoir on the robot bed according to the bed map, and add 15 ml 0.1N NaOH (Figure 101).
7. Place the sealed WG#-DNA plate on the vortexer and secure it with the provided Velcro straps (Figure 102).

Figure 102 Securing Plates to Vortexer Platform with Velcro Straps

8. Vortex the sealed WG#-DNA plate at 1600 pm for 1 minute.
9. Centrifuge to 280 xg for 1 minute at 22°C.
10. Place the AMP3 and WG#-DNA plates on the robot bed according to the bed map (Figure 101). Remove all plate seals.

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.

11. In the lab tracking form, record the plate positions on the robot bed.
12. 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.
13. (Non-Infinium LIMS only) At the robot PC, click **Run**. Observe the robot start to run to ensure that there are no problems.

    After the robot adds the 0.1N NaOH to the DNA in the AMP3 plate, the Make AMP3 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.
14. (Infinium LIMS only) Make sure the **Use Barcodes** check box is checked and click **Run**.
    a. Log in when prompted.

       After the robot initializes, the Make AMP3 screen appears after a moment.

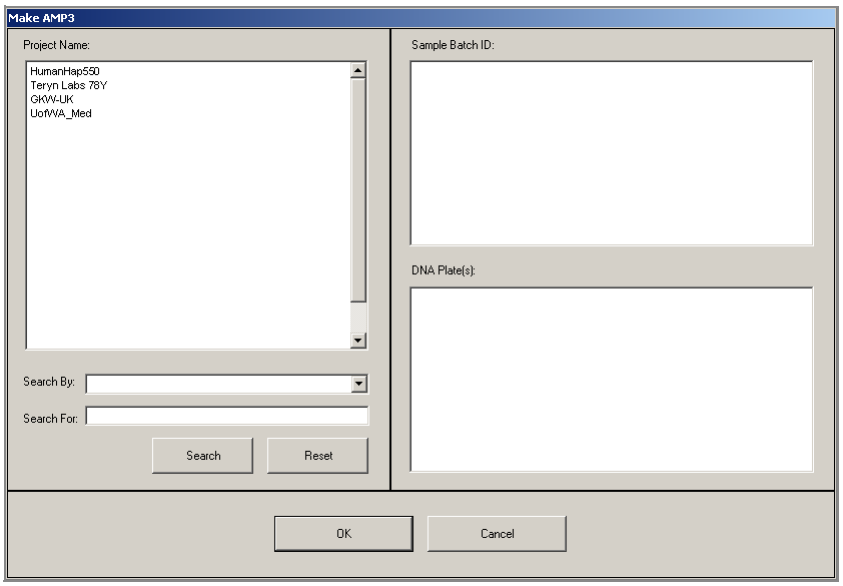


Figure 103 Selecting Project or Batch for Make AMP3

    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 pane.*

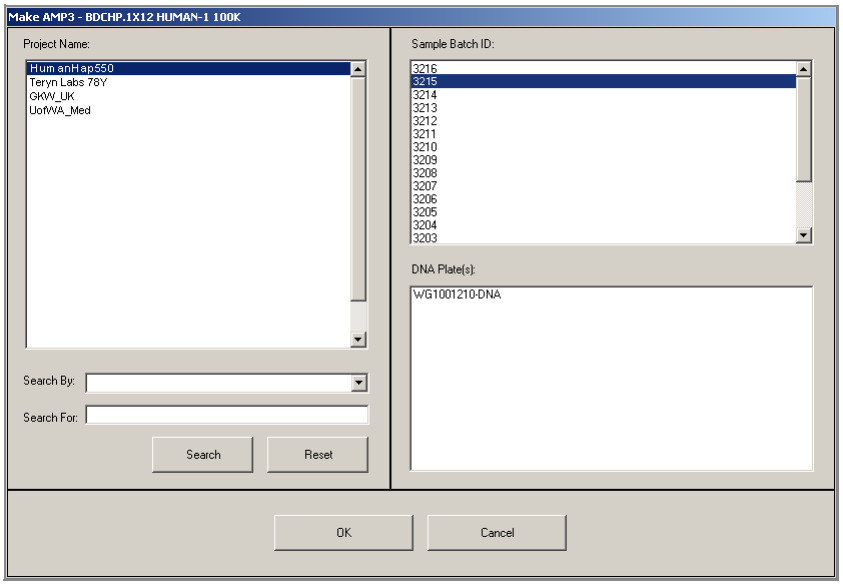


Figure 104 Make AMP3 Screen with Project and Batch Selected

— *Use the* **Search** *box to search for a specific Batch ID or DNA Plate.*

c. Select the batches you want to run and click **OK**.

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.

15. Click **OK** in the message box. Remove the AMP3 plate from the robot bed and seal with the 96-well cap mat.

16. Invert the sealed AMP3 plate at least 10 times to mix the contents.

17. Pulse centrifuge to 280 xg.

18. Record each DNA sample in the lab tracking form.

19. Discard unused reagents in accordance with facility standards.

20. Proceed immediately to *Incubate the AMP3 Plate* on page 119.

Make Multiple AMP3 Plates

This process creates up to two AMP3 plates for DNA amplification, allowing you to run 48 or 96 samples. First, DNA samples are added to designated well locations, and then 0.1N NaOH is added, denaturing the DNA. Neutralization occurs when the MP1 reagent is added. AMM (Amplification Master Mix) is then added to the DNA samples.

This feature may not be available on systems that are not running Infinium LIMS (Laboratory Information Management System). For information about how to use Infinium LIMS, see the *Infinium LIMS User Guide*.

Estimated Time

Robot:

- 70 minutes for 48 samples
- 140 minutes for 96 samples

Consumables

Item	Quantity	Storage	Supplied By
MP1	3 tubes per 48 samples	-15 to -25°C	Illumina
AMM	3 tubes per 48 samples	-15 to -25°C	Illumina
0.1N NaOH	15 ml per 48 samples	2 to 8°C	User
WG#-DNA plate, thawed to room temperature, with 48–96 DNA samples	1 plate	-15 to -25°C	User
96-well 0.2 ml microtiter plate (MIDI or TCY)	1 plate for up to 48 samples; up to 2 plates total	-15 to -25°C	User

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 AMP3 process, preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- Apply an AMP3 barcode label to each new storage plate.
- In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - WG#-DNA plate barcode
  - AMP3 plate barcodes
  - MP1 tube barcode(s)
  - AMM tube barcode(s)

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

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

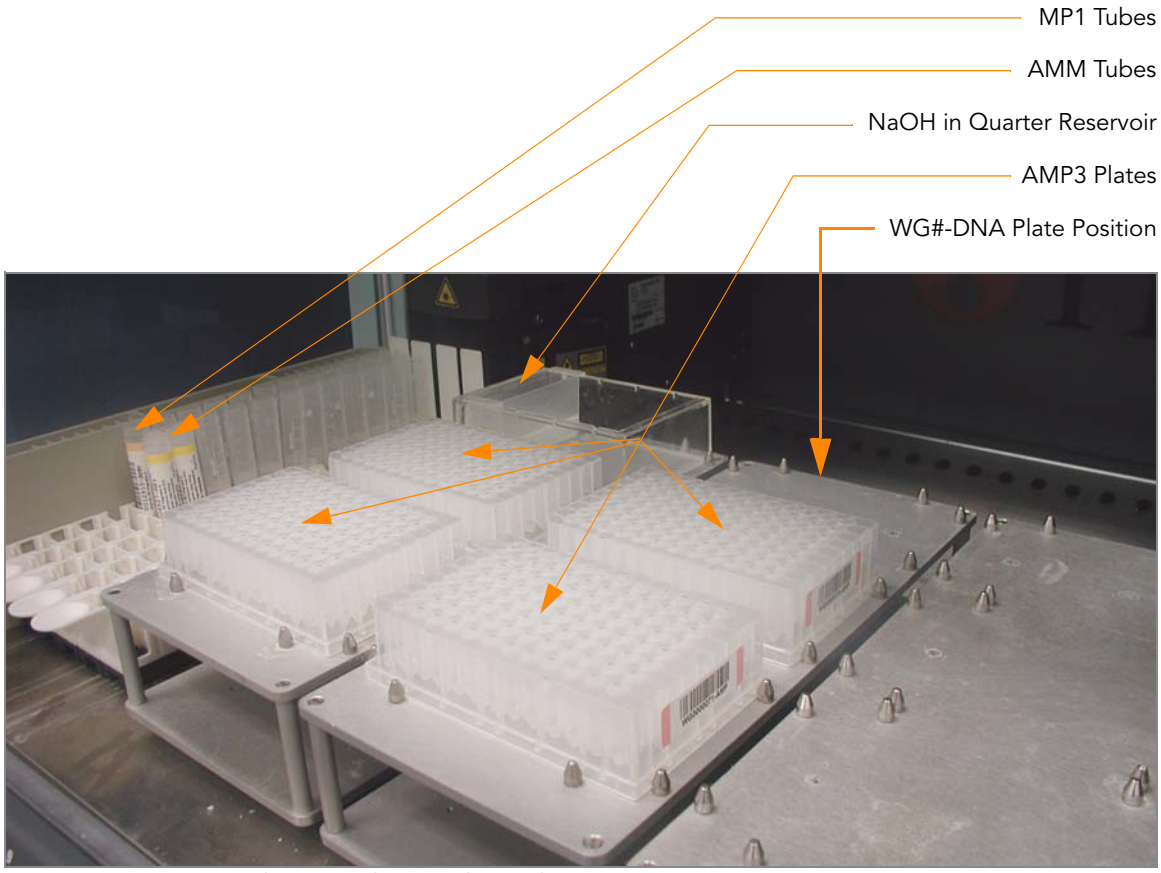


Figure 105 Tecan Eight-Tip Robot (Make Multi AMP3 Setup)

Steps

1. If you do not already have a WG#-DNA plate, dispense DNA into either a:
   - MIDI plate: 40 µl to each WG#-DNA plate well
   - TCY plate: 30 µl to each WG#-DNA plate well

   Apply a barcode label to the new WG#-DNA plate.
2. At the robot PC, select **AMP3 Tasks | Make Multi AMP3**.
3. In the DNA Plate Selection dialog box, select the plate type.

Do not mix plate types on the robot bed.

4. If you are not using Infinium LIMS, skip ahead to step 6.
5. If you are using Infinium LIMS, click **Run**.
   a. Log in when prompted.
      The Make Multi AMP3 screen appears after a moment.

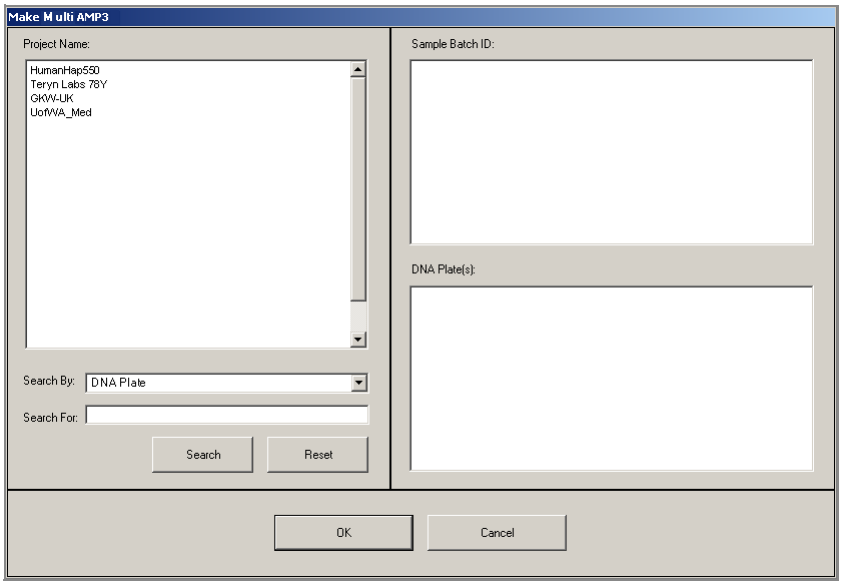


Figure 106 Selecting Project for Make Multi AMP3

b. Scan or type the DNA plate barcode into the **Search For** text box, and then click **Search**.

c. Select up to four batches and click **OK**.

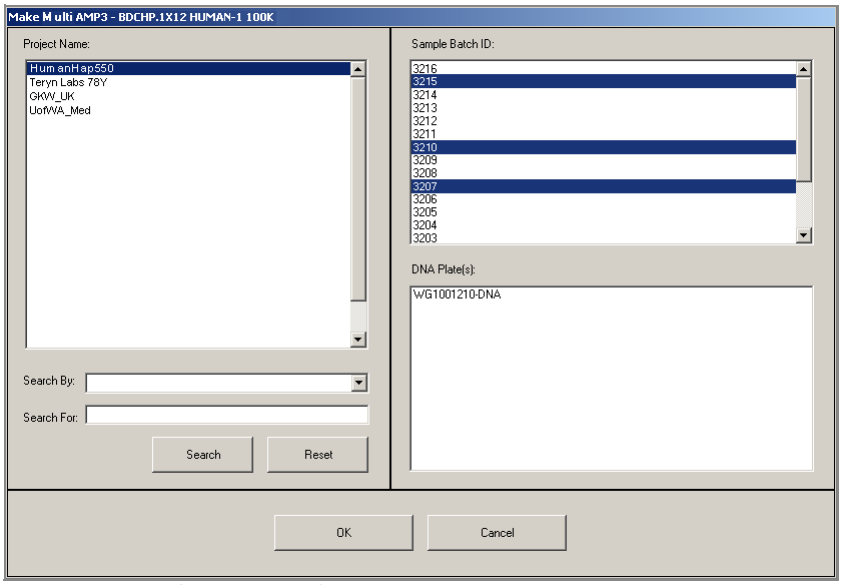


Figure 107 Selecting Batches

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.

6. (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** (48 or 96).

NOTE

If you are using Infinium 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.

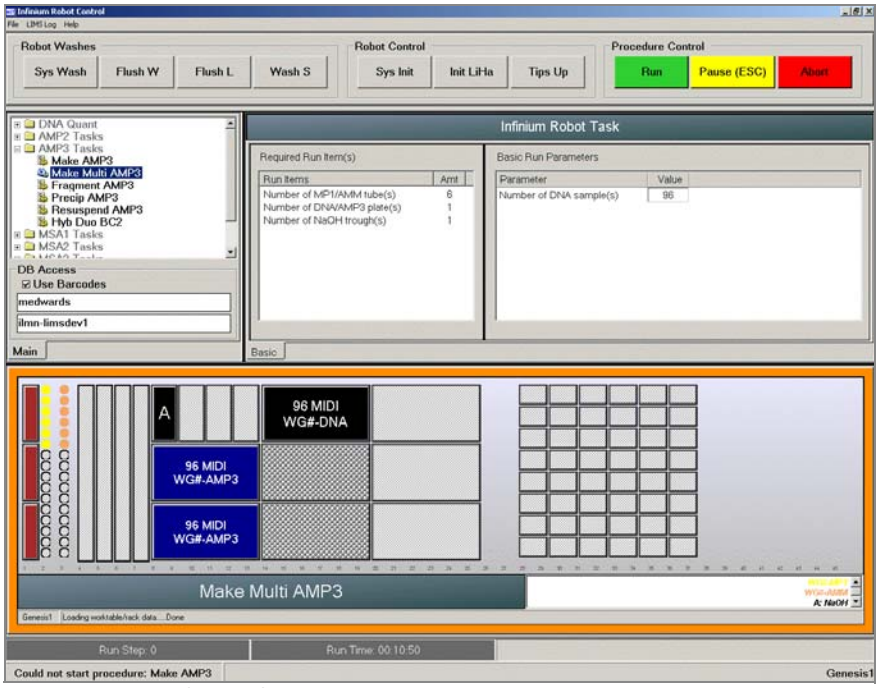


Figure 108 Make Multi AMP3 Screen

7. Vortex the sealed WG#-DNA plate at 1600 rpm for 1 minute.
8. Centrifuge to 280 xg for 1 minute at 22°C.
9. Remove the cap mat.
10. Place the MP1 and AMM tubes in the robot tube rack according to the bed map (Figure 108). Remove the caps.
11. Add 15 ml NaOH to the quarter reservoir, and then place the reservoir on the robot bed according to the bed map (Figure 108).
12. Place the AMP3 and WG#-DNA plates on the robot bed according to the bed map (Figure 108).

    Infinium LIMS only: The robot starts the process when the plates are in place.
13. In the lab tracking form, record the plate positions on the robot bed.

    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.

14. (Non-Infinium LIMS only) At the robot PC, clear the **Use Barcodes** check box and click **Run**. Observe the robot start to run to ensure that there are no problems.

    After the robot has added the 0.1N NaOH to the DNA in the AMP3 plates, the Make Multi AMP3 Wait for reaction time message appears. Wait time for this reaction is 10 minutes per plate.

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

15. Click **OK** in the message box. Remove the AMP3 plates from the robot bed and seal them with the 96-well cap mats.

16. Invert each sealed AMP3 plate at least 10 times to mix contents.

17. Pulse centrifuge the plates to 280 xg.

18. Discard unused reagents in accordance with facility standards.

19. Proceed immediately to *Incubate the AMP3 Plate* on page 119.

Incubate the AMP3 Plate

This process incubates the AMP3 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 twice in the Infinium HD Assay Gemini.

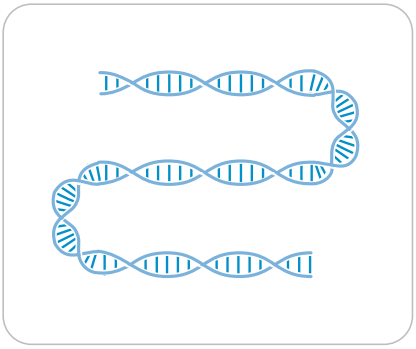


Figure 109 Incubating DNA to Amplify

Estimated Time

20–24 hours.

Preparation

Verify AMP3 for Incubation (Infinium LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Incubate AMP3**.
2. Scan the barcode of the AMP3 plate and click **Verify**.

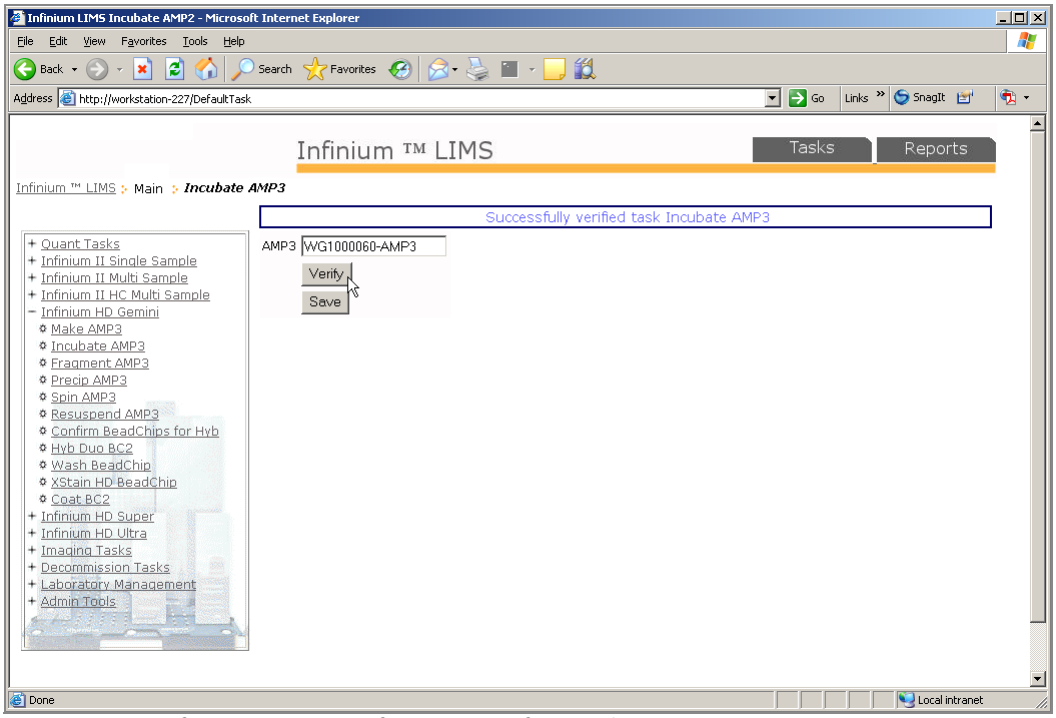


Figure 110 (Infinium LIMS) Verifying AMP3 for Incubation

3. If the AMP3 plate is queued for incubation, a blue confirmation message appears at the top of the window. Proceed with incubation.
4. If the AMP3 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 Infinium LIMS, see the *Infinium LIMS User Guide*.

Steps

1. Incubate the AMP3 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.
3. If you are using Infinium LIMS:
   a. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Incubate AMP3**.
   b. Scan the barcode of the AMP3 plate and click **Save**. Infinium LIMS records the data and queues the plate for fragmentation.
4. Proceed to *Fragment the AMP3 Plate* on page 121.

Fragment the AMP3 Plate

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

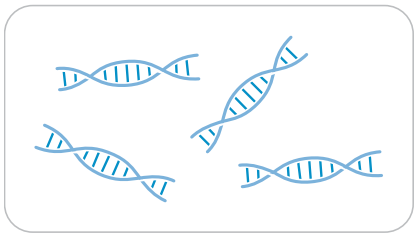


Figure 111 Fragmenting DNA

Estimated Time

Robot time:

- 10 minutes per 16 samples
- 15 minutes per 32 samples
- 35 minutes per 48 samples

Consumables

Item	Quantity	Storage	Supplied By
FRG	1 tube per 16 samples	-15 to -25°C	Illumina

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.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - FRG tube barcode(s)

NOTE

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

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 112 throughout this protocol. Note that the barcodes all face to the right.

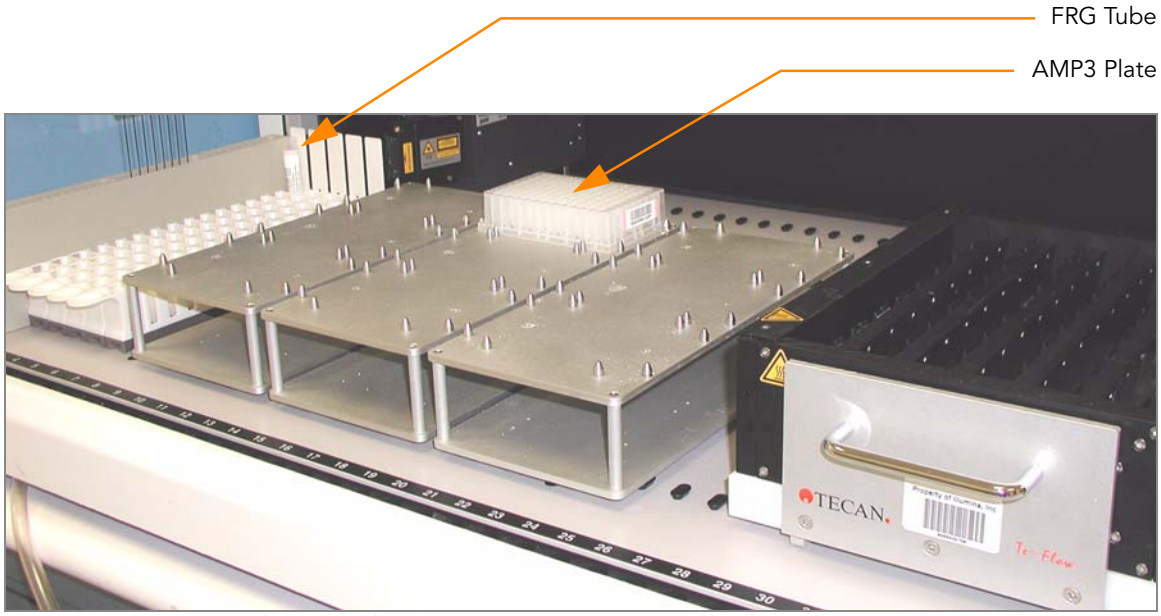


Figure 112 Tecan Eight-Tip Robot (Fragment AMP3 Setup)

Steps Set Up the Robot

1. Centrifuge the AMP3 plate to 50 xg for 1 minute.
2. At the robot PC, select **AMP3 Tasks | Fragment AMP3**.
3. (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP3 Plates**.

NOTE

If you are using Infinium 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.

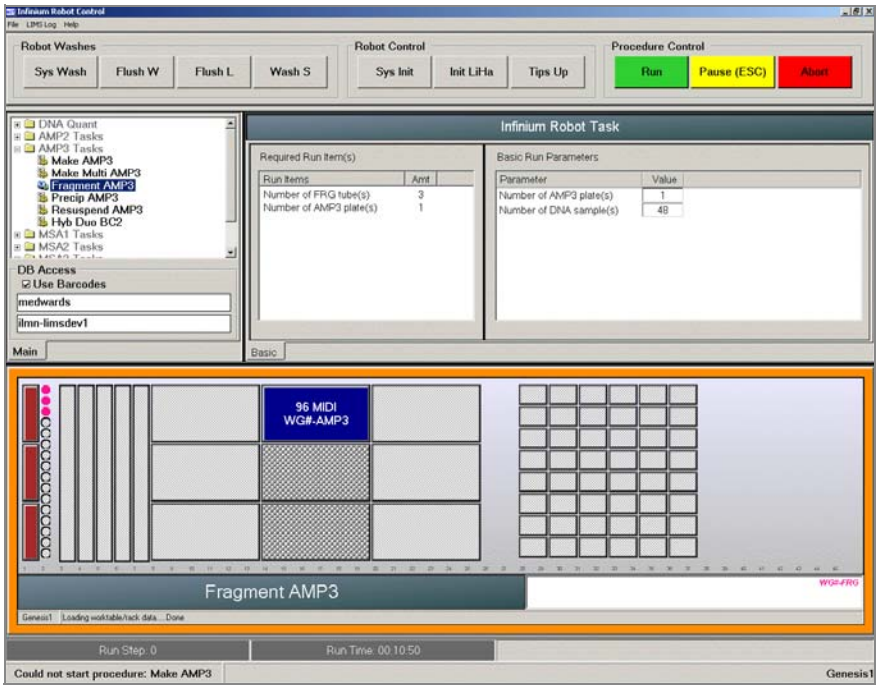


Figure 113 Fragment AMP3 Screen

4. Remove any plate seals. Place the AMP3 plate on the robot bed according to the robot bed map (Figure 113).
5. Remove the caps. Place the FRG tubes in the robot tube rack according to the robot bed map.
6. In the lab tracking form, record the plate positions on the robot bed.
7. Make sure to properly place all items 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 Infinium LIMS, clear the **Use Barcodes** check box.
   b. Click **Run** to start the process.
   c. Log in if prompted.
   d. 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.
2. Click **OK** in the message box. Remove the AMP3 plate from the robot bed and seal with the 96-well cap mat.
3. Vortex the plate at 1600 rpm for 1 minute.
4. Centrifuge to 50 xg for 1 minute at 22°C.
5. Incubate on the heat block for 1 hour at 37°C.
6. On the lab tracking form, record the start and stop times.

7. Discard unused reagents in accordance with facility standards.
8. Do one of the following:
   - Proceed to *Precipitate the AMP3 Plate* on page 125. Leave plate in the 37°C heat block until preparation is complete.
   - Store the sealed AMP3 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 AMP3 Plate

In this process, PA1 and 2-propanol are added to the AMP3 plate to precipitate the DNA samples.

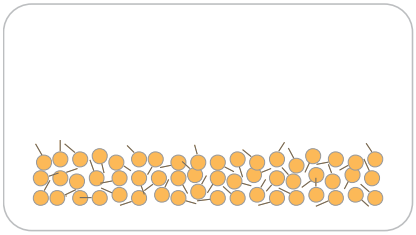


Figure 114 Precipitating DNA

Estimated Time

Robot time:

- 15 minutes per 16 samples
- 20 minutes per 32 samples
- 30 minutes per 48 samples

Dry time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
PA1	1 tube per 16 samples	2 to 8°C	Illumina
100% 2-propanol	40 ml	Room temperature	User

Preparation

- If you froze the AMP3 plate after fragmentation, thaw it to room temperature. Centrifuge to 50 xg for 1 minute.
- Preheat the heat block to 37°C, if it is not already.
- In preparation for the 4°C spin, set the centrifuge to 4°C.
- Thaw PA1 to room temperature. Centrifuge to 280 xg for 1 minute.
- Preheat the heat sealer. Allow 20 minutes.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - PA1 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 provided on your documentation CD. This form can be filled out and saved online, or printed and filled in by hand.

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

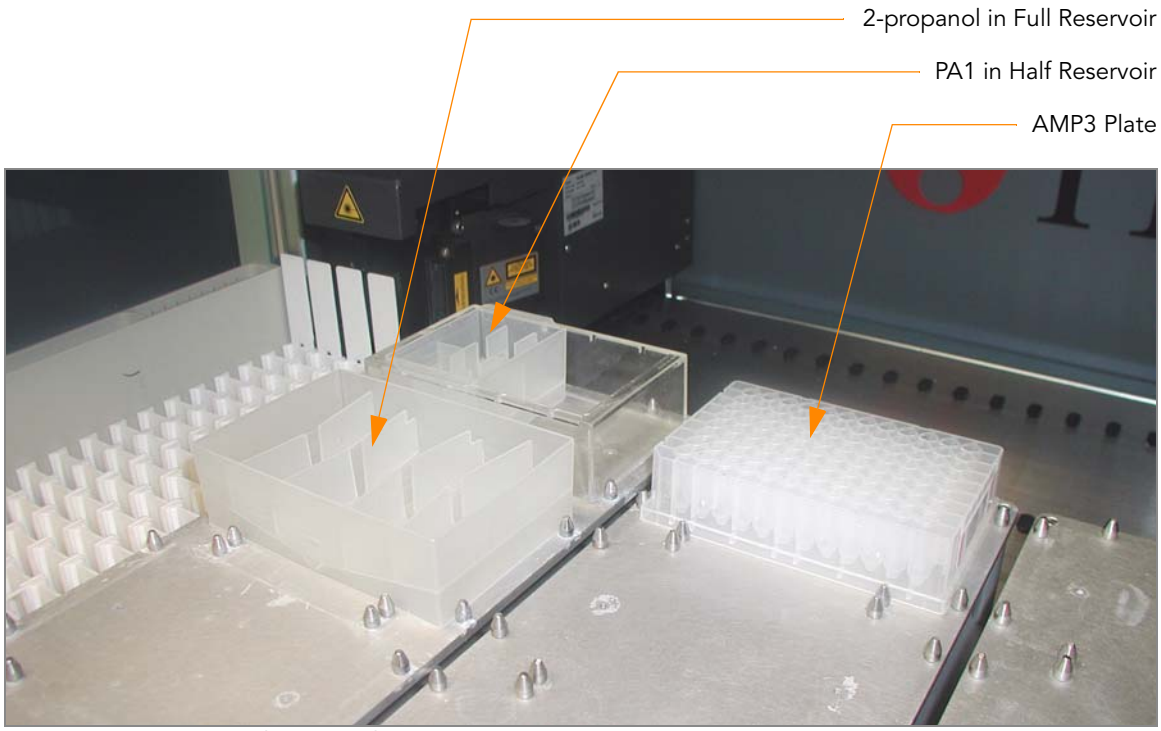


Figure 115 Tecan Eight-Tip Robot (Precip AMP3 Setup)

Verify AMP3 for Centrifugation (Infinium LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Spin AMP3**.
2. Scan the barcode of the AMP3 plate and click **Verify**. You can scan up to four plates.

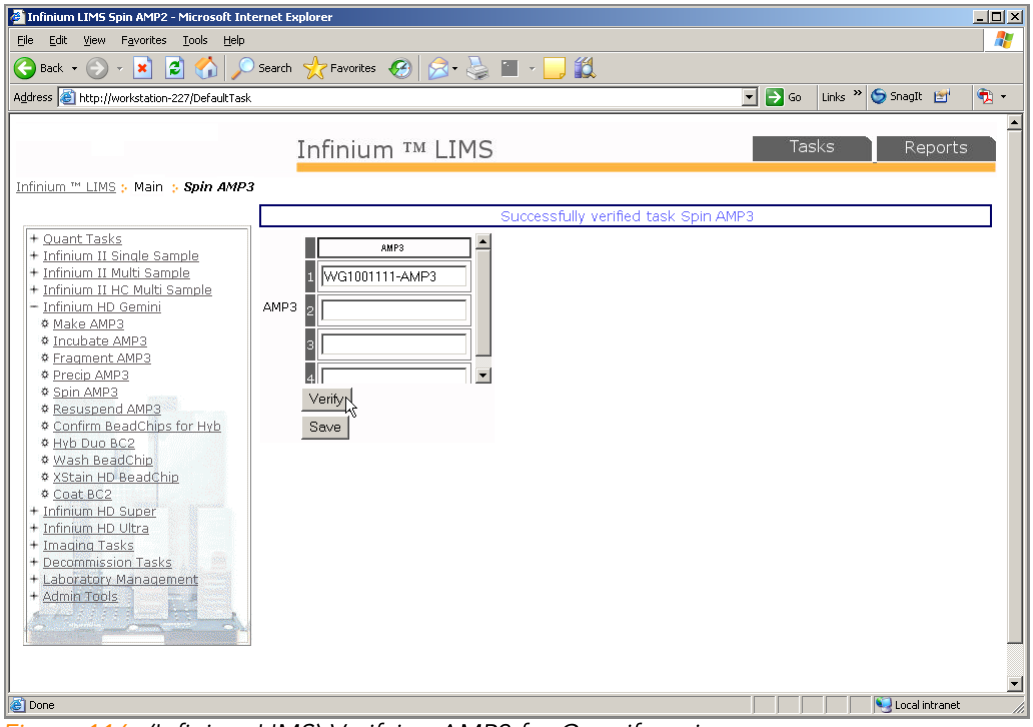


Figure 116 (Infinium LIMS) Verifying AMP3 for Centrifugation

3. If the AMP3 plate is queued for centrifugation, a blue confirmation message appears at the top of the window. Proceed to precipitation.
4. If the AMP3 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 Infinium LIMS, see the *Infinium LIMS User Guide*.

Steps Set Up the Robot

1. At the robot PC, select **AMP3 Tasks | Precip AMP3**.
2. (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP3 plates** (1 to 4).

If you are using Infinium 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.

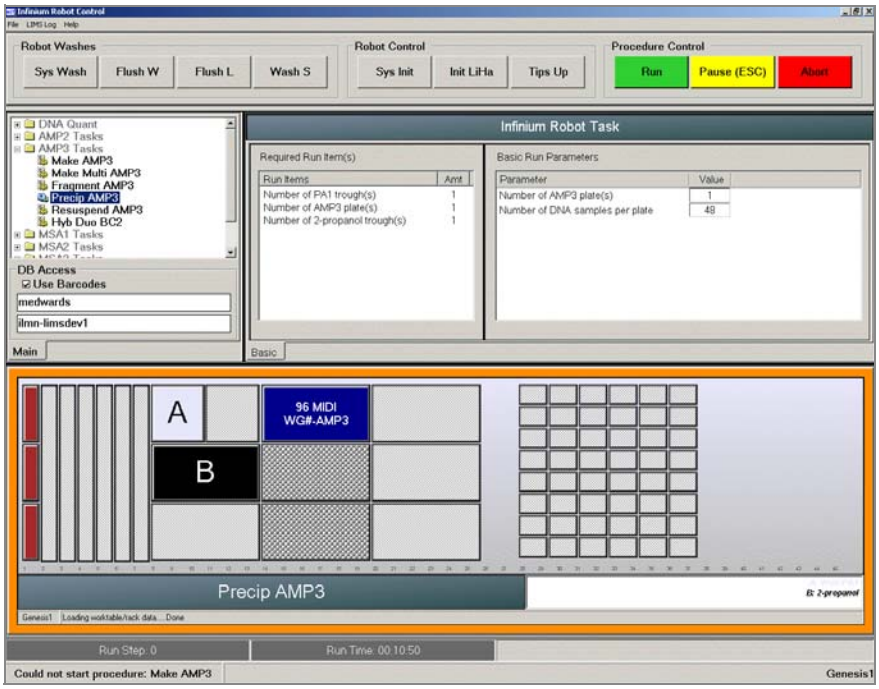


Figure 117 Precip AMP3 Screen

3. Centrifuge the sealed AMP3 plate to 50 xg for 1 minute at 22°C.
4. Place the AMP3 plate on the robot bed according to the robot bed map (Figure 117). Remove the plate seal.
5. Place a half reservoir in the reservoir frame, according to the robot bed map (Figure 117), and add PA1 as follows:
   - 16 samples: 1 tube
   - 32 samples: 2 tubes
   - 48 samples: 3 tubes

6. Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:
   - 16 samples: 20 ml
   - 32 samples: 30 ml
   - 48 samples: 40 ml
7. In the lab tracking form, record the plate position on the robot bed.
8. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. Click **Run** to start the process.
   c. Log in if prompted.
   d. Observe the robot start to run to ensure that there are no problems.
2. When prompted, remove the AMP3 plate from the robot bed. Do ***not*** click **OK** in the message box yet.
3. Seal the AMP3 plate with the same cap mat removed earlier.
4. Vortex the plate at 1600 rpm for 1 minute.
5. Incubate at 37°C for 5 minutes.
6. Centrifuge to 50 xg at room temperature for 1 minute.
7. Return the AMP3 plate to the robot bed according to the robot bed map (Figure 117). Remove the plate seal and discard it.
8. At the robot PC, click **OK** to restart the run.

   The robot PC sounds an alert and displays a message when the process is complete.
9. In preparation for the 4°C spin, set centrifuge to 4°C.
10. Click **OK** in the message box. Remove the AMP3 plate from the robot bed and carefully seal with a ***new, dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.
11. Invert each plate at least 10 times to mix contents thoroughly.
12. Incubate for 30 minutes at 4°C.
13. Place the sealed AMP3 plate in the centrifuge opposite another plate of equal weight (Figure 118).

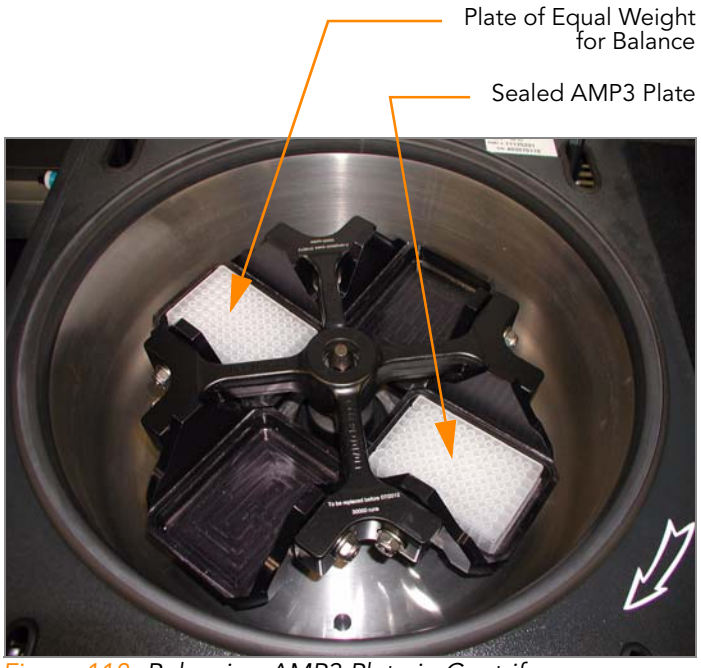


Figure 118 Balancing AMP3 Plate in Centrifuge

14. Centrifuge to 3000 xg for 20 minutes at 4°C.
15. Immediately remove the AMP3 plate from the centrifuge.

    Perform the next step immediately to avoid dislodging the blue pellet. If any delay occurs, repeat steps 14 through 15 before proceeding.
16. Remove the cap mat and discard it.
17. Decant supernatant by quickly inverting the AMP3 plate and smacking it down onto an absorbent pad appropriate for 2-propanol disposal.

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

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.
19. Place the inverted, uncovered plate on a tube rack for 1 hour at 22°C to air dry the pellet (Figure 119).

    At this point, blue pellets should be present at the bottoms of the wells.

Figure 119 Uncovered AMP3 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. If you are using Infinium LIMS:

a. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Spin AMP3**.

b. Scan the barcode of the AMP3 plate and click **Save**. Infinium LIMS records the data and queues the plate for resuspension.

22. Discard unused reagents in accordance with facility standards.

23. Do one of the following:

- Proceed immediately to *Resuspend the AMP3 Plate* on page 132.
- Heat-seal the AMP3 plate and store it at -15 to -25°C for the following day or -80°C for long-term storage.

This is a good stopping point in the process.

Resuspend the AMP3 Plate

In this process, RA1 is added to the AMP3 plate to resuspend the precipitated DNA samples.

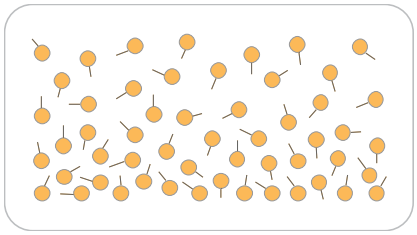


Figure 120 Resuspending DNA

Estimated Time

Robot time: 15 minutes per 48 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	9 ml per 48 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.

Preparation

- Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve any crystals.
- If you stored the AMP3 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 provided on your documentation CD. This form can be filled out and saved online, or printed and filled in by hand.

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

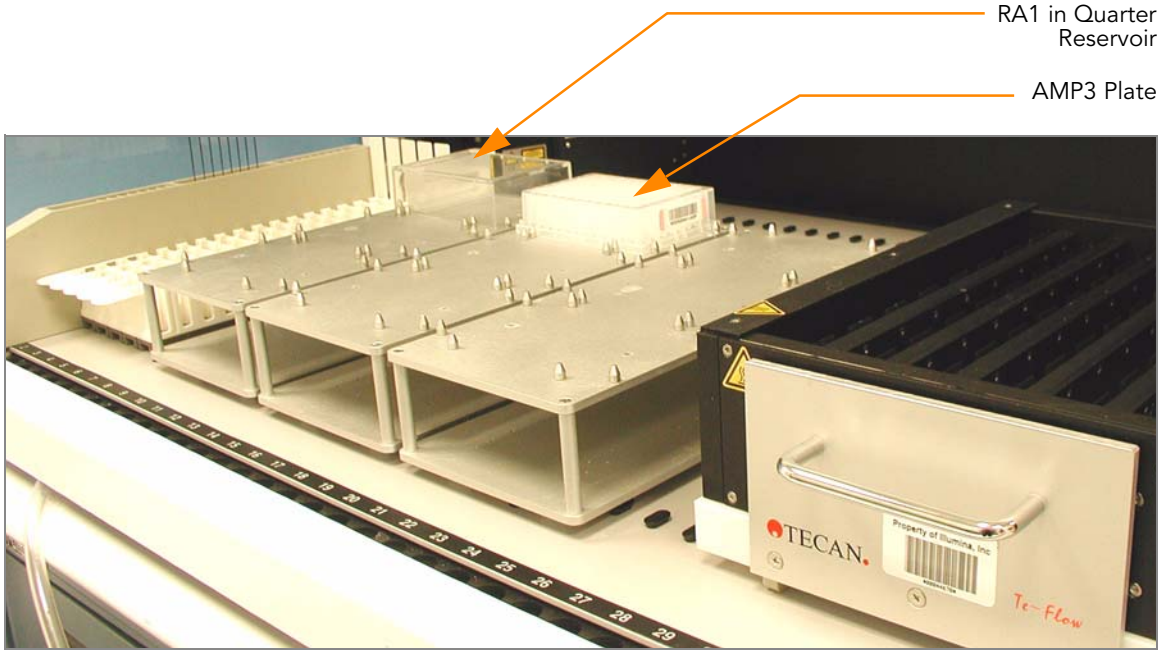


Figure 121 Tecan Eight-Tip Robot (Resuspend AMP3 Setup)

Steps Set Up the Robot

1. At the robot PC, select **AMP3 Tasks | Resuspend AMP3**.
2. (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP3 plates** (1 to 4).

NOTE

If you are using Infinium 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.

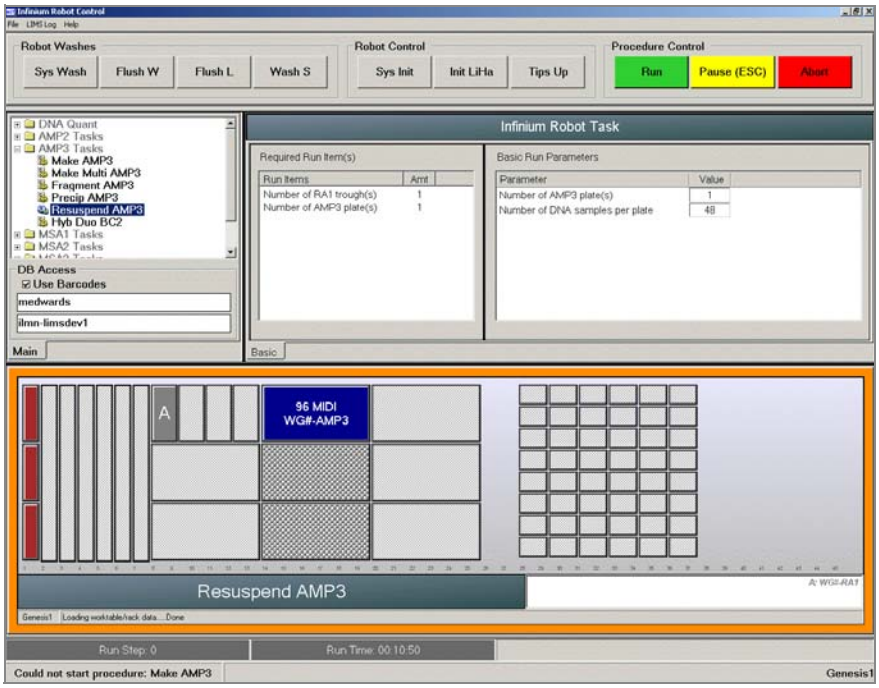


Figure 122 Resuspend AMP3 Screen

3. Place the AMP3 plate on the robot bed according to the robot bed map (Figure 122). Remove the plate seal.
4. Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add RA1 as follows:
   - 16 samples: 4 ml
   - 32 samples: 7 ml
   - 48 samples: 9 ml
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. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. Click **Run** to start the process.
   c. Log in if prompted.
   d. 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.
2. When prompted, remove the AMP3 plate from the robot bed and click **OK**.

3. Apply a foil heat seal to the AMP3 plate by firmly holding the heat sealer sealing block down for 3 seconds.
4. Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
5. In the lab tracking form, record the start and stop times.
6. Vortex the sealed plate at 1800 rpm for 1 minute.
7. Pulse centrifuge to 280 xg.

If you stored the DNA pellets at -15 to -25°C for more than 72 hours after Precip AMP3, you may need to repeat the vortexing and centrifugation until the pellets are completely resuspended.

8. Discard unused reagents in accordance with facility standards.
9. Do one of the following:
   - Proceed to *Hybridize Duo BeadChip* on page 136. If you do so immediately, it is safe to leave the RA1 at room temperature.
   - Store the sealed AMP3 plate and the RA1 at -15 to -25°C (-80°C if storing for more than 24 hours).

This is a good stopping point in the process.

Hybridize Duo BeadChip

In this process, the resuspended DNA samples are denatured and dispensed onto BeadChips using the robot. DNA-loaded BeadChips are placed into inserts, which are then positioned into the Hyb Chambers.

The Hyb Chambers are hybridized in the Illumina Hybridization Oven for 16–24 hours at 48°C.

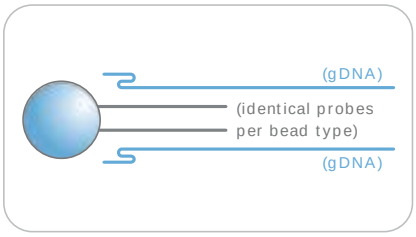


Figure 123 Hybridizing DNA to BeadChip

Estimated Time

Robot time:

- 10 minutes per 8 BeadChips (16 samples)
- 30 minutes per 16 BeadChips (32 samples)
- 60 minutes per 24 BeadChips (48 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 16 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 Fixture	4		Illumina
Robot Tip Alignment Guide	4		Illumina
1% aqueous Alconox	As needed		User

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.
- If you plan to perform the XStain process tomorrow, begin thawing the XC4 reagent. For instructions, see *Resuspend XC4 Reagent for XStain HD BeadChip* on page 150.
- 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(s)

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

Steps

Prepare Robot Tip Alignment Guides

1. Wash and dry the Robot Tip Alignment Guides prior to each use. See *Wash Robot Tip Alignment Guides* at the end of the Hyb Duo BeadChip steps (page 151) for washing instructions.
2. Make sure you have the correct Robot Tip Alignment Guide for the Gemini assay. The guide barcode should say Guide-A on it (Figure 124).

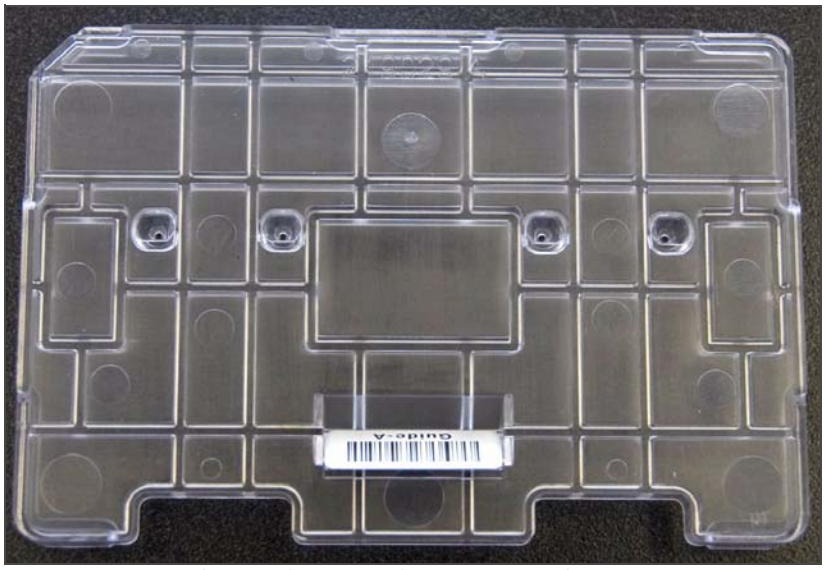


Figure 124 Guide-A Robot Tip Alignment Guide

Prepare Hyb Chambers

NOTE

Perform the Hyb Chamber assembly near the Robot to minimize the distance 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. For every 8 BeadChips, place the following items on the bench top (Figure 125):
   - BeadChip Hyb Chambers (2)
   - Hyb Chamber Gaskets (2)
   - Robot BeadChip Alignment Fixtures (4)
   - BeadChip Hyb Chamber Inserts (8)
   - Robot Tip Alignment Guides (4)

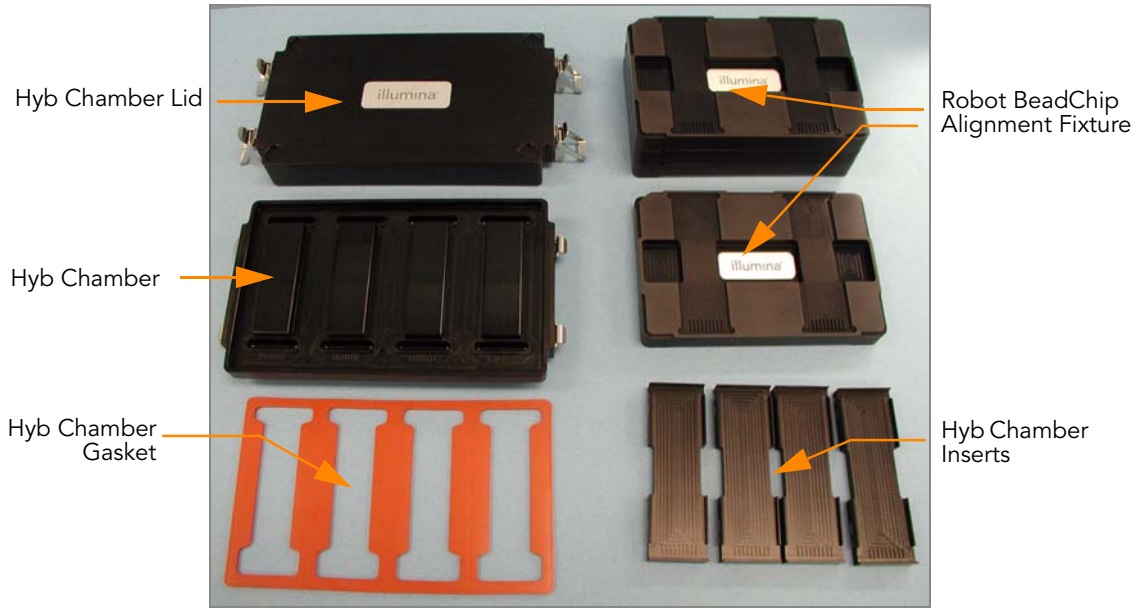


Figure 125 BeadChip Hyb Cartridge Components

2. Place the gasket into the Hyb Chambers:
   a. Match the wider edge of the gasket to the barcode-ridge side of the Hyb Chamber (Figure 126).

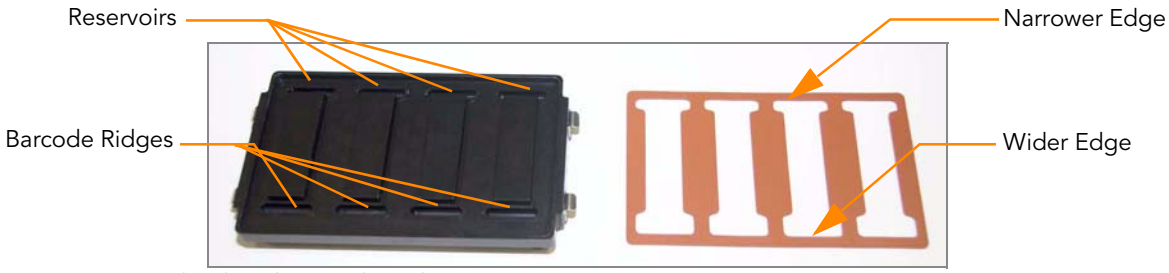


Figure 126 Hyb Chamber and Gasket

   b. Lay the gasket into the Hyb Chamber (Figure 127), and then press it down all around.

Figure 127 Placing Gasket into Hyb Chamber

c. Make sure the gasket is properly seated (Figure 128).

Figure 128 Hyb Chamber with Gasket in Place

3. Dispense 400 µl PB2 into each of the 8 humidifying buffer reservoirs in the Hyb Chamber (Figure 129).

Figure 129 Dispensing PB2 into Hyb Chamber Reservoirs

4. Remove the BeadChips from 2 to 8°C storage but do not unpackage.
5. Close and lock the BeadChip Hyb Chamber lid (Figure 130).
   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 130 Sealing the Hyb Chamber

6. Leave the closed Hyb Chamber on the bench at room temperature until the BeadChips are loaded with DNA sample.

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 131 throughout this protocol. Note that all of the plate barcodes face to the right.

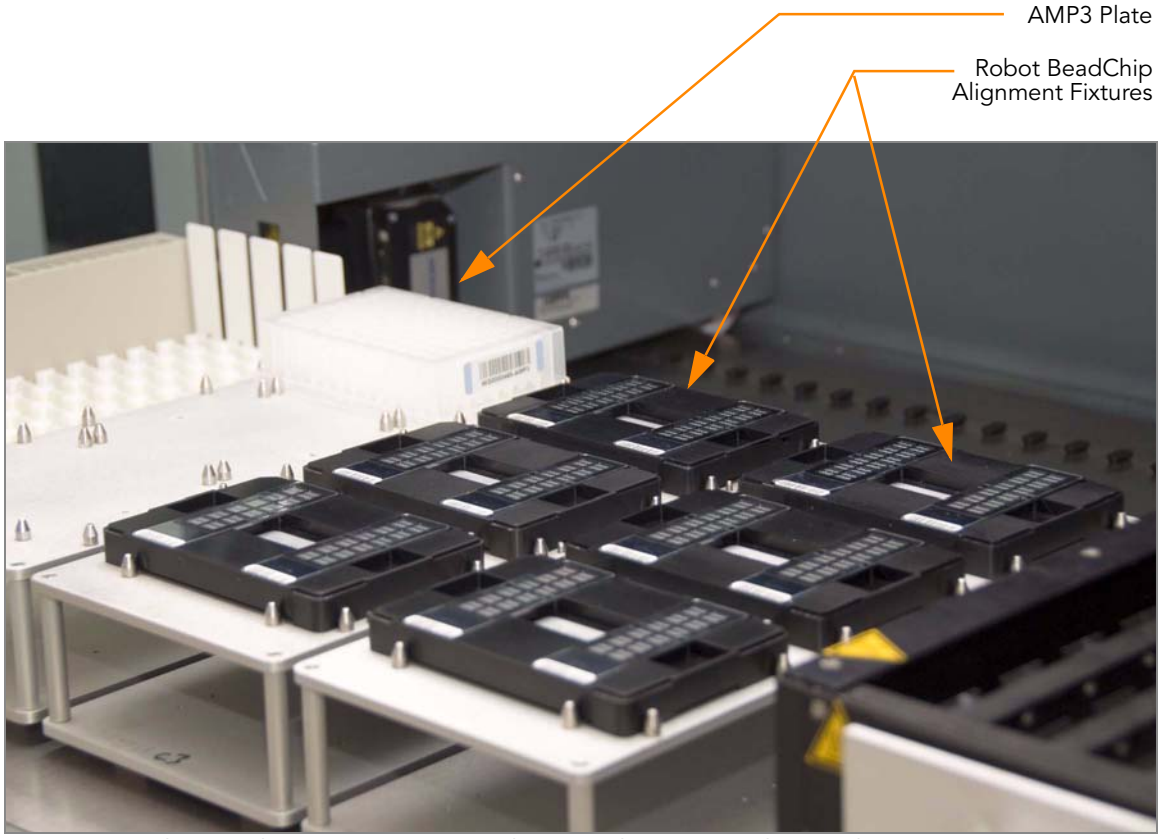


Figure 131 Placing Alignment Fixtures and AMP3 Plate onto Robot Bed

Verify AMP3 and BeadChips for Hyb (Infinium LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Confirm BeadChips for Hyb**.
2. Scan the barcode of the AMP3 plate.
3. Scan the barcodes of all the BeadChips you plan to hybridize with the plate. You can scan up to 24 BeadChips.

NOTE

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

4. Click **Verify**.

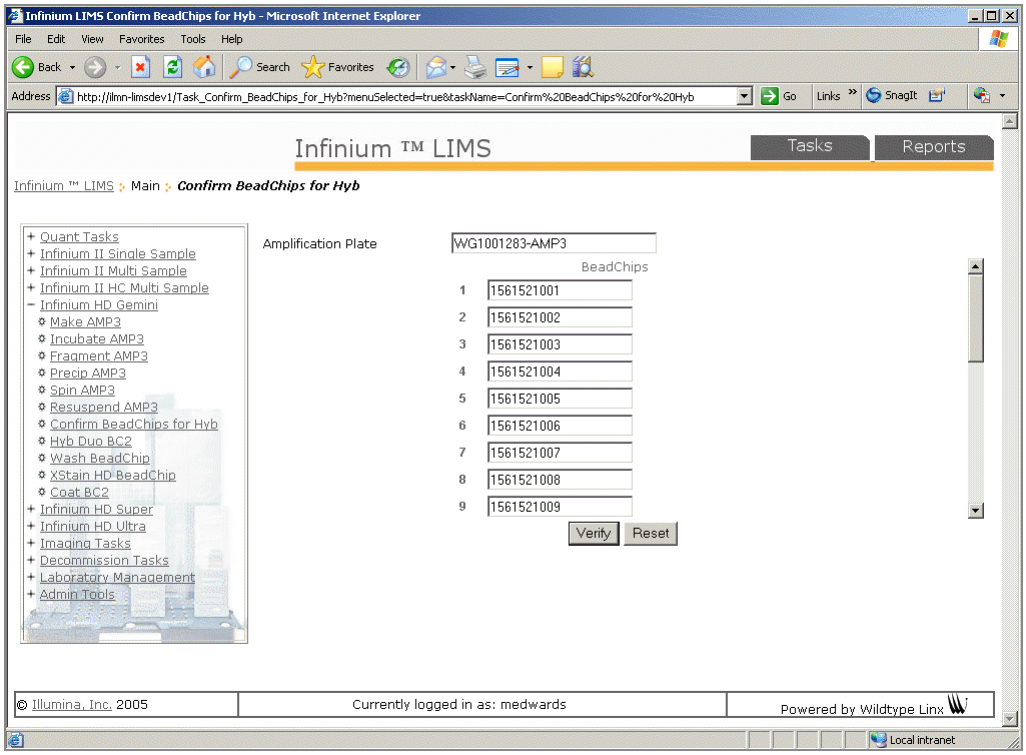


Figure 132 (Infinium LIMS) Verifying AMP3 and BeadChips for Hyb

5. If the AMP3 plate and BeadChips are queued for hybridization, a blue confirmation message appears at the top of the window. Proceed to loading the BeadChips.

   If the AMP3 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 the BeadChips* on page 144.

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

Load the BeadChips

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 Guides* at the end of the Hyb Multi BeadChip section.

1. Place the resuspended AMP3 plate on the heat block at 95°C for 20 minutes, to denature the samples.
2. Pulse centrifuge the AMP3 plate to 280 xg for 1 minute.

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

3. Remove all BeadChips from their packages.
4. Place two BeadChips into each Robot BeadChip Alignment Fixture so that the barcode lines up with the ridges on the fixture.

CAUTION Handle BeadChips only by the edges or by the barcode end.

Figure 133 Placing BeadChips into Robot Alignment Fixture

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

Figure 134 Four Stacked Robot Alignment Fixtures

Set Up the Robot

1. At the robot PC, select **AMP3 Tasks | Hyb Duo BeadChip**.
2. (Non-LIMS only) In the Basic Run Parameters pane, enter the **Number of BeadChips**.

   You can only dispense samples from one AMP3 plate at a time. To process additional AMP3 plates, repeat the *Load the BeadChips* section on page 144.

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

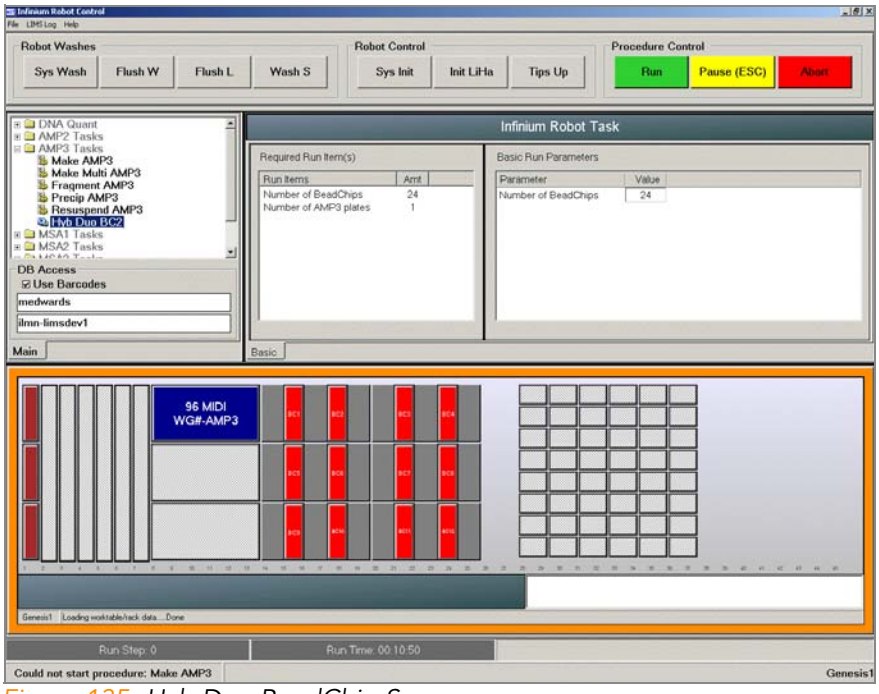


Figure 135 Hyb Duo BeadChip Screen

3. Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map (Figure 135). Do one of the following:
   - For 8 samples, position the two Robot BeadChip alignment fixtures in the top row.
   - For 24 samples, use all six positions on the robot bed.
4. Place the AMP3 plate onto the robot bed according to the bed map and remove the foil seal.
5. On the lab tracking form, record the plate and fixture positions on the robot bed, and record the BeadChip barcodes associated with each well.
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, click **Run**.
2. (Infinium 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.
3. Place a Robot Tip Alignment Guide on top of each Robot BeadChip Alignment Fixture. The Guide-A barcode should be upside down and facing away from you when properly placed on the Robot BeadChip Alignment Fixture.
4. At the robot PC, click **OK** to confirm you have placed a Robot Tip Alignment Guide on top of each Robot BeadChip Alignment Fixture.

Figure 136 Full set of Robot Tip Alignment Guides on Robot Bed

5. (Infinium LIMS only) The Robot scans the barcode on the Robot Tip Alignment Guide to confirm the correct guide is being used.

6. The robot dispenses the sample onto the BeadChips, following the layout shown in the lab tracking form. The robot PC sounds an alert and displays a message when the process is complete.

   Click **OK** in the message box.

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.

7. 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.
8. If you are running 12 BeadChips or fewer, discard the AMP3 plate and proceed to *Set Up Duo BeadChip for Hyb* on page 148.
9. If you are running 13 BeadChips or more, the Infinium Robot Control application prompts you to place a new set of BeadChips on the robot deck. Before you place the next set of samples on the Robot bed, immediately assemble the Hyb Chambers for the first set of 12 BeadChips as described in the steps on page 148. Leave the assembled Hyb Chambers of the first 12 BeadChips on the lab bench in a horizontal position.
10. Replace the Robot Tip Alignment Guides used for the first set of BeadChips with a new set of Robot Tip Alignment Guides that have been washed and dried.
11. After you have completed the steps for the first set of 12 BeadChips, place the new set of BeadChips on the robot bed according to the bed map and click **OK.**
12. When prompted, place a new set of Robot Tip Alignment Guides that have been washed and dried on top of each Robot BeadChip Alignment Fixture and click **OK** to start the next run.

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

13. Continue on to *Set Up Duo BeadChip for Hyb* on page 148.

Set Up Duo BeadChip for Hyb

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

CAUTION

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

2. Carefully remove each BeadChip from the Robot BeadChip Alignment Fixtures when the robot finishes. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the insert (Figure 137).

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 beadstripe area and sample inlets.

Figure 137 Placing Hyb Chamber Inserts into Hyb Chamber

3. Place the back side of the lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts (Figure 138).
4. Close the clamps on both sides of the Hyb Chamber.

Figure 138 Securing Hyb Chamber Lid

NOTE

For optimal performance, take care to 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 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 139 Hyb Chamber Correctly Placed in Hyb Oven

Figure 140 Two Hyb Chambers Correctly Placed in Hyb Oven

Figure 141 Incorrectly Placed Hyb Chamber

6. If you are loading multiple Hyb Chambers, stack them on top of each other, up to 3 per stack, for a total of 6 in the Hyb Oven. The Illumina logo on top of the Hyb Chamber should be facing you.

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 bottom Hyb Chamber. This will hold the Hyb Chambers in place while they are rocking.

7. (Optional) Set the rocker speed to 5 and start the rocker.
8. Incubate the Hyb Chamber(s) in the Illumina Hybridization Oven for 16–24 hours at 48°C.
9. Proceed to *Wash BeadChips* on page 152.

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.

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. You can store it at 2 to 8°C overnight, but thaw it again before use.

Wash Robot Tip Alignment Guides

1. Soak the Robot Tip Alignment Guides 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 Robot Tip Alignment Guides.

2. After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the Robot Tip Alignment Guides with DiH_2O at least three times to remove any residual detergent. Make sure the DiH_2O runs through all the tip guide channels.
3. Dry the Robot Tip Alignment Guides, especially the channels, using a Kimwipe or lint-free paper towels. Use a laboratory air gun to ensure they are dry. Be sure to inspect the channels, including the top and bottom of the insert. Robot Tip Alignment Guides should be completely dry and free of any residual contaminates before next use.

Wash BeadChips

In this process you prepare the BeadChips for the XStain HD BeadChip process. First, you remove the IntelliHyb Seals from the BeadChips and wash the BeadChips in two separate PB1 reagent washes to remove unhybridized and non-specifically hybridized DNA. The BeadChips are then assembled into Flow-Through Chambers under the PB1 buffer.

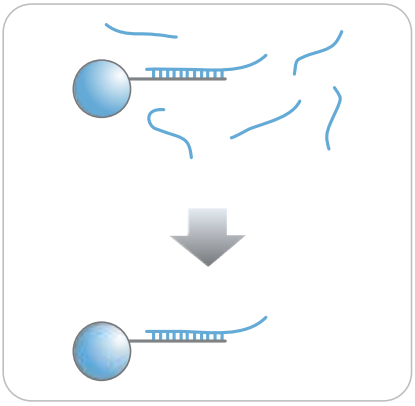


Figure 142 Washing BeadChip

Estimated Time

Hands-on time:

- 20 minutes per 8 BeadChips (16 samples)
- 30 minutes per 16 BeadChips (32 samples)
- 50 minutes per 24 BeadChips (48 samples)

Consumables

Item	Quantity (per 8 BeadChips)	Storage	Supplied By
PB1	550 ml	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1		Illumina
Te-Flow Flow-Through Chambers (with Black Frames, Spacers, Glass Back Plates, and Clamps)	1 per BeadChip		Illumina
Wash Dish	8 BeadChips: 2 dishes 24 BeadChips: 6 dishes		Illumina
Wash Rack	8 BeadChips: 1 rack 24 BeadChips: 3 racks		Illumina

Only pour out the recommended volume of PB1 needed for the suggested number of samples listed in the consumables table. Additional PB1 is used later in the XStain HD BeadChip step. One bottle of PB1 should be used per 8 BeadChips.

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.

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

- Fill 2 wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".
- Fill the BeadChip Alignment Fixture with 150 ml PB1.
- Separate the clear plastic spacers from the white backs.
- Clean the glass back plates according to the directions in the *Infinium Assay Lab Setup and Procedures Guide*.
- 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 provided on your documentation CD. This form can be filled out and saved online, or printed and filled in by hand.

Verify Reagents and BeadChips for Washing (Infinium LIMS only)

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

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

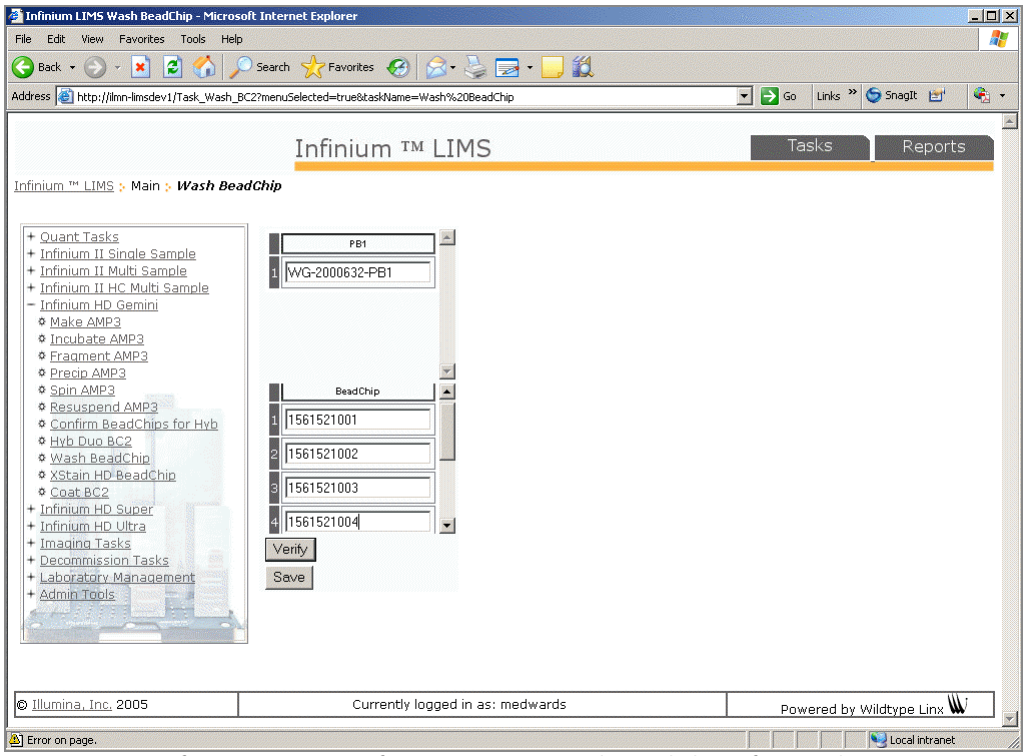


Figure 143 (Infinium LIMS) Verifying Reagents and BeadChips for Washing

5. If the reagents are correct and BeadChips are queued for washing, a blue confirmation message appears at the top of the window. Proceed to the wash step.
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.

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

Steps Wash BeadChip

1. Remove each Hyb Chamber from the Illumina Hybridization Oven.
2. Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1 (Figure 144).
3. Remove the Hyb Chamber inserts from the Hyb Chambers.
4. Remove BeadChips from the Hyb Chamber inserts one at a time.

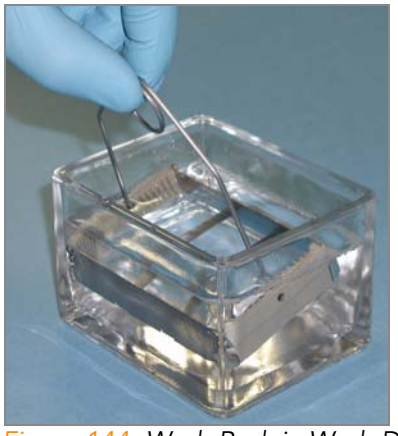


Figure 144 Wash Rack in Wash Dish Containing PB1

5. Remove the IntelliHyb seal from each BeadChip as follows:

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. Wearing powder-free gloves, 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, rapid 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.

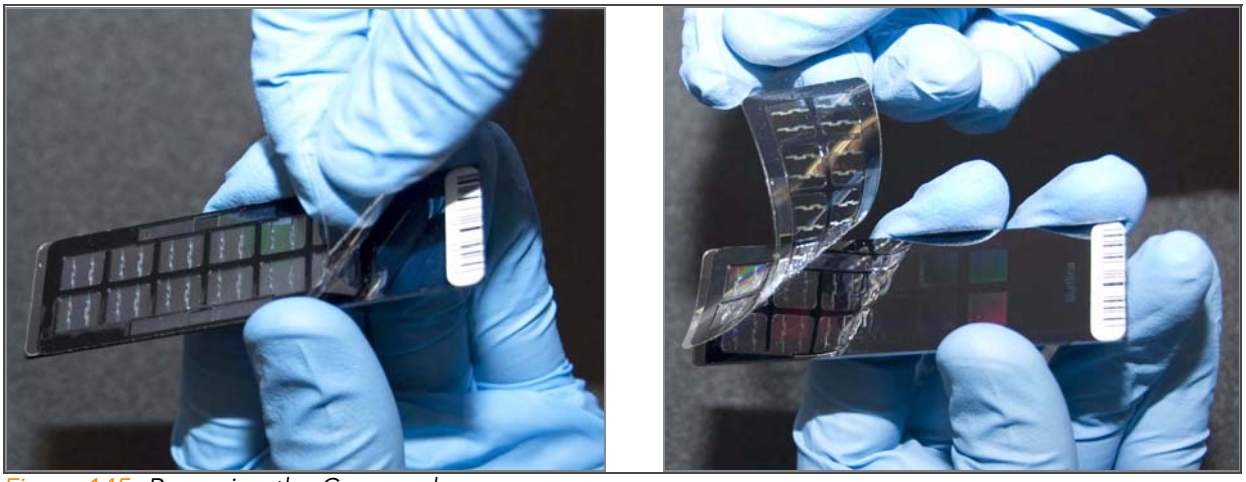


Figure 145 Removing the Coverseal

CAUTION

Do not touch the arrays!

6. 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 146).

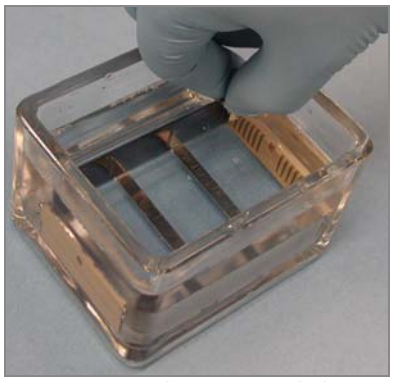


Figure 146 Placing BeadChips in Wash Dish Containing PB1

7. Repeat steps 5 and 6 until all BeadChips are transferred to the submerged wash rack. The wash rack holds up to 8 BeadChips.
8. 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.
9. Move the wash rack to the other wash dish containing PB1. Make sure the BeadChips are completely submerged.
10. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

11. If you are processing more than 8 BeadChips:
    a. Complete the steps in the next section, *Assemble Flow-Through Chambers*, for the first eight BeadChips.
    b. Place the assembled Flow-Through Chambers of the first eight BeadChips on the lab bench in a horizontal position.

NOTE

Keep them in a horizontal position on the lab bench until all assembled Flow-Through Chambers are ready to be loaded into the Chamber Rack. Do not place the Flow-Through Chambers in the Chamber Rack until all necessary steps are completed.

    c. Repeat steps 3 through 11 from this section for any additional BeadChips. Use new PB1 for each set of eight BeadChips.

CAUTION

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

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 for every additional set of eight BeadChips.

1. If you have not done so yet, fill the 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 147) pre-filled with PB1 (see *Preparation* on page 153).

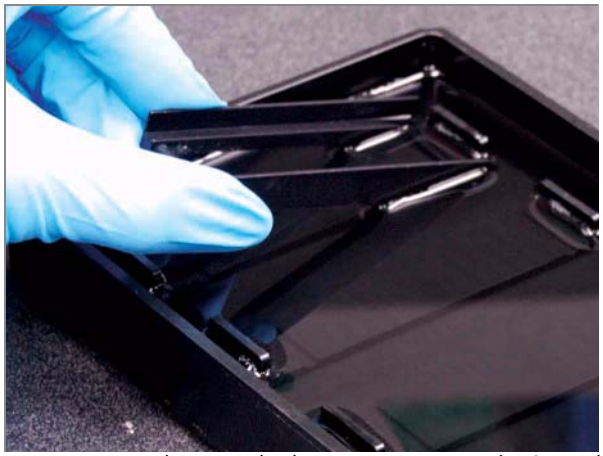


Figure 147 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

3. Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture (Figure 148). Each BeadChip should be fully immersed in PB1.

Figure 148 Placing BeadChip into Black Frame on Alignment Fixture

4. Place a clear spacer onto the top of each BeadChip to be processed (Figure 149). 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.

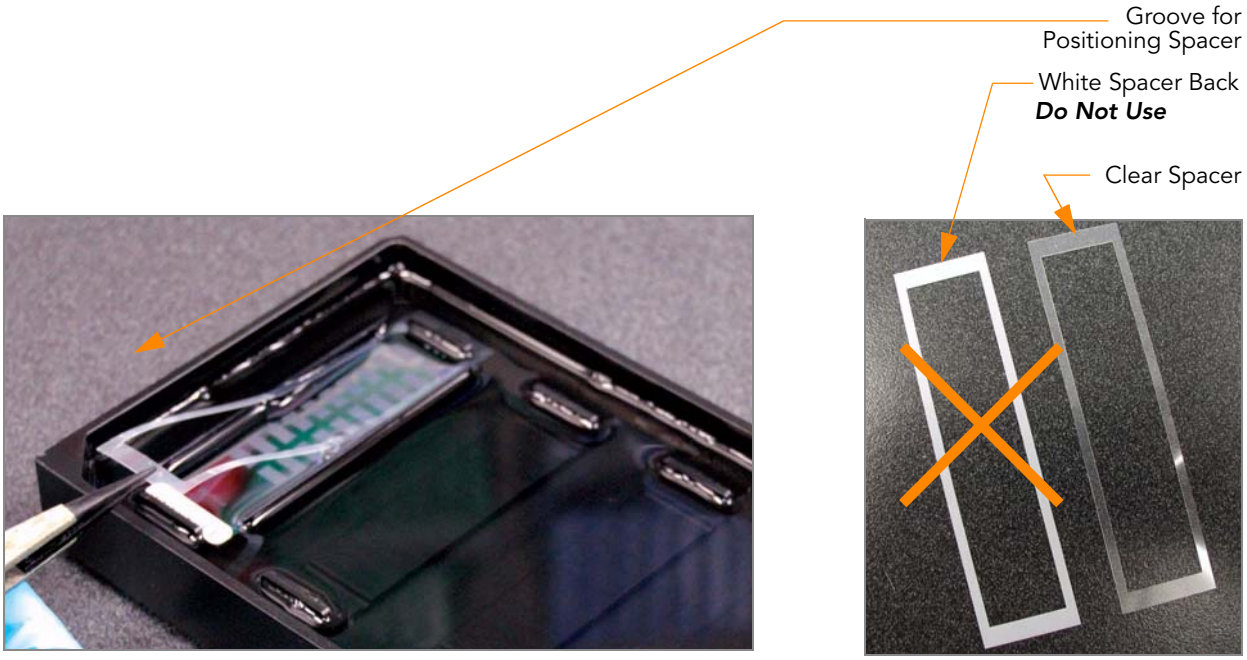


Figure 149 Placing Clear Plastic Spacer onto BeadChip

5. Place the Alignment Bar onto the Alignment Fixture (Figure 150).

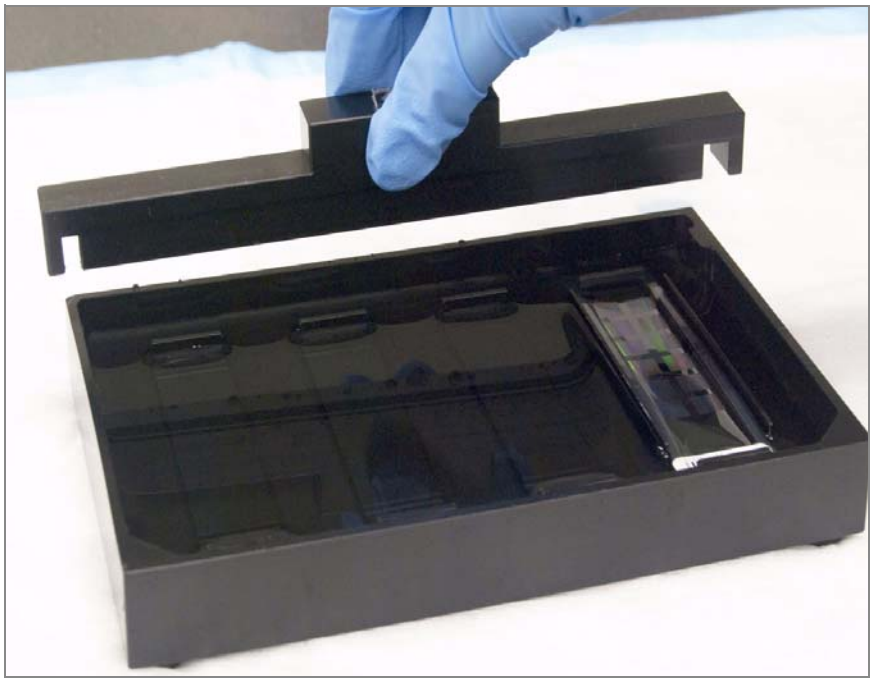


Figure 150 Placing Alignment Bar onto Alignment Fixture

6. Use a 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 151).

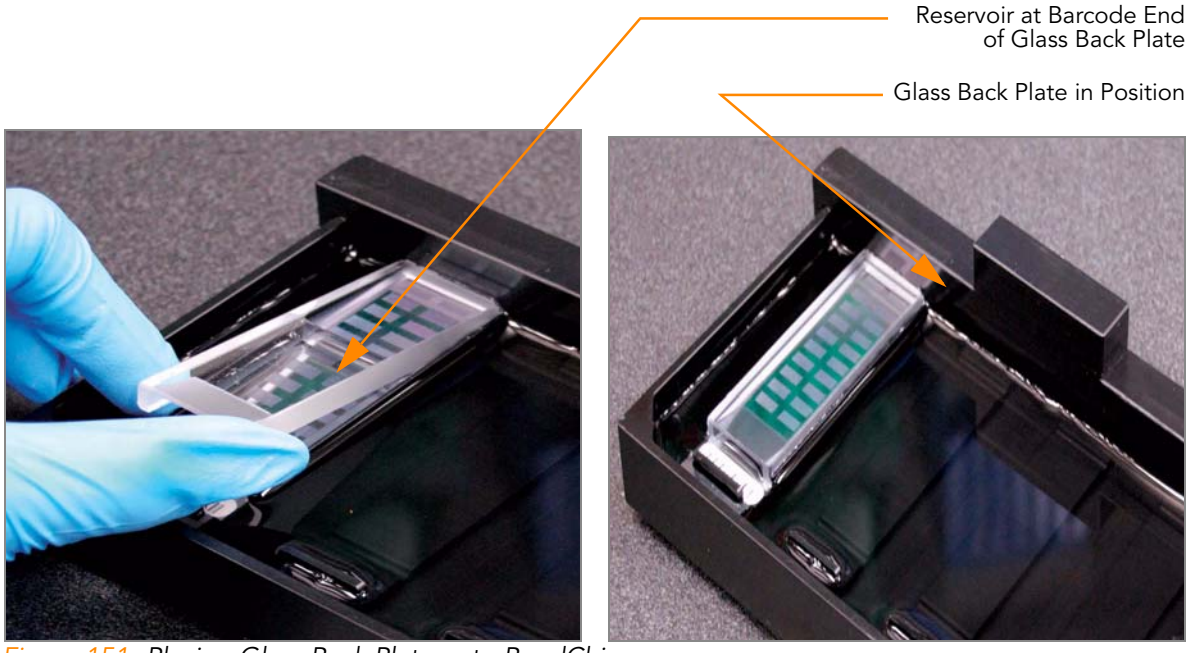


Figure 151 Placing Glass Back Plate onto BeadChip

8. Attach the metal clamps to the Flow-Through Chambers as follows (Figure 152):
   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.

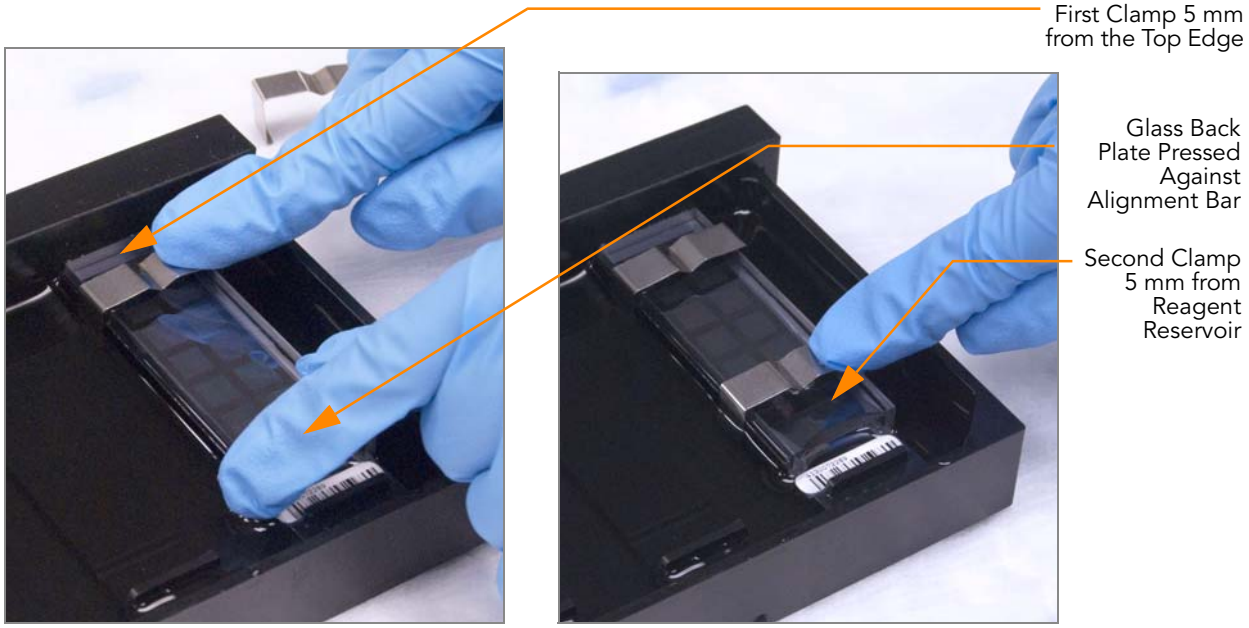


Figure 152 Securing Flow-Through Chamber Assembly with Metal Clamps

9. Using scissors, trim the ends of the clear plastic spacers from the Flow-Through Chamber assembly (Figure 153):

Figure 153 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.

11. If you are using Infinium LIMS:
    a. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Wash BeadChip**.
    b. Scan the reagent barcodes and the BeadChip barcodes. Click **Save**. Infinium LIMS records the data and queues the BeadChips for the next step.

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.

12. Discard unused reagents in accordance with facility standards.

13. If you are using Infinium LIMS:
    a. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Wash BeadChip**.

b. Scan the reagent barcodes and BeadChip barcodes. Click **Save**. Infinium LIMS records the data and queues the BeadChips for the next step, *Single-Base Extension and Stain HD BeadChip*.

14. Proceed to *Single-Base Extension and Stain HD BeadChip* on page 163.

Single-Base Extension and Stain HD BeadChip

Following hybridization, RA1 reagent is used to wash away any remaining 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.

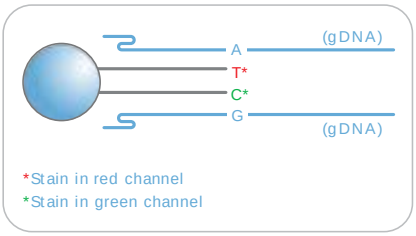


Figure 154 Extending and Staining BeadChip

Estimated Time

Robot time:

- 2 hours and 30 minutes per 8 BeadChips (16 samples)
- 2 hours and 40 minutes per 16 BeadChips (32 samples)
- 2 hours and 50 minutes per 24 BeadChips (48 samples)

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	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
XC2	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
TEM	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina

Item	Quantity	Storage	Supplied By
XC3	50 ml for 1–8 BeadChips 100 ml for 9–16 BeadChips 150 ml for 24 BeadChips	Room temperature	Illumina
STM (Make sure that all STM tubes indicate the same stain temperature on the label)	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
ATM	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
PB1	310 ml for 1–8 BeadChips 285 ml for 9– 24 BeadChips	Room temperature	Illumina
XC4	310 ml for 1–8 BeadChips 285 ml for 9– 24 BeadChips	-15 to -25°C	Illumina
Alconox Powder Detergent	as needed		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.

Preparation

- RA1 is shipped frozen. Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- Place all reagent tubes to be used in the assay in a tube rack; if frozen, thaw to room temperature and centrifuge to 3000 xg for 3 minutes.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - RA1 barcode

- XC3 barcode
- XC1 barcode(s)
- XC2 barcode(s)
- TEM barcode(s)
- STM barcode(s)
- ATM barcode(s)
- PB1 barcode
- XC4 barcode(s)

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

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.

   This temperature may vary depending on facility ambient conditions.

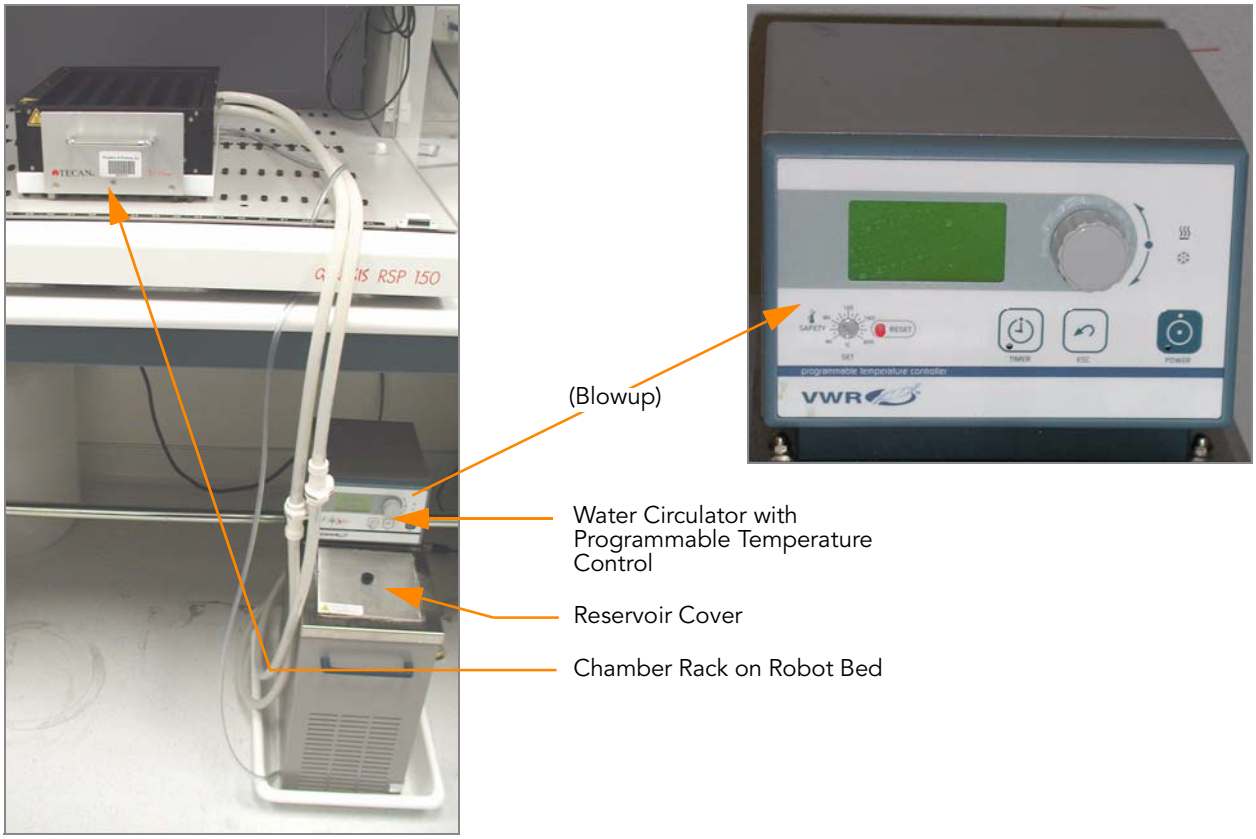


Figure 155 Water Circulator Connected to Chamber Rack

3. The temperature displayed on the water circulator LCD screen may differ from the actual temperature on the Chamber Rack. Confirm this using the temperature probe for the Chamber Rack.
4. Remove the bubbles trapped in the Chamber Rack. You must do this every time you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*.
5. Use the Illumina Temperature Probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 156).

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

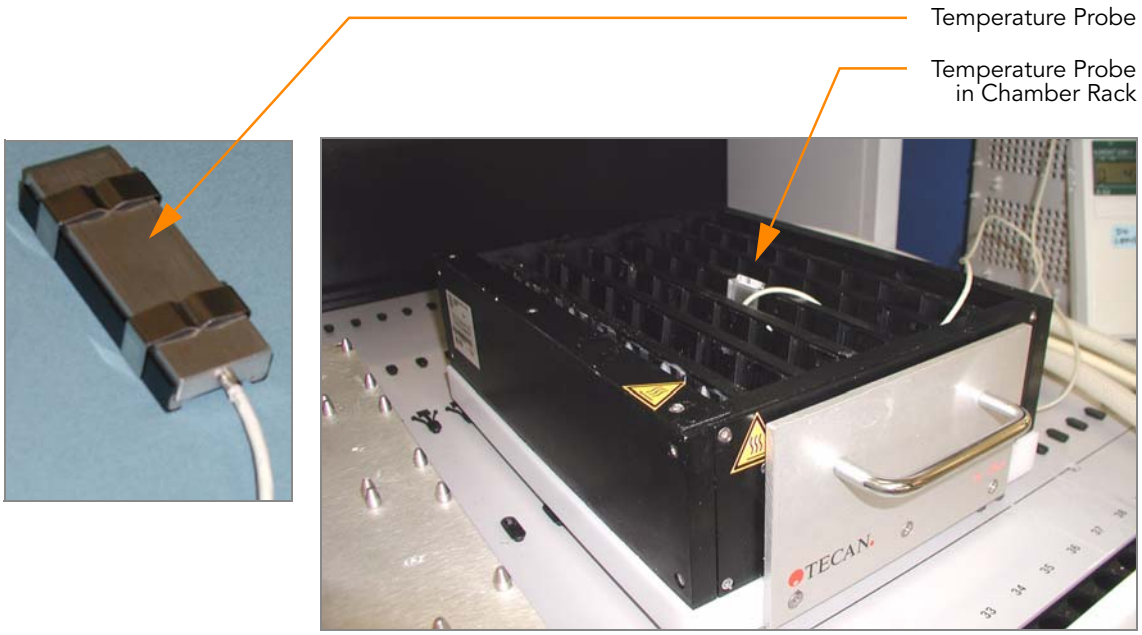


Figure 156 Illumina Temperature Probe in Chamber Rack

6. For accurate temperature measurement, ensure the Temperature Probe is touching the base of the Chamber Rack.

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 157 throughout this protocol.

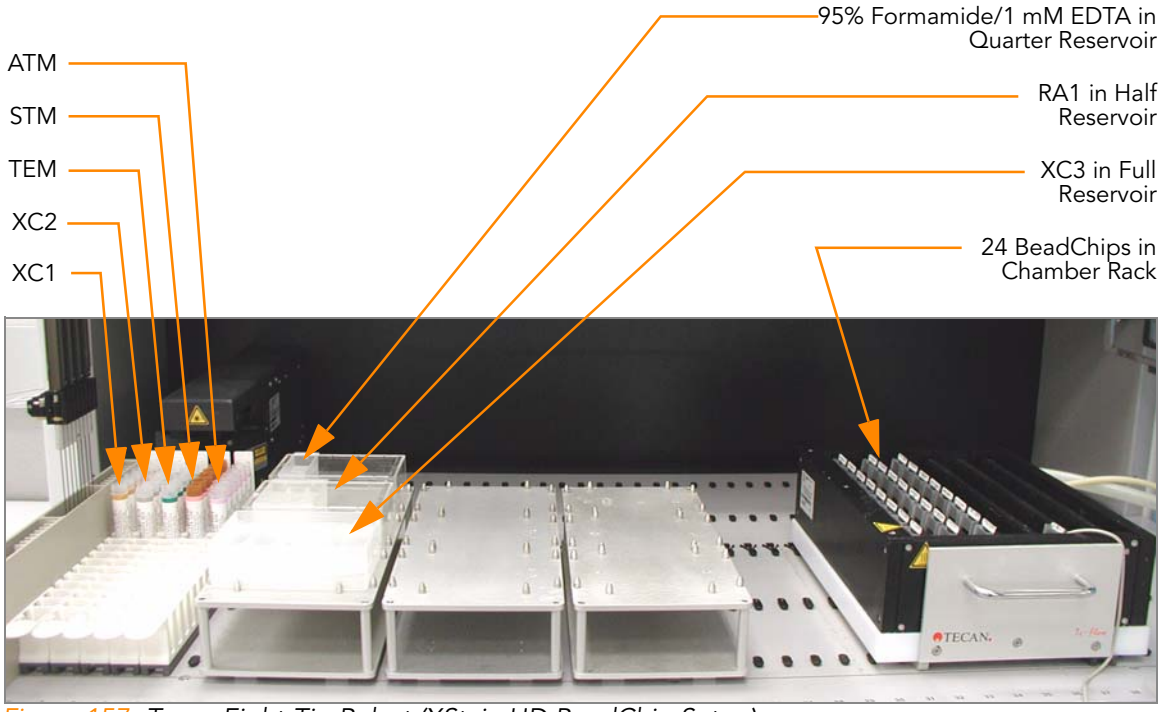


Figure 157 Tecan Eight-Tip Robot (XStain HD BeadChip Setup)

Steps Single-Base Extension and Stain (XStain)

CAUTION The remaining steps must be performed without interruption.

1. Slide the Chamber Rack into column 28 on the robot bed. Ensure that it is seated properly.
2. At the robot PC, select **Infinium II Chemistry | XStain HD BeadChip**.
3. (Non–LIMS only) In the Basic Run Parameters pane, enter the **Number of BeadChips** (up to 24).

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

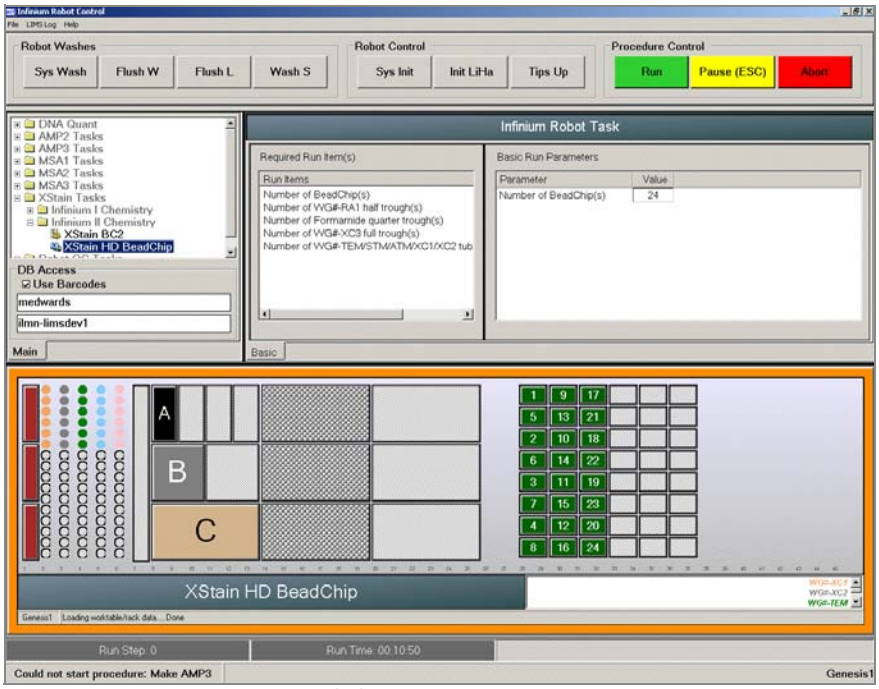


Figure 158 XStain HD BeadChip Screen

4. Place a quarter reservoir in the reservoir frame according to the robot bed map (Figure 158), and add 95% formamide/1 mM EDTA as follows:
   - 8 BeadChips: 15 ml
   - 16 BeadChips: 20 ml
   - 24 BeadChips: 25 ml
5. Place a half reservoir in the reservoir frame according to the robot bed map, and add RA1 as follows:
   - 8 BeadChips: 10 ml
   - 16 BeadChips: 20 ml
   - 24 BeadChips: 30 ml
6. Place a full reservoir in the reservoir frame according to the robot bed map, and add XC3 as follows:
   - 8 BeadChips: 50 ml
   - 16 BeadChips: 100 ml
   - 24 BeadChips: 150 ml
7. Place the XC1, XC2, TEM, STM, and ATM tubes in the robot tube rack according to the robot bed map. Remove the caps.
8. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. Click **Run** to start the process.
   c. Log in if prompted.
   d. When prompted (Figure 159), enter the stain temperature. The correct temperature is indicated on the STM tube. If no temperature is listed, enter 37°C.

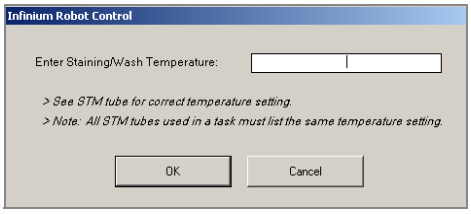


Figure 159 Entering Stain Temperature

   e. Observe the robot start to run to ensure that there are no problems.

2. When prompted (Figure 160), wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.

Figure 160 Adjusting Chamber Rack to 44°C Message

3. When the temperature probe registers 44°C, click **OK**.
4. When prompted (Figure 161), load the BeadChips click **OK**.

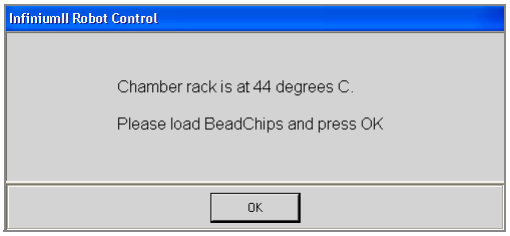


Figure 161 Load BeadChips Message

5. Quickly place each Flow-Through Chamber into the Chamber Rack according to the robot bed map.
6. Ensure each Flow-Through Chamber is properly seated to allow adequate heat exchange between the rack and the chamber.
7. On the lab tracking form, record the chamber rack position for each BeadChip.

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

Table 15 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

9. When prompted, immediately remove the Flow-Through Chambers from the Chamber Rack. Place them horizontally on the lab bench at room temperature.

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

10. Click **OK** to finish the process.

Verify Reagents and BeadChips for Coating (Infinium LIMS only)

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

3. Scan the barcode(s) of the XC4.
4. Scan the BeadChip barcodes.
5. Click **Verify**.

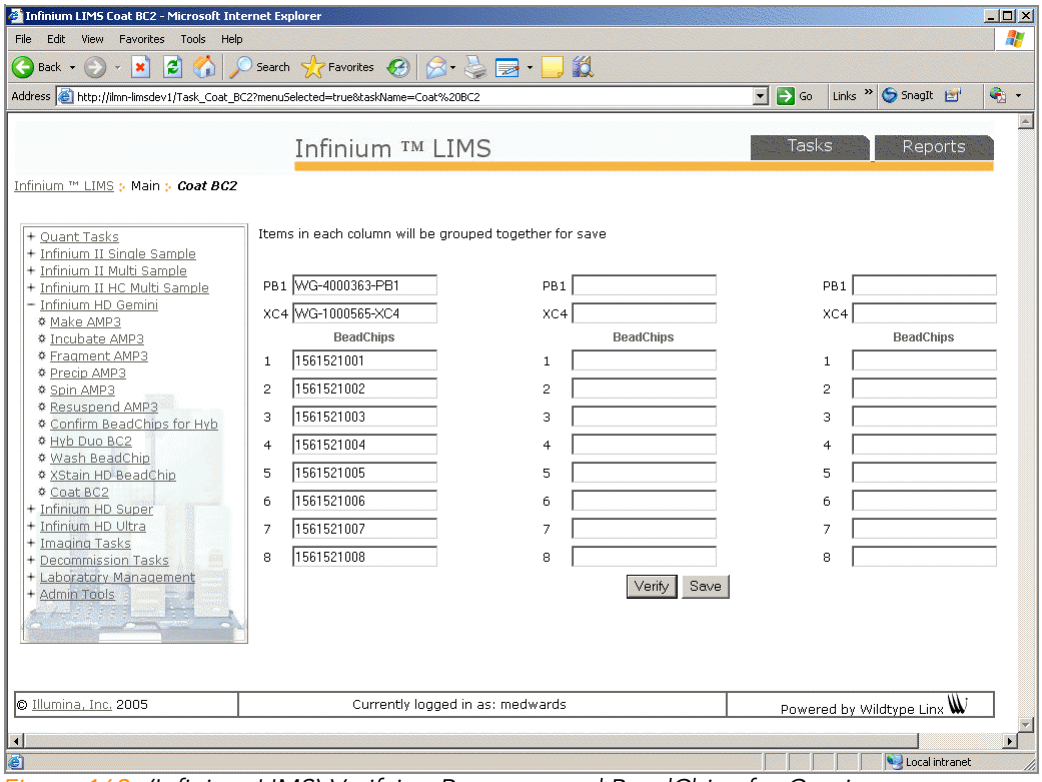


Figure 162 (Infinium LIMS) Verifying Reagents and BeadChips for Coating

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 the wash and coat step.
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.

Wash and Coat 8 BeadChips

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. Lay out the following equipment on the lab bench:
   - 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 as shown in Figure 163.
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. 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.

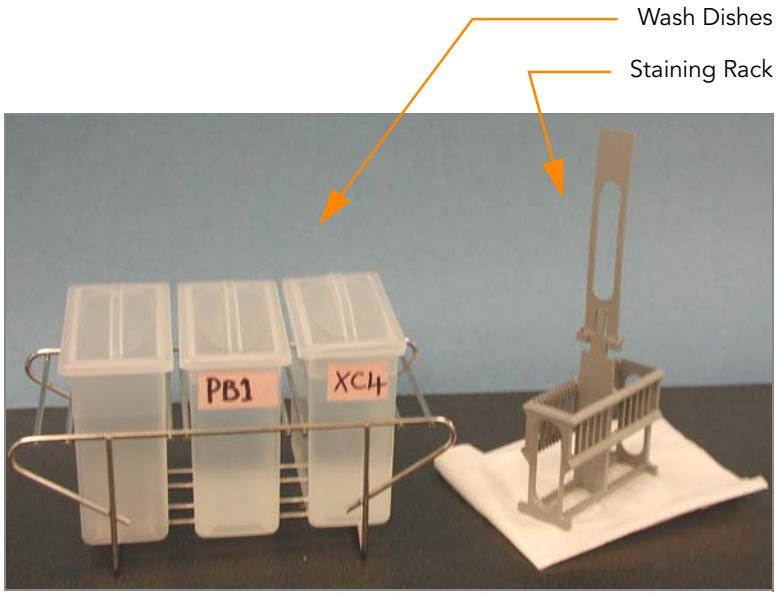


Figure 163 PB1 & XC4 Wash Dishes with Staining Rack

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 164). This orients the staining rack so that you can safely remove the BeadChips.

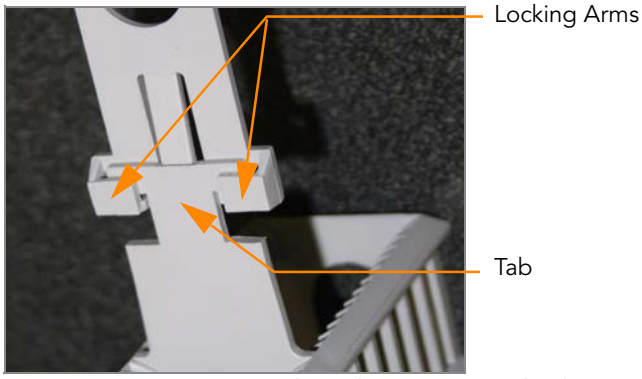


Figure 164 Staining Rack Locking Arms and Tabs

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. Using the dismantling tool, remove the two metal clamps (Figure 165).

CAUTION

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

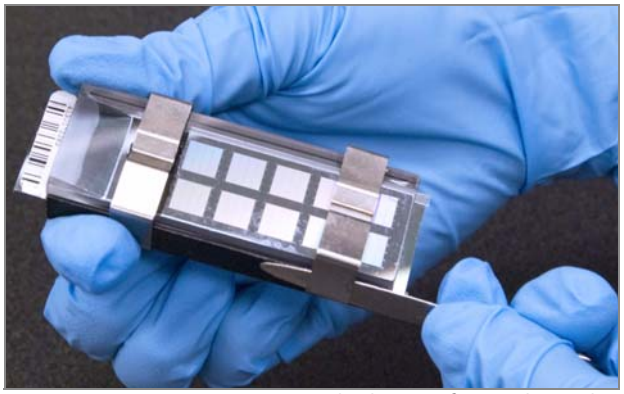


Figure 165 Removing Metal Clamps from Flow-Through Chamber

b. Remove the glass back plate.
c. Set the glass back plates aside. When you finish the XStain 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.

7. Place BeadChips in the staining rack while it is submerged in PB1. Put all four BeadChips above the staining rack handle. 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 166).

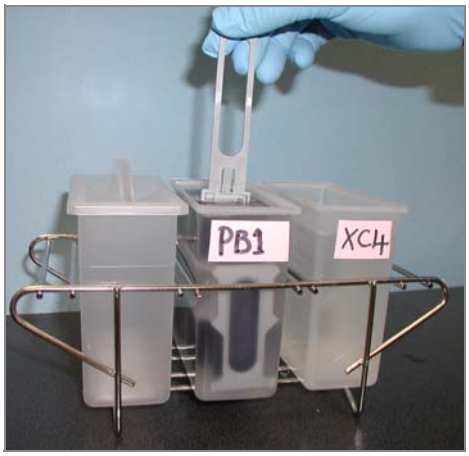


Figure 166 Washing BeadChips in PB1

NOTE

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

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

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 dish containing PB1 and place it directly into the wash dish containing XC4 (Figure 167). 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.

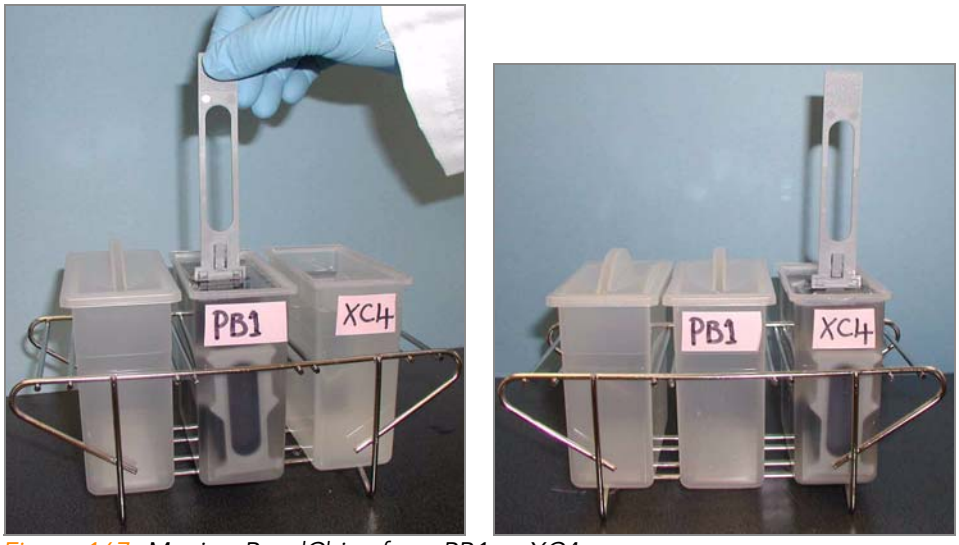


Figure 167 Moving BeadChips from PB1 to XC4

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

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

14. Soak for 5 minutes.

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

CAUTION Use XC4 only once. To process subsequent 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 (Illumina-provided from VWR catalog # 60916-748) 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 168, showing 8 BeadChips).

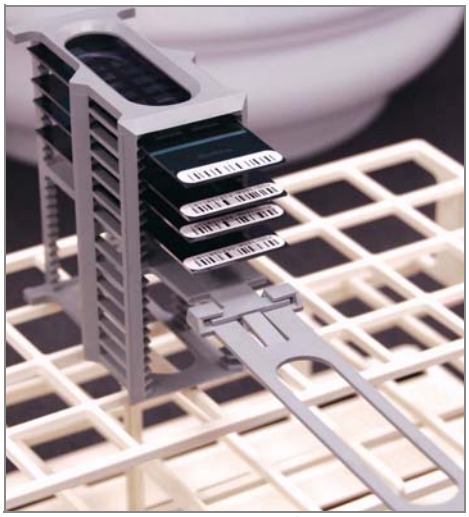


Figure 168 Staining Rack in Correct Orientation

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

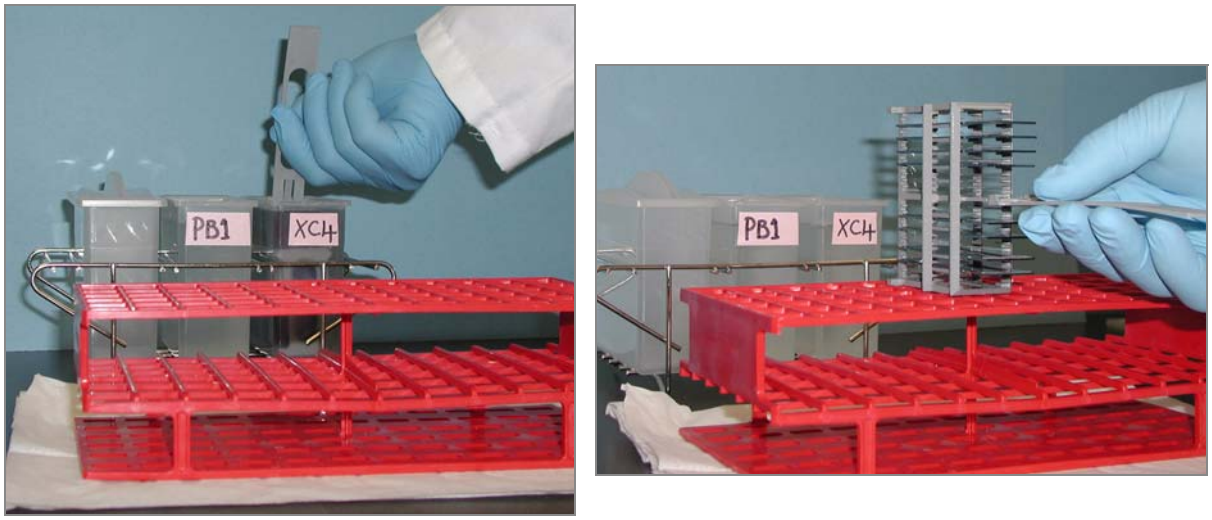


Figure 169 Moving BeadChip Carrier from XC4 to Tube Rack

19. For each of the 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 the BeadChip horizontally on a tube rack with the barcode facing up and towards you (Figure 170).

Figure 170 Placing 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.

20. Holding the top of the staining rack in position, grasp the handle between your thumb and forefinger. Push the tab up and push the handle away from you to unlock it. Pull up the handle and remove (Figure 171).

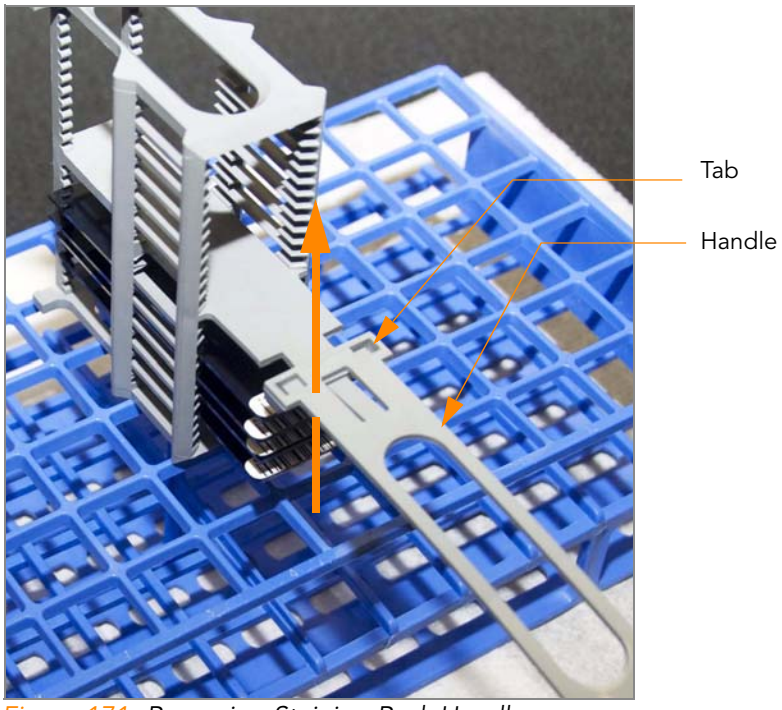


Figure 171 Removing Staining Rack Handle

21. Dry the BeadChips:
    a. Place the tube rack with the BeadChips (Figure 179) 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 dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.
    d. Dry under vacuum for 50–55 minutes.

    Drying times may vary according to room temperature and humidity.

22. Proceed to step 37, page 188.

Wash and Coat 24 BeadChips

Equipment Needed

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 as shown in Figure 172.
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.

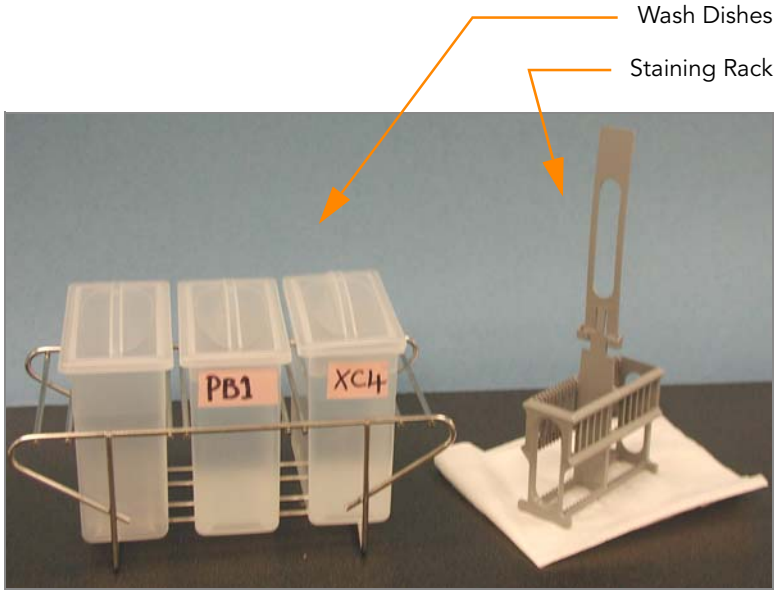


Figure 172 PB1 and XC4 Wash Dishes with Staining Rack

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 ***you*** (Figure 173). This orients the staining rack so that you can safely remove the BeadChips.

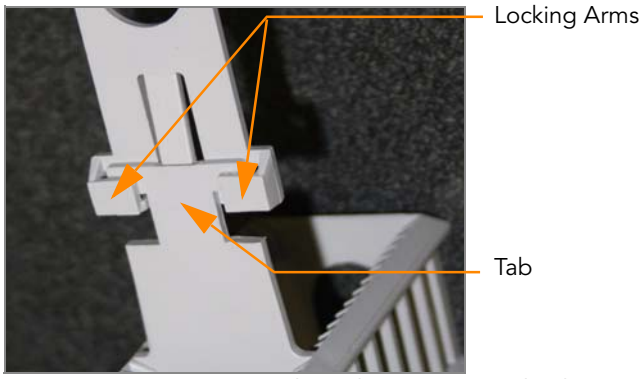


Figure 173 Staining Rack Locking Arms and Tabs

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 174).

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

Figure 174 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. Place 12 BeadChips 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 175).

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.

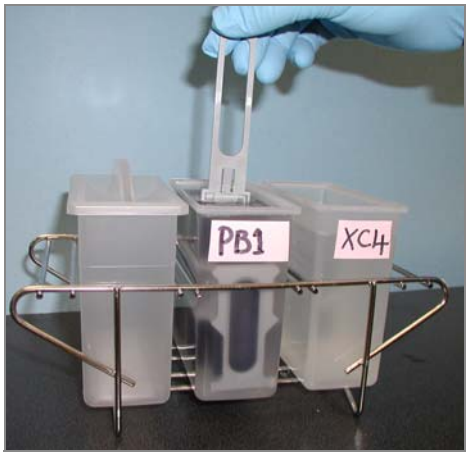


Figure 175 Washing BeadChips in PB1

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 176). 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.

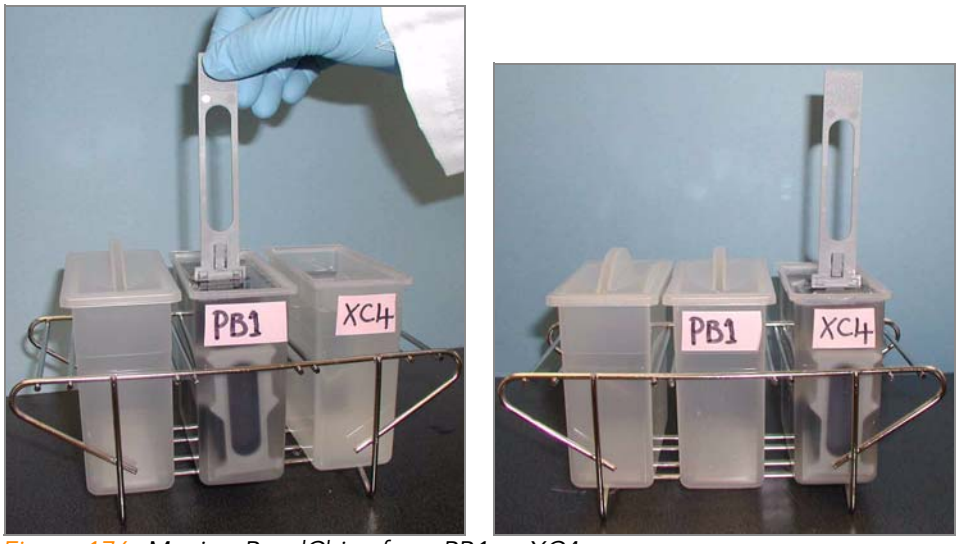


Figure 176 Moving BeadChips from PB1 to XC4

NOTE

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

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

CAUTION

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

16. Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.
17. Prepare one additional tube rack per 8 BeadChips (Illumina-provided from VWR catalog # 60916-748) that fits the internal dimensions of the vacuum desiccator.
18. Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes face ***up*** and the locking arms and tab face ***down*** (Figure 178).

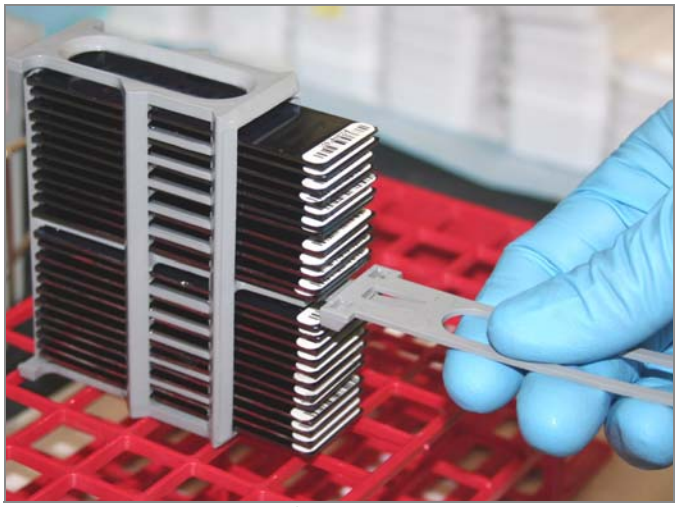


Figure 177 Staining Rack in Correct Orientation

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

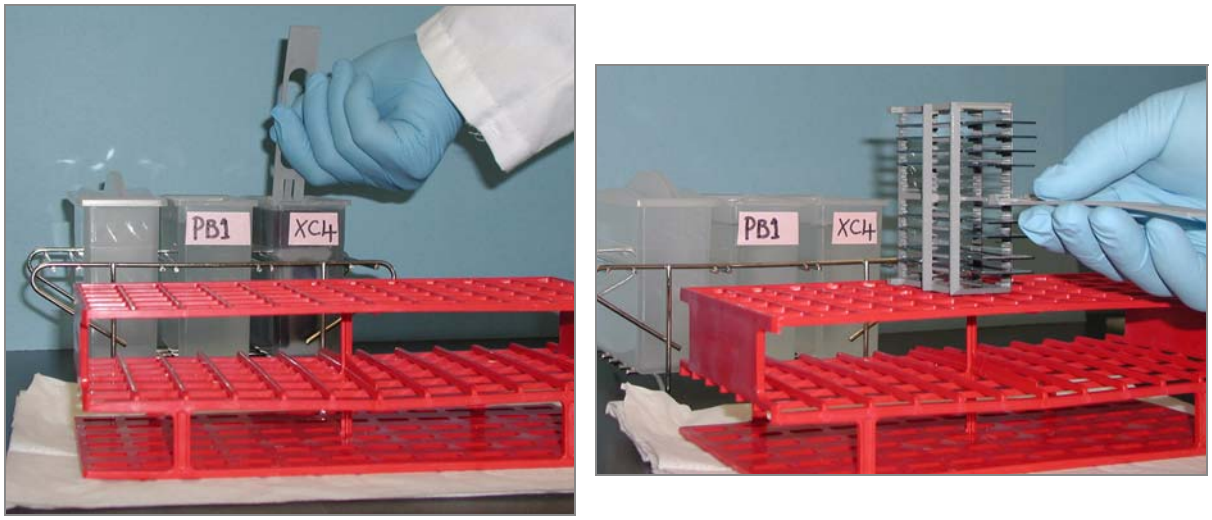


Figure 178 Moving BeadChip Carrier from XC4 to Tube Rack

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

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

NOTE

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

b. Put the eight BeadChips on the tube rack as shown in Figure 179, 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 179 Placing BeadChips on Tube Rack

21. Return the staining rack to the XC4 wash dish and top off the dish until the BeadChips are completely covered with remaining XC4 reagent.
22. Soak the BeadChips for 10 seconds.
23. Dry the **first 8** BeadChips:
    a. Place the tube rack with the first 8 BeadChips (Figure 179) 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 dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.
    d. Dry under vacuum for 50–55 minutes.

    Drying times may vary according to room temperature and humidity.
24. Remove the staining rack with the remaining BeadChips in one rapid motion from the XC4 wash dish and place it directly on the tube rack. Ensure that the BeadChips are horizontal with the barcodes facing *up*.

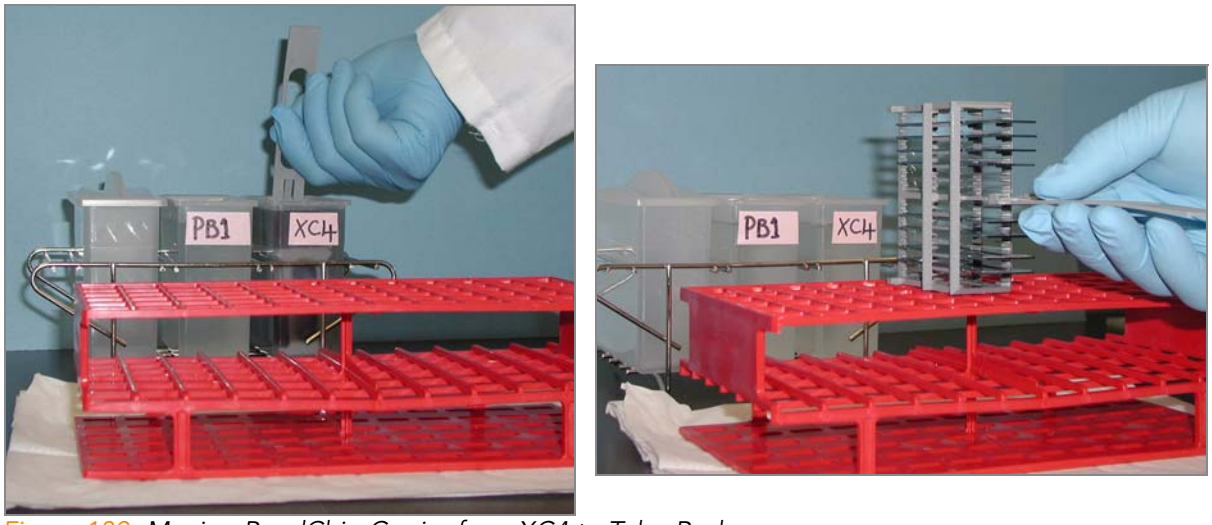


Figure 180 Moving BeadChip Carrier from XC4 to Tube Rack

25. Remove the four BeadChips that remain above the staining rack handle and place them on the tube rack.
26. 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 181).

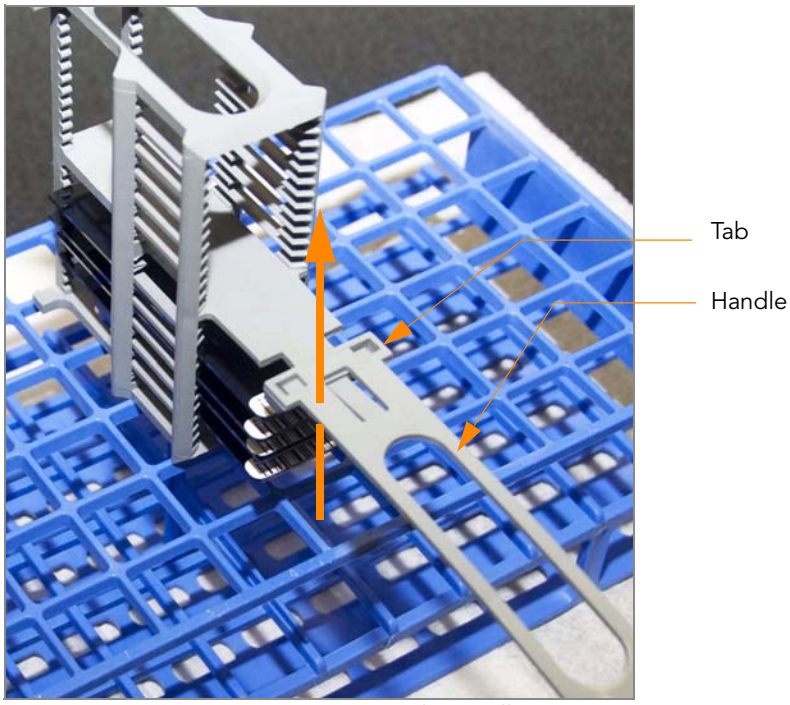


Figure 181 Removing Staining Rack Handle

27. Place BeadChips on the tube rack as shown in Figure 179 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.
28. 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.
29. Soak BeadChips for 10 seconds.
30. Dry the **second set of 8** BeadChips:
    a. Place the tube rack with the second set of 8 BeadChips (Figure 179) 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 dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.
    d. Dry under vacuum for 50–55 minutes.
31. 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.
32. Place BeadChips on the tube rack as shown in Figure 179 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.

33. Place the tube rack with the **third set** of 8 BeadChips (Figure 179) into the desiccator. Check the vacuum pressure and make sure that the valve is securely attached.
34. Start the vacuum, using at least 508 mm Hg (0.68 bar).
35. To ensure that the dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.

Figure 182 Testing Vacuum Seal

36. Dry under vacuum for 50–55 minutes.
37. 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.

38. 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.
39. 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.
40. If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom two BeadChips are the most likely to have some excess.
    a. Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
    b. Wrap a pre-saturated Prostat EtOH Wipe around your index finger.

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

Do not touch the stripes.

41. If you are using Infinium LIMS:
    a. In the Infinium LIMS left sidebar, click **Infinium HD Gemini | Coat BeadChip**.
    - Scan the reagent barcodes and BeadChip barcodes and click **Save**. Infinium LIMS records the data and queues the BeadChips for the next step, *Image BeadChip on the iScan System* on page 190 or *Image BeadChip on the BeadArray Reader* on page 198.
42. Clean the glass back plates. For instructions, see the *Infinium Assay Lab Setup and Procedures Guide*.
43. 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 humidifying buffer reservoirs.
44. Discard unused reagents in accordance with facility standards.
45. Do one of the following:
    - Proceed to *Image BeadChip on the iScan System* on page 190 or *Image BeadChip on the BeadArray Reader* on page 198.
    - 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: 35 minutes per BeadChip

Preparation

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

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

- For more information about the iScan System, GenomeScan software, or AutoLoader2, see the *iScan and AutoLoader2 System Guide*.

Steps

Overview

The GenomeScan 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 GenomeScan application.

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

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

Starting Up the iScan System

1. Turn on the iScan Reader and the attached PC.
2. Let the iScan Reader warm up for at least 5 minutes before beginning a scan. It is fine to use the GenomeScan software during this time.
3. For each BeadChip you plan to scan, copy the contents of the mini-CD provided with the BeadChip 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 195.

4. Double-click the GenomeScan icon on the desktop.

   The Welcome window appears (Figure 183). The GenomeScan 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.

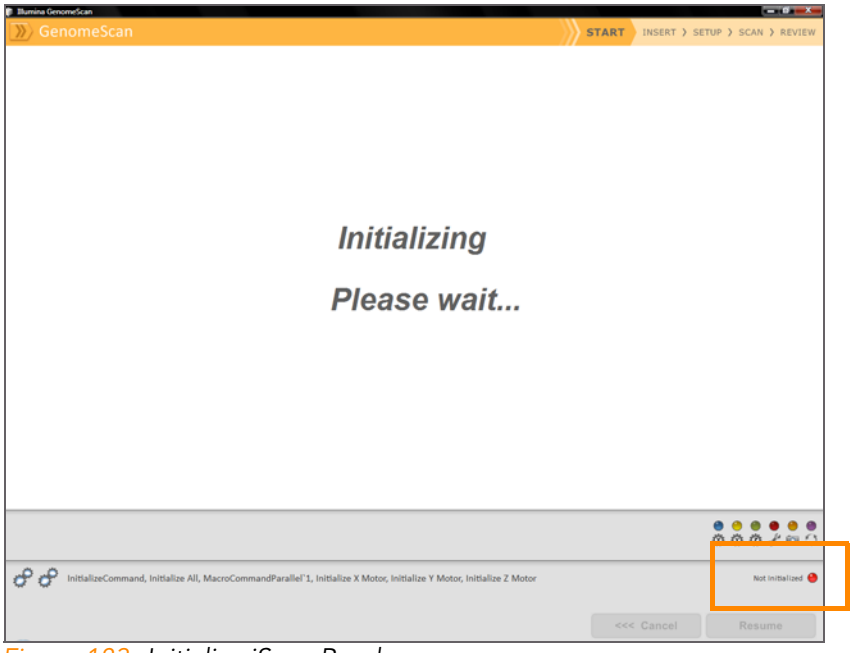


Figure 183 Initialize iScan Reader

5. Set the **LIMS** dropdown list to **None** and enter your Windows user name (Figure 184).

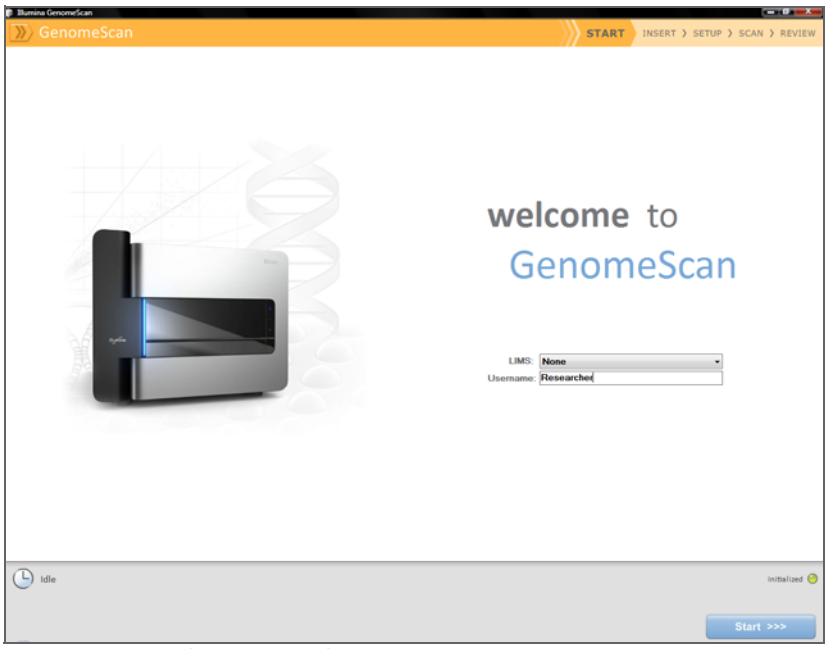


Figure 184 Welcome Window

6. 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 185).

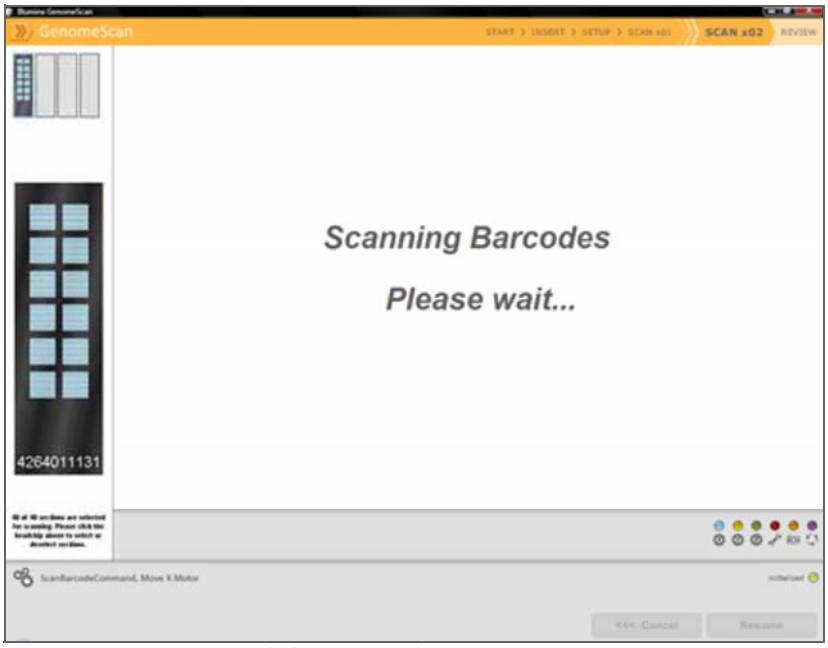


Figure 185 Scan BeadChip Barcodes

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.

For more information about the scan settings, see the *iScan and AutoLoader2 System Guide*.

2. 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 186). The section will no longer be highlighted blue.

Figure 186 Deselect BeadChip Sections

3. If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.
4. To begin scanning the BeadChips, click **Scan**.

   Scanning should take 8–10 minutes per BeadChip.

   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 196.
5. At the end of the scan, a Review window appears (Figure 187). The Scan Metrics table at the top shows the intensity values, registration, and focus metrics for each stripe on the BeadChip.

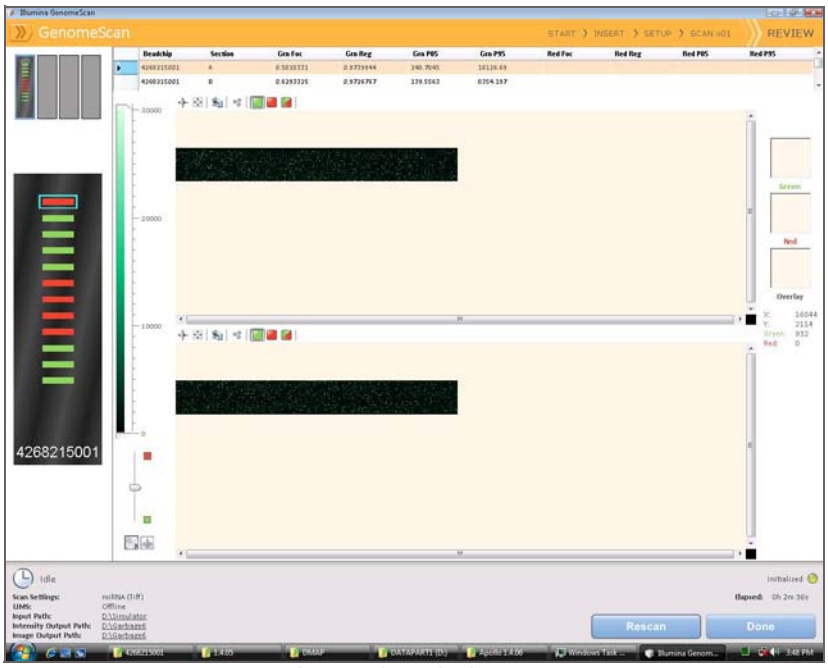


Figure 187 Review Window with Failed Stripes

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.

6. 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.

7. 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, no *.idat files will be saved for those sections. 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 GenomeScan software. Use another program such as Illumina's GenomeStudio software to view images from the scan.

Setting Up Input and Output Paths

This step should only occur once, when you install the GenomeScan 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 came on each BeadChip CD (for

example, D:\Decode). The GenomeScan software refers to this folder as the Input Path.

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

   During the scan, GenomeScan automatically creates subfolders 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 GenomeScan 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 188).

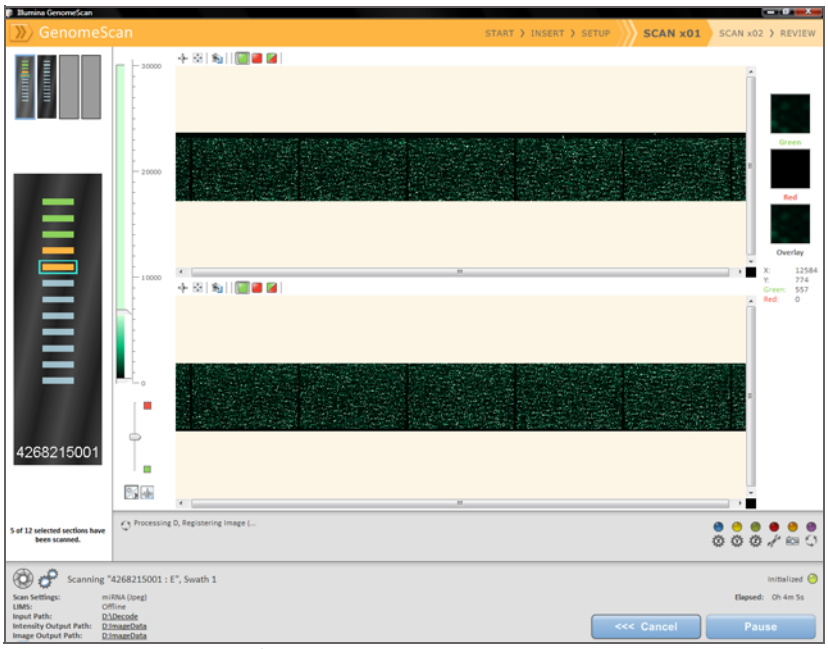


Figure 188 Monitor the Scan

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 register 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 warmup for the BeadArray Reader (first use of the day only)

45 minutes to scan each BeadChip using BeadScan 3.2 FastScan settings

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

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

Initializing the BeadArray Reader (Daily)

If this is the first time the scanner is being used today, follow these steps.

Refer to Figure 189 throughout this protocol.

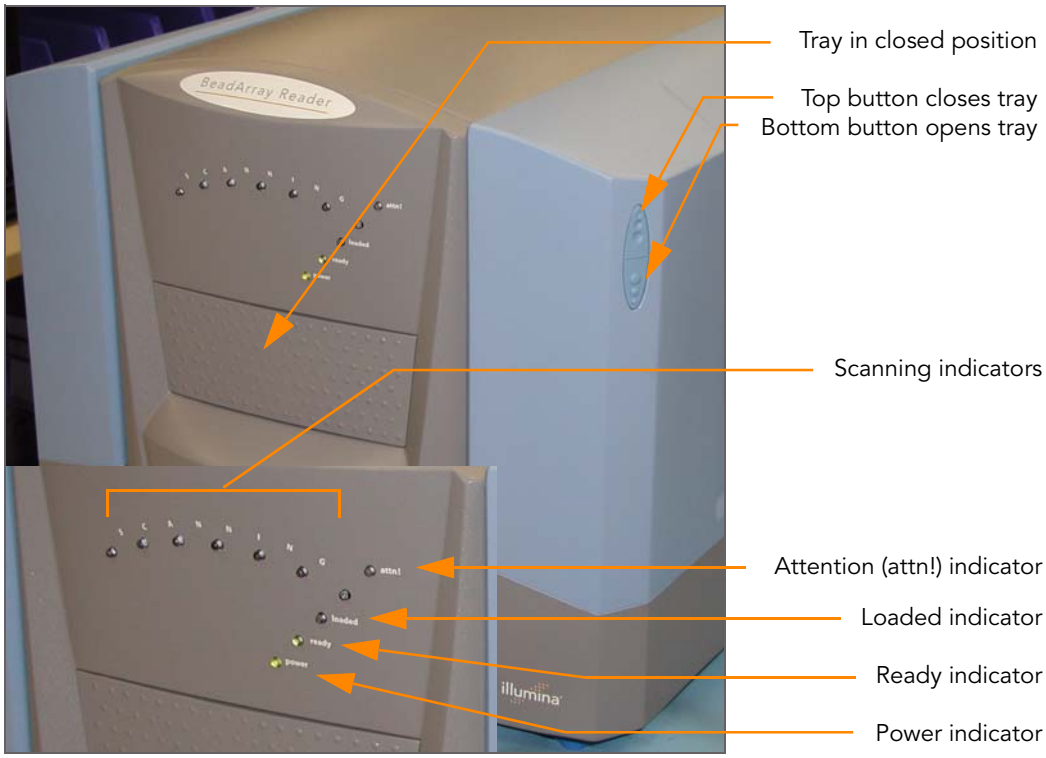


Figure 189 Illumina BeadArray Reader

Table 16 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 190).

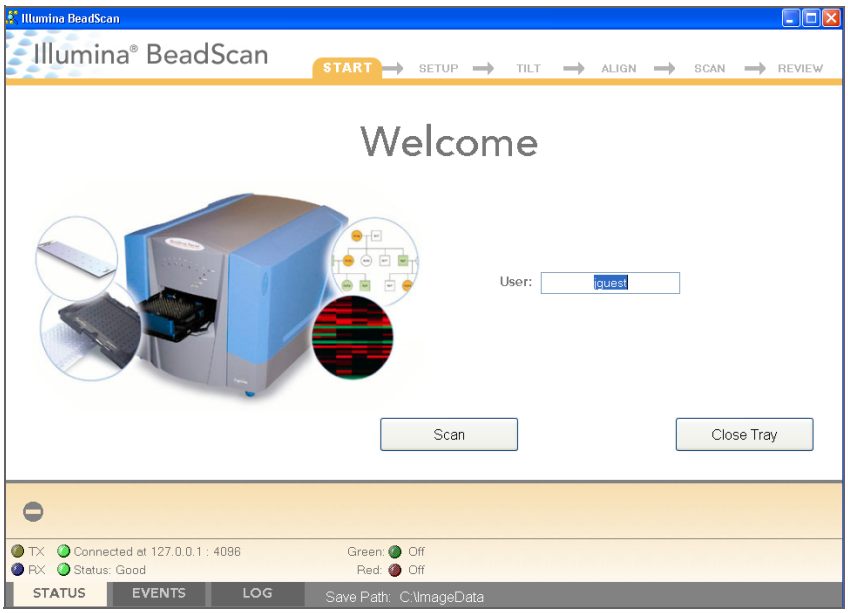


Figure 190 BeadScan Welcome Screen

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 191).

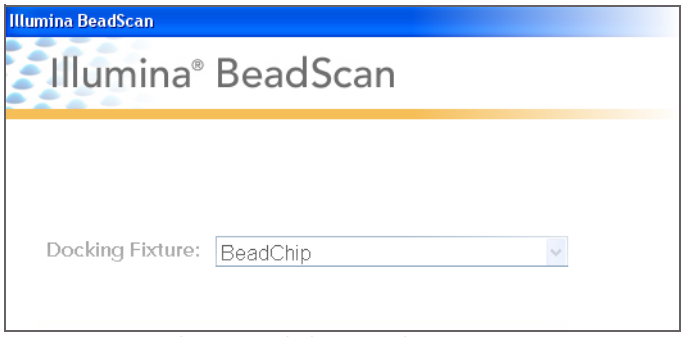


Figure 191 Select BeadChip Docking Fixture

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 from the BeadChip CD. The default path is C:\DecodeData.
3. If either path is not correct:
   a. Click **Edit** to open the Options dialog box.

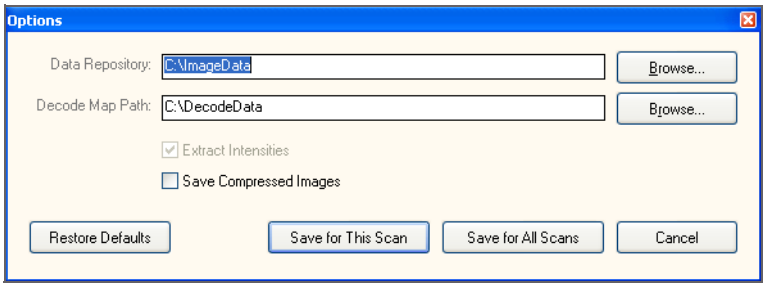


Figure 192 Enter Data Repository and Decode Data Paths

b. Click **Browse** to navigate to and select the Data Repository path and the Decode Map path.

c. Select or clear the **Save Compressed Images** check box. Compressed images use the JPG format. Uncompressed images use the TIFF format and may be 75 MB or more.

d. After changing settings, click either **Save for this Scan** or **Save for All Scans**.

4. Copy the decode map (*.dmap) files for each BeadChip from the BeadChip CD to the Decode Map path directory.

5. For each BeadChip:

a. Place the BeadChip into the BeadArray Reader tray.

b. Using the hand-held barcode scanner, scan the BeadChip barcode. The barcode appears on the screen in the position corresponding to the BeadChip position in the tray.

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

d. Select the appropriate scan method and click **Select**.

6. Make sure that the BeadChips are properly seated in the BeadArray Reader tray (Figure 193).

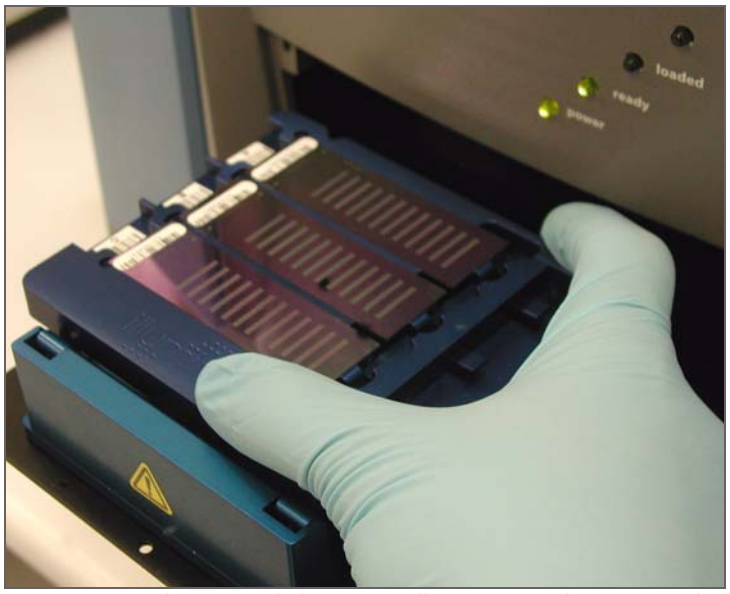


Figure 193 Place BeadChips into Illumina BeadArray Reader Tray

7. Click **Scan**.

Scanning Process

BeadScan begins the BeadArray Reader Tilt and Align processes (Figure 195):

- **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.

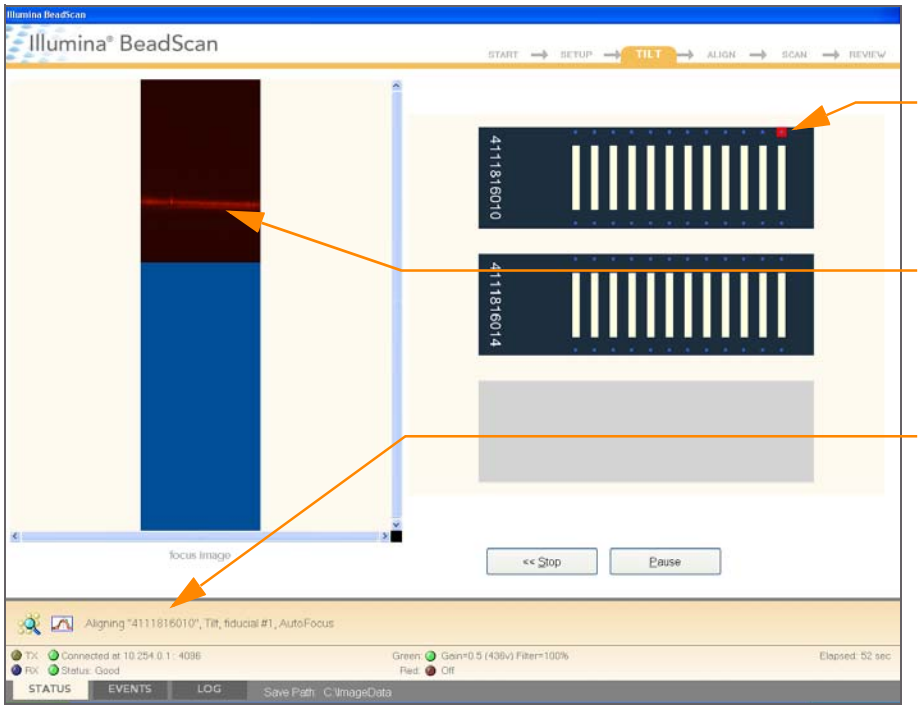


Figure 194 BeadArray Reader Tile Process

- **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.

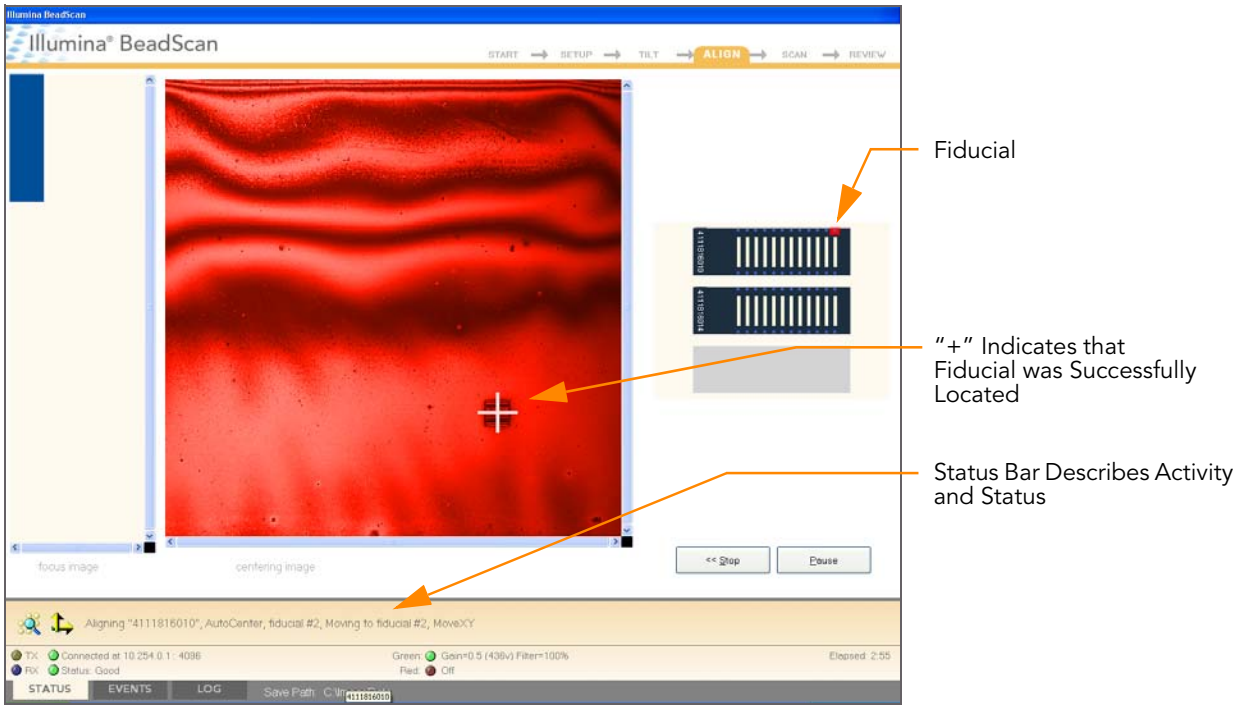


Figure 195 BeadArray Reader Tilt & Align Processes

Once the Tilt and Align processes are complete, the Scan process begins (Figure 196). Hover over any of the green dots in the closeup image to see the relative intensity and the XY position.[1] The red value should be at or close to zero, because this is a one-color assay.

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

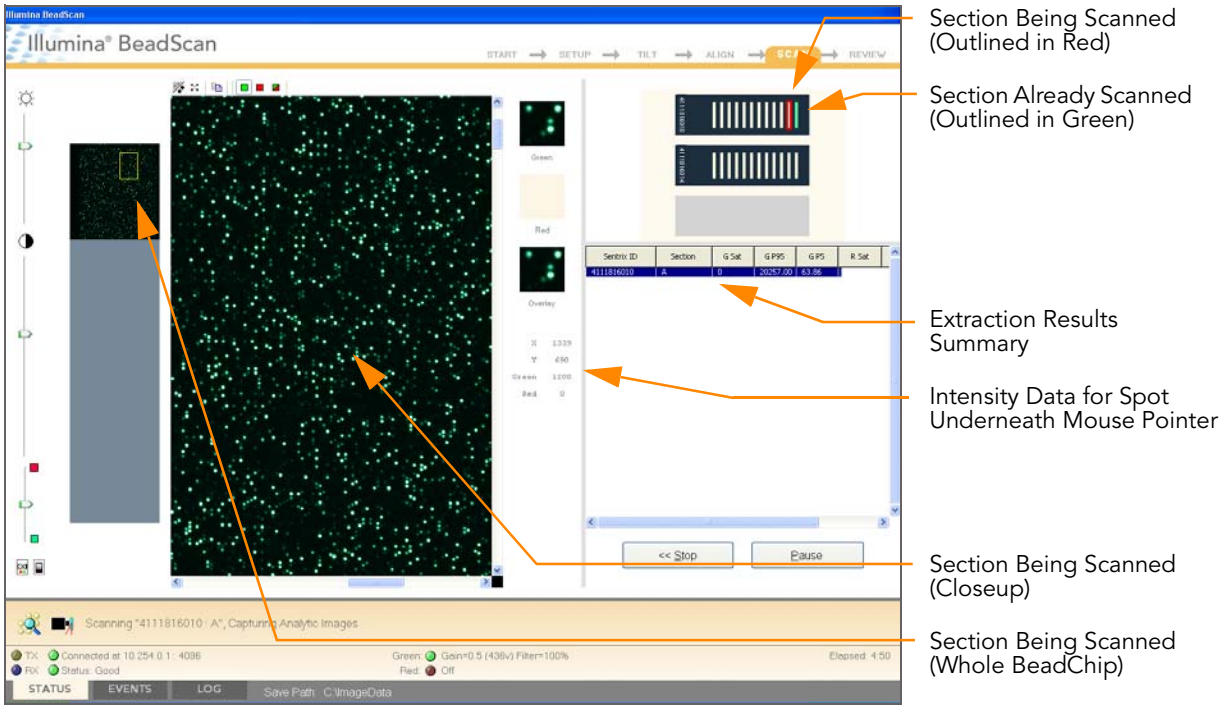


Figure 196 BeadArray Reader Scan Process

As the BeadArray Reader scans, the front panel blue Scanning indicator lights flash in sequence (see Figure 10 on page 25).

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.

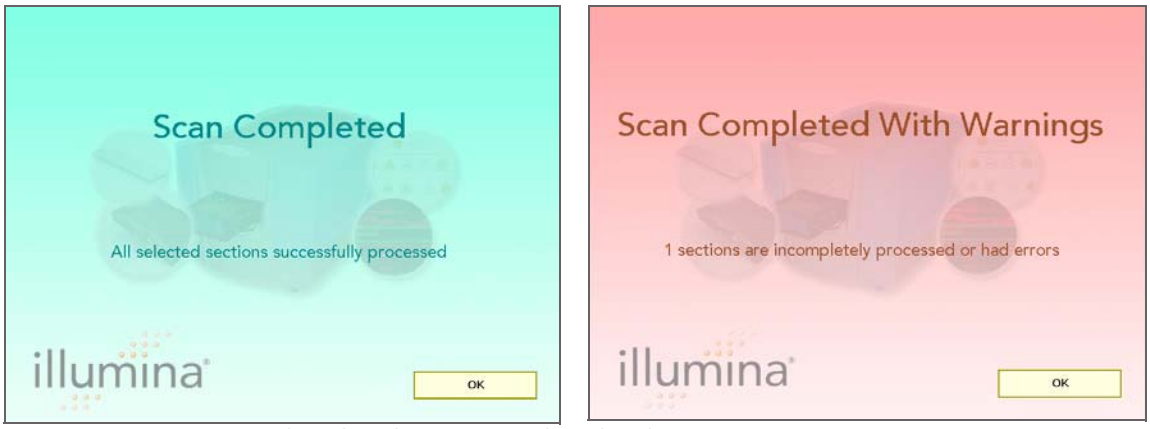


Figure 197 Scan Completed and Scan Completed with Warnings Screens

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 198).

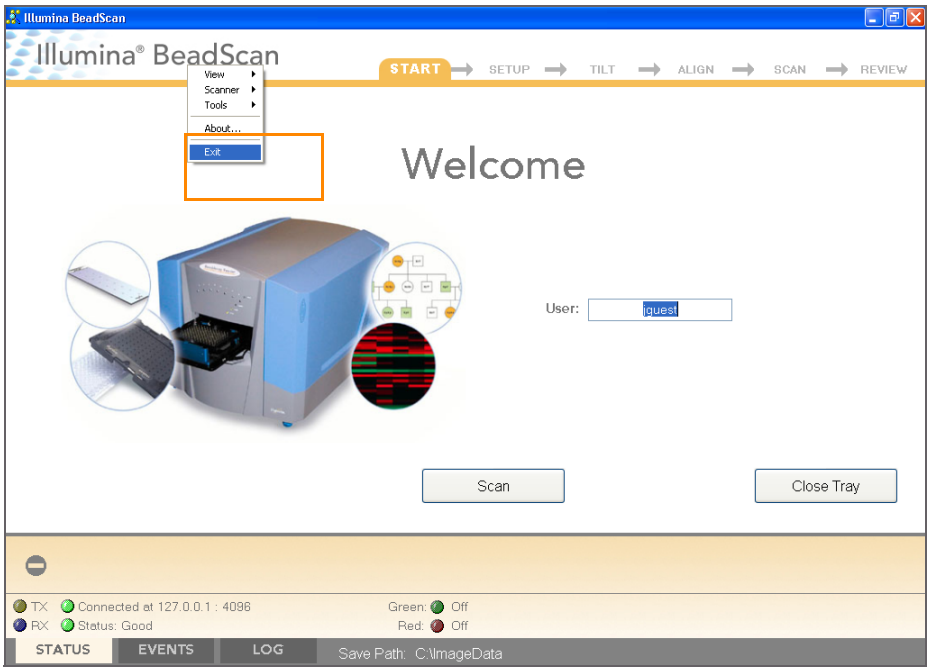


Figure 198 Exit the BeadArray Reader Software

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 199 inset).

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

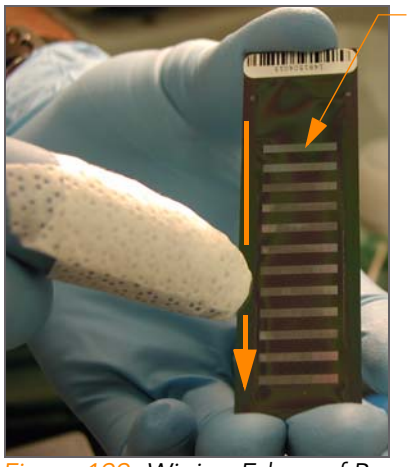


Figure 199 Wiping Edges of BeadChip

2. Wipe along the edge of each BeadChip five or six times (Figure 199), 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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