

Infinium XT Assay

Reference Guide for the HT Workflow



This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

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).

© 2017 Illumina, Inc. All rights reserved.

Illumina, Infinium, iScan, and the streaming bases design are registered or pending trademarks of Illumina, Inc. and/or its affiliate(s) in the U.S. and/or other countries. All other names, logos, and other trademarks are the property of their respective owners.

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, NON-INFRINGEMENT, 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

Document	Date	Description of Change
Document # 1000000016440 v01	June 2017	Added instructions to support the automated workflow with Illumina LIMS.
Document # 1000000016440 v00	September 2016	Initial release.

Table of Contents

Revision History	vii
Chapter 1 Overview	1
Introduction to the Infinium XT Assay	1
Important Note	1
DMAP Files	2
Infinium XT HT Automated Workflow	3
Tips and Techniques	4
Prepare and Store Reagents	5
Prepare and Store PB20	5
Illumina LIMS	6
No Illumina LIMS	6
Additional Resources	6
Chapter 2 Automated Protocol with Illumina LIMS	8
Introduction	8
Amplify DNA (Pre-Amp)	8
Incubate DNA	10
Fragment DNA	10
Precipitate DNA	12
Resuspend DNA	14
Hybridize to BeadChip	16
Wash BeadChips	19
Extend and Stain (XStain)	22
Image BeadChip	27
Chapter 3 Automated Protocol without Illumina LIMS	28
Introduction	28
Amplify DNA (Pre-Amp)	28
Incubate DNA	30
Fragment DNA	30
Precipitate DNA	31
Resuspend DNA	33
Hybridize to BeadChip	35
Wash BeadChips	38
Extend and Stain (XStain)	41
Image BeadChip	46
Appendix A Supporting Information	47
Introduction	47
Acronyms	47
Kit Contents	47
Consumables and Equipment	50

Technical Assistance 54

Chapter 1 Overview

Introduction to the Infinium XT Assay	1
Important Note	1
DMAP Files	2
Infinium XT HT Automated Workflow	3
Tips and Techniques	4
Prepare and Store Reagents	5
Prepare and Store PB20	5
Illumina LIMS	6
No Illumina LIMS	6
Additional Resources	6

Introduction to the Infinium XT Assay

The Infinium family of genotyping assays harnesses proven chemistry and a robust BeadChip platform to produce exceptional data quality, superior call rates, and high reproducibility. Infinium XT 96-Sample BeadChips build on the success of the Infinium product family to provide the highest throughput array format to date from Illumina. This assay was developed for customers who require up to 50,000 SNPs to perform large scale screening applications on any species with either prevalidated or novel custom content.

The Infinium XT Assay optimizes the user experience as customers transition large-scale genotyping studies to the next level of throughput. Infinium XT simplifies the custom assay design process, reduces overall hands-on time, and enhances automation robot performance and utilization. If the time to answer is more important than maximum weekly output for a given batch of samples, the workflow offers an option to reduce the overall turnaround time to generate genotyping data. Also, the Infinium XT Assay introduces a new data analysis software solution for real-time data generation and on-demand QC report functionality so production issues can be identified and corrected earlier.

The Infinium XT Assay offers:

- ▶ Ultrahigh throughput
- ▶ Flexible content
- ▶ High-efficiency workflow
- ▶ Low DNA input—200 ng per sample
- ▶ 96-sample BeadChip format

The workflows are as follows:

- ▶ **HT**—The Infinium XT HT workflow supports production-scale laboratories with a seamless, fully automated workflow, XStain batch sizing of 2,304 or 4,608 samples (24 or 48 BeadChips) per run, optional integrated LIMS solution, and convenient reagent kit packaging with plate-based X-Stain reagents supporting 4,608 and 23,040 samples per kit.
- ▶ **ST**—The Infinium XT ST workflow supports high-throughput laboratories with optimized-for-throughput manual and automated workflow options, XStain batch sizing options of 384, 768, or 1152 samples (4, 8, or 12 BeadChips) per run, optional integrated LIMS solution, and convenient reagent kit packaging with tube-based reagents supporting 1152 samples per kit.

Important Note

Before using the procedures in this guide, read the *Infinium Lab Setup and Procedures Guide*. The guide explains how to equip and run an Infinium XT Assay laboratory, including information on the following topics:

- ▶ Prevention of amplification product contamination
- ▶ Safety precautions
- ▶ Equipment, materials, and reagents to purchase in advance
- ▶ Standard lab procedures
- ▶ Robot use for automation
- ▶ Preparation for BeadChip imaging
- ▶ System maintenance
- ▶ Troubleshooting

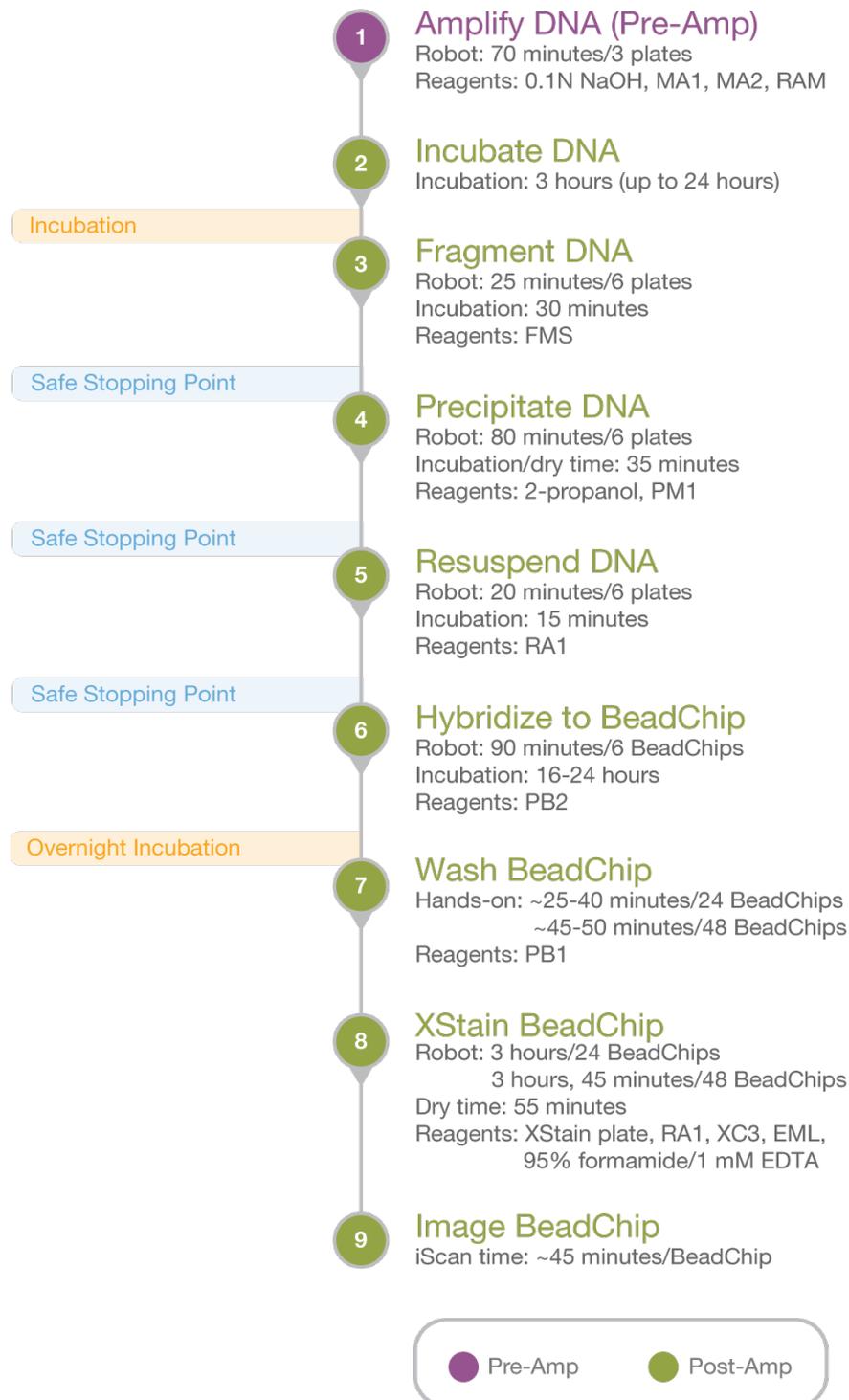
The instructions apply equally to all Infinium BeadChips provided by Illumina. All Infinium XT documentation assumes that you have already set up the laboratory space, and are familiar with the standard procedures and safety precautions.

DMAP Files

Before run setup, download the DMAP files for the arrays, and prepare a sample sheet. The DMAP files can be downloaded using the Decode File Client.

Infinium XT HT Automated Workflow

Figure 1 Illumina Infinium XT HT Automated Workflow



Tips and Techniques

Unless a safe stopping point is specified, proceed immediately to the next step.

Avoid Cross-Contamination

When adding or transferring samples, change tips between *each sample*.

Measure Volumes Carefully

When measuring volumes of reagent to put in reservoirs, make sure that the measurements are exactly as specified in the instructions. Do not estimate volumes.

Inspect XCG Glass Back Plates

For optimal performance, use XCG glass back plates free of chips and cracks along the beveled edge and the long edges. Chipped or cracked XCG glass back plates risk volume leakage and affect overall performance.

Seal the Plate

Always seal plates before the following steps in the assay:

- ▶ Vortexing steps
- ▶ Centrifuge steps

Apply the cap mat and seal tightly using a rubber roller.

Heat Sealer

If you are using the ALPS 50 V model heat sealer, set it to 165°C and 2.5 seconds.

Tip Alignment

Make sure that robot tips align with Illumina XT tip guides for accurate volume transfer to BeadChips.

Prepare to Wash and Coat BeadChips

Before starting the wash and coat process:

- ▶ Place wash dish covers on wash dishes when not in use.
- ▶ Clean wash dishes with low-pressure air to remove particulates before use.
- ▶ Wash tube racks and wash dishes thoroughly before and after use. Rinse with DI H₂O. Place upside down on wash rack to dry.
- ▶ Prepare an additional clean tube rack that fits the internal dimensions of the vacuum desiccator. Allow 1 rack per 8 BeadChips.

Prepare and Store Reagents

Conserve Reagents

- ▶ Kits contain reagents in exact quantities needed for the assay. Measure reagents carefully to avoid shortages.
- ▶ This protocol and the supporting kits are designed for 24–48 plates per XStain batch. If you process fewer than 24 plates or between 25–47 plates, surplus reagents cannot be reused.
- ▶ Use fresh reagents for each batch of plates and empty reservoirs between batches.

Use Fresh RA1 Reagent for Each Step

It is important to use fresh RA1 for each 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 exposed to room temperature air for extended periods of time, it is no longer fresh.

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

Prepare Batches of 95% Formamide/1 mM EDTA

To minimize errors in preparing 95% formamide/1 mM EDTA, prepare it in large batches and aliquot it into 15 ml or 50 ml sealed tubes. Store aliquots for 6 months at -25°C to -15°C and use in the protocol as needed. After you open an aliquot, use it on the same day. Discard leftover reagent.

Prepare Batches of 0.1N NaOH

To minimize errors in preparing 0.1N NaOH fresh each day, prepare it in large batches and aliquot it into 15 ml or 50 ml sealed tubes. Store aliquots up to 6 months at 2°C to 8°C and use them in the protocol as needed. After you open an aliquot, use it on the same day. Discard leftover reagent.

Prepare and Store PB20

Store PB20

- ▶ Room temperature.

Dilute PB20 to Make 1X PB1 Solution

- 1 Add 10 L DI H₂O to the 20 L carboy.
- 2 Pour the entire contents of PB20 (approximately 1 L) into the carboy.
- 3 Fill to the 20 L line with DI H₂O. Use a graduated cylinder or a gentle stream of DI H₂O to avoid creating bubbles.

Store PB1

- ▶ Room temperature.
- ▶ Keep PB1 for up to 3 months.

Clean the Carboy

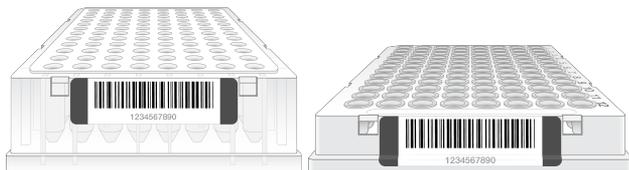
- ▶ Rinse the carboy with 10–20 L DI H₂O 3 times.
- ▶ Run 5 L DI H₂O through the spigot to flush it.

Illumina LIMS

If you are using Illumina LIMS:

- ▶ At each step in the protocol, make sure that the **Use Barcodes** checkbox is selected.
- ▶ The barcode must be located on the right side of the plate. Make sure the barcode label fits between the notches and does not cover the holes on the top of the plate.

Figure 2 Correctly Placed Barcodes



- ▶ Each time you click **Run** to start a new process, you are prompted to log in to LIMS.
- ▶ At each step of the protocol that operates on the robot, you are prompted to select the project, and the batch ID or DNA plate. Do either 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.
 - ▶ Use **Search** to search for a specific Batch ID or DNA plate.
- ▶ Some of the protocol steps require verification in LIMS before you start the step.
 - ▶ If verification is successful, a blue confirmation message appears at the top of the window.
 - ▶ If the verification fails, a red error message appears at the top of the window. Do **not** proceed. Instead, follow these steps to troubleshoot the problem:
 - Click the Reports tab in the upper-right corner.
 - In the left pane, click **Tracking Reports | Get Queue Status**.
 - Scan the plate barcode and click **Go**.
 - Note which step the plate is queued to run, and proceed with that step.

No Illumina LIMS

If you are using the automated protocol without Illumina LIMS:

- ▶ At each step in the protocol, make sure that the **Use Barcodes** checkbox is **cleared**.
- ▶ At each step in the protocol, you are prompted to enter the number of samples, plates, or BeadChips. Enter the requested information, and then click **OK**.

Additional Resources

Visit the Infinium XT Assay support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

The following documentation is available for download from the Illumina website.

Resource	Description
<i>Infinium XT - Checklist for the HT Workflow with Illumina LIMS (document # 1000000034877)</i>	Provides a checklist of steps for users who are experienced using the Infinium XT HT workflow, with Illumina LIMS.
<i>Infinium XT - Checklist for the HT Workflow without Illumina LIMS (document # 1000000026232)</i>	Provides a checklist of steps for users who are experienced at using the Infinium XT HT workflow, without Illumina LIMS.
<i>Infinium Lab Setup and Procedures Guide (document # 11322460)</i>	Describes how to set up an Infinium lab, and best practices for lab operation.

Chapter 2 Automated Protocol with Illumina LIMS

Introduction	8
Amplify DNA (Pre-Amp)	8
Incubate DNA	10
Fragment DNA	10
Precipitate DNA	12
Resuspend DNA	14
Hybridize to BeadChip	16
Wash BeadChips	19
Extend and Stain (XStain)	22
Image BeadChip	27

Introduction

This section describes pre- and post-amplification automated laboratory protocols for the Infinium XT Assay. Follow the protocols in the order shown.

This section includes instructions for performing the protocol using the Illumina Laboratory Information Management System (LIMS) to track barcodes and other project information. If you are not running LIMS, see [Automated Protocol without Illumina LIMS on page 28](#) for protocol instructions. For information about how to use LIMS, see the *LIMS User Guide*.

Amplify DNA (Pre-Amp)

This process adds the DNA samples to the plates, then denatures and neutralizes the samples to prepare them for amplification.

Consumables

- ▶ MA1
- ▶ MA2
- ▶ RAM
- ▶ 0.1N NaOH
- ▶ 96-well 0.8 ml microplates (MIDI)
- ▶ WG#-DNA plates with 96 DNA samples (10 µl at 50 ng/µl) (MIDI or TCY)
- ▶ Cap mats

Preparation

- 1 Thaw DNA samples to room temperature.
- 2 Thaw the following consumables at 4°C for approximately 24 hours. If necessary, use a room temperature water bath to complete thawing and bring the reagents to room temperature.

Item	Storage
MA2	-25°C to -15°C
RAM	-25°C to -15°C

- 3 Invert to mix.
- 4 Preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- 5 Apply MSA7 barcode labels to new MIDI plates.
- 6 Vortex DNA samples at 1600 rpm for 1 minute.
- 7 Centrifuge DNA samples at 280 × g at room temperature for 1 minute.

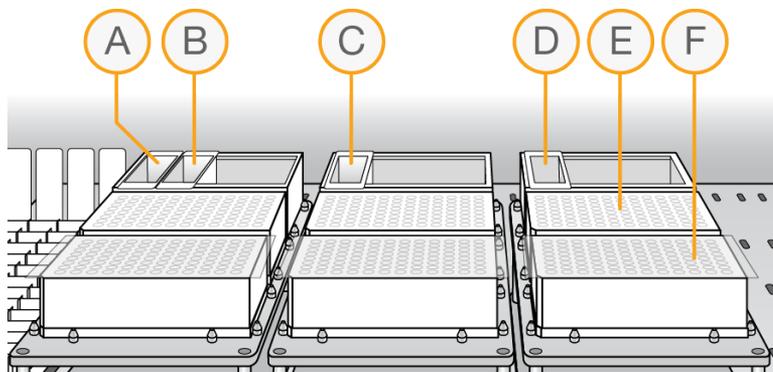
Procedure

- 1 At the robot PC, select **MSA7 HT Tasks | Make MSA7 HT**.
- 2 Select the WG#-DNA plate type (MIDI or TCY). Do not mix plate types on the robot.
- 3 Place 4 quarter reservoirs on the robot bed, according to the bed map.
- 4 Use a serological pipette to add reagents to reservoirs. Use exactly the following amounts for every 3 plates:

Reagent	Volume
MA1	9 ml
MA2	13.5 ml
RAM	13.5 ml
0.1N NaOH	5 ml

- 5 Place WG#-DNA source and MSA7 MIDI plates on the robot bed according to the bed map.
- 6 At the robot PC, click **Run**.
- 7 Select the project, the batch ID or DNA plate, and the batch.
- 8 Click **OK** to confirm the required DNAs.

Figure 3 Robot Setup for Amplify DNA



- A MA1 Reservoir
- B 0.1N NaOH Reservoir
- C MA2 Reservoir
- D RAM Reservoir
- E MSA7 Plates
- F DNA Plates

- 9 After the robot has completed the run, apply cap mats to the MSA7 plates and vortex at 1600 rpm for 1 minute.
- 10 Centrifuge at $280 \times g$ at room temperature for 1 minute.

Incubate DNA

This step uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used in the Infinium XT Assay.



NOTE

This and all remaining steps in the workflow are performed in the post-amp lab.

- 1 In Illumina LIMS, select **Infinium XT | Incubate MSA7 HT**.
- 2 Scan the MSA7 plate barcode, click **Verify**, and then click **Save**.
- 3 Incubate the MSA7 plates in the Illumina Hybridization Oven for 3–24 hours at 37°C.

Fragment DNA

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

Consumables

- ▶ FMS

Preparation

- 1 Prepare the following consumables.

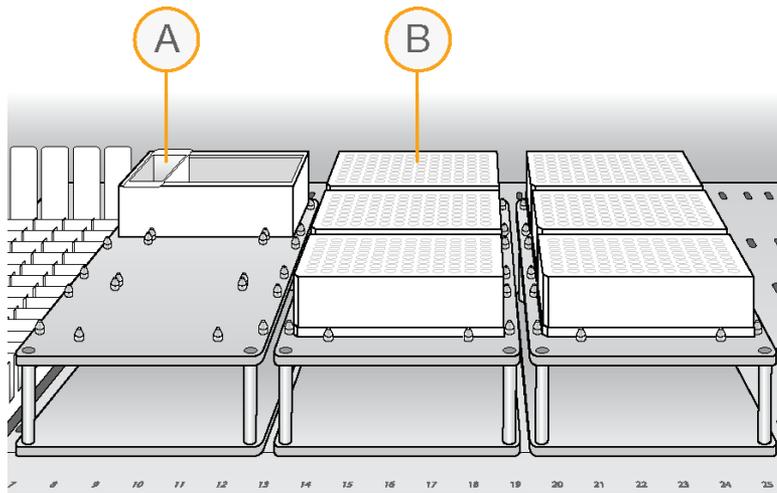
Item	Storage	Instructions
FMS	-25°C to -15°C	Thaw to room temperature and invert to mix.

- 2 Preheat the Illumina Hybridization Oven to 37°C.
- 3 If you plan to resuspend the MSA7 plates today, remove the RA1 from the freezer to thaw.

Procedure

- 1 Centrifuge the MSA7 plates at $280 \times g$ at room temperature for 1 minute.
- 2 At the robot PC, select **MSA7 HT Tasks | Fragment MSA7 HT**.
- 3 Place the MSA7 plates on the robot bed according to the bed map. Remove the cap mats.
- 4 Place a quarter reservoir on the robot bed, according to the bed map, and add 20 ml FMS for 6 plates.

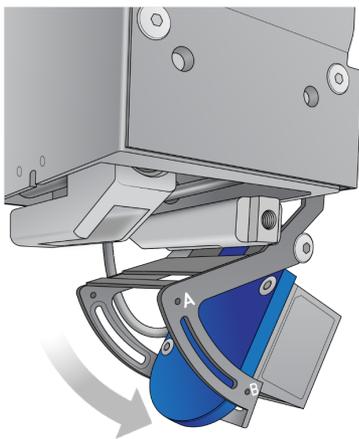
Figure 4 Robot Setup for Fragment MSA7



- A FMS Reservoir
- B MSA7 Plates

- 5 Adjust the Tecan scanner bracket to **Position B**.

Figure 5 Move Tecan Scanner Bracket to Position B



- 6 At the robot PC, click **Run**.
- 7 When the robot finishes, click **OK** in the message box.
- 8 Remove the plates from the robot bed and apply cap mats.
- 9 Vortex at 1600 rpm for 1 minute.
- 10 Centrifuge at $280 \times g$ at room temperature for 1 minute.
- 11 Place into 37°C Illumina Hybridization Oven for 30 minutes.
If you are continuing, you can leave the plates in the 37°C Illumina Hybridization Oven until you have completed preparation for the next step, no longer than 2 hours.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C.

Precipitate DNA

This process begins with an isopropanol precipitation, then centrifuges to collect the fragmented DNA.

Consumables

- ▶ PM1
- ▶ 100% 2-propanol
- ▶ Cap mats

Preparation

- 1 Prepare the following consumables.

Item	Storage	Instructions
PM1	2°C to 8°C	Bring to room temperature

- 2 Cool refrigerated centrifuge to 4°C.
- 3 If you froze the MSA7 plates, thaw to room temperature.
- 4 Centrifuge at 280 × g at room temperature for 1 minute.

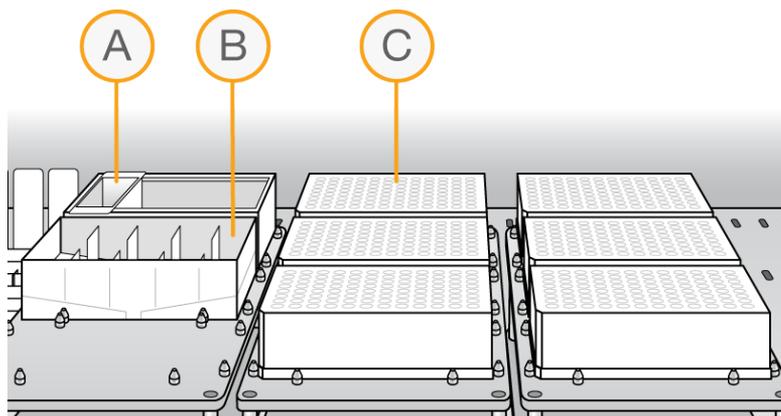
Precipitate the MSA7 Plate

- 1 At the robot PC, select **MSA7 HT Tasks | Precip MSA7 HT**.
- 2 Scan the MSA7 plate barcodes, click **Verify**, and then click **Save**.
- 3 Remove the cap mats and place the MSA7 plates on the robot bed according to the bed map.
- 4 Place a quarter reservoir on the robot bed, according to the bed map, and add PM1:

Number of Plates	Volume
1	8 ml
2	14 ml
3	21 ml
4	27 ml
5	34 ml
6	40 ml

- 5 Place a full reservoir on the robot bed, according to the bed map, and add 2-propanol:

Number of Plates	Volume
1	25 ml
2	50 ml
3	75 ml
4	100 ml
5	125 ml
6	150 ml

Figure 6 Robot Setup for Precipitate MSA7

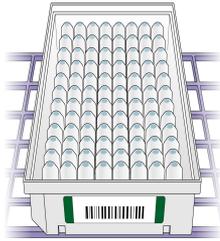
- A PM1 Reservoir
- B 2-Propanol Reservoir
- C MSA7 Plates

- 6 At the robot PC, click **Run**.
- 7 When prompted, scan the reagent barcode.
- 8 When prompted, click **OK**. Remove the plates from the robot bed and apply fresh cap mats.
- 9 Invert the plates 10 times to mix.
- 10 In Illumina LIMS, select **Infinium XT HT | Spin MSA7 HT**.
- 11 Scan the MSA7 plate barcodes, click **Verify**, and then click **Save**.
- 12 Centrifuge at 3000 × g at 4°C for 20 minutes.
Perform the next steps immediately to avoid dislodging the blue pellets. If any delay occurs, repeat centrifugation before proceeding.
- 13 Remove the plates from the centrifuge and remove the cap mats.
- 14 Quickly invert the plates and drain liquid to decant the supernatant. Then smack the plates down on a dry pad.
- 15 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

**CAUTION**

Keep the plates inverted. Do not allow supernatant in wells to pour into other wells.

- 16 Leave uncovered, inverted plate on the tube rack for 15 minutes at room temperature to air dry pellets. Look for blue pellets at the bottom of the wells. Keeping the plate inverted, use a Kimwipe to remove any residual alcohol draining from the wells or remaining on the surface.

Figure 7 Uncovered MSA7 Plate Inverted for Air Drying**CAUTION**

Do not overdry the pellets. Pellets that are overdried are difficult to resuspend and can lead to poor data quality.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C .

Resuspend DNA

This process adds RA1 to the MSA7 plate to resuspend the precipitated DNA samples.

**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/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Consumables

- ▶ RA1
- ▶ Foil heat seals

Preparation

- 1 Prepare the following consumable.

Item	Storage	Instructions
RA1	-25°C to -15°C	Warm to room temperature in a 20°C to 25°C water bath. Alternatively, thaw overnight at 4°C then let come to room temperature. Mix to dissolve any remaining crystals.

**NOTE**

Use fresh RA1 for each step where it is required. RA1 that has been stored properly and has not been dispensed is considered fresh. To conserve RA1, only pour out the amount needed for the current step.

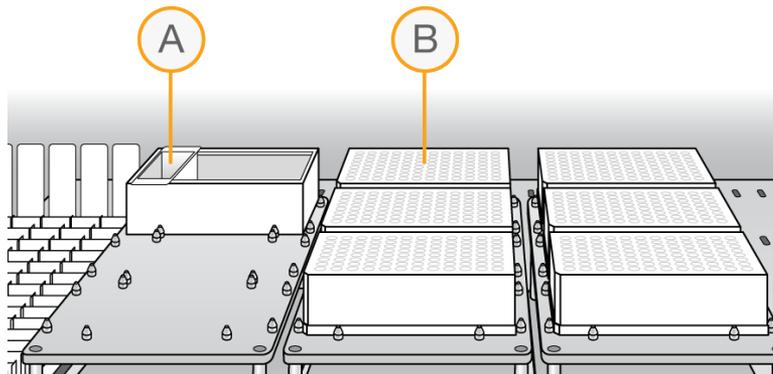
- 2 If you stored the MSA7 plates at -25°C to -15°C , thaw to room temperature, pulse centrifuge, and then remove the cap mats.
- 3 Preheat the Illumina Hybridization Oven to 48°C .
- 4 Preheat the heat sealer for 20 minutes before use.

Resuspend the MSA7 Plate

- 1 At the robot PC, select **MSA7 HT Tasks | Resuspend MSA7 HT**.
- 2 In the Basic Run Parameters pane, change the value for the **Number of MSA7 plates**.
- 3 Place the MSA7 plates on the robot bed according to the bed map.
- 4 Place a quarter reservoir on the robot bed, according to the bed map, and add RA1:

Number of Plates	Volume
1	5 ml
2	8 ml
3	11 ml
4	14 ml
5	17 ml
6	20 ml

Figure 8 Robot Setup for Resuspend MSA7



- A RA1 Reservoir
- B MSA7 Plates

- 5 At the robot PC, click **Run**.
- 6 When prompted, click **OK**. Remove the MSA7 plates from the robot bed.
- 7 Apply foil heat seals to the MSA7 plates using the heat sealer.
- 8 Incubate in the Illumina Hybridization Oven for 15 minutes at 48°C. If the plates were frozen, incubate for 1 hour.
- 9 Vortex at 1800 rpm for 1 minute.
- 10 Check to make sure that the pellets are resuspended. If necessary, repeat the incubation and vortexing steps.
- 11 Centrifuge at 280 × g at room temperature for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA7 plates at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C. Store RA1 at -25°C to -15°C. If RA1 will be used the next day, store it overnight at 4°C.

Hybridize to BeadChip

In this process, the fragmented and resuspended DNA samples are dispensed onto the BeadChips. The BeadChips are incubated in the Illumina Hybridization Oven, enabling each sample to hybridize to an individual section of the BeadChip.

Consumables

- ▶ PB2
- ▶ 1% aqueous Alconox solution
- ▶ DI H₂O

Preparation

- 1 If you froze the MSA7 plates, thaw to room temperature, then pulse centrifuge at 280 × g at room temperature for 1 minute.
- 2 Remove BeadChips from storage but do not unpackage.
- 3 Preheat the heat block to 95°C.
- 4 Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.

Procedure

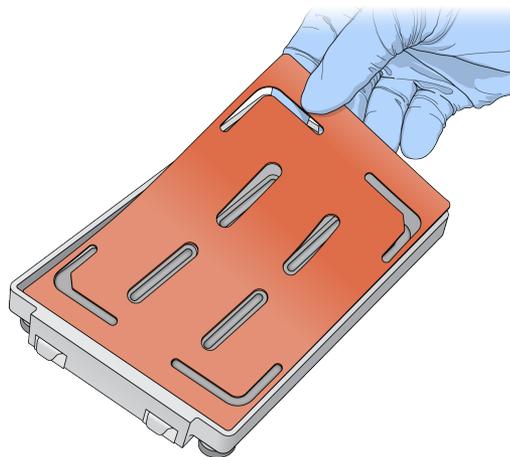
Denature DNA

- 1 Place the MSA7 plates on the heat block at 95°C for 20 minutes to denature samples.
- 2 Cool the MSA7 plates on the benchtop at room temperature for 30 minutes.
- 3 Centrifuge at 280 × g at room temperature for 1 minute.

Assemble Hybridization Chambers

- 1 Place the gaskets into the XT Hyb chambers.
Press the gasket down all around the edges to make sure that it is properly seated.

Figure 9 XT Hyb Chamber and Gasket

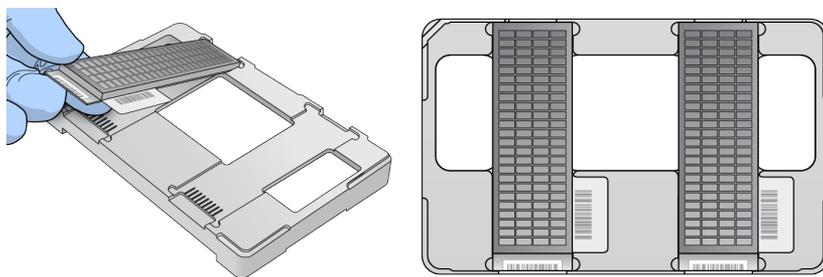


- 2 Dispense 800 µl PB2 into each of the 4 humidifying buffer reservoirs in the XT Hyb chambers.
- 3 Close the XT Hyb chamber immediately to prevent evaporation.
- 4 Leave closed XT Hyb chambers on the bench at room temperature until BeadChips are loaded with DNA sample.
- 5 In Illumina LIMS, select **Infinium XT | Confirm BeadChips for Hyb**.
- 6 Scan the barcodes of the MSA7 plates, and all the BeadChips you plan to hybridize. Click **Verify**.

Prepare the Robot

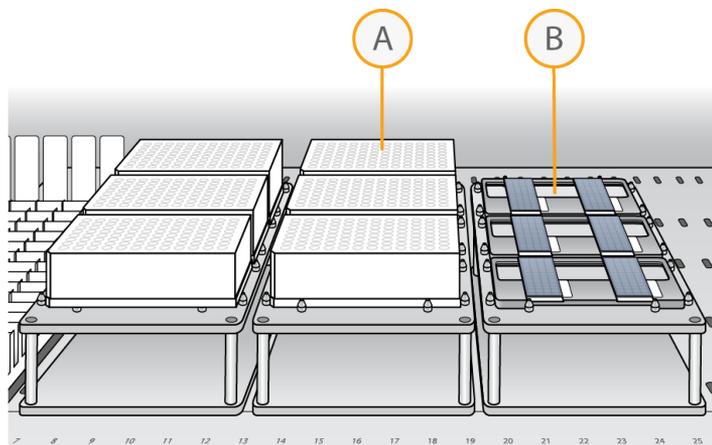
- 1 Remove all BeadChips from packaging.
- 2 Place up to 2 BeadChips onto each XT dual Hyb insert and baseplate, making sure the BeadChip is flush with the baseplate.

Figure 10 Placing BeadChips on Baseplates



- 3 At the robot PC, select **MSA7 HT Tasks | Hyb Multi-BC2**.
- 4 In the BeadChip Selection dialog box, select the 96-sample BeadChip.
- 5 Place the XT dual Hyb insert and baseplates onto the robot bed according to the bed map.
- 6 Place the MSA7 plates onto the robot bed according to the bed map, and remove the heat seals.

Figure 11 Robot Setup for Hybridization



- A MSA7 Plates
- B XT Dual Hyb Insert and Baseplates

Start the Robot

- 1 At the robot PC, click **Run**.
- 2 After the robot scans the BeadChip barcodes, place an XT tip guide #1 on each XT dual Hyb insert and baseplate.
- 3 Click **OK**.
 - ▶ The robot dispenses DNA sample to the BeadChips.
 - ▶ Allow ~30 minutes for each tip guide.
- 4 When prompted, remove XT tip guide #1 and replace it with XT tip guide #2.
- 5 At the robot PC, click **OK**.
- 6 When prompted, remove XT tip guide #2 and replace it with XT tip guide #3.
- 7 At the robot PC, click **OK**.
When the process is complete, the robot PC sounds an alert and opens a message.
- 8 Click **OK** in the message box.
- 9 Remove XT tip guide #3 from the XT dual Hyb insert and baseplates.
- 10 Remove the XT dual Hyb insert and baseplates from the robot bed and inspect the BeadChips. Note any sections that are not covered with DNA sample.

Set Up and Incubate BeadChips

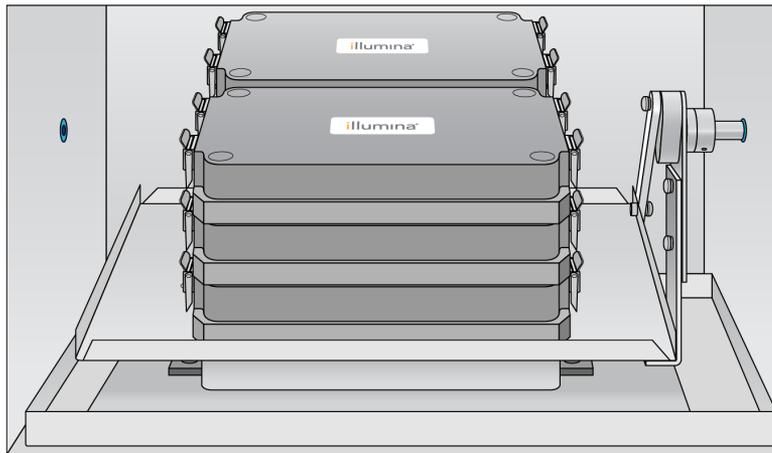
- 1 Make sure that the Illumina Hybridization Oven is set to 48°C.



WARNING

Keep Hyb chambers at room temperature when you load the BeadChips. Do not place the Hyb chambers in the Illumina Hybridization Oven before loading the BeadChips.

- 2 Load the XT dual Hyb insert and baseplates containing BeadChips inside the XT Hyb chambers. You can stack up to 3 XT dual Hyb insert and baseplates in each XT Hyb chamber.
- 3 In Illumina LIMS, select **Infinium XT | Prepare Hyb Chamber**.
- 4 Scan the barcodes of the PB2 and BeadChips.
- 5 Click **Verify**, and then click **Save**.
- 6 Close each XT Hyb chamber and secure the clamps.
- 7 Place the XT Hyb chambers in the Illumina Hybridization Oven with the Illumina logo facing you. If you are stacking multiple XT Hyb chambers in the Illumina Hybridization Oven, fit the feet of each XT Hyb chamber into the matching indents on the lid of the XT Hyb chamber below it. You can stack up to 3 XT Hyb chambers for a maximum of 6 total in the Illumina Hybridization Oven.

Figure 12 XT Hyb Chambers Correctly Placed in Hyb Oven

OVERNIGHT INCUBATION

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

Resuspend XC4 Reagent

- 1 Add 330 ml 100% EtOH to the XC4 bottle.
Final volume is approximately 350 ml. Each XC4 bottle can be used to process up to 48 BeadChips.
- 2 Shake the XC4 bottle vigorously.
- 3 Leave the bottle upright on the lab bench overnight.



NOTE

If XC4 was not left to resuspend overnight, you can still proceed with the assay.

Wash Robot XT Tip Guides

For optimal performance, wash and dry the XT tip guides after each use.

- 1 Soak the XT tip guides in 1% aqueous Alconox solution (1 part Alconox to 99 parts water) for 5 minutes.
Do not use bleach or ethanol to clean XT tip guides.
- 2 Thoroughly rinse the XT tip guides with DI H₂O at least 3 times to remove any residual detergent.
- 3 Dry the XT tip guides and make sure that they are free of any residual contaminants before next use.

Wash BeadChips

In this procedure, you prepare BeadChips for the XStain process.



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/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Consumables

- ▶ 1X PB1

Preparation

- 1 Make sure that you have diluted 1X PB1 ready for use.
- 2 Remove the XT Hyb chambers from the Illumina Hybridization Oven. Cool for 30 minutes at room temperature before opening.
- 3 While the XT Hyb chambers are cooling:
 - a Fill 2 wash dishes with 1X PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
 - b Make sure that the XCG glass back plates have no chips or cracks.
 - c Clean the XCG glass back plates if necessary.
- 4 Make sure that additional XCG Flow-Through Chamber frames and clips are ready for use.

Procedure

Wash BeadChips

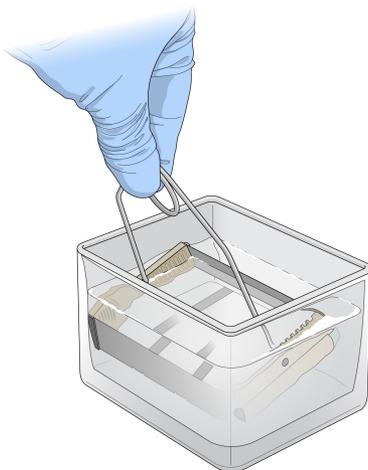
- 1 Attach the wire handle and submerge the wash rack in Wash 1 containing 1X PB1.



NOTE

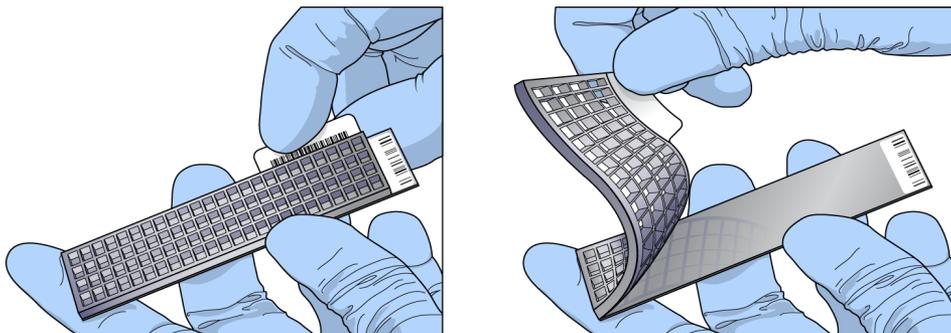
Replace 1X PB1 in Wash 1 after every 12 BeadChips.

Figure 13 Wash Rack in Wash Dish



- 2 Remove the XT dual Hyb insert and baseplates from the XT Hyb chambers.
- 3 Remove the BeadChips from the XT dual Hyb insert and baseplates.
- 4 Remove the cover seals from the BeadChips.

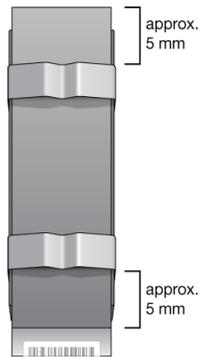
Using powder-free gloved hands, hold the BeadChip securely and by the edges in one hand. Remove the entire seal in a single, continuous motion. Do not touch exposed arrays.

Figure 14 Removing the Cover Seal

- 5 Immediately and carefully slide each BeadChip into the wash rack in Wash 1, making sure that the BeadChip is submerged in 1X PB1.
- 6 Repeat these steps until all BeadChips are transferred to the submerged wash rack in Wash 1.
- 7 Move the wash rack up and down for 1 minute, breaking the surface of the 1X PB1 with gentle, slow agitation.
- 8 Move the wash rack to wash 2 containing clean 1X PB1, making sure that the BeadChips are submerged.
- 9 Move the wash rack up and down for 1 minute, breaking the surface of the 1X PB1 with gentle, slow agitation.
- 10 Remove the BeadChips from the wash rack and inspect them for remaining residue.
If you see residue, submerge the BeadChip in 1X PB1 and carefully use a pipette tip to remove the remaining residue.

Assemble Flow-Through Chambers

- 1 Orient the stamped bar code ridges in the XCG Flow-Through Chamber assembly tray towards you.
- 2 Fill the XCG Flow-Through Chamber assembly tray with 1X PB1.
- 3 For each BeadChip to be processed, place an XCG Flow-Through Chamber frame into the XT Flow-Through Chamber assembly tray.
- 4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.
- 5 Place an XCG glass back plate onto a submerged BeadChip with spacers facing down and beveled edge towards bar code.
- 6 Attach XCG Flow-Through Chamber clips to each XCG Flow-Through Chamber frame.
 - a Gently push the XCG glass back plate against the far stop in the alignment position.
 - b Place the first XCG Flow-Through Chamber clip around the XCG Flow-Through Chamber frame, approximately 5 mm from the top edge.
 - c Place the second XCG Flow-Through Chamber clip around the XCG Flow-Through Chamber frame at the barcode end, approximately 5 mm from the reagent reservoir.

Figure 15 Assembled XCG Flow-Through Chamber

- 7 In Illumina LIMS, select **Wash BeadChip XT HT**.
- 8 Scan the BeadChip barcodes, click **Verify**, and then click **Save**.

Extend and Stain (XStain)

In this process, 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.

Consumables

- ▶ RA1
- ▶ EML
- ▶ XC3
- ▶ PB1
- ▶ XC4
- ▶ XStain plates
- ▶ Alconox powder detergent
- ▶ 95% formamide/1 mM EDTA



WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

Preparation

- 1 Thaw XStain plates for 24 hours at 4°C.
- 2 Use a room temperature water bath for 1 hr to complete thawing and to bring the reagents to room temperature.
 - ▶ The contents of the XStain plates are fully thawed when the bottom of the plate is no longer cool to the touch.

- 3 Invert the XStain plates 10 times to mix the reagents.
- 4 Centrifuge at 280 x g at room temperature for 1 minute.
- 5 Carefully remove the seal to avoid cross-contamination among wells.
- 6 Thaw RA1 to room temperature. Mix to dissolve any remaining crystals.



NOTE

Use fresh RA1 for each step where it is required. RA1 that has been stored properly and has not been dispensed is considered fresh. To make best use of RA1, only pour out the amount needed for the current step.

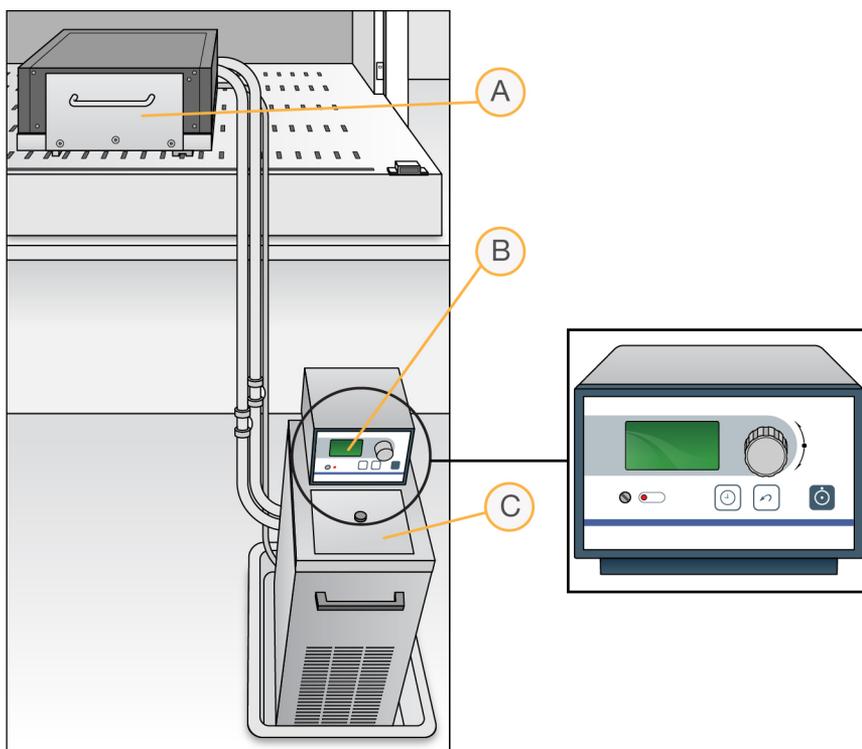
- 7 Thaw the EML tubes at room temperature.
- 8 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.

Procedure

Set Up the Chamber Rack

- 1 Make sure that the water circulator is filled to the appropriate level. See the *VWR Operator Manual*, VWR part # 110-229.
- 2 At the robot PC, select **Robot QC Tasks | Circulator Manager** to set the water circulator to 44°C.

Figure 16 Water Circulator Connected to Chamber Rack



- A Chamber Rack
- B Water Circulator

C Reservoir Cover

- 3 Remove bubbles trapped in the chamber rack **each time** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.
- 4 Use a temperature probe to confirm that the chamber rack temperature is at 44°C ± 0.5°C at all locations.

Single Base Extension and Stain

This process uses a robot to process the BeadChips.



CAUTION

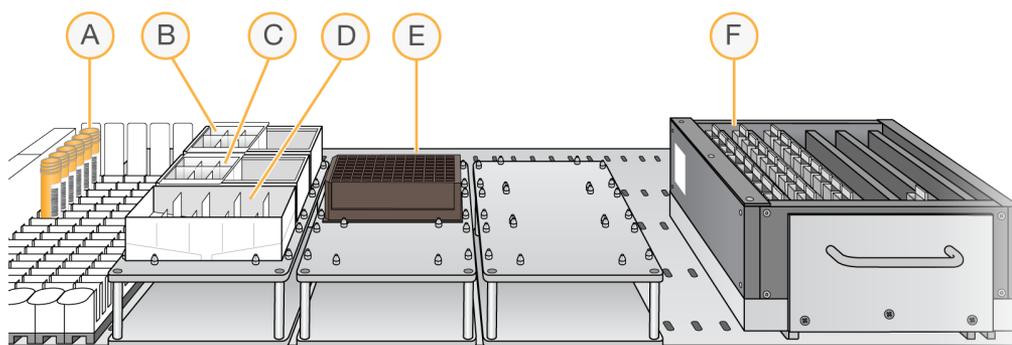
The following steps must be performed without interruption.

- 1 At the robot PC, select **XStain Tasks | XStain XCG BeadChip HT**.
- 2 Turn on the iScan systems to allow the lasers to stabilize.
- 3 Place reservoirs on the robot bed, according to the bed map, and add reagents to reservoirs as follows:

Reagent	# BeadChips	Volume
95% formamide/1 mM EDTA	24	30 ml
	48	60 ml
RA1	24	30 ml
	48	60 ml
XC3	24	150 ml
	48	250 ml

- 4 Place the XStain plates on the robot bed, according to the bed map. Remove the seals.
- 5 Invert the EML tubes to mix, remove the caps, and place on the robot bed, according to the robot bed map.
- 6 In the Basic Run Parameters pane, enter the number of BeadChips, up to 48.

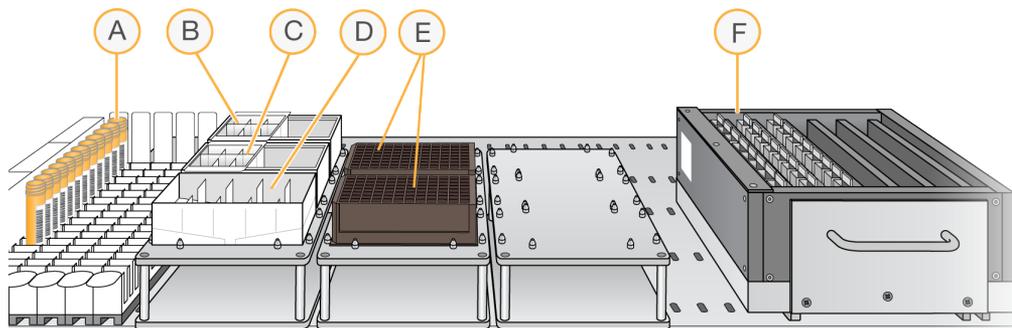
Figure 17 XStain Robot Setup for 24 BeadChips



- A EML Tubes
- B 95% Formamide/1 mM EDTA Reservoir
- C RA1 Reservoir
- D XC3 Reservoir
- E XStain Plate

F BeadChips in Flow-Through Chamber Frame

Figure 18 XStain Robot Setup for 48 BeadChips



- A EML Tubes
- B 95% Formamide/1 mM EDTA Reservoir
- C RA1 Reservoir
- D XC3 Reservoir
- E XStain Plates
- F BeadChips in Flow-Through Chamber Frame

- 7 Click **Run**.
- 8 When prompted, enter the stain temperature listed on the XStain plate. Do not load the BeadChips yet.
- 9 When the chamber rack reaches 44°C, place the XCG Flow-Through Chamber assemblies into the chamber rack, according to the robot bed map.
- 10 At the robot PC, click **OK**.
- 11 When the robot finishes, remove the XCG Flow-Through Chamber assemblies from the chamber rack and place them horizontally on the lab bench at room temperature.

Wash and Coat BeadChips

- 1 Set up 2 top-loading PB1 and XC4 wash dishes, labeled PB1 and XC4.
- 2 To indicate fill volume, pour 310 ml water into the wash dishes and mark the water level. Empty the water from the wash dish.
- 3 Pour 310 ml PB1 into a wash dish labeled PB1.
- 4 Place a staining rack inside the wash dish with locking arms and tab facing towards you.
- 5 One at a time, disassemble each XCG Flow-Through Chamber:
 - a Use the dismantling tool to remove the metal clamps. It is important to use the dismantling tool to avoid chipping XCG glass back plates.
 - b Remove the XCG glass back plate, then the BeadChip.
- 6 Place BeadChips into a staining rack in the PB1 wash dish. Make sure that all barcodes face the same direction and that all BeadChips are submerged.



CAUTION

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

- 7 Submerge the XCG glass back plates in the DI H₂O wash basin for later cleaning as detailed in the *Infinium Lab Setup and Procedures Guide*.
- 8 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.



NOTE

If BeadChip edges begin to touch during washing, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

- 9 Soak the BeadChips for an additional 5 minutes.



CAUTION

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

- 10 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.
- 11 Pour 310 ml XC4 into a wash dish.
- 12 Move the staining rack from the PB1 dish to the XC4 wash dish.
- 13 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

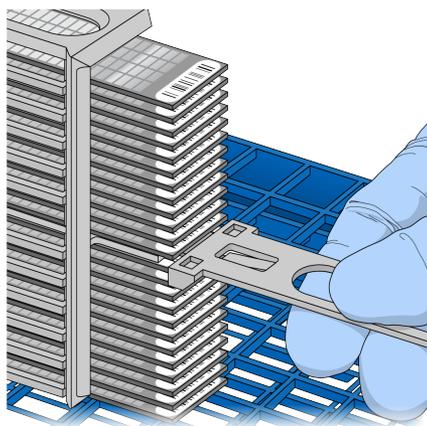


NOTE

If BeadChip edges begin to touch during washing, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

- 14 Soak the BeadChips for an additional 5 minutes.
- 15 Remove the staining rack and place it on the prepared tube rack with the array side of the BeadChips facing up.

Figure 19 Staining Rack in Correct Orientation



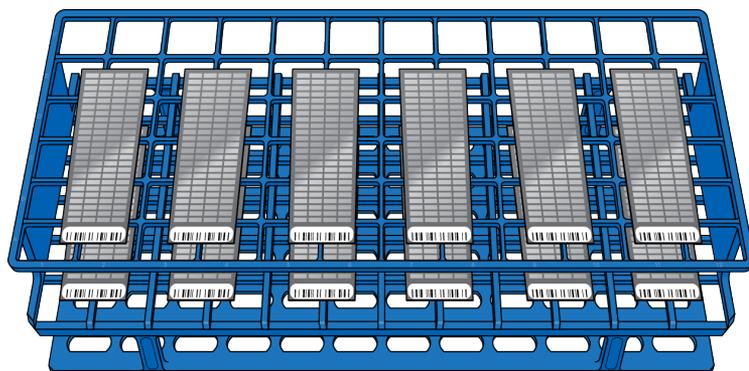
- 16 Remove the handle from the staining rack for easier access to the BeadChips.
- 17 For each BeadChip, working top to bottom:
 - a Use self-locking tweezers to grip the BeadChip at its barcode end.
 - b Place the BeadChip on the tube rack with the array side facing up.



CAUTION

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

Figure 20 BeadChips on Tube Rack



- 18 Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 675 mm Hg (0.9 bar). Drying times can vary according to room temperature and humidity.
- 19 Release the vacuum by turning the handle 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.

- 20 Touch the edges of the BeadChips (**do not touch arrays**) to make sure etched, barcoded sides are dry.
- 21 If the back of the BeadChip feels tacky, wipe it with a Prostat wipe or a Kimwipe wetted with 70% EtOH.
- 22 Image the BeadChips immediately or store protected from light.

Image BeadChip

Follow the instructions in the *iScan System User Guide* to scan your BeadChips. Use the **Infinium XT** scan setting for your BeadChips.

Chapter 3 Automated Protocol without Illumina LIMS

Introduction	28
Amplify DNA (Pre-Amp)	28
Incubate DNA	30
Fragment DNA	30
Precipitate DNA	31
Resuspend DNA	33
Hybridize to BeadChip	35
Wash BeadChips	38
Extend and Stain (XStain)	41
Image BeadChip	46

Introduction

This section describes pre- and post-amplification automated laboratory protocols for the Infinium XT Assay. Follow the protocols in the order shown.

This section includes instructions for performing the protocol without Illumina Laboratory Information Management System (LIMS). Robot automation steps use the automation control software. If you are using Illumina LIMS, see *Automated Protocol with Illumina LIMS on page 8* for protocol instructions.

Amplify DNA (Pre-Amp)

This process adds the DNA samples to the plates, then denatures and neutralizes the samples to prepare them for amplification.

Consumables

- ▶ MA1
- ▶ MA2
- ▶ RAM
- ▶ 0.1N NaOH
- ▶ 96-well 0.8 ml microplates (MIDI)
- ▶ WG#-DNA plates with 96 DNA samples (10 µl at 50 ng/µl) (MIDI or TCY)
- ▶ Cap mats

Preparation

- 1 Thaw DNA samples to room temperature.
- 2 Thaw the following consumables at 4°C for approximately 24 hours. If necessary, use a room temperature water bath to complete thawing and bring the reagents to room temperature.

Item	Storage
MA2	-25°C to -15°C
RAM	-25°C to -15°C

- 3 Invert to mix.

- 4 Preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- 5 Apply MSA7 barcode labels to new MIDI plates.
- 6 Vortex DNA samples at 1600 rpm for 1 minute.
- 7 Centrifuge DNA samples at 280 × g at room temperature for 1 minute.

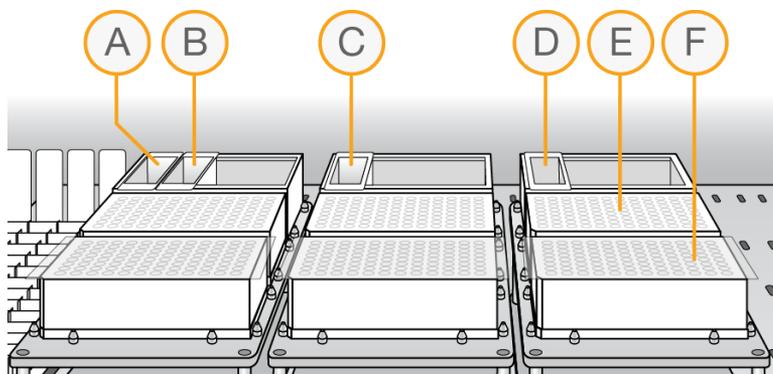
Procedure

- 1 At the robot PC, select **MSA7 HT Tasks | Make MSA7 HT**.
- 2 Select the WG#-DNA plate type (MIDI or TCY). Do not mix plate types on the robot.
- 3 In the Basic Run Parameters pane, enter the **Number of DNA plates**.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed.
- 4 Place 4 quarter reservoirs on the robot bed, according to the bed map.
- 5 Use a serological pipette to add reagents to reservoirs. Use exactly the following amounts for every 3 plates:

Reagent	Volume
MA1	9 ml
MA2	13.5 ml
RAM	13.5 ml
0.1N NaOH	5 ml

- 6 Place WG#-DNA source and MSA7 MIDI plates on the robot bed according to the bed map.
- 7 At the robot PC, click **Run**.

Figure 21 Robot Setup for Amplify DNA



- A MA1 Reservoir
- B 0.1N NaOH Reservoir
- C MA2 Reservoir
- D RAM Reservoir
- E MSA7 Plates
- F DNA Plates

- 8 After the robot has completed the run, apply cap mats to the MSA7 plates and vortex at 1600 rpm for 1 minute.
- 9 Centrifuge at $280 \times g$ at room temperature for 1 minute.

Incubate DNA

This step uniformly amplifies the genomic DNA, generating a sufficient quantity of each individual DNA sample to be used in the Infinium XT Assay.



NOTE

This and all remaining steps in the workflow are performed in the post-amp lab.

- 1 Incubate the MSA7 plates in the Illumina Hybridization Oven for 3–24 hours at 37°C.

Fragment DNA

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

Consumables

- ▶ FMS

Preparation

- 1 Prepare the following consumables.

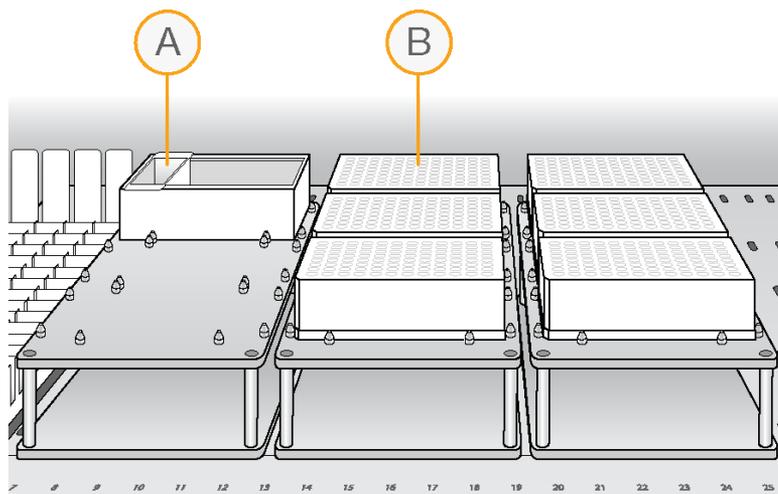
Item	Storage	Instructions
FMS	-25°C to -15°C	Thaw to room temperature and invert to mix.

- 2 Preheat the Illumina Hybridization Oven to 37°C.
- 3 If you plan to resuspend the MSA7 plates today, remove the RA1 from the freezer to thaw.

Procedure

- 1 Centrifuge the MSA7 plates at $280 \times g$ at room temperature for 1 minute.
- 2 At the robot PC, select **MSA7 HT Tasks | Fragment MSA7 HT**.
- 3 In the Basic Run Parameters pane, enter the **Number of MSA7plates**.
- 4 Place the MSA7 plates on the robot bed according to the bed map. Remove the cap mats.
- 5 Place a quarter reservoir on the robot bed, according to the bed map, and add 20 ml FMS for 6 plates.

Figure 22 Robot Setup for Fragment MSA7



- A FMS Reservoir
- B MSA7 Plates

- 6 At the robot PC, click **Run**.
- 7 Remove the plates from the robot bed and apply cap mats.
- 8 Vortex at 1600 rpm for 1 minute.
- 9 Centrifuge at 280 × g at room temperature for 1 minute.
- 10 Place into 37°C Illumina Hybridization Oven for 30 minutes.
If you are continuing, you can leave the plates in the 37°C Illumina Hybridization Oven until you have completed preparation for the next step, no longer than 2 hours.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C.

Precipitate DNA

This process begins with an isopropanol precipitation, then centrifuges to collect the fragmented DNA.

Consumables

- ▶ PM1
- ▶ 100% 2-propanol
- ▶ Cap mats

Preparation

- 1 Prepare the following consumables.

Item	Storage	Instructions
PM1	2°C to 8°C	Bring to room temperature

- 2 Cool refrigerated centrifuge to 4°C.

- 3 If you froze the MSA7 plates, thaw to room temperature.
- 4 Centrifuge at $280 \times g$ at room temperature for 1 minute.

Precipitate the MSA7 Plate

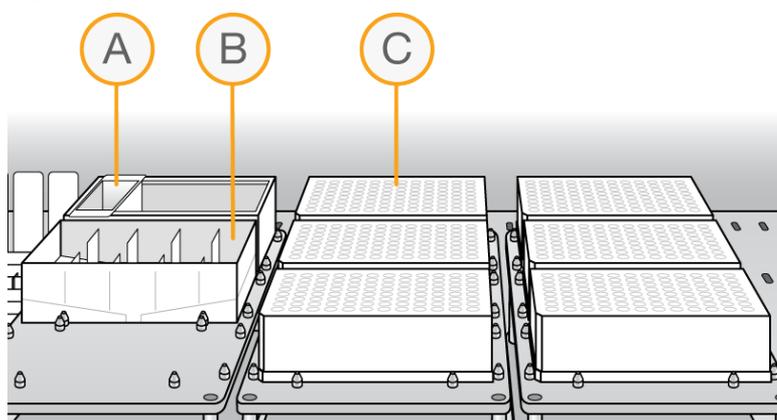
- 1 At the robot PC, select **MSA7 HT Tasks | Precip MSA7 HT**.
- 2 In the Basic Run Parameters pane, change the value for the **Number of MSA7 plates**.
- 3 Remove the cap mats and place the MSA7 plates on the robot bed according to the bed map.
- 4 Place a quarter reservoir on the robot bed, according to the bed map, and add PM1:

Number of Plates	Volume
1	8 ml
2	14 ml
3	21 ml
4	27 ml
5	34 ml
6	40 ml

- 5 Place a full reservoir on the robot bed, according to the bed map, and add 2-propanol:

Number of Plates	Volume
1	25 ml
2	50 ml
3	75 ml
4	100 ml
5	125 ml
6	150 ml

Figure 23 Robot Setup for Precipitate MSA7



- A PM1 Reservoir
- B 2-Propanol Reservoir
- C MSA7 Plates

- 6 At the robot PC, click **Run**.

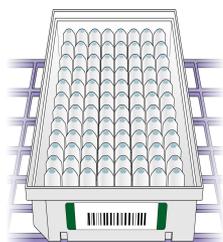
- 7 When prompted, click **OK**. Remove the plates from the robot bed and apply fresh cap mats.
- 8 Invert the plates 10 times to mix.
- 9 Centrifuge at $3000 \times g$ at 4°C for 20 minutes.
Perform the next steps immediately to avoid dislodging the blue pellets. If any delay occurs, repeat centrifugation before proceeding.
- 10 Remove the plates from the centrifuge and remove the cap mats.
- 11 Quickly invert the plates and drain liquid to decant the supernatant. Then smack the plates down on a dry pad.
- 12 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

**CAUTION**

Keep the plates inverted. Do not allow supernatant in wells to pour into other wells.

- 13 Leave uncovered, inverted plate on the tube rack for 15 minutes at room temperature to air dry pellets. Look for blue pellets at the bottom of the wells. Keeping the plate inverted, use a Kimwipe to remove any residual alcohol draining from the wells or remaining on the surface.

Figure 24 Uncovered MSA7 Plate Inverted for Air Drying

**CAUTION**

Do not overdry the pellets. Pellets that are overdried are difficult to resuspend and can lead to poor data quality.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C .

Resuspend DNA

This process adds RA1 to the MSA7 plate to resuspend the precipitated DNA samples.

**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/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Consumables

- ▶ RA1
- ▶ Foil heat seals

Preparation

- 1 Prepare the following consumable.

Item	Storage	Instructions
RA1	-25°C to -15°C	Warm to room temperature in a 20°C to 25°C water bath. Alternatively, thaw overnight at 4°C then let come to room temperature. Mix to dissolve any remaining crystals.



NOTE

Use fresh RA1 for each step where it is required. RA1 that has been stored properly and has not been dispensed is considered fresh. To conserve RA1, only pour out the amount needed for the current step.

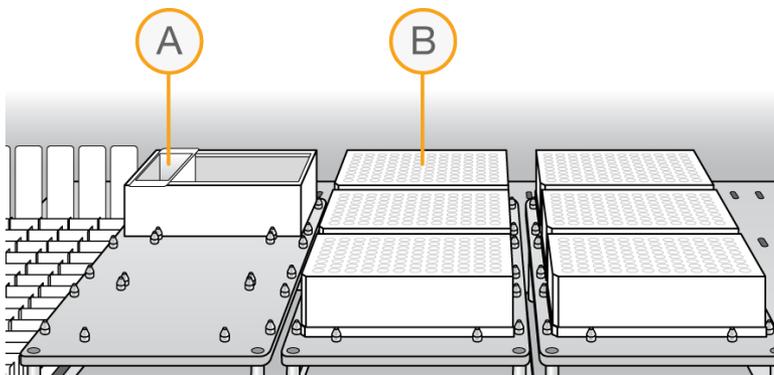
- 2 If you stored the MSA7 plates at -25°C to -15°C, thaw to room temperature, pulse centrifuge, and then remove the cap mats.
- 3 Preheat the Illumina Hybridization Oven to 48°C.
- 4 Preheat the heat sealer for 20 minutes before use.

Resuspend the MSA7 Plate

- 1 At the robot PC, select **MSA7 HT Tasks | Resuspend MSA7 HT**.
- 2 In the Basic Run Parameters pane, change the value for the **Number of MSA7 plates**.
- 3 Place the MSA7 plates on the robot bed according to the bed map.
- 4 Place a quarter reservoir on the robot bed, according to the bed map, and add RA1:

Number of Plates	Volume
1	5 ml
2	8 ml
3	11 ml
4	14 ml
5	17 ml
6	20 ml

Figure 25 Robot Setup for Resuspend MSA7



- A RA1 Reservoir
- B MSA7 Plates

- 5 At the robot PC, click **Run**.
- 6 When prompted, click **OK**. Remove the MSA7 plates from the robot bed.
- 7 Apply foil heat seals to the MSA7 plates using the heat sealer.
- 8 Incubate in the Illumina Hybridization Oven for 15 minutes at 48°C. If the plates were frozen, incubate for 1 hour.
- 9 Vortex at 1800 rpm for 1 minute.
- 10 Check to make sure that the pellets are resuspended. If necessary, repeat the incubation and vortexing steps.
- 11 Centrifuge at 280 × g at room temperature for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA7 plates at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C. Store RA1 at -25°C to -15°C. If RA1 will be used the next day, store it overnight at 4°C.

Hybridize to BeadChip

In this process, the fragmented and resuspended DNA samples are dispensed onto the BeadChips. The BeadChips are incubated in the Illumina Hybridization Oven, enabling each sample to hybridize to an individual section of the BeadChip.

Consumables

- ▶ PB2
- ▶ 1% aqueous Alconox solution
- ▶ DI H₂O

Preparation

- 1 If you froze the MSA7 plates, thaw to room temperature, then pulse centrifuge at 280 × g at room temperature for 1 minute.
- 2 Remove BeadChips from storage but do not unpackage.
- 3 Preheat the heat block to 95°C.
- 4 Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.

Procedure

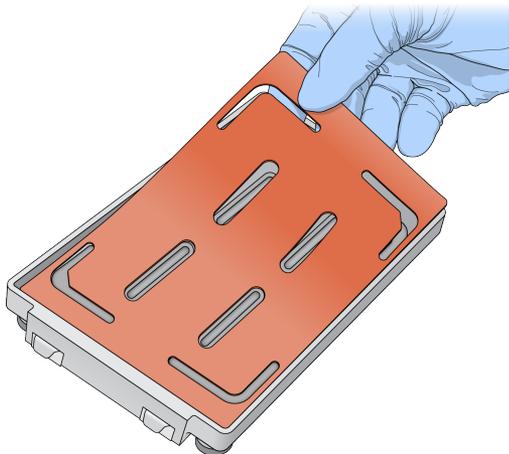
Denature DNA

- 1 Place the MSA7 plates on the heat block at 95°C for 20 minutes to denature samples.
- 2 Cool the MSA7 plates on the benchtop at room temperature for 30 minutes.
- 3 Centrifuge at 280 × g at room temperature for 1 minute.

Assemble Hybridization Chambers

- 1 Place the gaskets into the XT Hyb chambers.
Press the gasket down all around the edges to make sure that it is properly seated.

Figure 26 XT Hyb Chamber and Gasket

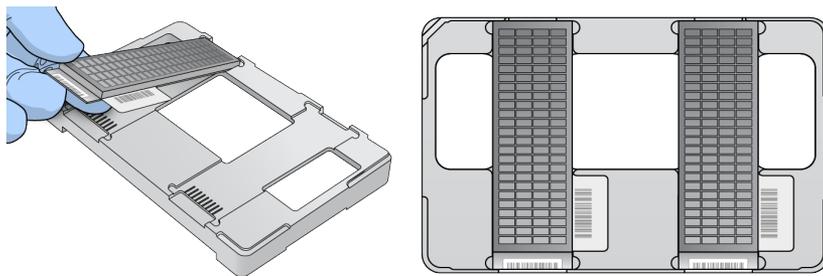


- 2 Dispense 800 μ l PB2 into each of the 4 humidifying buffer reservoirs in the XT Hyb chambers.
- 3 Close the XT Hyb chamber immediately to prevent evaporation.
- 4 Leave closed XT Hyb chambers on the bench at room temperature until BeadChips are loaded with DNA sample.

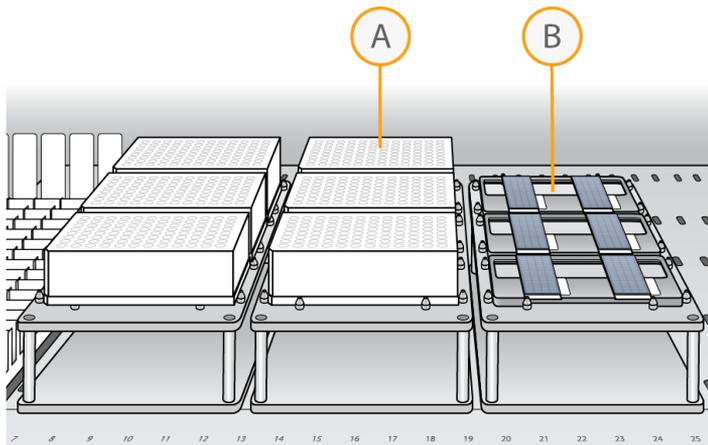
Prepare the Robot

- 1 Remove all BeadChips from packaging.
- 2 Place up to 2 BeadChips onto each XT dual Hyb insert and baseplate, making sure the BeadChip is flush with the baseplate.

Figure 27 Placing BeadChips on Baseplates



- 3 At the robot PC, select **MSA7 HT Tasks | Hyb Multi-BC2**.
- 4 In the BeadChip Selection dialog box, select the 96-sample BeadChip.
- 5 In the Basic Run Parameters pane, change the value for the **Number of MSA7 plates**.
- 6 Place the XT dual Hyb insert and baseplates onto the robot bed according to the bed map.
- 7 Place the MSA7 plates onto the robot bed according to the bed map, and remove the heat seals.

Figure 28 Robot Setup for Hybridization

- A MSA7 Plates
- B XT Dual Hyb Insert and Baseplates

Start the Robot

- 1 Place an XT tip guide #1 on top of each XT dual Hyb insert and baseplate.
- 2 At the robot PC, click **Run**, then click **OK**.
 - ▶ The robot dispenses DNA sample to the BeadChips.
 - ▶ Allow ~30 minutes for each tip guide.
- 3 When prompted, remove XT tip guide #1 and replace it with XT tip guide #2.
- 4 At the robot PC, click **OK**.
- 5 When prompted, remove XT tip guide #2 and replace it with XT tip guide #3.
- 6 At the robot PC, click **OK**.
When the process is complete, the robot PC sounds an alert and opens a message.
- 7 Click **OK** in the message box.
- 8 Remove XT tip guide #3 from the XT dual Hyb insert and baseplates.
- 9 Remove the XT dual Hyb insert and baseplates from the robot bed and inspect the BeadChips. Note any sections that are not covered with DNA sample.

Set Up and Incubate BeadChips

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



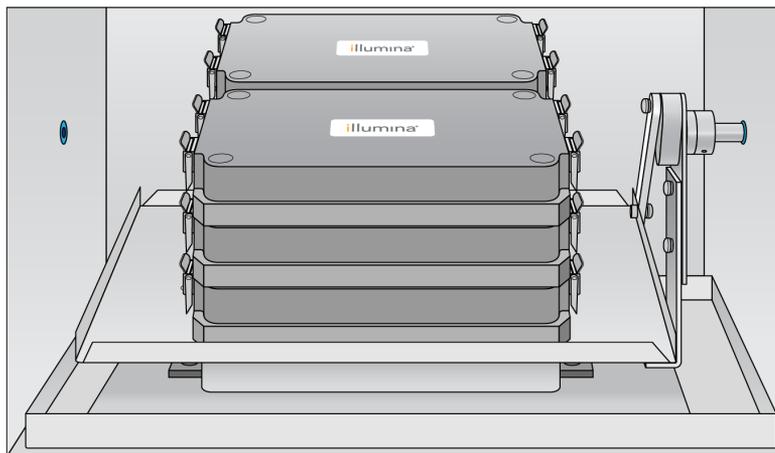
WARNING

Keep Hyb chambers at room temperature when you load the BeadChips. Do not place the Hyb chambers in the Illumina Hybridization Oven before loading the BeadChips.

- 2 Load the XT dual Hyb insert and baseplates containing BeadChips inside the XT Hyb chambers. You can stack up to 3 XT dual Hyb insert and baseplates in each XT Hyb chamber.
- 3 Close each XT Hyb chamber and secure the clamps.

- Place the XT Hyb chambers in the Illumina Hybridization Oven with the Illumina logo facing you. If you are stacking multiple XT Hyb chambers in the Illumina Hybridization Oven, fit the feet of each XT Hyb chamber into the matching indents on the lid of the XT Hyb chamber below it. You can stack up to 3 XT Hyb chambers for a maximum of 6 total in the Illumina Hybridization Oven.

Figure 29 XT Hyb Chambers Correctly Placed in Hyb Oven



OVERNIGHT INCUBATION

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

Resuspend XC4 Reagent

- Add 330 ml 100% EtOH to the XC4 bottle. Final volume is approximately 350 ml. Each XC4 bottle can be used to process up to 48 BeadChips.
- Shake the XC4 bottle vigorously.
- Leave the bottle upright on the lab bench overnight.



NOTE

If XC4 was not left to resuspend overnight, you can still proceed with the assay.

Wash Robot XT Tip Guides

For optimal performance, wash and dry the XT tip guides after each use.

- Soak the XT tip guides in 1% aqueous Alconox solution (1 part Alconox to 99 parts water) for 5 minutes. Do not use bleach or ethanol to clean XT tip guides.
- Thoroughly rinse the XT tip guides with DI H₂O at least 3 times to remove any residual detergent.
- Dry the XT tip guides and make sure that they are free of any residual contaminants before next use.

Wash BeadChips

In this procedure, you prepare BeadChips for the XStain process.



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/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Consumables

- ▶ 1X PB1

Preparation

- 1 Make sure that you have diluted 1X PB1 ready for use.
- 2 Remove the XT Hyb chambers from the Illumina Hybridization Oven. Cool for 30 minutes at room temperature before opening.
- 3 While the XT Hyb chambers are cooling:
 - a Fill 2 wash dishes with 1X PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
 - b Make sure that the XCG glass back plates have no chips or cracks.
 - c Clean the XCG glass back plates if necessary.
- 4 Make sure that additional XCG Flow-Through Chamber frames and clips are ready for use.

Procedure

Wash BeadChips

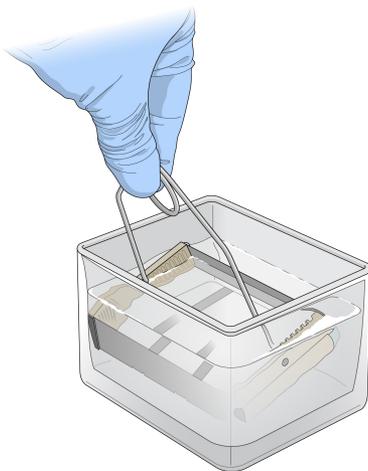
- 1 Attach the wire handle and submerge the wash rack in Wash 1 containing 1X PB1.



NOTE

Replace 1X PB1 in Wash 1 after every 12 BeadChips.

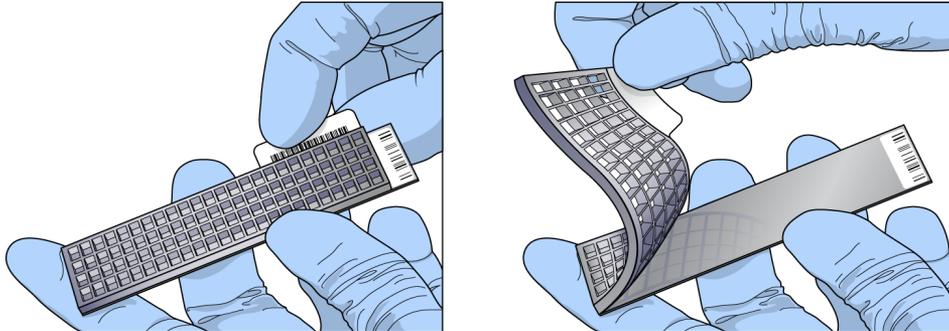
Figure 30 Wash Rack in Wash Dish



- 2 Remove the XT dual Hyb insert and baseplates from the XT Hyb chambers.

- 3 Remove the BeadChips from the XT dual Hyb insert and baseplates.
- 4 Remove the cover seals from the BeadChips.
Using powder-free gloved hands, hold the BeadChip securely and by the edges in one hand. Remove the entire seal in a single, continuous motion. Do not touch exposed arrays.

Figure 31 Removing the Cover Seal



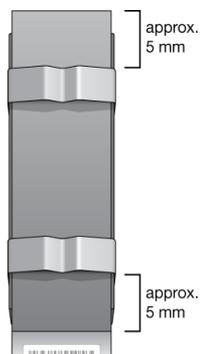
- 5 Immediately and carefully slide each BeadChip into the wash rack in Wash 1, making sure that the BeadChip is submerged in 1X PB1.
- 6 Repeat these steps until all BeadChips are transferred to the submerged wash rack in Wash 1.
- 7 Move the wash rack up and down for 1 minute, breaking the surface of the 1X PB1 with gentle, slow agitation.
- 8 Move the wash rack to wash 2 containing clean 1X PB1, making sure that the BeadChips are submerged.
- 9 Move the wash rack up and down for 1 minute, breaking the surface of the 1X PB1 with gentle, slow agitation.
- 10 Remove the BeadChips from the wash rack and inspect them for remaining residue.
If you see residue, submerge the BeadChip in 1X PB1 and carefully use a pipette tip to remove the remaining residue.

Assemble Flow-Through Chambers

- 1 Orient the stamped bar code ridges in the XCG Flow-Through Chamber assembly tray towards you.
- 2 Fill the XCG Flow-Through Chamber assembly tray with 1X PB1.
- 3 For each BeadChip to be processed, place an XCG Flow-Through Chamber frame into the XT Flow-Through Chamber assembly tray.
- 4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.
- 5 Place an XCG glass back plate onto a submerged BeadChip with spacers facing down and beveled edge towards bar code.
- 6 Attach XCG Flow-Through Chamber clips to each XCG Flow-Through Chamber frame.
 - a Gently push the XCG glass back plate against the far stop in the alignment position.
 - b Place the first XCG Flow-Through Chamber clip around the XCG Flow-Through Chamber frame, approximately 5 mm from the top edge.

- c Place the second XCG Flow-Through Chamber clip around the XCG Flow-Through Chamber frame at the barcode end, approximately 5 mm from the reagent reservoir.

Figure 32 Assembled XCG Flow-Through Chamber



Extend and Stain (XStain)

In this process, 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.

Consumables

- ▶ RA1
- ▶ EML
- ▶ XC3
- ▶ PB1
- ▶ XC4
- ▶ XStain plates
- ▶ Alconox powder detergent
- ▶ 95% formamide/1 mM EDTA



WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

Preparation

- 1 Thaw XStain plates for 24 hours at 4°C.
- 2 Use a room temperature water bath for 1 hr to complete thawing and to bring the reagents to room temperature.
 - ▶ The contents of the XStain plates are fully thawed when the bottom of the plate is no longer cool to the touch.
- 3 Invert the XStain plates 10 times to mix the reagents.

- 4 Centrifuge at 280 x g at room temperature for 1 minute.
- 5 Carefully remove the seal to avoid cross-contamination among wells.
- 6 Thaw RA1 to room temperature. Mix to dissolve any remaining crystals.



NOTE

Use fresh RA1 for each step where it is required. RA1 that has been stored properly and has not been dispensed is considered fresh. To make best use of RA1, only pour out the amount needed for the current step.

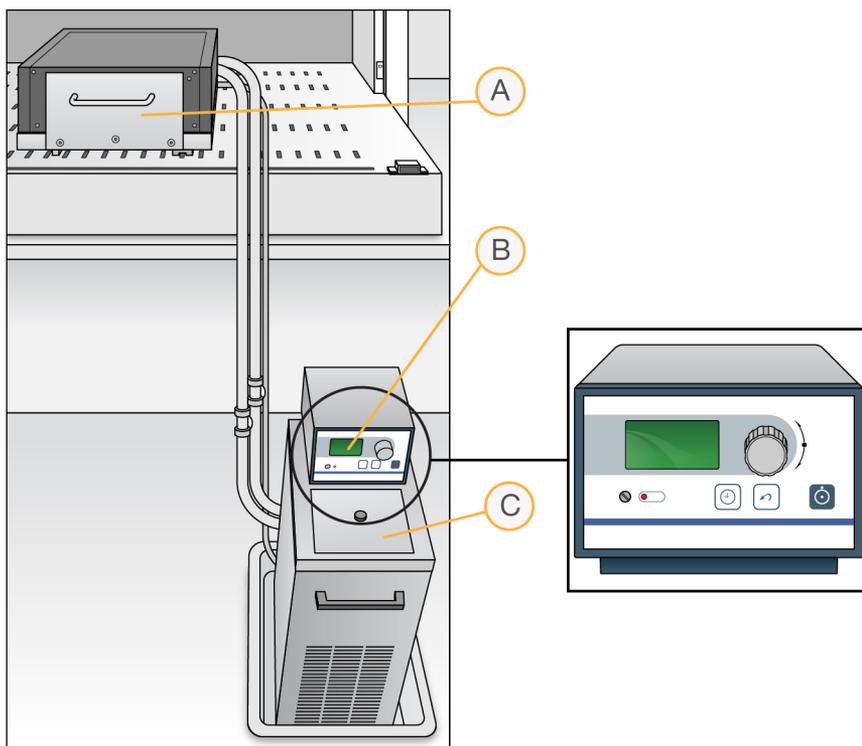
- 7 Thaw the EML tubes at room temperature.
- 8 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.

Procedure

Set Up the Chamber Rack

- 1 Make sure that the water circulator is filled to the appropriate level. See the *VWR Operator Manual*, VWR part # 110-229.
- 2 At the robot PC, select **Robot QC Tasks | Circulator Manager** to set the water circulator to 44°C.

Figure 33 Water Circulator Connected to Chamber Rack



- A Chamber Rack
- B Water Circulator
- C Reservoir Cover

- Remove bubbles trapped in the chamber rack **each time** you run this process. Follow instructions in the *Te-Flow (Tecan Flow-Through Module) Operating Manual*, Tecan Doc ID 391584.
- Use a temperature probe to confirm that the chamber rack temperature is at $44^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ at all locations.

Single Base Extension and Stain

This process uses a robot to process the BeadChips.



CAUTION

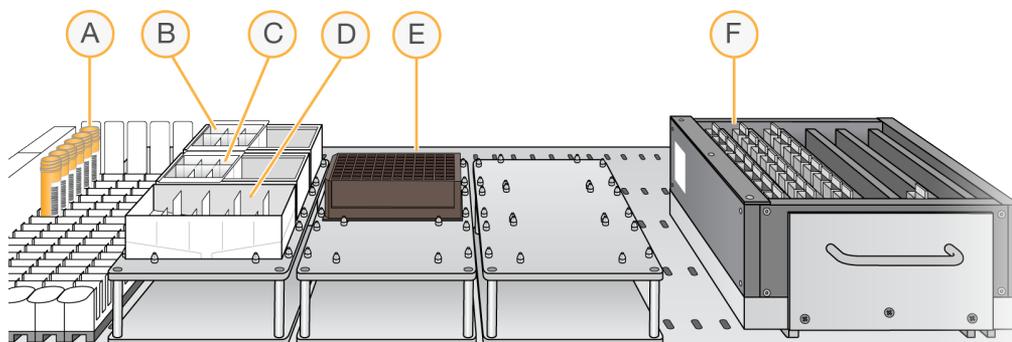
The following steps must be performed without interruption.

- At the robot PC, select **XStain Tasks | XStain XCG BeadChip HT**.
- Turn on the iScan systems to allow the lasers to stabilize.
- Place reservoirs on the robot bed, according to the bed map, and add reagents to reservoirs as follows:

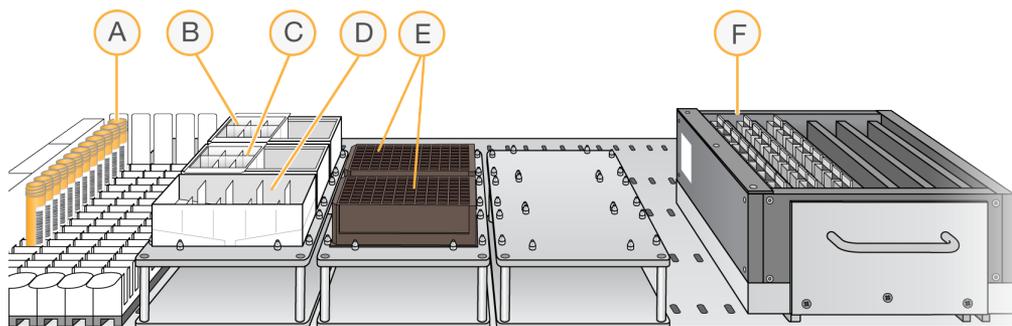
Reagent	# BeadChips	Volume
95% formamide/1 mM EDTA	24	30 ml
	48	60 ml
RA1	24	30 ml
	48	60 ml
XC3	24	150 ml
	48	250 ml

- Place the XStain plates on the robot bed, according to the bed map. Remove the seals.
- Invert the EML tubes to mix, remove the caps, and place on the robot bed, according to the robot bed map.
- In the Basic Run Parameters pane, enter the number of BeadChips, up to 48.

Figure 34 XStain Robot Setup for 24 BeadChips



- A EML Tubes
- B 95% Formamide/1 mM EDTA Reservoir
- C RA1 Reservoir
- D XC3 Reservoir
- E XStain Plate
- F BeadChips in Flow-Through Chamber Frame

Figure 35 XStain Robot Setup for 48 BeadChips

- A EML Tubes
- B 95% Formamide/1 mM EDTA Reservoir
- C RA1 Reservoir
- D XC3 Reservoir
- E XStain Plates
- F BeadChips in Flow-Through Chamber Frame

- 7 Click **Run**.
- 8 When prompted, enter the stain temperature listed on the XStain plate. Do not load the BeadChips yet.
- 9 When the chamber rack reaches 44°C, place the XCG Flow-Through Chamber assemblies into the chamber rack, according to the robot bed map.
- 10 At the robot PC, click **OK**.
- 11 When the robot finishes, remove the XCG Flow-Through Chamber assemblies from the chamber rack and place them horizontally on the lab bench at room temperature.

Wash and Coat BeadChips

- 1 Set up 2 top-loading PB1 and XC4 wash dishes, labeled PB1 and XC4.
- 2 To indicate fill volume, pour 310 ml water into the wash dishes and mark the water level. Empty the water from the wash dish.
- 3 Pour 310 ml PB1 into a wash dish labeled PB1.
- 4 Place a staining rack inside the wash dish with locking arms and tab facing towards you.
- 5 One at a time, disassemble each XCG Flow-Through Chamber:
 - a Use the dismantling tool to remove the metal clamps. It is important to use the dismantling tool to avoid chipping XCG glass back plates.
 - b Remove the XCG glass back plate, then the BeadChip.
- 6 Place BeadChips into a staining rack in the PB1 wash dish. Make sure that all barcodes face the same direction and that all BeadChips are submerged.



CAUTION

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

- 7 Submerge the XCG glass back plates in the DI H₂O wash basin for later cleaning as detailed in the *Infinium Lab Setup and Procedures Guide*.

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



NOTE

If BeadChip edges begin to touch during washing, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

- 9 Soak the BeadChips for an additional 5 minutes.



CAUTION

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

- 10 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.
- 11 Pour 310 ml XC4 into a wash dish.
- 12 Move the staining rack from the PB1 dish to the XC4 wash dish.
- 13 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

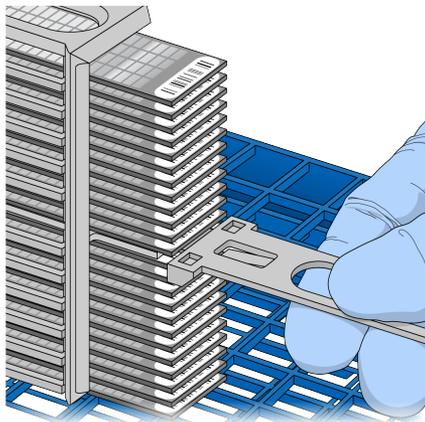


NOTE

If BeadChip edges begin to touch during washing, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

- 14 Soak the BeadChips for an additional 5 minutes.
- 15 Remove the staining rack and place it on the prepared tube rack with the array side of the BeadChips facing up.

Figure 36 Staining Rack in Correct Orientation



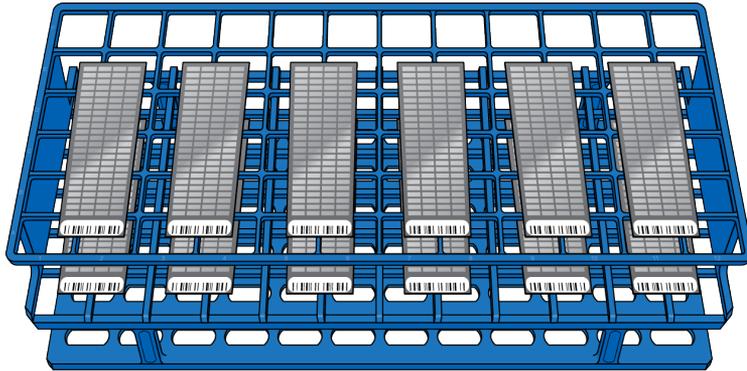
- 16 Remove the handle from the staining rack for easier access to the BeadChips.
- 17 For each BeadChip, working top to bottom:
 - a Use self-locking tweezers to grip the BeadChip at its barcode end.
 - b Place the BeadChip on the tube rack with the array side facing up.



CAUTION

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

Figure 37 BeadChips on Tube Rack



- 18 Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 675 mm Hg (0.9 bar). Drying times can vary according to room temperature and humidity.
- 19 Release the vacuum by turning the handle 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.

- 20 Touch the edges of the BeadChips (**do not touch arrays**) to make sure etched, barcoded sides are dry.
- 21 If the back of the BeadChip feels tacky, wipe it with a Prostat wipe or a Kimwipe wetted with 70% EtOH.
- 22 Image the BeadChips immediately or store protected from light.

Image BeadChip

Follow the instructions in the *iScan System User Guide* to scan your BeadChips. Use the **Infinium XT** scan setting for your BeadChips.

Appendix A Supporting Information

Introduction	47
Acronyms	47
Kit Contents	47
Consumables and Equipment	50

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

Acronyms

Acronym	Definition
DI H ₂ O	Deionized water
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
EML	Extension mix long
FMS	Fragmentation solution
LX1	Long XStain solution 1
LX2	Long XStain solution 2
MA1	Multi-sample amplification mix 1
MA2	Multi-sample amplification mix 2
Midi plate	Acceptable 96-well plate for MSA7 plate
MSA7 plate	MIDI plate used in the amplification through hybridization steps
NaOH	Sodium hydroxide
1X PB1	Wash buffer
PB2	Humidifying buffer used during hybridization
PB20	Concentrated PB1
PM1	Precipitation solution
RA1	Resuspension, hybridization, and wash solution
RAM	Rapid amplification mix
TCY plate	Acceptable 96-well plate for DNA plate
WG#-DNA	Whole genome-DNA plate
XC3	XStain BeadChip solution 3
XC4	XStain BeadChip solution 4

Kit Contents

Make sure that you have all the kit contents identified in this section before proceeding to the protocol procedures.

Kit Name	Catalog #
Infinium XT iSelect-96 Kit (4608 samples)	20006614
Infinium XT iSelect-96 Kit (23,040 samples)	20006615

Infinium XT iSelect-96 Kit (4608 Samples)

Some kit components require a different temperature for storage than for shipping. On receipt of the kit, remove all components and store them at the specified temperature.

Package	Quantity	Description	Storage Temperature
Box 1 (Pre-Amp)	2	MA2	-25°C to -15°C
	2	RAM	-25°C to -15°C
Box 2 (Pre-Amp)	2	MA1	15°C to 30°C
	48	Labels for MSA7 plates	
	48	Labels for DNA plates	
Box 3	2	FMS	-25°C to -15°C
	2	RA1	-25°C to -15°C
	2	PM1	2°C to 8°C
Box 4	2	XStain (XP1) plate	-25°C to -15°C
Box 5	2	PB2	15°C to 30°C
	2	XC3	15°C to 30°C
	1	XC4	15°C to 30°C
Box 6	14	EML	-25°C to -15°C
Box 7	2	Infinium XT iSelect Custom BeadChips (24)	2°C to 8°C

Infinium XT iSelect-96 Kit (23,040 Samples)

Some kit components require a different temperature for storage than for shipping. On receipt of the kit, remove all components and store them at the specified temperature.

Package	Quantity	Description	Storage Temperature
Box 1 (Pre-Amp)	10	MA2	-25°C to -15°C
	240	Labels for DNA plates	
Box 2 (Pre-Amp)	10	RAM	-25°C to -15°C
	240	Labels for MSA7 plates	
Box 3 (Pre-Amp)	10	MA1	15°C to 30°C
Box 4	10	FMS	-25°C to -15°C
Box 5	10	RA1	-25°C to -15°C
Box 6	10	PM1	2°C to 8°C
Box 7	10	XStain (XP1) plate	-25°C to -15°C
Box 8	10	PB2	15°C to 30°C
	5	XC4	15°C to 30°C

Package	Quantity	Description	Storage Temperature
Box 9	10	XC3	15°C to 30°C
Box 10	72	EML	-25°C to -15°C
Box 11	5	Infinium XT iSelect Custom BeadChips (48)	2°C to 8°C

Infinium XT Starter Packages

Package	Description	Illumina Catalog #
Infinium XT starter kit (24 BeadChip batches)	This kit includes accessory equipment required to support 24 BeadChip batch XStain processing of the Infinium XT assay. Tip guides, AutoLoader 2.x, iScan, and automation robot sold separately.	20011100
Infinium XT starter kit (48 BeadChip batches)	This kit includes accessory equipment required to support 48 BeadChip batch XStain processing of the Infinium XT assay. Tip guides, AutoLoader 2.x, iScan, and automation robot sold separately.	20011069
Infinium XT upgrade kit (24 BeadChip batches)	This kit supports existing Infinium customers looking to add the Infinium XT workflow to scale their targeted genotyping applications. It includes Infinium XT specific accessories required to process 24 BeadChip XStain batches through the Infinium XT workflow. Infinium XT tip guides are included. AutoLoader 2.x, iScan, and automation robot sold separately.	20011101
Infinium XT tip guide set	This set supports the hybridization step of the Infinium XT Assay for manual and automated protocols. This set includes 6 sets of 3 tip guides (K, L, M).	20011102
Infinium XT PB20 Kit	This kit includes concentrated PB20 reagent required to run the Infinium XT workflow. Each kit supports approximately 20 Infinium XT iSelect-96 (1152 samples) kits. User-supplied carboy required for dilution.	20007420

Infinium XT Additional Items Sold Separately

Items available as sold separately for increased or customized throughput needs.

Hardware	Description	Illumina Catalog #
Infinium BeadChip storage boxes	Stores BeadChips used in Illumina array-based assays. Includes 10 BeadChip storage boxes. Each box holds 25 BeadChips.	BD-60-500
Infinium BeadChip wash rack and glass tray	The BeadChip wash rack and glass tray (purchased separately) are used to handle BeadChip arrays following hybridization during Infinium and Infinium XT assays.	BD-60-450 (wash rack) BD-60-460 (glass tray)
Infinium hybridization oven	Hybridization oven used in the Infinium and Infinium XT Assays.	SE-901-1001 (110 V) SE-901-1002 (220 V)
Infinium XT dual hyb chamber insert and baseplate	Dual function part serves as both the hyb chamber insert and the robot base plate for the Infinium XT BeadChips on the Tecan during the sample hybridization process.	20011759

Hardware	Description	Illumina Catalog #
Infinium XT flow-through chamber assembly tray	Tray for assembling XCG flow through chambers.	20011760
Infinium XT flow through chamber (8-Pack)	Contains XCG glass back plates (8), frames (8), and clips (16) to enable assembly of 8 complete XCG flow through chambers used in the Infinium XT XStain process.	20012129
Infinium XT hybridization chamber	Infinium XT-hybridization chamber supporting the hybridization of DNA samples to BeadChips. Includes the Infinium XT hybridization chamber gasket (1).	20011755
Infinium XT hybridization chamber replacement gasket	This gasket fits inside the Infinium XT hybridization chamber to ensure proper BeadChip retention and optimal exposure to reagents. 1 gasket is included with the purchase of an Infinium XT hybridization chamber. Replacement gaskets are available for purchase separately.	20012127
Infinium staining set (staining rack and wash dish)	The Infinium staining set includes a wash dish and staining rack that accommodates up to 24 BeadChip arrays at a time. This set is used in both Infinium and Infinium XT Assays during the wash and coat step of BeadChip processing.	WG-10-207
Infinium TeFlow thermometer assembly	The Infinium TeFlow thermometer assembly, which includes the thermometer and a temperature probe, reads the temperature in the TeFlow rack during Infinium and Infinium XT Assays.	A1-99-109

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

The protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

User-Supplied Materials

Material	Supplier
Absorbent pads/towels	General lab supplier
Aluminum foil	General lab supplier
Cap mats, 96-well, pierceable, nonautoclavable	Thermo Fisher Scientific, catalog # AB-0566
Compressed air can	VWR, Int'l, catalog # 16650-027
Container, 1 L, for diluting bleach	General lab supplier
Heat sealing foil sheets, Thermo-Seal	Thermo Fisher Scientific, catalog # AB-0559
Kimwipes or any lint-free tissue	General lab supplier
Lab coats • 2 supplies: 1 for pre- and 1 for post-amplification processes	General lab supplier

Material	Supplier
Pipette tips <ul style="list-style-type: none"> • 20 µl aerosol filter • 200 µl aerosol filter • 1000 µl aerosol filter 	General lab supplier
Pipettes, serological, 50 ml	General lab supplier
Pipetting troughs, disposable	VWR, Int'l, catalog # 21007-970
Powder-free gloves <ul style="list-style-type: none"> • 2 supplies: 1 for pre- and 1 for post-amplification processes 	General lab supplier
ProStat EtOH presaturated wipes <ul style="list-style-type: none"> • Recommend 1 wipe per 2 chips; 30 wipes per package • Substitute with Kimwipes and 70% EtOH 	Contec, catalog # PS-911EB/EtOH
Pyrex glass container, 9" x 13"	General lab supplier
Safety glasses <ul style="list-style-type: none"> • 2 supplies: 1 for pre- and 1 for post-amplification processes 	General lab supplier
Skirted microplates, 96-well, 0.2 ml <ul style="list-style-type: none"> • Microseal 96-well skirted polypropylene microplates, 8x12 well array • TCY plates, 1 per run • Substitute with 0.8 ml storage plate (midi plate), conical well bottom, if desired 	MJ Research, catalog # MSP-9601, www.mjr.com
Storage microplates, 96-well, 0.8 ml <ul style="list-style-type: none"> • Midi plates, 1 per run 	Thermo Fisher Scientific, catalog # AB0765
Tubes <ul style="list-style-type: none"> • 15 ml conical • 50 ml conical 	General lab supplier

User-Supplied Reagents

Consumable	Supplier
Bleach	General lab supplier
Deionized water (DI H ₂ O)	General lab supplier
EDTA, 0.5 M	EMD Chemicals, catalog # 4056 Sigma-Aldrich, catalog # E7889
Ethanol, 100%	General lab supplier
Formamide, OmniPur	VWR, Int'l, catalog # EM-4650
Isopropanol (2-propanol), 100%	General lab supplier
Mild detergent, such as Alconox® Powder Detergent	VWR, Int'l, catalog # 21835
Sodium hydroxide, purchase as solid and prepare a 0.1N NaOH solution in DI H ₂ O	Sigma-Aldrich, catalog # 221465
TE, 1X <ul style="list-style-type: none"> • 10 mM Tris-HCl, pH 8.0, 1 mM EDTA • For diluting DNA 	General lab supplier

Illumina-Supplied Equipment

Equipment	Supplier
Autoloader 2.X, single-scanner configuration (110 V/220 V)	Illumina catalog # SY-201-1001
Autoloader 2.X, dual-scanner configuration (110 V/220 V)	Illumina catalog # SY-201-1002
BeadChip wash dish	Illumina catalog # 198205
BeadChip wash rack	Illumina catalog # 203676
Braided silicone tubing, 0.375" ID x 0.625" OD, 0.125" inch wall thickness, 25' long	Illumina, catalog # 11188141
Chamber rack, 48-position	Te-Flow, Tecan, catalog # 11176950
Combi heat sealing unit	Marsh Bio Products, catalog # AB-0384
Flow-Through Chamber dismantling tool	Illumina, catalog # WG-10-204
Flow-Through Chambers	Illumina, catalog # 20012129
Hyb oven with rocker platform	Illumina, catalog # 11277582 (115 VAC) or # 11277591 (230 VAC)
iScan System (110 V/220 V)	Illumina, catalog # SY-101-1001
Midi plate heat block insert	Illumina, catalog # 211191
Staining wash dishes and rack (1 staining rack per 24 BeadChips processed simultaneously)	Illumina, catalog # WG-10-207
TeFlow additive	Tecan, catalog # 760-801
TeFlow cell storage box	Te-Flow, Illumina, catalog # 1190268
Temperature probe	Illumina, catalog # 11191228
Temperature probe block	Illumina, catalog # 211262
Vacuum desiccator (1 per 8 BeadChips processed simultaneously)	VWR, Int'l, catalog # 24988-197
Vortex mixer (for use in the post-amplification area only) Signature™ High-Speed Microplate Shaker	VWR, Int'l, catalog # 13500-890 (110 V) or # 14216-214 (220 V)
Water circulator (6 L heating/cooling)	Illumina, catalog # 1188125 (110 V 60 Hz) and # 1188133 (240 V 50 Hz)
XCG Flow-Through Chamber clips (16 pack)	Illumina, catalog # 20011758
XCG Flow-Through Chamber frames (8 pack)	Illumina, catalog # 20011757
XCG glass back plates (8 pack)	Illumina, catalog # 20011756
XCG glass drying rack	Illumina, catalog # 20011754
XT dual Hyb insert and baseplates	Illumina, catalog # 20011759
XT Flow-Through Chamber assembly tray	Illumina, catalog # 20011760
XT Hyb chamber	Illumina, catalog # 20011755
XT Hyb chamber gasket	Illumina, catalog # 20012127
XT tip guide set (6 of each guide)	Illumina, catalog # 20011102

User-Supplied Equipment

Equipment	Supplier
Adapters to centrifuge 96-well plates and tubes (2 sets)	General lab supplier
Autodesiccator cabinet	VWR, Int'l, 74950-342, www.vwr.com
Cap mat sealer (recommended)	Corning, 3081
Centrifuge, benchtop 120 V, for pre-amplification processes	General lab supplier
Centrifuge, benchtop refrigerated 120 V (8–3000 × g), for post-amplification processes	General lab supplier
Forceps <ul style="list-style-type: none"> • Included with system, only needed if additional pairs are required 	VWR, Int'l, 25601-008, www.vwr.com
Micropipettors <ul style="list-style-type: none"> • 2 separate sets: 1 for pre- and 1 for post-amplification processes • P-20 • P-200 • P-1000 	General lab supplier
Multichannel precision pipettes <ul style="list-style-type: none"> • 2 separate sets: 1 for pre- and 1 for post-amplification processes • P-20 • P-200 	General lab supplier
Optical tachometer/stroboscope (recommended)	Cole-Parmer, A-87700-06, www.coleparmer.com
Serological pipette aid	General lab supplier
Stop watches/timers <ul style="list-style-type: none"> • 2 separate sets: 1 for pre- and 1 for post-amplification processes 	General lab supplier
Tube rack <ul style="list-style-type: none"> • 2 separate sets: 1 for pre- and 1 for post-amplification processes • Any rack fitting the Infinium reagent 17 mm tube diameter 	VWR, Int'l, catalog # 66023-540
Tube vortexers <ul style="list-style-type: none"> • 2 separate sets: 1 for pre- and 1 for post-amplification processes 	General lab supplier
Vacuum source, hose, or pump capable of pulling greater than 508 mm Hg	General lab supplier

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.635.9898	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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

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



Illumina

5200 Illumina Way

San Diego, California 92122 U.S.A.

+1.800.809.ILMN (4566)

+1.858.202.4566 (outside North America)

techsupport@illumina.com

www.illumina.com

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

© 2017 Illumina, Inc. All rights reserved.

illumina[®]