illumina®

Infinium® HTS Assay Protocol Guide

FOR RESEARCH USE ONLY

ILLUMINA PROPRIETARY
Part # 15045738 Rev. A
October 2013

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY ILLUMINA IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

FOR RESEARCH USE ONLY

Read Before Using this Product

This Product, and its use and disposition, is subject to the following terms and conditions. If Purchaser does not agree to these terms and conditions then Purchaser is not authorized by Illumina to use this Product and Purchaser must not use this Product.

1 **Definitions**. "**Application Specific IP**" means Illumina owned or controlled intellectual property rights that pertain to this Product (and use thereof) only with regard to specific field(s) or specific application(s). Application Specific IP excludes all Illumina owned or controlled intellectual property that cover aspects or features of this Product (or use thereof) that are common to this Product in all possible applications and all possible fields of use (the "**Core IP**"). Application Specific IP and Core IP are separate, non-overlapping, subsets of all Illumina owned or controlled intellectual property. By way of non-limiting example, Illumina intellectual property rights for specific diagnostic methods, for specific forensic methods, or for specific nucleic acid biomarkers, sequences, or combinations of biomarkers or sequences are examples of Application Specific IP. "**Consumable(s)**" means Illumina branded reagents and consumable items that are intended by Illumina for use with, and are to be consumed through the use of, Hardware. "**Documentation**" means Illumina's user manual for this Product, including without limitation, package inserts, and any other documentation that accompany this Product or that are referenced by the Product or in the packaging for the Product in effect on the date of shipment from Illumina. Documentation includes this document. "**Hardware**" means Illumina branded instruments, accessories or peripherals. "**Illumina**" means Illumina, Inc. or an Illumina affiliate, as applicable. "**Product**" means the product that this document accompanies (e.g., Hardware, Consumables, or Software). "**Purchaser**" is the person or entity that rightfully and legally acquires this Product from Illumina or an Illumina authorized dealer. "**Software**" means Illumina branded software (e.g., Hardware operating software, data analysis software). All Software is licensed and not sold and may be subject to additional terms found in the Software's end user license agreement. "**Specifications**" means Illumina's written specifications for this Product in effect on the date that the Product ships from Illumina.

2 **Research Use Only Rights**. Subject to these terms and conditions and unless otherwise agreed upon in writing by an officer of Illumina, Purchaser is granted only a non-exclusive, non-transferable, personal, non-sublicensable right under Illumina's Core IP, in existence on the date that this Product ships from Illumina, solely to use this Product in Purchaser's facility for Purchaser's internal research purposes (which includes research services provided to third parties) and solely in accordance with this Product's Documentation, **but specifically excluding any use that** (a) would require rights or a license from Illumina to Application Specific IP, (b) is a re-use of a previously used Consumable, (c) is the disassembling, reverse-engineering, reverse-compiling, or reverse-assembling of this Product, (d) is the separation, extraction, or isolation of components of this Product or other unauthorized analysis of this Product, (e) gains access to or determines the methods of operation of this Product, (f) is the use of non-Illumina reagent/consumables with Illumina's Hardware (does not apply if the Specifications or Documentation state otherwise), or (g) is the transfer to a third-party of, or sub-licensing of, Software or any third-party software. All Software, whether provided separately, installed on, or embedded in a Product, is licensed to Purchaser and not sold. Except as expressly stated in this Section, no right or license under any of Illumina's intellectual property rights is or are granted expressly, by implication, or by estoppel.

Purchaser is solely responsible for determining whether Purchaser has all intellectual property rights that are necessary for Purchaser's intended uses of this Product, including without limitation, any rights from third parties or rights to Application Specific IP. Illumina makes no guarantee or warranty that purchaser's specific intended uses will not infringe the intellectual property rights of a third party or Application Specific IP.

3 **Regulatory**. This Product has not been approved, cleared, or licensed by the United States Food and Drug Administration or any other regulatory entity whether foreign or domestic for any specific intended use, whether research, commercial, diagnostic, or otherwise. This Product is labeled For Research Use Only. Purchaser must ensure it has any regulatory approvals that are necessary for Purchaser's intended uses of this Product.

4 **Unauthorized Uses**. Purchaser agrees: (a) to use each Consumable only one time, and (b) to use only Illumina consumables/reagents with Illumina Hardware. The limitations in (a)-(b) do not apply if the Documentation or Specifications for this Product state otherwise. Purchaser agrees not to, nor authorize any third party to, engage in any of the following activities: (i) disassemble, reverse-engineer, reverse-compile, or reverse-assemble the Product, (ii) separate, extract, or isolate components of this Product or subject this Product or components thereof to any analysis not expressly authorized in this Product's Documentation, (iii) gain access to or attempt to determine the methods of operation of this Product, or (iv) transfer to a third-party, or grant a sublicense, to any Software or any third-party software. Purchaser further agrees that the contents of and methods of operation of this Product are proprietary to Illumina and this Product contains or embodies trade secrets of Illumina. The conditions and restrictions found in these terms and conditions are bargained for conditions of sale and therefore control the sale of and use of this Product by Purchaser.

5 **Limited Liability. TO THE EXTENT PERMITTED BY LAW, IN NO EVENT SHALL ILLUMINA OR ITS SUPPLIERS BE LIABLE TO PURCHASER OR ANY THIRD PARTY FOR COSTS OF PROCUREMENT OF SUBSTITUTE PRODUCTS OR SERVICES, LOST PROFITS, DATA OR BUSINESS, OR FOR ANY INDIRECT, SPECIAL, INCIDENTAL, EXEMPLARY, CONSEQUENTIAL, OR PUNITIVE DAMAGES OF ANY KIND ARISING OUT OF OR IN CONNECTION WITH, WITHOUT LIMITATION, THE SALE OF THIS PRODUCT, ITS USE, ILLUMINA'S PERFORMANCE HEREUNDER OR ANY OF THESE TERMS AND CONDITIONS, HOWEVER ARISING OR CAUSED AND ON ANY THEORY OF LIABILITY (WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT LIABILITY OR OTHERWISE).**

6 **ILLUMINA'S TOTAL AND CUMULATIVE LIABILITY TO PURCHASER OR ANY THIRD PARTY ARISING OUT OF OR IN CONNECTION WITH THESE TERMS AND CONDITIONS, INCLUDING WITHOUT LIMITATION, THIS PRODUCT (INCLUDING USE THEREOF) AND ILLUMINA'S PERFORMANCE HEREUNDER, WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT LIABILITY OR OTHERWISE, SHALL IN NO EVENT EXCEED THE AMOUNT PAID TO ILLUMINA FOR THIS PRODUCT.**

7 **Limitations on Illumina Provided Warranties. TO THE EXTENT PERMITTED BY LAW AND SUBJECT TO THE EXPRESS PRODUCT WARRANTY MADE HEREIN ILLUMINA MAKES NO (AND EXPRESSLY DISCLAIMS ALL) WARRANTIES, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THIS PRODUCT, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NONINFRINGEMENT, OR ARISING FROM COURSE OF PERFORMANCE, DEALING, USAGE OR TRADE. WITHOUT LIMITING THE GENERALITY OF THE FOREGOING, ILLUMINA MAKES NO CLAIM, REPRESENTATION, OR WARRANTY OF ANY KIND AS TO THE UTILITY OF THIS PRODUCT FOR PURCHASER'S INTENDED USES.**

8 **Product Warranty**. All warranties are personal to the Purchaser and may not be transferred or assigned to a third-party, including an affiliate of Purchaser. All warranties are facility specific and do not transfer if the Product is moved to another facility of Purchaser, unless Illumina conducts such move.

a **Warranty for Consumables**. Illumina warrants that Consumables, other than custom Consumables, will conform to their Specifications until the later of (i) 3 months from the date of shipment from Illumina, and (ii) any expiration date or the end of the shelf-life pre-printed on such Consumable by Illumina, but in no event later than 12 months from the date of shipment. With respect to custom Consumables (i.e., Consumables made to specifications or designs made by Purchaser or provided to Illumina by, or on behalf of, Purchaser), Illumina only warrants that the custom Consumables will be made and tested in accordance with Illumina's standard manufacturing and quality control processes. Illumina makes no warranty that custom Consumables will work as intended by Purchaser or for Purchaser's intended uses.

b **Warranty for Hardware**. Illumina warrants that Hardware, other than Upgraded Components, will conform to its Specifications for a period of 12 months after its shipment date from Illumina unless the Hardware includes Illumina provided installation in which case the warranty period begins on the date of installation or 30 days after the date it was delivered, whichever occurs first ("Base Hardware Warranty"). "Upgraded Components" means Illumina provided components, modifications, or enhancements to Hardware that was previously acquired by Purchaser. Illumina warrants that Upgraded Components will conform to their Specifications for a period of 90 days from the date the Upgraded Components are installed. Upgraded Components do not extend the warranty for the Hardware unless the upgrade was conducted by Illumina at Illumina's facilities in which case the upgraded Hardware shipped to Purchaser comes with a Base Hardware Warranty.

c **Exclusions from Warranty Coverage**. The foregoing warranties do not apply to the extent a non-conformance is due to (i) abuse, misuse, neglect, negligence, accident, improper storage, or use contrary to the Documentation or Specifications, (ii) improper handling, installation, maintenance, or repair (other than if performed by Illumina's personnel), (iii) unauthorized alterations, (iv) Force Majeure events, or (v) use with a third party's good not provided by Illumina (unless the Product's Documentation or Specifications expressly state such third party's good is for use with the Product).

d **Procedure for Warranty Coverage**. In order to be eligible for repair or replacement under this warranty Purchaser must (i) promptly contact Illumina's support department to report the non-conformance, (ii) cooperate with Illumina in confirming or diagnosing the non-conformance, and (iii) return this Product, transportation charges prepaid to Illumina following Illumina's instructions or, if agreed by Illumina and Purchaser, grant Illumina's authorized repair personnel access to this Product in order to confirm the non-conformance and make repairs.

e **Sole Remedy under Warranty**. Illumina will, at its option, repair or replace non-conforming Product that it confirms is covered by this warranty. Repaired or replaced Consumables come with a 30-day warranty. Hardware may be repaired or replaced with functionally equivalent, reconditioned, or new Hardware or components (if only a component of Hardware is non-conforming). If the Hardware is replaced in its entirety, the warranty period for the replacement is 90 days from the date of shipment or the remaining period on the original Hardware warranty, whichever is shorter. If only a component is being repaired or replaced, the warranty period for such component is 90 days from the date of shipment or the remaining period on the original Hardware warranty, whichever ends later.

The preceding states Purchaser's sole remedy and Illumina's sole obligations under the warranty provided hereunder.

f **Third-Party Goods and Warranty**. Illumina has no warranty obligations with respect to any goods originating from a third party and supplied to Purchaser hereunder. Third-party goods are those that are labeled or branded with a third-party's name. The warranty for third-party goods, if any, is provided by the original manufacturer. Upon written request Illumina will attempt to pass through any such warranty to Purchaser.

9 **Indemnification**.

a **Infringement Indemnification by Illumina**. Subject to these terms and conditions, including without limitation, the Exclusions to Illumina's Indemnification Obligations (Section 9(b) below), the Conditions to Indemnification Obligations (Section 9(d) below), Illumina shall (i) defend, indemnify and hold harmless Purchaser against any third-party claim or action alleging that this Product when used for research use purposes, in accordance with these terms and conditions, and in accordance with this Product's Documentation and Specifications infringes the valid and enforceable intellectual property rights of a third party, and (ii) pay all settlements entered into, and all final judgments and costs (including reasonable attorneys' fees) awarded against Purchaser in connection with such infringement claim. If this Product or any part thereof, becomes, or in Illumina's opinion may become, the subject of an infringement claim, Illumina shall have the right, at its option, to (A) procure for Purchaser the right to continue using this Product, (B) modify or replace this Product with a substantially equivalent non-infringing substitute, or (C) require the return of this Product and terminate the rights, license, and any other permissions provided to Purchaser with respect this Product and refund to Purchaser the depreciated value (as shown in Purchaser's official records) of the returned Product at the time of such return; provided that, no refund will be given for used-up or expired Consumables. This Section states the entire liability of Illumina for any infringement of third party intellectual property rights.

b **Exclusions to Illumina Indemnification Obligations**. Illumina has no obligation to defend, indemnify or hold harmless Purchaser for any Illumina Infringement Claim to the extent such infringement arises from: (i) the use of this Product in any manner or for any purpose outside the scope of research use purposes, (ii) the use of this Product in any manner not in accordance with its Specifications, its Documentation, the rights expressly granted to Purchaser hereunder, or any breach by Purchaser of these terms and conditions, (iii) the use of this Product in combination with any other products, materials, or services not supplied by Illumina, (iv) the use of this Product to perform any assay or other process not supplied by Illumina, or (v) Illumina's compliance with specifications or instructions for this Product furnished by, or on behalf of, Purchaser (each of (i) – (v), is referred to as an "Excluded Claim").

c **Indemnification by Purchaser**. Purchaser shall defend, indemnify and hold harmless Illumina, its affiliates, their non-affiliate collaborators and development partners that contributed to the development of this Product, and their respective officers, directors, representatives and employees against any claims, liabilities, damages, fines, penalties, causes of action, and losses of any and every kind, including without limitation, personal injury or death claims, and infringement of a third party's intellectual property rights, resulting from, relating to, or arising out of (i) Purchaser's breach of any of these terms and conditions, (ii) Purchaser's use of this Product outside of the scope of research use purposes, (iii) any use of this Product not in accordance with this Product's Specifications or Documentation, or (iv) any Excluded Claim.

d **Conditions to Indemnification Obligations**. The parties' indemnification obligations are conditioned upon the party seeking indemnification (i) promptly notifying the other party in writing of such claim or action, (ii) giving the other party exclusive control and authority over the defense and settlement of such claim or action, (iii) not admitting infringement of any intellectual property right without prior written consent of the other party, (iv) not entering into any settlement or compromise of any such claim or action without the other party's prior written consent, and (v) providing reasonable assistance to the other party in the defense of the claim or action; provided that, the party reimburses the indemnified party for its reasonable out-of-pocket expenses incurred in providing such assistance.

e **Third-Party Goods and Indemnification**. Illumina has no indemnification obligations with respect to any goods originating from a third party and supplied to Purchaser. Third-party goods are those that are labeled or branded with a third-party's name. Purchaser's indemnification rights, if any, with respect to third party goods shall be pursuant to the original manufacturer's or licensor's indemnity. Upon written request Illumina will attempt to pass through such indemnity, if any, to Purchaser.

Revision History

Part #	Revision	Date	Description of Change
15045738	A	October 2013	Initial release.

Table of Contents

List of Tables

Overview

Chapter 1

Introduction to Infinium HTS assay

The Illumina Infinium HTS assay is designed to maximize content flexibility with a High-Throughput Screening (HTS) capacity for genotyping and CNV analysis. Using Infinium I and Infinium II probe designs and a dual color channel approach, the Infinium HTS assay enables the DNA analysis of up to 750,000 SNPs and CNV markers per sample.

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

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

The Infinium HTS assay offers:

- High multiplexing
- High call rate and accuracy
- Unlimited genome-wide marker selection
- Single tube amplification—single chip—no PCR
- Minimal risk of carryover contamination
- Low DNA input—200 ng per sample.
- Walkaway automation using Tecan Genesis or Freedom Evo Robots and Tecan GenePaint system
- Compatibility with both Illumina iScan and HiScan Systems
- Multiple-sample BeadChip format

Audience and Purpose

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

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

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

Important Note

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

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

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

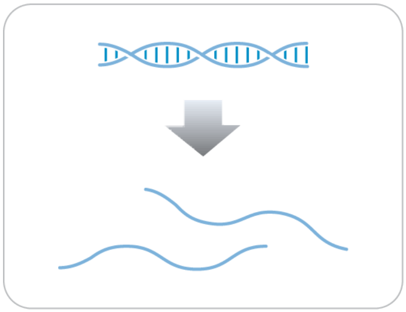
Infinium HTS assay

This section describes and illustrates the assay protocol. The assay requires 200 ng of original DNA sample as input.

Amplify DNA (Pre-Amp)

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

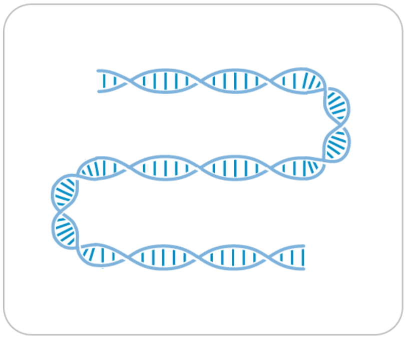
Figure 1 Denaturing and Neutralizing DNA



Incubate DNA (Post-Amp)

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

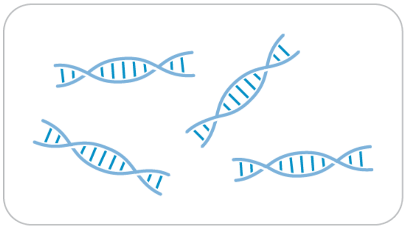
Figure 2 Incubating DNA to Amplify



Fragment DNA (Post-Amp)

A controlled enzymatic process fragments the amplified product. The process uses end-point fragmentation to avoid over-fragmenting the sample.

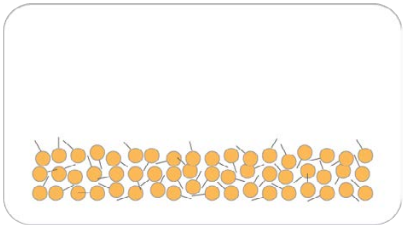
Figure 3 Fragmenting DNA



Precipitate DNA (Post-Amp)

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

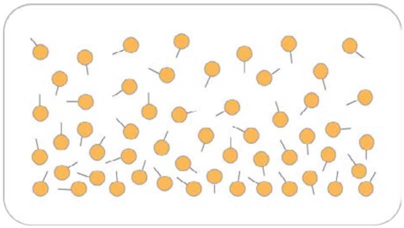
Figure 4 Precipitating DNA



Resuspend DNA (Post-Amp)

The precipitated DNA is resuspended in hybridization buffer.

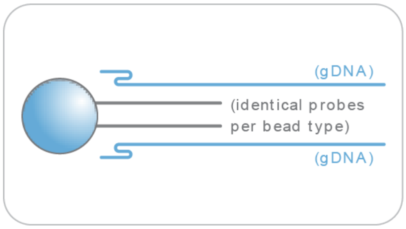
Figure 5 Resuspending DNA



Hybridize to BeadChip (Post-Amp)

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

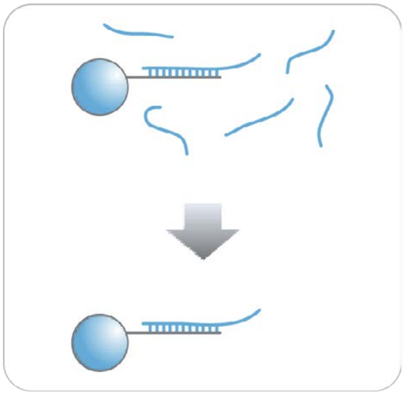
Figure 6 Hybridize to BeadChip



Wash BeadChip (Post-Amp)

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

Figure 7 Washing BeadChip



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

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

Figure 8 Extending and Staining BeadChip

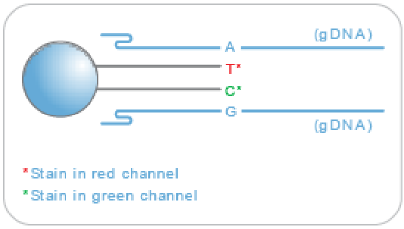
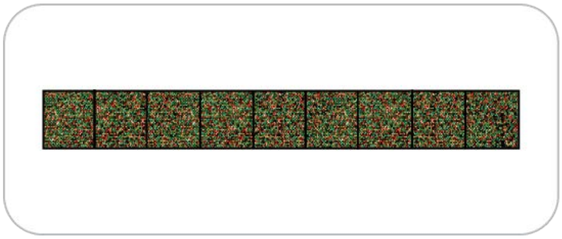


Image BeadChip (Post-Amp)

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

Figure 9 Imaging BeadChip



Illumina Infinium BeadChips

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

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

Illumina Lab Protocols

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

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

Tracking Tools

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

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

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

Sample Sheet

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

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

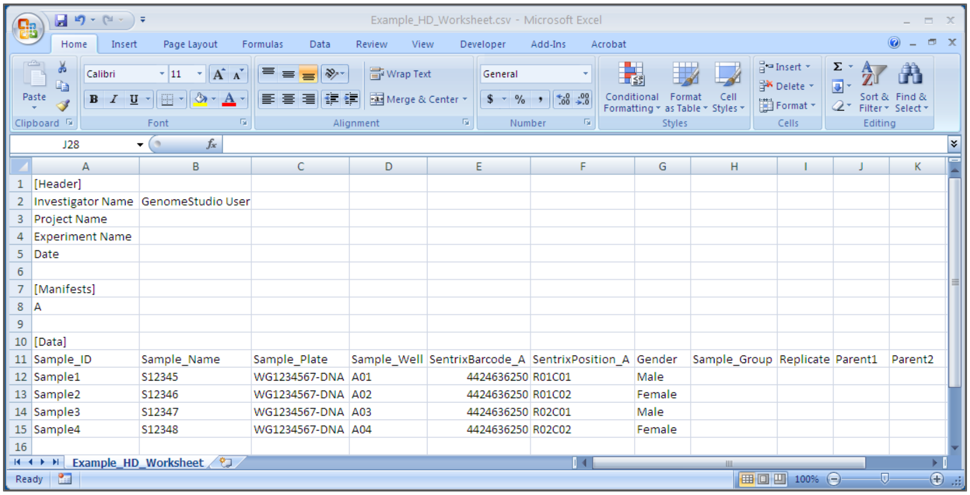
Table 1 Sample Sheet Guidelines

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

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

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

Figure 10 Sample Sheet Example



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

Tecan GenePaint

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

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

Imaging Systems

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

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

Loading and unloading the iScan System can be automated with the optional AutoLoader2 or AutoLoader 2x for the HiScan System. All AutoLoaders support unattended processing by placing BeadChip carriers in the tray of the imaging system, so that it can scan the BeadChips. AutoLoader features include those items listed in the table.

Table 2 AutoLoader2 and AutoLoader2x Features

Feature	AutoLoader2	AutoLoader2x
Integrated with iScan Control Software	●	●
Integrated with Illumina LIMS	●	●
Email alert system	●	●
Single-reader or dual-reader configuration	●	●
Number of BeadChips supported per carrier	4	4
Number of carriers processed at a time	48	48

GenomeStudio Integrated Informatics Platform

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

Data analysis features of the GenomeStudio Genotyping Module include:

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

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

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

Manual Protocol

Chapter 2

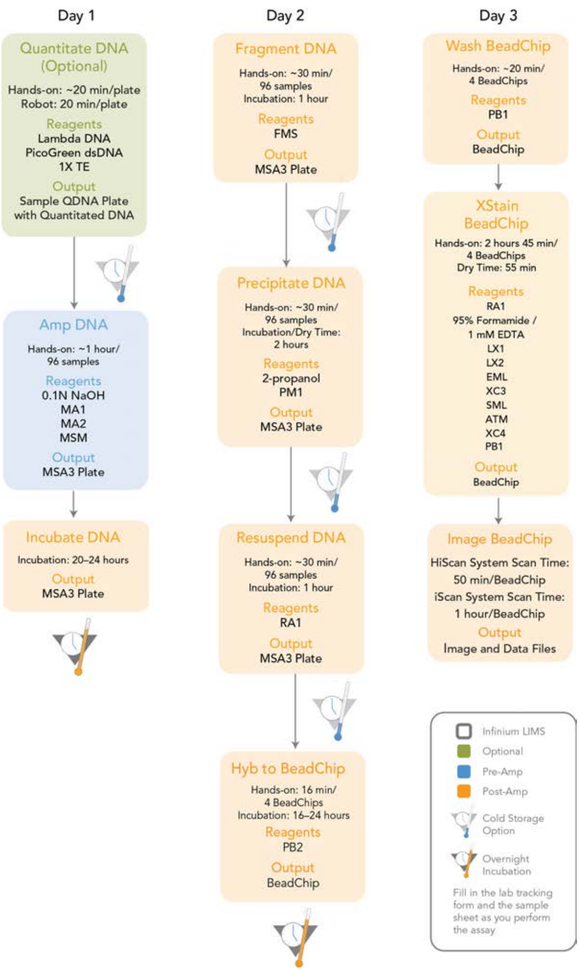
Introduction to Infinium HTS Manual Protocol

This chapter describes pre- and post-amplification manual laboratory protocols for the Infinium HTS assay. Follow the protocols in the order shown.

Infinium HTS Manual Workflow

The following figure graphically represents the Infinium HTS assay manual workflow for use with the 24x1 HTS BeadChip. These protocols describe the procedure for preparing 96 DNA samples.

Figure 11 Illumina Infinium HTS Protocol Manual Workflow



Equipment, Materials, and Reagents

These materials are specific to the manual Infinium HTS. For a list of other equipment, materials, and reagents needed in a lab performing Illumina Infinium assays, see the *Infinium Lab Setup and Procedures Guide*.

User-Supplied Equipment

Table 3 User-Supplied Equipment Infinium HTS Assay, Manual Protocol

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

Illumina-Supplied Equipment

Table 4 Illumina-Supplied Equipment Infinium HTS Assay, Manual Protocol

Item	Catalog #
Multi-Sample BeadChip Alignment Fixture	218528

User-Supplied Materials

Table 5 User Supplied Materials Infinium HTS Assay, Manual Protocol

Item	Catalog #
Tube vortexer	General lab supplier
Tube rack	VWR
Combination optical tachometer/stroboscope	Cole-Parmer, catalog # A-87700-06

Item	Catalog #
Microplate centrifuge with g-force range 8–3000 × g, for dedicated pre- and post-amp use	General lab supplier
Adapter for centrifuge plates and tubes	General lab supplier
Pipettes (two separate sets)	2 each of P-20, P-200, and P-1000
Eight-channel precision pipette (two separate sets)	50 µl to 300 µl
Adjustable spacer multichannel pipette (recommended)	Rainin LA8-50XLS; www.mt.com
Stop watch/timer	General lab supplier
Forceps	VWR, catalog # 25601-008
Powder-free gloves (two separate stocks)	General lab supplier
Lab coats (separate pre-PCR and post-PCR)	General lab supplier
Safety glasses (two separate stocks)	General lab supplier
15 ml conical tubes	General lab supplier
96-well 0.2 ml skirted microplates	MJ Research, catalog # MSP-9601
0.8 ml storage plate (MIDI plate), conical well-bottom	Abgene, catalog # AB-0765
Heat sealing foil sheets, Thermo-Seal	Abgene, catalog # AB-0559
96-well cap mats (pierceable, non-autoclavable)	Abgene, catalog # AB-0566
Absorbent pads	General lab supplier
Kimwipes	General lab supplier
Mild detergent, such as Alconox Powder	VWR, catalog ## 21835-032
Detergent	General lab supplier

Item	Catalog #
Aerosol filter pipette tips (two separate stocks)	20 µl, 200 µl, 1000 µl
Disposable pipetting troughs	VWR, catalog # 21007-970

Illumina-Supplied Materials

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

Illumina-Supplied Reagents

Table 6 Illumina-Supplied Reagents, Infinium HTS Assay, Manual Protocol

Item	Part #
ATM—Anti-Stain Two-Color Master Mix	11208317
FMS—Fragmentation solution	11203428
MA1—Multi-Sample Amplification 1 Mix	11202880
MA2—Multi-Sample Amplification 2 Mix	11203401
MSM—Multi-Sample Amplification Master Mix	11203410
PB1—Reagent used to prepare BeadChips for hybridization	11191922
PB2—Humidifying buffer used during hybridization	11191130
PM1—Precipitation solution	11203436
RA1—Resuspension, hybridization, and wash solution	11222442
SML—Superior Two-Color Master Mix	11288046

Item	Part #
EML—Two-Color Extension Master Mix	11208309
LX1—XStain BeadChip solution 1	11208288
LX2—XStain BeadChip solution 2	11208296
XC3—XStain BeadChip solution 3	11208421
XC4—XStain BeadChip solution 4	11208430

Quantitate DNA (Optional) (Pre-Amp)

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

Estimated Time

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

Spectrofluorometer read time: ~5 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	2° to 8°C	User
1X TE	See Instructions	Room temperature	General lab supplier
Lambda DNA	See Instructions	2° to 8°C	User
96-well 0.65 ml microplate	1 per 96 samples		General lab supplier
FLUOTRAC 200 96-well flat-bottom plate	1 per Standard DNA plate 1 per Sample DNA plate		General lab supplier

Preparation

- Thaw PicoGreen to room temperature for 60 minutes in a light-impermeable container.
- Hand-label the microplate "Standard DNA".
- Hand-label one of the FLUOTRAC plates "Standard QDNA".
- Hand-label the other FLUOTRAC plate "Sample QDNA". This plate is for the quantitated DNA.
- In the sample sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Make a Standard DNA Plate

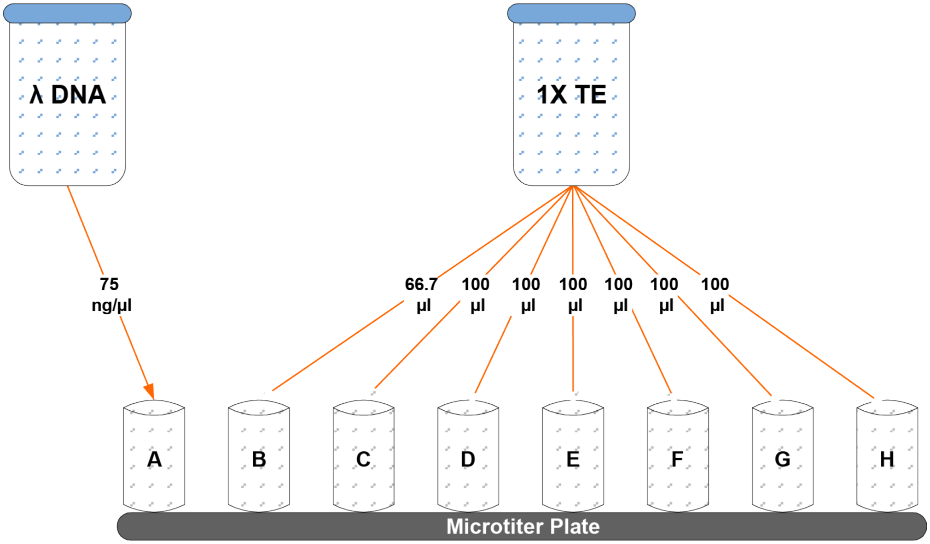
In this process, you create a Standard DNA plate with serial dilutions of stock Lambda DNA in the wells of column 1.

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

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

   b. Dilute the stock DNA in well A1 using the following formula:
      µl of 1X TE to add to A1 = 233.3 µl - µl of stock Lambda DNA in well A1
2. Add 66.7 µl 1X TE to well B1.
3. Add 100 µl 1X TE to wells C, D, E, F, G, and H of column 1.

Figure 12 Dilution of Stock Lambda DNA Standard



4 Transfer 133.3 µl of Lambda DNA from well A1 into well B1. Pipette up and down several times.

5 Change tips. Transfer 100 µl from well B1 into well C1. Pipette up and down several times.

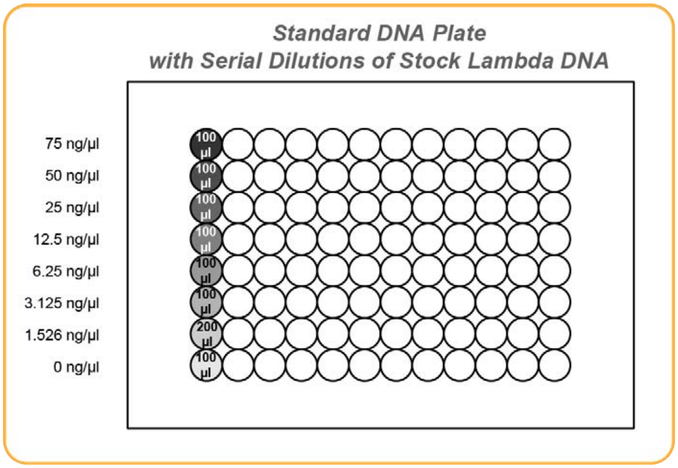
6 Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

Table 7 Concentrations of Lambda DNA

Row-Column	Concentration (ng/µl)	Final Volume in Well (µl)
A1	75	100
B1	50	100
C1	25	100
D1	12.5	100

Row-Column	Concentration (ng/µl)	Final Volume in Well (µl)
E1	6.25	100
F1	3.125	100
G1	1.5262	200
H1	0	100

Figure 13 Serial Dilutions of Lambda DNA



7 Cover the Standard DNA plate with a cap mat.

Dilute PicoGreen

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

CAUTION

Do not use glass containers for the PicoGreen reagent. PicoGreen degrades quickly in the presence of light and can adhere to glass, which lowers its effective concentration in solution and effects the upper response range accuracy.

1 Prepare a 1:200 dilution of PicoGreen into 1X TE using a sealed 100 ml or 250 ml Nalgene bottle wrapped in aluminum foil.

Refer to the following table to identify the volumes needed to produce diluted reagent for multiple 96-well QDNA plates. For fewer than 96 DNA samples, scale down the volumes.

Table 8 Volumes for PicoGreen Reagents

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

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

Create QDNA Standard Plate with Diluted PicoGreen

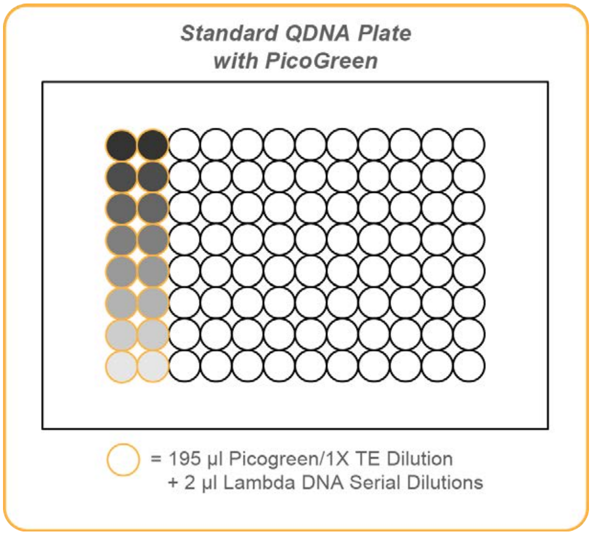
In this process, you transfer the serial dilutions from the Standard DNA plate into the Standard QDNA FLUOTRAC plate and add diluted PicoGreen.

1 Pour the PicoGreen/1X TE dilution into a clean reagent reservoir.

2 Using a multichannel pipette, transfer 195 µl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the FLUOTRAC plate labeled "Standard QDNA".

3 Add 2 µl of each stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA FLUOTRAC plate.

Figure 14 Standard QDNA Plate with PicoGreen



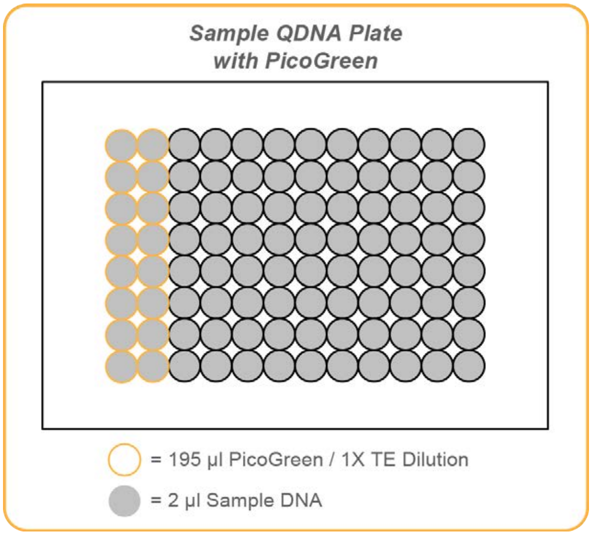
4 Immediately cover the plate with an adhesive aluminum seal.

Prepare QDNA Sample Plate with PicoGreen and DNA

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

1. Using a multichannel pipette, transfer 195 µl PicoGreen/1xTE dilution into each well of the FLUOTRAC plate labeled "Sample QNT" for each well that will contain sample.
2. Add 2 µl of DNA sample to each well containing PicoGreen/1xTE.

Figure 15 Sample QDNA Plate with PicoGreen



3 Immediately cover the plate with an adhesive aluminum seal.

Read QDNA Plate

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

NOTE
Depending on the software version that you are running, the SoftMax Pro screens and menu options can vary.

1 Turn on the spectrofluorometer. At the PC, open the SoftMax Pro program.

2 Load the Illumina QDNA.ppr file from the installation CD that came with your system.

3 Select **Protocols | GTS_QDNA**.

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

5 Click the blue arrow next to **Illumina QDNA | SQDNA_STD**.

6. Click **Read**.
7. When the software finishes reading the data, remove the plate from the drawer.
8. Click the blue arrow next to **Standard Curve** to view the standard curve graph.
9. If the standard curve is acceptable, continue with the sample plate. Otherwise, click **Standard Curve** again.
10. Place the first Sample QDNA plate in the spectrofluorometer with well A1 in the upper left corner.
11. Click the blue arrow next to **SQDNA** and click **Read**.
12. When the software finishes reading the plate, remove the plate from the drawer.
13. Repeat steps 10 through 12 to quantitate all Sample QDNA plates.
14. After all plates have been read, click **File | Save** to save the output data file (*.pda).
15. When you have saved the *.pda file, click **File | Import/Export | Export** and export the file as a *.txt file. You can open the *.txt file in Microsoft Excel for data analysis.
16. Do one of the following:
    - Proceed to *Amplify DNA (Pre-Amp*.
    - Store the quantitated DNA at 2°C to 8°C for up to one month.

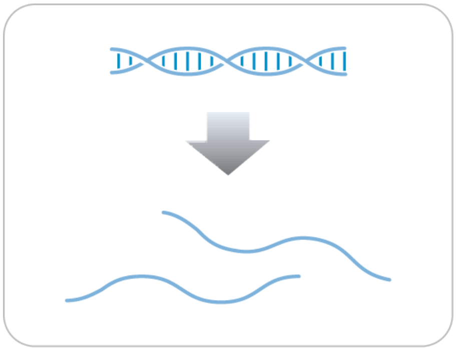
SAFE STOPPING POINT

Now is a good stopping point in the process.

Amplify DNA (Pre-Amp)

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

Figure 16 Denaturing and Neutralizing BCD



Estimated Time

Hands-on time:

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

Incubation time: 20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MA1	1 tube (per 96 samples)	-15°C to -25°C	Illumina
MA2	1 tube (per 96 samples)	-15°C to -25°C	Illumina

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

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

Preparation

- In preparation for the Incubate MSA3 process, preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.

17 Apply an MSA3 barcode label to a new 0.8 ml microplate (MIDI).

18 Thaw MA1, MA2, and MSM tubes to room temperature. Gently invert at least 10 times to mix contents.

19 Thaw DNA samples to room temperature.

- In the sample sheet, enter the Sample_Name and Sample_Plate for each Sample_Well.
- On the lab tracking form, record:
  - Date/Time
  - Operator
  - DNA plate barcode
  - MSA3 plate barcodes
  - MA1 tube barcodes
  - MA2 tube barcodes
  - MSM tube barcodes

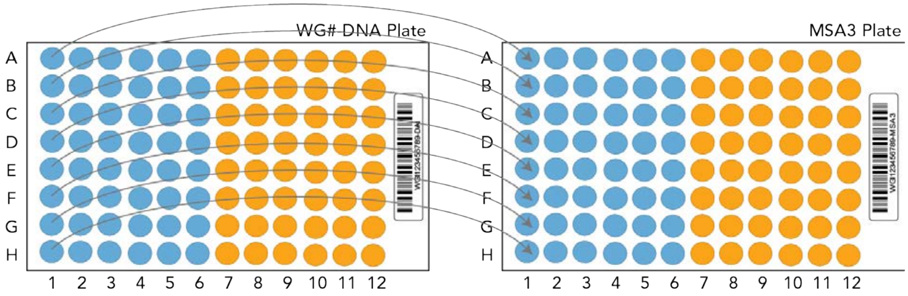
NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Steps to Make MSA3 Plate

1. If you do not already have a WG#-DNA plate, add DNA into one of the following:
   - MIDI plate: 20 µl to each WG#-DNA plate well
   - TCY plate: 10 µl to each WG#-DNA plate well

   Apply a barcode label to the new WG#-DNA plate.
2. Dispense 20 µl MA1 into the MSA3 plate wells.
3. Transfer 4 µl of the DNA sample from the WG#-DNA plate to the corresponding wells in the MSA3 plate.
4. Record the location of the original DNA sample ID for each well in the MSA3 plate.
5. Dispense 4 µl 0.1N NaOH into each well of the MSA3 plate that contains MA1 and sample.
   Refer to the following figure throughout the Make MSA3 process.

Figure 17 Distributing Sample to Wells



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

6. Seal the MSA3 plate with the 96-well cap mat.

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

7 Vortex the plate at 1600 rpm for 1 minute.

8 Centrifuge to 280 × g for 1 minute.

9 Incubate for 10 minutes at room temperature.

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

11 Dispense 34 µl MA2 into each well of the MSA3 plate containing sample.

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

12 Dispense 38 µl MSM into each well of the MSA3 plate containing sample.

13 Seal MSA3 plate with cap mat.
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

14 Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.

15 Pulse centrifuge to 280 × g.

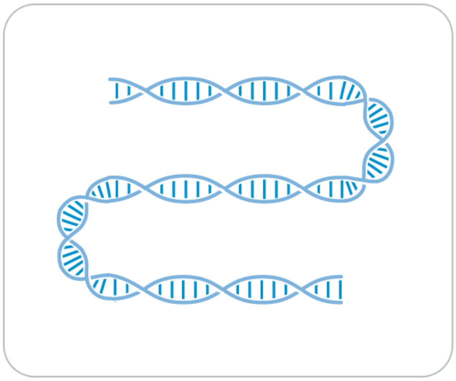
16 Discard unused reagents in accordance with facility standards.

17 Proceed immediately to the next step.

Incubate DNA (Post-Amp)

This process incubates the MSA3 plate for 20–24 hours at 37°C in the Illumina Hybridization Oven. The process uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used when in the Infinium HTS assay.

Figure 18 Incubating DNA to Amplify



Estimated Time

Incubation time: 20–24 hours

Steps to Incubate the MSA3 Plate

OVERNIGHT INCUBATION
Incubate MSA3 plate in the Illumina Hybridization Oven for at least 20 hours but no more than 24 hours at 37°C.

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

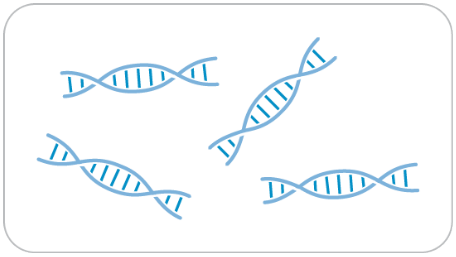
NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

19 Proceed to the next step.

Fragment DNA (Post-Amp)

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

Figure 19 Fragmenting DNA



Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation time: 1 hour

Consumables

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

Preparation

1 Preheat the heat block with the MIDI plate insert to 37°C.

2 Thaw FMS tubes to room temperature.

3 Gently invert the FMS tubes at least 10 times to mix contents. Pulse centrifuge to 280 × g.

4 Remove the MSA3 plate from the Illumina Hybridization Oven.

5 On the lab tracking form, record:

- Date/Time

- Operator
- FMS tube barcodes

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

Steps to Fragment the MSA3 Plate

1. Pulse centrifuge the plate to 50 × g.
2. Carefully remove the cap mat.
3. Add 25 µl FMS to each well containing sample.
4. Seal the MSA3 plate with the 96-well cap mat.

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

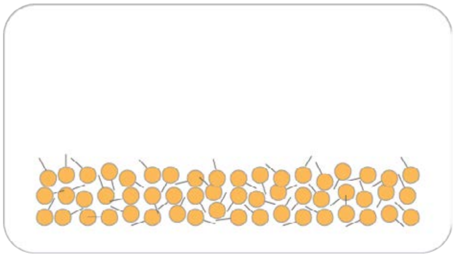
5. Vortex the plate at 1600 rpm for 1 minute.
6. Centrifuge the plate to 50 × g at 22°C for 1 minute.
7. Place the sealed plate on the 37°C heat block for 1 hour.
8. Record the start and stop times on the lab tracking form.
9. Discard unused reagents in accordance with facility standards.
10. Do one of the following:
    - Continue to the next step, *Precipitate the DNA (Post-Amp)*. Leave plate in 37°C heat block until setup is complete. Do not leave the plate in the 37°C heat block for longer than 2 hours.
    - If you do not plan to proceed to the next step immediately, store the sealed WG#-DNA plate at -15°C to -25°C.

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

Precipitate DNA (Post-Amp)

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

Figure 20 Precipitating DNA



Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation and dry time: 2 hours

Consumables

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

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

Preparation

1 Do one of the following:
  - If you froze the MSA3 plate after fragmentation, thaw it to room temperature, then pulse centrifuge to 280 × g.

- If you proceeded immediately from *Fragment the* MSA3 *Plate*, leave the plate in the 37°C heat block until setup is complete.

2. Preheat heat block to 37°C.
3. Thaw PM1 to room temperature. Centrifuge to 280 × g for 1 minute.
4. Remove the 96-well cap mat.

▶ On the lab tracking form, record:

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

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

Steps to Precipitate the MSA3 Plate

1. Add 50 µl PM1 to each MSA3 plate well containing sample.
2. Seal the plate with the cap mat.
3. Vortex the plate at 1600 rpm for 1 minute.
4. Incubate at 37°C for 5 minutes.
5. Pulse centrifuge to 280 × g for 1 minute.

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

6. Carefully remove the cap mat and discard it.
7. Add 155 µl 100% 2-propanol to each well containing sample.
8. Carefully seal the MSA3 plate with a new, ***dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.
9. Invert the plate at least 10 times to mix contents thoroughly.
10. Incubate at 4°C for 30 minutes.

11 Place the sealed MSA3 plate in the centrifuge opposite another plate of equal weight.

Figure 21 Sealed MSA3 Plate and Plate of Equal Balance in Centrifuge

12 Centrifuge to 3,000 × g at 4°C for 20 minutes. Immediately remove the MSA3 plate from centrifuge.

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

13 Remove the cap mat and discard it.

14 Quickly invert the MSA3 plate and drain the liquid onto an absorbent pad to decant the supernatant. Then smack the plate down on a dry area of the pad, avoiding the liquid that was drained onto the pad.

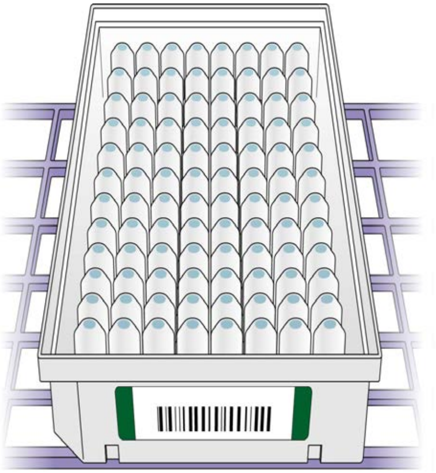
15 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

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

16 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.

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

Figure 22 Uncovered MSA3 Plate Inverted for Air Drying



CAUTION

Do not over-dry the pellet. Pellets that are over-dried are difficult to resuspend. Poorly resuspended samples leads to poor data quality.

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

18 Discard unused reagents in accordance with facility standards.

19 Do one of the following:

- Continue to the next step, *Resuspend DNA (Post-Amp).*
- If you do not plan to proceed to the next step immediately, seal the MSA3 plate with a new cap mat and store it at -15°C to -25°C.

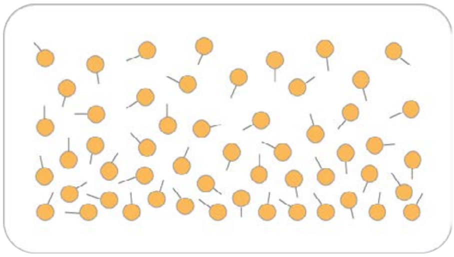
SAFE STOPPING POINT

Now is a good stopping point in the process.

Resuspend DNA (Post-Amp)

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

Figure 23 Resuspending DNA



Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation time: 1 hour

Consumables

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

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

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

Preparation

1. If you stored the MSA3 plate at -15°C to -25°C, thaw it to room temperature. Remove the cap mat and discard it.
2. Preheat the Illumina Hybridization Oven to 48°C.
3. Turn on the heat sealer to preheat. Allow 20 minutes.
4. RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that can be present.
5. On the lab tracking form, record:
   - Date/Time
   - Operator
   - RA1 bottle barcodes

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

Use Fresh RA1 Reagent for Each Step

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

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

Steps to Resuspend the MSA3 Plate

1. Add 23 µl RA1 to each well of the MSA3 plate containing a DNA pellet.
   Reserve any leftover reagent for **XStain BeadChip**.

2. Apply a foil heat seal to the MSA3 plate by firmly and evenly 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 × g.

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

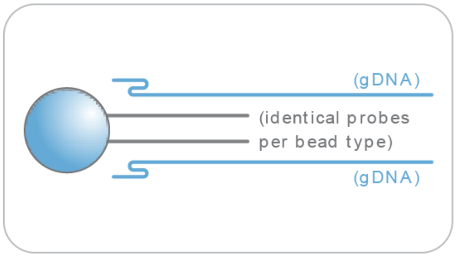
6. Discard unused reagents in accordance with facility standards.
7. Do one of the following:
   - Continue to the next step, *Hybridize Multi BeadChip*. If you plan to do so immediately, it is safe to leave the MSA3 plate at room temperature for up to 1 hour.
   - If you do not plan to proceed to the next step immediately, store the sealed MSA3 plate at -15°C to -25°C for no more than 24 hours. For more than 24 hours, store at 80°C. Store RA1 at -15°C to -25°C.

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

Hybridize to BeadChip (Post-Amp)

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

Figure 24 Hybridize Multi-BeadChip



Estimated Time

Hands-on time:

- 24x1 HTS BeadChip: ~16 minutes for 4 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

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

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

Preparation

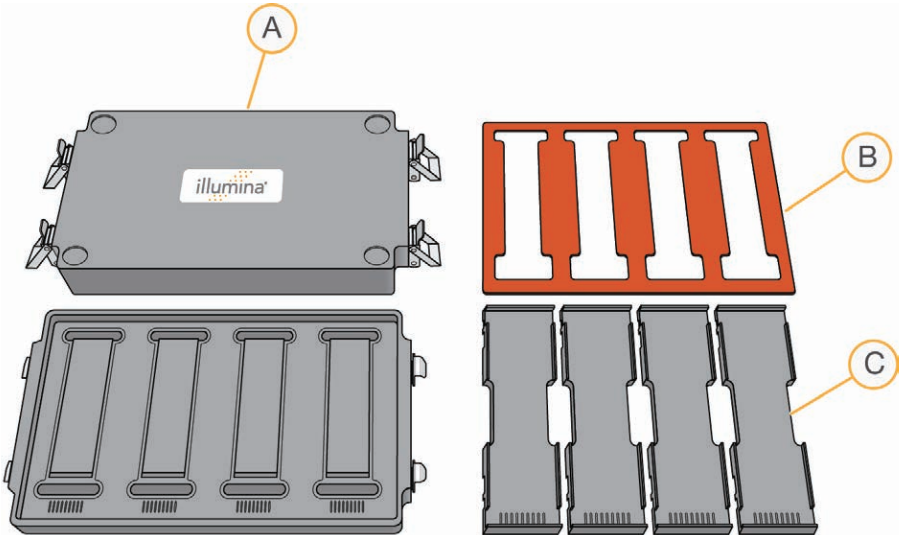
1. If frozen, thaw MSA3 plate to room temperature, and then pulse centrifuge the MSA3 plate to 280 × g.
2. Preheat the heat block to 95°C.
3. Prepare the Illumina Hybridization Oven as follows:
   a. Preheat the oven to 48°C:
      - Press the "F" button one time to change the display to **TSET**.
      - Press the "S" button to enter the set-temperature mode, and then use the Increment/Decrement dial to set the oven to 48°C.
      - Press the "S" button again to set 48°C as the temperature.
   b. Set the rocker speed to 5:
      - Press the "F" button twice until **SPd** is indicated on the display.
      - Press the "S" button to enter the rocker speed mode.
      - Use the Increment/Decrement dial to set the rocker speed to "5".
      - Press the "S" button again.
4. Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
5. On the lab tracking form, record:
   - Date/Time
   - Operator
   - PB2 tube lot number

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

Assemble the Hybridization Chambers

1. Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.
2. Remove the BeadChips from 2°C to 8°C storage, leaving the BeadChips in their plastic bags and mylar packages until you are ready to begin hybridization.
3. During the 20 minute incubation, prepare the Hyb Chambers.
   Place the following items on the benchtop for use in this procedure:

Figure 25 BeadChip Hyb Chamber Components

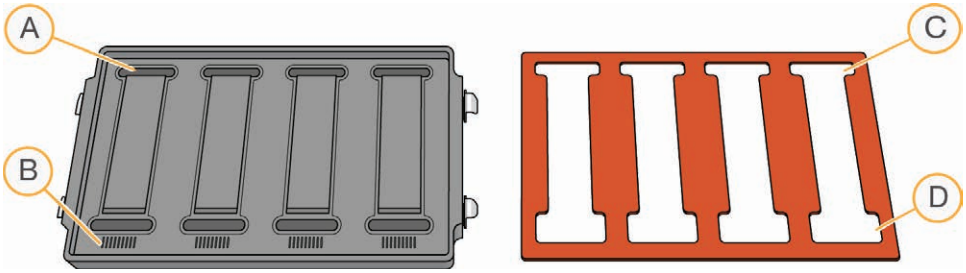


A	BeadChip Hyb Chambers
B	Hyb Chamber Gaskets
C	Hyb Chamber Inserts

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

a Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers.
  — Match the wider edge of the Hyb Chamber Gasket to the barcode-ridge side of the Hyb Chamber.

Figure 26 Hyb Chamber and Gasket



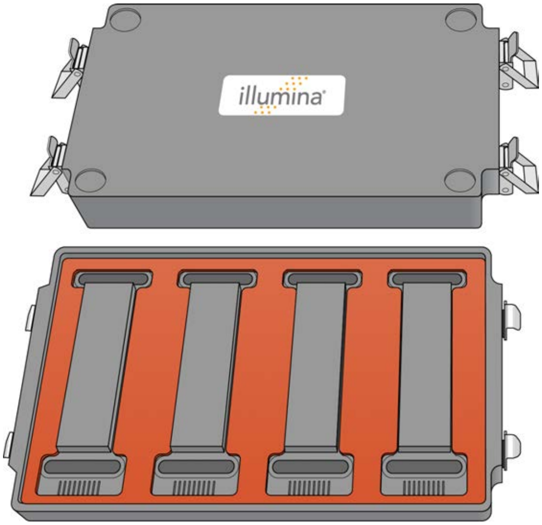
A	Reservoirs
B	Barcode Ridges
C	Narrower Edges
D	Wider Edges

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

Figure 27 Placing Gasket into Hyb Chamber

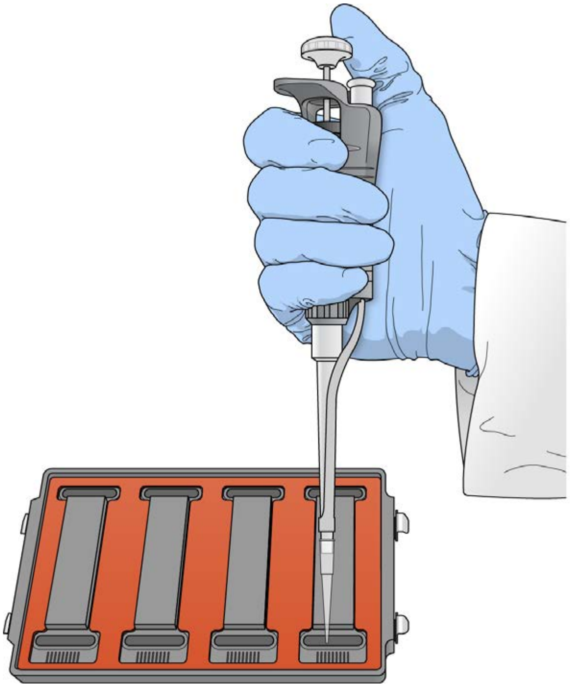
- Make sure the Hyb Chamber gaskets are properly seated.

Figure 28 Hyb Chamber with Gasket in Place



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

Figure 29 Dispensing PB2 into Hyb Chamber Reservoir



WARNING

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

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

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

NOTE

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

4 After the 20 minute incubation, remove the MSA3 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.

5 After the 30 minute cool down, pulse centrifuge the MSA3 plate to 280 × g. Remove the foil seal.

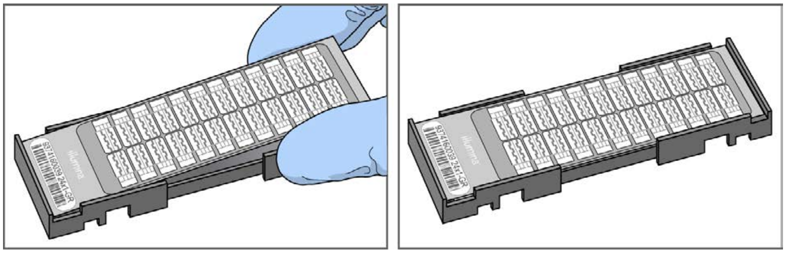
Load BeadChip

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

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

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

Figure 30 Placing BeadChips into Hyb Chamber Inserts



3 Use a single-channel pipette or an adjustable spacer multichannel pipette to load HTS BeadChips. Do not use a standard multichannel pipette because the spacing is not compatible with the HTS BeadChip.

For the Single-Channel Pipette

Using a single-channel precision pipette, dispense 14 µl of each DNA sample onto the appropriate BeadChip section. Make sure that the pipette tip is in the sample inlet before dispensing.

Follow the color-coded sections shown in the chart on the following page for sample loading assistance.

- Load sample A1 from the MSA3 plate into sample inlet A1 of the BeadChip. Make sure that the pipette tip is in the sample inlet before dispensing.
- Load sample B1 from the MSA3 plate into sample inlet B1 of the BeadChip.
- Load sample C1 from the MSA3 plate into sample inlet C1 of the BeadChip.
- Load sample D1 from the MSA3 plate into sample inlet D1 of the BeadChip.
- Continue in this manner, using the color-coded chart until all samples are loaded.

Figure 31 Distributing Sample from the MSA3 Plate with a Single-Channel Pipette

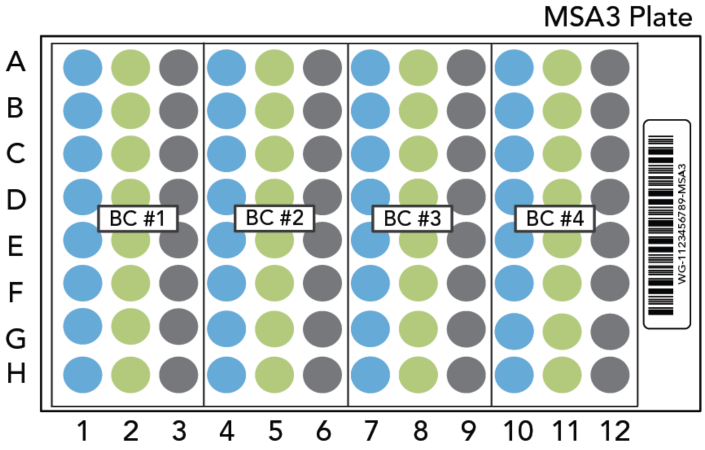
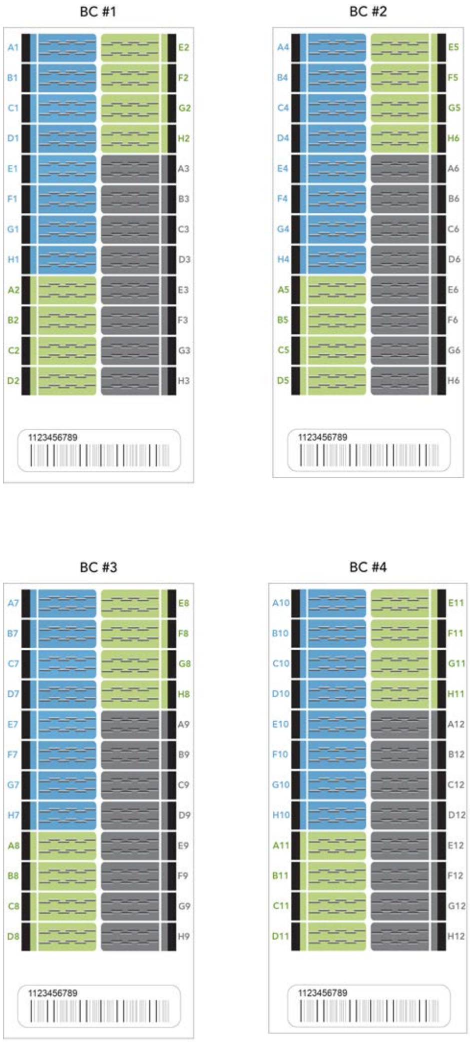


Figure 32 Distributing Sample to the BeadChips with a Single-Channel Pipette



For the MultiChannel Pipette

Using an adjustable spacer multichannel precision pipette, dispense 14 µl of each DNA sample onto the appropriate BeadChip section. Make sure that the pipette tip is in the sample inlet before dispensing.

Follow the color-coded sections shown in the chart on the following page for sample loading assistance.

- Load samples A1-A6 from the MSA3 plate into sample inlet ports A1-A6, on the left side of the BeadChip, in every other inlet port.
  Make sure that the pipette tip is in the sample inlet before dispensing.
- Load samples B1-B6 from the MSA3 plate into sample inlet ports B1-B6, on the left side of the BeadChip, in every other inlet port.
- Load samples C1-C6 from the MSA3 plate into sample inlet ports C1-C6, on the right side of the BeadChip, in every other inlet port.
- Load samples D1-D6 from the MSA3 plate into sample inlet ports D1-D6, on the right side of the BeadChip, in every other inlet port.
- Continue in this manner, using the color-coded chart until all samples are loaded.

Figure 33 Distributing Sample from the MSA3 Plate with an Adjustable Multi--Channel Pipette

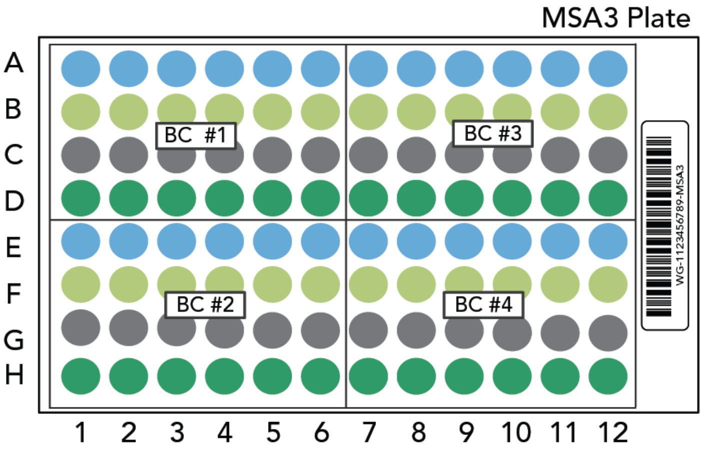
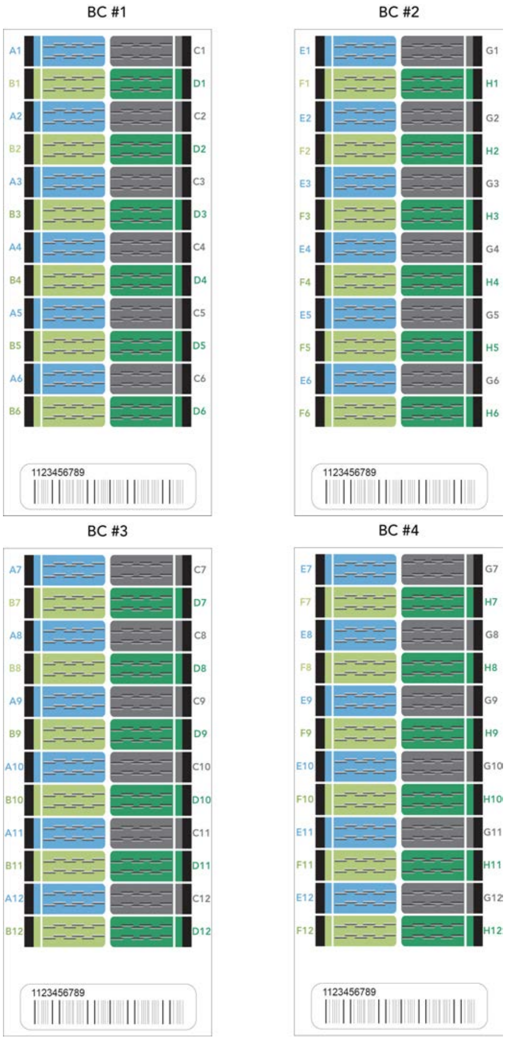


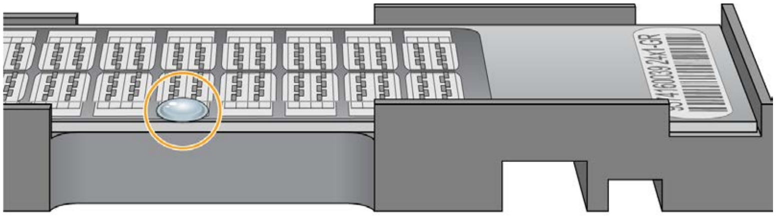
Figure 34 Distributing Sample to the BeadChips with an Adjustable Multichannel Pipette



For Both Single-Channel Pipette and Adjustable Spacer Multichannel Pipette

1. In the Sentrix ID column of the sample sheet, record the sample ID and position on the BeadChip. See the Sample Section Naming Diagram in the Lab Tracking Form.
2. After loading all DNA onto the BeadChip, wait for the sample to disperse over the entire surface.
3. Inspect the loading port to see if a large bolus of liquid remains. Excess sample volume in the BeadChip loading port helps prevent low-intensity areas resulting from evaporation.

   Figure 35 Bolus of Liquid at Loading Port

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

   NOTE
   Do not top off with RA1 (sample hybridization buffer) because RA1 dilutes the sample.

4. Record the top-off activity on the lab tracking form.
5. Heat-seal any residual sample in the MSA3 plate with foil, and store at -15°C to -25°C. Store at -80°C if you do not plan to use the sample again within 24 hours.

Set up Multi BeadChip for Hybridization

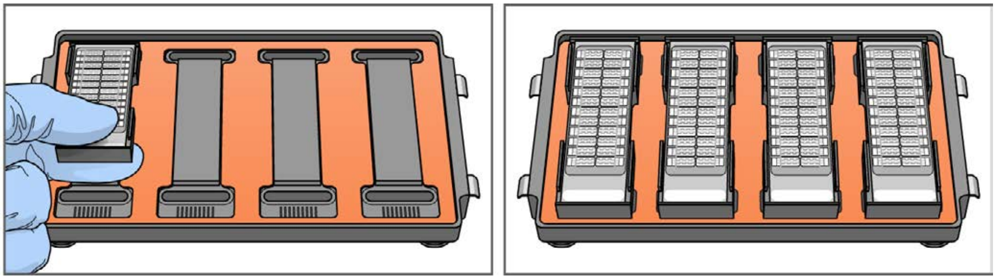
CAUTION
For optimal performance, take care to keep the Hyb Chamber inserts containing BeadChips steady and level when lifting or moving. Avoid shaking and keep parallel to the lab bench always. Do not hold by the sides near the sample inlets.

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

WARNING

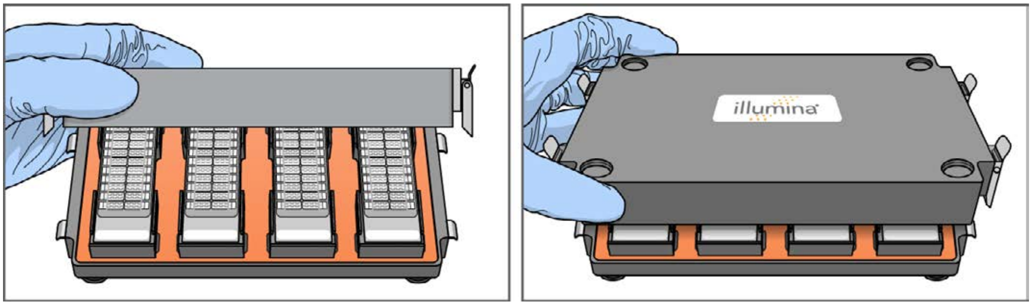
Keep Hyb Chambers at room temperature when you load the BeadChips. Do not precondition the Hyb Chambers in the Illumina Hybridization Oven before loading the BeadChips. If you heat the PB2 and then open the Hyb Chamber to add BeadChips, some of the PB2 evaporates, leading to a change in the osmolality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

Figure 36 Placing Hyb Chamber Inserts into the Hyb Chamber



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

Figure 37 Seating Lid onto Hyb Chamber



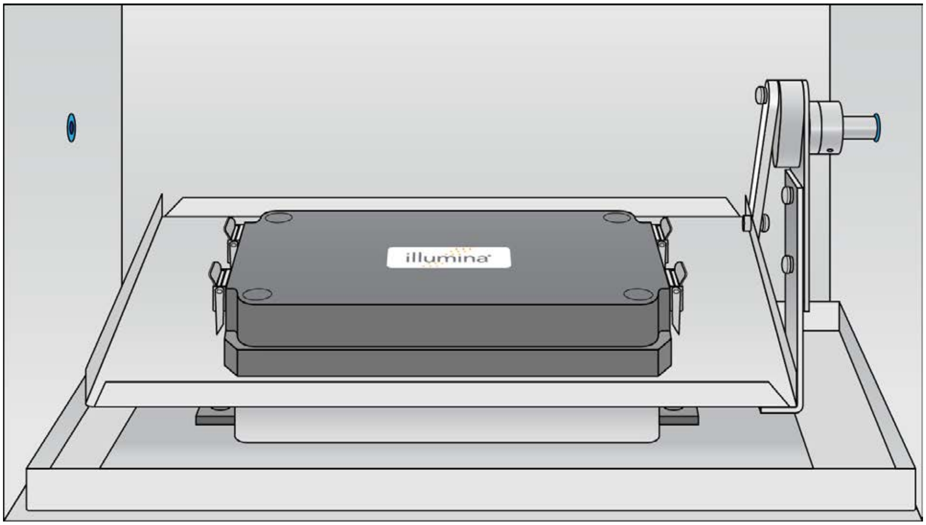
3 Close the clamps on both sides of the Hyb Chamber so that the lid is secure and even on the base (no gaps). It is best to close the clamps in a kitty-corner fashion, closing first the top left clamp, then the bottom right, then the top right followed by the bottom left.

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

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

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

Figure 38 Hyb Chamber Correctly Placed in Hyb Oven



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

Figure 39 Two Hyb Chambers Correctly Placed in Hyb Oven

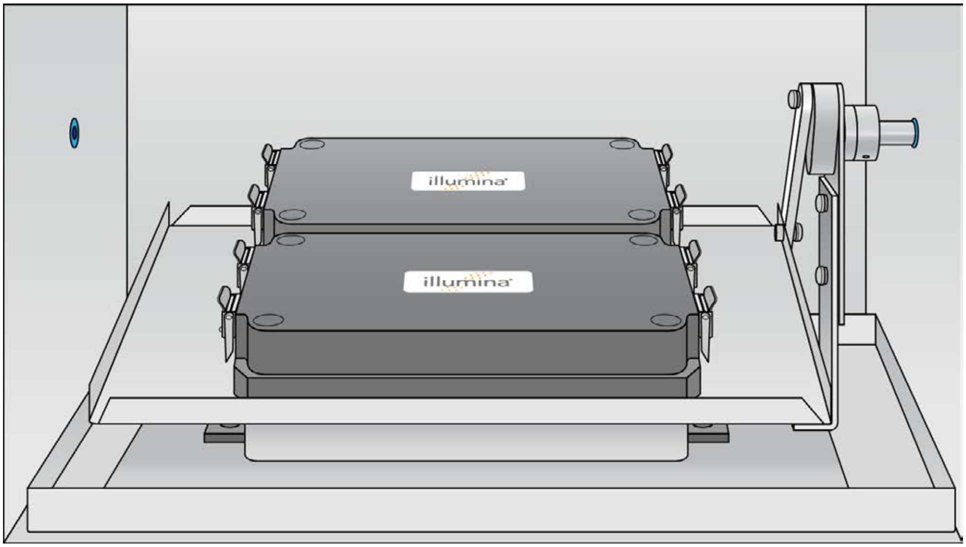
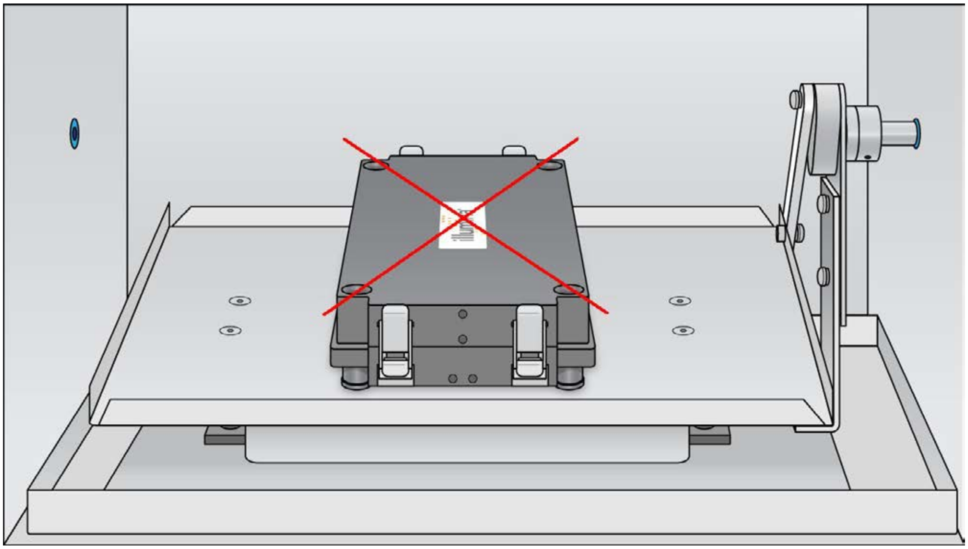


Figure 40 Incorrectly Placed Hyb Chamber



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

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

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

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

Resuspend XC4 Reagent for XStain BeadChip

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

1 Add 330 ml 100% EtOH to the XC4 bottle.
The final volume will be 350 ml. Each XC4 bottle has enough solution to process up to 24 BeadChips.

2 Shake vigorously for 15 seconds.

3 Leave the bottle upright on the lab bench overnight.

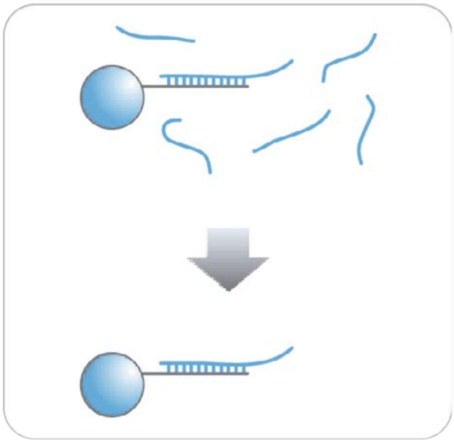
NOTE
If the XC4 was not left to resuspend overnight, you can still proceed with the assay. Add the EtOH and put the XC4 on its side on a rocker to resuspend. Leave it there until the BeadChips are ready for coating.

4 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until it is in complete suspension. After it is resuspended, use XC4 at room temperature.

Wash BeadChip (Post-Amp)

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

Figure 41 Washing BeadChip



Estimated Time

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

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml for 1 to 8 BeadChips 700 ml for 9 to 16 BeadChips 850 ml for 17 to 24 BeadChips	Room temperature	Illumina
Multi-sample BeadChip alignment fixture	1 (per 8 BeadChips)		Illumina
Te-Flow LCG flow-through chambers, with black frames, LCG spacers, LCG glass back plates, and clamps	1 (per BeadChip)		Illumina
Wash dish	2 (up to 8 BeadChips)		Illumina
Wash rack	1 (up to 8 BeadChips)		Illumina

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

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

Preparation

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

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

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

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

Steps to Wash BeadChip

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

Figure 42 Wash Rack in Wash Dish Containing PB1

2 Remove the Hyb Chamber inserts from the Hyb Chambers.

3 Remove BeadChips from the Hyb Chamber inserts one at a time.

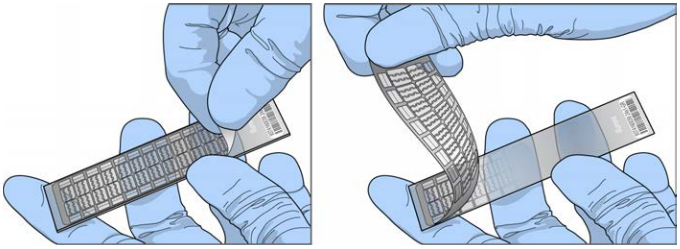
4 Remove the cover seal from each BeadChip.

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

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

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

Figure 43 Removing the Cover Seal



c Discard the cover seal.

CAUTION
Do not touch the arrays!

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

Figure 44 Submerging BeadChips in Wash Dish Containing PB1

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

NOTE
You can use the two 200 ml PB1 wash dishes for up to 24 BeadChips. However, use 150 ml of fresh PB1 for every 8 BeadChips in the Multi-Sample BeadChip Alignment Fixture.

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

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

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

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

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

11 If you are processing more than 8 BeadChips

a Assemble the flow-through chambers for the first 8 BeadChips, as described in the next section, and place them on the lab bench in a horizontal position.

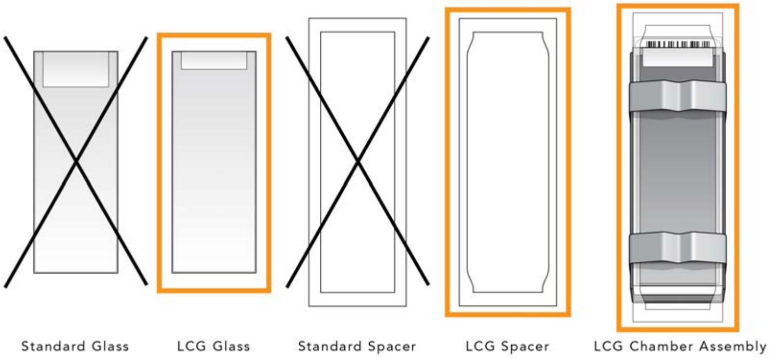
NOTE
Keep the flow-through chambers in a horizontal position on the lab bench until all assembled flow-through chambers are ready to be loaded into the chamber rack. Do not place the flow-through chambers in the chamber rack until all BeadChips are prepared in flow-through chambers.

b Return to this procedure and follow the steps described above to wash the next set of 8 BeadChips.

c Repeat for each remaining set of 8 BeadChips.

Assemble Flow-Through Chambers

NOTE
Confirm that you are using the correct Infinium LCG glass back plates and spacers before assembling the flow-through chambers. Refer to the following image for the correct flow-through chamber components.



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

2 For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture pre-filled with PB1.

Figure 45 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

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

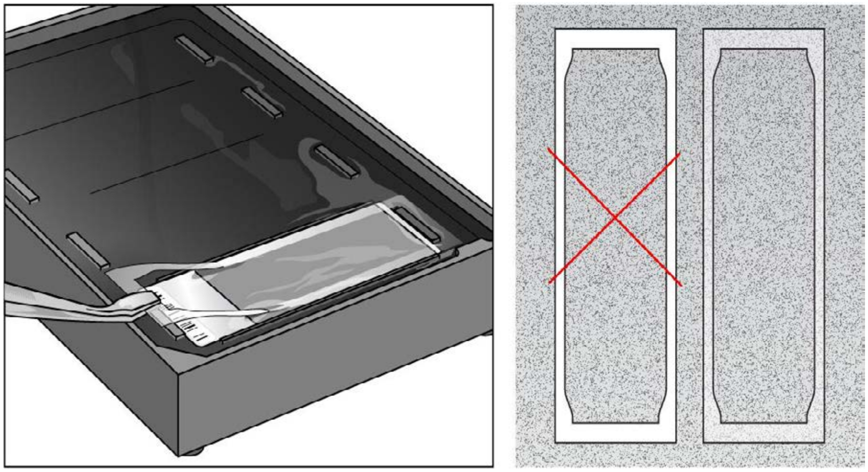
NOTE
Inspect the surface of each BeadChip for residue left by the seal. Use a pipette tip to remove any residue under buffer and be careful not to scratch the bead area.

Figure 46 Placing BeadChip into Black Frame on Alignment Fixture

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

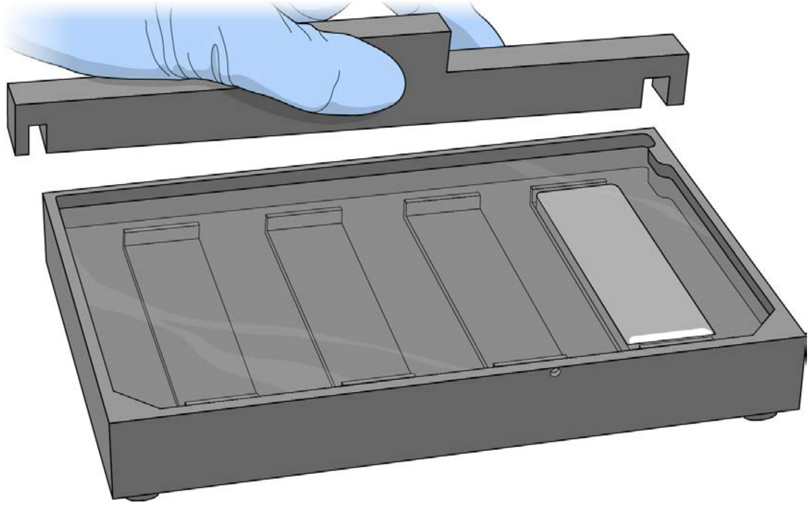
NOTE
Be sure to use the clear plastic spacers, not the white ones.

Figure 47 Placing Clear Plastic Spacer onto BeadChip



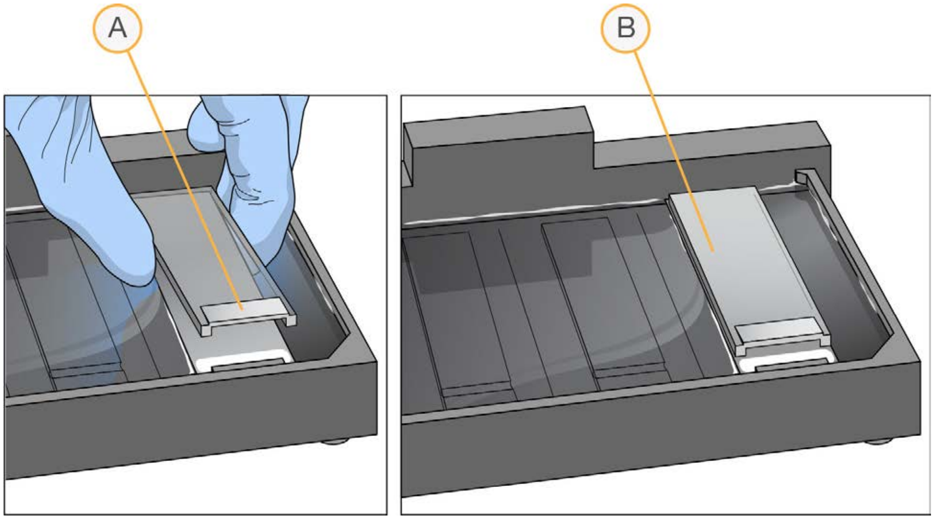
5 Place the alignment bar onto the alignment fixture.
The groove in the alignment bar fits over the tab on the alignment fixture.

Figure 48 Placing Alignment Bar onto Alignment Fixture



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

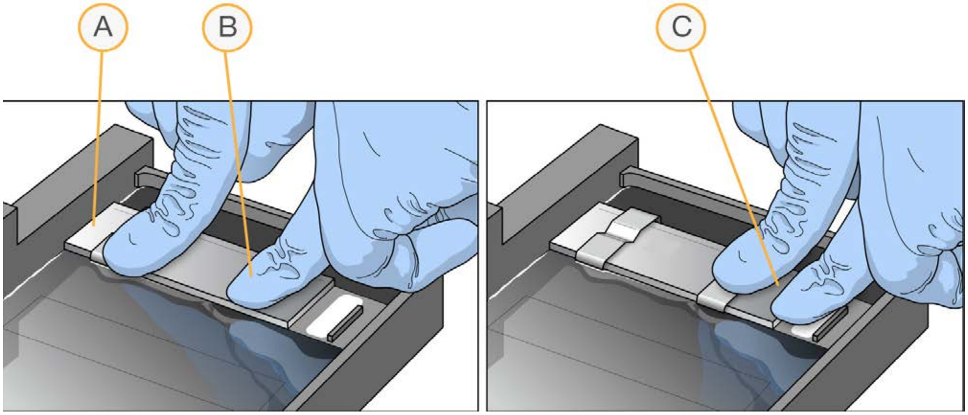
Figure 49 Placing Glass Back Plate onto BeadChip



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

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

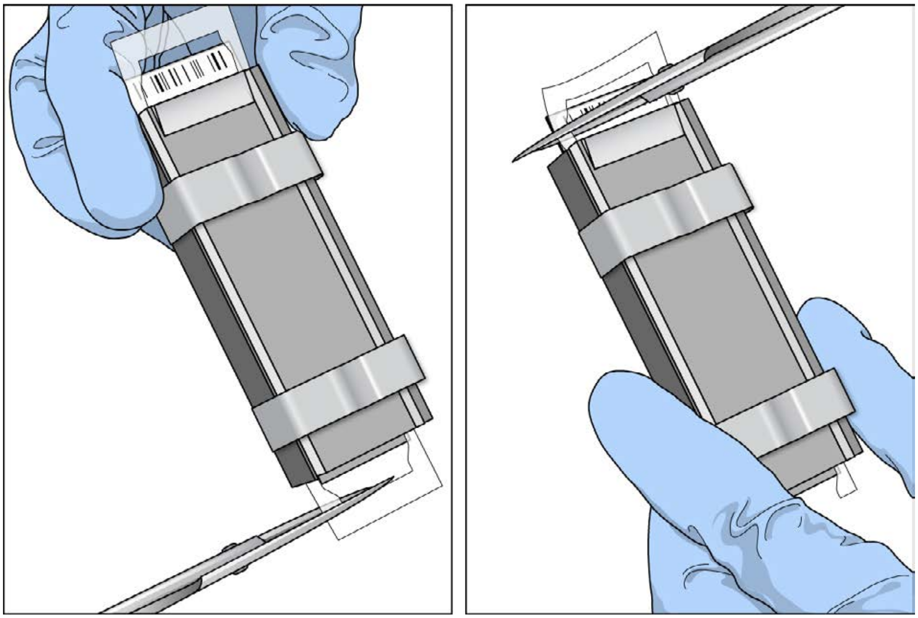
Figure 50 Securing Flow-Through Chamber Assembly with Metal Clamps



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

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

Figure 51 Trimming Spacer Ends from Flow-Through Chamber Assembly



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

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

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

10. Discard unused reagents in accordance with facility standards.

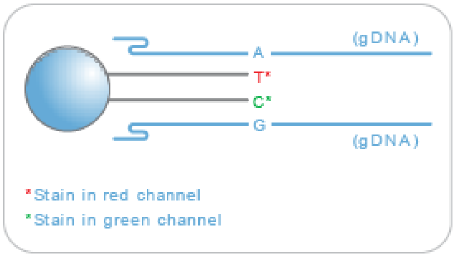
11. Proceed to the next step.

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

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

In this process, you use RA1 reagent to wash away unhybridized and non-specifically hybridized DNA sample. LX1 and LX2 are added to condition the BeadChip surface for the extension reaction. Dispense EML 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 52 Extending and Staining BeadChip



Estimated Time

Hands-on time: ~2 hours and 45 minutes for 8 BeadChips

Dry time: 55 minutes

Consumables

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

Item	Quantity	Storage	Supplied By
LX1	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
LX2	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
EML	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
XC3	50 ml for 1–8 BeadChips 100 ml for 9–16 BeadChips 150 ml for 17–24 BeadChips	Room temperature	Illumina
SML (Make sure that all SML tubes indicate the same stain temperature on the label)	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
ATM	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
PB1	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina
XC4	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina
Alconox Powder Detergent	As needed	Room temperature	General lab supplier
EtOH	As needed	Room temperature	General lab supplier
95% formamide/1 mM EDTA	15 ml for 1–8 BeadChips 17 ml for 9–16 BeadChips 25 ml for 17–24 BeadChips	-15°C to -25°C	General lab supplier

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

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

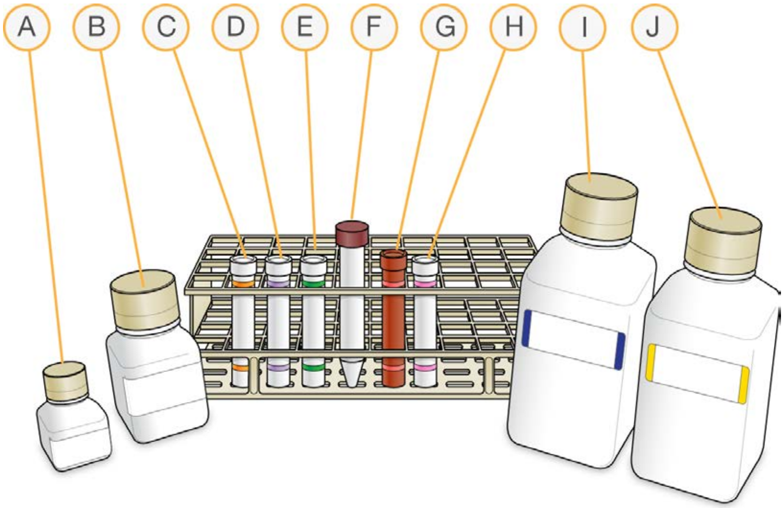
WARNING
This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and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

Preparation

1 RA1 is shipped and stored at -15C° to -25°C. Gradually warm the reagent to room temperature, preferably in a 20C° to 25°C water bath. Gently mix to dissolve any crystals that can present.

2 Place all reagent tubes in a rack in the order you plan to use them. If frozen, allow them to thaw to room temperature, and then gently invert the reagent tubes at least 10 times to mix contents.

Figure 53 XStain BeadChip Reagent Tubes and Bottles



A	RA1
B	XC3
C	LX1
D	LX2
E	EML
F	95% Formamide / 1mM EDTA
G	SML
H	ATM
I	PB1
J	XC4

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

4 On the lab tracking form, record:

- Date/Time
- Operator
- RA1 barcode
- XC3 barcode
- LX1 barcodes
- LX2 barcodes
- EML barcodes

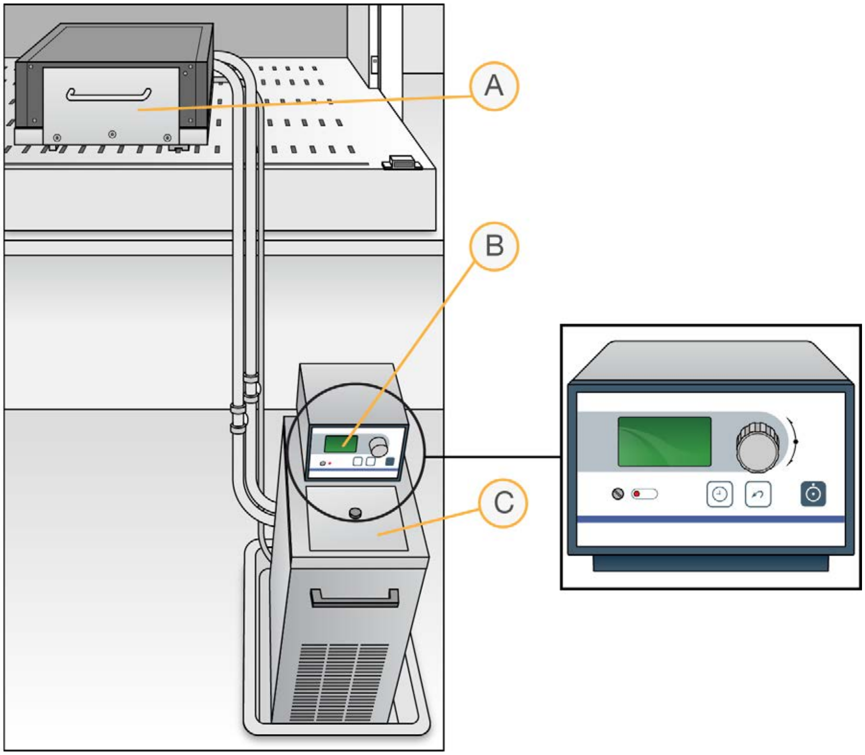
- SML barcodes
- ATM barcodes
- PB1 barcode
- XC4 barcodes

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

Set Up Chamber Rack

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

Figure 54 Water Circulator Connected to Chamber Rack



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

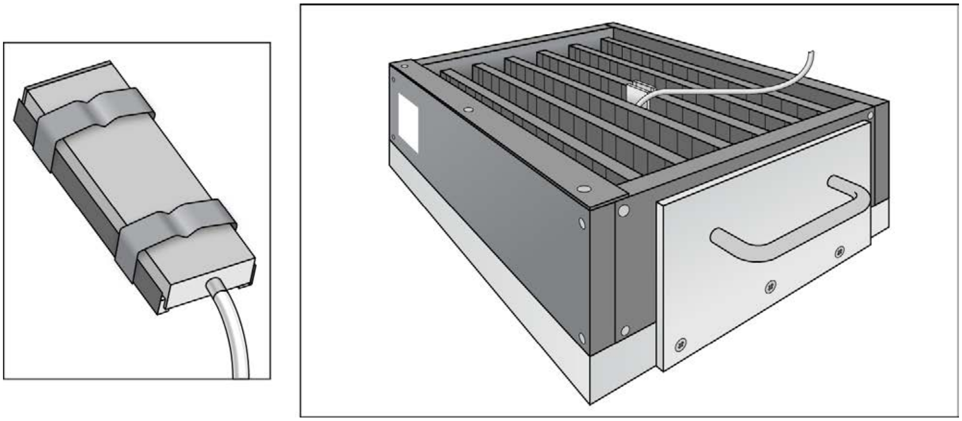
3 The temperature displayed on the water circulator LCD screen may differ from the actual temperature on the chamber rack. Confirm the actual temperature using the temperature probe for the chamber rack.

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

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

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

Figure 55 Illumina Temperature Probe and Temperature Probe in Chamber Rack



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

Single-Base Extension

CAUTION
The remaining steps must be performed without interruption.

NOTE
If you are processing more than 8 BeadChips, complete the reagent dispensing step for each reagent for the first set of 8 BeadChips. Then continue the same reagent dispensing steps for the second set of 8 BeadChips. Finally, move to the last set of 8 BeadChips before you start the incubation time.

Steps marked with an asterisk (*) indicate when to follow this reagent dispensing method.

1 When the chamber rack reaches 44°C, quickly place each flow-through chamber assembly into the chamber rack.

For 4 BeadChips, place the flow-through chambers in every other position, starting at 1, in the first row of the chamber rack. For larger numbers of BeadChips, fill all positions in the first row, then the second and third.

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

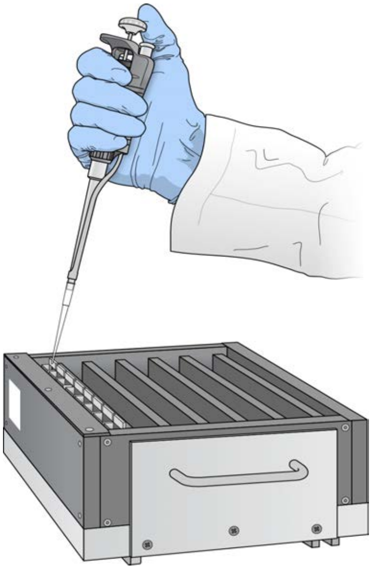
3 On the lab tracking form, record the chamber rack position for each BeadChip.

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

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

5 Into the reservoir of each flow-through chamber, dispense:
   a 150 µl RA1. Incubate for 30 seconds. Repeat 5 times.

Figure 56 Dispensing RA1 into Each Flow-Through Chamber



CAUTION
Pipette tip must not contact BeadChip surface.

   b 225 µl LX1. Repeat one time*. Incubate for 10 minutes.
   c 225 µl LX2. Repeat one time*. Incubate for 10 minutes.
   d 300 µl EML. Incubate for 15 minutes.
   e 250 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat twice.

  f Incubate 5 minutes.
  g Begin ramping the chamber rack temperature to the temperature indicated on the SML tube.
  h 250 µl XC3. Incubate for 1 minute. Repeat twice*.

6 Wait until the chamber rack reaches the correct temperature.

Stain BeadChip

NOTE

If you are processing more than 8 BeadChips, complete the reagent dispensing step for each reagent for the first set of 8 BeadChips. Then continue the same reagent dispensing steps for the second set of 8 BeadChips. Finally, move to the last set of 8 BeadChips before you start the incubation time.

Steps marked with an asterisk (*) indicate when to follow this reagent dispensing method.

1 If you plan to image the BeadChip immediately after the staining process, turn on the scanner now to allow the lasers to stabilize.

2 Into the reservoir of each flow-through chamber, dispense:
  a 250 µl SML. Incubate for 10 minutes.
  b 250 µl XC3. Incubate for 1 minute. Repeat twice*. Wait 5 minutes.
  c 250 µl ATM. Incubate for 10 minutes.
  d 250 µl XC3. Incubate for 1 minute. Repeat twice*. Wait 5 minutes.
  e 250 µl SML. Incubate for 10 minutes.
  f 250 µl XC3. Incubate for 1 minute. Repeat twice*. Wait 5 minutes.
  g 250 µl ATM. Incubate for 10 minutes.
  h 250 µl XC3. Incubate for 1 minute. Repeat twice*. Wait 5 minutes.
  i 250 µl SML. Incubate for 10 minutes.
  j 250 µl XC3. Incubate for 1 minute. Repeat twice*. Wait 5 minutes.

3 Immediately remove the flow-through chambers from the chamber rack and place horizontally on a lab bench at room temperature.

Wash and Coat 8 BeadChips

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

- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates before use.

- In preparation for XC4 BeadChip coating, wash the tube racks and wash dishes thoroughly before and after use. Rinse with DI water. Immediately following wash, place racks and wash dishes upside down on a wash rack to dry.
- Place Kimwipes in three layers on the lab bench. Place a tube rack on top of these Kimwipe layers. Do not place on absorbent lab pads. You will place the staining rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

Equipment Needed

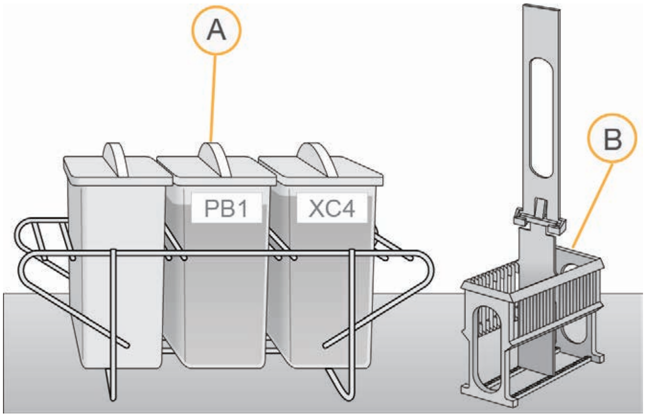
Place the following items on the bench:

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

Steps

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

Figure 57 PB1 and XC4 Wash Dishes with Staining Rack

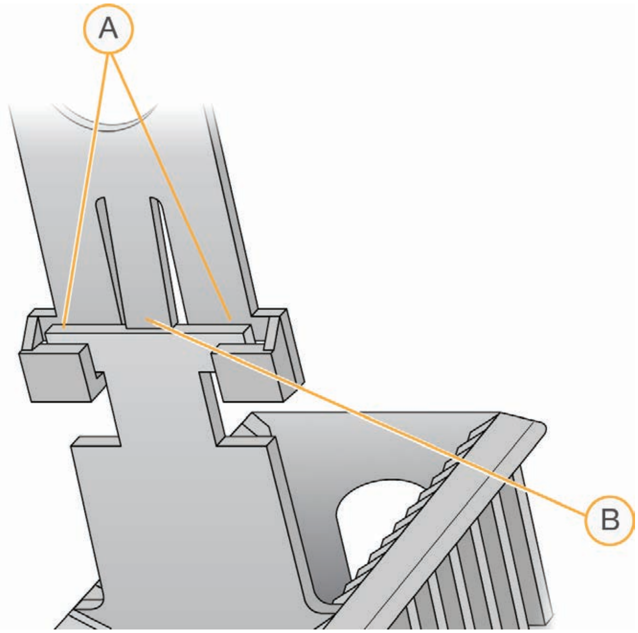


A	Wash Dishes
B	Staining Rack

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

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

Figure 58 Staining Rack Locking Arms and Tab



A	Locking Arms
B	Tab

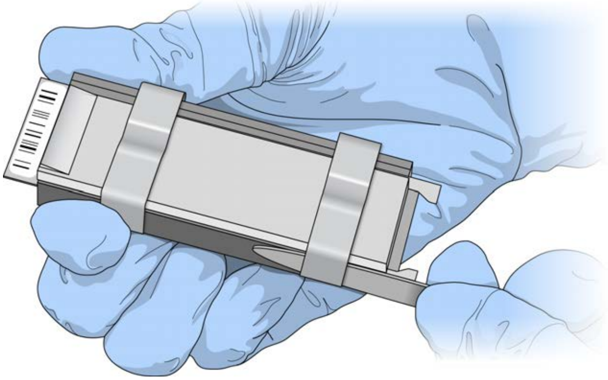
CAUTION
If the staining rack handle is not correctly oriented, the BeadChips can be damaged when you remove the staining rack handle before removing the BeadChips.

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

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

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

Figure 59 Removing the Metal Clamps from Flow-Through Chamber



b Remove the glass back plate.

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

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

e Remove the BeadChip.

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

6 Place the BeadChips in the staining rack while it is submerged in PB1. Put four BeadChips above the staining rack handle and four below. Make sure that the BeadChip barcodes ***face away*** from you and that the locking arms on the handle ***face towards*** you.
If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting each BeadChip.

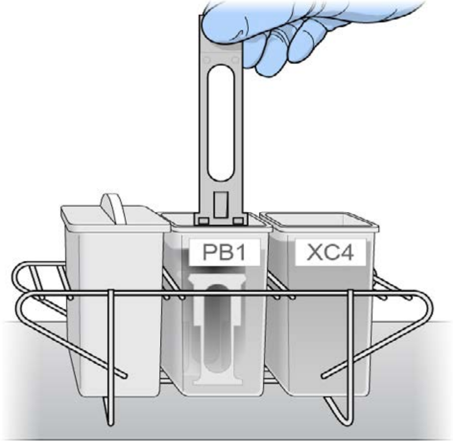
7 Make sure that the BeadChips are completely submerged.

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

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

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

Figure 60 Washing BeadChips in PB1



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

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

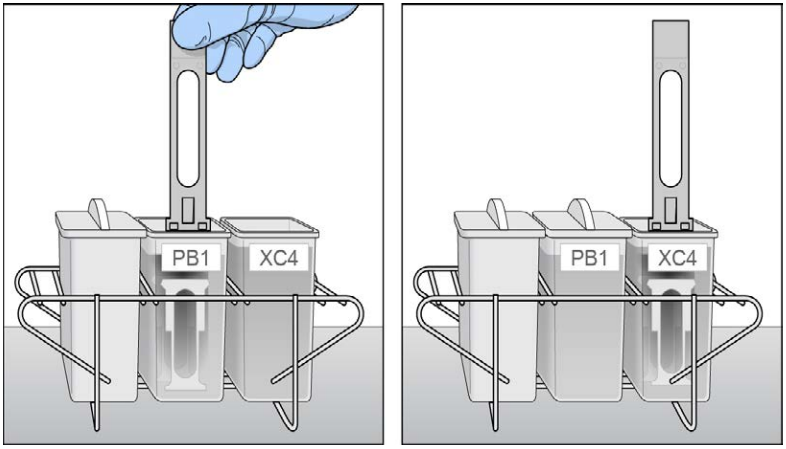
10 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

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

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

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

Figure 61 Moving BeadChips from PB1 to XC4



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

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

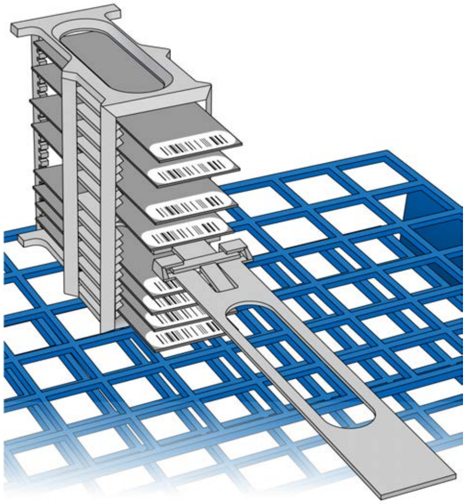
14 Allow the BeadChips to soak for an additional 5 minutes.

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

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

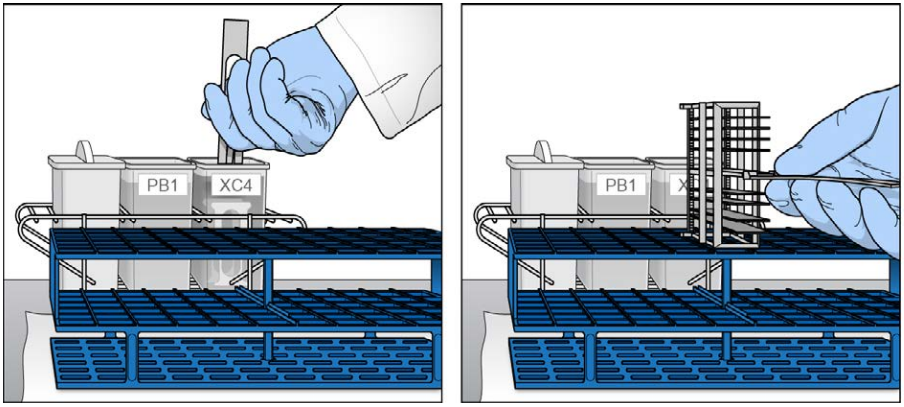
16 Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes *face up*, and the locking arms and tabs *face down*.

Figure 62 Staining Rack in Correct Orientation



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

Figure 63 Moving the Staining Rack from XC4 to Tube Rack



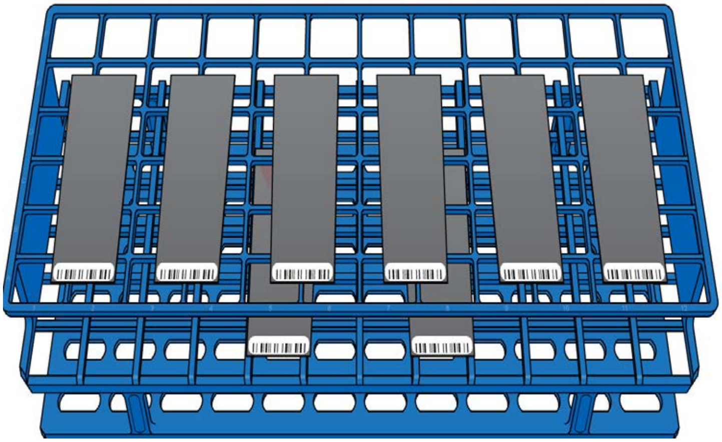
17 For each of the top four BeadChips, working top to bottom:

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.

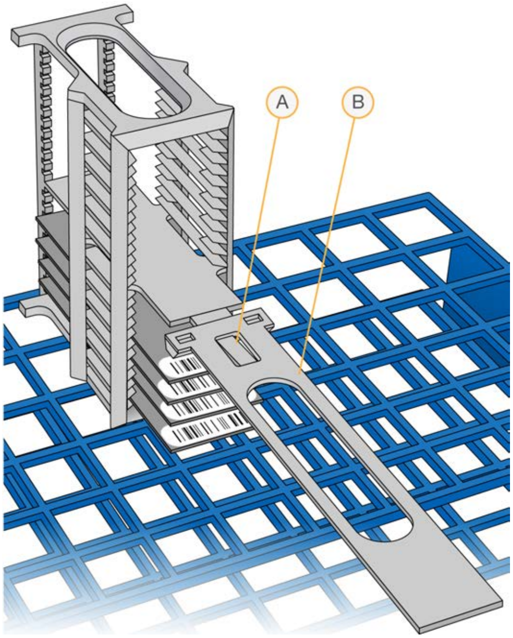
b Place each BeadChip on a tube rack with the barcode ***facing up and towards*** you.

Figure 64 BeadChips on Tube Rack



18 Holding the top of the staining rack in position, gently remove the staining rack handle by grasping the handle between the thumb and forefinger. Push 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 Removing Staining Rack Handle



A Tab
B Handle

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

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

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

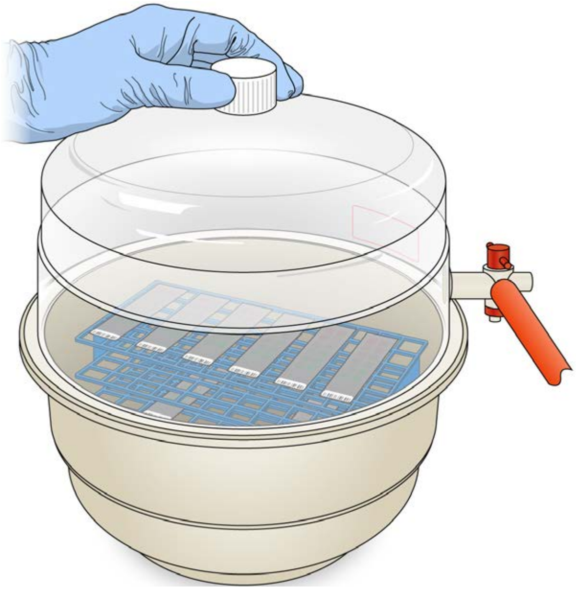
CAUTION
Make sure the vacuum valve is seated tightly and securely.

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

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

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

Figure 66 Testing Vacuum Seal



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

25 Release the vacuum by turning the handle very slowly.

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

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

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

28 If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom 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.
   b Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

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

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

30 Discard unused reagents in accordance with facility standards.

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

Image BeadChip (Post-Amp)

Follow the instructions in the *iScan System User Guide* or *HiScan System User Guide* to scan your BeadChips. Use the **Infinium LCG** scan setting for your BeadChip.

Illumina GenomeStudio

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

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

Automated Protocol

Introduction to Infinium HTS Automated Protocol

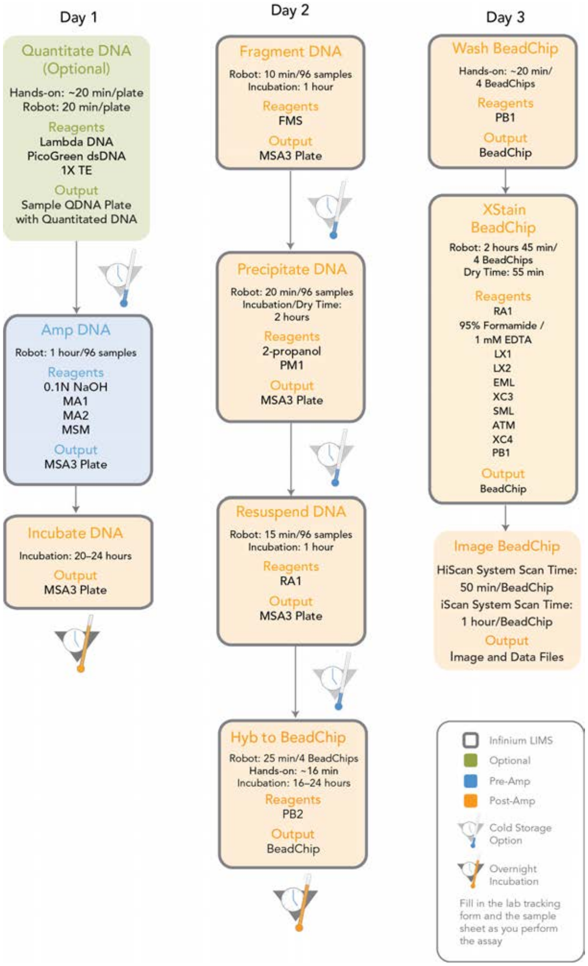
This chapter describes pre- and post-amplification automated laboratory protocols for the Infinium HTS assay. Follow the protocols in the order shown.

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

Infinium HTS Automated Workflow

The following figure graphically represents the Infinium HTS assay automated workflow for 4 BeadChips. These protocols describe the procedure for preparing 96 DNA samples.

Figure 67 Illumina Infinium HTS Automated Workflow



Equipment, Materials, and Reagents

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

User-Supplied Equipment

Table 9 User-Supplied Equipment Infinium HTS Assay, Automated Protocol

Item	Suggested Vendor
Vacuum desiccator (1 per 8 BeadChips processed simultaneously)	VWR, catalog # 24988-197
Vacuum tubing	VWR, catalog # 62995-335
2 Tecan eight-tip robots (one for pre- and one for post-amplification processes)	LIMS (other than Illumina) customers • SC-30-401 (110V) - North America and Japan • SC-30-402 (220V) - EU and Asia Pacific (Except Japan) Illumina LIMS customers • SC-30-403 (110V) - North America and Japan • SC-30-404 (220V) - EU and Asia Pacific (Except Japan)
Auto-desiccator cabinet (Optional—allows scanning of BeadChips up to three days after processing)	VWR, catalog # 74950-342

Illumina-Supplied Equipment

Table 10 Illumina-Supplied Materials Infinium HTS Assay, Automated

Item	Catalog or Part #
Multi-Sample BeadChip alignment fixture	218528
Robot BeadChip Alignment Fixture (6)	222691
Robot Tip Alignment Guide-G	One-piece: catalog # SE-104-1015, Part# 15044220

User-Supplied Materials

Table 11 User-Supplied Materials Infinium HTSAssay, Automated Protocol

Item	Suggested Vendor
96-well, black, flat-bottom Fluotrac 200 plates	Greiner, catalog # 655076
Forceps	VWR, catalog # 25601-008
Aluminum foil	
Foil adhesive seals (Microseal “F”)	MJ Research, catalog # MSF-1001
Reservoir, full, 150 ml	Beckman Coulter, catalog # 372784
Reservoir, half, 75 ml	Beckman Coulter, catalog # 372786
Reservoir, quarter, 40 ml	Beckman Coulter, catalog # 372790
Reservoir frame	Beckman Coulter, catalog # 372795
Tube racks for vacuum desiccator (1 for every 8 BeadChips to be processed simultaneously; must fit internal dimensions of vacuum desiccator)	VWR, catalog # 66023-526
Vacuum source (greater than 508 mm Hg (0.68 bar)	
Vacuum gauge for vacuum desiccator (recommended)	

Illumina-Supplied Materials

- MSA3 barcode labels
- WG#-QNT barcode labels

Illumina-Supplied Reagents

Table 12 Illumina-Supplied Reagents Infinium HTS Assay, Automated Protocol

Item	Part #
ATM—Anti-Stain Two-Color Master Mix	11208317
FMS—Fragmentation solution	11203428
MA1—Multi-Sample Amplification 1 Mix	11202880
MA2—Multi-Sample Amplification 2 Mix	11203401
MSM—Multi-Sample Amplification Master Mix	11203410
PB1—Reagent used to prepare BeadChips for hybridization	11291245
PB2—Humidifying buffer used during hybridization	11191130
PM1—Precipitation solution	11292436
RA1—Resuspension, hybridization, and wash solution	11292441
SML—Superior Two-Color Master Mix	11288046
EML—Two-Color Extension Master Mix	11208309
LX1—XStain BeadChip solution 1	11208288
LX2—XStain BeadChip solution 2	11208296
XC3—XStain BeadChip solution 3	11208392
XC4—XStain BeadChip solution 4	11208430

Quantitate DNA (Optional) (Pre-Amp)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples. You can quantitate up to three plates, each containing up to 96 samples. If you already know the concentration, proceed to *Amplify DNA (Pre-Amp)*.

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

Hands-on time: ~20 minutes per plate

Robot: 20 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	-15°C to -25°C	User
1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA (TE))	See Instructions	Room temperature	General lab supplier
Lambda DNA	See Instructions	2°C to 8°C	User
96-well 0.65 ml microtiter plate	1 per 96 samples		General lab supplier
Fluotrac 200 96-well flat-bottom plate	1 per Standard DNA plate 1 per Sample DNA plate		General lab supplier

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

Preparation

- Thaw PicoGreen to room temperature in a light-impermeable container.
- Follow the instructions for preparing the robot before each use in the *Infinium Lab Setup and Procedures Guide*.
- Thaw the sample DNA plates to room temperature.
- Apply a QDNA barcode label to a new Fluotrac plate for each GS#-DNA plate to be quantified.
- Hand-label the microtiter plate “Standard DNA.”
- Hand-label one of the Fluotrac plates “Standard QDNA.”
- In the sample sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Make a Standard DNA Plate

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

1 Add stock Lambda DNA to well A1 in the plate labeled "Standard DNA" and dilute it to 75 ng/µl in a final volume of 233.3 µl. Pipette up and down several times.

   a Use the following formula to calculate the amount of stock Lambda DNA to add to A1:

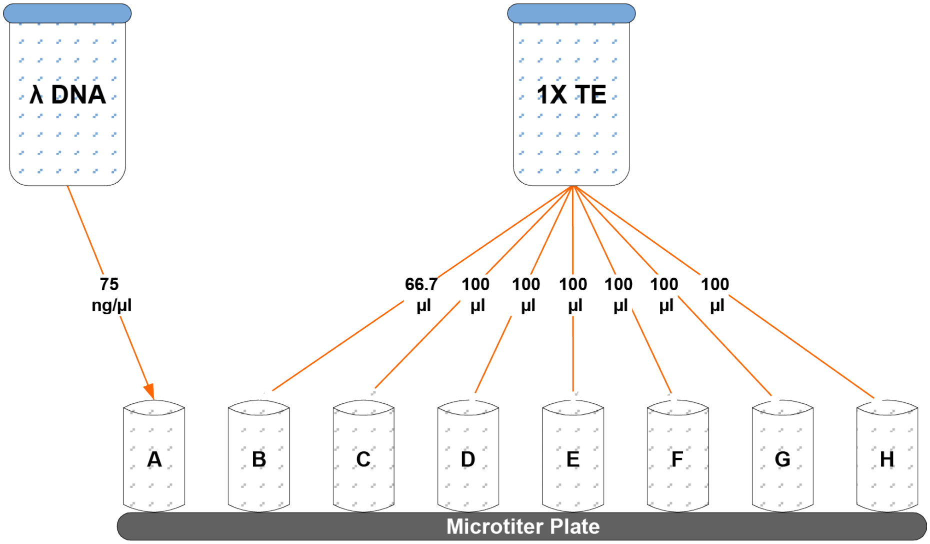
$$\frac{(233.3\ \mu\text{l}) \times (75\ \text{ng}/\mu\text{l})}{(\text{stock Lambda DNA concentration})} = \mu\text{l of stock Lambda DNA to add to A1}$$

   b Dilute the stock DNA in well A1 using the following formula:
   µl of 1X TE to add to A1 = 233.3 µl - µl of stock Lambda DNA in well A1

2 Add 66.7 µl 1X TE to well B1.

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

Figure 68 Dilution of Stock Lambda DNA Standard



4 Transfer 133.3 µl of Lambda DNA from well A1 into well B1. Pipette up and down several times.

5 Change tips. Transfer 100 µl from well B1 into well C1. Pipette up and down several times.

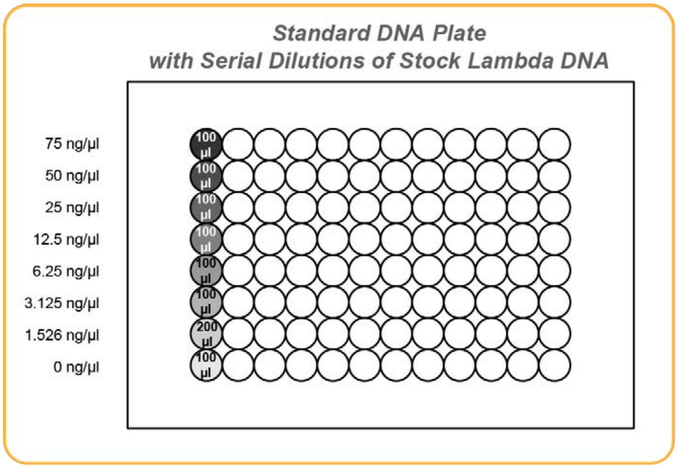
6 Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

Table 13 Concentrations of Lambda DNA

Row-Column	Concentration (ng/µl)	Final Volume in Well (µl)
A1	75	100
B1	50	100
C1	25	100
D1	12.5	100

Row-Column	Concentration (ng/µl)	Final Volume in Well (µl)
E1	6.25	100
F1	3.125	100
G1	1.5262	200
H1	0	100

Figure 69 Serial Dilutions of Lambda DNA



7 Cover the Standard DNA plate with a cap mat.

Dilute PicoGreen

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

CAUTION
Do not use glass containers for the PicoGreen reagent. PicoGreen degrades quickly in the presence of light and can adhere to glass, which lowers its effective concentration in solution and effects the upper response range accuracy.

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

Table 14 Volumes for PicoGreen Reagents

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

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

Create QDNA Standard and Sample Plates

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

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

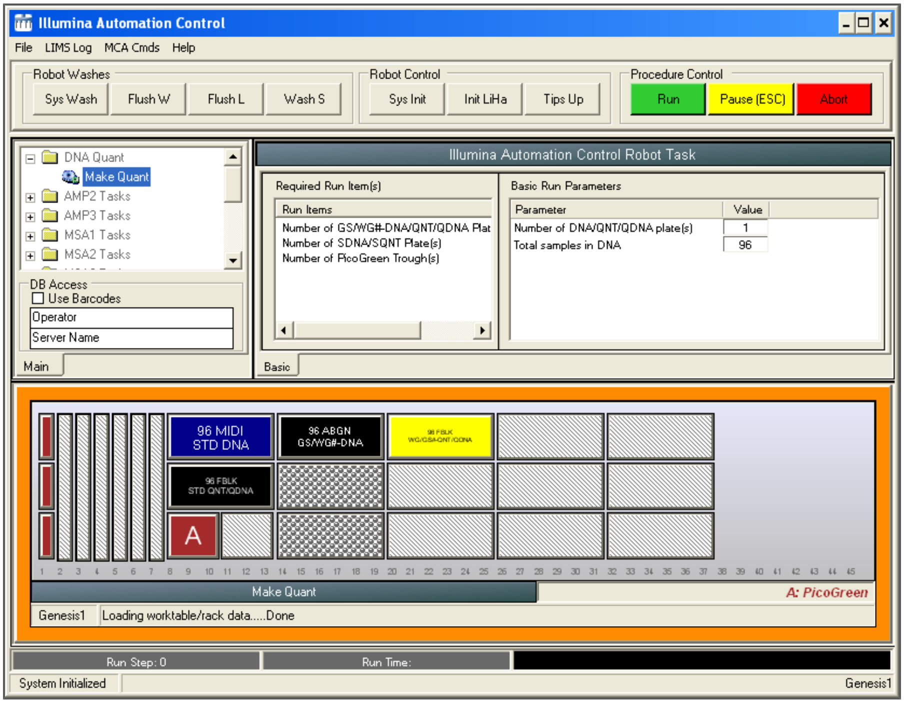
1 At the robot PC, select **DNA Quant | Make Quant**.

2 In the DNA Plate Selection dialog box, select the plate type of the Standard DNA and Sample DNA plates. They should all be MIDI plates, TCY plates or ABGN plates. Roll the mouse pointer over each picture to see a description of the plate.

Figure 70 DNA Plate Selection Dialog Box

3 In the Basic Run Parameters pane, enter the **Number of DNA/QDNA plates** (1, 2, or 3 pairs) and the **Number of DNA Samples**.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

Figure 71 Make QDNA Screen



☐ Use Barcodes

Required Run Item(s)

Run Items
Number of GS/WG#-DNA/QNT/QDNA Plat
Number of SDNA/SQNT Plate(s)
Number of PicoGreen Trough(s)

Basic Run Parameters

Parameter	Value
Number of DNA/QNT/QDNA plate(s)	1
Total samples in DNA	96

Genesis1 Loading worktable/rack data.....Done

System Initialized Genesis1

4 Vortex the GS#-DNA Sample plate at 1450 rpm for 1 minute.

5 Centrifuge the GS#-DNA Sample plate to 280 × g for 1 minute.

6 Vortex the Standard DNA plate at 1450 rpm for 1 minute.

7 Centrifuge the Standard DNA plate to 280 × g for 1 minute.

8 Place the GS#-DNA Sample, Standard DNA, Standard QDNA, and QDNA Sample plates on the robot bed according to the robot bed map. Place well A1 at the top-left corner of its robot bed carrier. Remove any plate seals.

9 Pour the PicoGreen dilution into half reservoir A and place it on the robot bed.

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

11 On the lab tracking form, record the position of the plates on the robot bed.

12 Clear the **Use Barcodes** checkbox.

13 Click **Run**. Observe the beginning of the robot run to ensure there are no problems.
The robot transfers 195 µl of diluted PicoGreen to all Fluotrac plates, then transfers 2 µl aliquots of DNA from Standard DNA plate to Standard QDNA plate and from GS#-DNA plate to sample QDNA plates.
The robot PC sounds an alert and opens a message when the process is complete.

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

15 On the lab tracking form, record:
- Date/Time
- Operator
- Robot
- The QDNA barcode that corresponds to each GS#-DNA barcode
- The Standard QDNA plate that corresponds to each Standard DNA plate

16 After the robot finishes, immediately seal all plates:
   a Place foil adhesive seals over Sample QDNA and Standard QDNA plates.
   b Place cap mats on GS#-DNA Sample and Standard DNA plates.

17 Discard unused reagents in accordance with facility requirements.

18 Store the GS#-DNA and Standard DNA plates at 2° to 8°C or -15° to -25°C.

19 Centrifuge the Sample QDNA Plate and Standard QDNA plates to 280 × g for 1 minute.

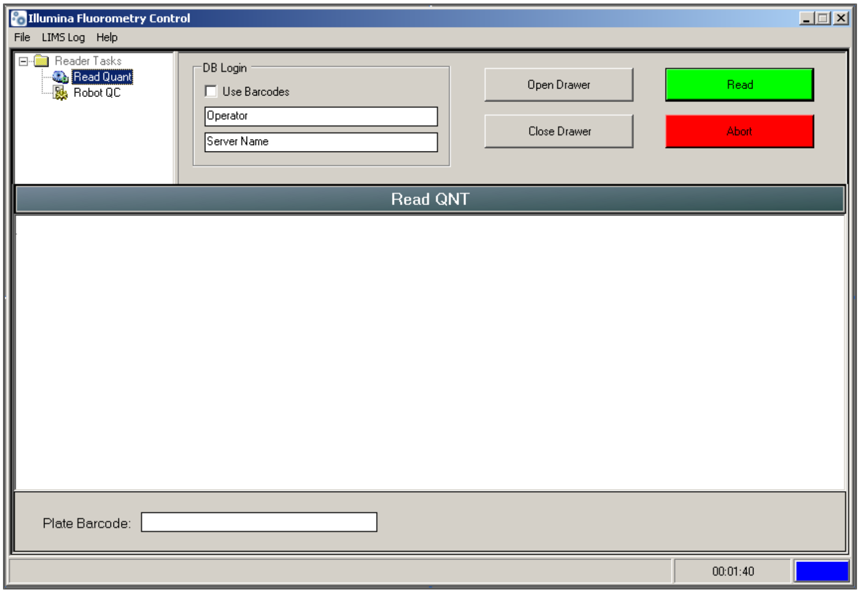
Read the QDNA Plate

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

1 Turn on the spectrofluorometer.

2 At the PC, open the Illumina Fluorometry Analysis program.

Figure 72 Illumina Fluorometry Analysis Main Screen



3 Select **Reader Tasks | Read Quant**.

4 (Other than Illumina LIMS) Clear the **Use Barcodes** checkbox.

5 (Illumina LIMS) Make sure that the **Use Barcodes** checkbox is checked.

6 Click **Read**.

7 (Illumina LIMS) When prompted, log in to the Illumina LIMS database.

8 When asked if you want to read a new Standard plate, click **Yes**.

9 Remove the plate seal and load the Standard QDNA plate into the fluorometry tray. Click **OK**. The spectrofluorometer will read the plate data.

10 Review the data from the Standard QDNA plate. Either accept it and go on to the next step, or reject it. Rejecting the data will stop the Read Quant process.

11 Remove the Standard QDNA place from the spectrofluorometer tray.

12 When prompted, enter the number of plates you want to read (1, 2, or 3). Do not include the Standard QDNA plate in this number. Click **OK**.

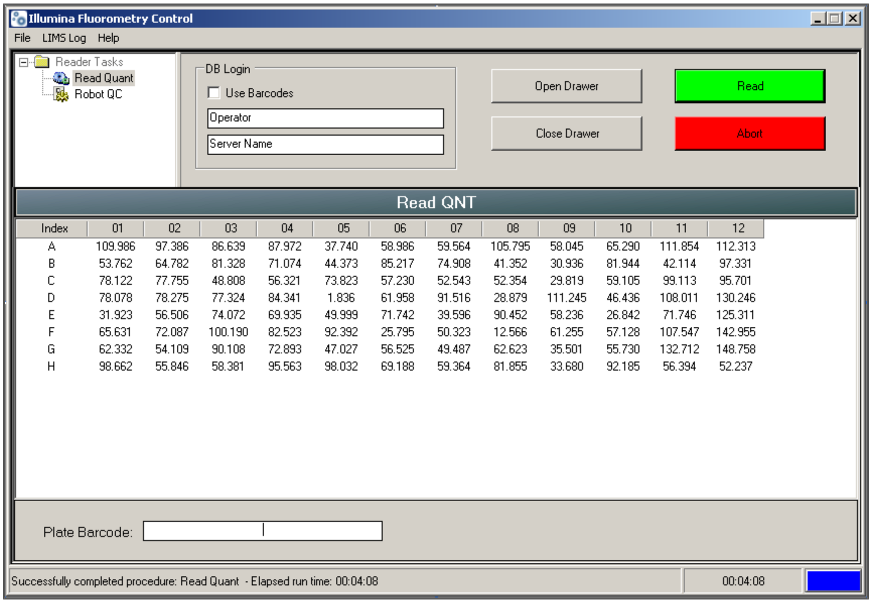
13 When prompted, hand-scan the Sample QDNA plate barcode. Click **OK**.

14 When prompted, remove the plate seal from the Sample QDNA plate and load it into the spectrofluorometer tray, with well A1 at the upper left corner. Click **OK**. The spectrofluorometer will read the plate data.

15 Remove the Standard QDNA plate from the spectrofluorometer tray.

16 When prompted, click **Yes** to review the raw Sample QDNA plate data.

Figure 73 Sample QDNA Data



Index	01	02	03	04	05	06	07	08	09	10	11	12
A	109.986	97.386	86.639	87.972	37.740	58.986	59.564	105.795	58.045	65.290	111.854	112.313
B	53.762	64.782	81.328	71.074	44.373	85.217	74.908	41.352	30.936	81.944	42.114	97.331
C	78.122	77.755	48.808	56.321	73.823	57.230	52.543	52.354	29.819	59.105	99.113	95.701
D	78.078	78.275	77.324	84.341	1.836	61.958	91.516	28.879	111.245	46.436	108.011	130.246
E	31.923	56.506	74.072	69.935	49.999	71.742	39.596	90.452	58.236	26.842	71.746	125.311
F	65.631	72.087	100.190	82.523	92.392	25.795	50.323	12.566	61.255	57.128	107.547	142.955
G	62.332	54.109	90.108	72.893	47.027	56.525	49.487	62.623	35.501	55.730	132.712	148.758
H	98.662	55.846	58.381	95.563	98.032	69.188	59.364	81.855	33.680	92.185	56.394	52.237

Successfully completed procedure: Read Quant - Elapsed run time: 00:04:08

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

- **SQDNA_STD**—Generates the standard curve by plotting the Relative Fluorescence (RF) values measured in the Standard QDNA plate against assumed concentrations in the Standard DNA Plate.
- **QDNA**—Plots the concentration (ng/µl) of each well of the Sample QDNA Plate as derived from the standard curve.

- **Data**—A readout of the raw data values for the Standard QDNA plate and the Sample QDNA Plate.

18 The Illumina Fluorometry Analysis software will prompt you to indicate whether you wish to save the QDNA data shown in an Excel file. Select the option you prefer:

- Click **Yes** to save.
  (Illumina LIMS only) The data will be sent to Illumina LIMS. In Illumina LIMS, the QDNA plate moves into the *Make Single-Use DNA (SUD) Plate (Pre-PCR)* queue.
- Click **No** to delete the quant data. You can read the same plate for quant data repeatedly.

19 If you entered more than one Sample QDNA plate to read, repeat steps 13 to 16 for each additional plate.

20 Discard the QDNA plates and reagents in accordance with facility requirements.

21 Do one of the following:

- Proceed to *Amplify DNA (Post-Amp)*.
- Store the Sample QDNA plate at 2° to 8°C for up to one month.

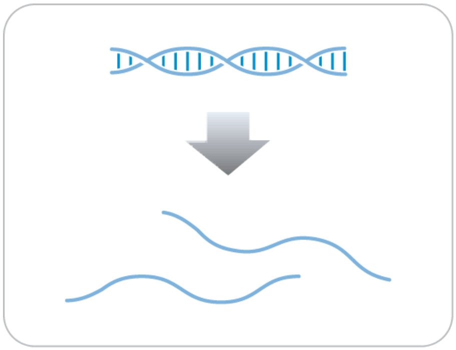
SAFE STOPPING POINT

Now is a good stopping point in the process.

Amplify DNA (Pre-Amp)

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

Figure 74 Denaturing and Neutralizing BCD



Estimated Time

Robot time:

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

Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MA1	1 tube (per 96 samples)	Room temperature	Illumina

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

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

Preparation

1. In preparation for the Incubate DNA process, preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate
2. In the Sample Sheet, enter the Sample_Name and Sample_Plate for each Sample_Well.
3. Apply an MSA3 barcode label to a new MIDI.
4. Thaw MA1, MA2, and MSM tubes to room temperature.
5. Thaw DNA samples to room temperature.

- On the Lab Tracking Form, record the following:
  - Date/Time
  - Operator
  - Robot
  - Batch number
  - Number of samples (48 or 96)
  - DNA plate barcodes

- MSA3 plate barcodes
- MA1 tube barcodes
- MA2 tube barcodes
- MSM tube barcodes

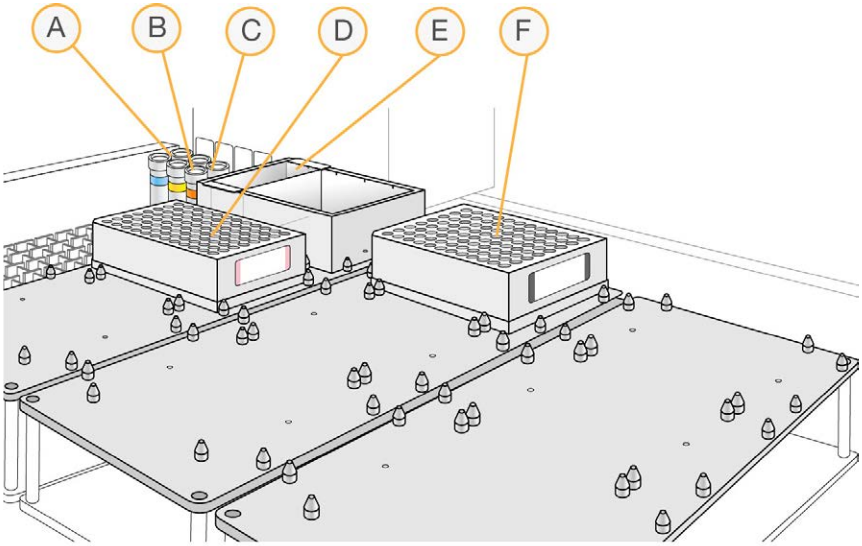
NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Prepare Robot

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

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

Figure 75 Eight-Tip Robot (Make MSA3 Setup)



A MA1 Tube
B MA2 Tube
C MSM Tube
D MSA3 Plate

E NaOH in Quarter Reservoir
F DNA Plate (MIDI)

Steps to Make the MSA3 Plate

1. If you do not already have a WG#-DNA plate, add DNA into one of the following:
   - MIDI plate: 20 µl to each WG#-DNA plate well
   - TCY plate: 10 µl to each WG#-DNA plate well

   Apply a barcode label to the new WG#-DNA plate.
2. At the robot PC, select **MSA3 Tasks | Make MSA3**.
3. In the Plate Selection dialog box, click on the plate type you wish to use. Roll the mouse pointer over each picture to see a description of the plate.

   Figure 76 Selecting the Plate Type

   NOTE
   Do not mix plate types on the robot bed.
4. (Other than Illumina LIMS) Make sure that the **Use Barcodes** checkbox is cleared. In the Basic Run Parameters pane, enter the **Number of DNA samples** (48 or 96) that are in the plate. This value must match the number of DNAs in the plate and the number of DNAs identified in the DNA manifest.

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

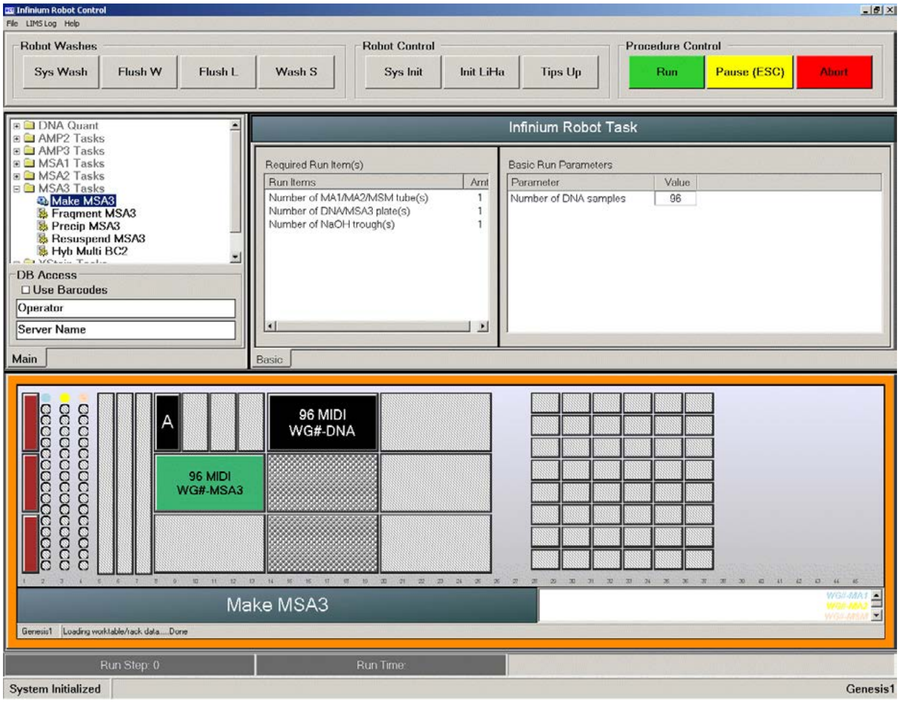
   You can process up to 96 DNA samples per robot run.

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

NOTE

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

Figure 77 Make MSA3 Screen



Required Run Item(s)

Run Items	Amt
Number of MA1/MA2/MSM tube(s)	1
Number of DNA/MSA3 plate(s)	1
Number of NaOH trough(s)	1

Basic Run Parameters

Parameter	Value
Number of DNA samples	96

Genesis1 Loading worktable/rack data.....Done

5 Remove caps from MA1, MA2, and MSM tubes, then place the tubes in the robot standoff tube rack according to the bed map.

6 Add 15 ml 0.1 N NaOH to the quarter reservoir, then place the reservoir on the robot bed according to the bed map.

7 Place the WG#-DNA and MSA3 plates on the robot bed according to the bed map.

8 In the Lab Tracking Form, record the plate positions on the robot bed.

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

10 (Other than Illumina LIMS) At the robot PC, click **Run**.

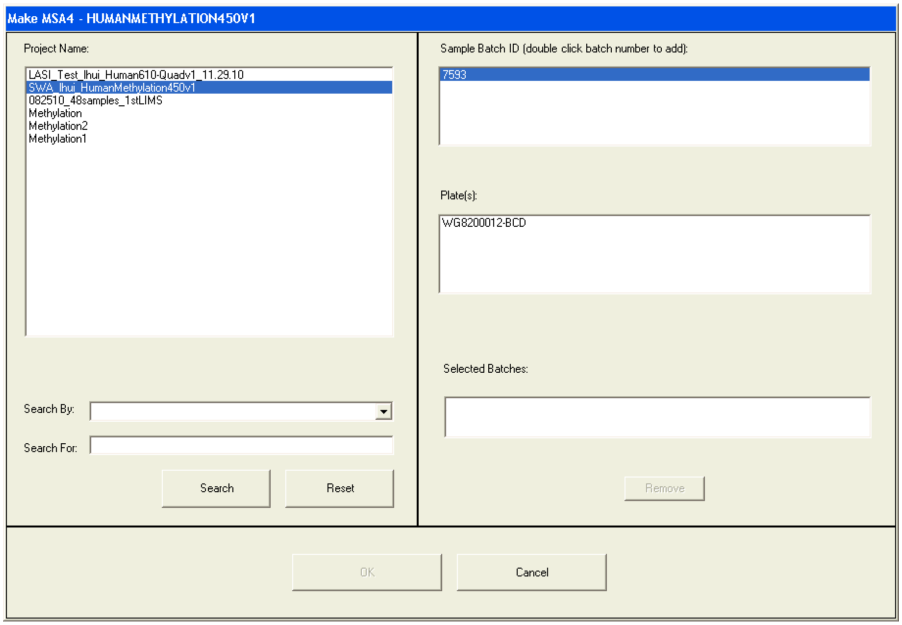
11 (Illumina LIMS) At the robot PC:
  a Make sure that the **Use Barcodes** checkbox is cleared.
  b Click **Run** to start the process. Log in if prompted.

12 After the robot initializes, the **Make MSA3** screen appears after a moment.

13 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 Plates pane.

Figure 78 Make MSA3 Screen with Project and Batch Selected



Make MSA4 - HUMANMETHYLATION450V1

Project Name:
LASI_Test_lhui_Human610-Quadv1_11.29.10
SWA_lhui_HumanMethylation450v1
082510_48samples_1stLIMS
Methylation
Methylation2
Methylation1

Sample Batch ID (double click batch number to add): 7593

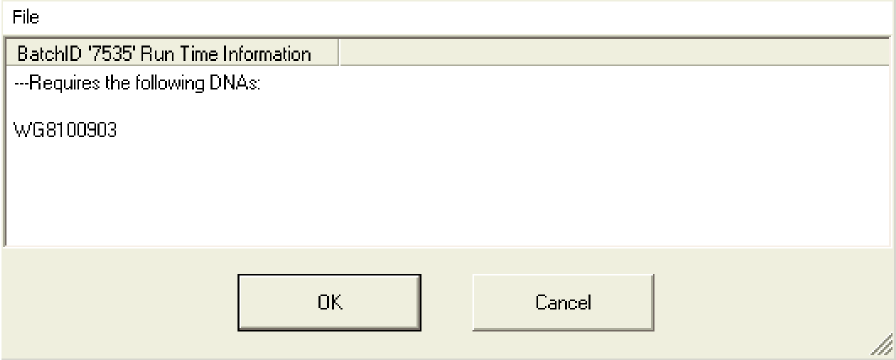
Plate(s): WG8200012-BCD

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

14 (Illumina LIMS only) Select the batch you want to run, and then click **OK**.

15 (Illumina LIMS only) Click **OK** to confirm the required DNAs.

Figure 79 Confirm DNAs



BatchID '7535' Run Time Information

---Requires the following DNAs:

WG8100903

16 When prompted, enter the barcode of each WG#-DNA plate. The robot bed map is updated with the WG#-DNA plate locations.

17 Place the WG#-DNA plates on the robot bed according to the bed map and click OK. The robot begins running when the plates are in place.

18 Observe the robot run to make sure that there are no problems.
After the robot adds the 0.1N NaOH to the DNA in the MSA3 plate, follow the instructions at the prompt.

19 Seal the plate with a cap mat.

20 Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.

21 Centrifuge to 280 × g at 22°C for 1 minute.

22 Remove the cap mat.

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

23 Place the MSA3 plate back on the robot bed in its original position, and then 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 opens a message when the process is complete.

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

25 Remove the MSA3 plate from the robot bed and seal with the 96-well cap mat.

26 Centrifuge to 280 × g for 1 minute.

27 (Illumina LIMS) In the Illumina LIMS left pane, click **Infinium HTS | Incubate** MSA3.

28 Scan the barcode of the MSA3 plate, click **Verify**, and then click **Save**.

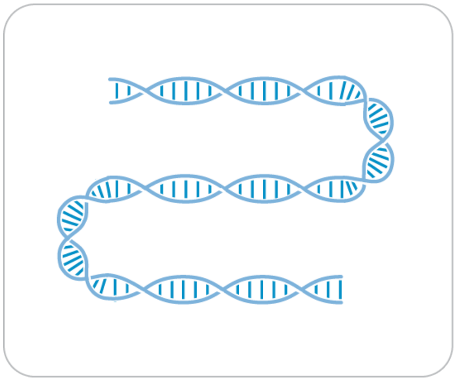
29 Discard unused reagents in accordance with facility standards.

30 Proceed immediately to the next step.

Incubate DNA (Post-Amp)

This process incubates the MSA3 plate for 20–24 hours at 37°C in the Illumina Hybridization Oven. The process uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used when in the Infinium HTS assay.

Figure 80 Incubating DNA to Amplify



Estimated Time

Incubation time: 20–24 hours

Verify MSA3 for Incubation (LIMS only)

1. In the Illumina LIMS left sidebar, click **Infinium HTS | Incubate MSA3**.
2. Scan the barcode of the MSA3 plate, click **Verify**, and then click **Save**.
3. If the MSA3 plate is queued for incubation, a blue confirmation message appears at the top of the window. Proceed to *Steps to Incubate the MSA3 Plate*.
4. If the MSA3 plate is not queued for incubation, a red error message appears at the top of the window. Do ***not*** proceed with incubation. Instead, follow these steps to troubleshoot the problem:
    a. Click the Reports tab in the upper-right corner.
    b. In the left sidebar, click **Tracking 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 Illumina LIMS, see the *Illumina LIMS User Guide*.

Steps to Incubate MSA3 Plate

OVERNIGHT INCUBATION
Incubate MSA3 plate in the Illumina Hybridization Oven for at least 20 hours but no more than 24 hours at 37°C.

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

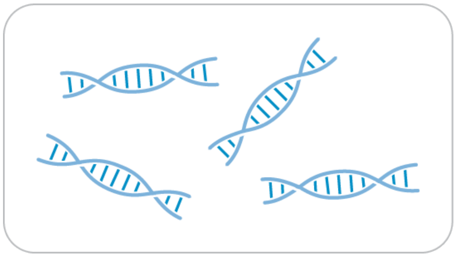
NOTE
To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

2 Proceed to the next step.

Fragment DNA (Post-Amp)

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

Figure 81 Fragmenting DNA



Estimated Time

Robot time:

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

Incubation time: 1 hour

Consumables

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

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

Preparation

1 Preheat the heat block with the MIDI plate insert to 37°C.

2 Thaw FMS tubes to room temperature. Gently invert at least 10 times to mix contents.

3 Pulse centrifuge to 280 × g.

4 Remove the MSA3 plate from the Illumina Hybridization Oven.

5 Remove the cap mat.

6 If you plan to Resuspend the MSA3 plate today, remove the RA1 from the freezer to thaw.

▸ On the lab tracking form, record:

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

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

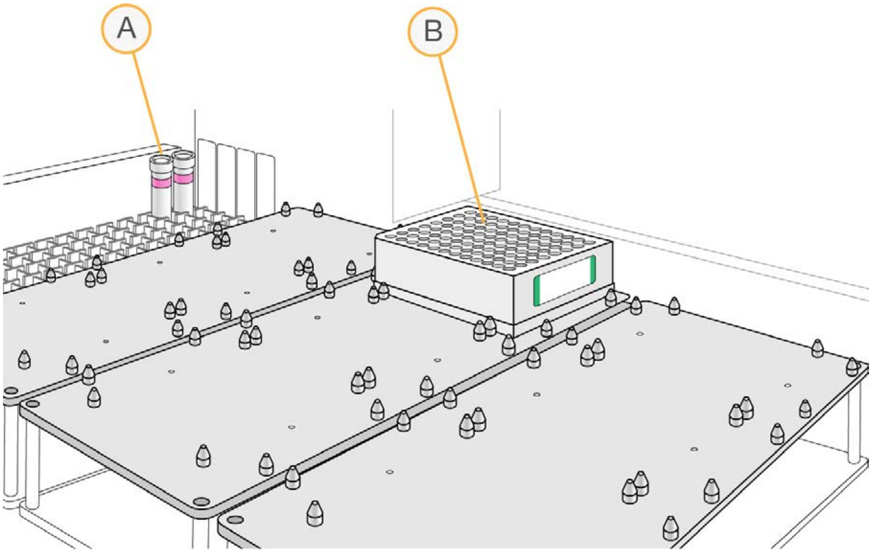
Prepare Robot

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

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

Refer to the figure shown below throughout this protocol.

Figure 82 Tecan Eight-Tip Robot (Fragment MSA3 Setup)



A FMS tubes
B MSA3 plate

Steps to Fragment the MSA3 Plate

1 Centrifuge the MSA3 plate to 50 × g for 1 minute.

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

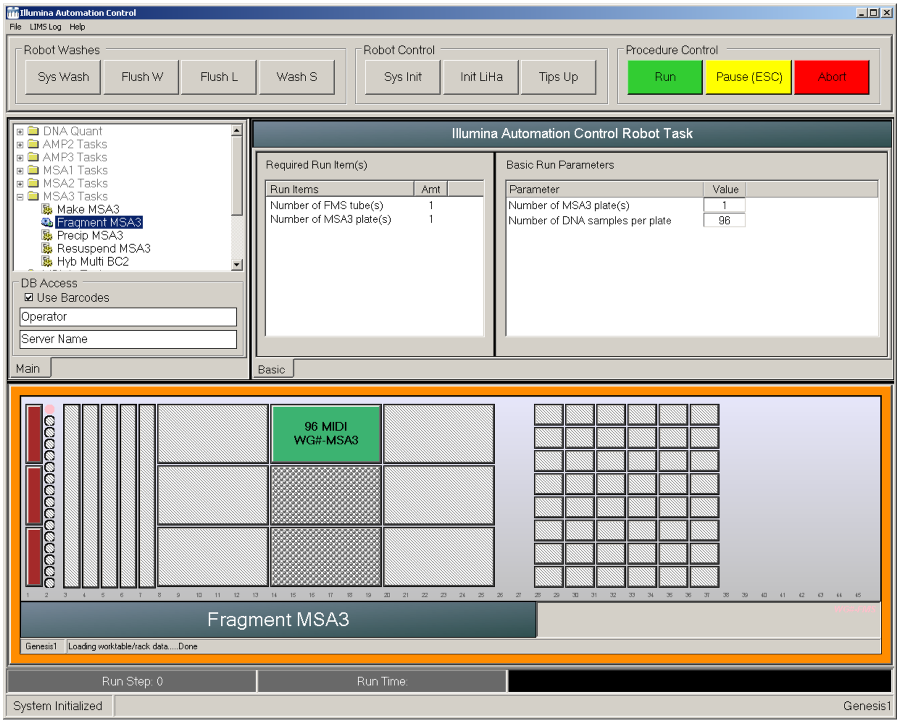
3 (Other than Illumina LIMS) Make sure that the **Use Barcodes** checkbox is cleared. In the **Basic Run Parameters** pane, change the value for **Number of MSA3 plates** and

Number of DNA samples per plate to indicate the number of samples being processed.

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

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

Figure 83 Fragment MSA3 Screen



☑ Use Barcodes

Operator

Server Name

Required Run Item(s)

Run Items	Amt
Number of FMS tube(s)	1
Number of MSA3 plate(s)	1

Basic Run Parameters

Parameter	Value
Number of MSA3 plate(s)	1
Number of DNA samples per plate	96

Loading worktable/rack data.....Done

Run Step: 0

Run Time:

System Initialized

4. Place the MSA3 plate on the robot bed according to the bed map.
5. Remove the plate seal.
6. Place FMS tubes in the robot tube rack according to the bed map. Remove the cap.
7. On the lab tracking form, record the plate positions on the robot bed.

Start the Robot

1. (Other than Illumina LIMS) At the robot PC, click **Run**.
2. (Illumina LIMS) At the robot PC:
    a. Make sure the **Use Barcodes** check box is checked.
    b. Click **Run** to start the process. Log in if prompted.

    The robot PC sounds an alert and displays a message when the process is done.
3. When the robot finishes, click **OK** in the message box.
4. Remove the MSA3 plate from the robot bed and seal it with a cap mat.
5. Vortex at 1600 rpm for 1 minute.
6. Centrifuge to 50 × g for 1 minute at 22°C.
7. Place the sealed plate on the 37°C heat block for 1 hour.
8. On the lab tracking form, record the start and stop times.
9. Discard unused reagents in accordance with facility standards.
10. Do one of the following:
    - Proceed to *Precipitate the MSA3 Plate*. Leave plate in 37°C heat block until you have completed the preparatory steps. Do not leave the plate in the 37°C heat block for longer than 2 hours.
    - If you do not plan to proceed to the next step immediately, store the sealed MSA3 plate at -15°C to -25°C.

SAFE STOPPING POINT

Now is a good stopping point in the process.

Precipitate DNA (Post-Amp)

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

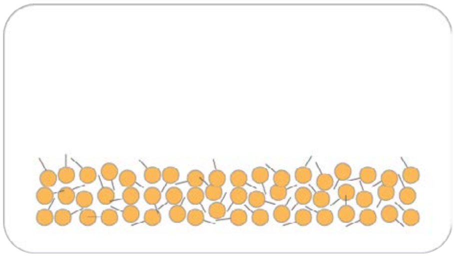


Figure 84 Precipitating DNA

Estimated Time

Robot time:

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

Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	1 tube (per 96 samples)	2°C to 8°C	Illumina
100% 2-propanol	32 ml (per 96 samples)	Room temperature	General lab supplier

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

Preparation

1. Preheat the heat block to 37°C.
2. If you froze the MSA3 plate, thaw it to room temperature, then pulse centrifuge to 50 × g.
3. Thaw PM1 to room temperature. Centrifuge to 280 × g for 1 minute.

- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - PM1 tube barcodes
  - 2-propanol lot number and date opened

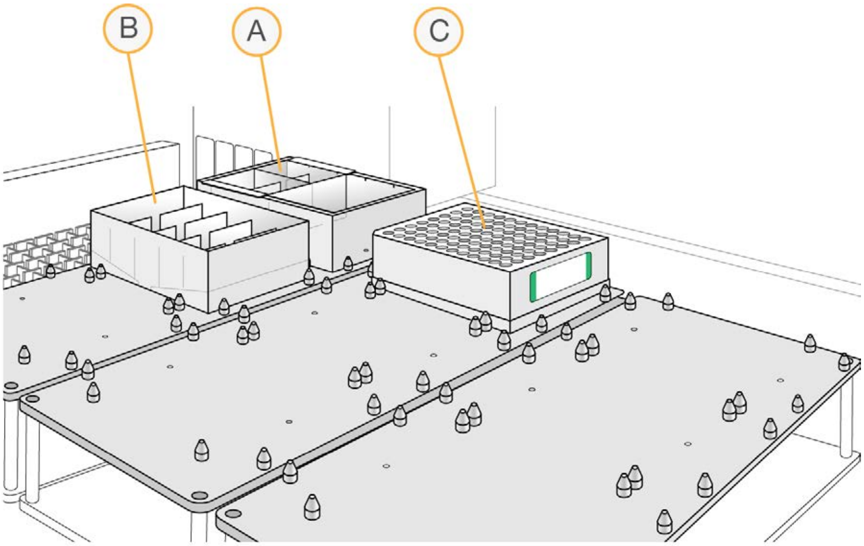
> NOTE
> To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form provided at www.illumina.com/documentation. This form can be filled out and saved online, or printed and filled in by hand.

Prepare Robot

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

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

Figure 85 Tecan Eight-Tip Robot (Precip MSA3 Setup)



A PM1 in Half Reservoir
B 2-propanol in full Reservoir
C MSA3 Plate

Verify MSA3 for Centrifugation (LIMS only)

1. In the Illumina LIMS left sidebar, click **Infinium HTS | Spin MSA3**.
2. Scan the barcodes of the MSA3 plates and click **Verify**, then click **Save**.
3. If the MSA3 plate is queued for centrifugation, a blue confirmation message appears at the top of the window.
4. If the MSA3 plate is not queued for centrifugation, a red error message appears at the top of the window. Do ***not*** proceed with centrifugation. Instead, follow these steps to troubleshoot the problem:
   a. Click the Reports tab in the upper-right corner.
   b. In the left sidebar, click **Tracking 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 Illumina LIMS, see the *Illumina LIMS User Guide*.

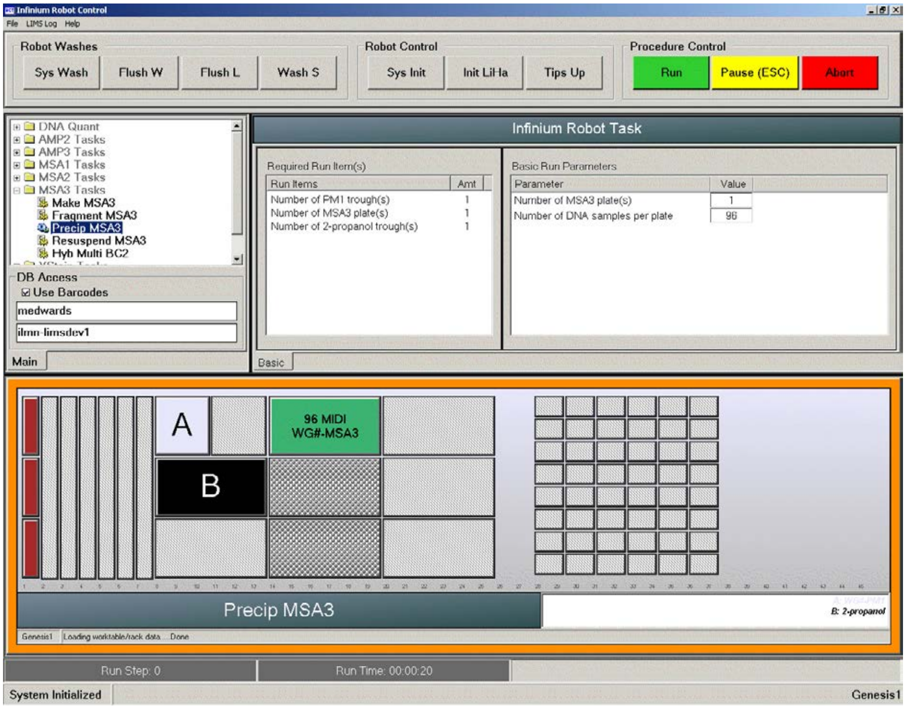
Steps to Precipitate the MSA3 Plate

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

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

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

Figure 86 Precip MSA3 Screen



☑ Use Barcodes

medwards

ilmn-limsdev1

Required Run Item(s)

Run Items	Amt
Number of PM1 trough(s)	1
Number of MSA3 plate(s)	1
Number of 2-propanol trough(s)	1

Basic Run Parameters

Parameter	Value
Number of MSA3 plate(s)	1
Number of DNA samples per plate	96

Genesis1 Loading worktable/rack data.....Done

Run Step: 0

Run Time: 00:00:20

System Initialized

Genesis1

3. Remove the cap mat and place the MSA3 plate on the robot bed according to the bed map.
4. Place a half reservoir in the reservoir frame, according to the robot bed map, and add PM1 as follows:
   - For 96 samples: 1 tube
5. Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:
   - For 96 samples: 32 ml
6. In the lab tracking form, record the plate positions on the robot bed.
7. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. (Other than Illumina LIMS) At the robot PC, click **Run**.
2. (Illumina LIMS) At the robot PC:
   a. Ensure the **Use Barcodes** check box is checked.
   b. Click **Run** to start the process. Log in if prompted.

   The robot PC sounds an alert and opens a message when the process is complete.
3. When prompted, remove the MSA3 plate from the robot bed. Do not click **OK** in the message box yet.
4. Seal the MSA3 plate with the same cap mat removed earlier.
5. Vortex the sealed plate at 1600 rpm for 1 minute.
6. Incubate at 37°C for 5 minutes.
7. Centrifuge to 50 × g at room temperature for 1 minute.

   NOTE
   Set centrifuge to 4°C in preparation for the next centrifuge step.
8. Remove the cap mat and discard it.
9. Place the MSA3 plate back on the robot bed according to the bed map.
10. Click **OK** in the message box.

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

11 Click **OK** in the message box. Remove the MSA3 plate from the robot bed and carefully seal with a ***new, dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.

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

13 Incubate at 4°C for 30 minutes.

14 (Illumina LIMS) In the Illumina LIMS left sidebar, click **Infinium HTS | Spin MSA3**. At the robot PC, click **Run**.

15 Place the sealed MSA3 plate in the centrifuge opposite another plate of equal weight.

Figure 87 Sealed MSA3 Plate and Plate of Equal Balance in Centrifuge

16 Centrifuge to 3,000 × g at 4°C for 20 minutes. Immediately remove the MSA3 plate from centrifuge.

CAUTION

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

17 Remove the cap mat and discard it.

18 Quickly invert the MSA3 plate and drain the liquid onto an absorbent pad to decant the supernatant. Then smack the plate down on a dry area of the pad, avoiding the liquid that was drained onto the pad.

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

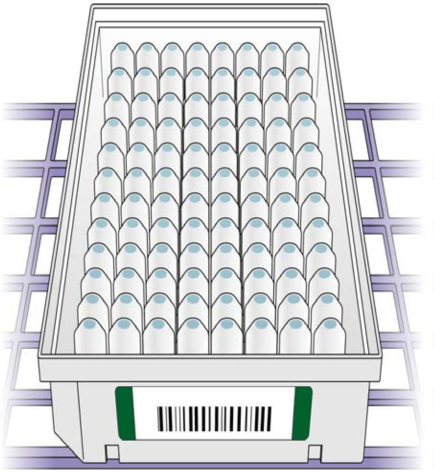
CAUTION

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

20 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.

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

Figure 88 Uncovered MSA3 Plate Inverted for Air Drying



CAUTION

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

21 Record the start and stop times onthe lab tracking form.

22 Discard unused reagents in accordance with facility standards.

23 Do one of the following:
- Proceed to *Resuspend DNA (Post-Amp)*.
- If you do not plan to proceed to the next step immediately, seal the MSA3 plate with a new cap mat and store at -15°C to -25°C.

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

Resuspend DNA (Post-Amp)

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

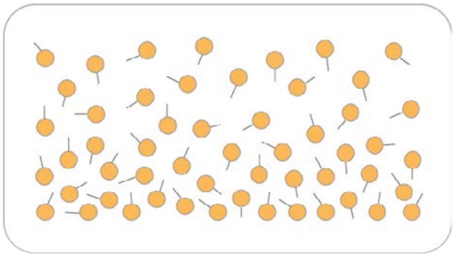


Figure 89 Resuspending DNA

Estimated Time

Robot time:

- 15 minutes for 96 samples

Incubation time: 1 hour

Consumables

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

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

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

Preparation

1. RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20C° to 25°C water bath. Gently mix to dissolve any crystals that may be present.
1. If you stored the MSA3 plate at -15°C to -25°C, thaw it to room temperature. Remove the cap mat and discard it.
2. Preheat the Illumina Hybridization Oven to 48°C.
3. Preheat the heat sealer. Allow 20 minutes.

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

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

Use Fresh RA1 Reagent for Each Step

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

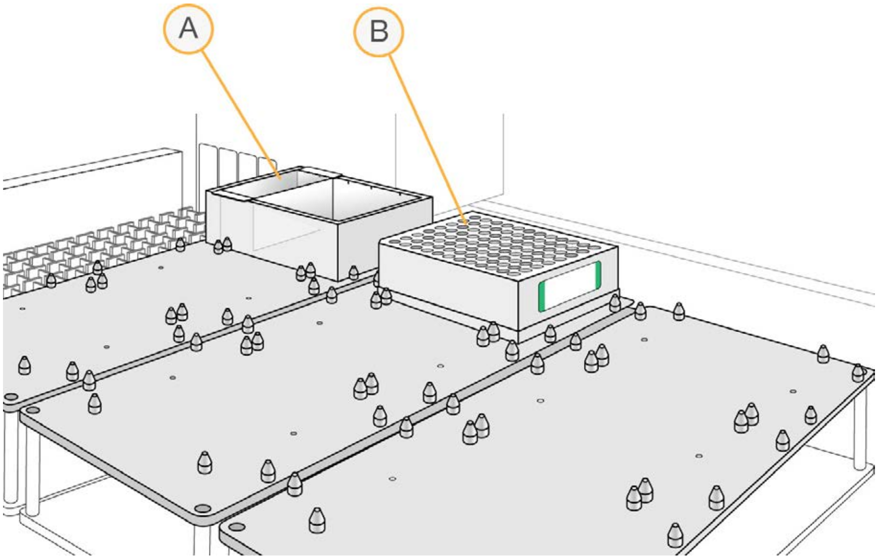
To make best use of RA1, only pour out the amount needed for the current step. If you plan to perform additional assay steps requiring RA1 that same day, then leave the remaining thawed reagent in the original, closed bottle at room temperature until it is needed. Otherwise, follow the standard RA1 storage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

Prepare Robot

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

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

Figure 90 Tecan Eight-Tip Robot (Resuspend MSA3 Setup)



A RA1 in Quarter Reservoir
B MSA3 Plate

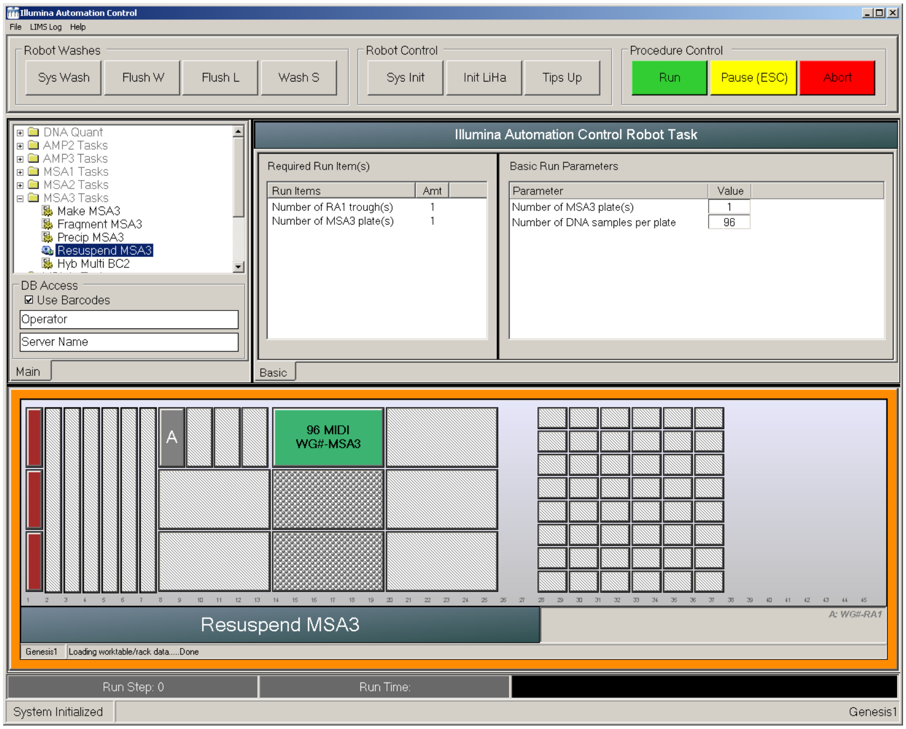
Steps to Resuspend the MSA3 Plate

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

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

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

Figure 91 Resuspend MSA3 Screen



Required Run Item(s)

Run Items	Amt
Number of RA1 trough(s)	1
Number of MSA3 plate(s)	1

Basic Run Parameters

Parameter	Value
Number of MSA3 plate(s)	1
Number of DNA samples per plate	96

Genesis1 Loading worktable/rack data.....Done

System Initialized Genesis1

3 Place the MSA3 plate on the robot bed according to the bed map.

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

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

6 Make sure that all items are placed 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 (Other than Illumina LIMS) At the robot PC, click **Run**.

2 (Illumina LIMS) At the robot PC:

   a. Ensure the **Use Barcodes** check box is checked.
   b. Click **Run** to start the process. Log in if prompted.

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

3. Click **OK** in the message box. Remove the MSA3 plate from the robot bed.
4. Apply a foil seal to the MSA3 plate by firmly holding the heat sealer block down for 5 full seconds.
5. Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
6. In the lab tracking form, record the start and stop times.
7. Vortex the plate at 1800 rpm for 1 minute.
8. Pulse centrifuge to 280 × g.

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

9. Discard unused reagents in accordance with facility standards.
10. Do one of the following:
    - Proceed to *Hybridize to BeadChip (Post-Amp)*. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
    - If you do not plan to proceed to the next step immediately, store the sealed MSA3 plate at -15°C to -25°C for no more than 24 hours. Store at -80°C if storing for more than 24 hours. Store RA1 at -15°C to -25°C.

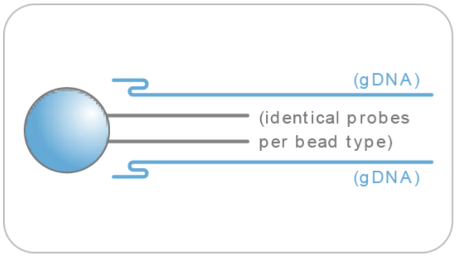
SAFE STOPPING POINT
Now is a good stopping point in the process.

Hybridize to BeadChip (Post-Amp)

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

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 92 Hybridizing DNA to BeadChip



Estimated Time

Robot time:

- 24x1 HTS BeadChip: ~25 minutes for 4 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	1 tube	Room temperature	Illumina
BeadChips	4		Illumina
Hyb chambers	1		Illumina

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

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

Preparation

1 If frozen, thaw MSA3 plate to room temperature, and then pulse centrifuge the MSA3 plate to 280 × g.

2 Preheat the heat block to 95°C.

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

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

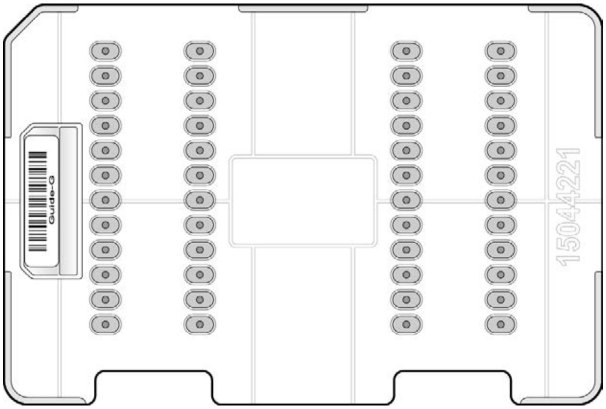
5 On the lab tracking form, record:
- Date/Time
- Operator
- PB2 tube lot number

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

Prepare the Robot Tip Alignment Guide

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

Figure 93 Guide-G Robot Tip Alignment Guide



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

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

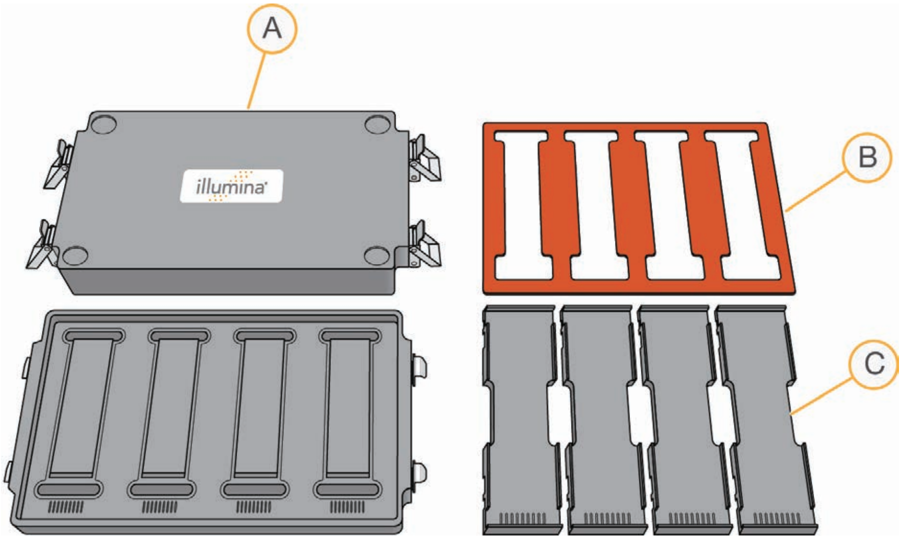
Assemble the Hybridization Chambers

1 Prepare the hyb chambers.

Place the following items on the bench top per 96 samples:

- For the 24 x 1 HTS BeadChip:
  - BeadChip hyb chambers (1)
  - Hyb chamber gaskets (1)
  - Robot BeadChip alignment fixtures (2)
  - BeadChip hyb chamber inserts (4)

Figure 94 BeadChip Hyb Chamber Components

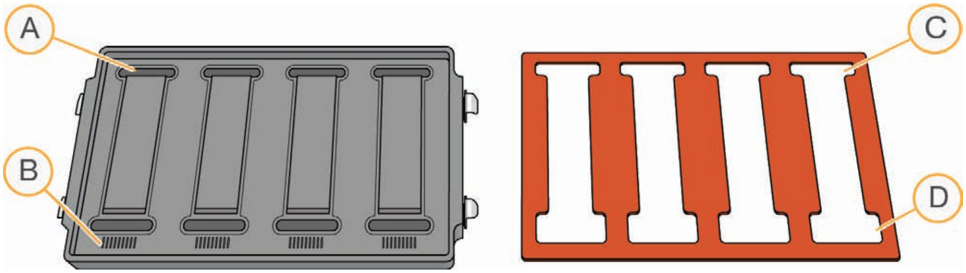


A BeadChip Hyb Chambers
B Hyb Chamber Gaskets
C Hyb Chamber Inserts

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

a Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers.
  - Match the wider edge of the Hyb Chamber Gasket to the barcode-ridge side of the Hyb Chamber.

Figure 95 Hyb Chamber and Gasket



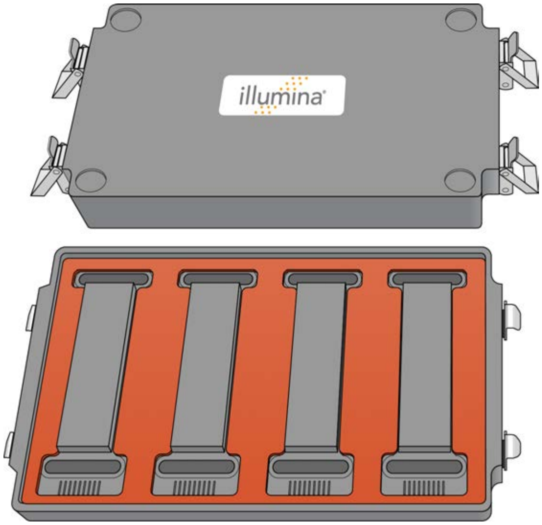
A Reservoirs
B Barcode Ridges
C Narrower Edges
D Wider Edges

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

Figure 96 Placing Gasket into Hyb Chamber

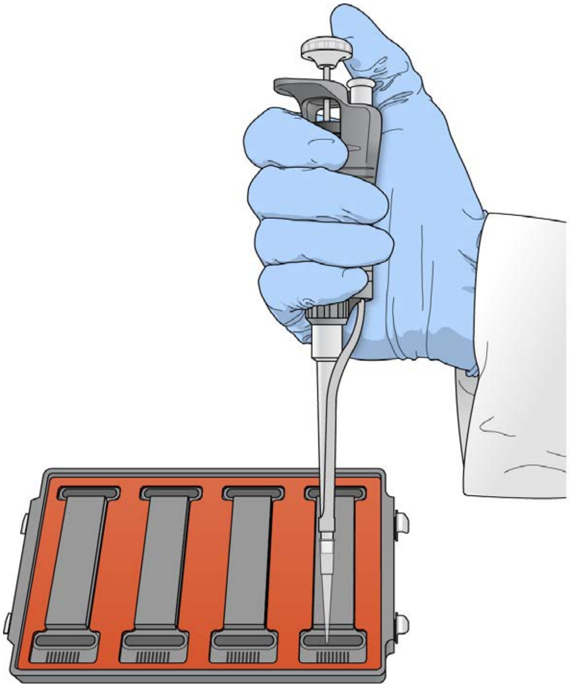
— Make sure the Hyb Chamber gaskets are properly seated.

Figure 97 Hyb Chamber with Gasket in Place



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

Figure 98 Dispensing PB2 into Hyb Chamber Reservoir



WARNING

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

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

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

NOTE

You can also prepare the hyb chambers later, during the 30 minute cool down.

2 Place the hyb chamber inserts into the hyb chambers.

3 Remove the BeadChips from 2°C to 8°C storage but do not unpackage.

4 (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Infinium HTS | Confirm BeadChips for Hyb**.

5 Scan the barcode of the MSA3 plate and all the BeadChips you plan to hybridize with the plate. Click **Verify**.

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

7 After the 20 minute incubation, remove the MSA3 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.

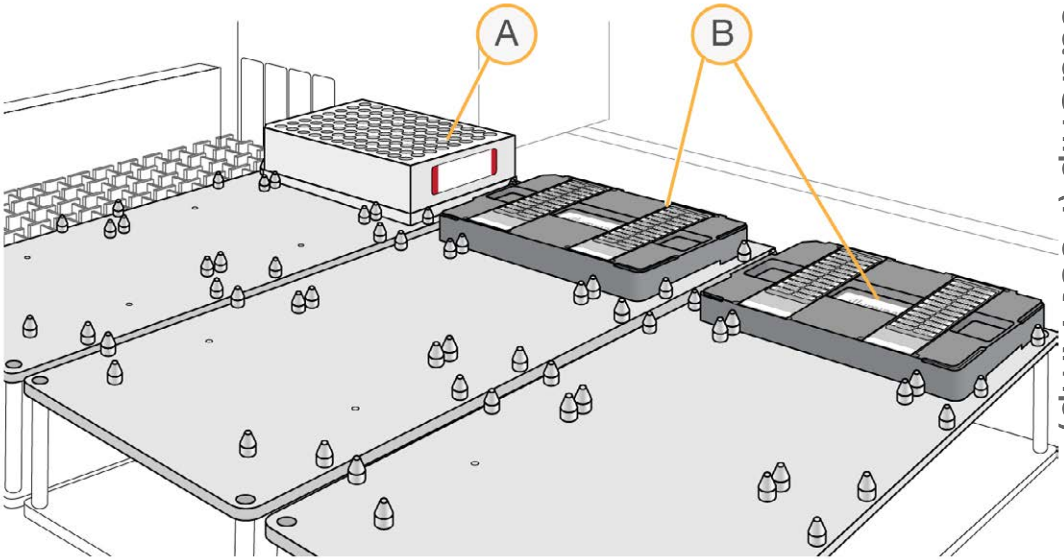
8 After the 30 minute cool down, pulse centrifuge the MSA3 plate to 280 × g.

Prepare the Robot

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

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

Figure 99 Placing Alignment Fixtures and MSA3 Plate onto Robot Bed



A MSA3 Plate
B Robot BeadChip Alignment Fixtures

Verify MSA3 and BeadChips for Hybridization (LIMS only)(Optional)

1. Scan the barcode of the MSA3 plate.
2. 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 Illumina LIMS.

3 Click **Verify**.

4 If the MSA3 plate and BeadChips are queued for hybridization, a blue confirmation message appears at the top of the window. Proceed to *Load BeadChips*.

If the MSA3 plate is not queued for hybridization, if any of the BeadChips have not been accessioned into the system, or if any of the BeadChips are the wrong type, a red error message appears at the top of the window. The error message indicates the first incorrect barcode it finds. Do ***not*** proceed with hybridization; complete the following steps instead:

  a Click the Reports tab in the upper-right corner.
  b In the left sidebar, click **Tracking | Get Queue Status**.
  c Scan the plate barcode and click **Go**.
  d If the plate is queued for another step, proceed with that step.

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

6 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.

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

Load BeadChips

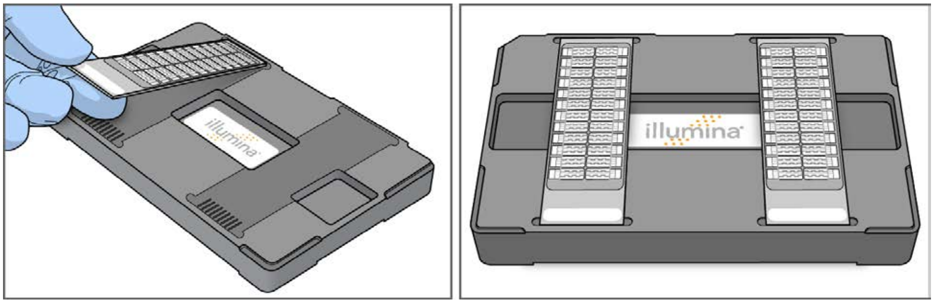
1 Remove all BeadChips from their plastic bags and mylar packages.

CAUTION

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

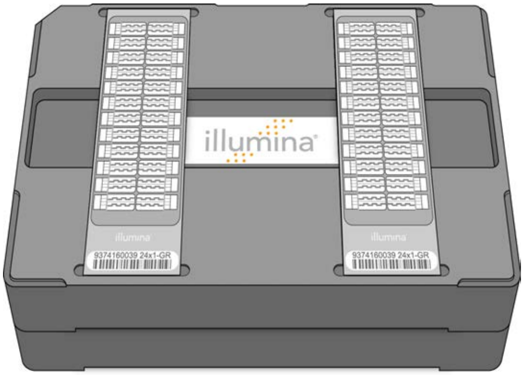
2 Place BeadChips into the Robot BeadChip alignment fixtures with the barcode end aligned to the ridges on the fixture.

Figure 100 Placing BeadChips in Robot BeadChip Alignment Fixture



3 Stack the Robot BeadChip alignment fixtures and carry them to the robot.

Figure 101 Stacked Robot BeadChip Alignment Fixtures



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

5 (Other than Illumina LIMS) In the Basic Run Parameters pane, change the value for **Number of MSA3 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.

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

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

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

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

8 Pulse centrifuge the MSA3 plate to 280 × g.

9 Place the MSA3 plate onto the robot bed according to the bed map. Remove the foil seal.

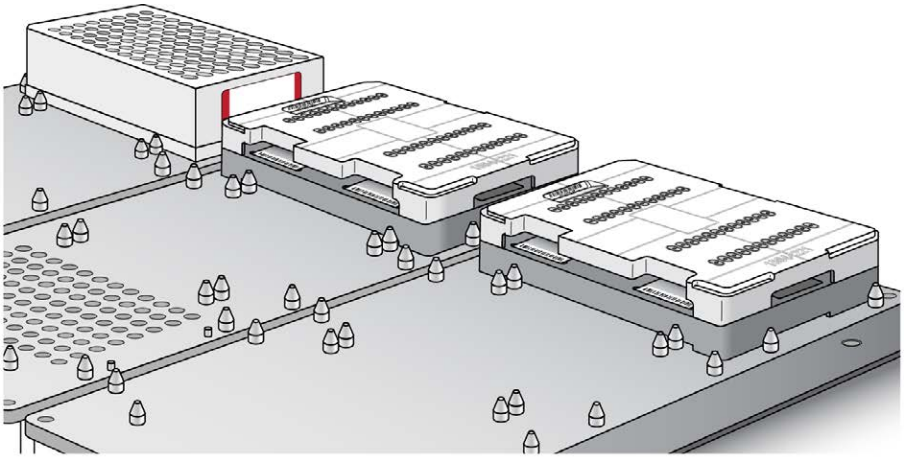
Start the Robot

1 (Other than Illumina LIMS) At the robot PC, click **Run**.

2 (Illumina LIMS) At the robot PC:
   - a Make sure the **Use Barcodes** check box is checked.
   - b Click **Run** to start the process. Log in if prompted.
     The robot scans the barcodes on the BeadChips to confirm the correct BeadChips are loaded. Once the correct BeadChips are confirmed, the robot pauses.

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

Figure 102 Robot Tip Alignment Guides on Robot Bed



The robot dispenses sample to the BeadChips.

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

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

Set up Multi-BeadChip for Hybridization

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

   WARNING

   Hyb Chambers should be at room temperature when you load the BeadChips. They should not be preconditioned in the Illumina Hybridization Oven prior to loading the BeadChips. Heating the PB2 and then opening the Hyb Chamber to add BeadChips causes some of the PB2 to evaporate, leading to a change in the osmolality of PB2 and an imbalance in the vapor pressure between PB2 and RA1 (sample hyb buffer).

   CAUTION

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

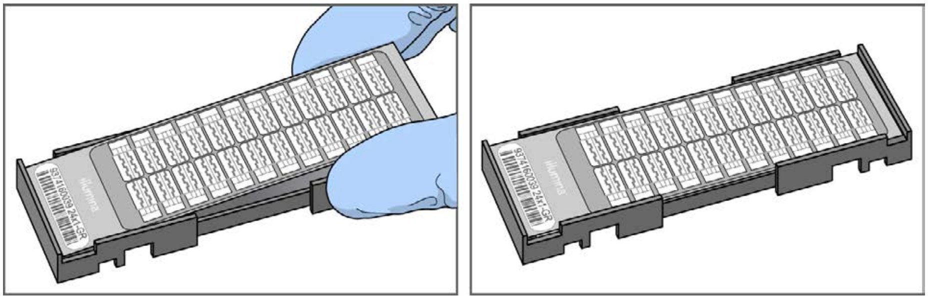
2. Carefully remove each BeadChip from the Robot BeadChip alignment fixtures when the robot finishes.

   CAUTION

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

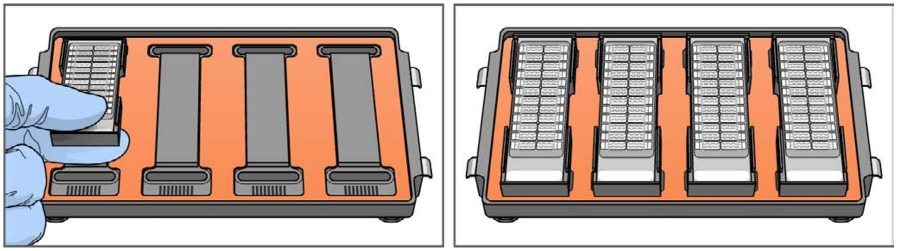
3. Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
4. Carefully place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the insert.

Figure 103 Matching the Barcode End to the Insert Fixture



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

Figure 104 Placing Hyb Chamber Inserts into Hyb Chamber



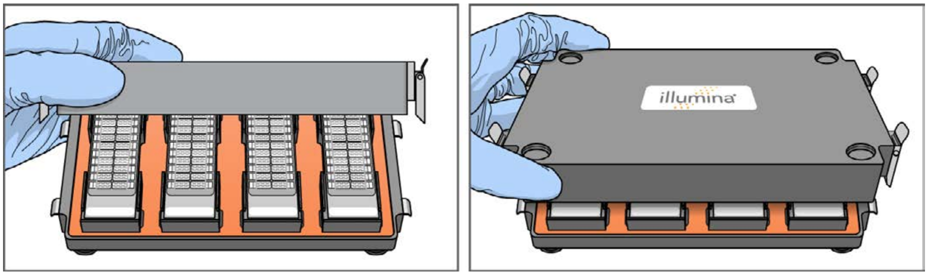
6 Ensure Hyb Chamber inserts are seated properly.

7 (Illumina LIMS) In the Illumina LIMS left sidebar click **Infinium HTS | Infinium Prepare Hyb Chamber**.

8 Scan the barcodes of the PB2 tubes and scan the BeadChip barcodes. Click **Verify**, and then click **Save**.

9 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 105 Seating Lid onto Hyb Chamber



10 Close the clamps on both sides of the Hyb Chamber so that the lid is secure and even on the base (no gaps).

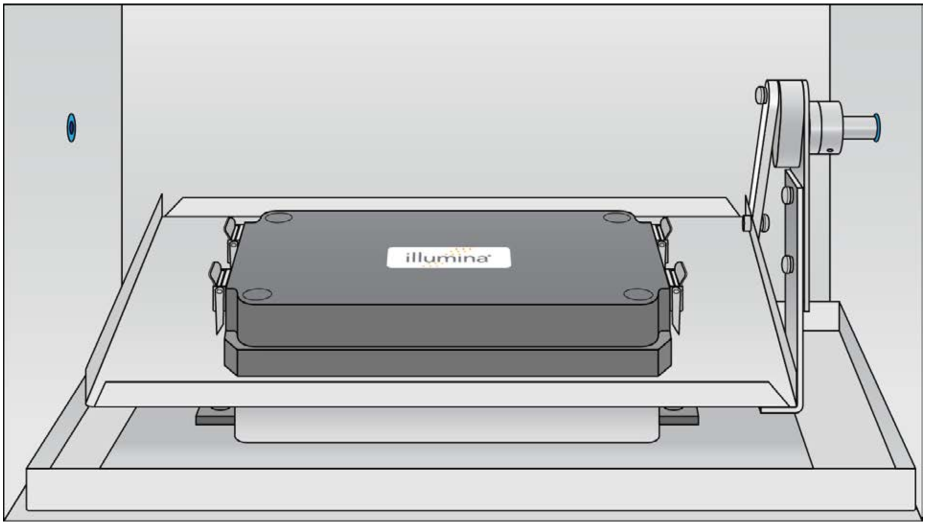
It is best to close them in a kitty-corner fashion, closing first the top left clamp, then the bottom right, then the top right followed by the bottom left.

NOTE

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

11 Place the Hyb Chamber in the 48°C Illumina Hybridization Oven so that the clamps of the Hyb Chamber face the left and right side of the oven and the Illumina logo on top of the Hyb Chamber is facing you.

Figure 106 Hyb Chamber Correctly Placed in Hyb Oven



NOTE

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

Figure 107 Two Hyb Chambers Correctly Placed in Hyb Oven

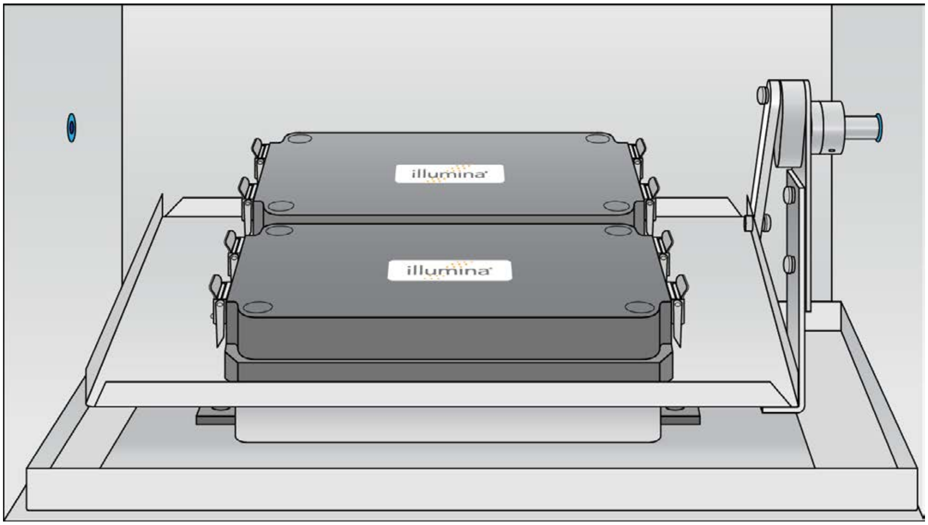
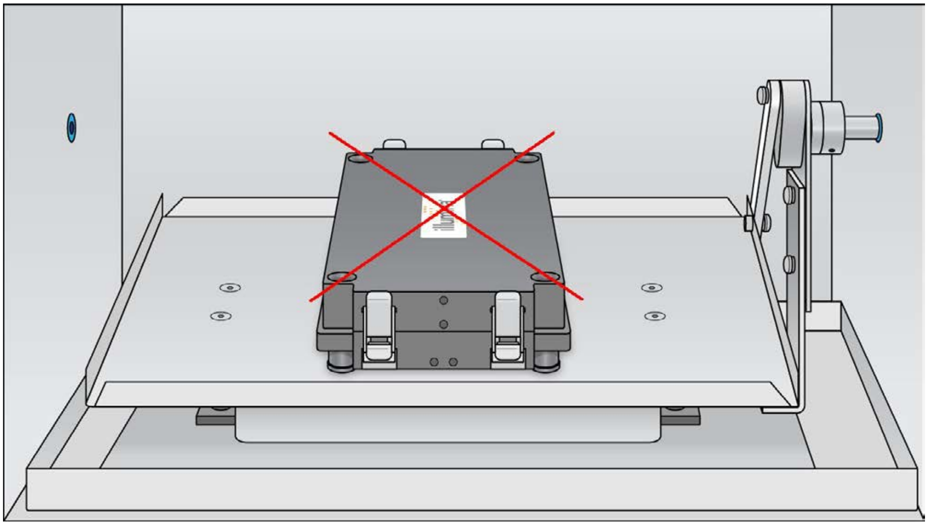


Figure 108 Incorrectly Placed Hyb Chamber



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

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

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

14 Proceed to *Wash BeadChip (Post-Amp)* after the overnight incubation.

Resuspend XC4 Reagent for XStain BeadChip

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

1 Add 330 ml 100% EtOH to the XC4 bottle.
The final volume will be 350 ml. Each XC4 bottle has enough solution to process up to 24 BeadChips.

2 Shake vigorously for 15 seconds.

3 Leave the bottle upright on the lab bench overnight.

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

4 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until it is in complete suspension. After it is resuspended, use XC4 at room temperature.

Wash the Robot Tip Alignment Guide

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

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

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

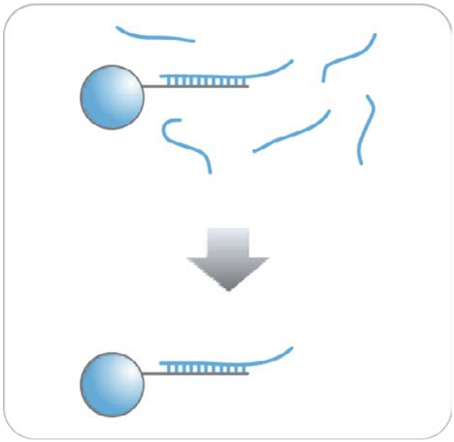
2 After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the tip guides with DiH_2O at least three times to remove any residual detergent.

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

Wash BeadChip (Post-Amp)

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

Figure 109 Washing BeadChip



Estimated Time

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

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml for 1 to 8 BeadChips 700 ml for 9 to 16 BeadChips 850 ml for 17 to 24 BeadChips	Room temperature	Illumina
Multi-sample BeadChip alignment fixture	1 (per 8 BeadChips)		Illumina
Te-Flow LCG flow-through chambers, with black frames, LCG spacers, LCG glass back plates, and clamps	1 (per BeadChip)		Illumina
Wash dish	2 (up to 8 BeadChips)		Illumina
Wash rack	1 (up to 8 BeadChips)		Illumina

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

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

Preparation

1. Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 30 minutes before opening.
2. Have ready on the lab bench:
   a. Two wash dishes:
   b. Containing 200 ml PB1, and labeled as such
   c. Multi-Sample BeadChip Alignment Fixture
   d. Using a graduated cylinder, fill with 150 ml PB1
      - Black frames
      - LCG spacers (separated for ease of handling)
      - Clean LCG glass back plates as directed in the *Infinium Lab Setup and Procedures Guide*.
      - Clamps
3. On the lab tracking form, record:
   - Date/Time
   - Operator
   - PB1 bottle barcode
   - Robot

NOTE

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

Verify Reagents and BeadChips for Washing (LIMS only)

4. Scan the barcodes of the PB1.
5. Scan the BeadChip barcodes.
6. Click **Verify** and then click **Save**.
7. 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 *Steps to Wash BeadChip*.

8 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.

9 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 Illumina LIMS, see the *Illumina LIMS User Guide*.

Steps to Wash BeadChip

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

Figure 110 Wash Rack in Wash Dish Containing PB1

2 Remove the Hyb Chamber inserts from the Hyb Chambers.

3 Remove BeadChips from the Hyb Chamber inserts one at a time.

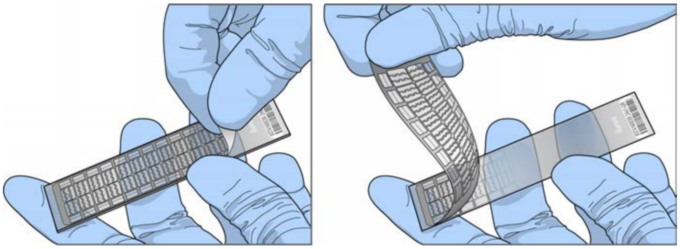
4 Remove the cover seal from each BeadChip.

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

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

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

Figure 111 Removing the Cover Seal



c Discard the cover seal.

CAUTION
Do not touch the arrays!

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

Figure 112 Submerging BeadChips in Wash Dish Containing PB1

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

NOTE
You can use the two 200 ml PB1 wash dishes for up to 24 BeadChips. However, use 150 ml of fresh PB1 for every 8 BeadChips in the Multi-Sample BeadChip Alignment Fixture.

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

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

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

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

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

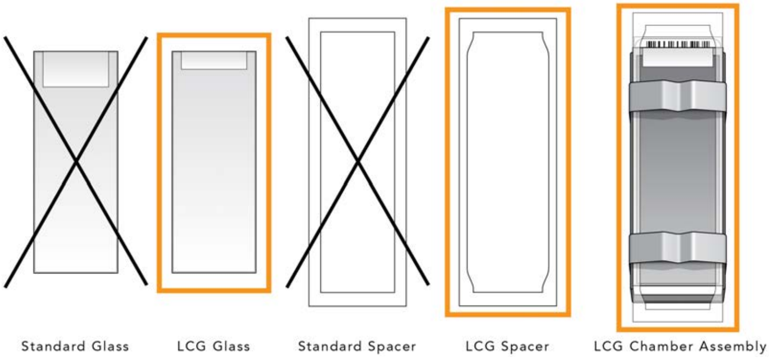
11 If you are processing more than 8 BeadChips
  a Assemble the flow-through chambers for the first 8 BeadChips, as described in the next section, and place them on the lab bench in a horizontal position.

  NOTE
  Keep the flow-through chambers in a horizontal position on the lab bench until all assembled flow-through chambers are ready to be loaded into the chamber rack. Do not place the flow-through chambers in the chamber rack until all BeadChips are prepared in flow-through chambers.

  b Return to this procedure and follow the steps described above to wash the next set of 8 BeadChips.
  c Repeat for each remaining set of 8 BeadChips.

Assemble Flow-Through Chambers

NOTE
Confirm that you are using the correct Infinium LCG glass back plates and spacers before assembling the flow-through chambers. Refer to the following image for the correct flow-through chamber components.



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

2 For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture pre-filled with PB1.

Figure 113 Placing Black Frames into Multi-Sample BeadChip Alignment Fixture

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

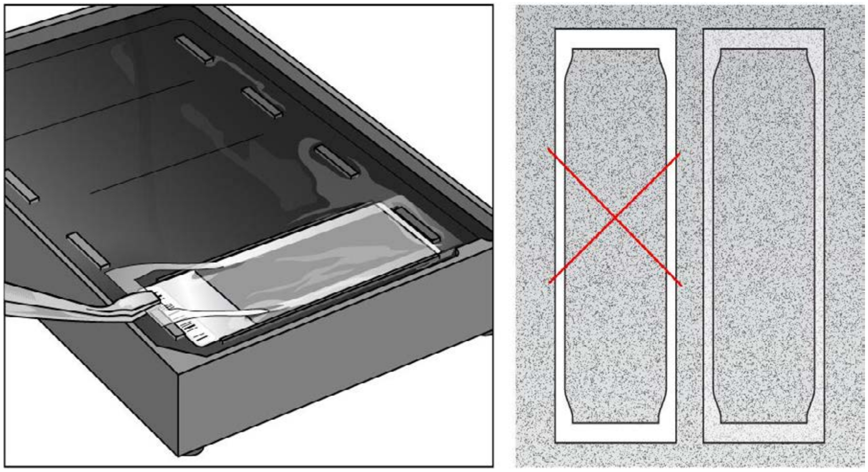
NOTE
Inspect the surface of each BeadChip for residue left by the seal. Use a pipette tip to remove any residue under buffer and be careful not to scratch the bead area.

Figure 114 Placing BeadChip into Black Frame on Alignment Fixture

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

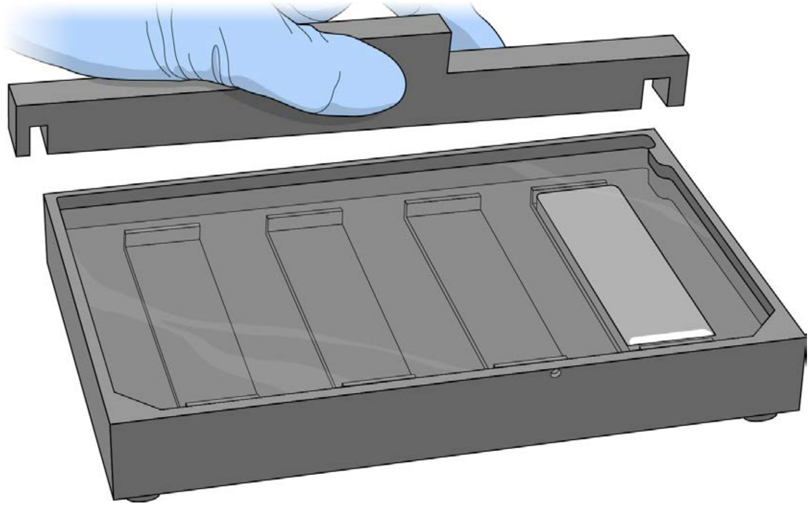
NOTE
Be sure to use the clear plastic spacers, not the white ones.

Figure 115 Placing Clear Plastic Spacer onto BeadChip



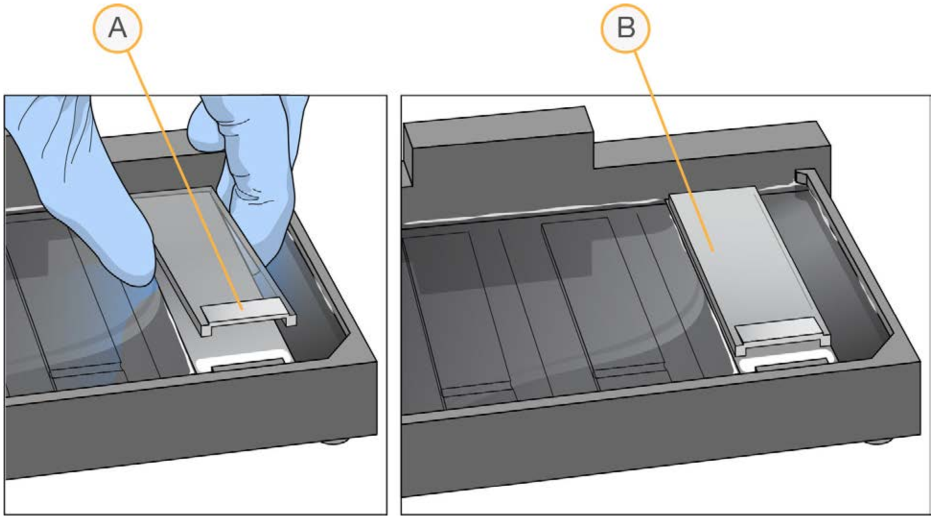
5 Place the alignment bar onto the alignment fixture.
The groove in the alignment bar fits over the tab on the alignment fixture.

Figure 116 Placing Alignment Bar onto Alignment Fixture



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

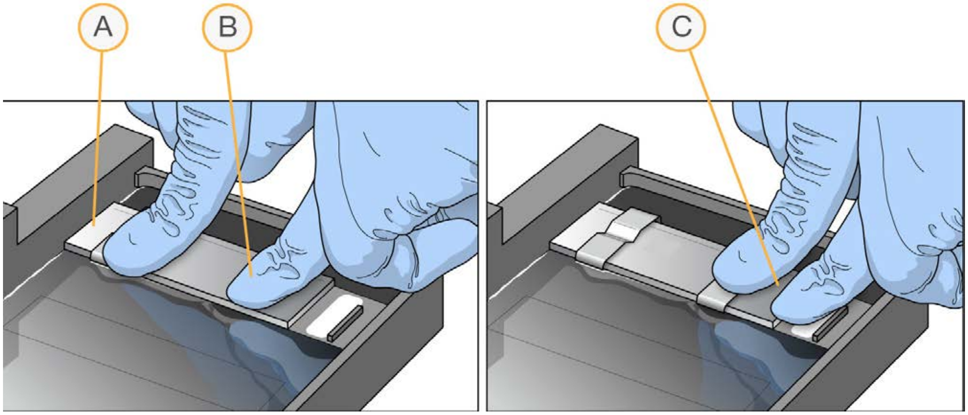
Figure 117 Placing Glass Back Plate onto BeadChip



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

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

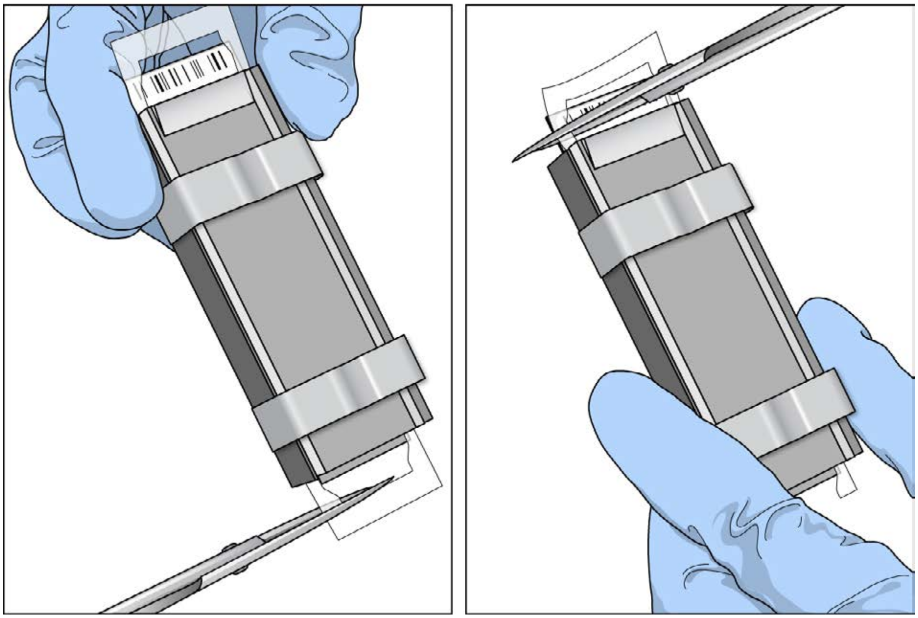
Figure 118 Securing Flow-Through Chamber Assembly with Metal Clamps



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

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

Figure 119 Trimming Spacer Ends from Flow-Through Chamber Assembly



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

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

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

10 Discard unused reagents in accordance with facility standards.

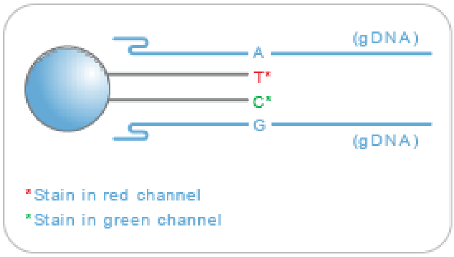
11 Proceed to *Extend and Stain (XStain) BeadChip (Post-Amp)*.

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

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

In this process, you use RA1 reagent to wash away unhybridized and non-specifically hybridized DNA sample. LX1 and LX2 are added to condition the BeadChip surface for the extension reaction. Dispense EML 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 120 Extending and Staining BeadChip



Estimated Time

Robot time:

- ~2 hours and 45 minutes for 8 BeadChips
- ~3 hours for 16 BeadChips
- ~3 hours and 10 minutes for 24 BeadChips

Dry time: 55 minutes

Consumables

Item	Quantity	Storage	Supplied By
RA1	10 ml for 1–8 BeadChips 20 ml for 9–16 BeadChips 30 ml for 17–24 BeadChips	-15°C to -25°C	Illumina
LX1	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
LX2	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
EML	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
XC3	50 ml for 1–8 BeadChips 100 ml for 9–16 BeadChips 150 ml for 17–24 BeadChips	Room temperature	Illumina
SML (Make sure that all SML tubes indicate the same stain temperature on the label)	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
ATM	2 tubes (per 8 BeadChips)	-15°C to -25°C	Illumina
PB1	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina
XC4	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina

Item	Quantity	Storage	Supplied By
Alconox Powder Detergent	As needed	Room temperature	General lab supplier
EtOH	As needed	Room temperature	General lab supplier
95% formamide/1 mM EDTA	15 ml for 1–8 BeadChips 17 ml for 9–16 BeadChips 25 ml for 17–24 BeadChips	-15°C to -25°C	General lab supplier

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

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

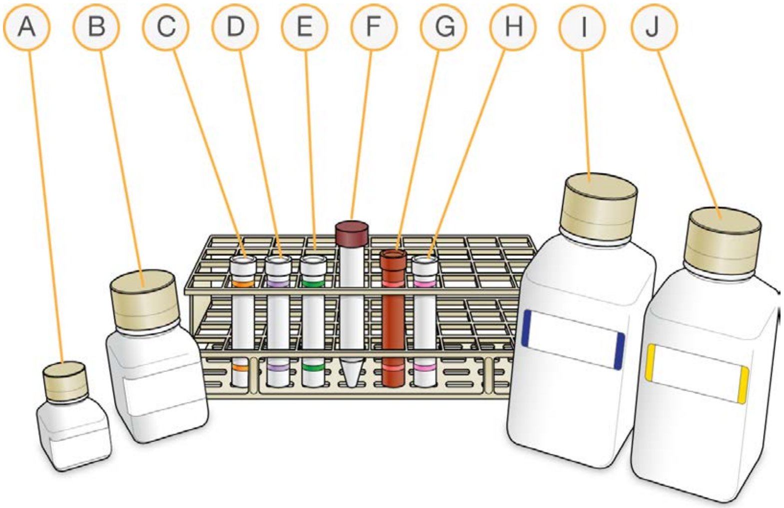
WARNING
This protocol uses an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/msds. Dispose of containers and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

Preparation

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

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

Figure 121 XStain BeadChip Reagent Tubes and Bottles



A	RA1
B	XC3
C	LX1
D	LX2
E	EML
F	95% Formamide / 1mM EDTA
G	SML
H	ATM
I	PB1
J	XC4

- On the lab tracking form, record:
  - Date/Time
  - Operator
  - Robot
  - RA1 barcode
  - XC3 barcode
  - LX1 barcodes
  - LX2 barcodes
  - EML barcodes
  - SML barcodes

- ATM barcodes
- PB1 barcode
- XC4 barcodes

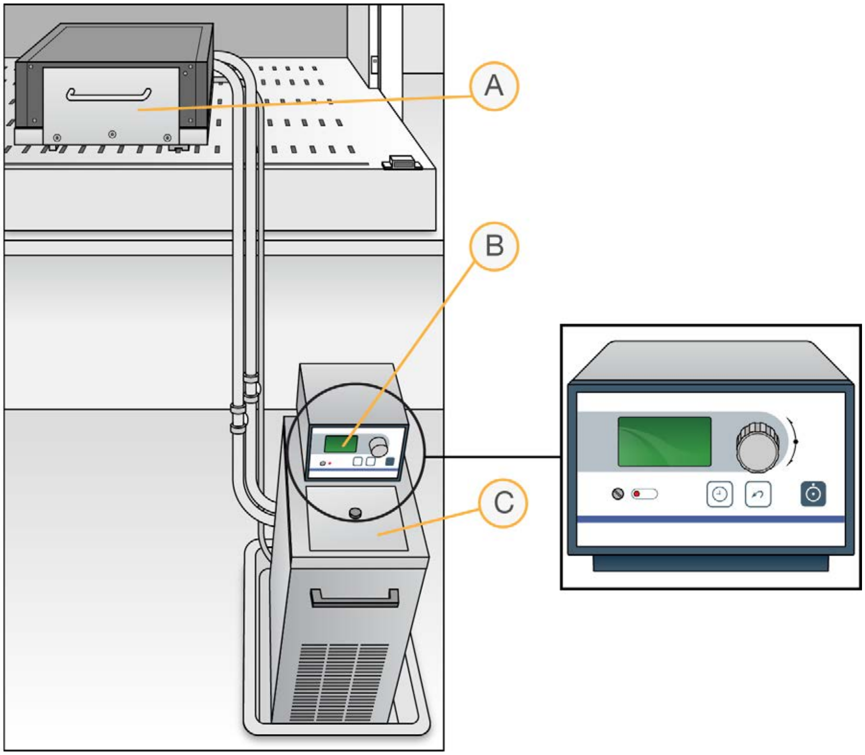
NOTE

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

Set Up Chamber Rack

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

Figure 122 Water Circulator Connected to Chamber Rack



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

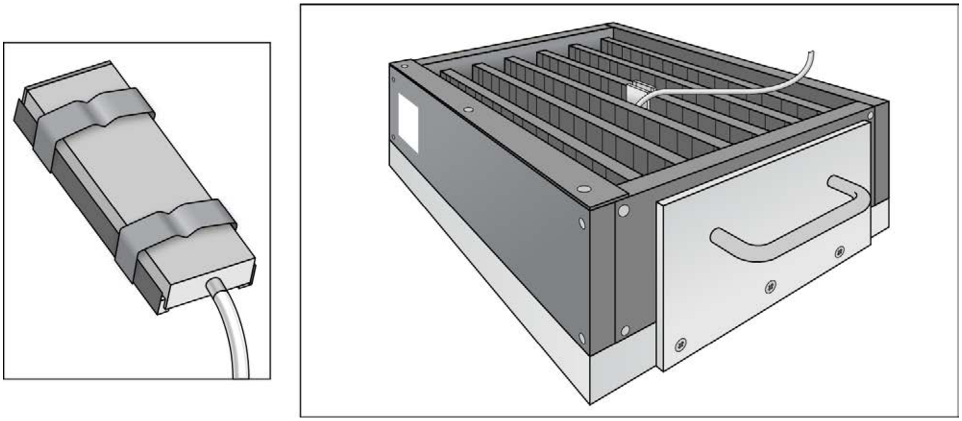
3 The temperature displayed on the water circulator LCD screen may differ from the actual temperature on the chamber rack. Confirm the actual temperature using the temperature probe for the chamber rack.

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

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

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

Figure 123 Illumina Temperature Probe and Temperature Probe in Chamber Rack



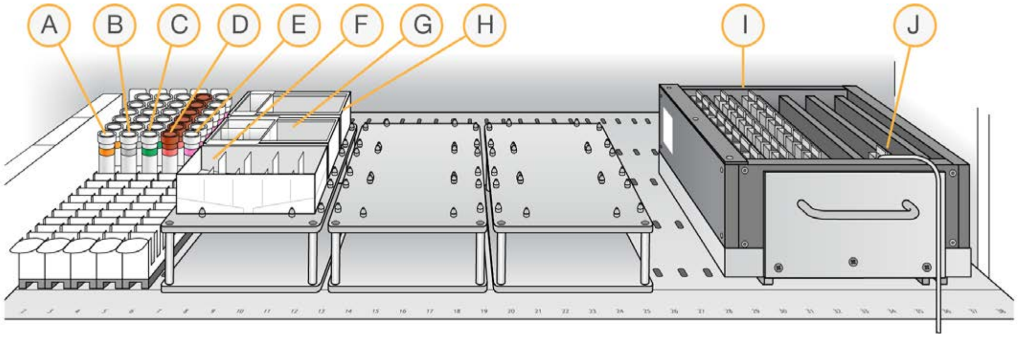
6 For accurate temperature measurement, ensure the Illumina Temperature Probe is touching the base of the chamber rack.

Prepare Robot

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

Refer to the figure shown below throughout this protocol.

Figure 124 Tecan Eight-Tip Robot (XStain BeadChip Setup)



A	LX1
B	LX2
C	EML
D	SML
E	ATM
F	XC3 in Full Reservoir
G	RA1 in Half Reservoir
H	95% Formamide / 1 mM EDTA in Quarter Reservoir
I	24 BeadChips in Chamber Rack
J	Temperature Probe

Single-Base Extension and Stain

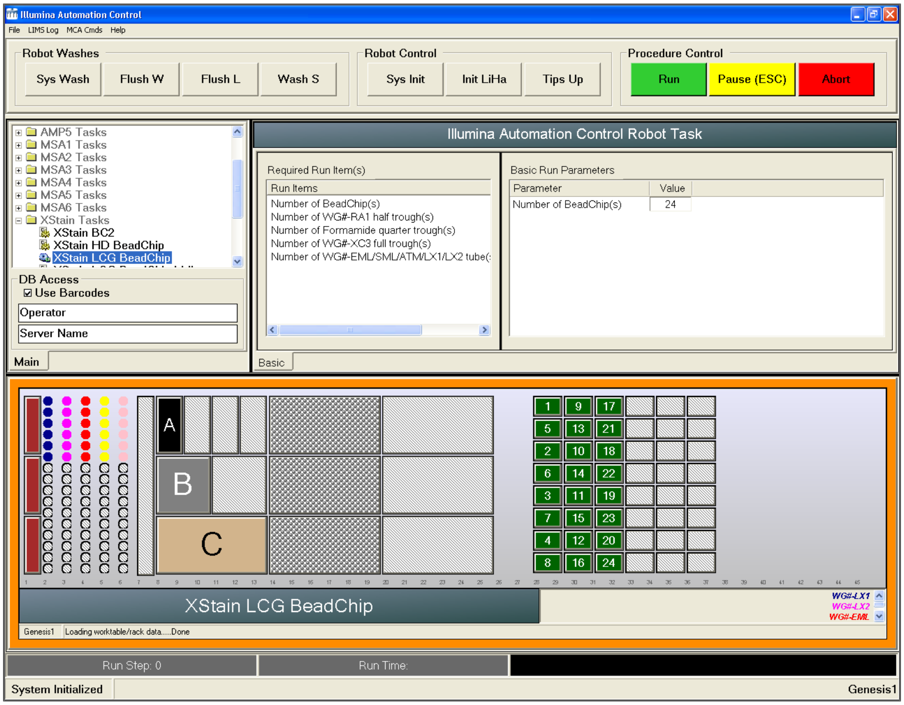
CAUTION
The remaining steps must be performed without interruption.

1. Slide the chamber rack into column 36 on the robot bed. Make sure that it is seated properly.
2. At the robot PC, select **XStain Tasks | XStain LCG BeadChip**.

3 In the Basic Run Parameters pane, enter the number of BeadChips.

You can process up to 24 BeadChips in the XStain BeadChip process.

The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed. All barcodes must face to the left.



Illumina Automation Control Robot Task

☑ Use Barcodes

Operator

Server Name

Required Run Item(s)

Run Items
Number of BeadChip(s)
Number of WG#-RA1 half trough(s)
Number of Formamide quarter trough(s)
Number of WG#-XC3 full trough(s)
Number of WG#-EML/SML/ATM/LX1/LX2 tube(

Basic Run Parameters

Parameter	Value
Number of BeadChip(s)	24

XStain LCG BeadChip

Genesis1 Loading worktable/rack data.....Done

Run Step: 0

Run Time:

System Initialized

Genesis1

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

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

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

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

- 10 ml to process 8 BeadChips

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

7. Place a full reservoir in the reservoir frame, according to the robot bed map, and add XC3 in the following volumes:
    - 50 ml to process 8 BeadChips
    - 100 ml to process 16 BeadChips
    - 150 ml to process 24 BeadChips
8. Place each reagent tube (LX1, LX2, EML, SML, ATM) in the robot tube rack according to the bed map, and remove their caps.
9. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start Robot

1. (Other than Illumina LIMS) At the robot PC, click **Run**.
2. (Illumina LIMS) At the robot PC:
    a. Ensure that the **Use Barcodes** check box is checked.
    b. In the **Basic Run Parameters** pane, change the value for **Number of BeadChips** to indicate the number of BeadChips being processed.
    c. Click **Run** to start the process. Log in if prompted.
3. When prompted, enter the stain temperature indicated on the SML tube.

    NOTE
    If you are using Illumina LIMS you will not be prompted to enter the staining temperature. Illumina LIMS will automatically set the correct temperature based on the SML tube barcodes.

4. When the prompt appears, wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.
5. Once the temperature probe registers 44°C, click **OK**.
6. When prompted, load the BeadChips and click **OK**.
7. Place each assembled flow-through chamber in the first row of the chamber rack. Refer to the robot bed map for the correct layout.
8. Ensure each flow-through chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.

9 On the lab tracking form, record the chamber rack position associated with each BeadChip.

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

Table 15 List of Reactions

#	Reagent	Wait Time
1	RA1	3 minutes
2	LX1	10 minutes
3	LX2	10 minutes
4	EML	15 minutes
5	Formamide/EDTA	7 minutes
6	XC3	2 minutes
7	SML	10 minutes
8	XC3	7 minutes
9	ATM	10 minutes
10	XC3	7 minutes
11	SML	10 minutes
12	XC3	7 minutes
13	ATM	10 minutes
14	XC3	7 minutes
15	SML	10 minutes
16	XC3	7 minutes

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

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

Verify Reagents and BeadChips for Coating (LIMS only)

1 Scan the barcodes of the PB1.

2 Scan the barcodes of the XC4.

3 Scan the BeadChip barcodes.

4 Click **Verify** and then click **Save**.

5 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*.

6 If any of the reagents are invalid, check the reagent type before re-scanning. The reagent name (e.g., PB1) appears at the end of the barcode. Make sure to scan the correct reagent into each box.

7 If any of the BeadChips are not queued for 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 Illumina LIMS, see the *Illumina LIMS User Guide*.

Wash and Coat 8 BeadChips

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

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

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

Equipment Needed

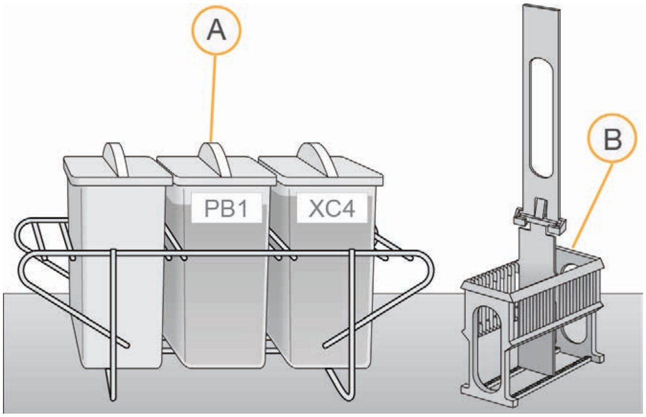
Place the following items on the bench:

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

Steps

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

Figure 125 PB1 and XC4 Wash Dishes with Staining Rack

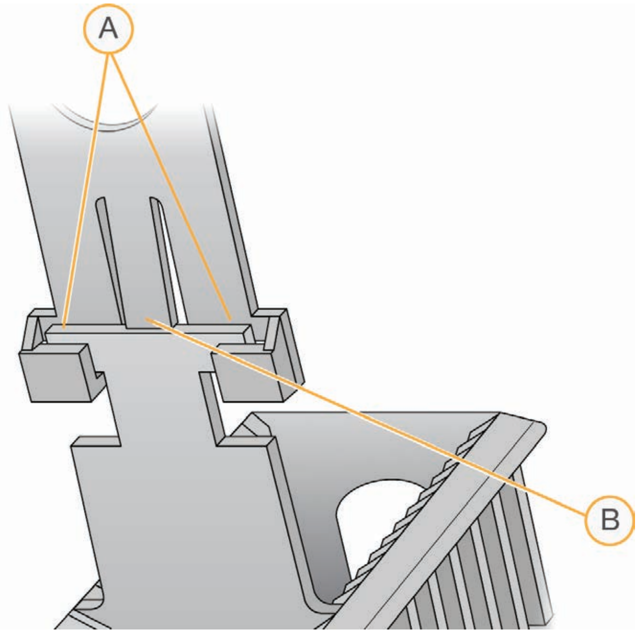


A Wash Dishes
B Staining Rack

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

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

Figure 126 Staining Rack Locking Arms and Tab



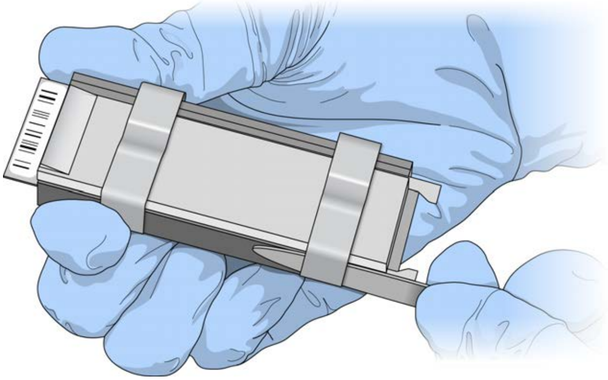
A Locking Arms
B Tab

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

5 One at a time, disassemble each flow-through chamber:
   a Use the dismantling tool to remove the two metal clamps.

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

Figure 127 Removing the Metal Clamps from Flow-Through Chamber



b Remove the glass back plate.

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

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

e Remove the BeadChip.

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

6 Place the BeadChips in the staining rack while it is submerged in PB1. Put four BeadChips above the staining rack handle and four below. Make sure that the BeadChip barcodes ***face away*** from you and that the locking arms on the handle ***face towards*** you.
If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting each BeadChip.

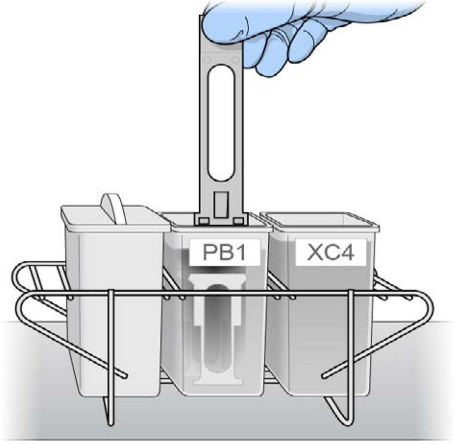
7 Make sure that the BeadChips are completely submerged.

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

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

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

Figure 128 Washing BeadChips in PB1



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

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

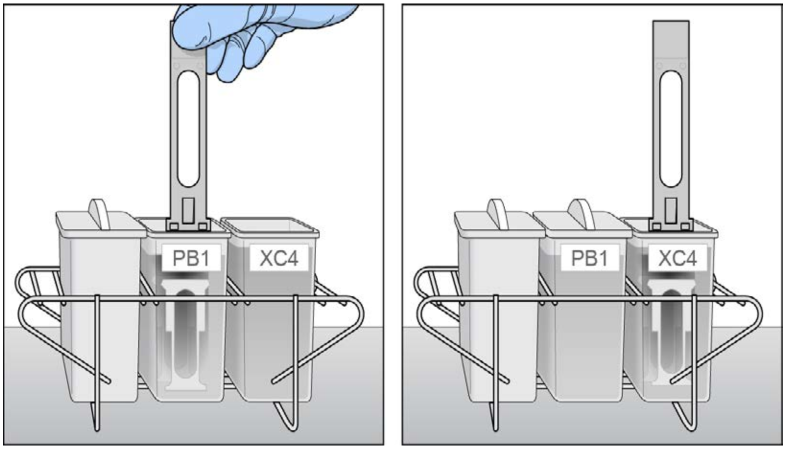
10 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

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

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

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

Figure 129 Moving BeadChips from PB1 to XC4



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

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

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

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

15 Prepare 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 tabs *face down*.

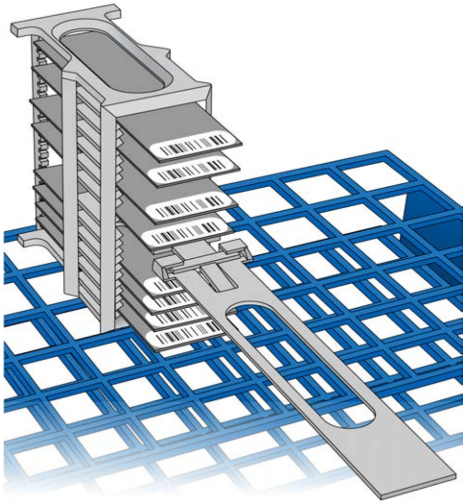


Figure 130 Staining Rack in Correct Orientation

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

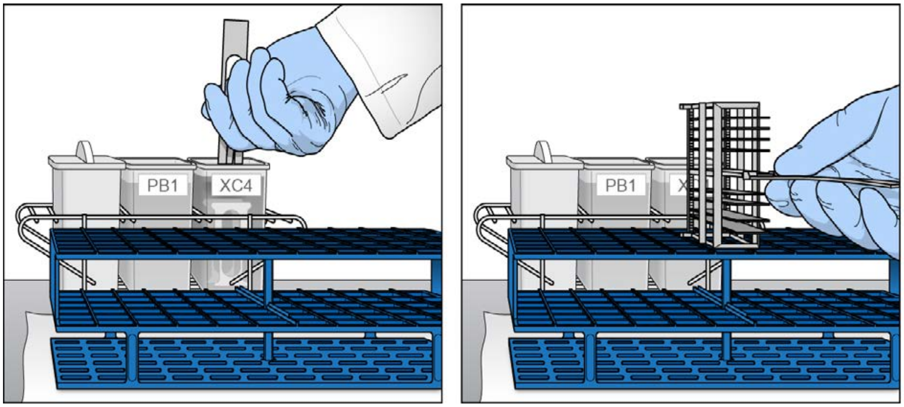


Figure 131 Moving the Staining Rack from XC4 to Tube Rack

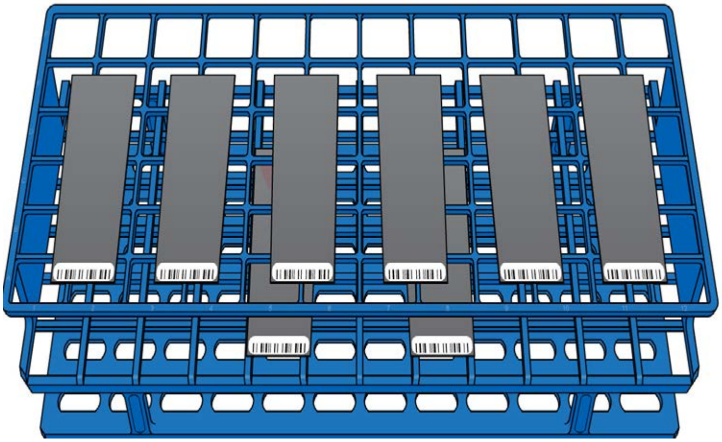
17 For each of the top four BeadChips, working top to bottom:

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

Figure 132 BeadChips on Tube Rack



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

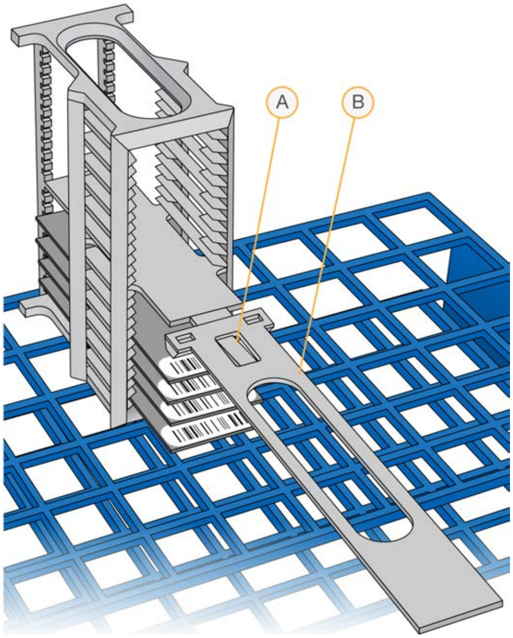


Figure 133 Removing Staining Rack Handle

A Tab
B Handle

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

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

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

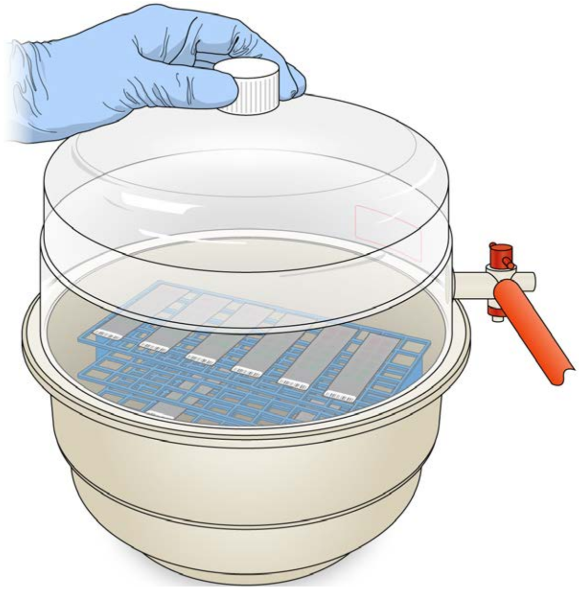
CAUTION
Make sure the vacuum valve is seated tightly and securely.

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

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

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

Figure 134 Testing Vacuum Seal



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

25 Release the vacuum by turning the handle very slowly.

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

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

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

28 If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom 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.
   b Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

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

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

30 Discard unused reagents in accordance with facility standards.

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

Image BeadChip (Post-Amp)

Follow the instructions in the *iScan System User Guide* or *HiScan System User Guide* to scan your BeadChips. Use the **Infinium LCG** scan setting for your BeadChip.

Illumina GenomeStudio

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

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

Index

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 16 Illumina General Contact Information

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

Table 17 Illumina Customer Support Telephone Numbers

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

MSDSs

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

Product Documentation

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

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