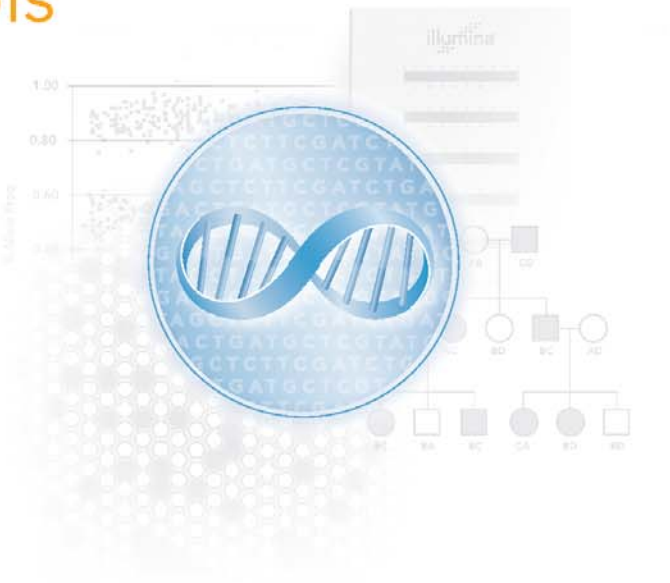
Infinium® II Assay
Multi-Sample Protocols

FOR RESEARCH ONLY



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Revision History

Revision Letter	Date
A	December 2006

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Chapter 1

Overview

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Introduction

The Illumina Infinium II Assay revolutionizes genotyping by streamlining sample preparation and enabling unlimited multiplexing. Using a single bead type and dual color channel approach, the Infinium II Assay scales genotyping from 10,000 to hundreds of thousands of SNPs per sample, dependent only on the number of features (bead types) on the array.

The Infinium II Assay accomplishes this unlimited multiplexing by combining whole-genome amplification (WGA) sample preparation with direct, array-based capture and enzymatic scoring of the SNP loci. Locus discrimination is provided by a combination of sequence-specific hybridization capture and array-based, single-base primer extension. A single primer is used to interrogate a SNP locus. The 3' end of the primer is positioned directly adjacent to the SNP site.

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 II Assay offers:

- Effectively unlimited multiplexing
- High call rate and accuracy
- Genome-wide SNP selection
- Single tube amplification—single chip—no PCR
- Minimal risk of carryover contamination
- Low DNA input mass of 750 ng
- Walk-away automation using Tecan Genesis or Freedom EVO Robots and Tecan GenePaint system
- Infinium LIMS automation
- Compatibility with both Illumina BeadLab and BeadStation systems
- Multiple-, duo-, and single-sample BeadChip formats
- Unlimited genotype multiplexing, scaling with the number of BeadChip features

Audience and Purpose

This guide is for laboratory technicians running the Infinium II Assay with the custom iSelect Genotyping BeadChip, also referred to as the multi-sample BeadChip. The guide documents the laboratory protocols associated with the assay. Follow all of the protocols in the order shown.

Chapter 2, *Multi-Sample BeadChip Manual Protocol* explains how to run the assay manually in the lab.

Chapter 3, *Multi-Sample BeadChip 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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963), which explains how to equip and run an Infinium II Assay laboratory. The guide includes important information on the following topics:

- Preventing amplification product contamination
- Safety precautions
- Equipment, materials, and reagents
- Standard lab procedures
- Using the robot
- Imaging the BeadChip
- System maintenance
- BeadStudio controls
- Troubleshooting

The instructions apply equally to all whole-genome genotyping chips provided by Illumina. All of the Infinium II Assay Protocol guides assume that you have already set up the laboratory space and are familiar with the standard procedures and safety precautions.

The Infinium II Assay

This section describes and illustrates the assay protocol. The assay requires only 750 ng of DNA sample as input.

Amplify DNA

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

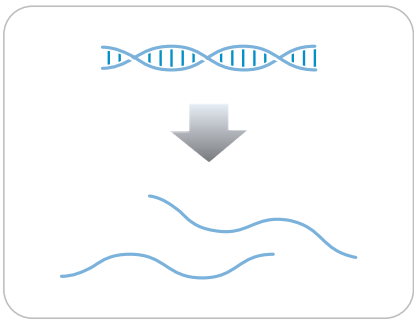


Figure 1 Denaturing and Neutralizing DNA

See Make the MSA2 Plate on page 26 for manual processing. See Make the MSA2 Plate on page 93 for automated processing.

Incubate DNA

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

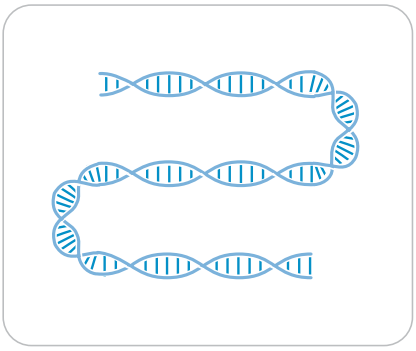


Figure 2 Incubating DNA to Amplify

See Incubate the MSA2 Plate on page 29 for manual processing. See Incubate the MSA2 Plate on page 100 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.

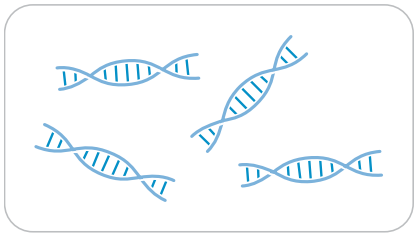


Figure 3 Fragmenting DNA

See Fragment the MSA2 Plate on page 30 for manual processing. See Fragment the MSA2 Plate on page 102 for automated processing.

Precipitate DNA

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

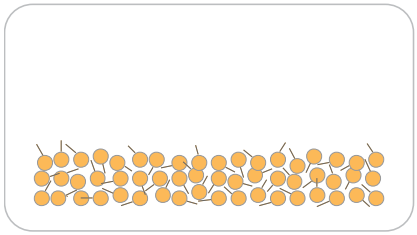


Figure 4 Precipitating DNA

See Precipitate the MSA2 Plate on page 32 for manual processing. See Precipitate the MSA2 Plate on page 105 for automated processing.

Resuspend DNA

The precipitated DNA is resuspended in hybridization buffer.

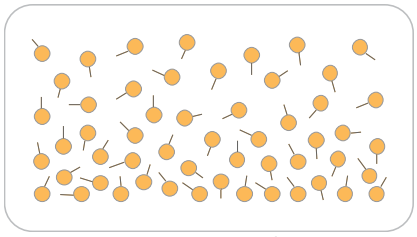


Figure 5 Resuspending DNA

See Resuspend the MSA2 Plate on page 35 for manual processing. See Resuspend the MSA2 Plate on page 111 for automated processing.

Hybridize to BeadChip

Twelve samples are applied to each BeadChip, which keeps them separate with an IntelliHyb seal. The prepared 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 500,000 bead types) during hybridization. One bead type corresponds to each allele per SNP locus.

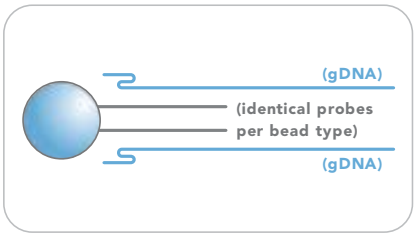


Figure 6 Hybridizing DNA to BeadChip

See Hyb Multi BC2 on page 37 for manual processing. See Hyb Multi BC2 on page 115 for automated processing.

Wash BeadChip

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

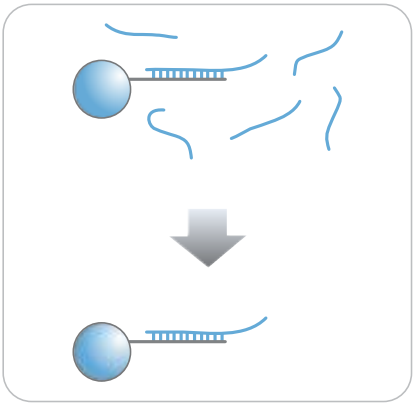


Figure 7 Washing BeadChip

See Wash BC2 on page 44 for manual processing. See Wash BC2 on page 125 for automated processing.

Extend and Stain (XStain) BeadChip

Unhybridized and non-specifically hybridized DNA is washed away. The chip undergoes staining and extension in capillary flow-through chambers. 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.

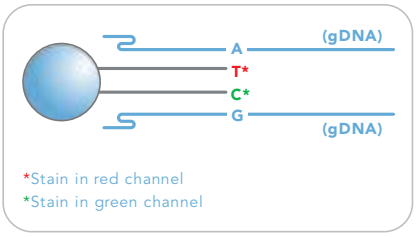


Figure 8 Extending and Staining BeadChip

See Single-Base Extension and Stain BC2 on page 52 for manual processing. See Single-Base Extension and Stain BC2 on page 134 for automated processing.
Multi-sample BeadChips also support allele-specific staining and extension, which uses the Infinium I chemistry. See Allele-Specific Extension & Stain BeadChip on page 66 for manual processing. See Allele-Specific Extension & Stain BeadChip on page 160 for automated processing.

Image BeadChip

The Illumina 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 II Assay Lab Setup and Procedures Guide (Illumina part # 11207963).

The Custom iSelect Genotyping BeadChip

The custom iSelect Genotyping BeadChip, or multi-sample BeadChip, is a sophisticated silicon-based array device composed of 12 individual arrays arranged in parallel on a single slide. The arrays are separated by a gasket that allows you to load 12 different DNA samples on a single chip.

Each individual array in the matrix may hold over 30,000 different oligonucleotide probe sequences. These are in turn attached to 3-micron beads assembled into the micro-wells of the BeadChip substrate. Because the micro-wells outnumber the probe sequences, multiple copies of each bead type are present in the array. This built-in redundancy improves robustness and measurement precision. The Sentrix 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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963) documents standard operating procedures and tools for an Infinium assay lab and explains how to set up and maintain separate pre- and post-amplification areas. Familiarize yourself with this guide before performing any Infinium assays.

Chapter 2, *Multi-Sample BeadChip Manual Protocol* and Chapter 3, *Multi-Sample BeadChip 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 Worksheet** 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 (Illumina part # 11230362) 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 BeadStudio application for data analysis. For instructions on data analysis, see the *BeadStudio Genotyping Module User Guide* (Illumina part # 11207066).

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

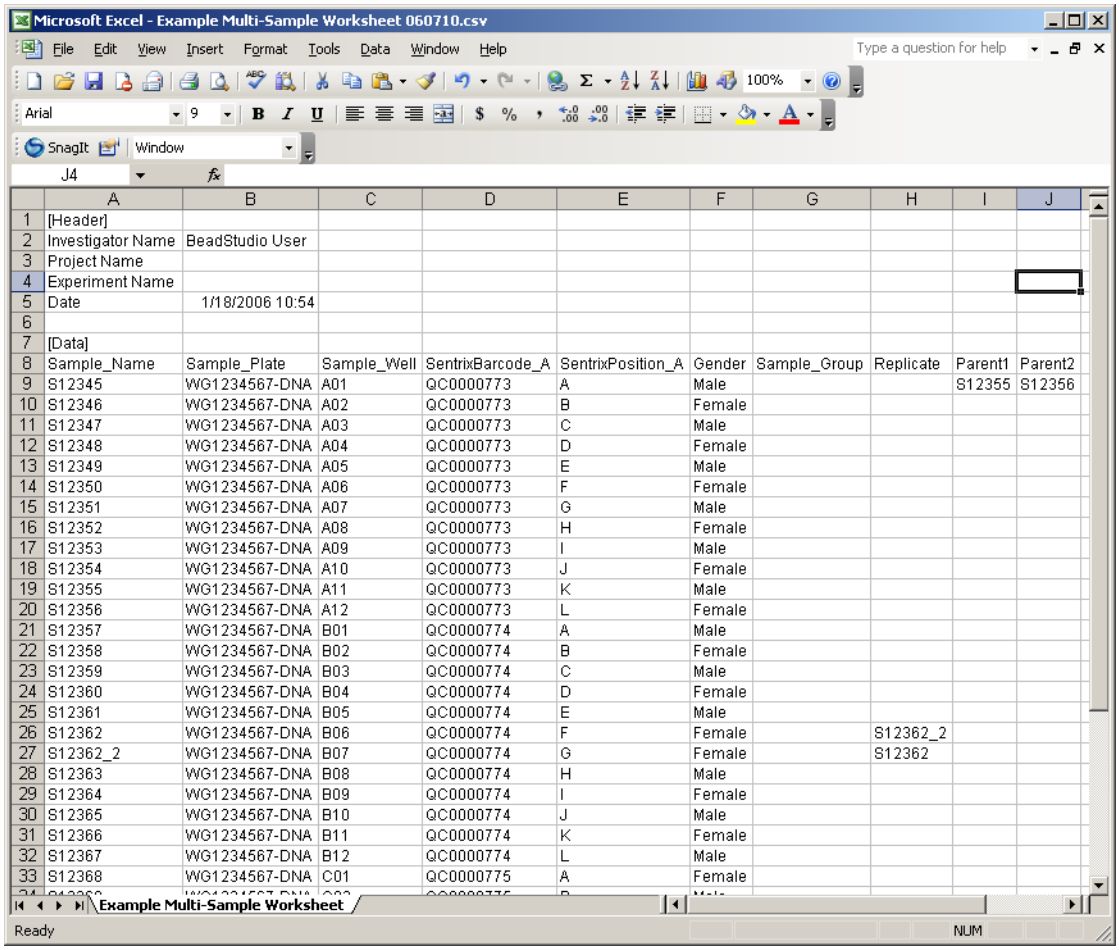
Table 1 Sample Sheet Guidelines

	Description	Optional (O) or Required (R)
Sample_Name	Name of the sample. Used only for display in the table.	O
Sample_ID	Unique identifier for the sample.	R
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
SentrixBarcocde_A	The barcode of the Sentrix array product 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

Table 1 Sample Sheet Guidelines (Continued)

	Description	Optional (O) or Required (R)
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 user-defined experiment name.	

Figure 10 provides an example of the Sample Sheet format. Your Documentation CD (Illumina part # 11230362) 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
1	[Header]									
2	Investigator Name	BeadStudio User								
3	Project Name									
4	Experiment Name									
5	Date	1/18/2006 10:54								
6										
7	[Data]									
8	Sample_Name	Sample_Plate	Sample_Well	SentrixBarcode_A	SentrixPosition_A	Gender	Sample_Group	Replicate	Parent1	Parent2
9	S12345	WG1234567-DNA	A01	QC0000773	A	Male			S12355	S12356
10	S12346	WG1234567-DNA	A02	QC0000773	B	Female				
11	S12347	WG1234567-DNA	A03	QC0000773	C	Male				
12	S12348	WG1234567-DNA	A04	QC0000773	D	Female				
13	S12349	WG1234567-DNA	A05	QC0000773	E	Male				
14	S12350	WG1234567-DNA	A06	QC0000773	F	Female				
15	S12351	WG1234567-DNA	A07	QC0000773	G	Male				
16	S12352	WG1234567-DNA	A08	QC0000773	H	Female				
17	S12353	WG1234567-DNA	A09	QC0000773	I	Male				
18	S12354	WG1234567-DNA	A10	QC0000773	J	Female				
19	S12355	WG1234567-DNA	A11	QC0000773	K	Male				
20	S12356	WG1234567-DNA	A12	QC0000773	L	Female				
21	S12357	WG1234567-DNA	B01	QC0000774	A	Male				
22	S12358	WG1234567-DNA	B02	QC0000774	B	Female				
23	S12359	WG1234567-DNA	B03	QC0000774	C	Male				
24	S12360	WG1234567-DNA	B04	QC0000774	D	Female				
25	S12361	WG1234567-DNA	B05	QC0000774	E	Male				
26	S12362	WG1234567-DNA	B06	QC0000774	F	Female		S12362_2		
27	S12362_2	WG1234567-DNA	B07	QC0000774	G	Female		S12362		
28	S12363	WG1234567-DNA	B08	QC0000774	H	Male				
29	S12364	WG1234567-DNA	B09	QC0000774	I	Female				
30	S12365	WG1234567-DNA	B10	QC0000774	J	Male				
31	S12366	WG1234567-DNA	B11	QC0000774	K	Female				
32	S12367	WG1234567-DNA	B12	QC0000774	L	Male				
33	S12368	WG1234567-DNA	C01	QC0000775	A	Female				

Figure 10 Sample Sheet Example

Tecan GenePaint

The Infinium II Assay 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 robot. The optional automated robotic processing and single-use reagent tube barcoding assure maximum consistency from slide to slide.

Illumina BeadArray Reader

BeadChips are imaged using the Illumina BeadArray Reader, a two-channel, 0.8 µm resolution confocal laser scanner. The BeadArray Reader scans BeadChips at two wavelengths simultaneously and creates an image file for each channel (i.e., two per array).

The BeadScan software determines intensity values for each bead type and creates data files for each color. BeadStudio uses this data file in conjunction with the individual bead pool map (*.bpm) file to analyse the data from the assay.

For instructions on imaging the BeadChip, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963). For instructions on using the BeadArray Reader, see the *Illumina BeadArray Reader User Guide* (Illumina part # 11179510).

Illumina BeadStudio Software

The BeadStudio software package included with the Illumina Infinium II Assay system extracts whole-genome genotyping data from data files created by the Illumina BeadArray Reader. You can export the resulting files to most standard databases and gene expression analysis programs.

For instructions on data analysis, see the *BeadStudio Genotyping Module User Guide* (Illumina part # 11207066).

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	www.illumina.com

Chapter 2

Multi-Sample BeadChip Manual Protocol

Topics

Introduction

This chapter describes pre- and post-amplification manual laboratory protocols for the Multi-Sample BeadChip. Follow the protocols in the order shown.

Multi-Sample BeadChip Manual Workflow

Figure 11 graphically represents the Illumina Infinium II Assay manual workflow for multi-sample BeadChips. These protocols describe the procedure for preparing 96 DNA samples.

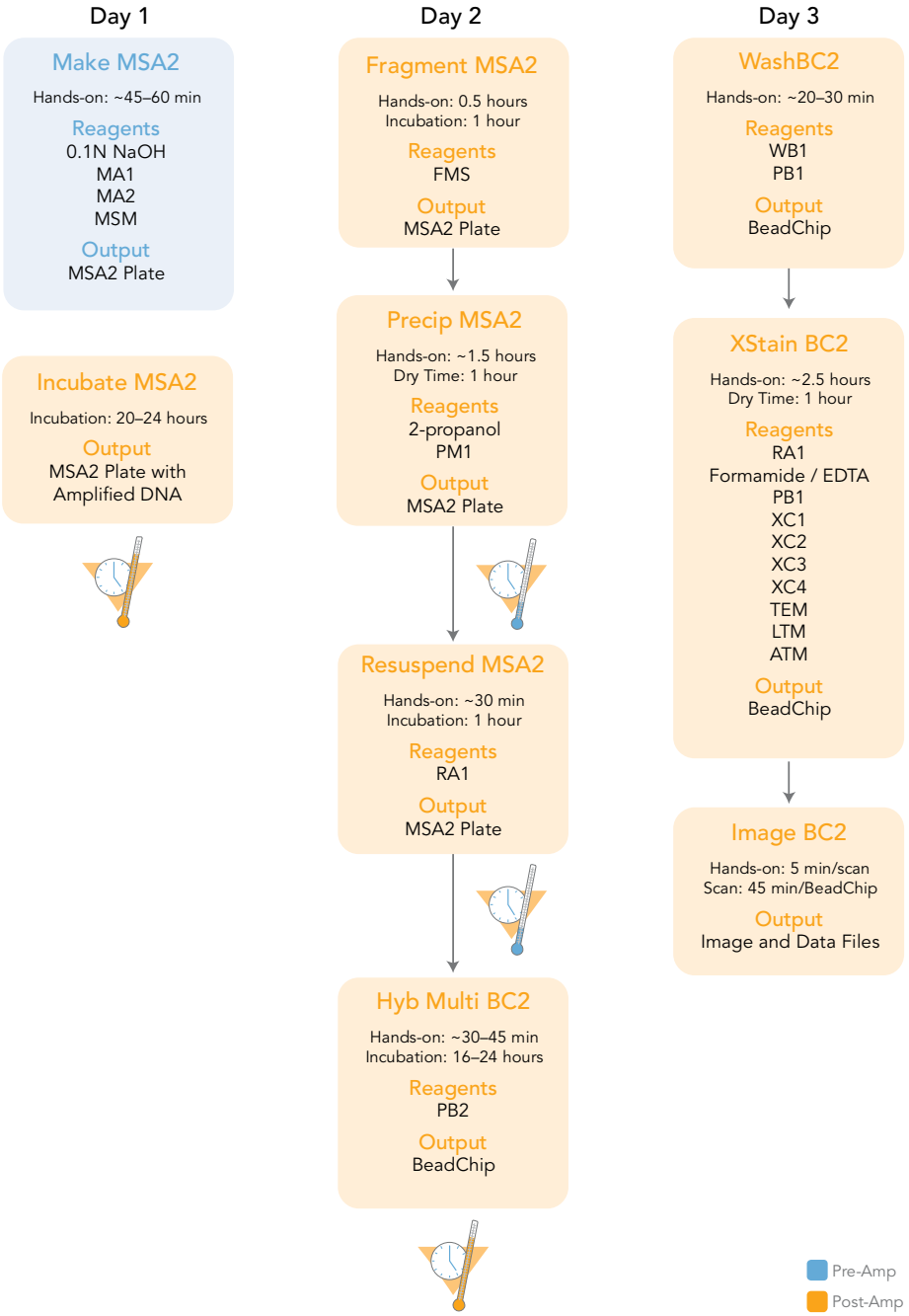


Figure 11 Multi-Sample BeadChip Manual Workflow

Equipment, Materials, and Reagents

These materials are specific to the multi-sample, manual BeadChip assay. For a list of other equipment, materials, and reagents needed in an Infinium II Assay lab, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Equipment

User-Supplied

- Forceps
  VWR International, catalog # 25601-008
- Desiccator (Optional)
  Auto-Desiccator Cabinet, VWR International, catalog # 74950-342

Illumina-Supplied

- Multi-Sample BeadChip Alignment Fixture
  Illumina part #218528

Materials

Illumina-Supplied

- MSA2 barcode labels
- WG#-DNA barcode labels

Reagents

Illumina-Supplied

Table 3 Reagents for Multi-Sample Manual Process

Reagent Name	Description	Illumina Part #
ATM	Anti-Stain Two-Color Master Mix	11208317
FMS	Fragmentation solution	11203428
LTM	Labeling Two-Color Master Mix	11208325
MA1	Multi-Sample Amplification 1 MIx	11202880
MA2	Multi-Sample Amplification 2 Mix	11203401
MSM	Multi-Sample Amplification Master Mix	11203410
PM1	Precipitation solution	11203436
RA1	Resuspension, hybridization, and wash solution	11222442
PB1	Used to wash Beadchips after hybridization	11221221
PB2	Humidifying buffer used during hybridization	11191130
TEM	Two-Color Extension Master Mix	11208309
WB1	Wash solution	11203444

Table 3 Reagents for Multi-Sample Manual Process (Continued)

Reagent Name	Description	Illumina Part #
XC1	XStain BeadChip solution 1	11208288
XC2	XStain BeadChip solution 2	11208296
XC3 (240 ml)	XStain BeadChip solution 3	11208421
XC4	XStain BeadChip solution 4	11208430

Make Standard DNA Plate (Optional/LIMS)

This process creates a Standard DNA plate with specific concentrations of DNA in the wells. Use this plate as input into the Make Quant process. If your DNA has already been quantified and you are not running Infinium LIMS (Laboratory Information Management System), skip ahead to *Prepare the WG#-DNA Plate* on page 25.

Illumina recommends the Molecular Probes PicoGreen assay for quantitation of dsDNA samples. The PicoGreen assay can quantitate small DNA volumes, and measures DNA directly.

For information about how to use Infinium LIMS, see the *Infinium II LIMS User Guide* (Illumina part # 11217344).

Reagents

User-Supplied

- 1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA)
- Lambda DNA
  Invitrogen, catalog # 25250-028

Dilute Standard DNA

To obtain quality genotypes, all DNA samples should be normalized to a concentration of 50 ng/µl.

1. Label a 96-well 0.65 ml MIDI plate "Standard DNA."
2. Dilute DNA in 10 mM Tris pH 8.0 /1 mM EDTA.
3. In well A1 of the Standard DNA plate, dilute stock Lambda DNA to 75 ng/µl in a final volume of 233.3 µl.
   a. Use the following formula to calculate dilution of stock Lambda DNA:

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

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

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

4. Transfer 66.7 µl 1X TE to well B of column 1 of the same plate.
5. Transfer 100 µl 1X TE to wells C, D, E, F, G, and H of column 1 of the same plate.

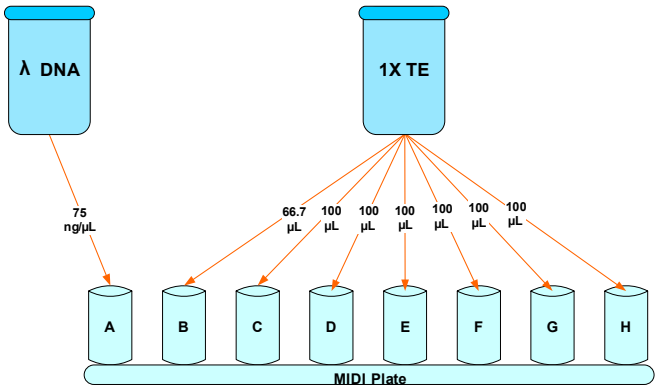


Figure 12 Standard DNA MIDI Plate Wells

Mix & Serially Dilute DNA

1. Pipet the contents of A1 up and down 10 times to mix.
2. Transfer 133.3 µl of Lambda DNA from well A1 into well B1, and then pipet the contents of well B1 up and down 10 times.
3. Transfer 100 µl from well B1 into well C1, and then pipet the contents of well C1 up and down 10 times.
4. Transfer 100 µl from well C1 into well D1, and then pipet the contents of well D1 up and down 10 times.
5. Transfer 100 µl from well D1 into well E1, and then pipet the contents of well E1 up and down 10 times.
6. Transfer 100 µl from well E1 into well F1, and then mix the contents of well F1 up and down 10 times.
7. Transfer 100 µl from well F1 into well G1, and then pipet the contents of well G1 up and down 10 times.
8. **Do not transfer solution from well G1 to well H1.** Well H1 serves as the blank 0 ng/µ Lambda DNA.

Table 4 Concentration of Lambda DNA Standards

Row-Column	Conc. (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	1.5625	200
H1	0	100

9. Seal the Standard DNA plate with the cap mat.

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

10. Do one of the following:
    - Proceed to *Make Quant (Optional/LIMS)* on page 20.
    - Store the sealed Standard DNA plate at 4°C if you do not plan to proceed to the next step immediately.

Make Quant (Optional/LIMS)

In this process, you create one to three QNT plates for use in the Molecular Dynamics Fluorometer (if available). The fluorometer quantifies the DNA present in the sample and enters that data into Infinium LIMS. Quantification ensures that there is enough sample DNA to generate good data. Use this procedure if you are running Infinium LIMS or if you have a fluorometer in your lab.

Reagents

User-Supplied

- PicoGreen dsDNA quantification reagent
  Molecular Probes, catalog # P7581

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

- 1X TE
  10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE)
- 8, 16, 24, 32, 48, or 96 DNA samples in up to three WG#-DNA plates

Setup

- Remove PicoGreen reagent from freezer and thaw at room temperature for 60 minutes in a light-impermeable container.
- For each WG#-DNA plate you plan to quantify, place a QNT barcode label on a new black Fluotrac plate. Position the label on the skirt of the plate, where the manufacturer's name appears.

Prepare PicoGreen Dilution Plates

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

1. Wrap aluminum foil around a sterile plastic container to prevent light penetration.
2. Make a 1:200 dilution of PicoGreen to 1X TE in the sterile plastic container.

   Table 5 outlines the required volumes for each reagent.

Table 5 PicoGreen Reagent Volumes

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

   You can prepare dilutions for up to three sample plates at a time.

3. Cap the sterile plastic container and mix thoroughly.
4. Pour the PicoGreen dilution into a sterile multichannel-pipet reservoir.
5. Transfer 195 µl PicoGreen dilution to each well in columns 1 and 2 of a new 96-well black flat-bottom plate (Figure 13). This is the Standard QNT plate.

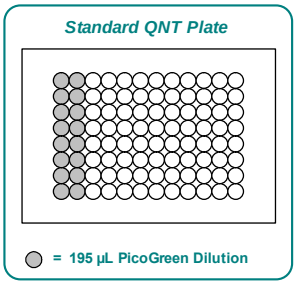


Figure 13 Standard QNT Plate

6. Add 2 µl of stock Lambda DNA from the Standard DNA plate to each well in columns 1 and 2 of the Standard QNT plate. Transfer from well A1 in the Standard DNA plate to well A1 in the Standard QNT plate, and so on for the rest of the wells.
7. Pipet the contents of the Standard QNT plate up and down several times.
8. Cover the plate with an aluminum adhesive seal and label as "Standard QNT Plate."
9. Transfer 195 µl PicoGreen dilution to all 96 wells of the QNT plate (Figure 14).

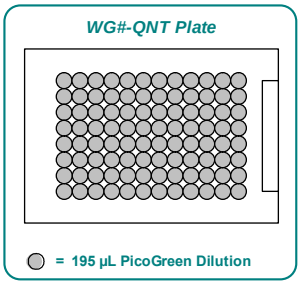


Figure 14 QNT Plate

10. Add 2 µl of sample DNA from the WG#-DNA plate to each well of the black, flat-bottom QNT plate. Transfer from well A1 in the WG#-DNA plate to well A1 in the QNT plate, and so on for the rest of the wells.
11. Pipet the contents of the QNT plate up and down several times.
12. Cover the QNT plate with an aluminum adhesive seal.
13. On the lab tracking worksheet, record:
    - Date/Time
    - Operator
    - The QNT barcode that corresponds to each WG#-DNA barcode
    - The Standard QNT plate that corresponds to each Standard DNA plate.
14. Proceed to *Read Quant (Optional/LIMS)* on page 22.

Read Quant (Optional/LIMS)

In this process, you use a fluorometer along with the Infinium Fluorometry Analysis software to interpret the quantified DNA and obtain the exact concentration of DNA in the sample. This information is saved with the other project data in Infinium LIMS. Illumina recommends using a fluorometer because fluorometry provides DNA-specific quantification. Spectrophotometry may also measure RNA, yielding values that are too high.

Equipment

User-Supplied

- Fluorometer
  Gemini XS or XPS and SoftMax Pro (Molecular Devices)

Read Quant

1. Turn on the fluorometer.
2. Open the Infinium Fluorometry Analysis software.

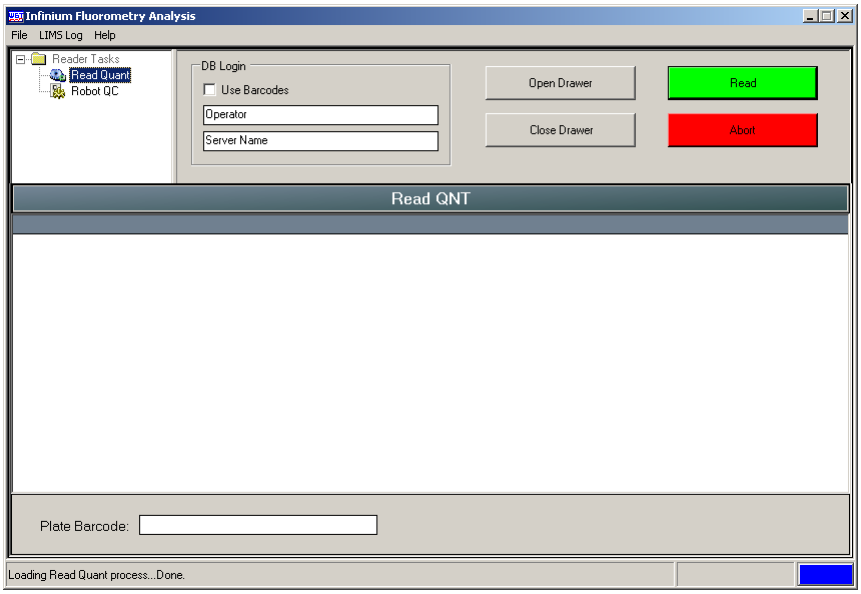


Figure 15 Infinium Fluorometry Analysis Opening Screen

3. Click **Read Quant**.
4. Select the **Use Barcodes** check box.
5. Click **Read**.
6. When prompted, log in to the Infinium LIMS database.
7. When asked if you want to read a new Standard QNT plate, click **Yes**. Load the Standard QNT plate in the open drawer of the fluorometer.
8. When the fluorometer finishes reading the Standard QNT data, unload the plate.
9. When prompted, enter the number of QNT plates you want to read. You can read up to three plates. Click **OK**.

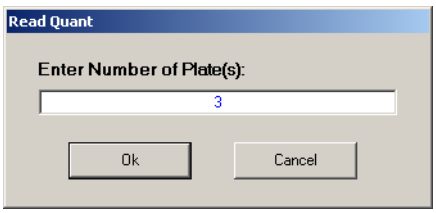


Figure 16 Number of Plates Dialog Box

10. When prompted, enter the QNT plate barcode. Load the plate in the open drawer of the fluorometer, and click **OK**.

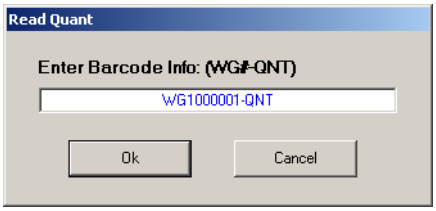


Figure 17 QNT Plate Barcode Dialog Box

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

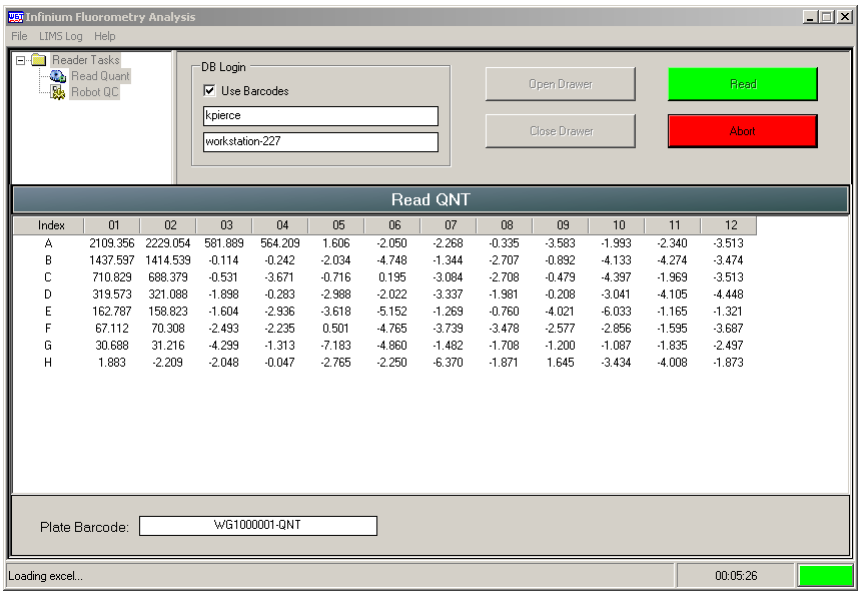


Figure 18 Fluorescence Data

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

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

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

11. Do one of the following:
    - Click **Yes** to send the data to Infinium LIMS. In Infinium LIMS, the plate moves into the Make MSA2 queue.
    - Click **No** to delete the quant data. You can read the quant data again for the same plate.
12. If you entered more than one QNT plate to read, repeat steps 10 and 11 for each additional plate.
13. Discard QNT plates and reagents in accordance with facility requirements.
14. Proceed to *Make the MSA2 Plate* on page 26.

Prepare the WG#-DNA Plate

1. Retrieve your DNA samples and thaw to room temperature (22°C).

NOTE

DNA samples must be 50 ng/µl, resuspended in TE (10 mM Tris, 1 mM EDTA).

2. Apply a WG#-DNA barcode label to a new 0.8 ml microtiter storage plate (MIDI) or a new 0.2 ml skirted microplate (TCY).
3. Dispense DNA according to Figure 19:
   - For MIDI plate: 20 µl DNA sample to each well
   - For TCY plate: 10 µl DNA sample to each well

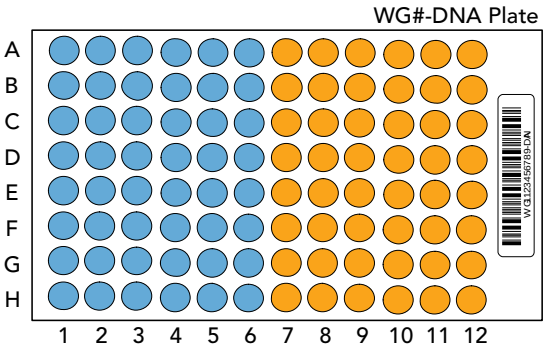


Figure 19 WG#-DNA Plate Sample Well Distribution

The example shown in Figure 19 applies to 96 samples. For 48 samples, fill the first half of the plate only (blue shaded section).

Make the MSA2 Plate

This process creates a MSA2 plate for DNA amplification. MA1 is first added to the MSA2 plate, followed by the DNA samples . Next, the 0.1N NaOH is added to denature the DNA samples. The MA2 reagent neutralizes the sample. Lastly, MSM (Multi-Sample Amplification Master Mix) is added to the plate.

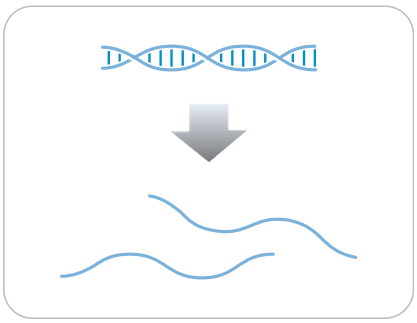


Figure 20 Denaturing and Neutralizing DNA

Estimated Time

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

Reagents

User-Supplied

- 0.1N NaOH
- 96 DNA samples in a labeled WG#-DNA plate, thawed to room temperature

Illumina-Supplied

Per 96 samples:

- MA1 (2 tubes)
- MA2 (2 tubes)
- MSM (2 tubes)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- In preparation for the Incubate MSA2 process (page 29), preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- Apply an MSA2 barcode label to a new 0.8 ml microtiter (MIDI) storage plate.
- On the lab tracking worksheet, record:
  - Date/Time

- Operator
- WG#-DNA plate barcode
- MSA2 plate barcode(s)
- MA1 tube barcode(s)
- MA2 tube barcode(s)
- MSM tube barcode(s)

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Populate Sample Sheet

In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well. You may also fill in the optional columns in the Sample Sheet: Gender, Sample_Group, Replicate, Parent1 and Parent2.

Make MSA2

1. Dispense 20 µl MA1 into the MSA2 plate wells.
2. Transfer 4 µl DNA sample, normalized to 50 ng/µl, from the WG#-DNA plate to the corresponding wells in the MSA2 plate.
3. On the lab tracking worksheet, record the original DNA sample ID for each well in the MSA2 plate.
4. Dispense 4 µl 0.1N NaOH into each well of the MSA2 plate that contains MA1 and sample.

Refer to Figure 21 throughout the Make MSA2 process.

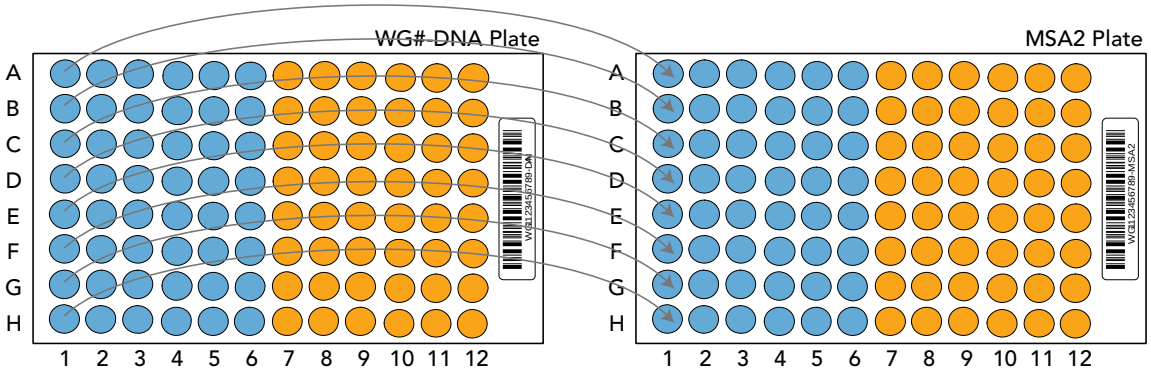


Figure 21 Distributing Sample to Wells

CAUTION

To avoid potential cross-contamination of sample, exchange tips between DNA samples and use aerosol filter tips when pipetting DNA.

5. Seal the MSA2 plate with the 96-well cap mat.

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

6. Vortex the plate at 1600 rpm for 1 minute.
7. Centrifuge to 280 xg for 1 minute.
8. Incubate for 10 minutes at room temperature (22°C).
9. Dispense 68 µl MA2 into each well of the MSA2 plate containing sample.

To avoid tip contamination, place the tips against the top edge of the wells. If you suspect the tips are contaminated with the contents of the well, discard the tips and use new tips. Use this technique for all subsequent dispensing steps.

10. Dispense 75 µl MSM into each well of the MSA2 plate containing sample.
11. Seal the storage plate with the 96-well cap mat.
12. Invert the sealed plate at least 10 times to mix contents.
13. Centrifuge to 280 xg for 1 minute.
14. Discard unused reagents in accordance with facility standards.
15. Proceed immediately to *Incubate the MSA2 Plate* on page 29.

Incubate the MSA2 Plate

This process incubates the MSA2 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 Multi-Sample Infinium II Assay.

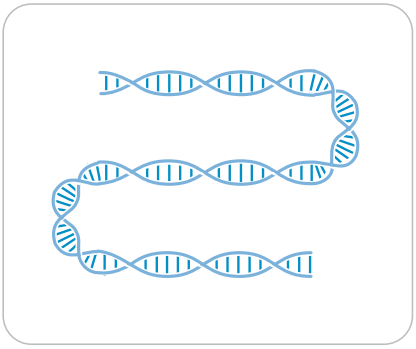


Figure 22 Incubating DNA to Amplify

Estimated Time

20–24 hours.

Incubate MSA2

1. Incubate MSA2 plate in the Illumina Hybridization Oven for at least 20 but no more than 24 hours at 37°C.
2. On the lab tracking worksheet, record the start and stop times.

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

3. Proceed to *Fragment the MSA2 Plate* on page 30.

Fragment the MSA2 Plate

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

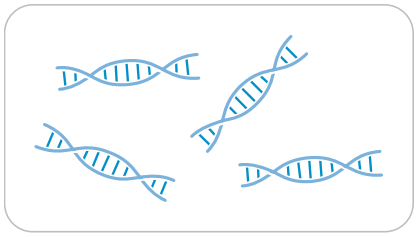


Figure 23 Fragmenting DNA

Estimated Time

Approximately 1.5 hours.

Reagents

Illumina-Supplied

Per 96 samples:

- FMS (2 tubes)

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- Preheat the heat block with the MIDI plate insert to 37°C.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - FMS tube barcode(s)

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Fragment MSA2

1. Remove the MSA2 plate from the Illumina Hybridization Oven.
2. Centrifuge the plate to 50 xg for 1 minute.
3. Carefully remove the cap mat.
4. 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.
5. Add 50 µl FMS to each well containing sample.
6. Seal the MSA2 plate with the 96-well cap mat.
7. Vortex the plate at 1600 rpm for 1 minute.
8. Centrifuge the plate to 50 xg for 1 minute at 22°C.
9. Place the sealed plate on the 37°C heat block for 1 hour.
10. On the lab tracking worksheet, record the start and stop times.
11. Discard unused reagents in accordance with facility standards.
12. Do one of the following:
    - Proceed to *Precipitate the MSA2 Plate* on page 32. Leave plate in 37°C heat block until setup is complete.
    - Store the sealed MSA2 plate at -20°C if you do not plan to proceed to the next step immediately.

This is a good stopping point in the process.

Precipitate the MSA2 Plate

Add PM1 and 2-propanol to the MSA2 plate to precipitate the DNA samples.

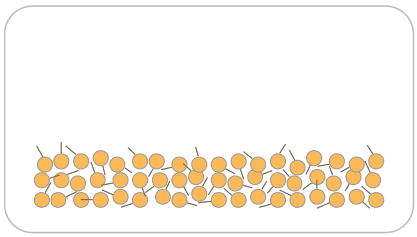


Figure 24 Precipitating DNA

Estimated Time

Approximately 2.5 hours

Reagents

User-Supplied

Per 96 samples:

- 2-propanol, 100% (40 ml)

Illumina-Supplied

Per 96 samples:

- PM1 (2 tubes)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- Do one of the following:
  - If you froze the AMP2 plate after fragmentation, thaw it to room temperature (22°C). Centrifuge to 280 xg for 1 minute.
  - If you are immediately from Fragment AMP2, leave the plate in the 37°C heat block until setup is complete.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - PM1 tube barcode(s)
  - 2-propanol lot number and date opened

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

- Preheat heat block to 37°C.

- In preparation for the 4°C spin (below), set centrifuge to 4°C.

Precip MSA2

1. Pulse centrifuge the sealed plate to 50 xg for 1 minute at 22°C.
2. Remove the 96-well cap mat.
3. Add 100 µl PM1 to each well containing sample.
4. Seal the plate with the cap mat.
5. Vortex the plate at 1600 rpm for 1 minute.
6. Incubate at 37°C for 5 minutes.
7. Centrifuge to 50 xg at 22°C for 1 minute.
8. Add 300 µl 100% 2-propanol to each well containing sample.
9. Carefully seal the MSA2 plate with a new, ***dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.
10. Invert at least 10 times to mix contents thoroughly.
11. Incubate at 4°C for 30 minutes.
12. Place the sealed MSA2 plate in the centrifuge opposite another plate of equal weight (Figure 25).

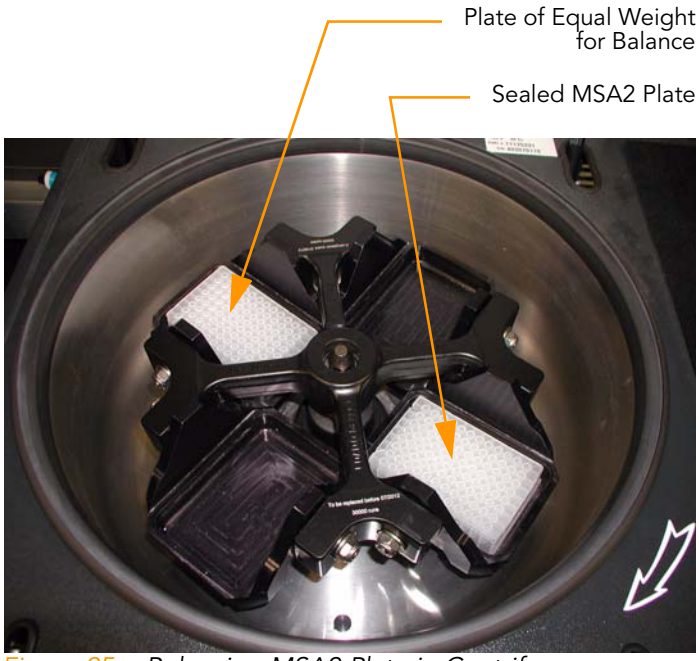


Figure 25 Balancing MSA2 Plate in Centrifuge

13. Centrifuge to 3,000 xg at 4°C for 20 minutes. Immediately remove the MSA2 plate from centrifuge.

    Perform the next step immediately, so that the blue pellet stays in place. If any delay occurs, repeat step 13 before proceeding.
14. Remove the cap mat and discard it.

15. Over an absorbent pad appropriate for 2-propanol disposal, decant supernatant by quickly inverting the AMP2 plate and smacking it down.
16. 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 prevent cross-contamination while decanting, do not allow supernatant in wells to pour into other wells.

17. Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature (22°C) to air dry the pellet (Figure 26).

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

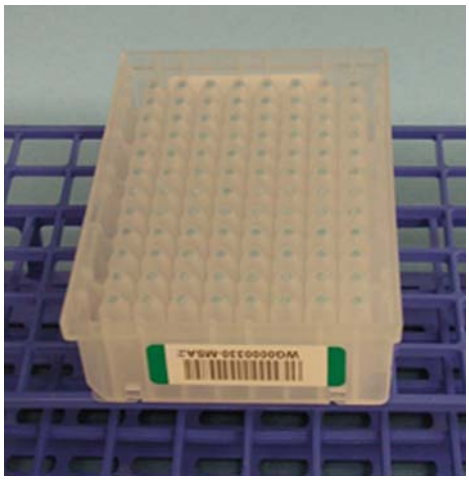


Figure 26 Uncovered MSA2 Plate Inverted for Air Drying

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.

18. On the lab tracking worksheet, enter the start and stop times.
19. Discard unused reagents in accordance with facility standards.
20. Do one of the following:
    - Proceed to *Resuspend the MSA2 Plate* on page 35.
    - Seal the MSA2 plate with a cap mat and store it at -20°C if you do not plan to proceed to the next step immediately.

This is a good stopping point in the process.

Resuspend the MSA2 Plate

Add RA1 to the MSA2 plate to resuspend the precipitated DNA samples.

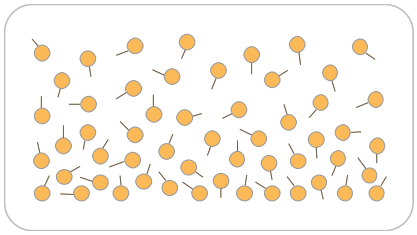


Figure 27 Resuspending DNA

Estimated Time

Approximately 1.5 hours.

Reagents

Illumina-Supplied

Per 96 samples:

- RA1 (bottle)

WARNING

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

Setup

- RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20–25°C water bath. Gently mix to dissolve any crystals that may be present.
- If you stored the MSA2 plate at -20°C, thaw it to room temperature. Remove the cap mat and discard it.
- Preheat oven to 48°C.
- Turn on the heat sealer to preheat. Allow 20 minutes.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - RA1 bottle barcode(s)

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Resuspend MSA2

1. Add 42 µl RA1 to each well of the MSA2 plate containing a DNA pellet.
2. Apply foil seal to MSA2 plate by firmly holding the heat-sealer sealing block down for 5 seconds.

3. Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
4. Vortex the plate at 1800 rpm for 1 minute.
5. Pulse centrifuge to 280 xg.

   If you stored the pellets at -20°C for extended periods of time after the Precip MSA2 process, you may need to repeat steps 3 to 5 until the pellets are completely resuspended.
6. Discard unused reagents in accordance with facility standards.
7. Do one of the following:
   - Proceed to *Hyb Multi BC2* on page 37. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
   - If you do not plan to proceed to the next step immediately, store the sealed MSA2 plate at -20°C. Store RA1 at -20°C.
   - If you plan to store the plate for more than 24 hours, store it at -80°C. Store RA1 at -20°C.

This is a good stopping point in the process.

Hyb Multi BC2

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

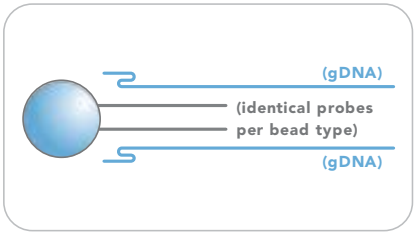


Figure 28 Hybridizing DNA to BeadChip

Estimated Time

- 30 minutes hands-on time for 48 samples
- 40 minutes for 96 samples
- 16–24 hours incubation

Reagents

Illumina-Supplied

Per 2 Hyb Chambers:

- PB2 (1 tube)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- Preheat the heat block to 95°C.
- Preheat the Illumina Hybridization Oven to 48°C.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - PB2 tube barcode

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Assemble the Hyb Chambers

1. Place the following items on the bench top (Figure 29):
   - BeadChip Hyb Chambers(2)
   - Hyb Chamber Gaskets (2)
   - BeadChip Hyb Chamber inserts (8)

Figure 29 BeadChip Hyb Chamber Components

2. Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers as shown.
   a. Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber (Figure 30).

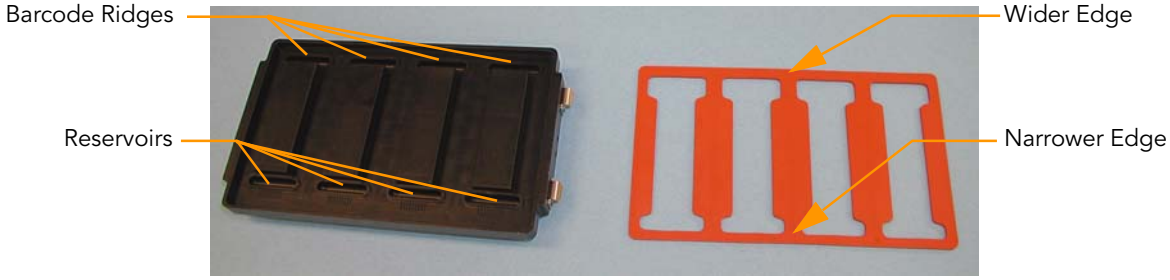


Figure 30 Hyb Chamber and Gasket

   b. Lay the gasket into the Hyb Chamber (Figure 31), and then press it down all around.

Figure 31 Placing Gasket into Hyb Chamber

   c. Make sure the Hyb Chamber gaskets are properly seated (Figure 32).

Figure 32 Hyb Chamber with Gasket in Place

3. Dispense 200 µL PB2 into the humidifying buffer reservoirs in the Hyb Chambers (Figure 33).

Figure 33 Dispensing PB2 into Hyb Chamber Reservoir

4. Close and lock the BeadChip Hyb Chamber lid (Figure 34).
   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 34 Sealing the Hyb Chamber

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

Load BeadChip

1. Place the resuspended MSA2 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.

Figure 35 Placing BeadChips into Hyb Chamber Inserts

2. Pulse centrifuge the plate to 280 xg. Remove the foil seal.
3. Just before loading DNA samples, remove all BeadChips from their packages.
4. 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 35).

NOTE For an alternative DNA loading protocol, see *Load BeadChip (Alternative Method)* on page 74.

5. Using a multi-channel precision pipet, dispense 12 µl of each DNA sample onto the appropriate BeadChip section, according to the chart

on the lab tracking worksheet (repeated in Figure 39) and the following illustrations:

a. Load samples A1–F1 from the MSA2 plate into the left side of the BeadChip (Figure 36).

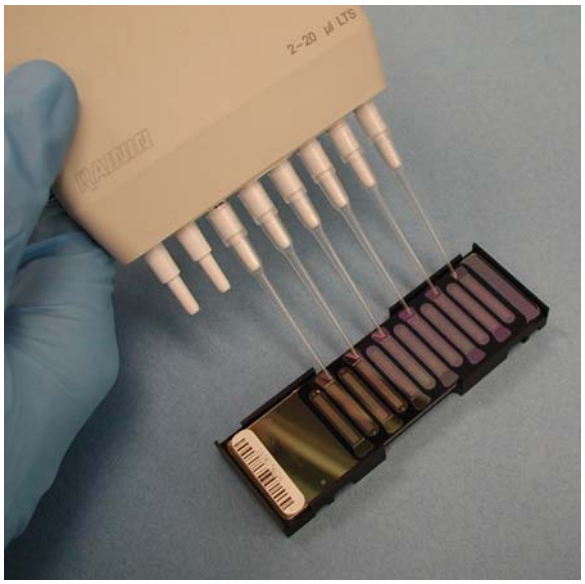


Figure 36 Loading Samples A1–F1

b. Load samples in G1 and H1 from the MSA2 plate into the top two inlets of the right side of the BeadChip (Figure 37).

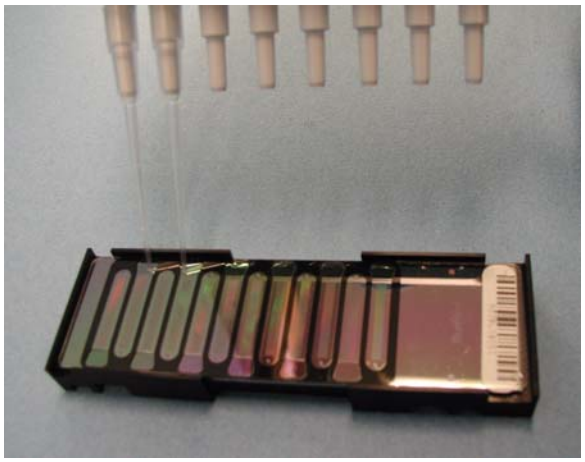


Figure 37 Loading Samples G1–H1

c. Load samples A2–D2 into the remaining four inlets on the right side of the BeadChip (Figure 38).

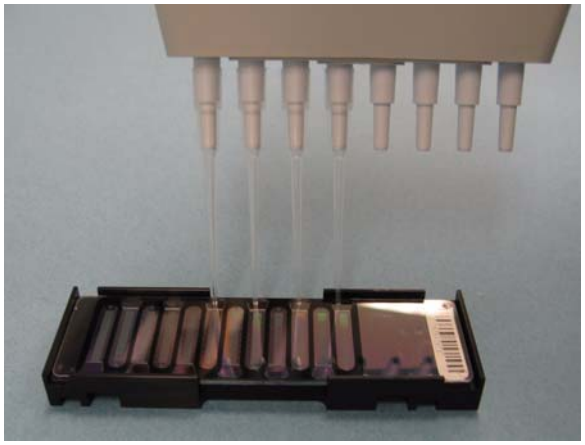


Figure 38 Loading Samples A2–D2

d. Continue in this manner, following the color-coded sections shown in Figure 39, until all samples are loaded.

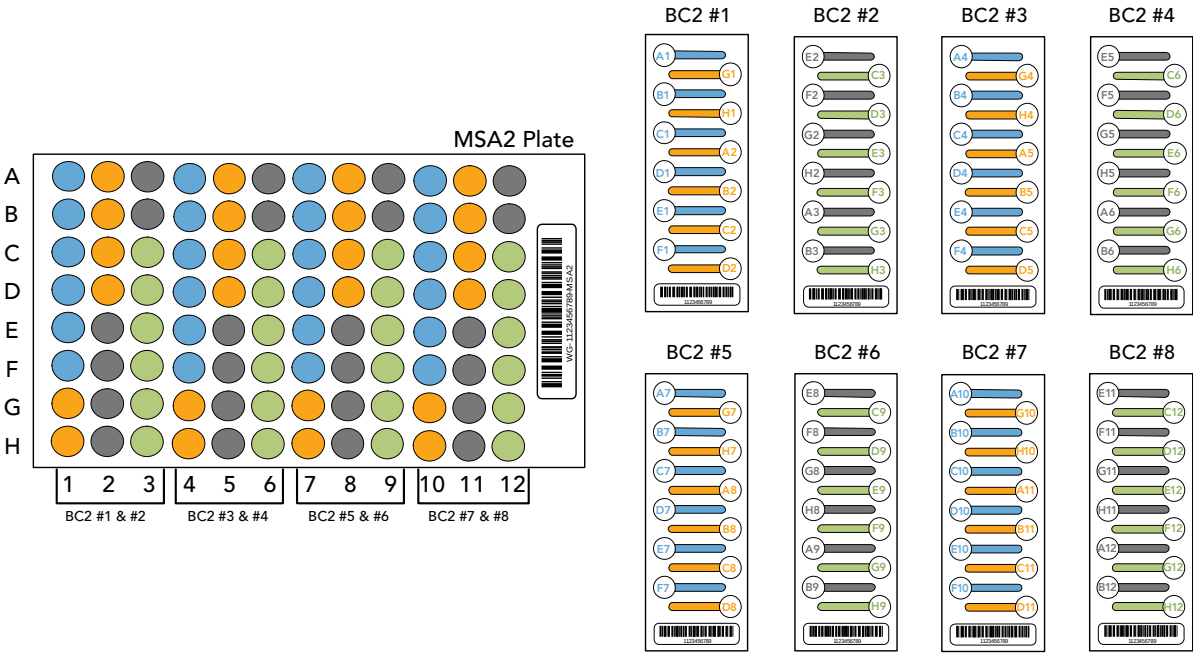


Figure 39 Distributing Sample in MSA2 Plate

CAUTION

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

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

7. Visually inspect all sections of the BeadChips. Ensure the DNA sample covers all of each bead stripe. Record any sections that are not completely covered.
8. Heat-seal any residual sample in the MSA2 plate with foil, and store at -20°C. Store at -80°C if you do not plan to use the sample again within 24 hours.

Hyb Multi BC2 Setup

1. Set the Illumina Hybridization Oven to 48°C.
2. Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
3. Load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chambers as shown (Figure 40). Position the barcode end over the ridges indicated on the Hyb Chamber.

Figure 40 Placing Hyb Chamber Inserts into the Hyb Chamber

4. Place the back side of lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts (Figure 41).
5. Close the clamps on both sides of the Hyb Chamber.

Figure 41 Seating Lid onto Hyb Chamber

6. Place the Hyb Chamber into the Illumina Hybridization Oven at 48°C.
7. Start the rocker (optional).
8. Incubate at 48°C for at least 16 hours but no more than 24 hours.
9. On the lab tracking worksheet, enter the start and stop times.
10. Place RA1 into the freezer at -20°C for use the next day.
11. Prepare the XC4 reagent for the XStain procedure (see *XStain BeadChip* on page 69).
12. Proceed to *Wash BC2* on page 44.

Wash BC2

This process prepares the BeadChips for the XStain BC2 process. First, remove the coverseals from the BeadChips and wash the BeadChips in WB1 reagent followed by PB1 reagent. Next, assemble the BeadChips into the Flow-Through Chambers under PB1 buffer.

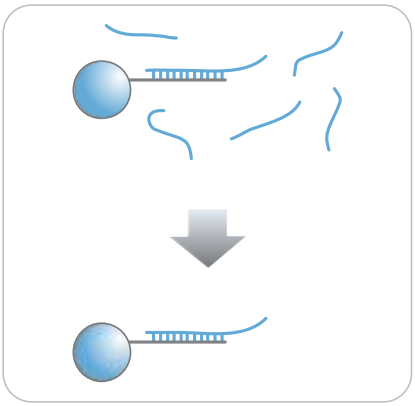


Figure 42 Washing BeadChip

Estimated Time

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

Reagents

Illumina-Supplied

- WB1 (bottle)
- PB1 (350 ml)
- Multi-Sample BeadChip Alignment Fixture
- Te-Flow Flow-Through Chambers (with black frames, spacers, glass back plates, and clamps)
- Wash dish (2)
- Wash rack

WARNING

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- Have ready on the lab bench:
  - Two wash dishes:
    - *One containing 200 ml WB1, and labeled as such*
    - *One containing 200 ml PB1, and labeled as such*
  - Multi-Sample BeadChip Alignment Fixture
    - *Using a graduated cylinder, fill with 150 ml PB1*
  - Te-Flow Flow-Through Chamber components:
    - *Black frames*
    - *Spacers (separated for ease of handling)*
    - *Clean glass back plates*
    - *Clamps*
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - WB1 bottle barcode
  - PB1 bottle barcode

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Wash BC2

1. Attach the wire handle to the rack and submerge the wash rack in the wash dish containing 200 ml WB1 (Figure 43).
2. Remove Hyb Chamber inserts from the Hyb Chambers.
3. Remove BeadChips from the Hyb Chamber inserts.

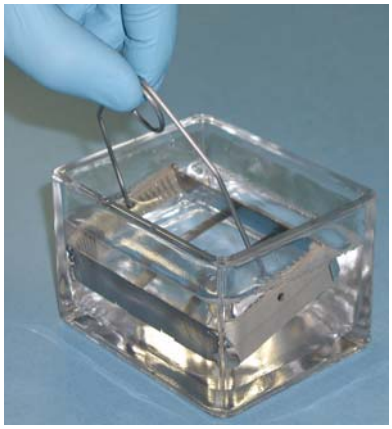


Figure 43 Wash Rack in Wash Dish Containing WB1

4. Remove the coverseal from the BeadChip (Figure 44) as follows:
   a. Using powder-free gloved hands, hold the BeadChip in one hand between thumb and forefinger, with the front side of the BeadChip facing away from you.
   b. Remove the entire seal by pulling it off in a downward direction, starting with the barcode end.
   c. Discard the seal.

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

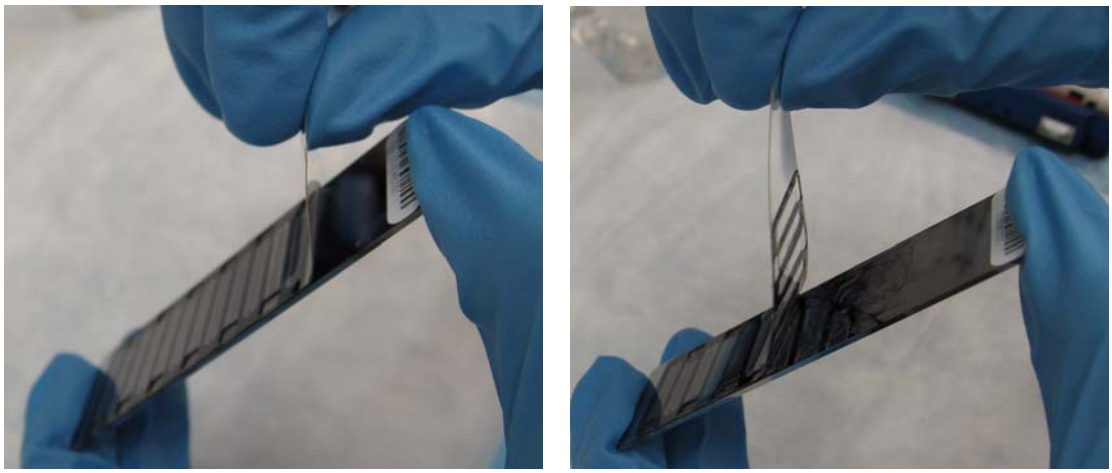


Figure 44 Removing the Coverseal

CAUTION

Do not touch the arrays!

5. Carefully slide each BeadChip into the wash rack, making sure that it is completely submerged in the WB1 (Figure 45).

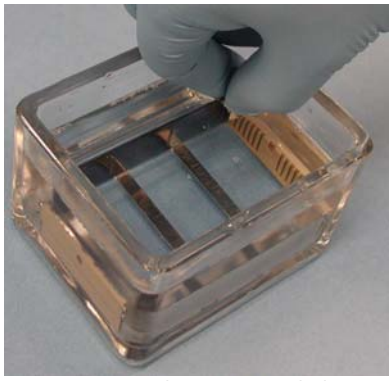


Figure 45 Placing BeadChips in Wash Dish Containing WB1

6. Repeat steps 4 and 5 for each BeadChip to be processed.
7. Once all BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the WB1 with gentle, slow agitation.
8. Remove the wash rack from the wash dish and place it immediately into the wash dish containing PB1. Make sure that the BeadChips are completely submerged.
9. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

Assemble Flow-Through Chamber

1. For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture (Figure 46). The Alignment Fixture should already be filled with PB1.

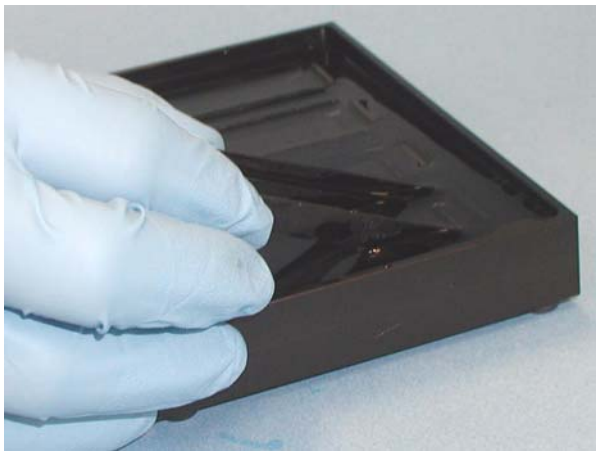


Figure 46 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

2. Place each BeadChip into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture (Figure 47).

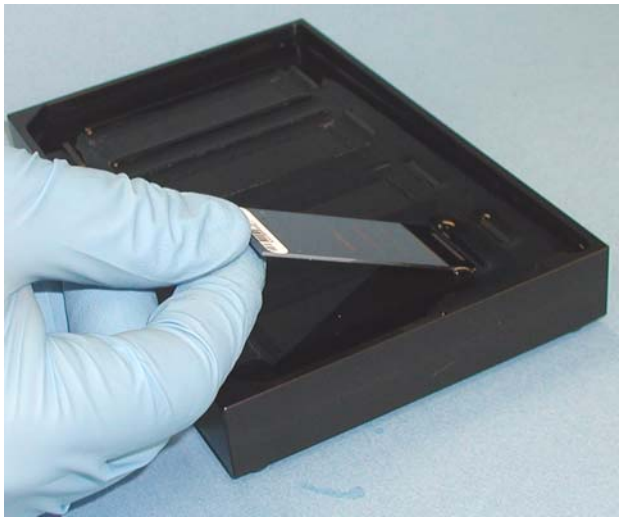


Figure 47 Placing BeadChip into Black Frame on Alignment Fixture

3. Place a clear spacer onto the top of each BeadChip (Figure 48). 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.

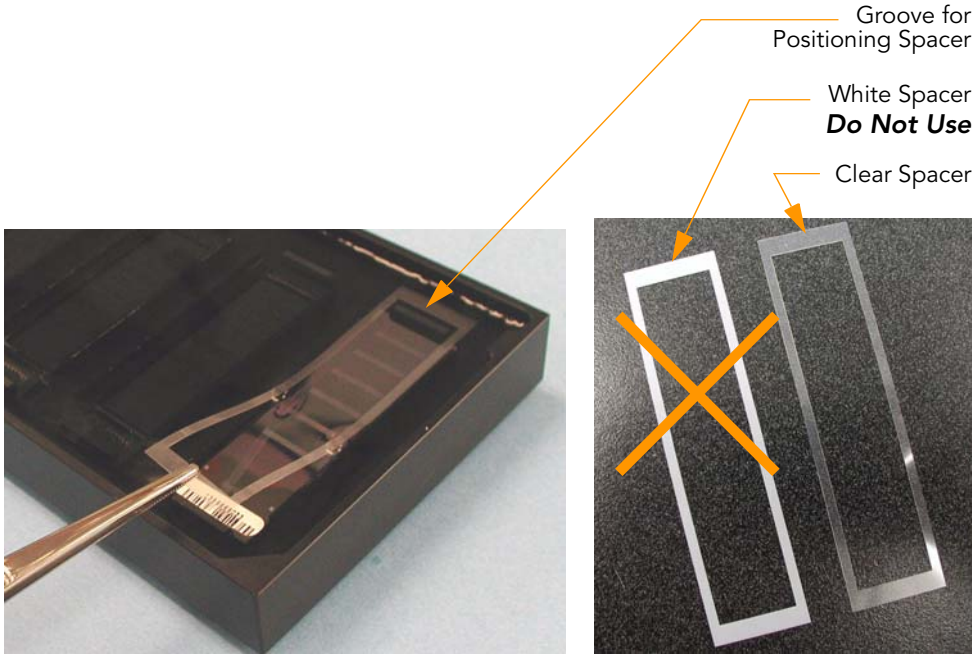


Figure 48 Placing Clear Plastic Spacer onto BeadChip

4. Place the Alignment Bar onto the Alignment Fixture (Figure 49). The groove in the Alignment bar should fit over the metal tab on the Alignment Fixture.

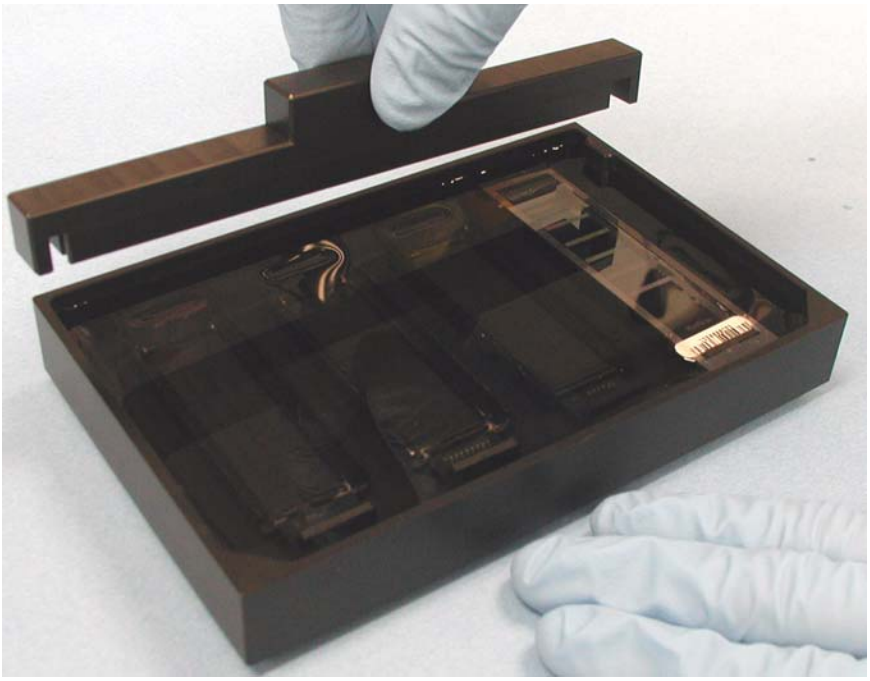


Figure 49 Placing Alignment Bar onto Alignment Fixture

5. Use a Whoosh duster or laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.
6. 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 50).

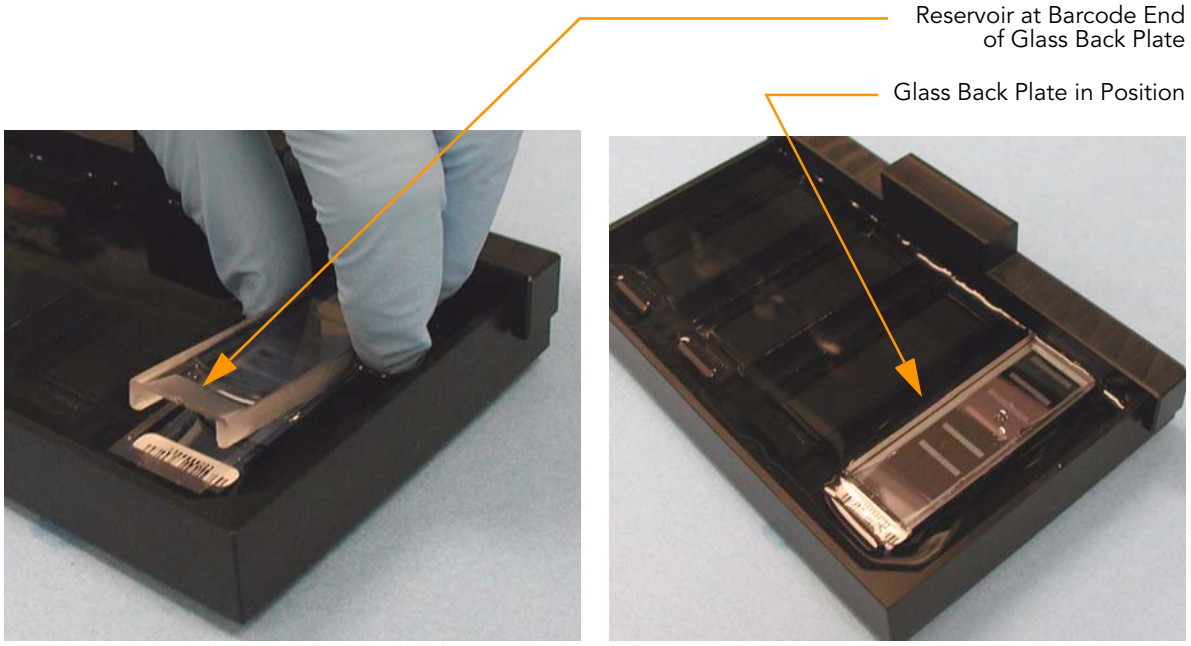


Figure 50 Placing Glass Back Plate onto BeadChip

7. Attach the metal clamps onto each Flow-Through Chamber as follows (Figure 51):
   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 one stripe shows between it and the Alignment Bar.
   c. Place the second metal clamp around the Flow-Through Chamber so that no stripes show between the clamp and the barcode.

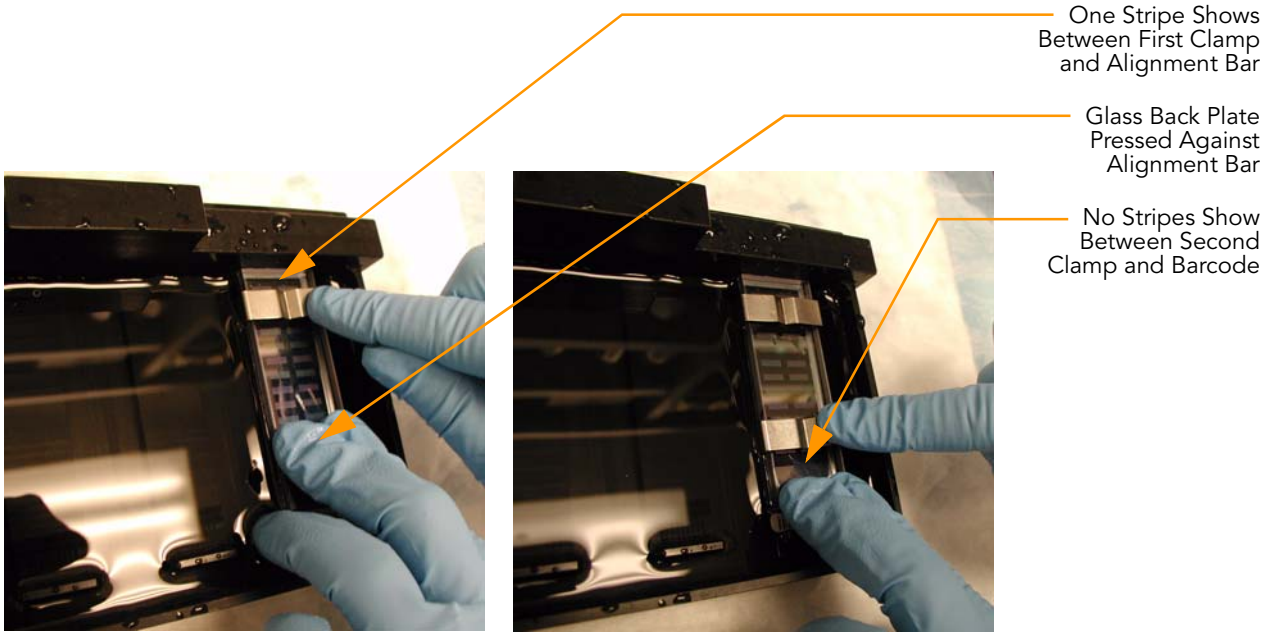


Figure 51 Securing Flow-Through Chamber Assembly with Metal Clamps

8. Using scissors, trim the ends of the clear plastic spacers from the Flow-Through Chamber assembly (Figure 52):
   a. Trim the spacer ends at the non-barcode end of the assembly.
   b. On the barcode end of the assembly, slip scissors up over the barcode to trim the spacer ends.

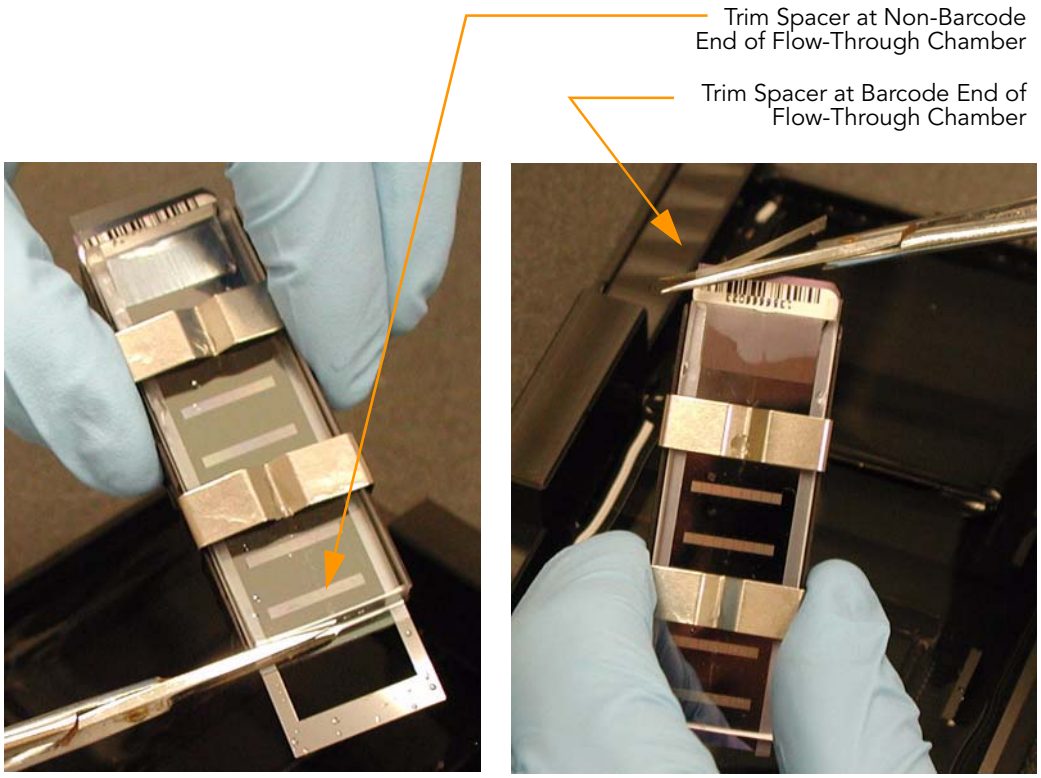


Figure 52 Trimming Spacer Ends from Flow-Through Chamber Assembly

9. Discard unused reagents in accordance with facility standards.
10. Proceed to *Single-Base Extension and Stain BC2* on page 52.

Single-Base Extension and Stain BC2

NOTE

If you are using HumanNS-12 BeadChips, do not use this procedure. Instead, follow the procedures in *Allele-Specific Extension & Stain BeadChip* on page 66.

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

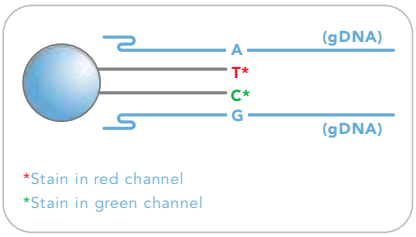


Figure 53 Extending and Staining BeadChip

Estimated Time

Approximately 2.75 hours for 8 BeadChips.

Reagents

User-Supplied

Per 8 BeadChips:

- 95% formamide/1 mM EDTA (15 ml)

Illumina Supplied

Per 8 BeadChips:

- RA1 (10 ml) (see *Setup* for special instructions)
- XC1 (2 tubes)
- XC2 (2 tubes)
- TEM (2 tubes)
- XC3 (75 ml)
- LTM (2 tubes) (make sure that both tubes indicate the same stain temperature on the label)
- ATM (2 tubes)
- PB1 (310 ml)
- XC4 (310 ml)

WARNING

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

Setup

- RA1 is shipped and stored at -20°C. Gradually warm the reagent to room temperature, preferably in a 20–25°C water bath. Gently mix to dissolve any crystals that may be present.
- Place all reagent standoff tubes in a rack in the order in which they will be used (Figure 54). If frozen, allow them to thaw to room temperature (22°C) and centrifuge to 3000 xg for 3 minutes.

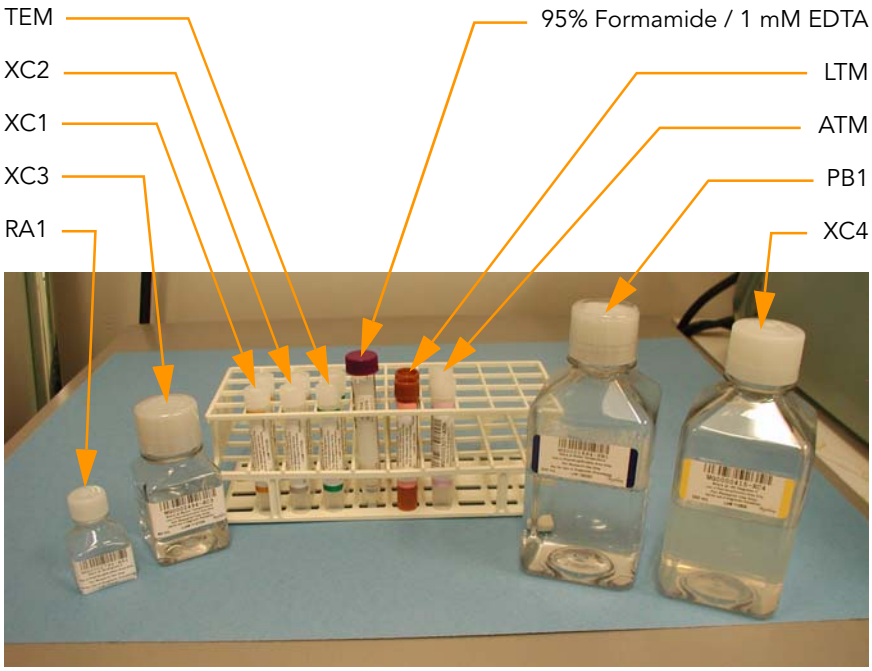


Figure 54 XStain BC2 Reagent Tubes and Bottles

- Dispense all bottled reagents into disposable reservoirs, as they are needed.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - RA1 barcode
  - XC3 barcode
  - XC1 barcode(s)
  - XC2 barcode(s)
  - TEM barcode(s)
  - LTM barcode(s)

- ATM barcode(s)
- PB1 barcode
- XC4 barcode(s)

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 (Figure 55).

   This temperature may vary depending on facility ambient conditions.

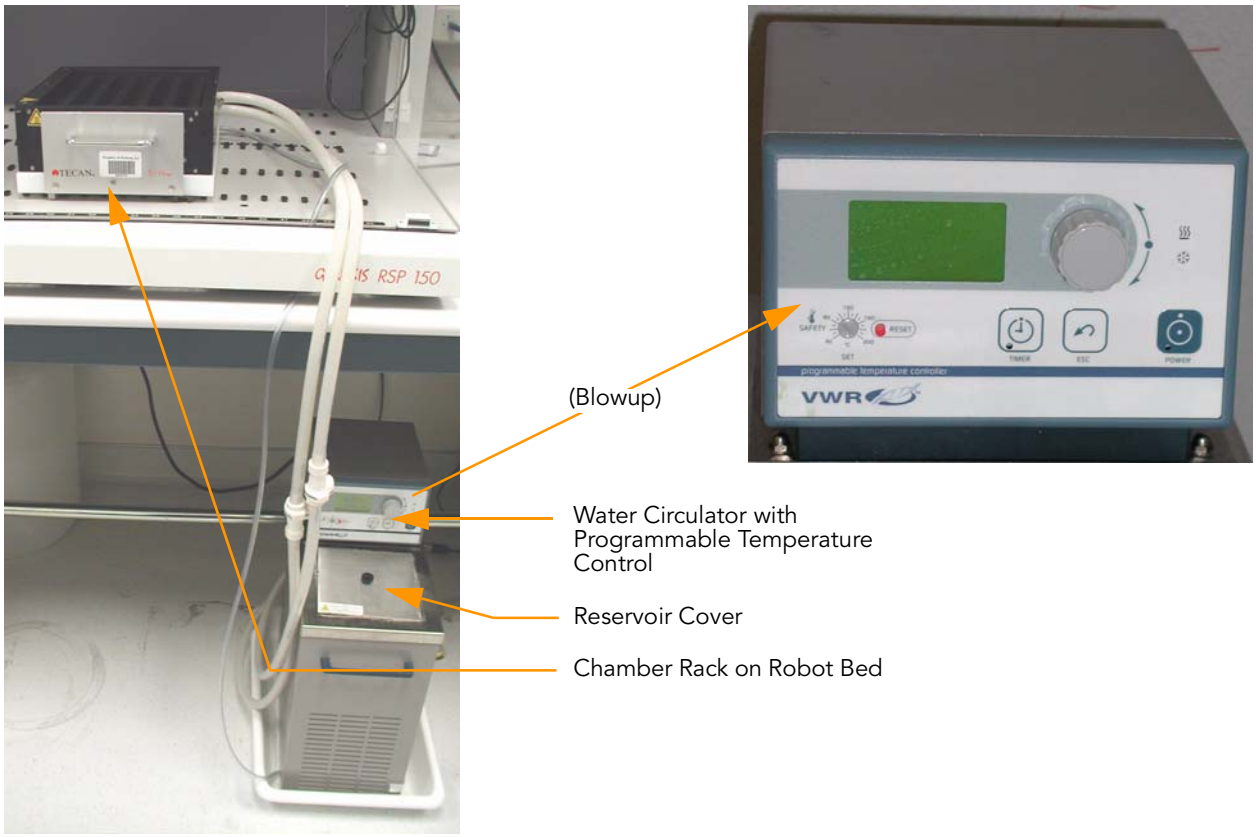


Figure 55 Water Circulator Connected to Chamber Rack

3. The temperature displayed on the water circulator LCD screen may differ from the actual temperature on the Chamber Rack. Confirm the actual temperature using the temperature probe for the Chamber Rack.
4. You must remove bubbles trapped in the Chamber Rack ***each time*** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.

5. Use the Illumina Temperature Probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 56).

NOTE

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

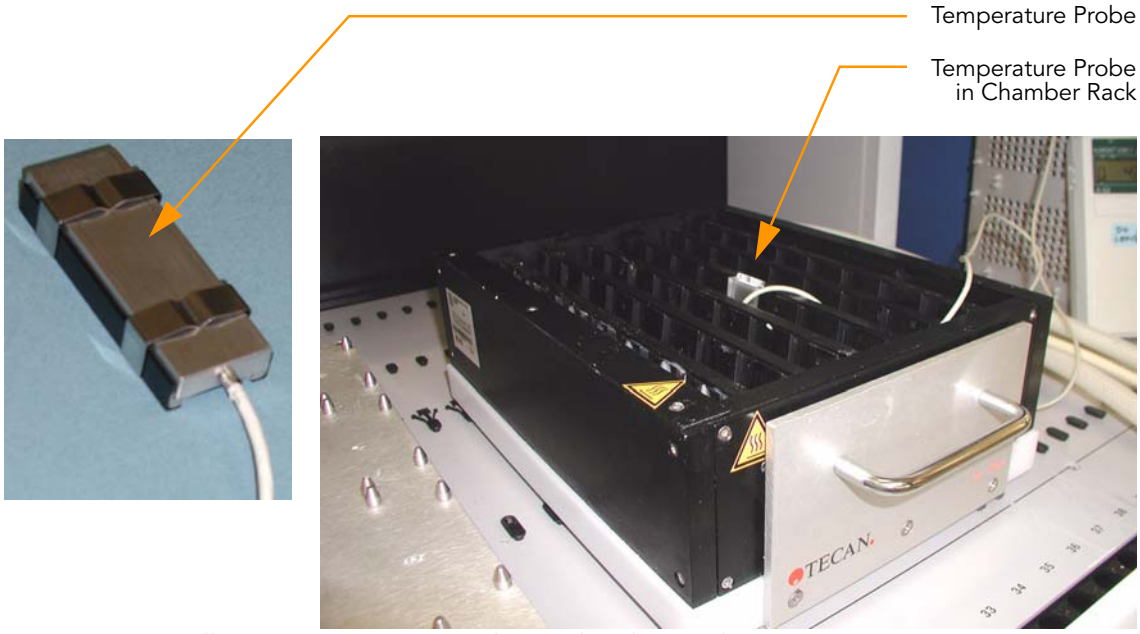


Figure 56 Illumina Temperature Probe in Chamber Rack

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

   The remaining steps in this protocol must be performed without interruption.

XStain BC2 Single-Base Extension

1. After equilibrating the Chamber Rack to 44°C, remove the Hyb Chamber from the oven and quickly place each assembled Flow-Through Chamber in the first rows of the Chamber Rack.

   If you are staining 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 worksheet, record the chamber rack position associated with each BeadChip.
4. Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until the solution is completely dissolved.
5. Into the reservoir of each Flow-Through Chamber, dispense:

a. 150 µl RA1 (Figure 57) and incubate for 30 seconds. Repeat this step five times.

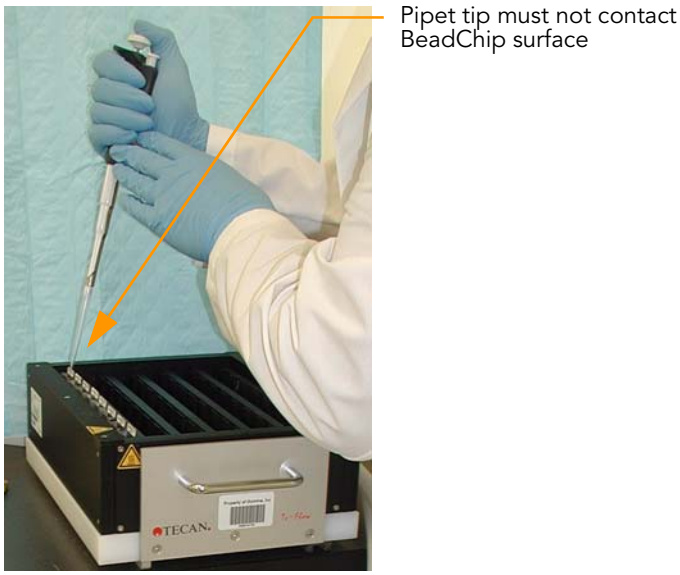


Figure 57 Dispensing RA1 into Each Flow-Through Chamber

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

b. 450 µl XC1 and incubate for 10 minutes.
c. 450 µl XC2 and incubate for 10 minutes.
d. 200 µl TEM and incubate for 15 minutes.
e. 450 µl 95% formamide/1 mM EDTA and incubate for 1 minute. Repeat this step once, and then incubate for an additional 5 minutes.

6. Ramp the Chamber Rack temperature to the temperature indicated on the LTM tube. If no temperature is listed, ramp it to 37°C.
7. As the Chamber Rack is ramping, dispense 450 µl XC3 into the reservoir of each Flow-Through Chamber and incubate for 1 minute. Repeat this step once.
8. Allow the Chamber Rack temperature to reach the correct temperature before proceeding.

Stain BeadChip

1. Into the reservoir of each Flow-Through Chamber, dispense:
   a. 250 µl LTM 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 LTM 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 LTM and incubate for 10 minutes.
j. 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

2. Immediately remove the Flow-Through Chambers from the Chamber Rack and place horizontally on a lab bench at room temperature (22°C).

Preparing Wash Dishes and Tube Racks

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 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 diapers. 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 (Illumina-provided from VWR catalog # 60916-748) 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.

Wash and Coat 8 BeadChips

Equipment Needed

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

1. Set up two top-loading wash dishes, labeled “PB1” and “XC4” (Figure 58).
2. To indicate the fill volume before filling wash dishes with PB1 and XC4, pour 310 ml water into the wash dishes and mark the water level on the side. Empty the water from the wash dish. 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.

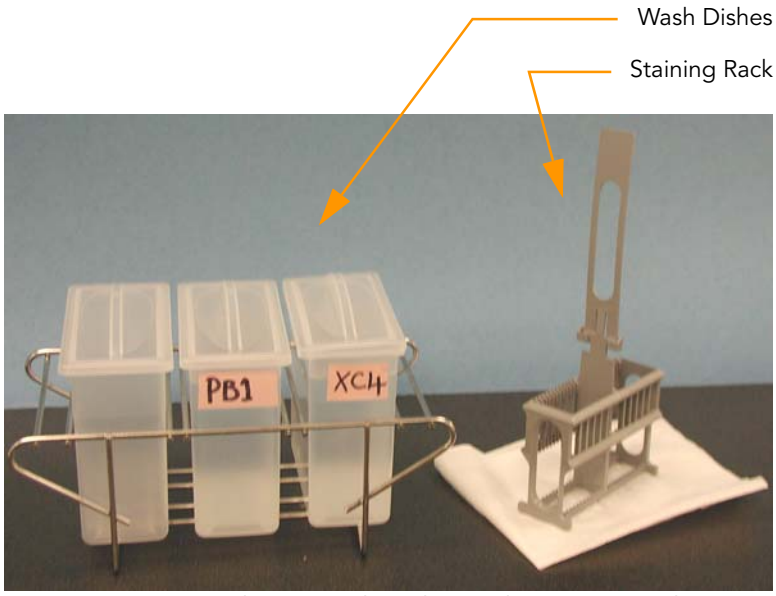


Figure 58 PB1 and XC4 Wash Dishes with Staining Rack

3. Pour 310 ml PB1 into the wash dish labeled "PB1."
4. Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing you*** (Figure 59). This orients the staining rack so that you can safely remove the BeadChips.

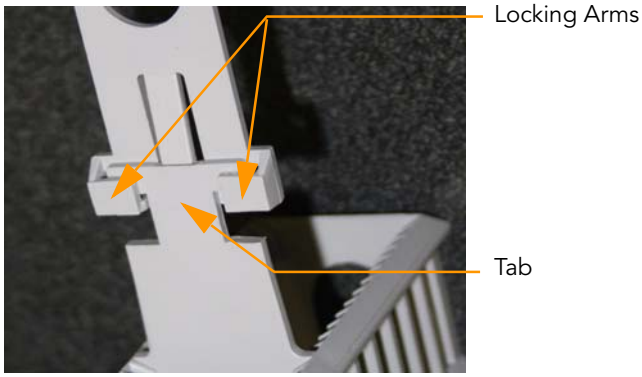


Figure 59 Staining Rack Locking Arms and Tabs

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.

5. One at a time, disassemble each Flow-Through Chamber:
   a. Use the dismantling tool to remove the two metal clamps (Figure 72).

CAUTION

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

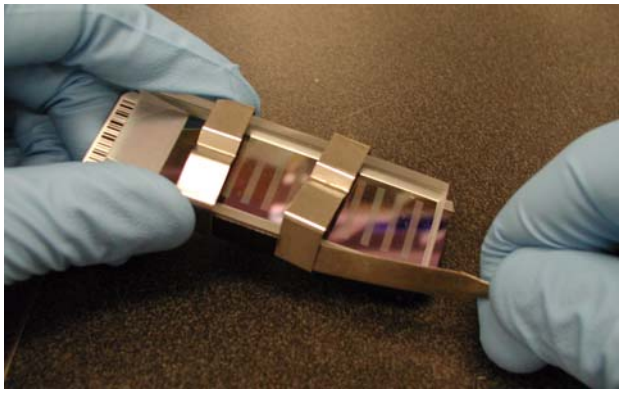


Figure 60 Removing Metal Clamps from Flow-Through Chamber

b. Remove the glass back plate.
c. Set the glass back plate aside. When you finish the XStain BC2 protocol, clean the glass back plates as described in the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
d. Remove the spacer. To avoid damaging the stripes on the BeadChip, pull the spacer out so that the long sides slide along the sides of the BeadChip.
e. Remove the BeadChip.

CAUTION

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

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

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

7. 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.

8. Move the staining rack up and down 10 times, breaking the surface of the PB1 (Figure 73).

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.

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

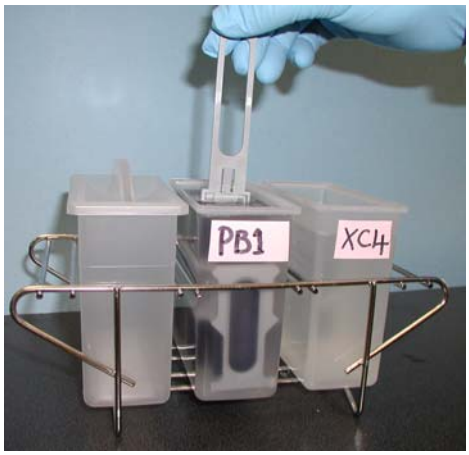


Figure 61 Washing BeadChips in PB1

NOTE

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

10. 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.

11. Remove the staining rack from the PB1 dish and place it directly into the wash dish containing XC4 (Figure 62). 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.

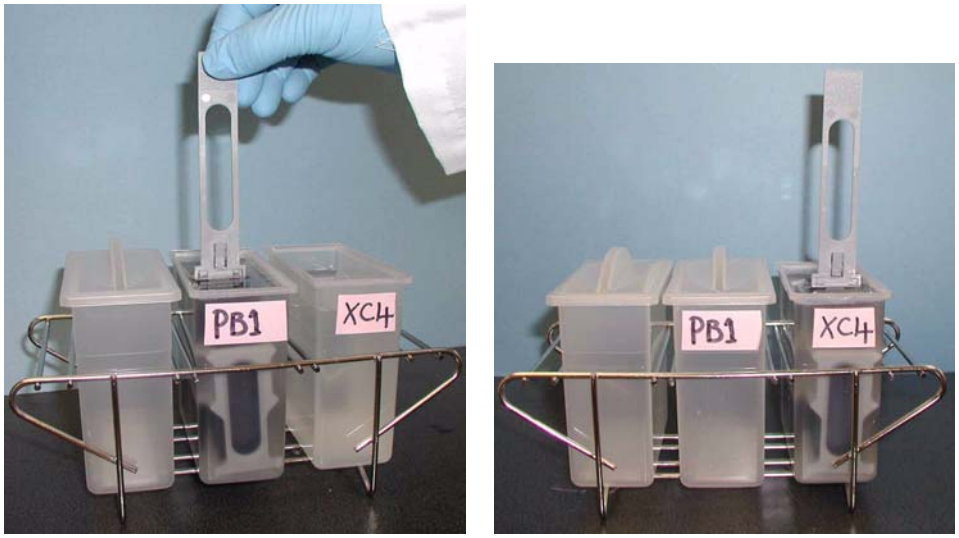


Figure 62 Moving BeadChips from PB1 to XC4

12. 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.

13. 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.

14. Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.
15. Prepare one additional tube rack per 8 BeadChips (Illumina-provided from VWR catalog # 60916-748) that fits the internal dimensions of vacuum desiccator
16. Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes ***face up*** and the locking arms and tab ***face down*** (Figure 63).

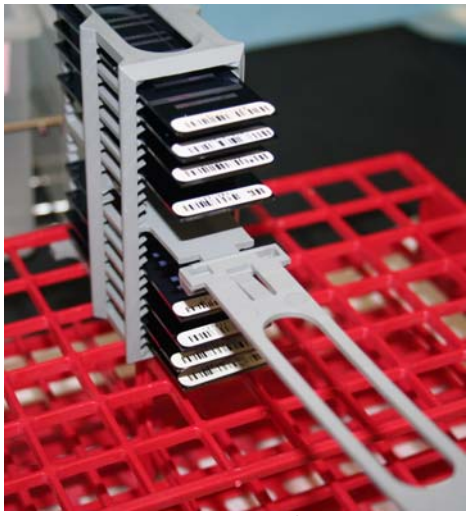


Figure 63 Staining Rack in Correct Orientation

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

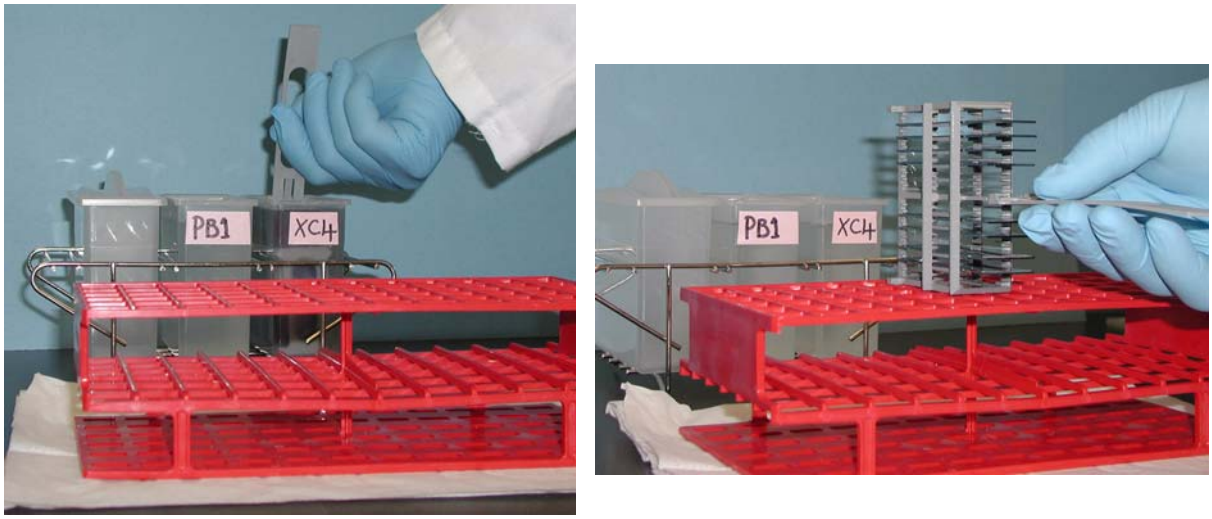


Figure 64 Moving the Staining Rack from XC4 to Tube Rack

18. For the top four BeadChips, working top to bottom:
    a. Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

NOTE

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

    b. Place each BeadChip on a tube rack with the barcode facing up and towards you (Figure 66).

19. 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 65).

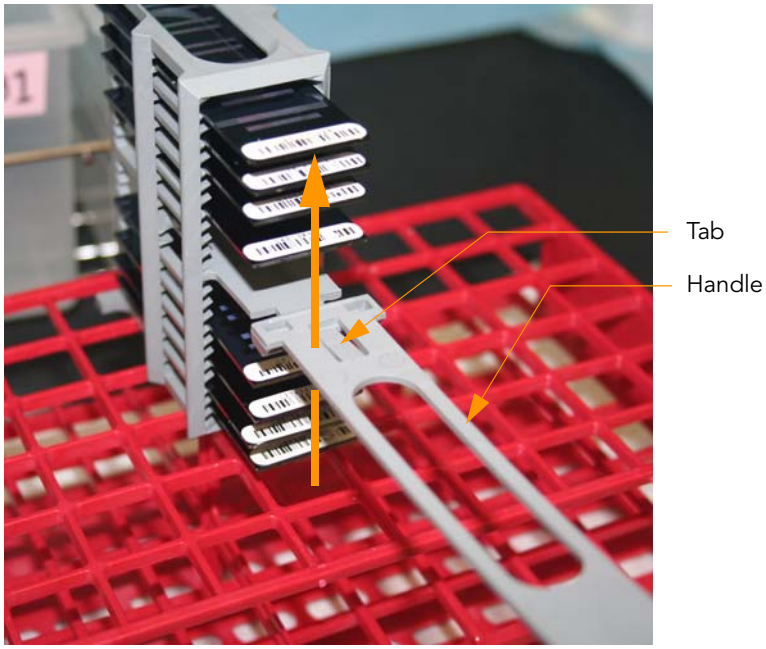


Figure 65 Removing Staining Rack Handle

20. Remove the remaining BeadChips to the tube rack as shown in (Figure 66), 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.

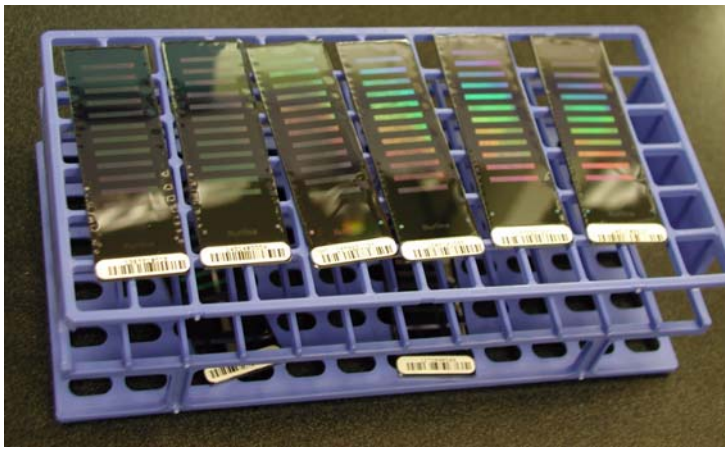


Figure 66 BeadChips on Tube Rack

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.

21. Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack (8 BeadChips).

CAUTION

Ensure the vacuum valve is seated tightly and securely.

22. Remove the red plug from the three-way valve before applying vacuum pressure.
23. Start the vacuum, using at least 508 mm Hg (0.68 bar).
24. To ensure that the dessicator is properly sealed, gently lift the lid of the vacuum desiccator (Figure 67). It should not lift off the desiccator base.

Figure 67 Testing Vacuum Seal

25. Dry under vacuum for 50–55 minutes.

    Drying times may vary according to room temperature and humidity.
26. 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.

27. 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.
28. Touch the borders of the chips (**do not touch the stripes**) to ensure that the etched, barcoded side of the BeadChips are dry to the touch.

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

Do not touch the stripes.

30. Clean the glass back plates. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
31. Clean the Hyb Chambers:
    a. Remove the rubber gaskets from the Hyb Chambers.
    b. Rinse all Hyb Chamber components with DI water.
    c. Thoroughly rinse the eight humidifying buffer reservoirs.
32. Discard unused reagents in accordance with facility standards.

Image BC2

The BeadChips are now ready for scanning. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963). Image the BeadChips within 72 hours.

Allele-Specific Extension & Stain BeadChip

Use this procedure only for HumanNS-12 BeadChips. If you are using any other sort of BeadChip, follow the procedures in *Single-Base Extension and Stain BC2* on page 52.

In this process, you use RA1 reagent to wash away unhybridized and non-specifically hybridized DNA sample. Dispense EMM reagent into the Flow-Through Chambers to extend primers hybridized to DNA on the BeadChip. The reaction incorporates labelled nucleotides into the extended primers. 95% formamide/1 mM EDTA is added to remove the hybridized DNA. After neutralization with the XB3 reagent, the labelled extended primers undergo a multi-layer staining process on the Chamber Rack. Finally, disassemble the Flow-Through Chambers, wash the BeadChips in the PB1 reagent, and then dry them.

Estimated Time

Approximately 2.5 hours.

Reagents

User-Supplied

- 95% formamide/1 mM EDTA (15 ml)

Illumina-Supplied

Per 8 BeadChips:

- RA1 (21 ml) (See *Setup* for special instructions)
- XB1 (2 tubes)
- XB2 (2 tubes)
- EMM (2 tubes)
- XB3 (75 ml)
- LMM (2 tubes)
- ASM (2 tubes)
- PB1 (200 ml)

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- RA1 is shipped and stored at -20°C. Gradually warm the RA1 reagent to room temperature (22°C), preferably in a 20–25°C water bath. Gently mix to dissolve any crystals that may be present.
- Place all reagent standoff tubes in a rack in the order in which they will be used (Figure 68).

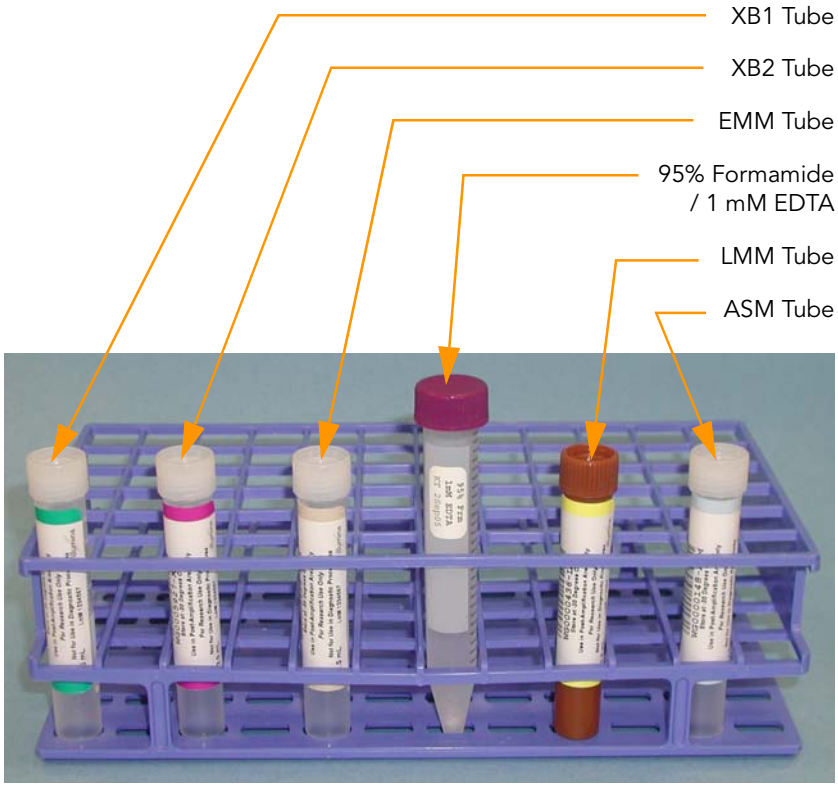


Figure 68 XStain BC2 Reagent Tubes

- Pour 15 ml 95% formamide/1 mM EDTA into a 15 ml conical tube.
- Dispense all bottled reagents into disposable reservoirs, as they are needed.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - RA1 barcode
  - XB3 barcode
  - XB1 barcode(s)
  - XB2 barcode(s)
  - EMM barcode(s)
  - LMM barcode(s)
  - ASM barcode(s)
  - PB1 barcode

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 (Figure 69).

   This temperature may vary depending on facility ambient conditions.

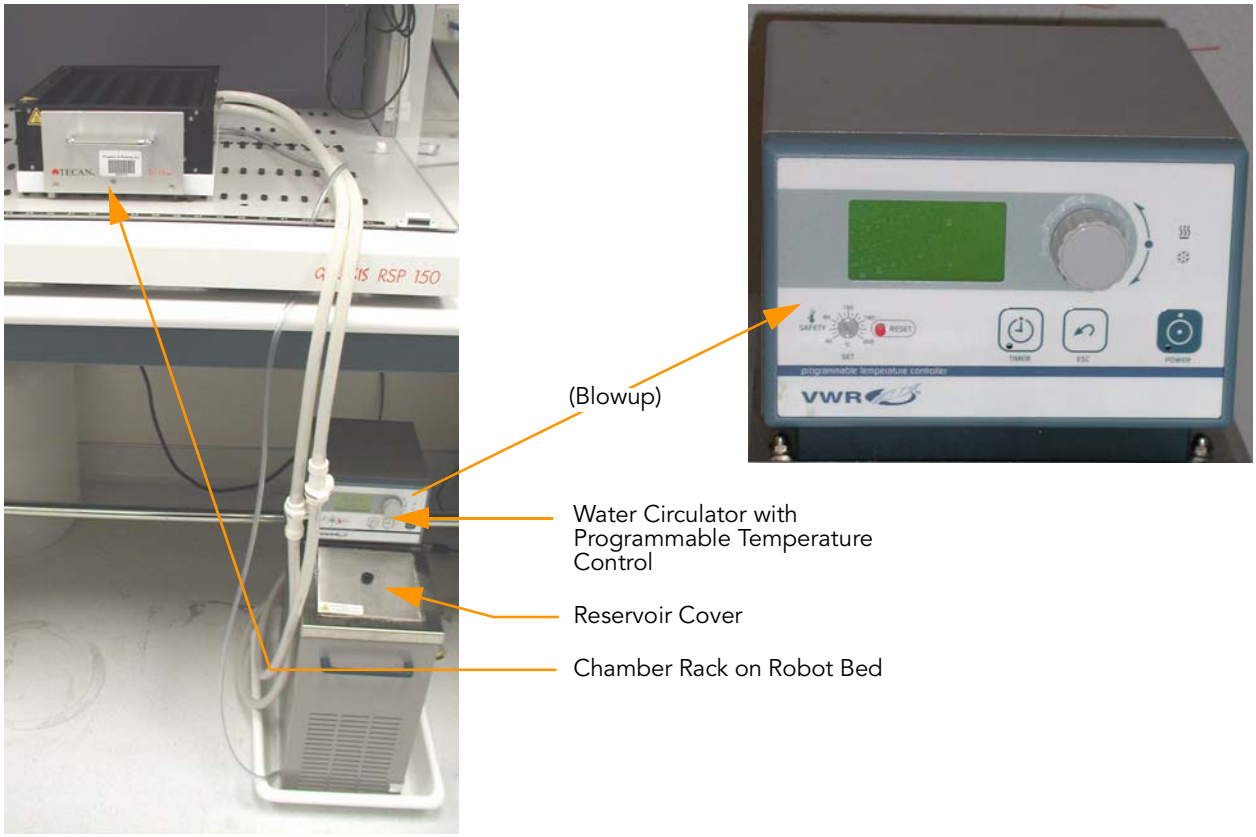


Figure 69 Water Circulator Connected to Chamber Rack

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. You must remove bubbles trapped in the Chamber Rack ***each time*** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.
5. Use the Illumina Temperature Probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 70).

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

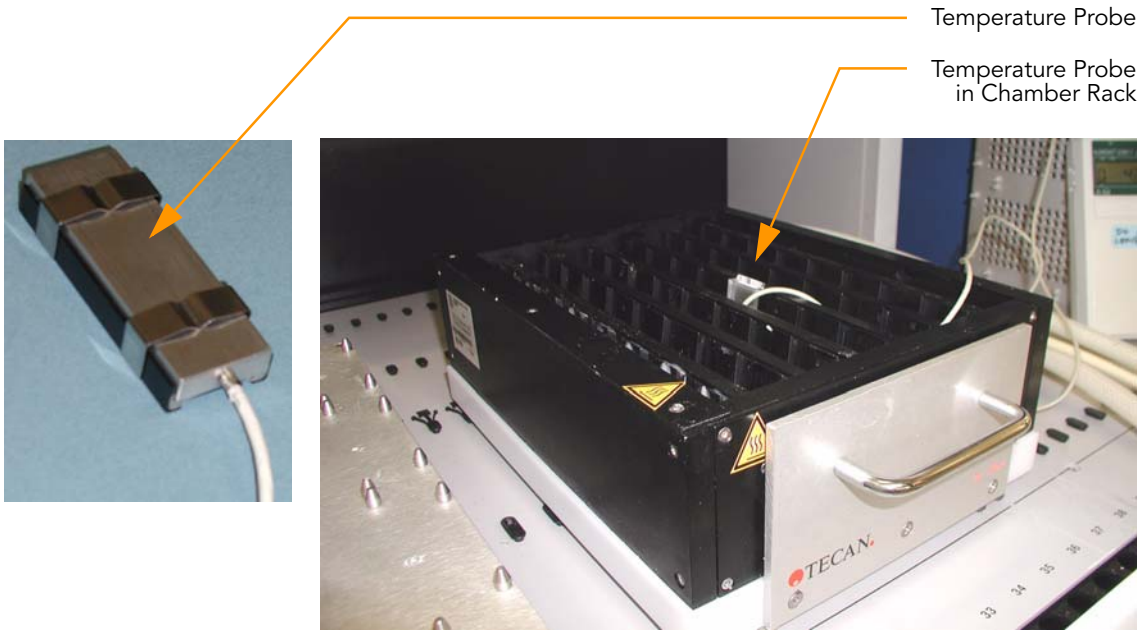


Figure 70 Illumina Temperature Probe in Chamber Rack

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

   The remaining steps in this protocol must be performed without interruption.

XStain BeadChip

Allele-Specific Extension

1. After equilibrating the Chamber Rack to 44°C, remove the Hyb Chambers from the oven and quickly place each assembled Flow-Through Chamber in the first row of the Chamber Rack.

   If you are staining 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 to allow adequate heat exchange between the rack and the chamber.

3. On the lab tracking worksheet, record the chamber rack position associated with each BeadChip.

4. ***Immediately*** wash the Hyb Chamber reservoirs with dH_2O and scrub them with a small cleaning brush.

   CAUTION

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

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

   a. 450 µl RA1 (Figure 71) and incubate for 1 minute. Repeat four times.

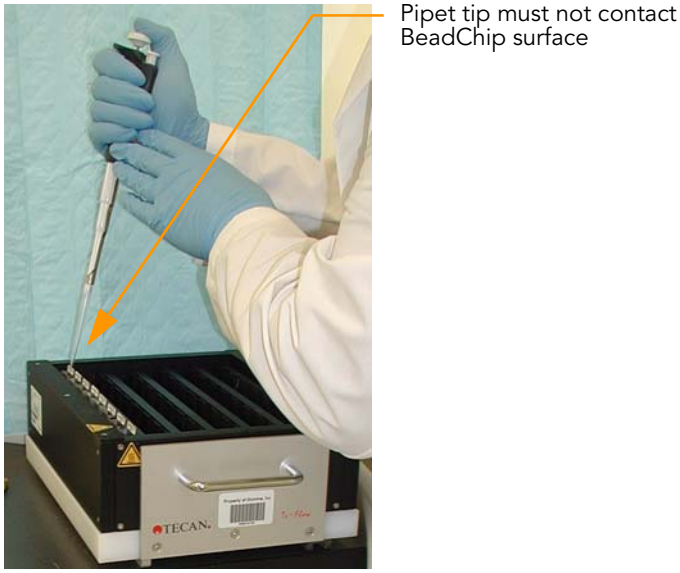


Figure 71 RA1 into Each Flow-Through Chamber

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

b. 450 µl XB1 and incubate for 10 minutes.
c. 450 µl XB2 and incubate for 5 minutes.
d. 200 µl EMM and incubate for 15 minutes.
e. 450 µl 95% formamide/1 mM EDTA and incubate for 1 minute. Repeat this step once.
f. 450 µl XB3 and incubate for 1 minute.

Stain BeadChip

1. Into the reservoir of each Flow-Through Chamber, dispense:
   a. 250 µl LMM and incubate for 10 minutes.
   b. 450 µl XB3 and incubate for 1 minute. Repeat this step twice.
   c. 250 µl ASM and incubate for 10 minutes.
   d. 450 µl XB3 and incubate for 1 minute. Repeat this step twice.
   e. Repeat steps a through d once.

1. Into the reservoir of each Flow-Through Chamber, dispense:
   a. 250 µl LMM and incubate for 10 minutes.
   b. 450 µl XB3 and incubate for 1 minute. Repeat this step twice.

2. Immediately remove the Flow-Through Chambers from the Chamber Rack and place horizontally on a lab bench at room temperature (22°C).

Wash & Spin

1. Pour 200 ml PB1 into a BeadChip wash dish and submerge a BeadChip wash rack in it.

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

a. Use the dismantling tool to remove the two metal clamps (Figure 72).

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

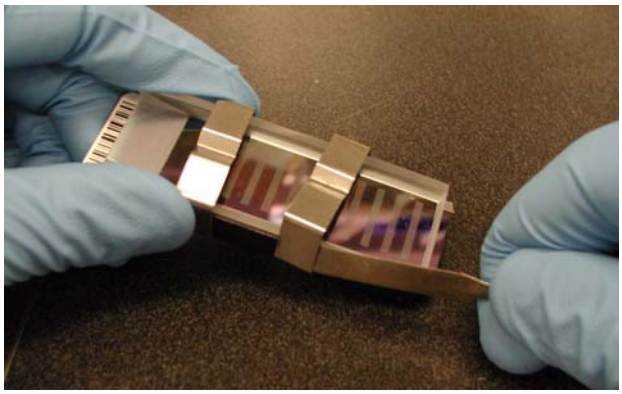


Figure 72 Removing the Metal Clamps from Flow-Through Chamber

b. Remove the glass back plate.
c. Set the glass back plates aside. When you finish the XStain BC2 protocol, clean the glass back plates as described in the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
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.

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

CAUTION Do not allow BeadChips to dry out.

4. Move the wash rack up and down for 30 seconds, breaking the surface of the PB1 with gentle, slow agitation at the beginning, middle, and end of a 5-minute period.

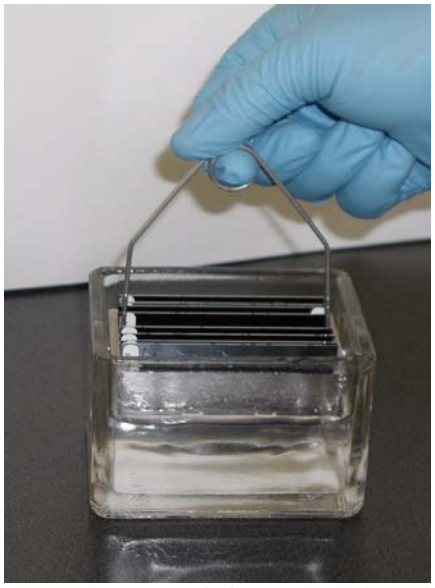


Figure 73 BeadChips in Wash Dish

NOTE

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

5. Transport the entire wash bath to the centrifuge.
6. Place a folded paper towel in the bottom of each of two opposite-facing centrifuge carriers.
7. Remove the rack with BeadChips from the bath. Carefully place the rackin the centrifuge on top of a paper towel. The towel will catch any excess liquid during the spin.
8. Place another rack loaded with empty BeadChips in the opposite carrier for balance (Figure 74).
9. Remove the handles from the racks.

CAUTION

Do not allow the Beadchips to dry in the rack without centrifugation.

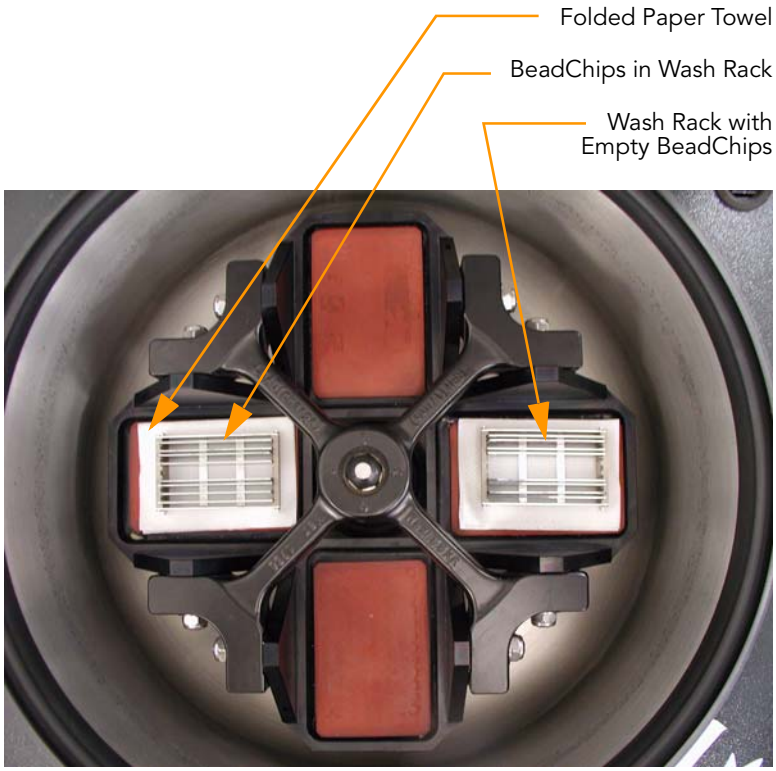


Figure 74 Balancing BeadChip Racks in Centrifuge

10. Spin at 280 xg for 1 minute to dry the BeadChips completely.
11. If you do not plan to scan the BeadChips immediately, place the BeadChips in the Illumina Slide Storage Box and place the box inside a dessicator until ready to proceed. You may store the BeadChips like this for up to 3 days at room temperature (22°C) before scanning them.
12. Clean the glass back plates. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
13. Clean the Hyb Chambers:
    a. Remove the rubber gaskets from the Hyb Chambers.
    b. Rinse all Hyb Chamber components with DI water.
    c. Ensure the eight humidifying buffer reservoirs are rinsed thoroughly.
14. Discard unused reagents in accordance with facility standards.

Image BC2

The BeadChips are now ready for scanning. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963). Image the BeadChips within 72 hours.

Load BeadChip (Alternative Method)

This section describes an alternative method of manually loading the DNA by rows instead of columns from the MSA2 plate. For the original method of loading DNA samples, see *Load BeadChip* on page 40.

1. Place the resuspended MSA2 plate on the heat block to denature the samples at 95°C for 20 minutes.
2. Remove all the BeadChips from their packages.
3. 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 75). Repeat for each slide.

Figure 75 Placing BeadChips into Hyb Chamber Inserts

4. After denaturation, remove the MSA2 plate from the heat block and pulse centrifuge to 280 xg.
5. Remove the MSA2 plate from the centrifuge. Remove the foil seal.
6. Using a multi-channel precision pipet, dispense 12 µl of each DNA sample onto the appropriate BeadChip stripe. Follow the alternate loading pattern on the lab tracking worksheet (also shown in Figure 77):
   a. Load samples A1–A6 from the MSA2 plate to the left side of the BeadChip (Figure 76).
   b. Load samples B1–B6 from the MSA2 plate to the right side of the BeadChip.
   c. Continue in this manner for Rows C through H.
   d. When you finish the left half of the plate, move to the right half of the plate. Load samples A7–A12 to the left side of the BeadChip and samples B7–B12 to the right side of the BeadChip. Continue in this manner for rows C through H.

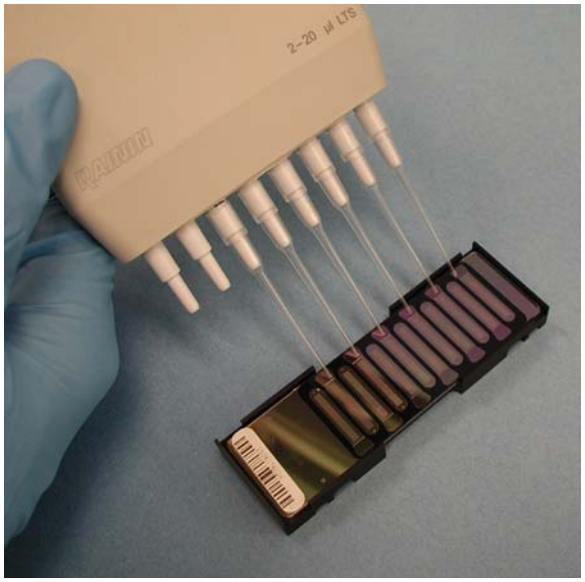


Figure 76 Dispensing Sample onto Arrays

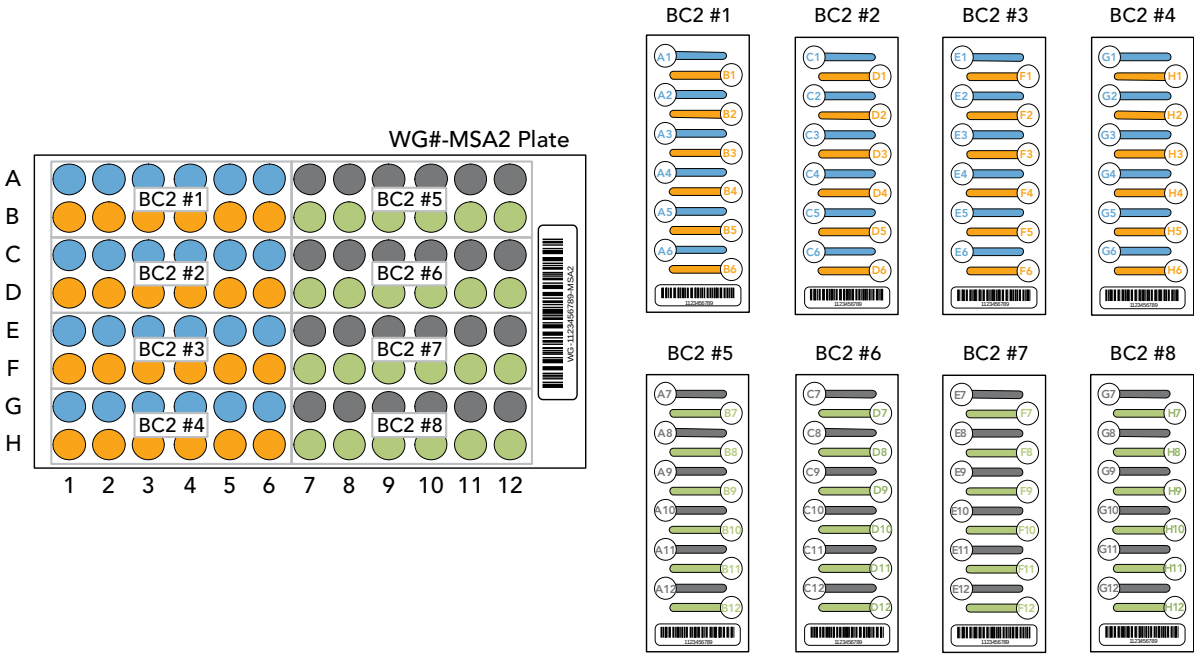


Figure 77 Distributing Sample to MSA2 Plate

7. On the lab tracking worksheet, record the BeadChip barcode for each group of samples.
8. Visually inspect all sections of the BeadChips to ensure the DNA sample covers all of the sections of each bead stripe. Record any sections that are not completely covered.
9. Heat-seal residual sample in the MSA2 plate with foil, and store at -20°C, or at -80°C if you do not plan to use it again within 24 hours.
10. Proceed to *Hyb Multi BC2 Setup* on page 43.

Chapter 3

Multi-Sample BeadChip Automated Protocol

Topics

Introduction

This chapter describes pre- and post-amplification automated laboratory protocols for the Multi-Sample BeadChip. 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 II LIMS User Guide* (Illumina part # 11217344).

Multi-Sample BeadChip Automated Workflow

Figure 78 graphically represents the Illumina Infinium II Assay automated workflow, with or without Infinium LIMS, for multi-sample BeadChips. These protocols describe the procedure for preparing 96 DNA samples.

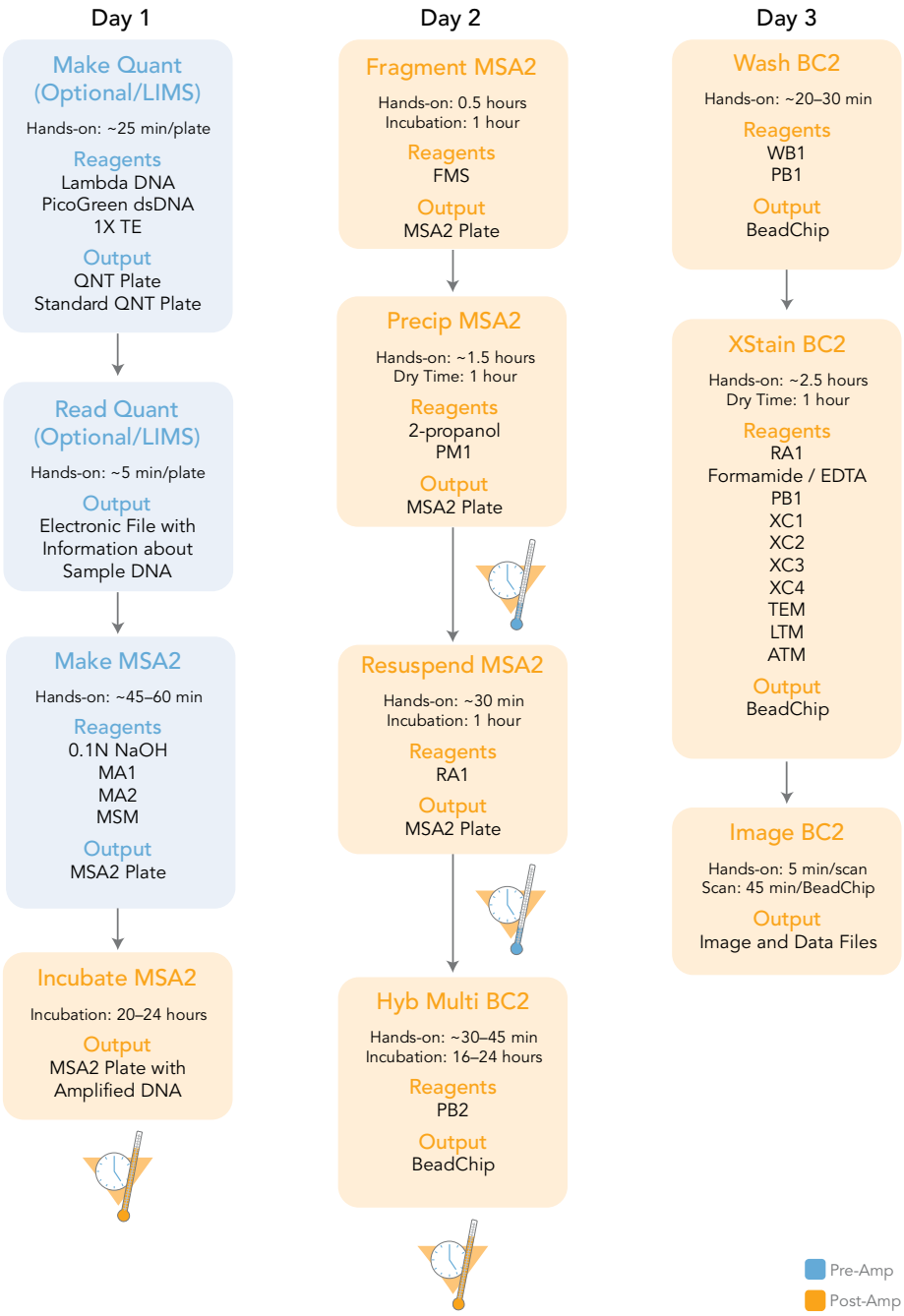


Figure 78 Multi-Sample BeadChip Automated Workflow

Equipment, Materials, and Reagents

These materials are specifically required for the multi-sample, automated BeadChip assay. For a list of other equipment, materials, and reagents needed in an Infinium II Assay lab, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Equipment

User-Supplied

- 2 Tecan eight-tip robots
  One for pre- and one for post-amplification processes
- Forceps
  VWR International, catalog # 25601-008
- Desiccator (Optional)
  Auto-Desiccator Cabinet, VWR International, catalog # 74950-342

Illumina-Supplied

- Multi-Sample BeadChip Alignment Fixture
  Illumina part # 218528
- Robot BeadChip Alignment Fixture (4)
  Illumina part # 222691

Materials

User-Supplied

- 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

Illumina-Supplied

- MSA2 barcode labels
- WG#-DNA barcode labels

Reagents

Illumina-Supplied

Table 6 Reagents for the Multi-Sample Protocol

Reagent Name	Description	Illumina Part #
ATM	Anti-Stain Two-Color Master Mix	11208317
FMS	Fragmentation solution	11203428
LTM	Labeling Two-Color Master Mix	11208325
MA1	Multi-Sample Amplification 1 MIx	11202880
MA2	Multi-Sample Amplification 2 Mix	11203401
MSM	Multi-Sample Amplification Master Mix	11203410
PM1	Precipitation solution	11203436
RA1	Resuspension, hybridization, and wash solution	11222442
PB1	Used to wash BeadChips after hybridization	11221221
PB2	Humidifying buffer used during hybridization	11191130
TEM	Two-Color Extension Master Mix	11208309
WB1	Wash solution	11203444
XC1	XStain BeadChip solution 1	11208288
XC2	XStain BeadChipsolution 2	11208296
XC3 (240 ml)	XStain BeadChip solution 3	11208421
XC4	XStain BeadChip solution 4	11208430

Make Standard DNA Plate (Optional/LIMS)

This process creates a Standard DNA plate with specific concentrations of DNA in the wells. Use this plate as input into the Make Quant process. If your DNA has already been quantified and you are not running Infinium LIMS (Laboratory Information Management System), skip ahead to *Prepare the WG#-DNA Plate (Required)* on page 92.

For information about how to use Infinium LIMS, see the *Infinium II LIMS User Guide* (Illumina part # 11217344).

Reagents

User-Supplied

- 1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA)
- Lambda DNA
  Invitrogen, catalog # 25250-028

Dilute Standard DNA

To obtain quality genotypes, DNA samples should be normalized to a concentration of 50 ng/µl.

1. Dilute DNA in 10 mM Tris pH 8.0 /1 mM EDTA.
2. In well A1 of a 96-well 0.65 ml MIDI plate (the Standard DNA plate), dilute stock Lambda DNA to 75 ng/µl in a final volume of 233.3 µl.
   a. Use the following formula to calculate dilution of stock Lambda DNA:

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

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

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

3. Transfer 66.7 µl 1X TE to well B of column 1 of the same plate.
4. Transfer 100 µl 1X TE to wells C, D, E, F, G, and H of column 1 of the same plate.

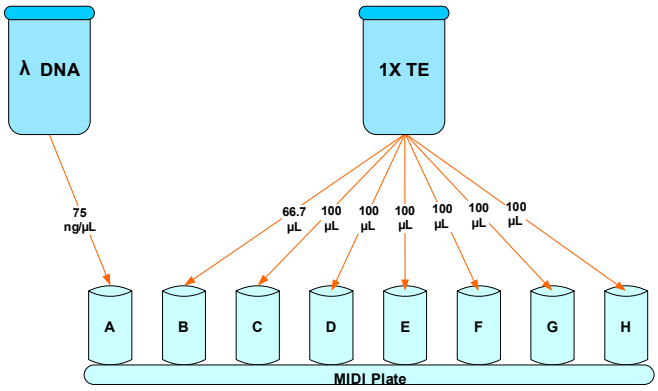


Figure 79 Standard DNA MIDI Plate Wells

Mix & Serially Dilute DNA

1. Pipet the contents of A1 up and down 10 times to mix.
2. Transfer 133.3 µl of Lambda DNA from well A1 into well B1, and then pipet the contents of well B1 up and down 10 times.
3. Transfer 100 µl from well B1 into well C1, and then pipet the contents of well C1 up and down 10 times.
4. Transfer 100 µl from well C1 into well D1, and then pipet the contents of well D1 up and down 10 times.
5. Transfer 100 µl from well D1 into well E1, and then pipet the contents of well E1 up and down 10 times.
6. Transfer 100 µl from well E1 into well F1, and then mix the contents of well F1 up and down 10 times.
7. Transfer 100 µl from well F1 into well G1, and then pipet the contents of well G1 up and down 10 times.
8. **Do not transfer solution from well G1 to well H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

Table 7 Concentration of Lambda DNA Standards

Row-Column	Conc. (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	1.5625	200
H1	0	100

9. Seal the Standard DNA plate with the cap mat.

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

10. Do one of the following:
    - Proceed to *Make Quant (Optional/LIMS)* on page 85.
    - Store the sealed Standard DNA plate at 4°C if you do not plan to proceed to the next step immediately.

Make Quant (Optional/LIMS)

In this process, you create one to three QNT plates for use in the Molecular Dynamics Fluorometer (if available). The fluorometer quantifies the DNA present in the sample and enters that data in to Infinium LIMS. Quantification ensures that there is enough sample DNA to generate good data. Use this procedure if you are running Infinium LIMS or if you have a fluorometer in your lab.

Estimated Robot Run Time

- 25 minutes for 8 samples
- 45 minutes for 16 samples
- 65 minutes for 24 samples

Reagents

User-Supplied

- PicoGreen dsDNA quantification reagent
  Molecular Probes, catalog # P7581

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

- 1X TE
  10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE)
- 8, 16, 24, 32, 48, or 96 DNA samples in up to three plates that are labelled with WG#-DNA barcode labels and have been accessioned into Infinium LIMS (if you are using Infinium LIMS)

Setup

- Remove PicoGreen reagent from freezer and thaw at room temperature for 60 minutes in a light-impermeable container.
- Remove the previously accessioned WG#-DNA plate(s) from the refrigerator or freezer. If frozen, thaw completely.
- For each WG#-DNA plate you plan to quantify, place a QNT barcode label on a new black Fluotrac plate. Position the label on the skirt of the plate, where the manufacturer's name appears.
- Obtain a new black Fluotrac plate to be the Standard QNT plate.

Populate Sample Sheet

In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 88 throughout this protocol. Note that all of the barcodes face to the right.

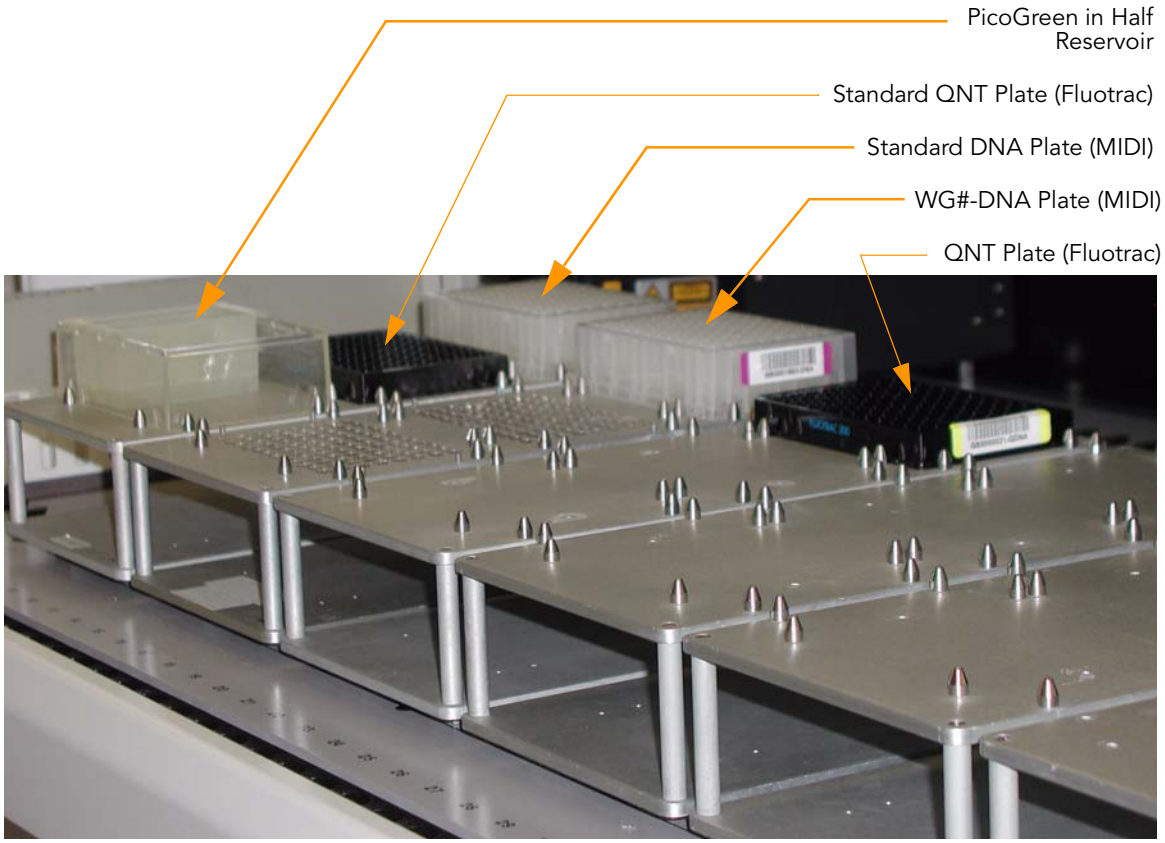


Figure 80 Tecan Eight-Tip Robot (Make Quant Setup)

Make QNT

1. At the robot PC, select **DNA Quant | Make Quant**.
2. In the DNA Plate Selection dialog box (Figure 89), select the plate type of the Standard DNA and WG#-DNA plates. Roll the mouse pointer over each picture to see a description of the plate. They should all be MIDI plates or all be TCY plates.
3. In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect the number of samples being processed.

   You can process up to 96 samples per robot run.

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.

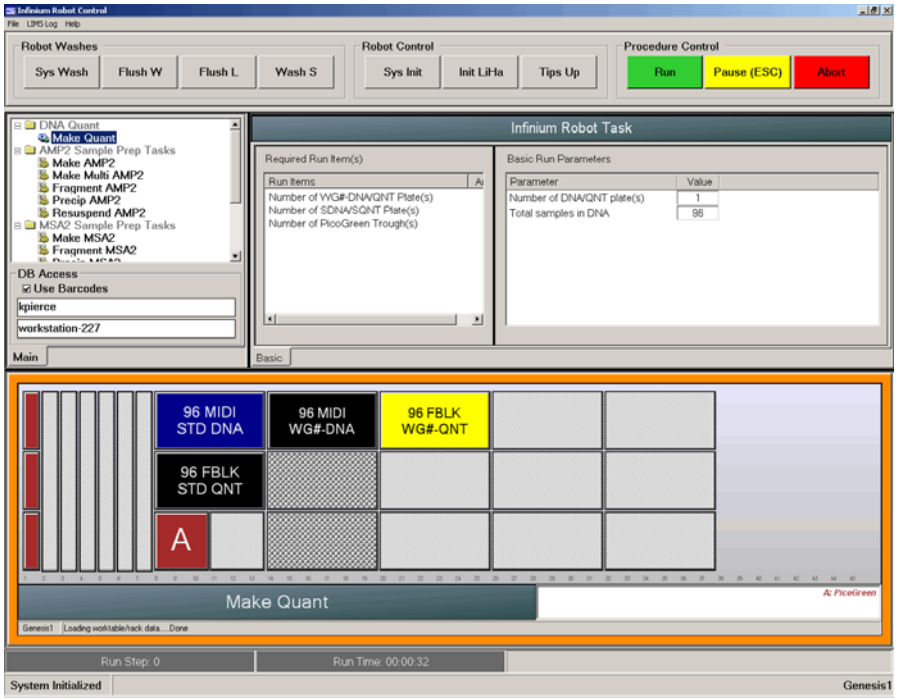


Figure 81 Make Quant Screen

4. Vortex:
   - WG#-DNA MIDI plate(s) at 1450 rpm for 1 minute
   - WG#-DNA TCY plate(s) at 1250 rpm for 1 minute
5. Centrifuge the WG#-DNA plate(s) to 280 xg for 1 minute.
6. Place the WG#-DNA plate(s) on the robot bed according to the bed map (Figure 81). Remove any plate seals.
7. Vortex the Standard DNA plate at 1450 rpm for 1 minute.
8. Centrifuge the Standard DNA plate to 280 xg for 1 minute.
9. Place the Standard DNA plate on the robot bed according to the bed map. Remove any plate seals.
10. Place the unlabeled black Fluotrac Standard QNT plate on the robot bed according to the bed map. Place well A1 at the top-left corner of its robot bed carrier. Quantified DNA from the Standard DNA plate will be transferred into the Standard QNT plate.
11. Place the black Fluotrac QNT plate(s) on the robot bed according to the bed map. You should have one QNT plate for each WG#-DNA plate.
12. Proceed to *Prepare PicoGreen Dilution*.

Prepare PicoGreen Dilution

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

1. Wrap aluminum foil around a sterile plastic container to prevent light penetration.
2. Make a 1:200 dilution of PicoGreen to 1X TE in the sterile plastic container.

Table 8 PicoGreen Reagent Volumes

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

You can prepare dilutions for up to three sample plates at a time.

3. Mix dilution thoroughly.
4. Pour PicoGreen dilution into a half reservoir.
5. Place the PicoGreen half reservoir on the robot bed according to the bed map.
6. Make sure that all plates are placed properly on the robot bed and that the seals have been removed from all plates.

All barcodes must face the right side of the robot bed.

Start the Robot

1. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. Observe the robot start to run to ensure that there are no problems.

   The robot transfers sample from the Standard DNA plate to the Standard Quant plate, and from the WG#-DNA plate(s) to the QNT plate(s).

   The robot PC sounds an alert and displays a message when the process is complete.
2. Click **OK** in the message box.
3. On the lab tracking worksheet, 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.

4. Immediately seal all plates:
   a. Place foil adhesive seals over QNT and Standard QNT plates.
   b. Place cap mats on WG#-DNA and Standard DNA plates.
5. Discard unused reagents in accordance with facility requirements.
6. Store the WG#-DNA and Standard DNA plates at 4°C.
7. Centrifuge the QNT and Standard QNT plates to 280 xg for 1 minute.
8. Proceed to *Read Quant (Optional/LIMS)* on page 89.

Read Quant (Optional/LIMS)

In this process, you use a fluorometer along with the Infinium Fluorometry Analysis software to interpret the quantified DNA in the QNT plate(s) and obtain the exact concentration of DNA in the sample. This information is saved with the other project data in Infinium LIMS. Illumina recommends using a fluorometer because fluorometry provides DNA-specific quantification. Spectrophotometry may also measure RNA, yielding values that are too high.

For information about how to use Infinium LIMS, see the *Infinium II LIMS User Guide* (Illumina part # 11217344).

Equipment

User-Supplied

- Fluorometer
  Gemini XS or XPS and SoftMax Pro (Molecular Devices)

Read Quant

1. Turn on the fluorometer.
2. Open the Infinium Fluorometry Analysis software.

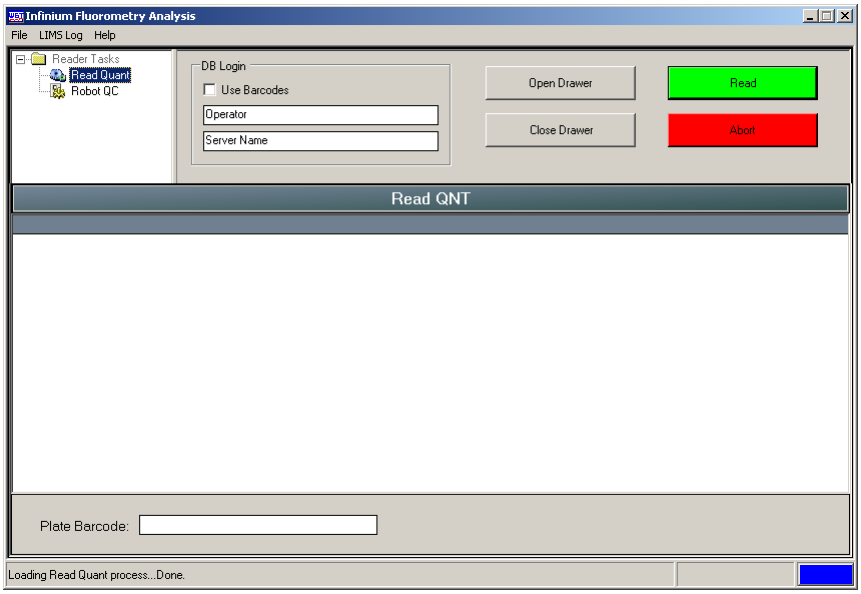


Figure 82 Infinium Fluorometry Analysis Opening Screen

3. Click **Read Quant**.
4. Select the **Use Barcodes** check box.
5. Click **Read**.
6. When prompted, log in to the Infinium LIMS database.
7. When asked if you want to read a new Standard QNT plate, click **Yes**. Load the Standard QNT plate in the open drawer of the fluorometer.
8. When the fluorometer finishes reading the Standard QNT data, unload the plate.
9. When prompted, enter the number of QNT plates you want to read. You can read up to three plates. Click **OK**.

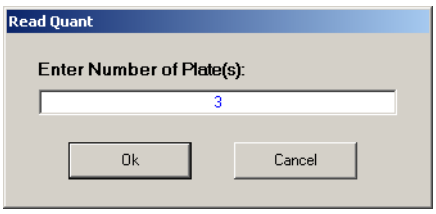


Figure 83 Number of QNT Plates Dialog Box

10. When prompted, enter the QNT plate barcode. Load the QNT plate in the open drawer of the fluorometer and click **OK**.

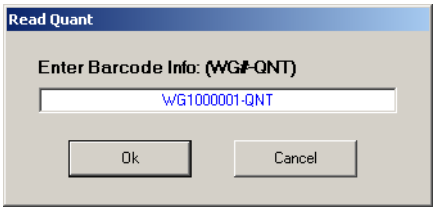


Figure 84 Plate Barcode Dialog Box

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

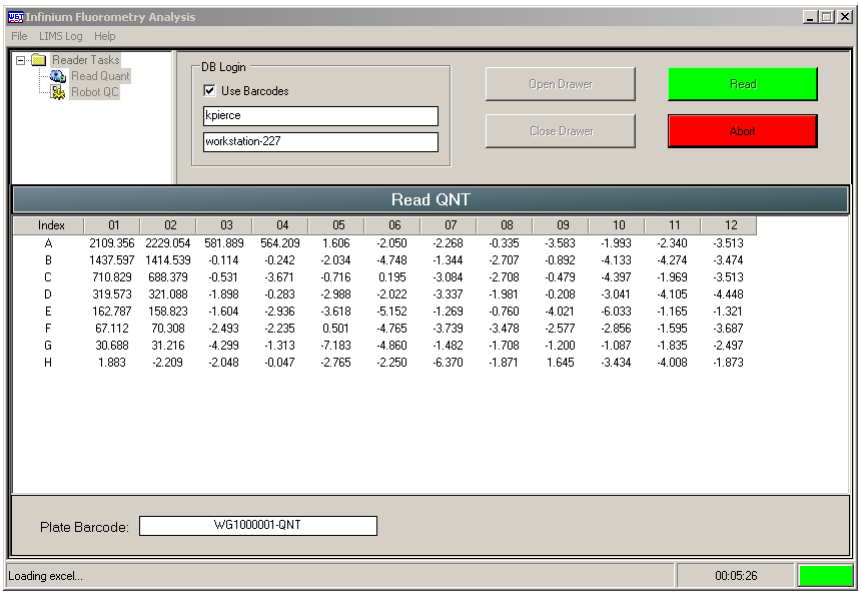


Figure 85 Fluorescence Data

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

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

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

11. Do one of the following:
    - Click **Yes** to send the data to Infinium LIMS. In Infinium LIMS, the QNT plate moves into the Make MSA2 queue.
    - Click **No** to delete the quant data. You can read the quant data again for the same plate.
12. If you entered more than one QNT plate to read, repeat steps 10 and 11 for each additional plate.
13. Discard the QNT plates and reagents in accordance with facility requirements.
14. Proceed to *Make the MSA2 Plate* on page 93.

Prepare the WG#-DNA Plate (Required)

1. Retrieve your DNA samples and thaw to room temperature (22°C).

   DNA samples must be 50 ng/µl, resuspended in TE (10 mM Tris, 1 mM EDTA).

2. Apply a WG#-DNA barcode label to a new 0.8 ml microtiter storage plate (MIDI) or a new 0.2 ml skirted microplate (TCY).

3. Dispense DNA according to Figure 86:
   - For MIDI plate: 20 µl DNA sample to each well
   - For TCY plate: 10 µl DNA sample to each well

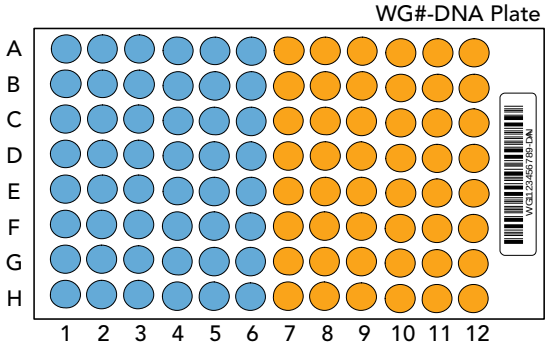


Figure 86 Distributing Sample to WG#-DNA Plate

The example shown in Figure 86 applies to 96 samples. For 48 samples, fill first half of the plate only (blue shaded section).

Make the MSA2 Plate

This process creates a MSA2 plate for DNA amplification. MA1 is first added to the MSA2 plate, followed by the DNA samples. 0.1N NaOH is added to denature the DNA samples. The MA2 reagent neutralizes the sample. MSM (Multi-Sample Amplification Master Mix) is then added to the DNA samples.

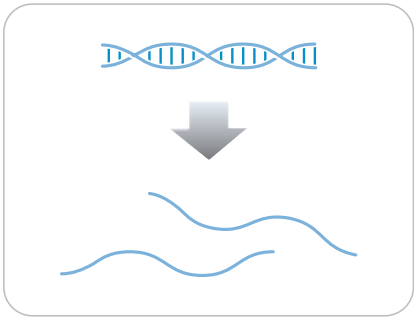


Figure 87 Denaturing and Neutralizing DNA

Estimated Robot Run Time

- 30 minutes for 48 samples
- 45 minutes for 96 samples

Reagents

User-Supplied

- 0.1N NaOH
- 96 DNA samples in a labeled WG#-DNA plate, thawed to room temperature

Illumina-Supplied

Per 96 samples:

- MA1 (2 tubes)
- MA2 (2 tubes)
- MSM (2 tubes)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- In preparation for the Incubate MSA2 process (page 100), preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow temperature to equilibrate.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - Batch number

- Number of samples (48 or 96)
- WG#-DNA plate barcode(s)
- MSA2 plate barcode(s)
- MA1 tube barcode(s)
- MA2 tube barcode(s)
- MSM tube barcode(s)

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Populate Sample Sheet

In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well. You may also fill in the other optional columns in the Sample Sheet: Gender, Sample_Group, Replicate, Parent1, and Parent2.

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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 88 throughout this protocol. Note that all of the barcodes face to the right.

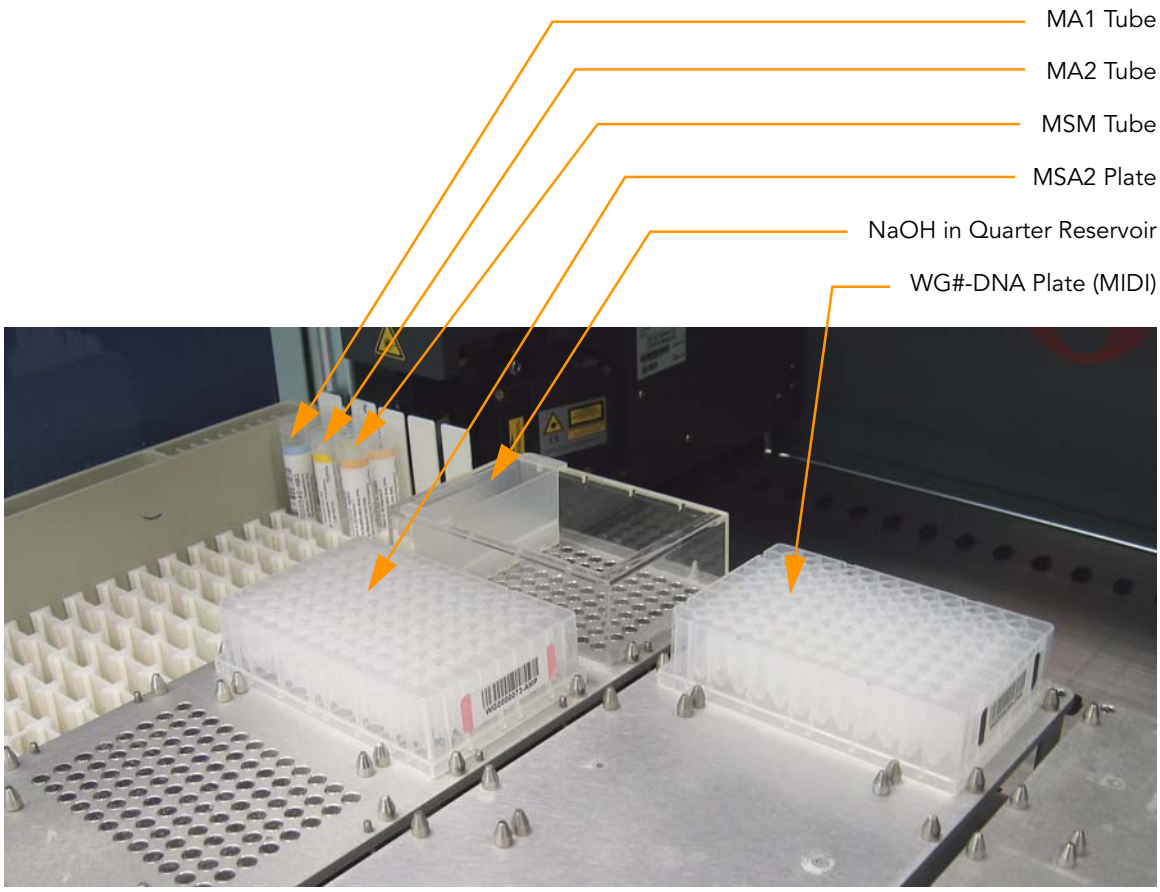


Figure 88 Eight-Tip Robot (Make MSA2 Setup)

Make MSA2

1. Apply an MSA2 barcode label to a new 0.8 ml microtiter (MIDI) storage plate.
2. Select **MSA2 Sample Prep Tasks | Make MSA2**.
3. In the DNA Plate Selection dialog box (Figure 89), click on the plate type you wish to use. Roll the mouse pointer over each picture to see a description of the plate.

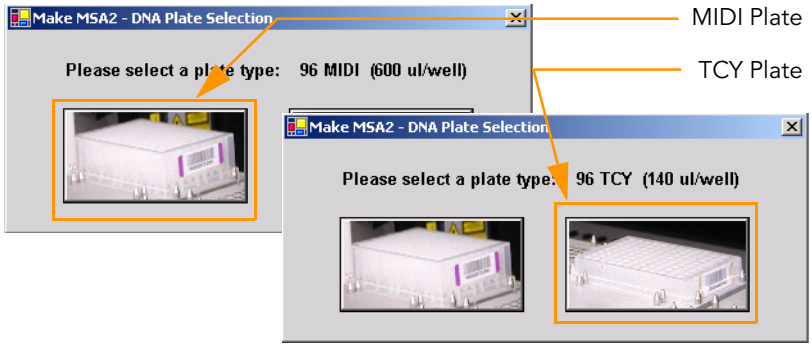


Figure 89 Selecting the DNA Plate Type

NOTE

Do not mix plate types on the robot bed.

4. In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect the number of DNAs being processed. 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.

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

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

NOTE

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

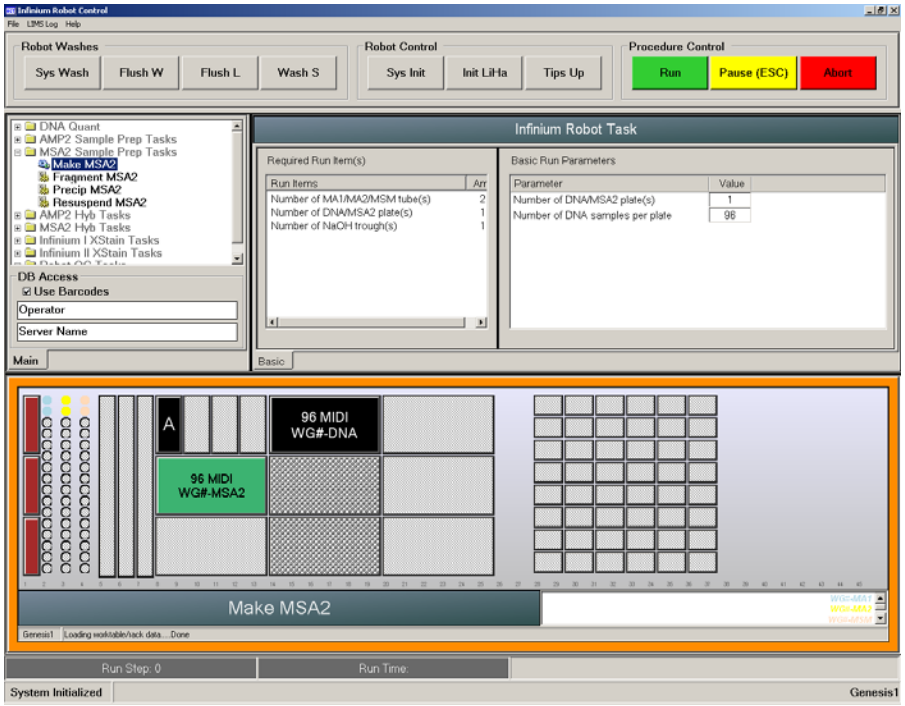


Figure 90 Make MSA2 Screen

5. Place the WG#-DNA and MSA2 plates on the robot bed according to the bed map (Figure 90).

6. Remove caps from MA1, MA2, and MSM tubes, then place the tubes in the robot standoff tube rack according to the bed map.
7. Add 15 ml NaOH to the quarter reservoir, then place the reservoir on the robot bed according to the bed map (Figure 90).
8. In the lab tracking worksheet, record the plate positions on the robot bed.
9. Make sure that all plates and tubes 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. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. Observe the robot start to run to ensure that there are no problems.
2. If you are not using Infinium LIMS, skip to step 7.

   If you are using Infinium LIMS, the Make MSA2 screen appears after a moment.

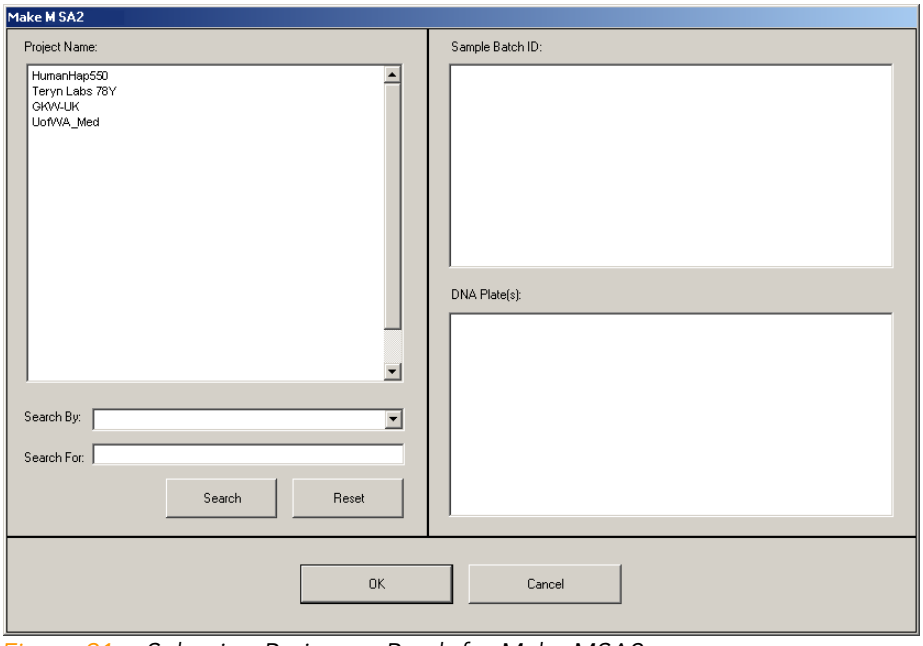


Figure 91 Selecting Project or Batch for Make MSA2

3. 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.

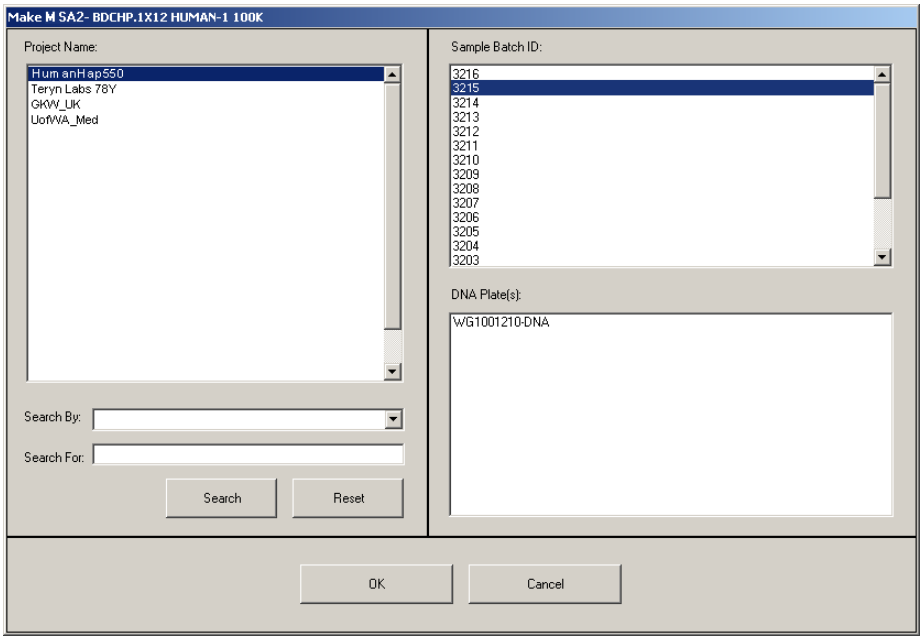


Figure 92 Make MSA2 Screen with Project and Batch Selected

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

4. Select the batch you want to run and click **OK**.
5. When prompted, enter the barcode of each WG#-DNA plate.
   The robot bed map is updated with the WG#-DNA plate locations.
6. Place the WG#-DNA plate(s) on the robot bed according to the bed map. The robot begins running when the plates are in place.
7. Observe the robot run to ensure there are no problems.
8. After the robot adds the 0.1N NaOH to the DNA in the MSA2 plate, follow the instructions at the prompt (Figure 93).

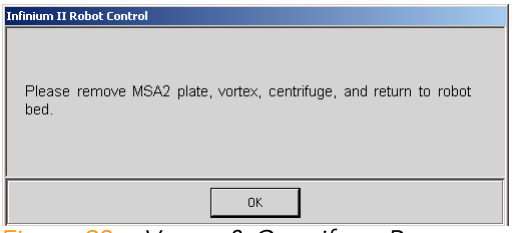


Figure 93 Vortex & Centrifuge Prompt

9. Seal the plate with a cap mat.
10. Vortex the sealed MSA2 plate at 1600 rpm for 1 minute.
11. Centrifuge to 280 xg for 1 minute at 22°C.
12. Remove the cap mat.

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

13. Place the MSA2 plate back on the robot bed in its original position and click **OK**.

    The Wait for reaction time message appears. The wait time for this reaction is 10 minutes.

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

14. Click **OK** in the message box. Remove the MSA2 plate from the robot bed and seal with the 96-well cap mat.

15. Invert the sealed MSA2 plate at least 10 times to mix contents.

16. Centrifuge to 280 xg for 1 minute.

17. Discard unused reagents in accordance with facility standards.

18. Proceed immediately to *Incubate the MSA2 Plate* on page 100.

Incubate the MSA2 Plate

This process incubates the MSA2 plate for at least 20 but no more than 24 hours at 37°C in the Illumina Hybridization Oven. It generates a sufficient quantity of each individual DNA sample to be used twice in the Multi-Sample Infinium II Assay.

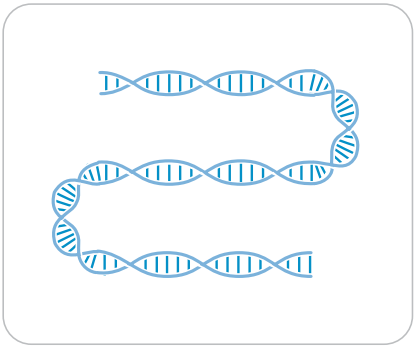


Figure 94 Incubating DNA to Amplify

Estimated Time

20–24 hours.

Verify MSA2 for Incubation (LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Incubate MSA2**.
2. Scan the barcode of the MSA2 plate and click **Verify**.

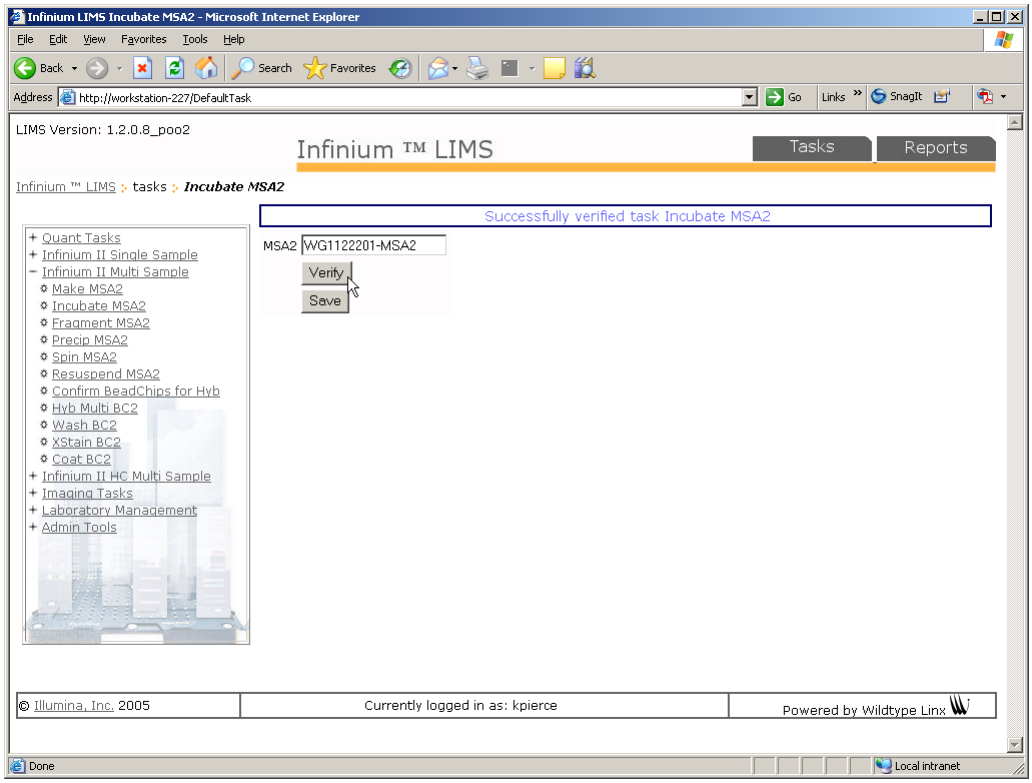


Figure 95 (Infinium LIMS) Verifying MSA2 for Incubation

3. If the MSA2 plate is queued for incubation, a blue confirmation message appears at the top of the window. Proceed to *Incubate MSA2*.
4. If the MSA2 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 Reports | 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 II LIMS User Guide* (Illumina part # 11217344).

Incubate MSA2

1. Incubate the MSA2 plate in the Illumina Hybridization Oven for at least 20 but no more than 24 hours at 37°C.
2. On the lab tracking worksheet, record the start and stop times.

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

3. If you are using Infinium LIMS:

a. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Incubate MSA2**.
b. Scan the barcode of the MSA2 plate and click **Save**. Infinium LIMS records the data and queues the plate for the next step, *Fragment MSA2*.

4. Proceed to *Fragment the MSA2 Plate* on page 102.

Fragment the MSA2 Plate

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

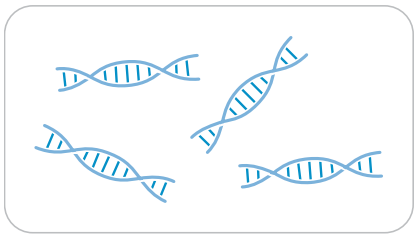


Figure 96 Fragmenting DNA

Estimated Robot Run Time

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

Reagents

Illumina-Supplied

Per 96 samples:

- FMS (2 tubes)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- Preheat the heat block with the MIDI plate insert to 37°C.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - FMS tube barcode(s)

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 97 throughout this protocol.

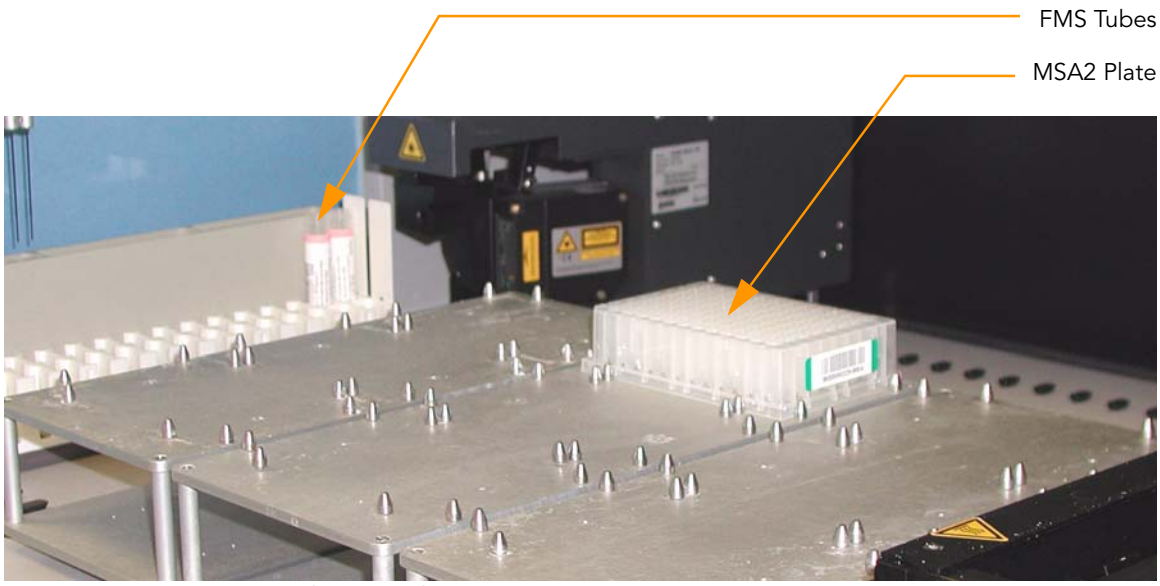


Figure 97 Tecan Eight-Tip Robot (Fragment MSA2 Setup)

Fragment MSA2

1. Centrifuge the MSA2 plate to 50 xg for 1 minute.
2. Remove the cap mat.
3. At the robot PC, select **MSA2 Sample Prep Tasks | Fragment MSA2**.
4. In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect the number of samples being processed.

NOTE

If you are using 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.

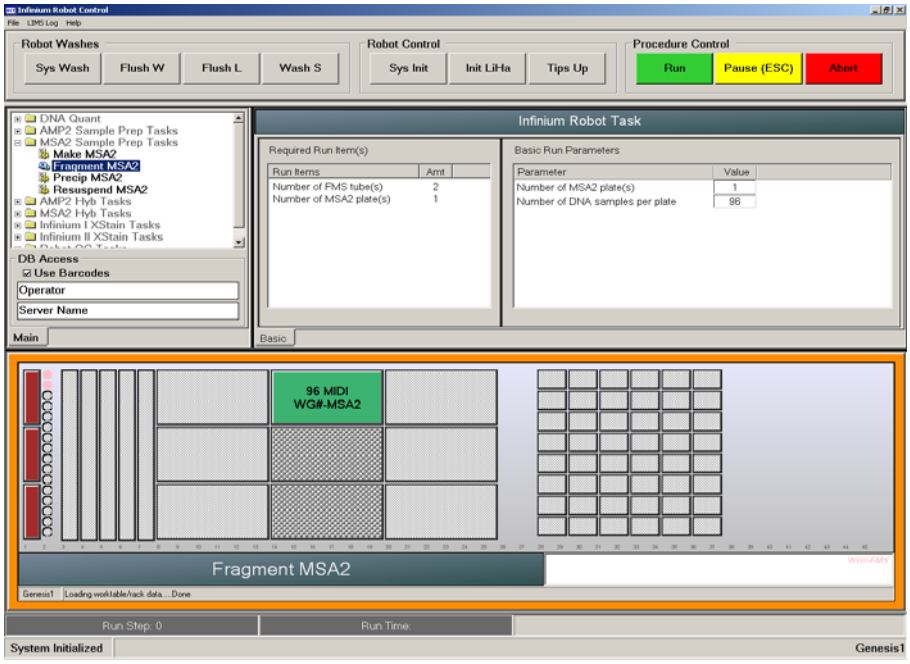


Figure 98 Fragment MSA2 Screen

5. Place the MSA2 plate on the robot bed according to the bed map (Figure 98).
6. Place the FMS tubes in the robot tube rack according to the bed map. Remove the cap.
7. In the lab tracking worksheet, record the plate positions on the robot bed.

Start the Robot

1. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. 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 MSA2 plate from the robot bed and seal with the 96-well cap mat.
3. Vortex at 1600 rpm for 1 minute.
4. Centrifuge to 50 xg for 1 minute at 22°C.
5. Place the sealed plate on the 37 °C heat block for 1 hour.
6. On the lab tracking worksheet, 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 MSA2 Plate* on page 105. Leave plate in 37°C heat block until setup is complete.
- Store the sealed MSA2 plate at -20°C if you do not plan to proceed to the next step immediately.

This is a good stopping point in the process.

Precipitate the MSA2 Plate

PM1 and 2-propanol are added to the MSA2 plate to precipitate the DNA samples.

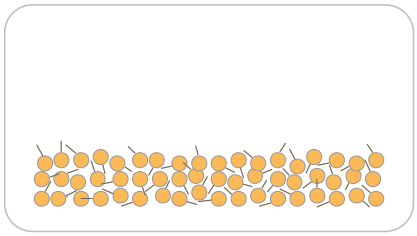


Figure 99 Precipitating DNA

Estimated Robot Run Time

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

Reagents

User-Supplied

- 2-propanol, 100%

Illumina-Supplied

Per 96 samples:

- PM1 (2 tubes)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- MSA2 plate:
  - If you froze the MSA2 plate after fragmentation, thaw it to room temperature (22°C). Centrifuge to 280 xg for 1 minute.
  - If you continued immediately from Fragment MSA2, leave the plate in the 37°C heat block until setup is complete.

- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - PM1 tube barcode(s)
  - 2-propanol lot number and date opened

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 100 throughout this protocol. Note that all of the barcodes face to the right.

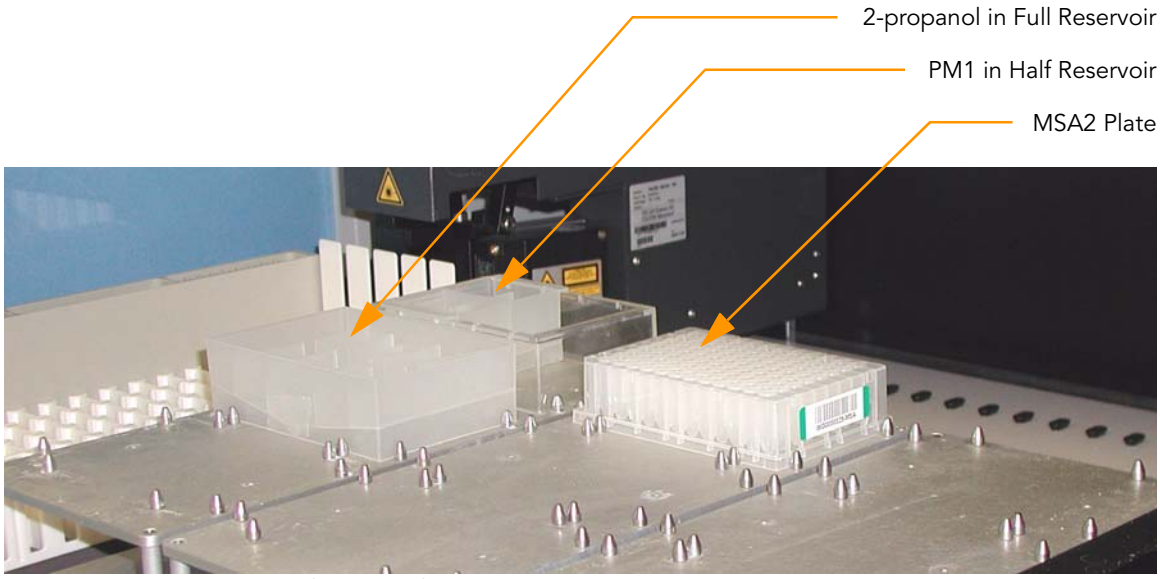


Figure 100 Tecan Eight-Tip Robot (Precip MSA2 Setup)

Verify MSA2 for Centrifugation (LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium Multi-Sample | Spin MSA2**.
2. Scan the barcode of the MSA2 plate and click **Verify**. You can scan up to four plates.

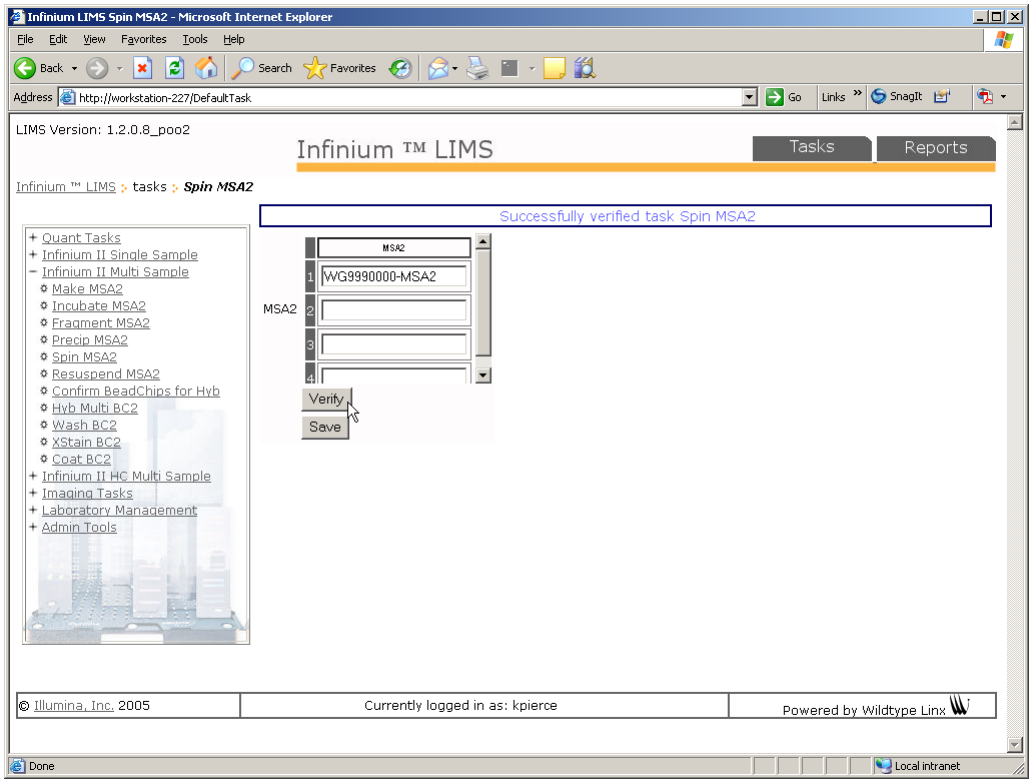


Figure 101 (Infinium LIMS) Verifying MSA2 for Centrifugation

3. If the MSA2 plate is queued for centrifugation, a blue confirmation message appears at the top of the window. Proceed to *Precip MSA2*.
4. If the MSA2 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 Reports | 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 II LIMS User Guide* (Illumina part # 11217344).

Precip MSA2

1. Centrifuge the sealed MSA2 plate to 50 xg for 1 minute at 22°C.
2. At the robot PC, select **MSA2 Sample Prep Tasks | Precip MSA2**.
3. In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect the number of DNAs being processed.

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.

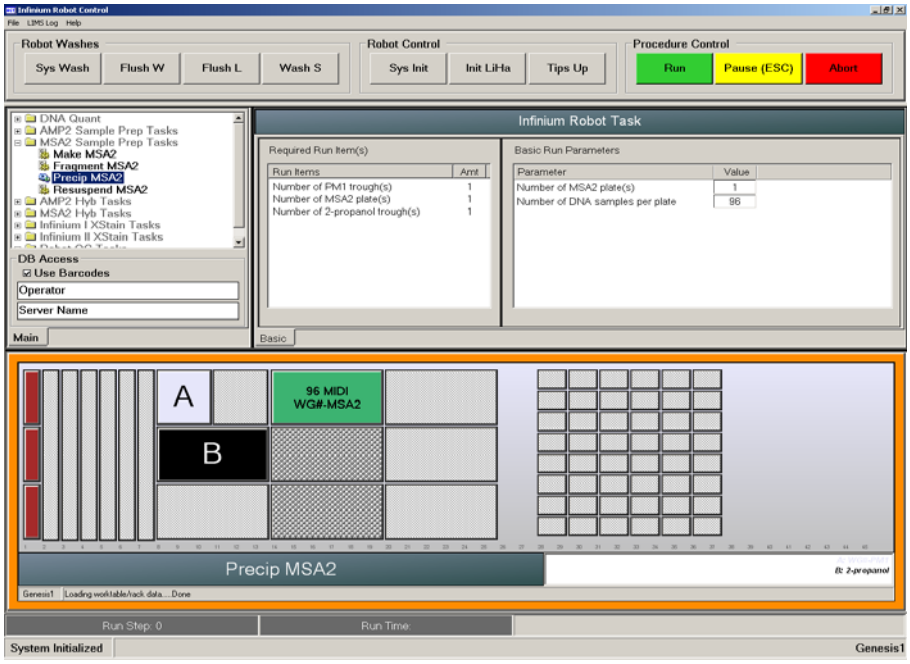


Figure 102 Precip MSA2 Screen

4. Remove the cap mat and place the MSA2 plate on the robot bed according to the bed map (Figure 102).
5. Place a half reservoir in the reservoir frame, according to the robot bed map, and add PM1 as follows:
   - 1 tube for 48 samples
   - 2 tubes for 96 samples
6. Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:
   - 20 ml for 48 samples
   - 40 ml for 96 samples
7. In the lab tracking worksheet, record the plate positions on the robot bed.
8. Make sure that all plates and tubes 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. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. Observe the robot start to run to ensure that there are no problems.
2. When prompted, remove the MSA2 plate from the robot bed. Do not click **OK** in the message box yet.

3. Seal the MSA2 plate with the same cap mat removed earlier.
4. Vortex the sealed plate at 1600 rpm for 1 minute.
5. Incubate at 37°C for 5 minutes.
6. Centrifuge 50 xg at room temperature (22°C) for 1 minute.
7. Remove the cap mat and place the MSA2 plate back on the robot bed according to the bed map (Figure 102).
8. Click **OK** in the Please Remove MSA2 Plate message box.
9. In preparation for the 4°C spin, set centrifuge to 4°C.

   The robot PC sounds an alert and displays a message when the process is complete.
10. Click **OK** in the message box. Remove the MSA2 plate from the robot bed and carefully seal with a ***new, dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.
11. Invert the plate at least 10 times to mix contents thoroughly.
12. Incubate at 4°C for 30 minutes.
13. Place the sealed MSA2 plate in the centrifuge opposite another plate of equal weight (Figure 103).

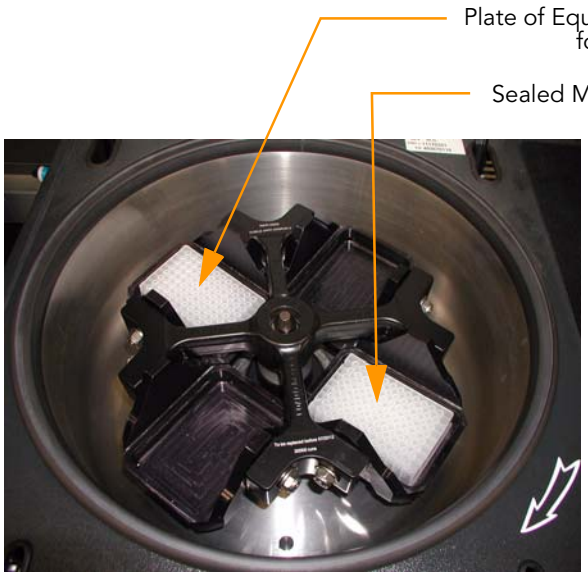


Figure 103 Balancing MSA2 Plate in Centrifuge

14. Centrifuge to 3000 xg at 4°C for 20 minutes.
15. Immediately remove MSA2 plate from centrifuge. Remove the cap mat and discard it.

    Perform the next step immediately to avoid dislodging the blue pellet. If any delay occurs, repeat steps 14 through 15 before proceeding.
16. Over an absorbent pad appropriate for 2-propanol disposal, decant supernatant by quickly inverting the MSA2 plate and smacking it down.

17. Tap the plate firmly on the pad several times over a period of 1 minute or until all wells are completely devoid of liquid.

Keep the plate inverted. To prevent cross-contamination while decanting, do not allow supernatant in wells to pour into other wells.

18. Leave the uncovered plate inverted on the tube rack for 1 hour at room temperature (22°C) to air dry the pellet (Figure 104).

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

Figure 104 Uncovered MSA2 Plate Inverted for Air Drying

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.

19. On the lab tracking worksheet, record the start and stop times.
20. If you are using Infinium LIMS:
    a. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Spin MSA2**.
    b. Scan the barcode of the MSA2 plate and click **Save**. Infinium LIMS records the centrifugation step and queues the plate for the next step, *Resuspend MSA2*.
21. Discard unused reagents in accordance with facility standards.
22. Do one of the following:
    - Proceed to *Resuspend the MSA2 Plate* on page 111.
    - If you do not plan to proceed to the next step immediately, seal the MSA2 plate with a new cap mat and store at -20°C .

This is a good stopping point in the process.

Resuspend the MSA2 Plate

RA1 is added to the MSA2 plate to resuspend the precipitated DNA samples.

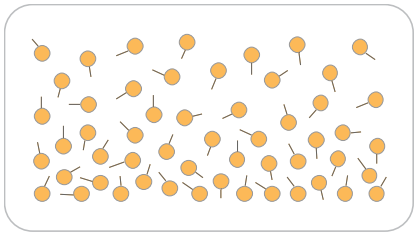


Figure 105 Resuspending DNA

Estimated Robot Run Time

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

Reagents

Illumina-Supplied

Per 96 samples:

- RA1 (9 ml)

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

Setup

- RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20–25°C water bath. Gently mix to dissolve any crystals that may be present.
- Preheat oven to 48°C.
- Preheat the heat sealer. Allow 20 minutes.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - RA1 bottle barcode(s)

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 106 throughout this protocol. Note that all of the barcodes face to the right.

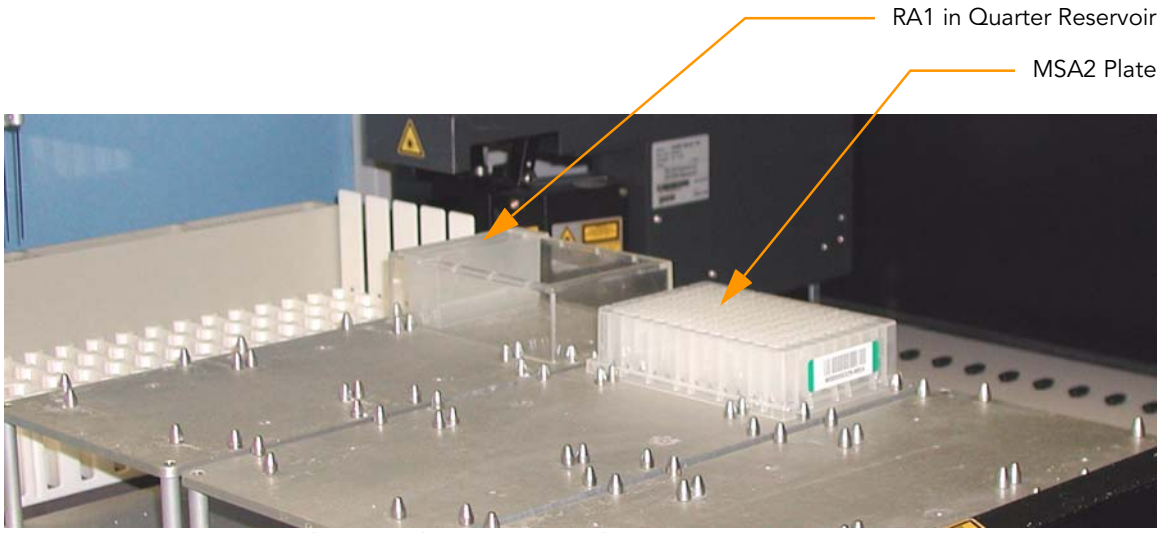


Figure 106 Tecan Eight-Tip Robot (Resuspend MSA2 Setup)

Resuspend MSA2

1. If you stored the MSA2 plate at -20°C, thaw it to room temperature. Remove the cap mat and discard it.
2. At the robot PC, select **MSA2 Sample Prep Tasks | Resuspend MSA2**.
3. In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect the number of DNAs being processed.

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.

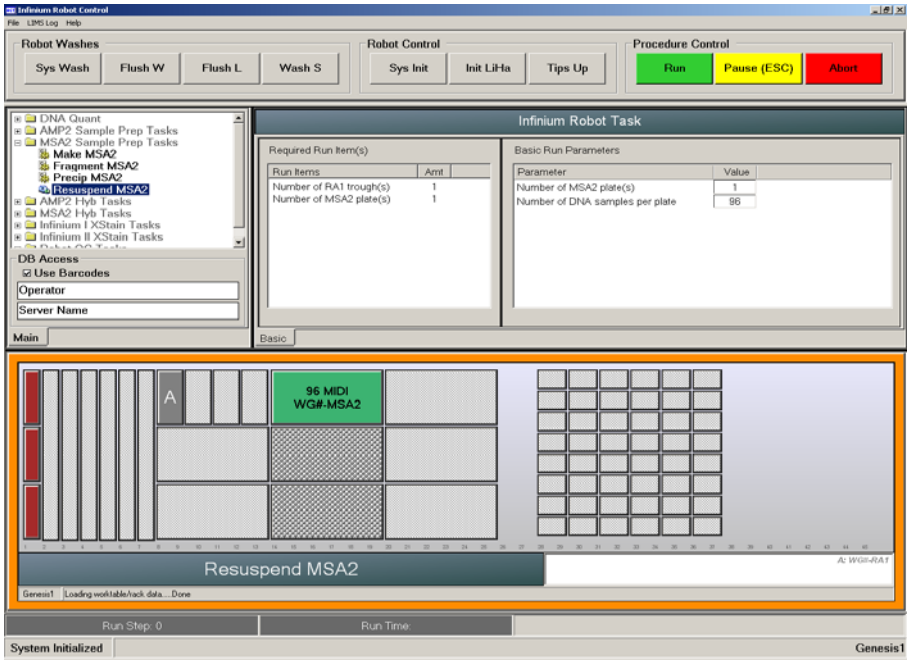


Figure 107 Resuspend MSA2 Screen

4. Place the MSA2 plate on the robot bed according to the bed map (Figure 107).
5. Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add RA1 as follows:
   - 4.5 ml for 48 samples
   - 9 ml for 96 samples
6. In the lab tracking worksheet, record the plate positions on the robot bed.
7. Make sure that all plates and tubes 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. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. 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 MSA2 plate from the robot bed.
3. Apply a foil seal to the MSA2 plate by firmly holding the heat sealer block down for 5 seconds.

4. Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
5. In the lab tracking worksheet, record the start and stop times.
6. Vortex the plate at 1800 rpm for 1 minute.
7. Pulse centrifuge to 280 xg.

If you stored the pellets at -20°C for extended periods of time after the Precip MSA2 process, you may need to repeat steps 4 through 7 until the pellets are completely resuspended.

8. Discard unused reagents in accordance with facility standards.
9. Do one of the following:
   - Proceed to *Hyb Multi BC2* on page 115. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
   - If you do not plan to proceed to the next step immediately, store the sealed MSA2 plate at -20°C (-80°C if storing for more than 24 hours). Store RA1 at -20°C.

This is a good stopping point in the process.

Hyb Multi BC2

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

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

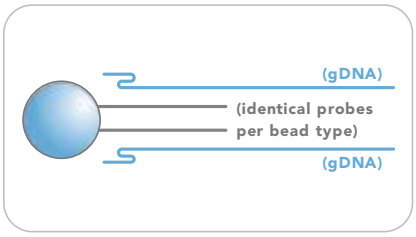


Figure 108 Hybridizing DNA to BeadChip

Estimated Robot Run Time

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

Reagents

Per 2 Hyb Chambers:

- PB2 (1 tube)

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- Preheat the heat block to 95 °C.
- Preheat the Illumina Hybridization Oven to 48°C.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - PB2 tube barcode

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Assemble the Hyb Chambers

1. Place the following items on the bench top (Figure 109):
   - BeadChip Hyb Chambers (2)
   - Hyb Chamber Gaskets (2)
   - Robot BeadChip Alignment Fixtures (4)
   - BeadChip Hyb Chamber Inserts (8)

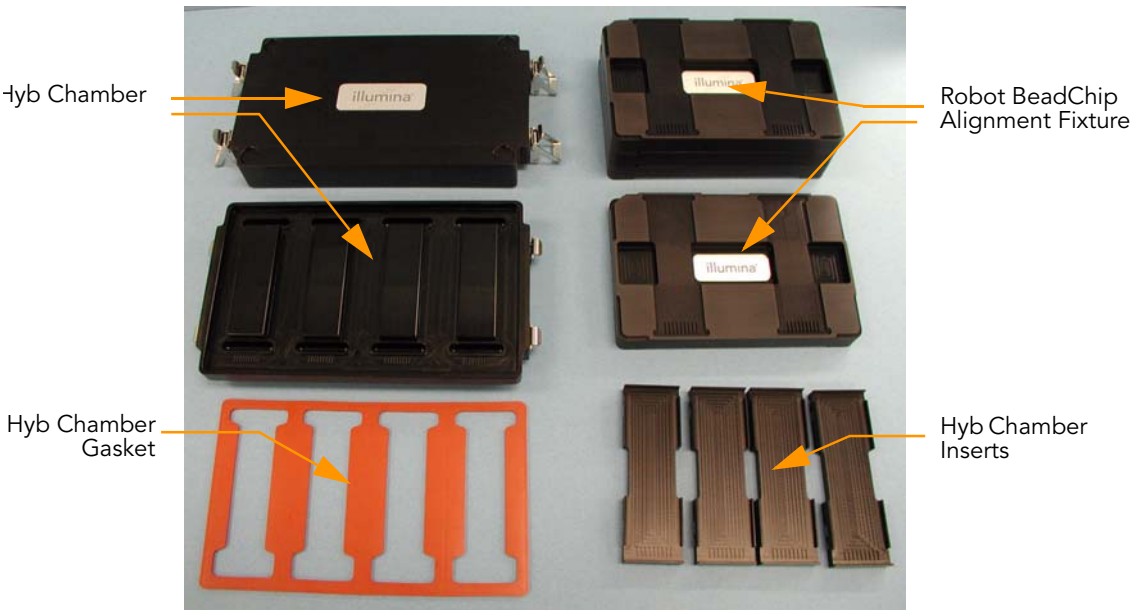


Figure 109 BeadChip Hyb Cartridge Components

2. Place the BeadChip Hyb Chamber gaskets 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 110).

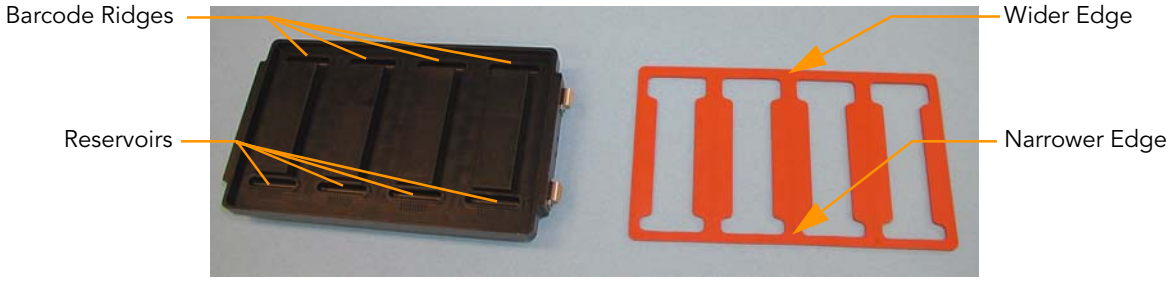


Figure 110 Hyb Chamber and Gasket

   b. Lay the gasket into the Hyb Chamber (Figure 111), and then press it down all around.

Figure 111 Placing Gasket into Hyb Chamber

c. Make sure the Hyb Chamber gaskets are properly seated (Figure 112).

Figure 112 Hyb Chamber with Gasket in Place

3. Dispense 200 µl PB2 into the eight humidifying buffer reservoirs in the Hyb Chambers (Figure 113).

Figure 113 Dispensing PB2 into Hyb Chamber Reservoirs

4. Close and lock the BeadChip Hyb Chamber lid (Figure 114).
   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 114 Sealing the Hyb Chamber

5. Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample (Figure 114).

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 II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

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

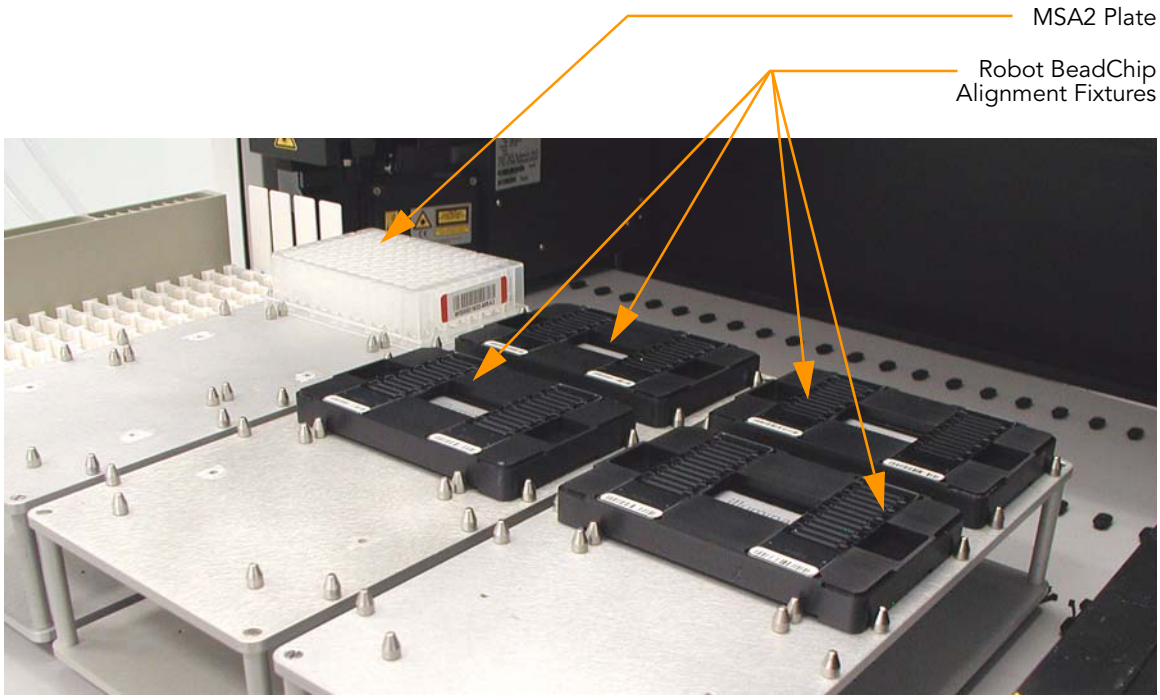


Figure 115 Placing Alignment Fixtures and MSA2 Plate onto Robot Bed

Verify MSA2 and BeadChips for Hyb (LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Confirm BeadChips for Hyb**.
2. Scan the barcode of the MSA2 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**.

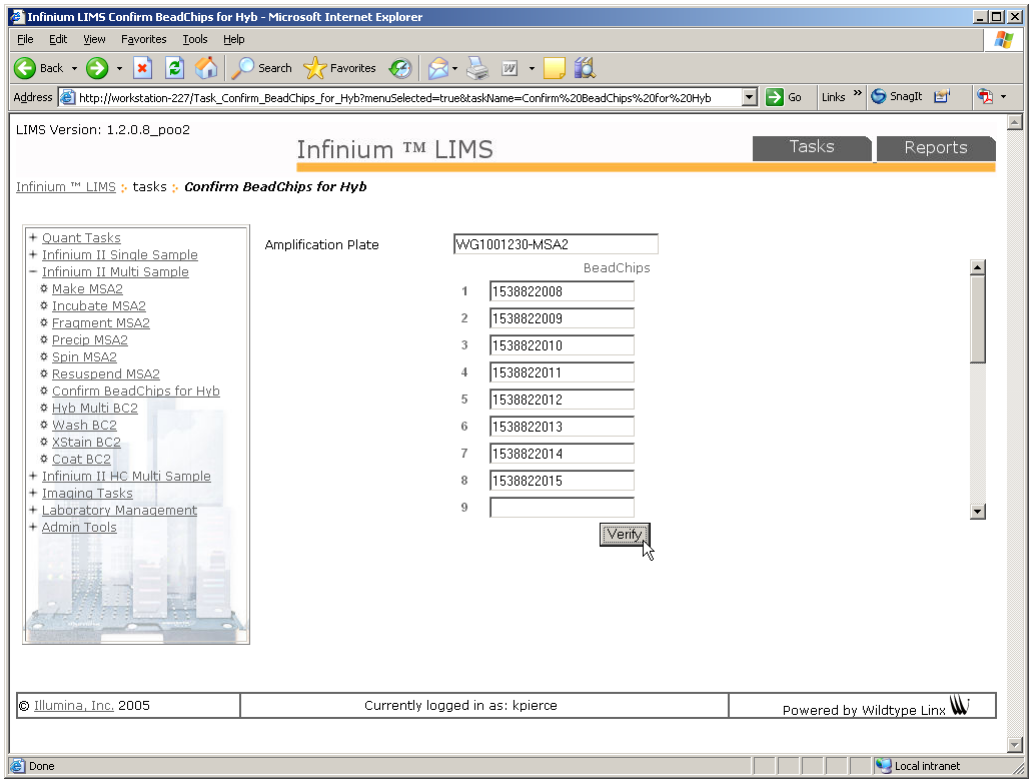


Figure 116 (Infinium LIMS) Verifying MSA2 and BeadChips for Hyb

5. If the MSA2 plate and BeadChips are queued for hybridization, a blue confirmation message appears at the top of the window. Proceed to *Load BeadChips*.

   If the MSA2 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 Reports | Get Queue Status**.
   c. Scan the plate barcode and click **Go**.
   d. If the plate is queued for another step, proceed with that step.

7. If one of the BeadChips is not accessioned into the system, accession it and then repeat the verification step.

8. If one of the BeadChips is not the right type for this batch, accession one that is the right type and repeat the verification step.

9. When the verification is successful, proceed to *Load BeadChips*.

For information about how to use Infinium LIMS, see the *Infinium II LIMS User Guide* (Illumina part # 11217344).

Load BeadChips

1. Place the resuspended MSA2 plate on the heat block to denature the samples at 95°C for 20 minutes.

CAUTION

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

2. Remove all BeadChips from their packages.
3. Place BeadChips into the Robot BeadChip Alignment Fixtures with the barcode end aligned to the ridges on the fixture (Figure 117).

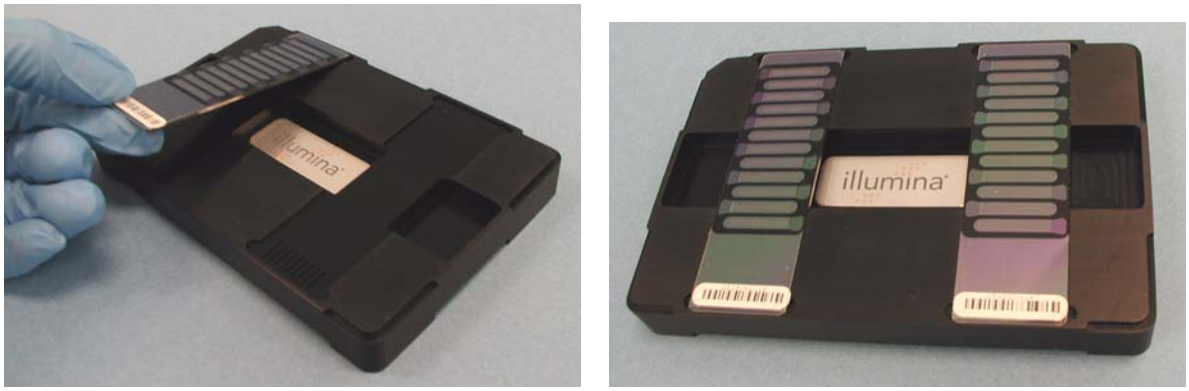


Figure 117 Placing BeadChips in Robot BeadChip Alignment Fixture

4. Place the appropriate number of BeadChips per sample number (4 BeadChips for 48 samples, 8 BeadChips for 96 samples) into the Robot BeadChip Alignment Fixtures.
5. Stack the Robot BeadChip Alignment Fixtures (Figure 118) and carry them to the robot.

Figure 118 Four Stacked Robot BeadChip Alignment Fixtures

6. At the robot PC, select **MSA2 Hyb Tasks | Hyb Multi BC2**.
7. In the Basic Run Parameters pane, change the value for the **Number of DNA Samples** to reflect the number of DNAs being processed.

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

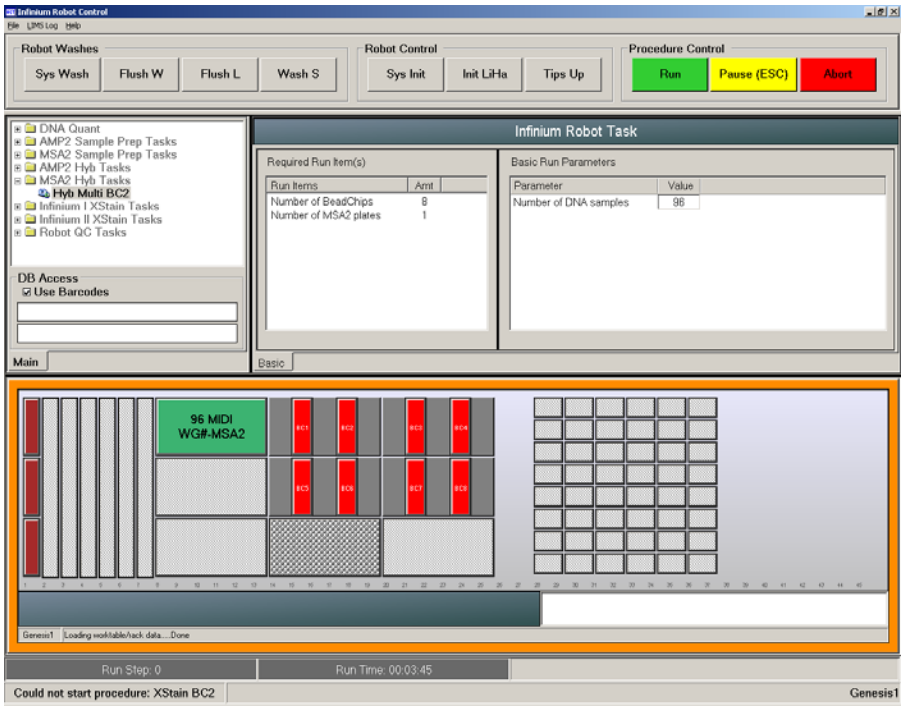


Figure 119 Hyb Multi BC2 Screen

8. Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map (Figure 119).
9. On the lab tracking worksheet, record the plate position on the robot bed.
10. Pulse centrifuge the MSA2 plate to 280 xg.
11. Place the plate onto the robot bed according to the bed map. Remove the foil seal.

Start the Robot

1. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. Observe the robot start to run to ensure that there are no problems.

   The robot dispenses the sample onto the BeadChips, following the lay-out shown in the lab tracking worksheet. The robot PC sounds an alert and displays a message when the process is complete.
2. Click **OK** in the message box.
3. 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.

4. Apply a foil seal to the MSA2 plate by firmly holding the heat sealer block down for 5 seconds.
5. Store MSA2 plate at -20°C. Store at -80°C if you do not plan to use it again within 24 hours.
6. Continue on to *Hyb Multi BC2 Setup* on page 123.

Hyb Multi BC2 Setup

1. Ensure the Illumina Hybridization Oven is set to 48°C.
2. Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
3. Remove slides from the Robot BeadChip Alignment Fixtures and place them carefully into the Hyb Chamber inserts, matching the barcode end to the ridges indicated on the insert fixture (Figure 120).

Figure 120 Matching the Barcode End to the Insert Fixture

4. Load the Hyb Chamber inserts containing the BeadChips inside the Illumina Hyb Chambers as shown (Figure 121). Position the barcode over the ridges.

Figure 121 Placing Hyb Chamber Inserts into Hyb Chamber

5. Ensure Hyb Chamber inserts are seated properly.
6. Position the lid onto the Hyb Chamber by applying the backside of the lid first and then slowly bringing down the front end to avoid dislodging the Hyb Chamber inserts (Figure 122).

Figure 122 Seating Lid onto Hyb Chamber

7. Secure the lid by closing down the clamps on both sides of the Hyb Chamber.
8. Place the Hyb Chambers into the Illumina Hybridization Oven at 48°C.
9. Start the rocker (optional).
10. Incubate at 48°C for at least 16 hours but no more than 24 hours.
11. On the lab tracking worksheet, record the start and stop times.
12. Place RA1 into the freezer at 20°C for use the next day.
13. Prepare the XC4 reagent for the XStain Procedure (*Single-Base Extension and Stain BC2* on page 134).
14. Proceed to *Wash BC2* on page 125.

Wash BC2

In this process, the BeadChips are prepared for the XStain BC2 process. Coverseals are removed from BeadChips and the BeadChips are washed in WB1 reagent followed by PB1 reagent. BeadChips are then assembled into Flow-Through Chambers under the PB1 buffer.

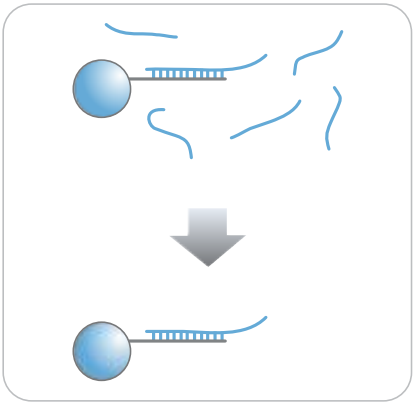


Figure 123 Washing BeadChip

Estimated Time

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

Reagents

Illumina-Supplied

- WB1 (bottle)
- PB1 (350 ml)
- Multi-Sample BeadChip Alignment Fixture
- Te-Flow Flow-Through Chambers (with black frames, spacers, glass back plates, and clamps)
- Wash dish (2)
- Wash rack

Setup

- Have ready on the lab bench:
  - Two wash dishes:
    - *One containing 200 ml WB1, and labeled as such*
    - *One containing 200 ml PB1, and labeled as such*
  - Multi-Sample BeadChip Alignment Fixture
    - *Using a graduated cylinder, fill with 150 ml PB1*
  - Te-Flow Flow-Through Chamber components:
    - *Black frames*
    - *Spacers (separated for ease of handling)*
    - *Clean glass back plates*
    - *Clamps*
- On the lab tracking worksheet, record:
  - Date/Time

- Operator
- WB1 bottle barcode
- PB1 bottle barcode

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

Verify Reagents and BeadChips for Washing (LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Wash BC2**.
2. Scan the barcode(s) of the PB1.
3. Scan the barcode(s) of the WB1.
4. Scan the BeadChip barcodes.
5. Click **Verify**.

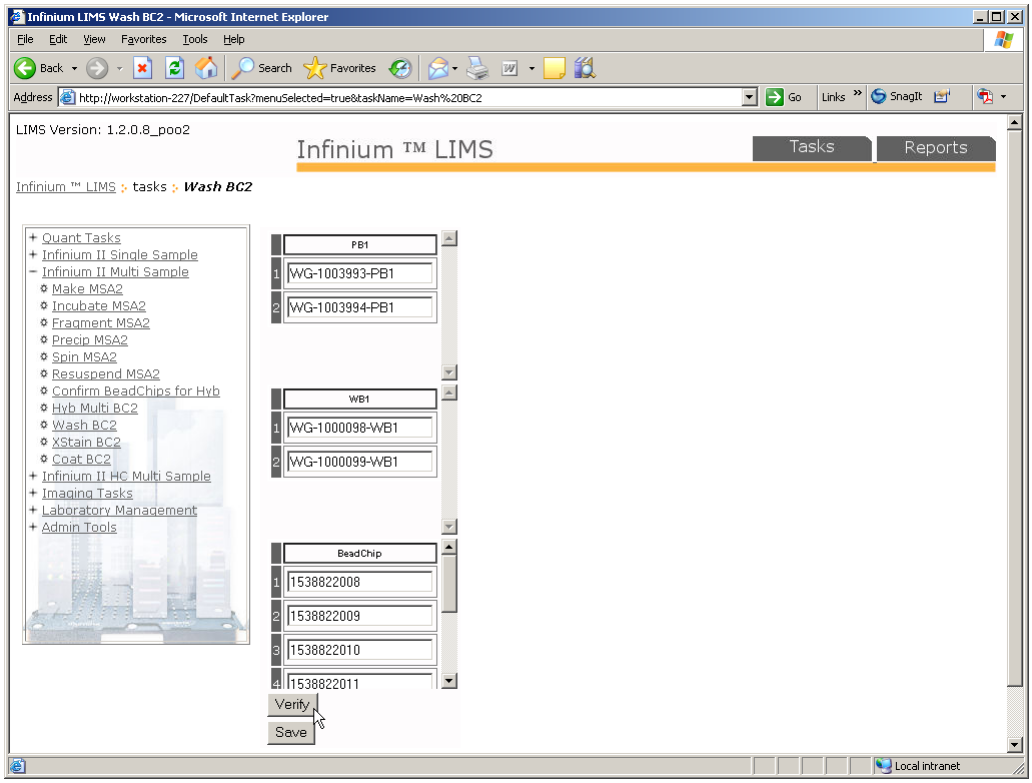


Figure 124 (Infinium LIMS) Verify Reagents and BeadChips for Washing

6. If the reagents are correct and the BeadChips are queued for washing, a blue confirmation message appears at the top of the window. Proceed to *Wash BC2*.
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 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 Reports | 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 II LIMS User Guide* (Illumina part # 11217344).

Wash BC2

1. Attach the wire handle to the rack and submerge the wash rack in the wash dish containing 200 ml WB1 (Figure 125).
2. Remove the Hyb Chamber inserts from the Hyb Chambers.
3. Remove BeadChips from the Hyb Chamber inserts.

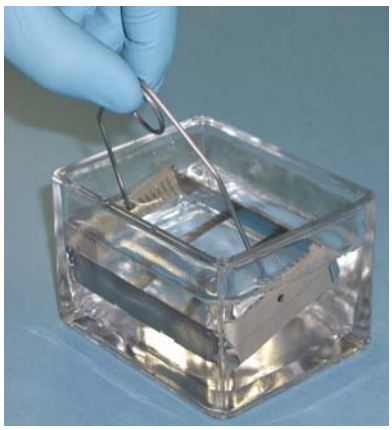


Figure 125 Wash Rack in Wash Dish Containing WB1

4. Remove the coverseal from the BeadChip as follows:
   a. Using powder-free, gloved hands, hold the BeadChip in one hand between thumb and forefinger, with the front side of BeadChip facing away from you.
   b. Remove entire seal by pulling it off in a downward direction, starting wit the barcode end (Figure 126).
   c. Discard the coverseal.

NOTE

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

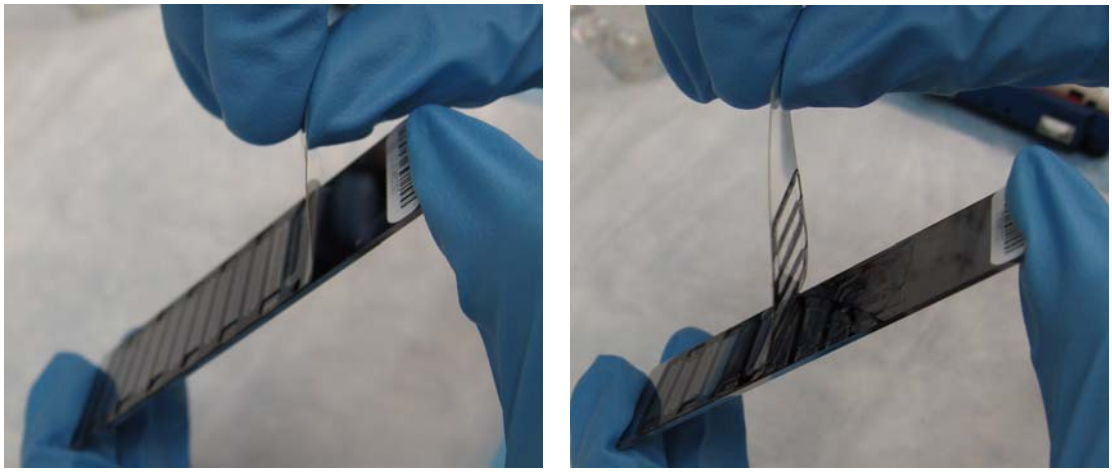


Figure 126 Removing the Coverseal

CAUTION

Do not touch the arrays!

5. Carefully slide the BeadChips into the wash rack, making sure that they are completely submerged in the WB1 (Figure 127).

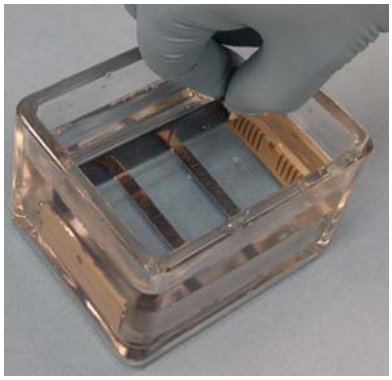


Figure 127 Placing BeadChips in Wash Dish Containing WB1

WARNING

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

6. Repeat steps 4 and 5 for each BeadChip to be processed.
7. Once all BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the WB1 with gentle, slow agitation.

8. Remove the wash rack from the wash dish containing WB#-WB1, and place it immediately into to the wash dish containing PB1. Make sure the BeadChips are completely submerged.
9. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

Assemble Flow-Through Chamber

1. For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture (Figure 128) pre-filled with PB1 (see *Setup* on page 125).

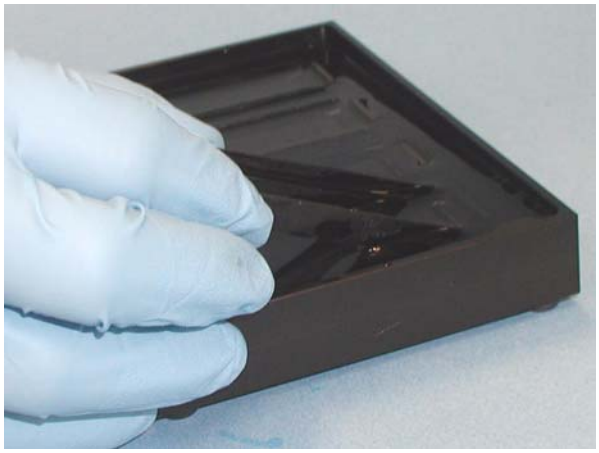


Figure 128 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

2. Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture (Figure 129).

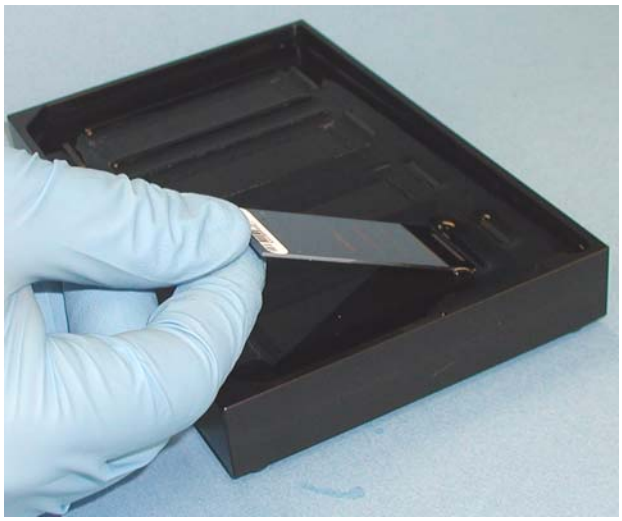


Figure 129 Placing BeadChip into Black Frame on Alignment Fixture

3. Place a clear spacer onto the top of each BeadChip to be processed (Figure 130). 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.

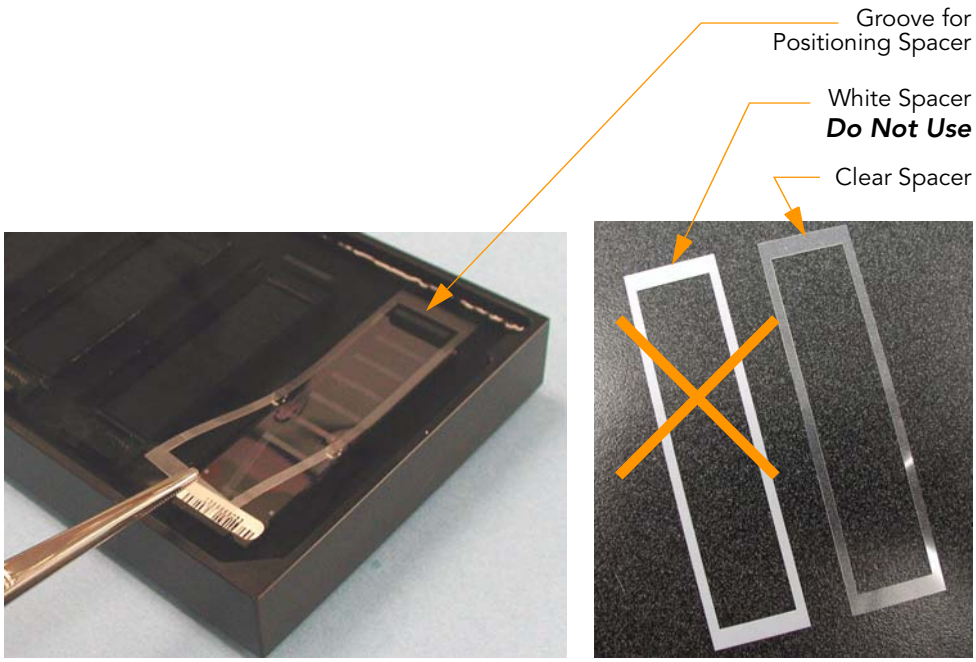


Figure 130 Placing Clear Plastic Spacer onto BeadChip

4. Place the Alignment Bar onto the Alignment Fixture (Figure 131).

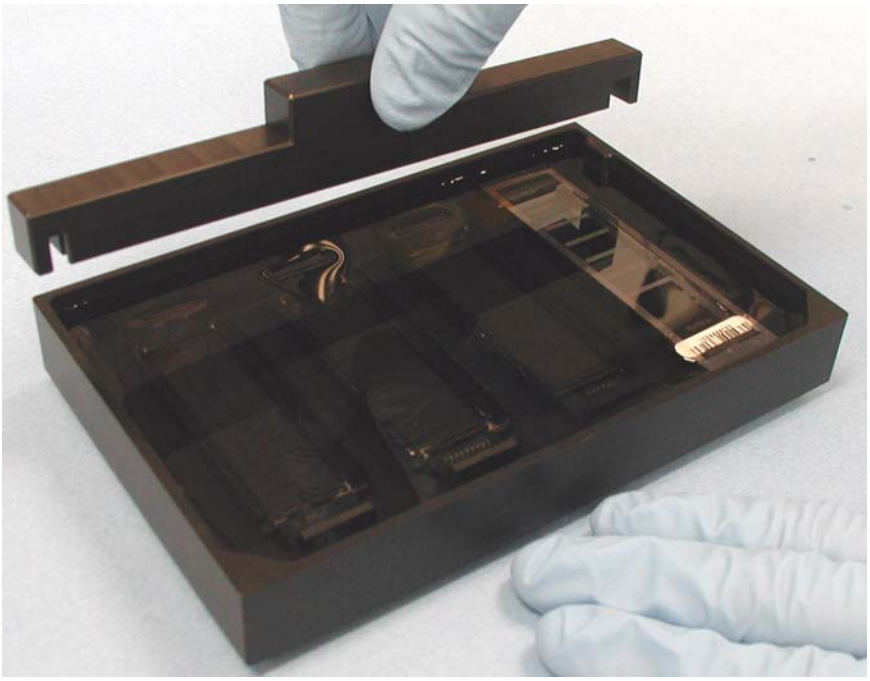


Figure 131 Placing Alignment Bar onto Alignment Fixture

5. Use a Whoosh duster or laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.
6. 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 132).

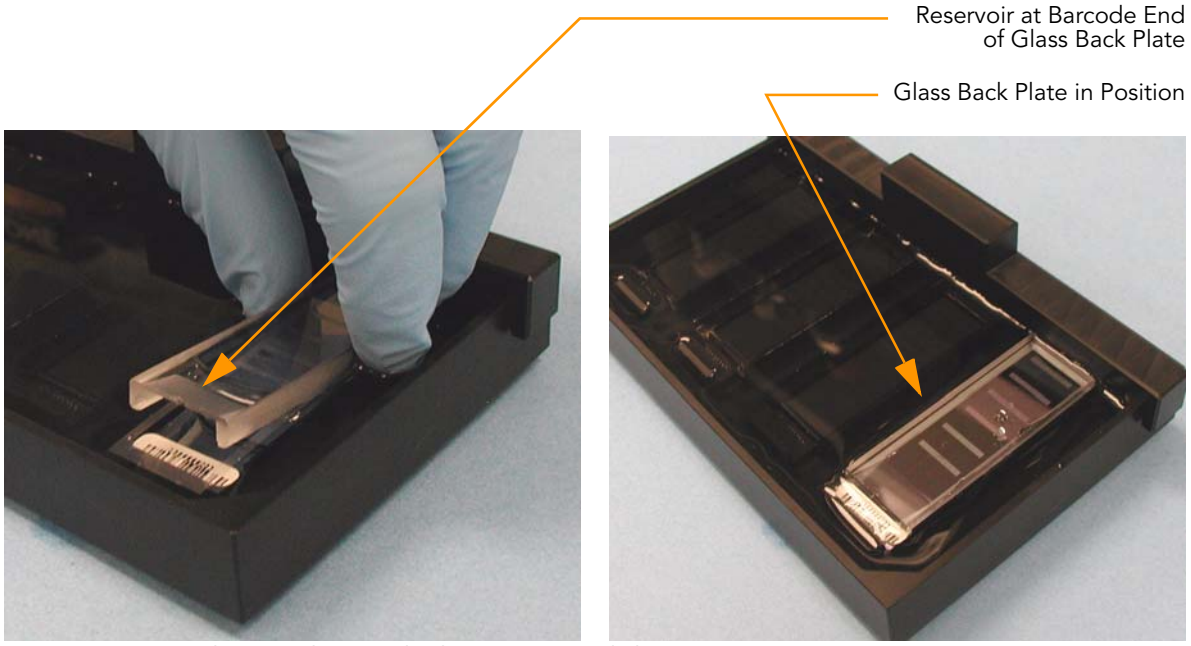


Figure 132 Placing Glass Back Plate onto BeadChip

7. Attach the metal clamps to the Flow-Through Chambers as follows (Figure 133):
   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 one stripe shows between it and the Alignment Bar.
   c. Place the second metal clamp around the Flow-Through Chamber so that no stripes show between the clamp and the barcode.

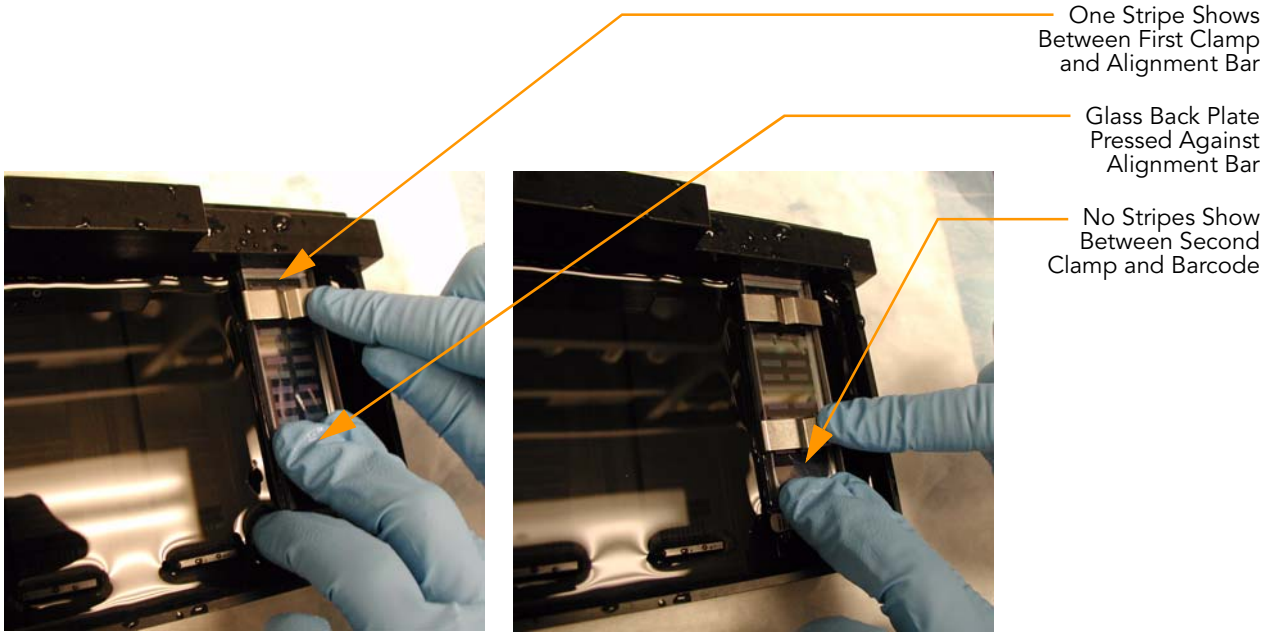


Figure 133 Securing Flow-Through Chamber Assembly with Metal Clamps

8. Using scissors, trim the ends of the clear plastic spacers from the Flow-Through Chamber assembly (Figure 134):
   a. Trim spacer ends at the non-barcode end of the assembly.
   b. On the barcode end of the assembly, slip scissors up over the barcode to trim spacer ends.

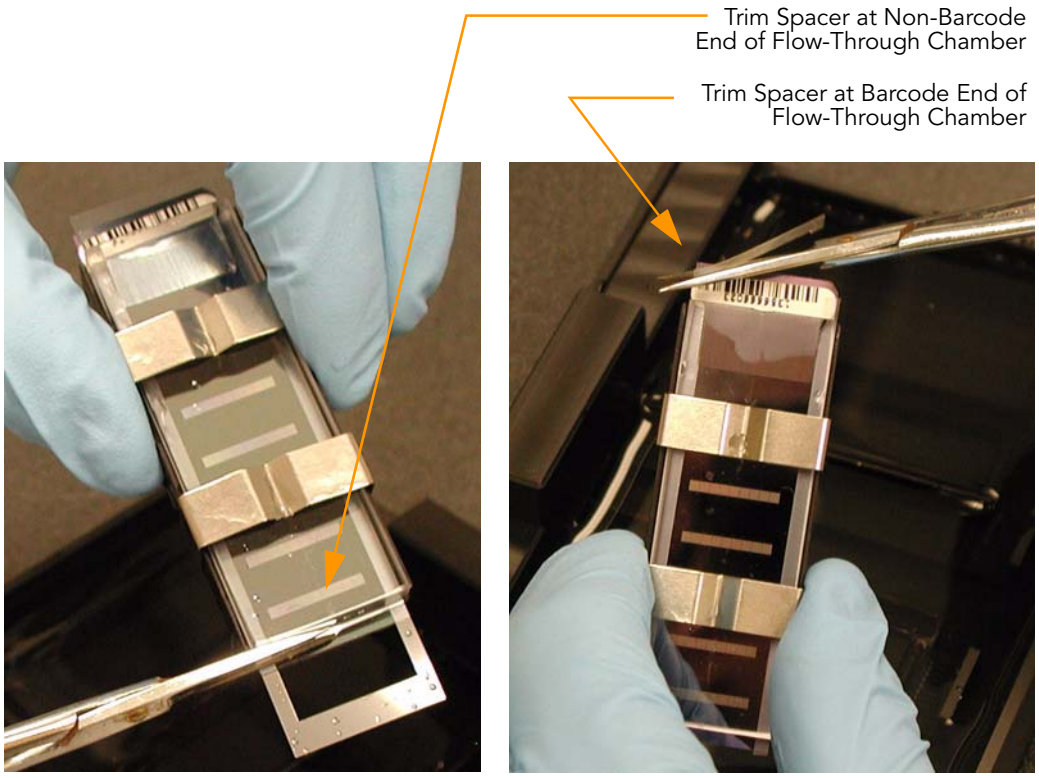


Figure 134 Trimming Spacer Ends from Flow-Through Chamber Assembly

9. If you are using Infinium LIMS:
   a. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Wash BC2**.
   b. Scan the reagent barcodes and the BeadChip barcodes. Click **Save**. Infinium LIMS records the data and queues the BeadChips for the next step, *XStain BC2*.
10. Discard unused reagents in accordance with facility standards.
11. Proceed to *Single-Base Extension and Stain BC2* on page 134.

Single-Base Extension and Stain BC2

NOTE

If you are using HumanNS-12 BeadChips, do not use this procedure. Instead, follow the procedures in *Allele-Specific Extension & Stain BeadChip* on page 160.

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

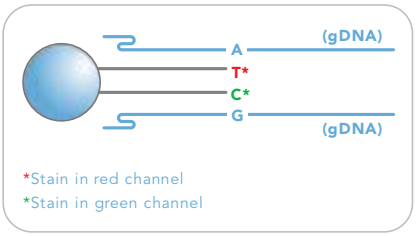


Figure 135 Extending and Staining BeadChip

Estimated Robot Run Time

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

Reagents

User-Supplied

Per 8 BeadChips:

- 95% formamide/1 mM EDTA (15 ml)

Illumina-Supplied

Per 8 BeadChips:

- RA1 (10 ml) (See *Setup* for special instructions)
- XC1 (2 tubes)
- XC2 (2 tubes)
- TEM (2 tubes)
- XC3 (75 ml)
- LTM (2 tubes) (make sure that both tubes have the same stain temperature indicated on the label)
- ATM (2 tubes)
- PB1 (200 ml)

- XC4 (310 ml)

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

Setup

- RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20–25°C water bath. Gently mix to dissolve any crystals that may be present.
- Place all reagent standoff tubes to be used in the assay in a tube rack; if frozen, thaw to room temperature (22°C) and centrifuge to 3000 xg for 3 minutes.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - RA1 barcode
  - XC3 barcode
  - XC1 barcode(s)
  - XC2 barcode(s)
  - TEM barcode(s)
  - LTM barcode(s)
  - ATM barcode(s)
  - PB1 barcode
  - XC4 barcode(s)

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 (Figure 136).

   This temperature may vary depending on facility ambient conditions.

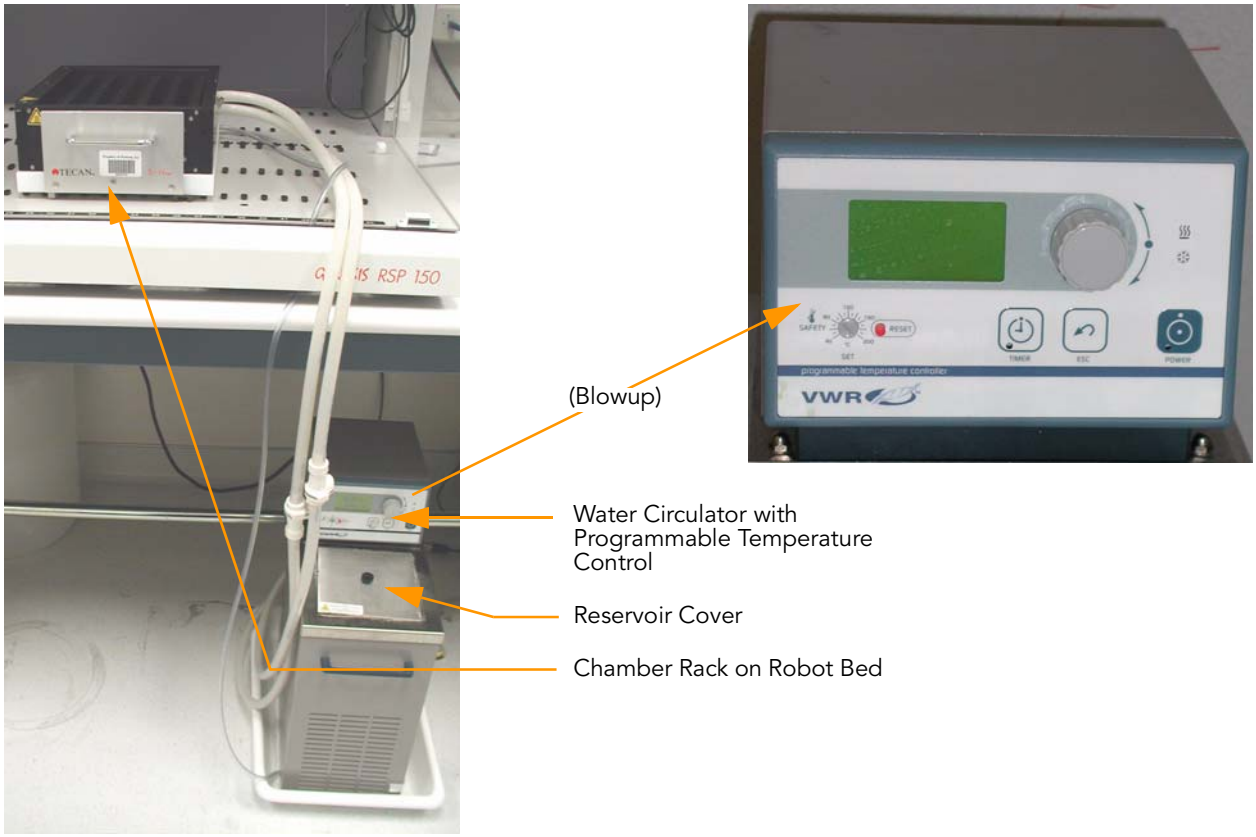


Figure 136 Water Circulator Connected to Chamber Rack

3. You must remove bubbles trapped in the Chamber Rack ***each time*** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.

4. Use the Illumina Temperature Probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 137).

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

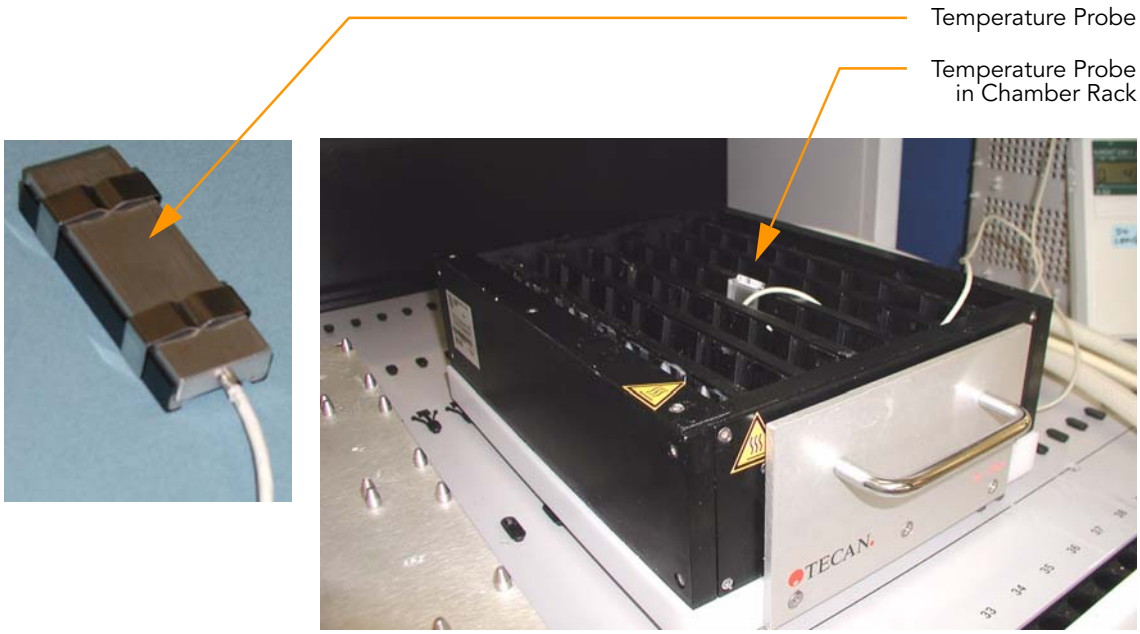


Figure 137 Illumina Temperature Probe in Chamber Rack

5. For accurate temperature measurement, ensure the Temperature Probe is touching the base of the Chamber Rack.
6. Confirm the Chamber Rack is seated in column 36 on the robot bed.
7. Slide the Chamber Rack back to ensure it is firmly seated.

Prepare the Robot

For instructions on preparing the robot for use in a protocol, and ensuring that the Chamber Rack is properly installed on the post-amplification robot bed, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 165 throughout this protocol.

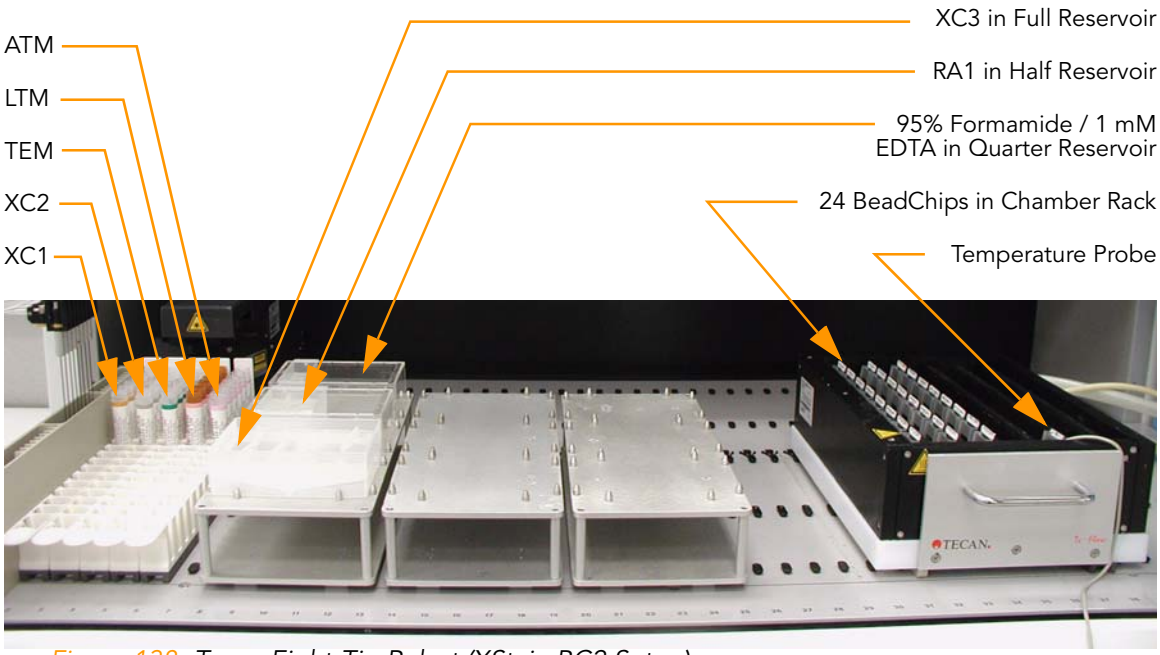


Figure 138 Tecan Eight-Tip Robot (XStain BC2 Setup)

XStain BC2

1. At the robot PC, select **Infinium II XStain Tasks | XStain BC2**.
2. In the Basic Run Parameters pane, enter the number of BeadChips.
   You can process up to 24 BeadChips in the XStain BC2 process.
   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed.

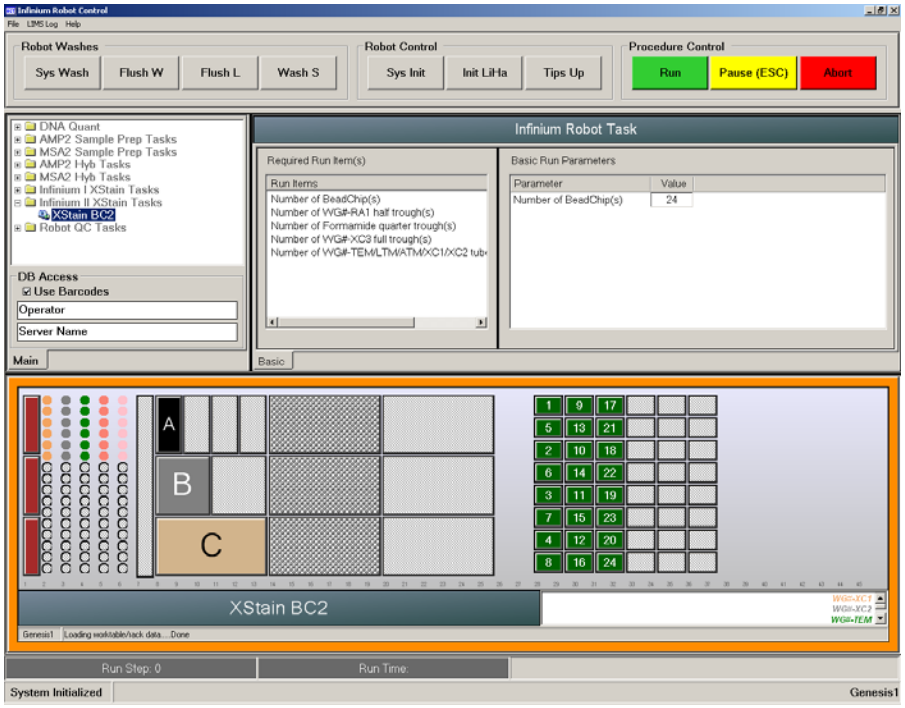


Figure 139 XStain BC2 Screen

3. Place a quarter reservoir in the reservoir frame, according to the robot bed map (Figure 166), and add 95% formamide/1 mM EDTA in the following volumes:
   - 15 ml to process 8 BeadChips
   - 17 ml to process 16 BeadChips
   - 25 ml to process 24 BeadChips
4. Place a half reservoir in the reservoir frame, according to the robot bed map, and add RA1 in the following volumes:
   - 10 ml to process 8 BeadChips
   - 20 ml to process 16 BeadChips
   - 30 ml to process 24 BeadChips
5. Place a full reservoir in the reservoir frame, according to the robot bed map, and add XC3 in the following volumes:
   - 49 ml to process 8 BeadChips
   - 97 ml to process 16 BeadChips
   - 145 ml to process 24 BeadChips
6. Place each reagent tube (XC1, XC2, TEM, LTM, ATM) in the robot tube rack according to the bed map, and remove their caps.
7. Make sure that all plates and tubes 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. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. At the prompt (Figure 140), enter the staining temperature. The correct temperature is listed on the LTM reagent label. If no temperature is listed, enter 37°C.

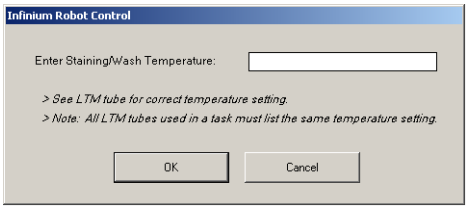


Figure 140 Entering XStain Temperature

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

2. When the prompt appears (Figure 167), wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.

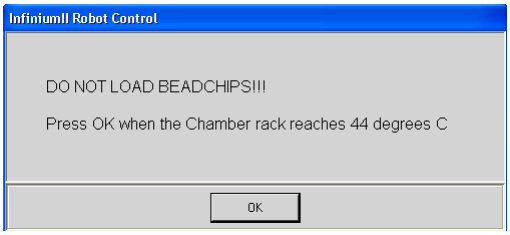


Figure 141 Adjusting Chamber Rack to 44°C Message

3. Once the temperature probe registers 44°C, click **OK** (Figure 167).
4. When prompted (Figure 168), remove the Hyb Chamber from the Illumina Hybridization Oven.

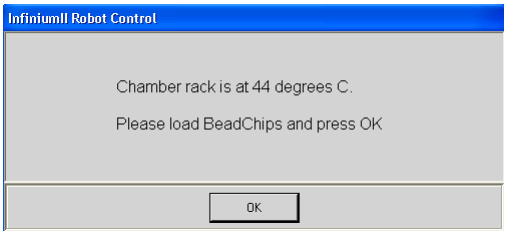


Figure 142 Load BeadChips Message

5. Place each assembled Flow-Through Chamber in the first row of the Chamber Rack. Refer to the robot bed map for the correct layout.
6. Ensure each Flow-Through Chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.
7. On the lab tracking worksheet, record the chamber rack position associated with each BeadChip.

8. ***Immediately*** wash the Hyb Chamber reservoirs with dH_2O and scrub them with a small cleaning brush.

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

9. Click **OK** (Figure 168). A series of reactions begins, each with a wait time. Message boxes on the robot PC tell you which reaction is occurring and how long the wait time is. The total wait time is 1 hour and 25 minutes.

Table 9 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	LTM	10 minutes
8	XC3	7 minutes
9	ATM	10 minutes
10	XC3	7 minutes
11	LTM	10 minutes
12	XC3	7 minutes
13	ATM	10 minutes
14	XC3	7 minutes
15	LTM	10 minutes
16	XC3	7 minutes

10. When prompted, remove the BeadChips from the Chamber Rack immediately and place them horizontally on the lab bench at room temperature (22°C). Click **OK** in the message box.

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

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

Preparing Wash Dishes and Tube Racks

Follow either the 8 BeadChips Process (*Wash and Coat 8 BeadChips* on page 144) or the 16–24 BeadChips Process (*Wash and Coat 16–24 BeadChips* on page 150).

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 diapers). 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.

Verify Reagents and BeadChips for Coating (LIMS only)

1. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Coat BC2**.
2. Scan the barcode(s) of the PB1.
3. Scan the barcode(s) of the XC4.
4. Scan the BeadChip barcodes.
5. Click **Verify**.

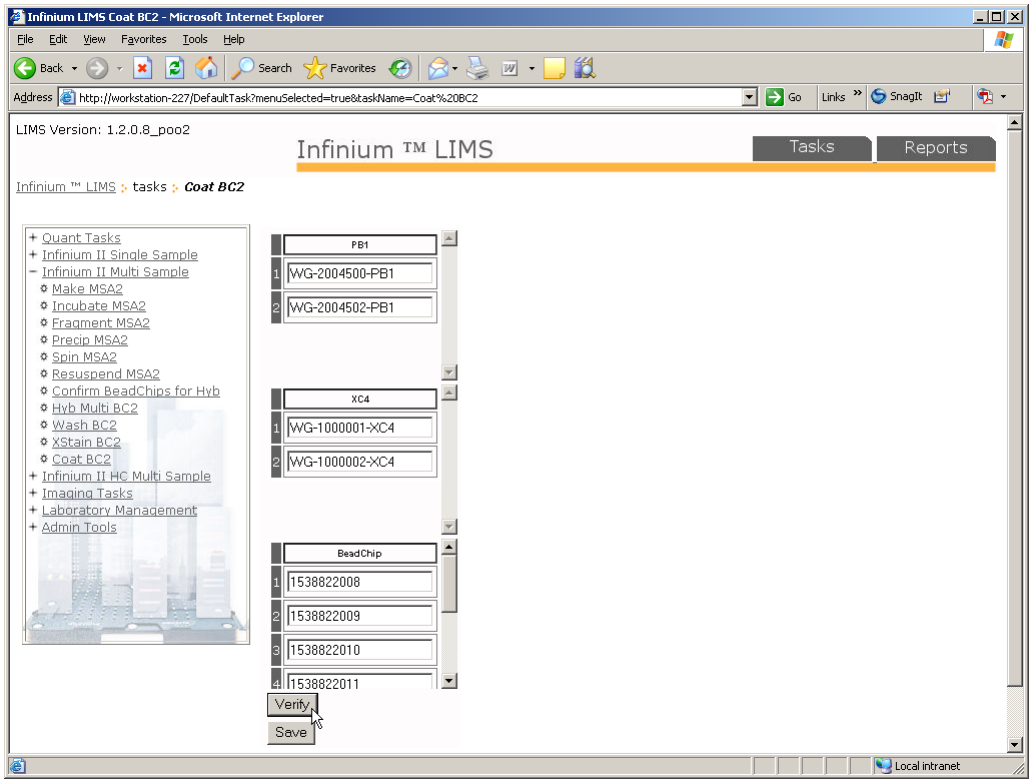


Figure 143 (Infinium LIMS) Verify Reagents and BeadChips for Coating

6. If the reagents are correct and the BeadChips are queued for coating, a blue confirmation message appears at the top of the window. Proceed to *Wash and Coat 8 BeadChips* on page 144 or *Wash and Coat 16–24 BeadChips* on page 150.
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 Reports | 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 II LIMS User Guide* (Illumina part # 11217344).

Wash and Coat 8 BeadChips

Equipment Needed

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

1. Set up two top-loading wash dishes, labeled "PB1" and "XC4" (Figure 144).
2. To indicate the fill volume before filling wash dishes with PB1 and XC4, pour 310 ml water into the wash dishes and mark the water level on the side. Empty the water from the wash dish. 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.

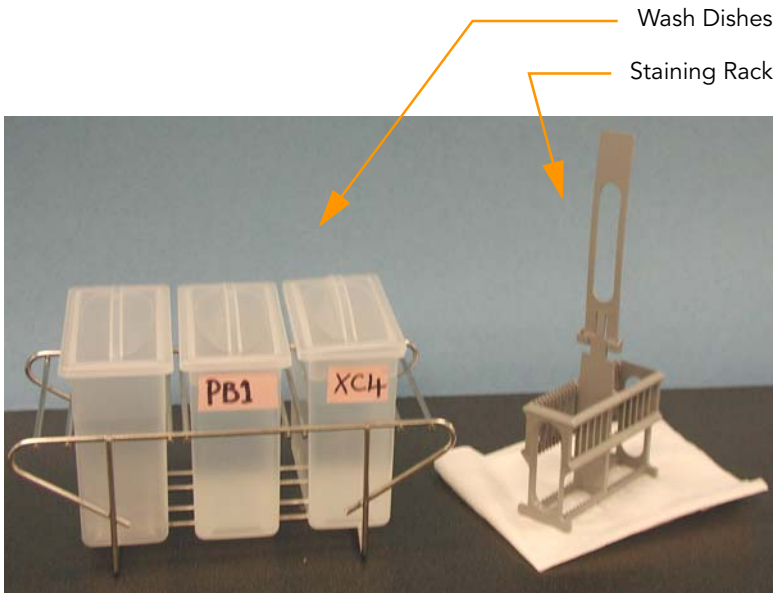


Figure 144 PB1 & XC4 Wash Dishes with BeadChip Rack

3. Pour 310 ml PB1 into the wash dish labeled "PB1."
4. Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing towards*** you (Figure 145). This orients the staining rack so that you can safely remove the BeadChips.

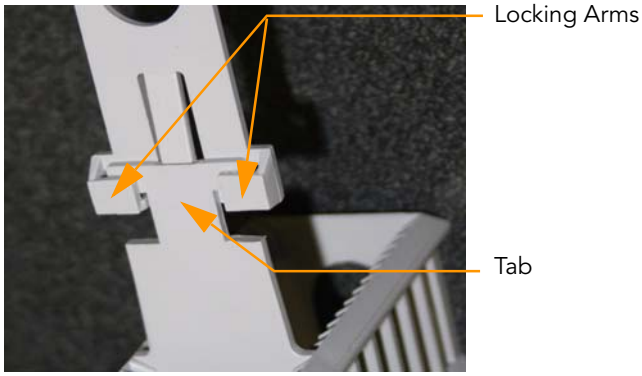


Figure 145 Staining Rack Locking Arms and Tabs

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.

5. One at a time, disassemble each Flow-Through Chamber:
   a. Using the dismantling tool, remove the two metal clamps (Figure 169).

CAUTION

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

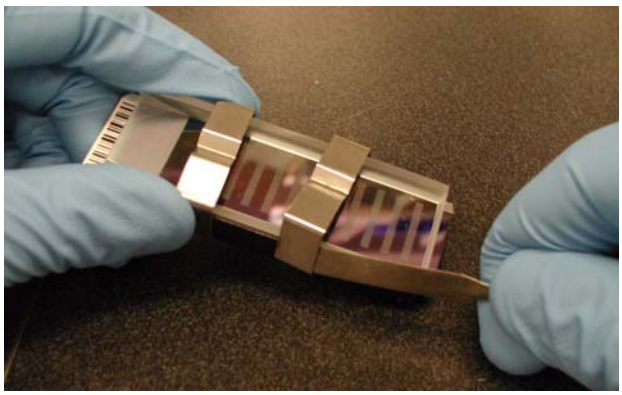


Figure 146 Removing the Metal Clamps from Flow-Through Chamber

   b. Remove the glass back plate.
   c. Set the glass back plates aside. When you finish the XStain BC2 protocol, clean the glass back plates as described in the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
   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.

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

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

7. 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.

8. Move the staining rack up and down 10 times, breaking the surface of the PB1 (Figure 170).

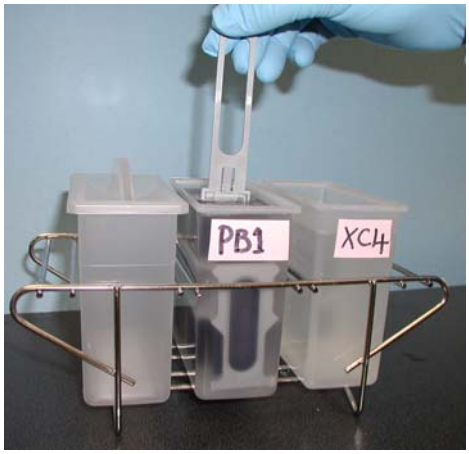


Figure 147 Washing BeadChips in PB1

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.

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

NOTE

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

10. 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.

11. Remove the staining rack from the dish containing PB1 and place it directly into the wash dish containing XC4 (Figure 148). 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.
12. Move the staining rack up and down 10 times, breaking the surface of the XC4.

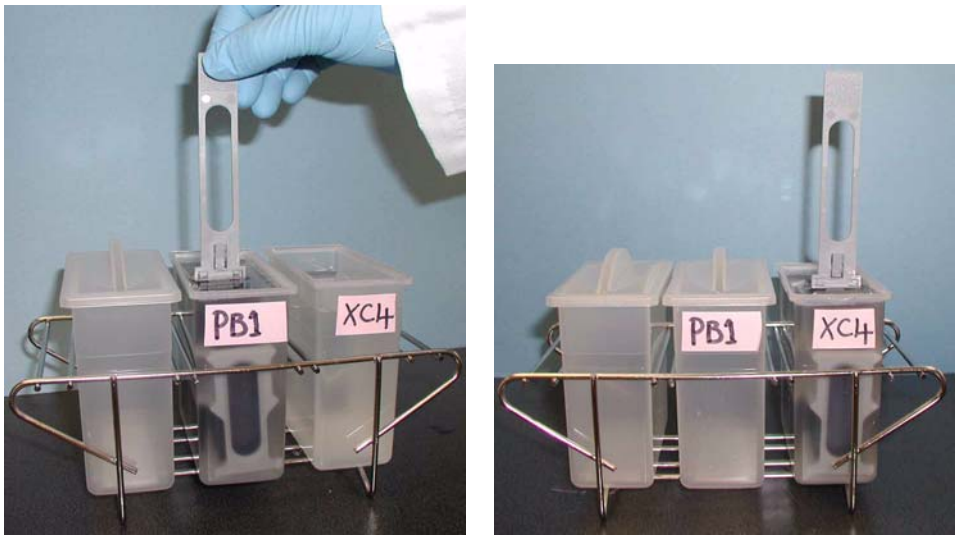


Figure 148 Moving BeadChips from PB1 to XC4

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.

13. 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.

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

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

16. Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes ***face upwards*** and the locking arms and tab ***face down*** (Figure 149).

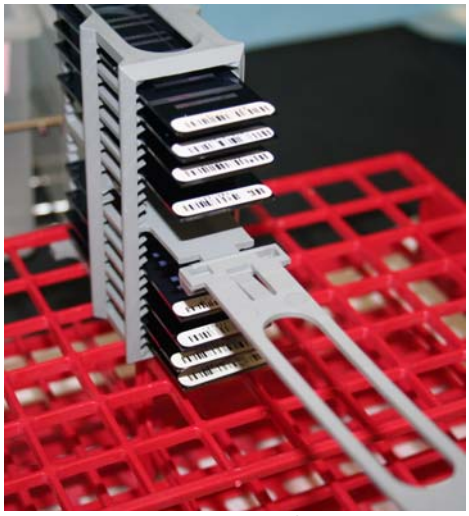


Figure 149 Staining Rack in Correct Orientation

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

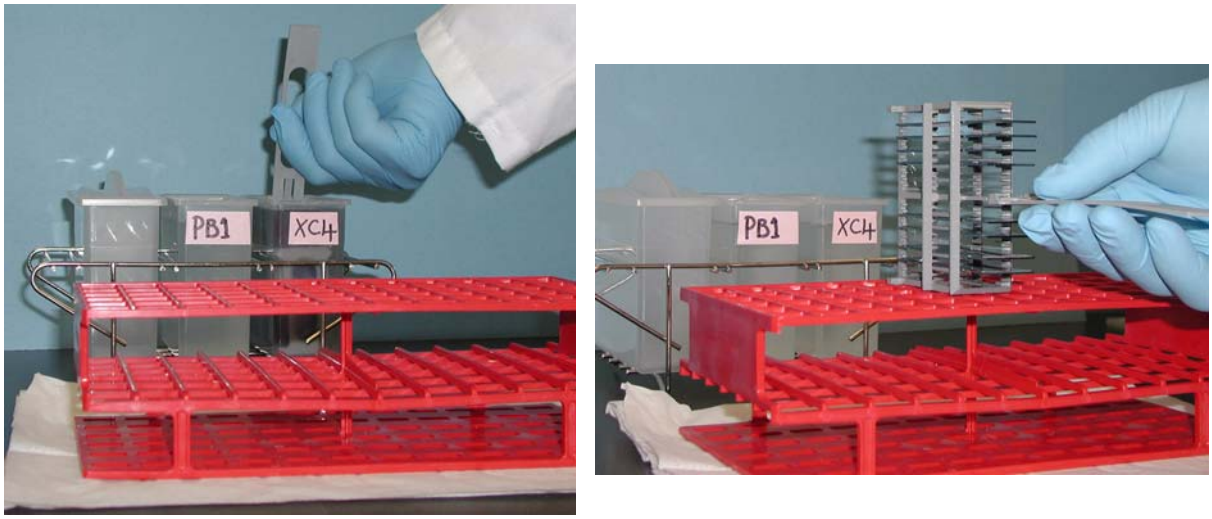


Figure 150 Moving BeadChip Carrier from XC4 to Tube Rack

18. For each of the top four BeadChips, working top to bottom:
    a. Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

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 152).

19. 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 151).

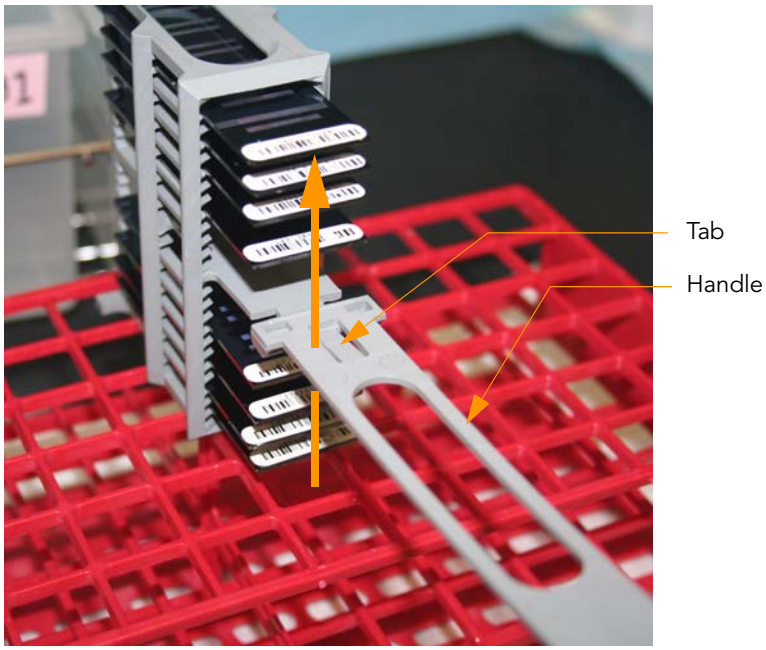


Figure 151 Removing Staining Rack Handle

20. Remove the remaining four BeadChips to the tube rack as shown in Figure 152, so that six BeadChips are on top of the rack and two BeadChips are on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.

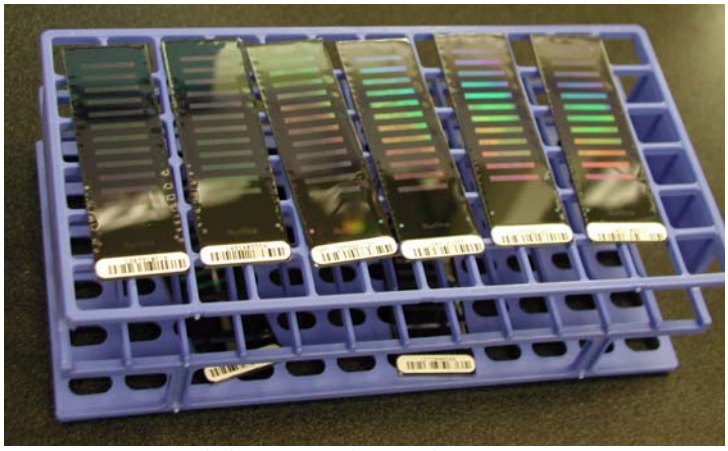


Figure 152 BeadChips on Tube Rack

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.

21. Dry the **first 8** BeadChips:

- **a.** Place the tube rack with the first 8 BeadChips (Figure 160) 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. If you are using Infinium LIMS:

- **a.** In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Coat BC2**.
- **b.** Scan the reagent barcodes and BeadChip barcodes. Click **Save**. Infinium LIMS records the data and queues the BeadChips for the next step, *Image BC2*.

23. Proceed to step 33, page 158.

Wash and Coat 16–24 BeadChips

Equipment Needed

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

1. Set up two top-loading wash dishes, labeled "PB1" and "XC4" (Figure 153).
2. 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.

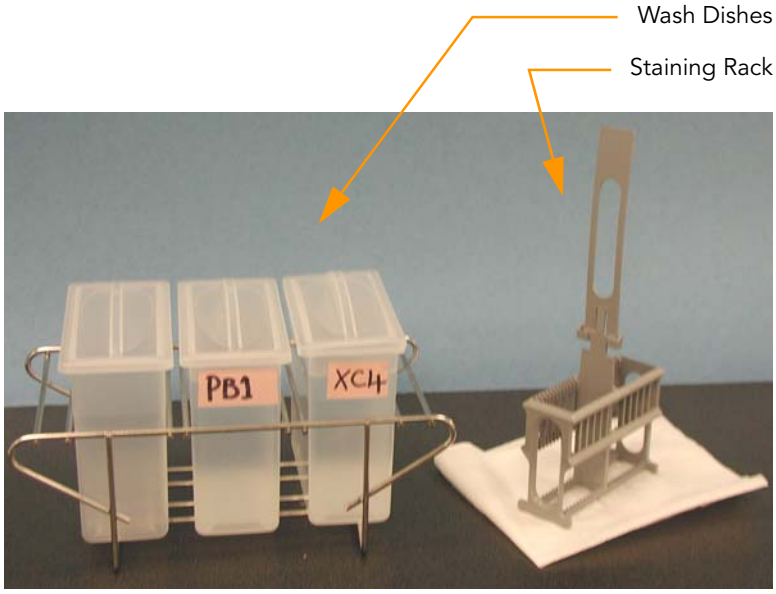


Figure 153 PB1 and XC4 Wash Dishes with Staining Rack

3. Pour 285 ml PB1 into the wash dish labeled "PB1."
4. Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing you*** (Figure 154). This orients the staining rack so that you can safely remove the BeadChips.

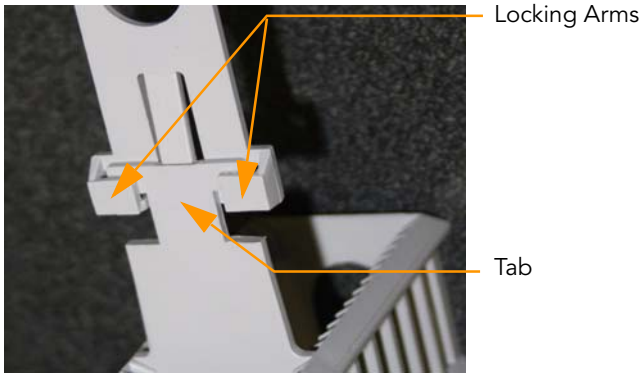


Figure 154 Staining Rack Locking Arms and Tabs

5. Let the staining rack sit in the wash dish. You will use it to carry the BeadChips after disassembling the Flow-Through Chambers.
6. Ensure the staining rack is in the proper orientation before loading BeadChips. If it is not correctly oriented, the BeadChips may be

damaged when you remove the staining rack handle before removing the BeadChips.

7. Ensure the staining rack is in the proper orientation before loading BeadChips. If it is not correctly oriented, the BeadChips may be damaged when you remove the staining rack handle before removing the BeadChips.

8. One at a time, disassemble each Flow-Through Chamber:
   a. Using the dismantling tool, remove the two metal clamps (Figure 155).

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

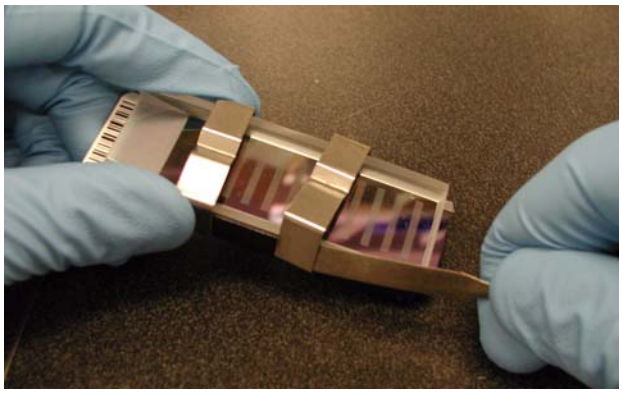


Figure 155 Removing Metal Clamps from Flow-Through Chamber

   b. Remove the glass back plate.
   c. Set the glass back plates aside. When you finish the XStain BC2 protocol, clean the glass back plates as described in the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
   d. Remove the spacer.
   e. Remove the BeadChip.

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

9. Place BeadChips in the staining rack while it is submerged in PB1.
   - For 16 BeadChips, place 8 above the handle and 8 below.
   - For 24 BeadChips, place 12 above the handle and 12 below.

   The BeadChip barcodes should ***face away*** from you, while the locking arms on the handle ***face*** you.

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

10. 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.

11. Move the staining rack up and down 10 times, breaking the surface of the PB1 (Figure 156).

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.

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

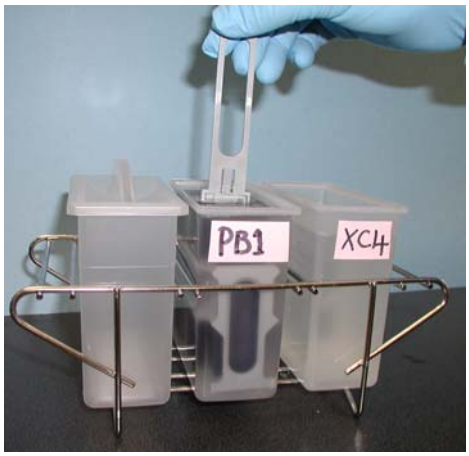


Figure 156 Washing BeadChips in PB1

NOTE

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

13. 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.

14. Remove the staining rack from the dish containing PB1 and place it directly into the wash dish containing XC4 (Figure 157). The barcode

labels on the BeadChips must ***face away*** from you, while the locking arms on the handle ***face*** you, for proper handling and coating.

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

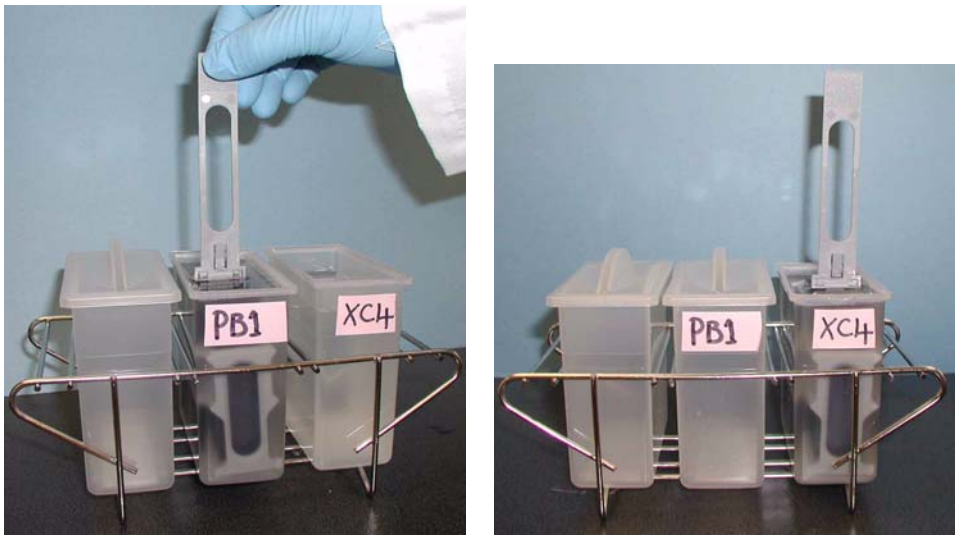


Figure 157 Moving BeadChips from PB1 to XC4

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.

16. 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.

17. Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.
18. Prepare one additional tube rack per 8 BeadChips (Illumina-provided from VWR catalog # 60916-748) that fits the internal dimensions of the vacuum desiccator.
19. 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 159).

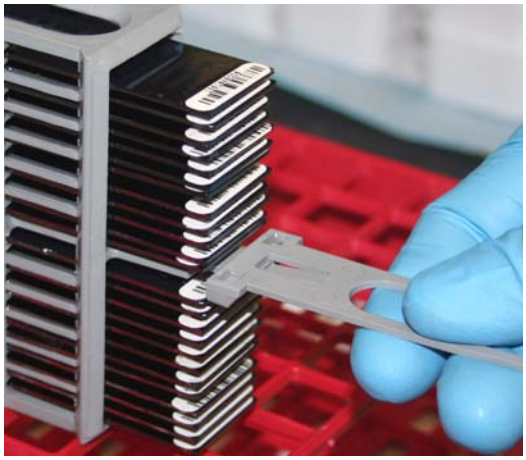
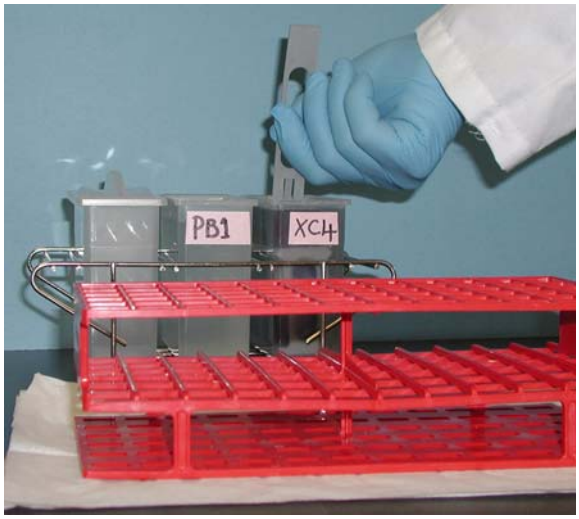


Figure 158 Staining Rack in Correct Orientation

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



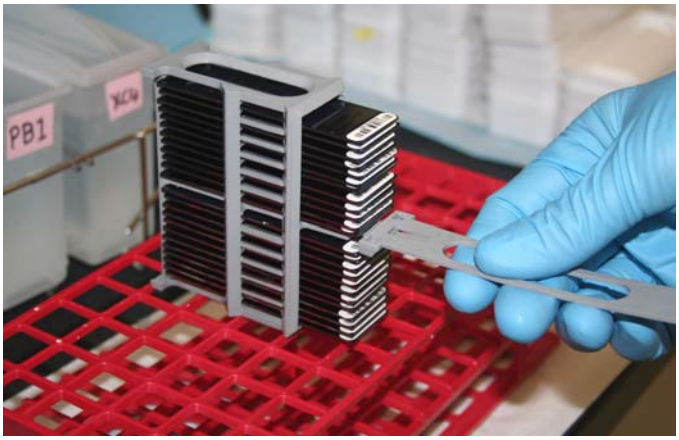


Figure 159 Moving the Staining Rack from XC4 to Tube Rack

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

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

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 160, 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.

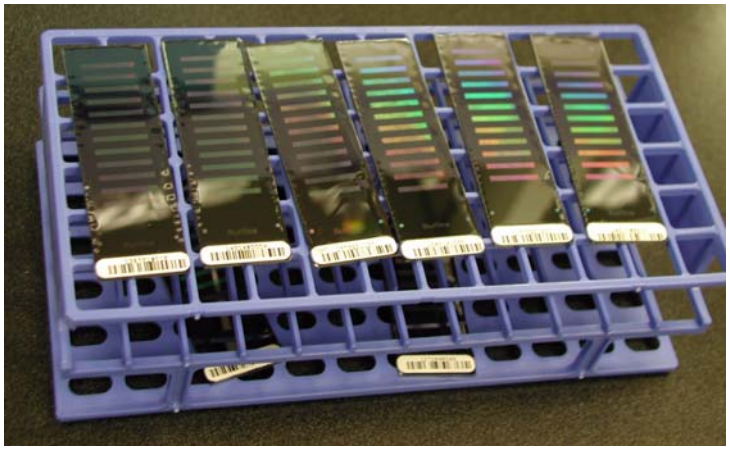


Figure 160 BeadChips on Tube Rack

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

22. Return the staining rack to the XC4 wash dish and top off wash dish until BeadChips are completely covered with remaining XC4 reagent.
23. Confirm that all BeadChips are covered with XC4 reagent, and soak the BeadChips for 10 seconds.
24. Dry the **first 8** BeadChips:
    a. Place the tube rack with the first 8 BeadChips (Figure 160) 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.
25. 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.
26. If you are processing 24 BeadChips, remove the 4 BeadChips that remain above the staining rack handle and place them on the tube rack.
27. (For both 16- and 24-BeadChip processes) Holding the top of the staining rack in position, grasp the handle between your thumb and forefinger. Push the tab up with your thumb and push the handle away from you to unlock it. Pull up the handle and remove (Figure 161).

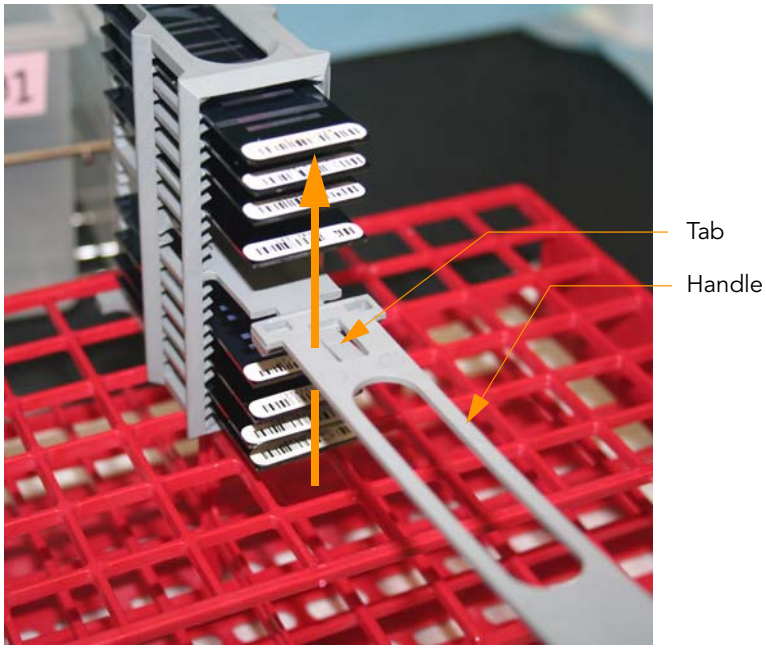


Figure 161 Removing Staining Rack Handle

28. Place BeadChips on the tube rack (Figure 160) until there are six BeadChips on top of the rack and two BeadChips on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.

    If you are processing 24 BeadChips, 8 remain in the staining rack.

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

30. Dry the **second set of 8** BeadChips:
    a. Place the tube rack with the second set of 8 BeadChips (Figure 160) 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. If you are processing 24 BeadChips:
    a. Remove staining rack with the remaining 8 BeadChips in one rapid motion from the 'XC4' wash dish and place it directly on tube rack. Ensure that the BeadChips are horizontal with the barcodes facing up.
    b. Place BeadChips on the tube rack as shown in Figure 160 until there are six BeadChips on top of the rack and two BeadChips on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.

a. Place the tube rack with the **third set of** 8 BeadChips (Figure 160) 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.

Figure 162 Testing Vacuum Seal

d. Dry under vacuum for 50–55 minutes.

32. If you are using Infinium LIMS:
    a. In the Infinium LIMS left sidebar, click **Infinium II Multi-Sample | Coat BC2**.
    b. Scan the reagent barcodes and BeadChip barcodes. Click **Save**. Infinium LIMS records the data and queues the BeadChips for the next step, *Image BC2*.
33. 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.

34. 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.
35. 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.

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

Do not touch the stripes.

37. Clean the glass back plates. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
38. Clean the Hyb Chambers:
    a. Remove the rubber gaskets from the Hyb Chambers.
    b. Rinse all Hyb Chamber components with DI water.
    c. Thoroughly rinse the eight humidifying buffer reservoirs.
39. Discard unused reagents in accordance with facility standards.

Image BC2

The BeadChips are now ready for scanning. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963). Image the BeadChips within 72 hours.

Allele-Specific Extension & Stain BeadChip

Use this procedure only for HumanNS-12 BeadChips. If you are using any other sort of BeadChip, follow the procedures in *Single-Base Extension and Stain BC2* on page 134.

Following hybridization, RA1 reagent is used to wash away unhybridized and non-specifically hybridized DNA sample. EMM 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 added to remove the hybridized DNA. After neutralization using the XB3 reagent, the labeled extended primers undergo a multi-layer staining process on the Chamber Rack. Finally, disassemble the Flow-Through Chambers, wash the BeadChips in the PB1 reagent, and then dry them.

Estimated Robot Run Time

Approximately 2.5 hours

Reagents

User-Supplied

- 95% formamide/1 mM EDTA (15 ml)

Illumina-Supplied

Per 8 BeadChips:

- RA1 (21 ml)
- XB1 (2 tubes)
- XB2 (2 tubes)
- EMM (2 tubes)
- XB3 (75 ml)
- LMM (2 tubes)
- ASM (2 tubes)
- PB1 (200 ml)

This protocol involves the use of formamide. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact with formamide. Dispose of formamide containers and any unused contents in accordance with the governmental safety standards for your region. Refer to the MSDS for formamide for complete information.

NOTE

Thaw all reagents completely at room temperature (22°C) 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.

Setup

- RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20–25°C water bath. Gently mix to dissolve any crystals that may be present.
- Place all reagent stand-off tubes to be used in the assay in a tube rack.
- On the lab tracking worksheet, record:
  - Date/Time
  - Operator
  - Robot
  - RA1 barcode
  - XB3 barcode
  - XB1 barcode(s)
  - XB2 barcode(s)
  - EMM barcode(s)
  - LMM barcode(s)
  - ASM barcode(s)
  - PB1 barcode

NOTE

You can print copies of the lab tracking worksheet from the Documentation CD you received with your system (Illumina part # 11230362).

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 (Figure 163).

   This temperature may vary depending on facility ambient conditions.

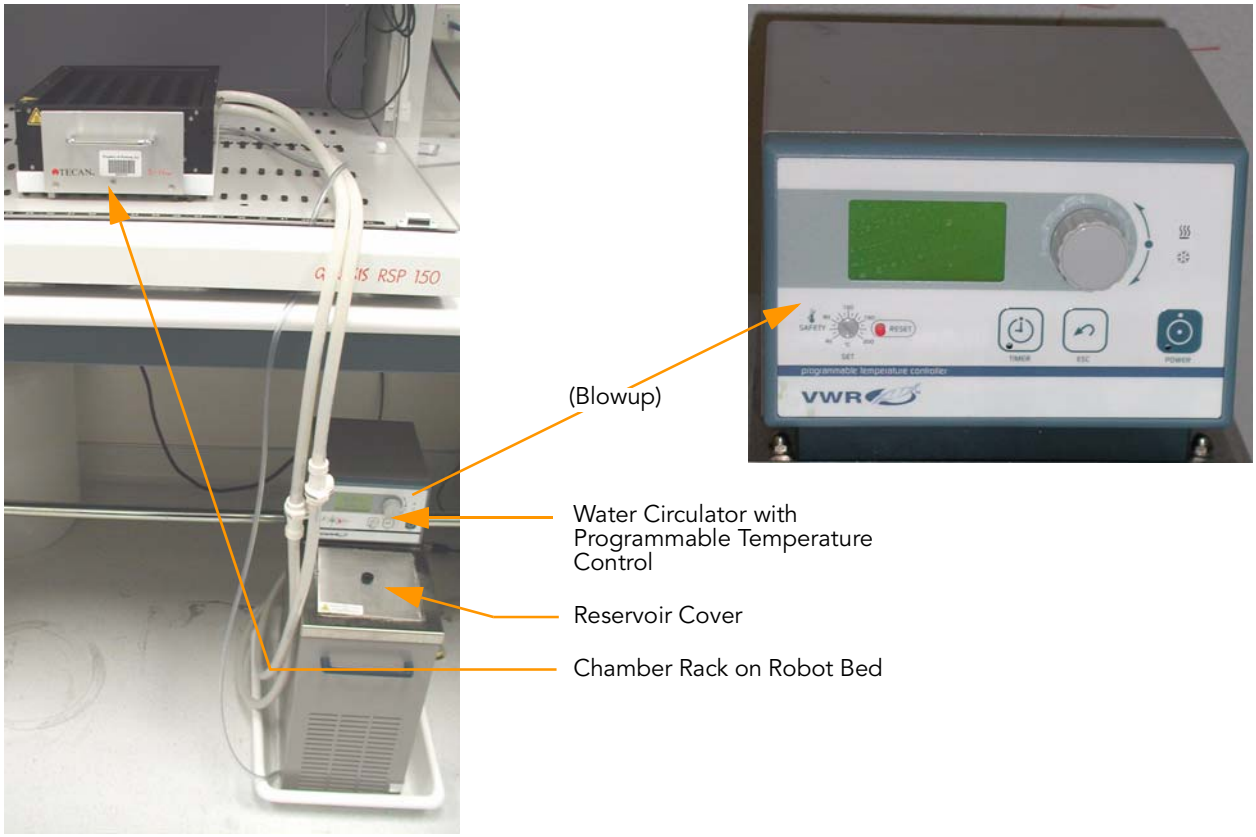


Figure 163 Water Circulator Connected to Chamber Rack

3. You must remove bubbles trapped in the Chamber Rack each time you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.
4. Use the Illumina Temperature Probe in several locations to ensure that the Chamber Rack is at 44°C (Figure 164).

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

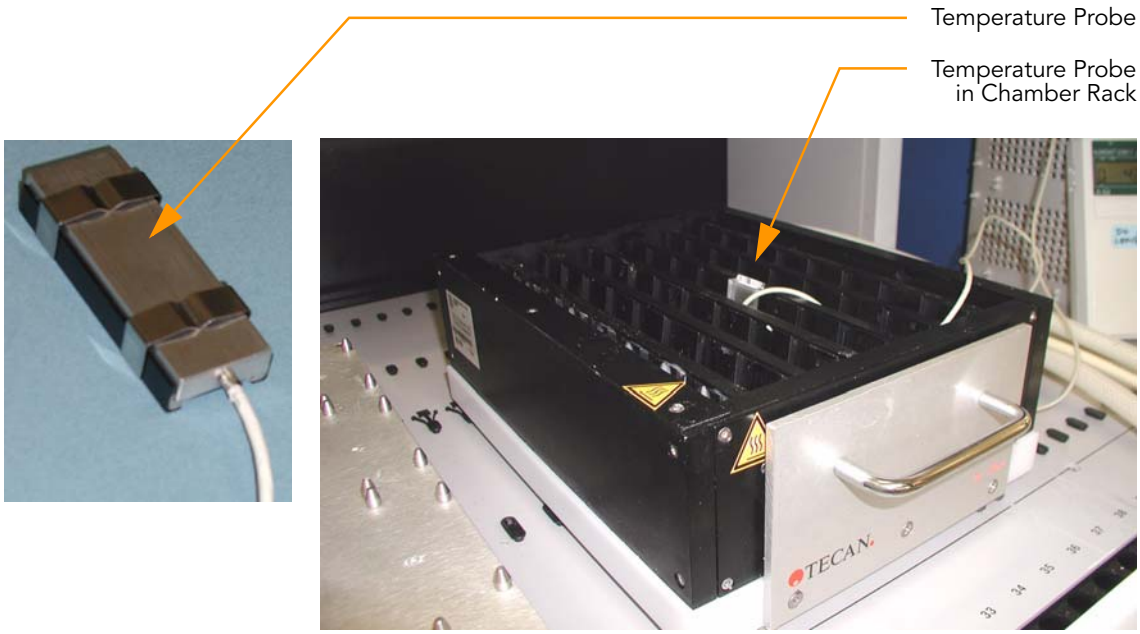


Figure 164 Illumina Temperature Probe in Chamber Rack

5. For accurate temperature measurement, ensure the Temperature Probe is touching the base of the Chamber Rack.
6. Slide the Chamber Rack into column 36 on the robot bed.
7. Slide the Chamber Rack back to ensure it is firmly seated.

Prepare the Robot

For instructions on preparing the robot for use in a protocol, and ensuring that the Chamber Rack is properly installed on the post-amplification robot bed, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).

Refer to Figure 165 throughout this protocol.

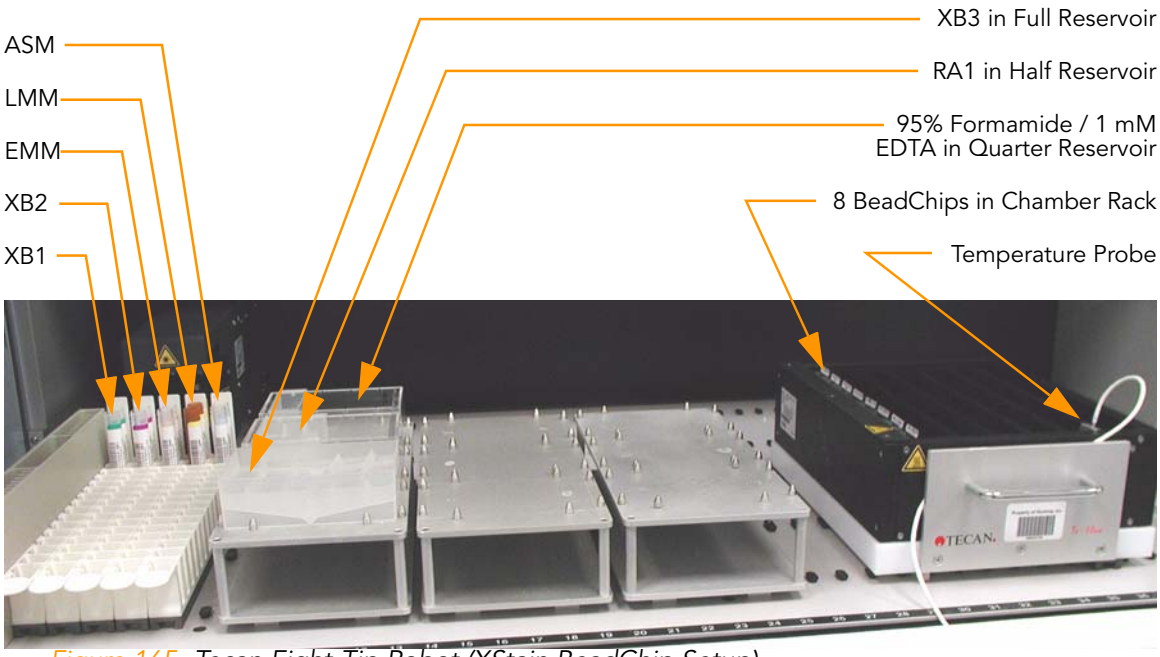


Figure 165 Tecan Eight-Tip Robot (XStain BeadChip Setup)

XStain BeadChip

1. At the robot PC, select **Infinium I XStain Tasks I XStain BeadChip**.
2. Enter the number of BeadChips.

   You can process up to 8 BeadChips in the XStain BC2 process.

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

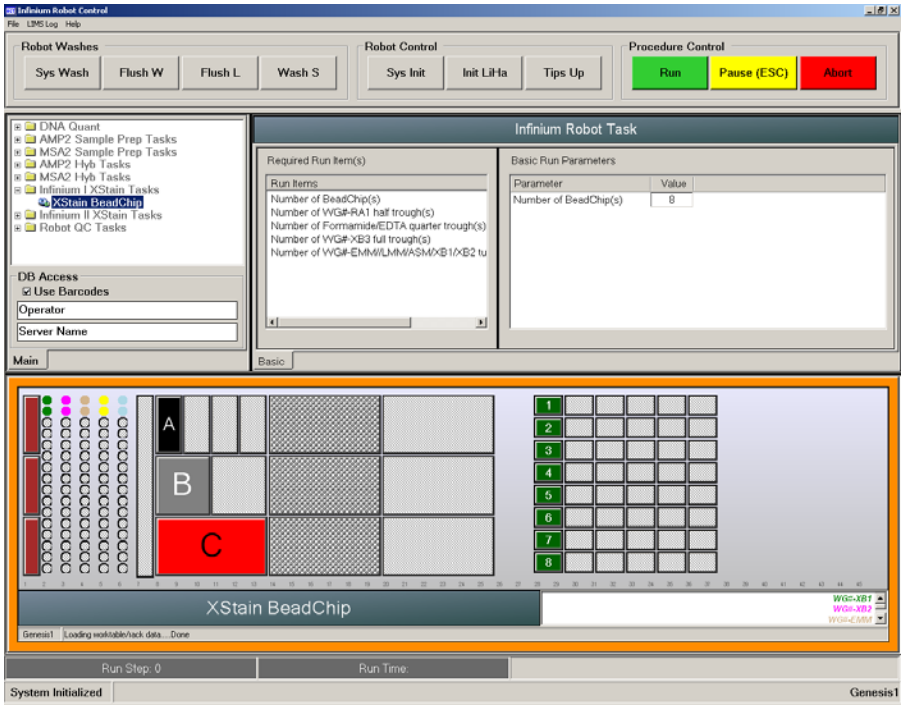


Figure 166 XStain BeadChip Screen

3. Place a quarter reservoir in the reservoir frame, according to the robot bed map (Figure 166), and add 15 ml 95% formamide/1 mM EDTA.
4. Place a half reservoir in the reservoir frame, according to the robot bed map, and add 21 ml RA1.
5. Place a full reservoir in the reservoir frame, according to the robot bed map, and add 75 ml XB3.
6. Place two of each reagent tube (XB1, XB2, EMM, LMM, ASM) in the robot tube rack according the bed map, and remove their caps.

Start the Robot

1. At the robot PC:
   a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
   b. If you are running Infinium LIMS, leave the check box selected.
   c. Click **Run** to start the process.
   d. Log in if prompted.
   e. Observe the robot start to run to ensure that there are no problems.
2. When the prompt appears (Figure 167), wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.

Figure 167 Adjusting Chamber Rack to 44°C Message

3. Once the temperature probe registers 44°C, click **OK** (Figure 167).
4. When prompted (Figure 168), remove Hyb Chamber from the Illumina Hybridization Oven.

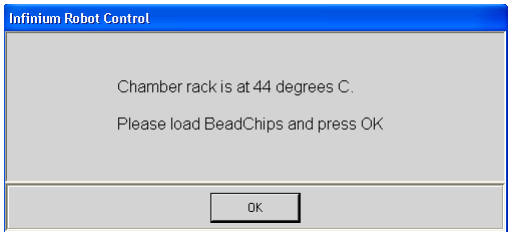


Figure 168 Load BeadChips Message

5. Place each assembled Flow-Through Chamber in the first row of the Chamber Rack. Refer to the robot bed map for the correct layout.
6. Ensure each Flow-Through Chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.
7. On the lab tracking worksheet, record the chamber rack position for each BeadChip.
8. Click **OK** (Figure 168). A series of reactions begins, each with a wait time. Message boxes on the robot PC tell you which reaction is occurring and how long the wait time is. The total wait time is 1 hour and 20 minutes.

Table 10 List of Reactions

#	Reagent	Wait Time
1	RA1	5 minutes
2	XB1	10 minutes
3	XB2	5 minutes
4	EMM	15 minutes
5	Formamide/EDTA	2 minutes
6	XB3	1 minute
7	LMM	10 minutes
8	XB3	3 minutes

Table 10 List of Reactions

#	Reagent	Wait Time
9	ASM	10 minutes
10	XB3	3 minutes
11	LMM	10 minutes
12	XB3	3 minutes
13	ASM	10 minutes
14	XB3	3 minutes
15	LMM	10 minutes
16	XB3	3 minutes

9. When prompted, remove the BeadChips from the Chamber Rack immediately and place them horizontally on the lab bench at room temperature (22°C). Click **OK** in the message box.

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

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

Wash & Spin

1. Pour 200 ml PB1 into a BeadChip wash dish and submerge a BeadChip wash rack in it. You will use it to carry the BeadChips after disassembling the Flow-Through Chamber.
2. One at a time, disassemble each Flow-Through Chamber:
   a. Remove the two metal clamps (Figure 169).

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

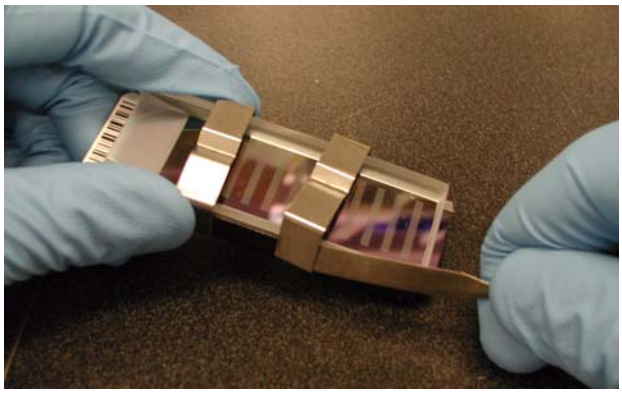


Figure 169 Removing Metal Clamps from Flow-Through Chamber

b. Remove the glass back plate.
c. Set the glass back plates aside. When you finish the XStain BC2 protocol, clean the glass back plates as described in the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
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.

3. Immediately place BeadChips in the wash rack while it is submerged in 200 ml PB1. Make sure the Beadchips are completely submerged.

CAUTION

Do not allow the BeadChips to dry.

4. Move the wash rack up and down for 30 seconds, breaking the surface of the PB1 with gentle, slow agitation at the beginning, middle, and end of a 5-minute period.

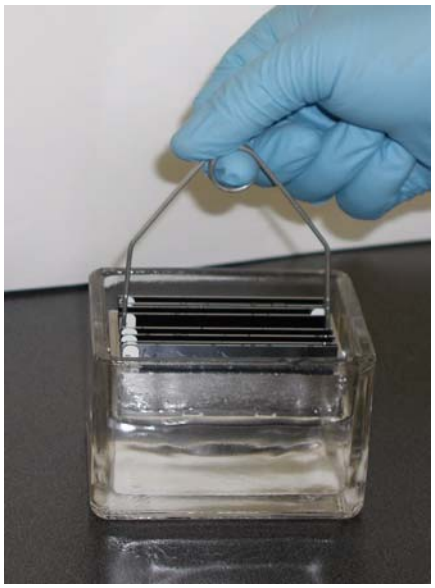


Figure 170 Placing BeadChips in Wash Dish

NOTE

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

5. Transport the entire wash bath to the centrifuge.

6. Place a folded paper towel in the bottom of each of two opposite-facing centrifuge carriers.
7. Remove the rack with BeadChips from the bath. Carefully place it in the centrifuge on top of the paper towel. The towel will catch any excess liquid during the spin.
8. Place another rack loaded with empty BeadChips in an opposite rotor position for balance (Figure 171).
9. Remove handles from the racks.

CAUTION Do not allow Beadchips in rack to dry without centrifugation.

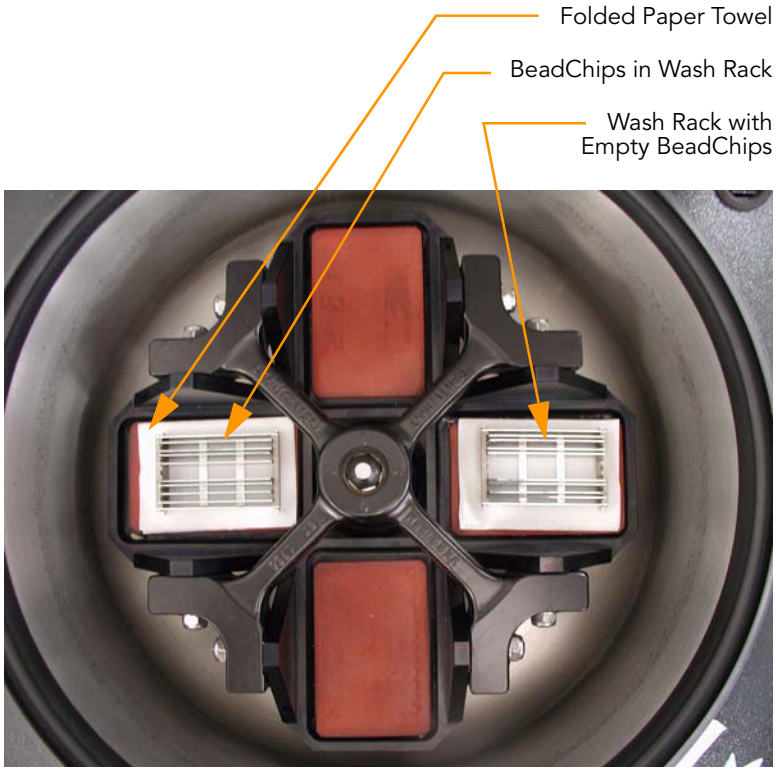


Figure 171 Balancing BeadChip Racks in Centrifuge

10. Centrifuge to 280 xg for 1 minute to dry the BeadChips completely.
11. If you do not plan to scan the BeadChips immediately, place the BeadChips in the Illumina Slide Storage Box and place the box inside a dessicator until ready to proceed. You may store the BeadChips like this for up to 3 days at room temperature (22°C) before scanning them.
12. Clean the glass back plates. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963).
13. Clean the Hyb Chambers:
    a. Remove the rubber gaskets from the Hyb Chambers.

b. Rinse all Hyb Chamber components with DI water.
c. Ensure the eight humidifying buffer reservoirs are rinsed thoroughly.

Image BC2

The BeadChips are now ready for scanning. For instructions, see the *Infinium II Assay Lab Setup and Procedures Guide* (Illumina part # 11207963). Image the BeadChips within 72 hours.

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