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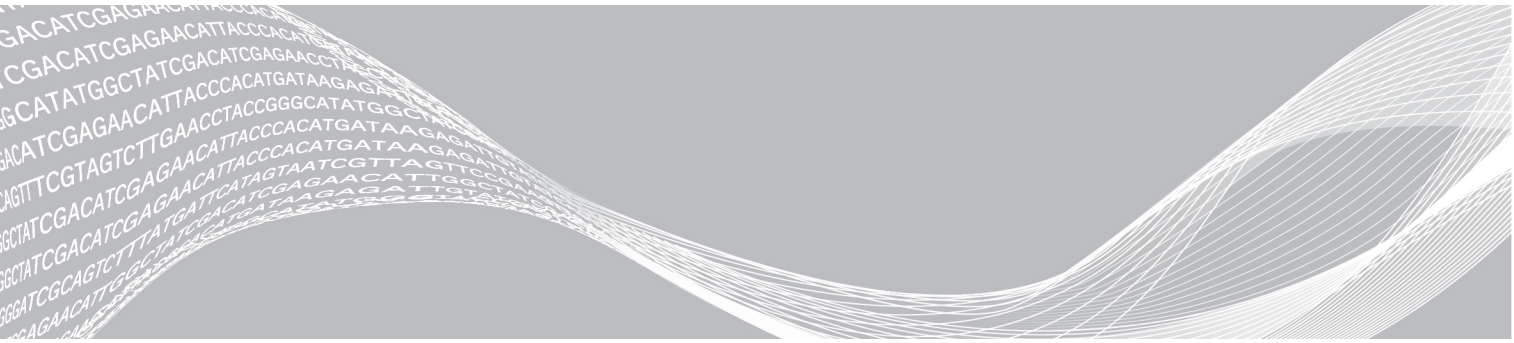
Infinium XT Assay

Reference Guide for the ST Workflow



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Revision History

Document	Date	Description of Change
Document # 1000000025687 v01	June 2017	Added instructions to support the automated workflows, with Illumina LIMS and without Illumina LIMS.
Document # 1000000025687 v00	December 2016	Initial release.

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Chapter 1 Overview

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 Assay 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.

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 H_2O. 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.
- 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 H_2O 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 H_2O. Use a graduated cylinder or a gentle stream of DI H_2O to avoid creating bubbles.

Store PB1

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

Clean the Carboy

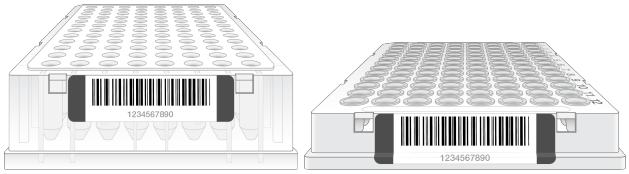
- Rinse the carboy with 10–20 L DI H_2O 3 times.
- Run 5 L DI H_2O through the spigot to flush it.

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 1 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:

    a Click the Reports tab in the upper-right corner.
    b In the left pane, click **Tracking Reports | Get Queue Status**.
    c Scan the plate barcode and click **Go**.
    d 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
Infinium XT - Checklist for the ST Workflow with Illumina LIMS (document # 1000000034878)	Provides a checklist of steps for users who are experienced using the Infinium XT ST workflow, with Illumina LIMS.
Infinium XT - Checklist for the ST Workflow without Illumina LIMS (document # 1000000034879)	Provides a checklist of steps for users who are experienced at using the Infinium XT ST workflow, without Illumina LIMS.
Infinium XT - ST Manual Workflow Checklist (document # 1000000025689)	Provides a checklist of steps for users who are experienced using the Infinium XT ST manual workflow.
Infinium Assay Lab Setup and Procedures Guide (document # 11322460)	Describes how to set up an Infinium lab, and best practices for lab operation.

Chapter 2 Manual Protocol

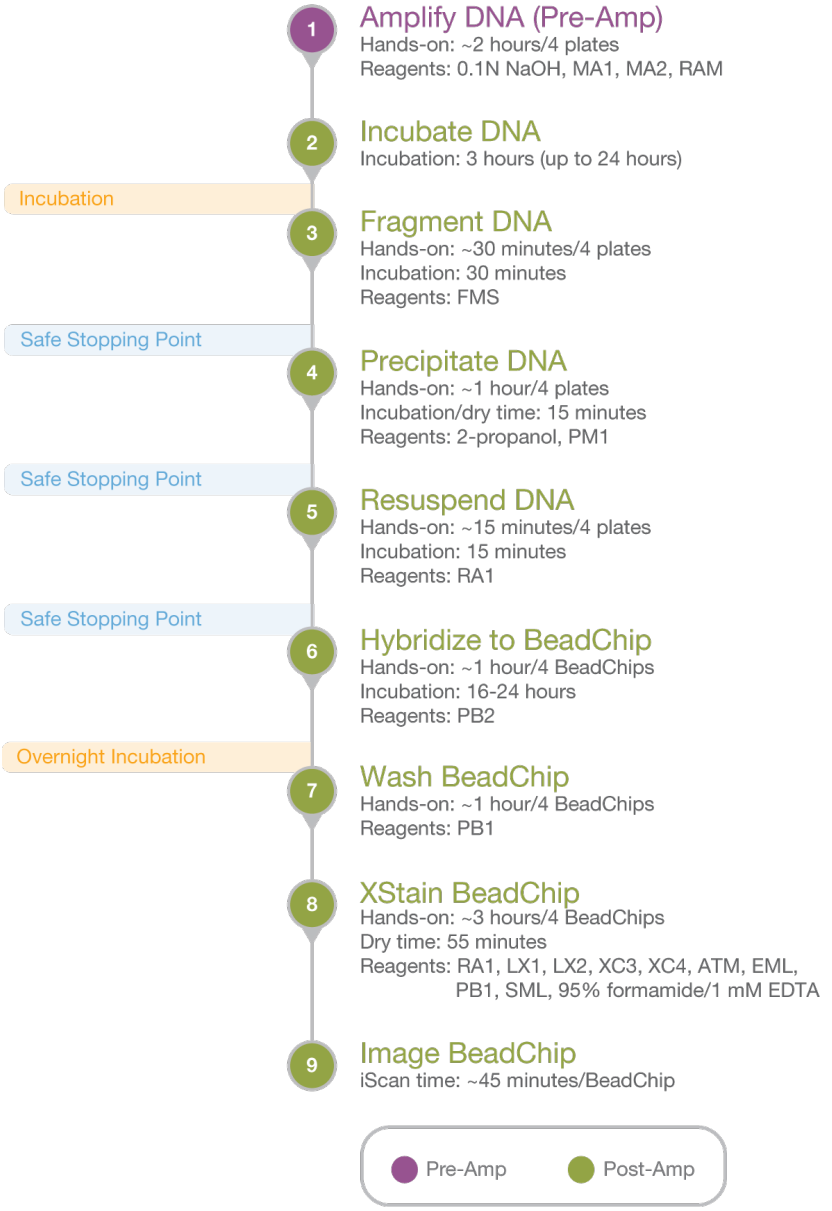
Introduction

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

Infinium XT ST Manual Workflow

The following figure graphically represents the Infinium XT Assay ST manual workflow for 4–12 BeadChips.

Figure 2 Infinium XT Protocol ST Manual Workflow



Amplify DNA (Pre-Amp)

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

If you are processing multiple plates, complete all amplification steps for one plate before starting another. You can then process plates in batches, starting with the incubation step. The recommended maximum batch size is plates per user.

Consumables

- MA1 (1 tube/plate)

- MA2 (1 tube/plate)
- RAM (1 tube/plate)
- 0.1N NaOH (5 ml/plate)
- 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 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 Use a 200 µl pipette to add 20 µl MA1 to each well of the MSA7 plate.

2 Add 4 µl 0.1N NaOH to the bottom of each well, beneath the MA1.

3 Transfer 4 µl of the DNA sample from each well of the WG#-DNA plate to the corresponding well in the MSA7 plate.

4 Apply a cap mat to the MSA7 plate and vortex at 1600 rpm for 1 minute.

5 Centrifuge at 280 × g at room temperature for 1 minute.

6 Incubate at room temperature for 10 minutes.

7 Remove the cap mat and set it aside for use later in the protocol.

8 Add 35 µl MA2 per well.

9 Add 35 µl RAM per well.

10 Apply a cap mat to the MSA7 plate and vortex at 1600 rpm for 1 minute.

11 Centrifuge at 280 × 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 (1 tube/plate)

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 × g at room temperature for 1 minute.

2 Carefully remove the cap mats.

3 Add 25 µl FMS per well.

4 Apply cap mats and vortex at 1600 rpm for 1 minute.

5 Centrifuge at 280 × g at room temperature for 1 minute.

6 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.

Procedure

1 Remove the cap mats.

2 Add 50 µl PM1 per well.

3 Add 155 µl 100% 2-propanol per well.

4 Apply fresh cap mats.

5 Invert the plates 10 times to mix.

6 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.

7 Remove the plates from the centrifuge and remove the cap mats.

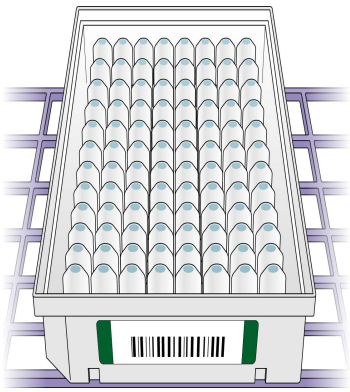
8 Quickly invert the plates and drain liquid to decant the supernatant. Then smack the plates down on a dry pad.

9 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.

10 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 3 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.

Procedure

1 Add 23 µl RA1 to each well of the MSA7 plate.

2 Apply foil heat seals to the MSA7 plates using the heat sealer.

3 Incubate in the Illumina Hybridization Oven for 15 minutes at 48°C. If the plates were frozen, incubate for 1 hour.

4 Vortex at 1800 rpm for 1 minute.

5 Check to make sure that the pellets are resuspended. If necessary, repeat the incubation and vortexing steps.

6 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 H_2O

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

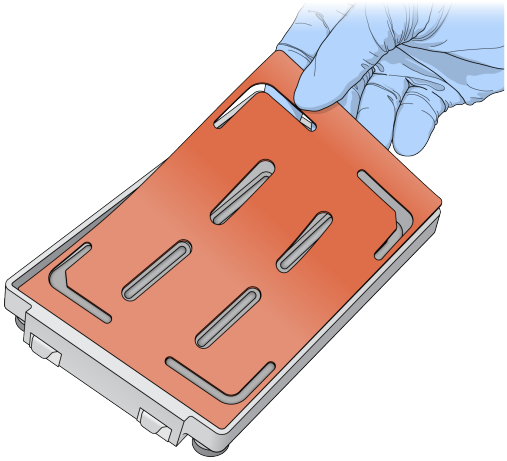
Denature DNA

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 4 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.

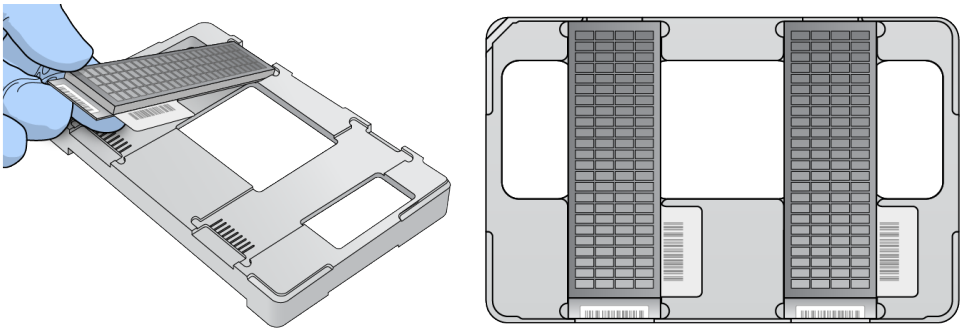
4 Leave closed XT Hyb chambers on the bench at room temperature until BeadChips are loaded with DNA sample.

Load BeadChips

1 Remove all BeadChips from packaging.

2 Place 2 BeadChips onto each XT dual Hyb insert and baseplate, making sure the BeadChip is flush with the baseplate.

Figure 5 Placing BeadChips on Baseplates



3 Place XT Tip Guide #1 on top of each XT dual Hyb insert and baseplate.

NOTE

96 samples from 1 MSA7 plate are loaded onto the 96 sample sections of 1 XT BeadChip according to the diagram. XT tip guides #1, #2, and #3 are used to guide the pipette to the appropriate sections for BeadChip loading as described.

Figure 6 Load 1 MSA7 Plate to 1 BeadChip

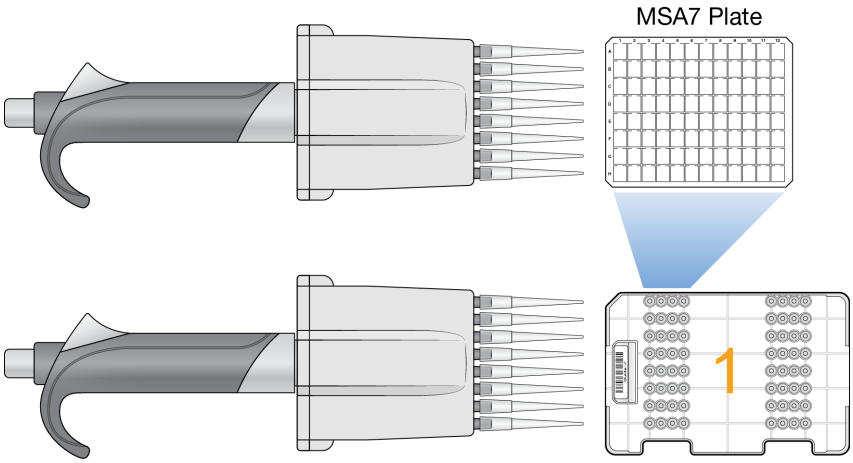
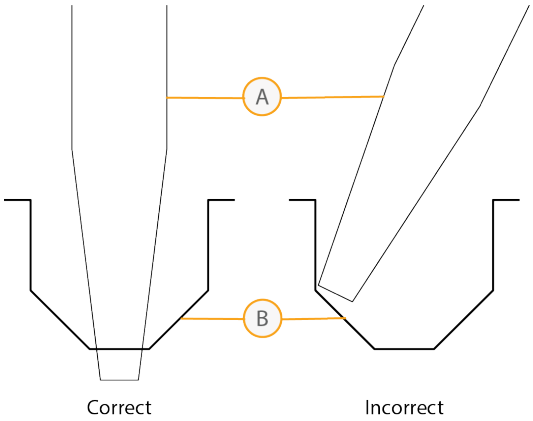


Figure 7 Correct and Incorrect Orientation of a Micropipette Tip in the XT Tip Guide



A Micropipette Tip
B Well of the XT Tip Guide

4 Using a 20 µl, 8-channel precision pipette, dispense 15 µl DNA sample into the appropriate BeadChip sections. Make sure that the pipette tip is in the bottom of the XT tip guide before dispensing.

a Load samples A01-H01 from the MSA7 plate into column C01 of tip guide #1.
b Load samples A02-H02 from the MSA7 plate into column C02 of tip guide #1.
c Load samples A03-H03 from the MSA7 plate into column C03 of tip guide #1.
d Load samples A04-H04 from the MSA7 plate into column C04 of tip guide #1.

Figure 8 Color Code for Each XT Tip Guide

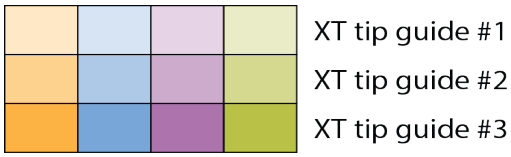
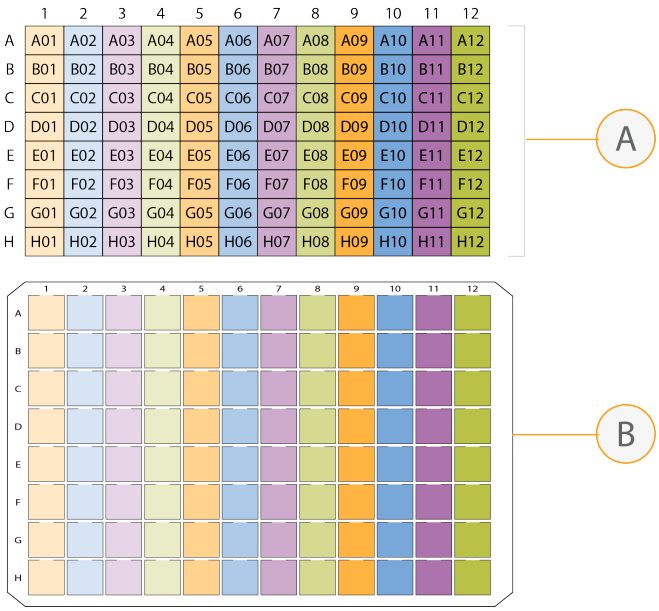
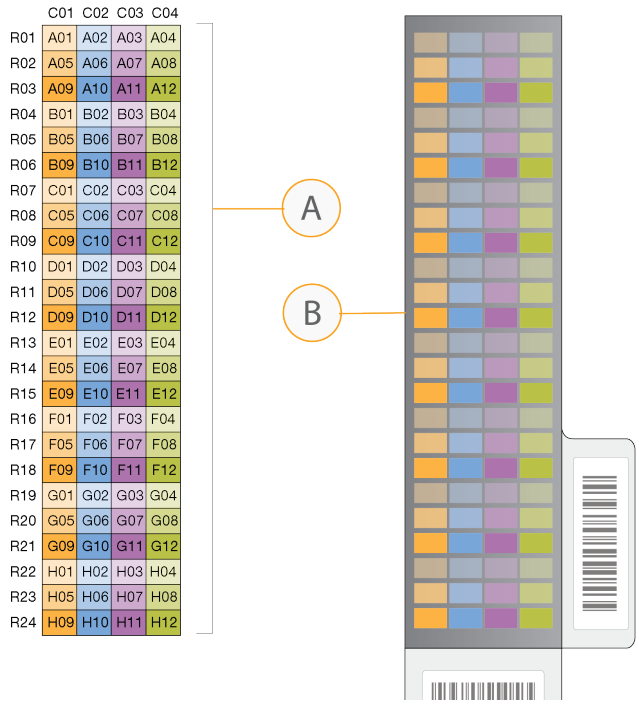


Figure 9 Color-Coded Transfer Matrix for 1 MSA7 Plate.



A Loading Matrix Calling Out Every Well
B Loading Matrix Overlaid on an MSA7 Plate

Figure 10 Color-Coded Transfer Matrix for 1 BeadChip



A Loading Matrix Calling Out Every Well
B Loading Matrix Overlaid on a BeadChip

5 Remove XT tip guide #1 and replace with XT tip guide #2. Pipette 15 µl of each DNA sample into the appropriate BeadChip sections. Make sure that the pipette tip is in the bottom of the tip guide before dispensing.

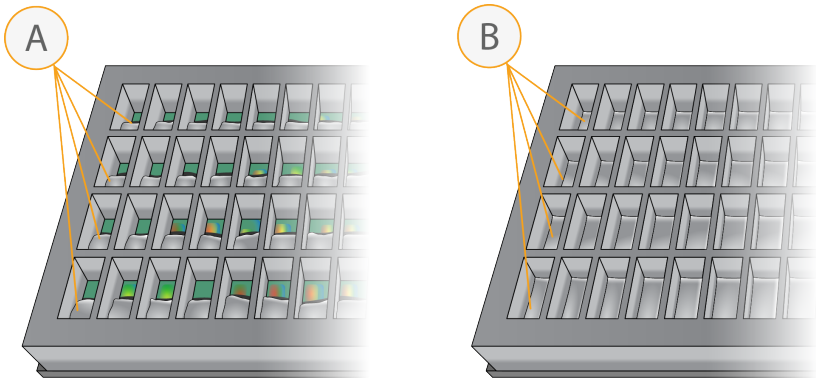
   a Load samples A05-H05 from the MSA7 plate into column C01 of tip guide #2.
   b Load samples A06-H06 from the MSA7 plate into column C02 of tip guide #2.
   c Load samples A07-H07 from the MSA7 plate into column C03 of tip guide #2.
   d Load samples A08-H08 from the MSA7 plate into column C04 of tip guide #2..

6 Remove XT tip guide #2 and replace with XT tip guide #3. Pipette 15 µl of each DNA sample into the appropriate BeadChip sections. Make sure that the pipette tip is in the bottom of the tip guide before dispensing.

   a Load samples A09-H09 from the MSA7 plate into column C01 of tip guide #3.
   b Load samples A10-H10 from the MSA7 plate into column C02 of tip guide #3.
   c Load samples A11-H11 from the MSA7 plate into column C03 of tip guide #3.
   d Load samples A12-H12 from the MSA7 plate into column C04 of tip guide #3.

7 Remove XT tip guide #3 from the XT dual Hyb insert and baseplate. Inspect the BeadChips. Note any sections that are not covered with DNA sample.

Figure 11 Sample in a BeadChip Before and After Incubation



A Uneven Sample Distribution after Manual Loading
B Even Sample Distribution after Incubation

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.

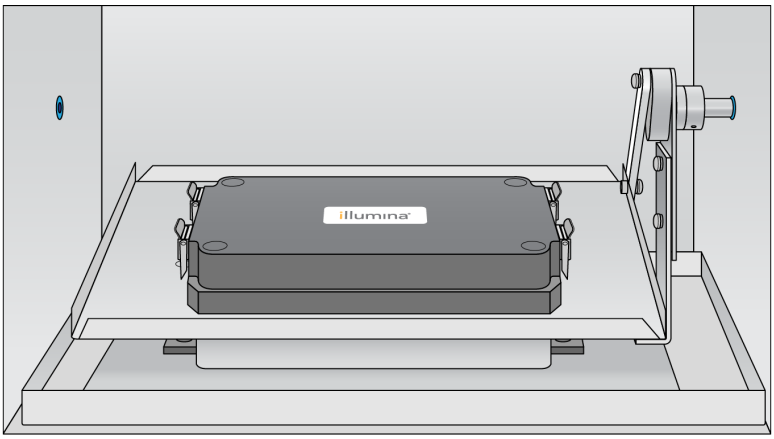
2 Load the XT dual Hyb insert and baseplates containing BeadChips inside the XT Hyb chambers. You can stack up to 3 XT dual Hyb insert and baseplates in each XT Hyb chamber.

3 Close each XT Hyb chamber and secure the clamps.

4 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 Chamber 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 H_2O 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 contaminates 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

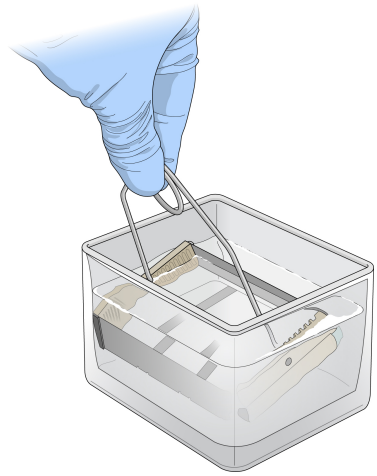
Wash BeadChips

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

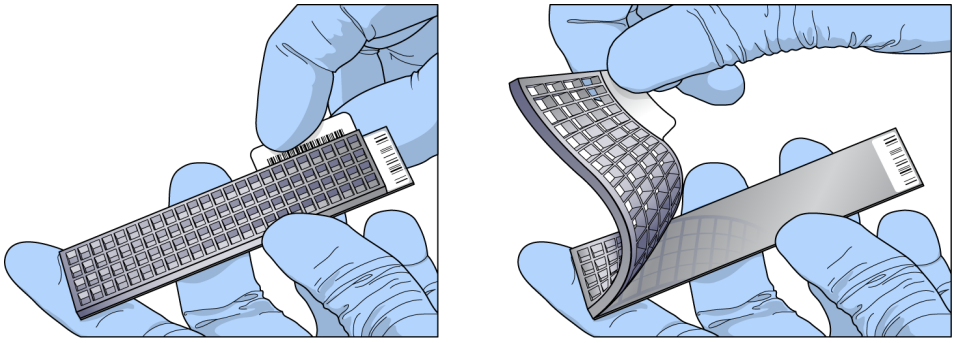


2. Remove the XT dual Hyb insert and baseplates from the XT Hyb chambers.

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.

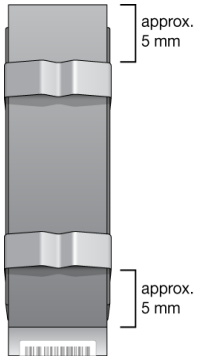
5 Place an XCG glass back plate onto a submerged BeadChip with spacers facing down and beveled edge towards bar code.

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



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
- LX1
- LX2
- EML
- XC3
- SML
- ATM
- PB1
- XC4
- 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 To prepare the following consumables, thaw to room temperature.
   - LX1

- LX2
- EML
- XC3
- SML
- ATM

2 Thaw RA1 and 95% formamide/1 mM EDTA to room temperature, preferably in a 20°C to 25°C water bath. 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.

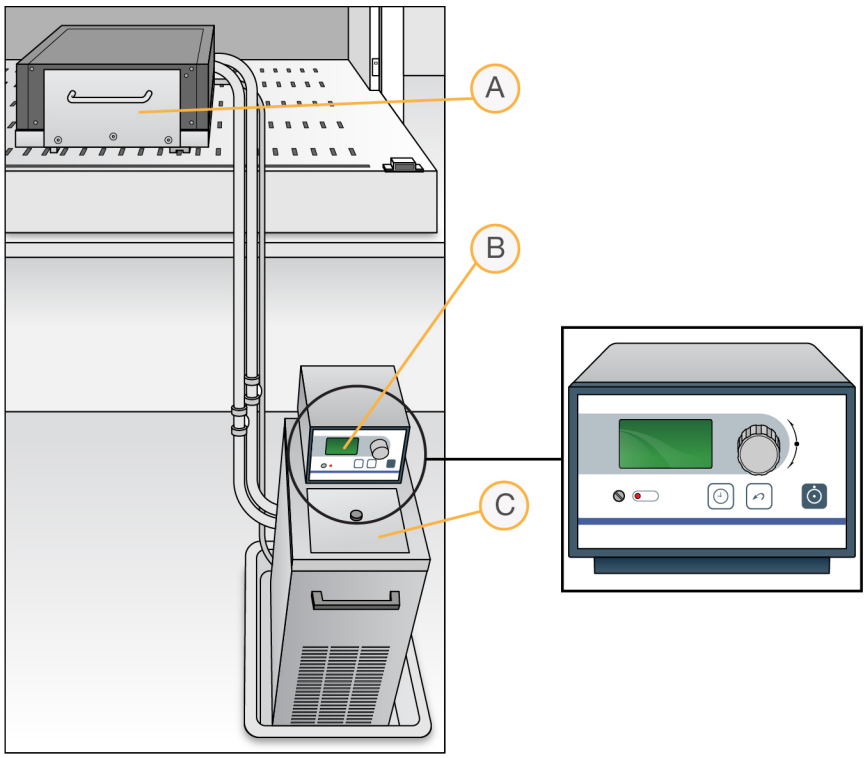
Procedure

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 Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium. This temperature can vary depending on facility ambient conditions.

Figure 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 in several locations to confirm that the chamber rack is at 44°C ± 0.5°C at all locations.
For accurate temperature measurement, confirm that the temperature probe is touching the base of the chamber rack. The temperature shown on the water circulator LCD screen can differ from the temperature on the chamber rack.

Single-Base Extension

CAUTION
The remaining steps must be performed without interruption.

1 When the chamber rack reaches 44°C, quickly place the Flow-Through Chamber assemblies into the chamber rack.

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

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

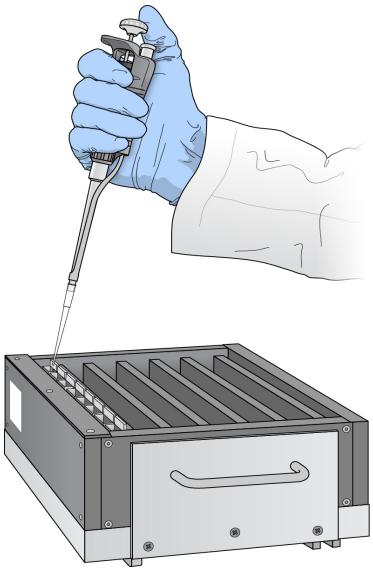
a 150 µl RA1. Incubate for 30 seconds. Repeat 5 times.

CAUTION
Pipette tip must not contact BeadChip surface.

b 225 µl LX1. Repeat 1 time. Incubate for 10 minutes.
c 225 µl LX2. Repeat 1 time. Incubate for 10 minutes.
d 300 µl EML. Incubate for 15 minutes.
e 250 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat twice.
f Incubate 5 minutes.
g Begin ramping the chamber rack temperature to the temperature indicated on the SML tube.
h 250 µl XC3. Incubate for 1 minute. Repeat twice.

4 Wait until the chamber rack reaches the correct temperature.

Figure 17 Dispensing RA1 into Each Flow-Through Chamber



Stain BeadChip

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

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

   a 250 µl SML. Incubate for 10 minutes.
   b 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.
   c 250 µl ATM. Incubate for 10 minutes.
   d 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.
   e 250 µl SML. Incubate for 10 minutes.
   f 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.
   g 250 µl ATM. Incubate for 10 minutes.
   h 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.
   i 250 µl SML. Incubate for 10 minutes.
   j 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.

3 Immediately remove the Flow-Through Chambers from the chamber rack and place horizontally on a lab bench at room temperature.

Wash and Coat BeadChips

1 Set up 2 top-loadingPB1 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 H_2O wash basin for later cleaning as detailed in the *Infinium Assay 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.

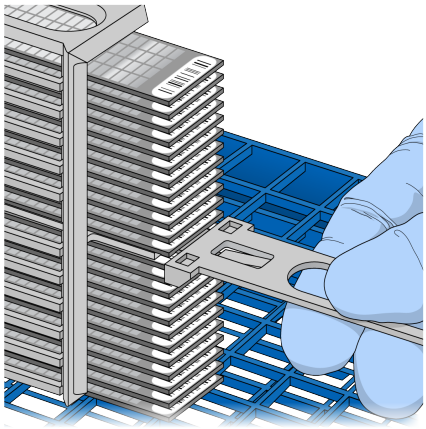
13 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

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 18 Staining Rack in Correct Orientation



16 Remove the handle from the staining rack for easier access to the BeadChips.

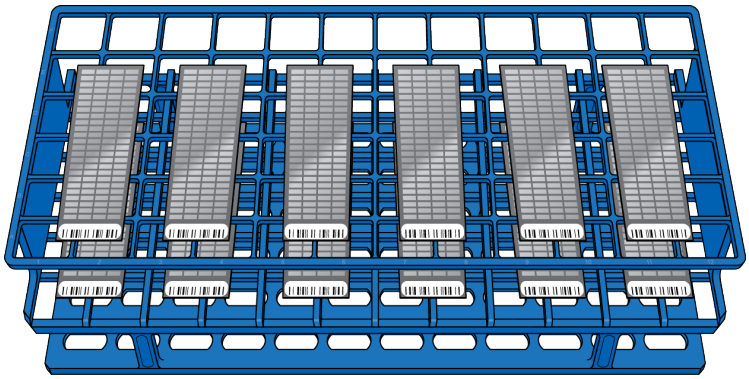
17 For each BeadChip, working top to bottom:

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 19 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 with Illumina LIMS

Introduction

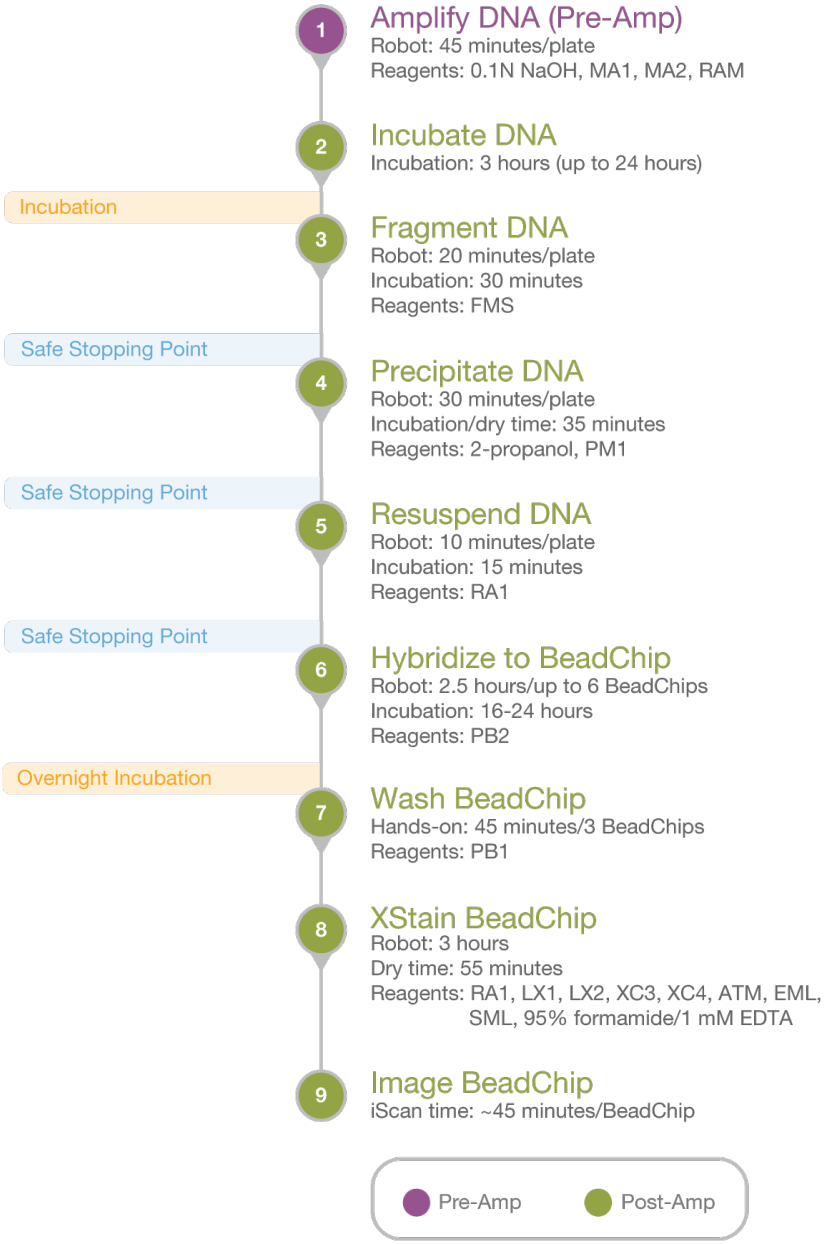
This section describes pre- and post-amplification automated laboratory protocols for the Infinium XT Assay. Follow the protocols in the order shown.

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 48 for protocol instructions. For information about how to use LIMS, see the *LIMS User Guide.*

Infinium XT ST Automated Workflow

The following figure graphically represents the Infinium XT Assay ST automated workflow for 4–12 BeadChips.

Figure 20 Infinium XT Protocol ST Automated Workflow



Amplify DNA (Pre-Amp)

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

If you are processing multiple plates, complete all amplification steps for one plate before starting another. You can then process plates in batches, starting with the incubation step. The recommended maximum batch size is 6 plates per user.

Consumables

- MA1 (1 tube/plate)
- MA2 (1 tube/plate)
- RAM (1 tube/plate)
- 0.1N NaOH (5 ml/plate)
- 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 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 ST Tasks | Make MSA7 ST**.

2 Select the WG#-DNA plate type (MIDI or TCY). Do not mix plate types on the robot.

3 Place MA1, MA2, and RAM tubes into the tube rack according to the robot bed map. Remove the caps.

4 Add 0.1 N NaOH to a quarter reservoir (5 ml per plate), then place on the robot bed according to the bed map.

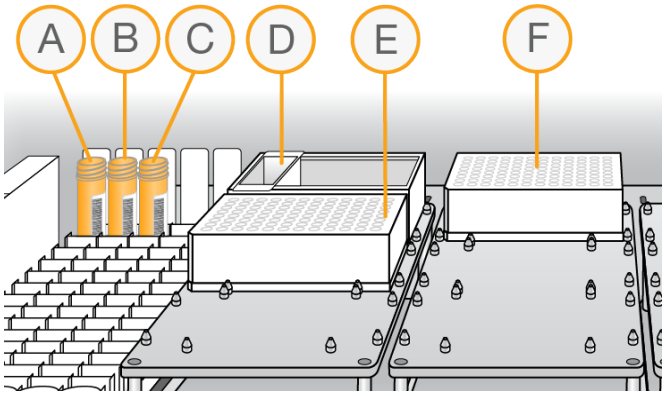
5 Place WG#-DNA source and MSA7 MIDI plates on the robot bed according to the bed map.

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 21 Robot Setup for Amplify DNA



A MA1 Tube
B MA2 Tube
C RAM Tube
D 0.1N NaOH Reservoir
E MSA7 Plate
F DNA Plate

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 × 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 ST**.

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 (1 tube/plate)

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

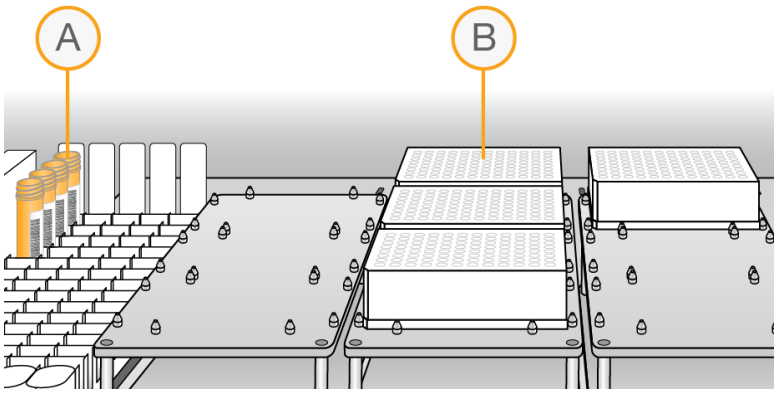
1 Centrifuge the MSA7 plates at 280 × g at room temperature for 1 minute.

2 At the robot PC, select **MSA7 ST Tasks | Fragment MSA7 ST**.

3 Place the MSA7 plates on the robot bed according to the bed map. Remove the cap mats.

4 Place FMS tubes into the tube rack according to the robot bed map. Remove the caps.

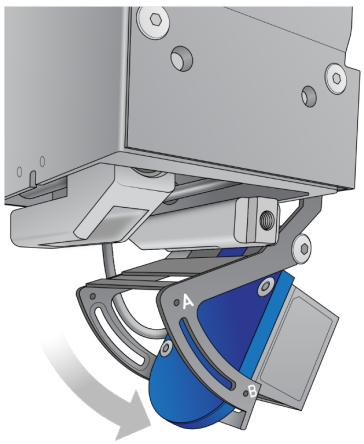
Figure 22 Robot Setup for Fragment MSA7



A FMS Tubes
B MSA7 Plates

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

Figure 23 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 × 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 ST Tasks | Precip MSA7 ST**.

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.

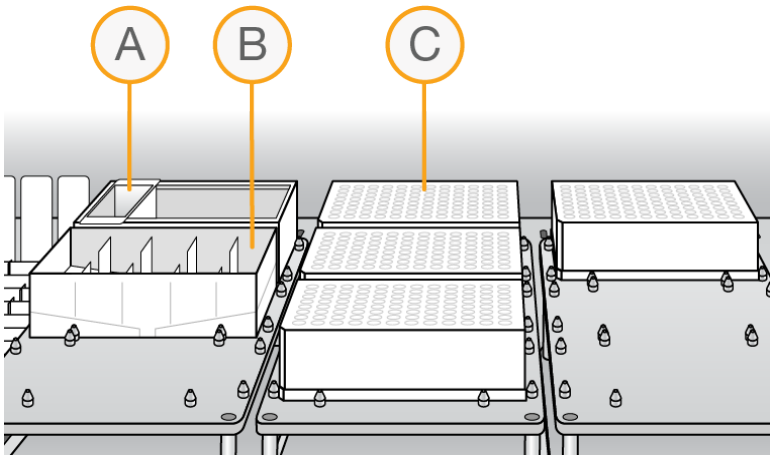
4 Place a quarter reservoir on the robot bed, according to the bed map, and add PM1:

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 24 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 ST | Spin MSA7 ST**.

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.

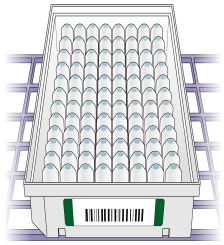
15 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

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 25 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 ST Tasks | Resuspend MSA7 ST**.

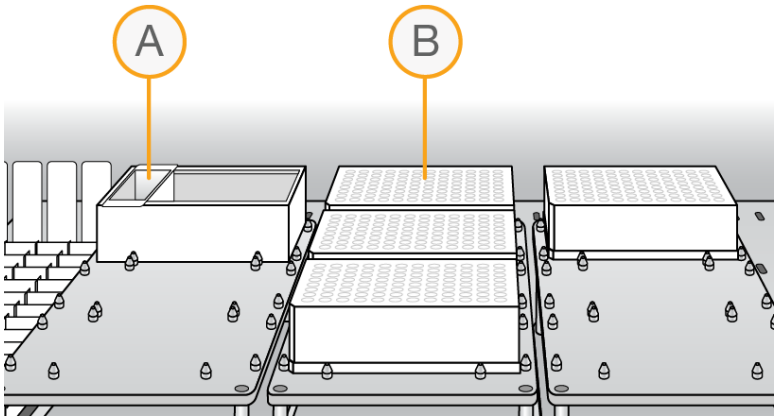
2 In the Basic Run Parameters pane, change the value for the **Number of MSA7 plates**.

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 26 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 H_2O

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

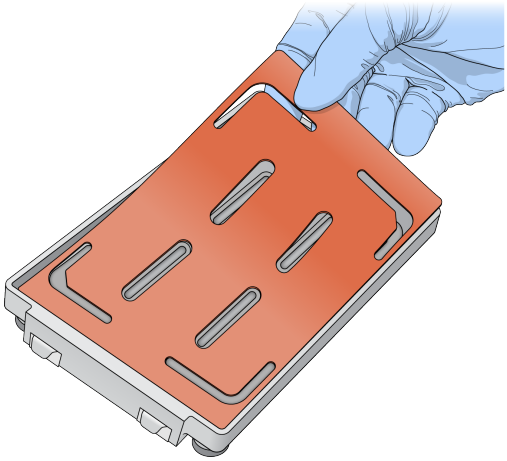
Denature DNA

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 27 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**.

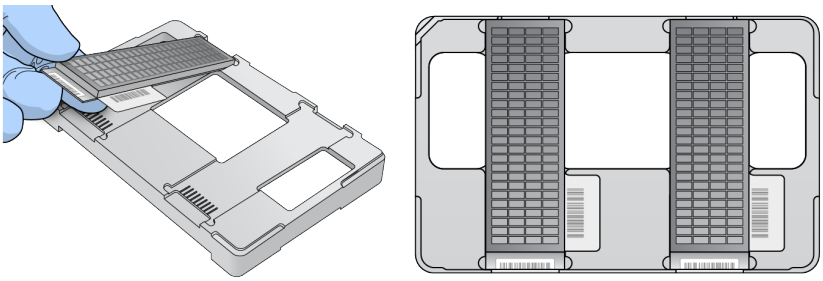
6 Scan the barcodes of the MSA7 plates, and all the BeadChips you plan to hybridize. Click **Verify**.

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 28 Placing BeadChips on Baseplates



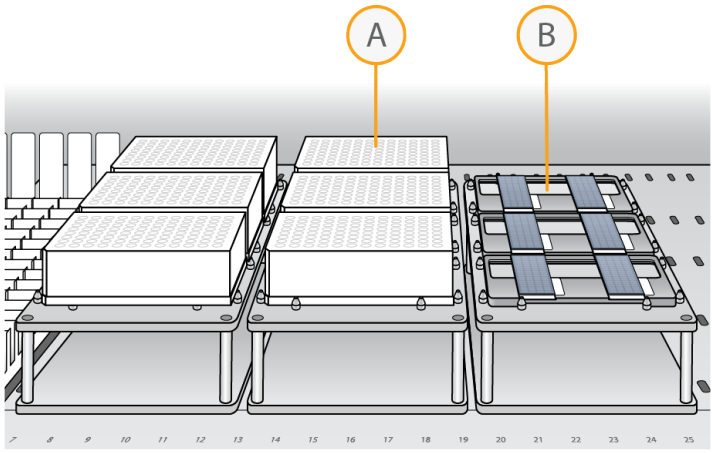
3 At the robot PC, select **MSA7 ST Tasks | Hyb Multi-BC2**.

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 29 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**.

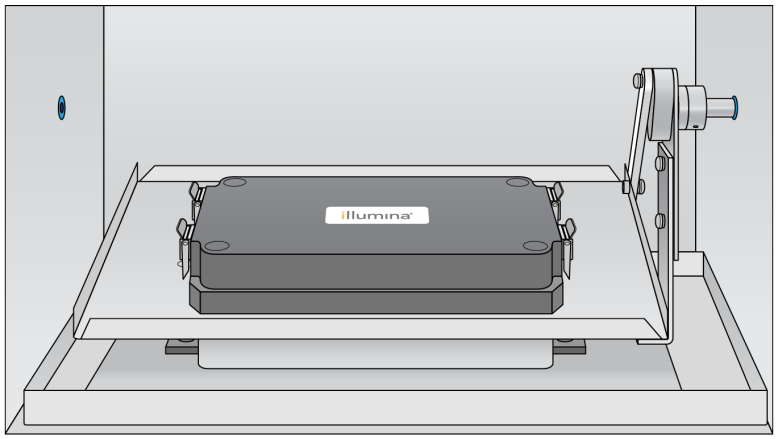
4 Scan the barcodes of the PB2 and BeadChips.

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 30 XT Hyb Chamber 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 H_2O 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 contaminates 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

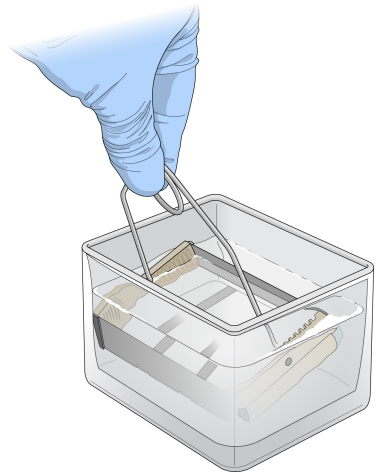
Wash BeadChips

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 31 Wash Rack in Wash Dish

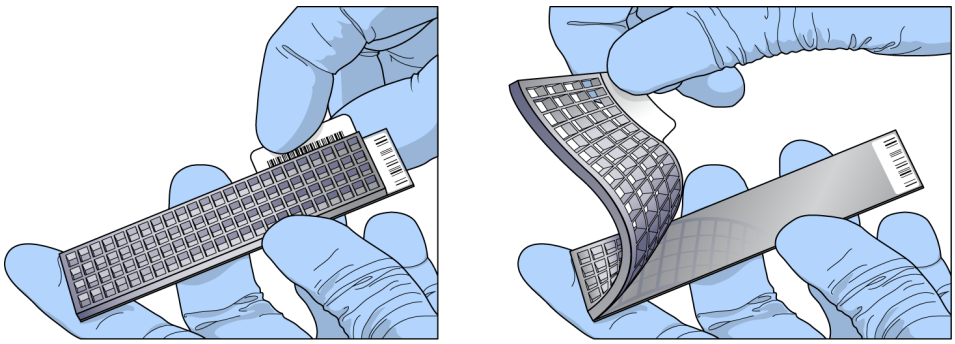


2 Remove the XT dual Hyb insert and baseplates from the XT Hyb chambers.

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 32 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.

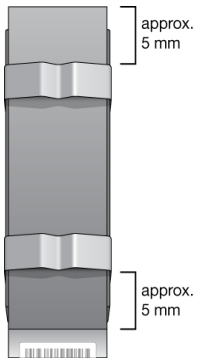
4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.

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 33 Assembled XCG Flow-Through Chamber



7 In Illumina LIMS, select **Wash BeadChip XT ST**.

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
- LX1
- LX2
- EML
- XC3
- SML

- ATM
- PB1
- XC4
- 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 To prepare the following consumables, thaw to room temperature.
   - LX1
   - LX2
   - EML
   - XC3
   - SML
   - ATM

2 Thaw RA1 and 95% formamide/1 mM EDTA to room temperature, preferably in a 20°C to 25°C water bath. 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.

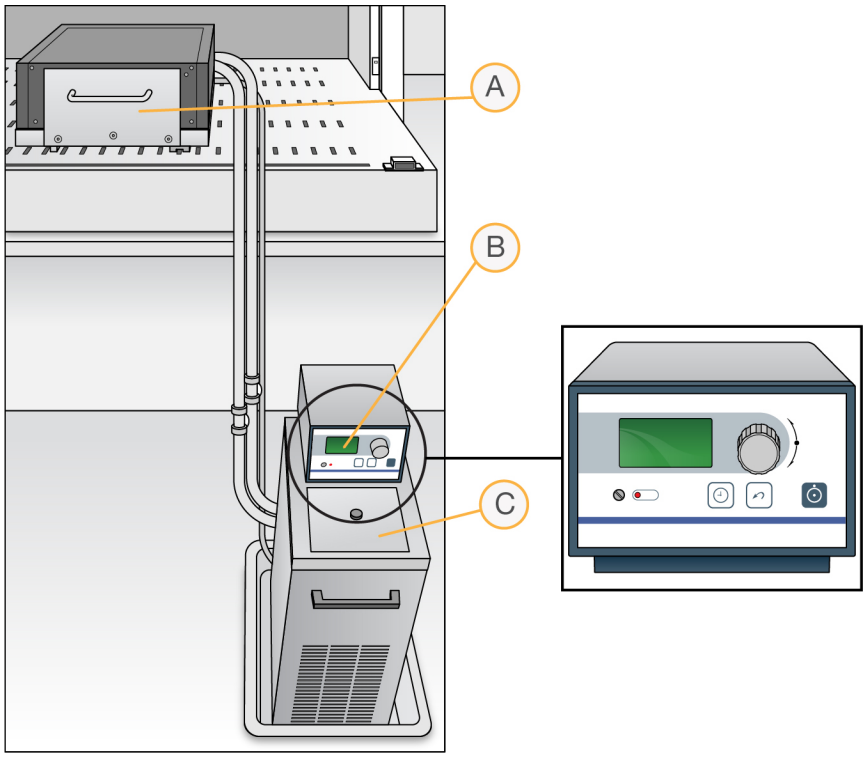
Procedure

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 34 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 ST**.

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	1–8	15 ml
	9–16	17 ml
RA1	1–8	10 ml

Reagent	# BeadChips	Volume
	9–16	20 ml
XC3	1–8	50 ml
	9–16	100 ml

4 Invert the LX1, LX2, EML, SML, and ATM tubes to mix. Remove the caps, and place on the robot bed, according to the bed map.

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

6 Click **Run**.

7 When prompted, enter the stain temperature listed on the XStain plate. Do not load the BeadChips yet.

8 When the chamber rack reaches 44°C, place the XCG Flow-Through Chamber assemblies into the chamber rack, according to the robot bed map.

9 At the robot PC, click **OK**.

10 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-loadingPB1 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 H_2O wash basin for later cleaning as detailed in the *Infinium Assay 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.

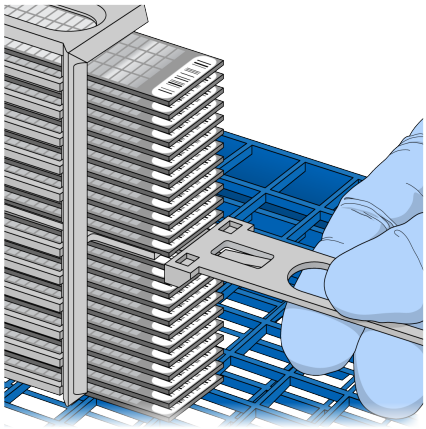
NOTE

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 35 Staining Rack in Correct Orientation



16 Remove the handle from the staining rack for easier access to the BeadChips.

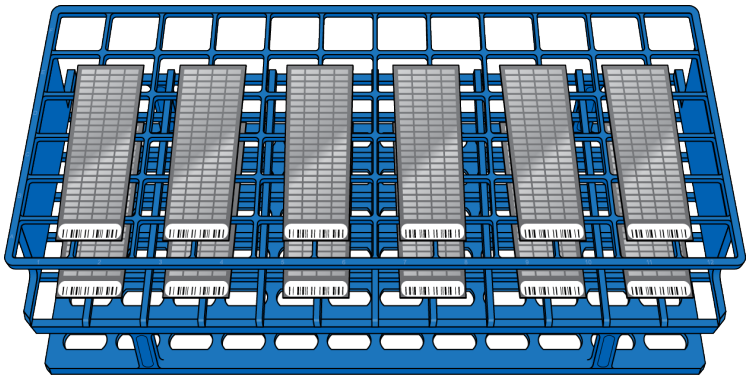
17 For each BeadChip, working top to bottom:

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 36 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 4 Automated Protocol without Illumina LIMS

Introduction

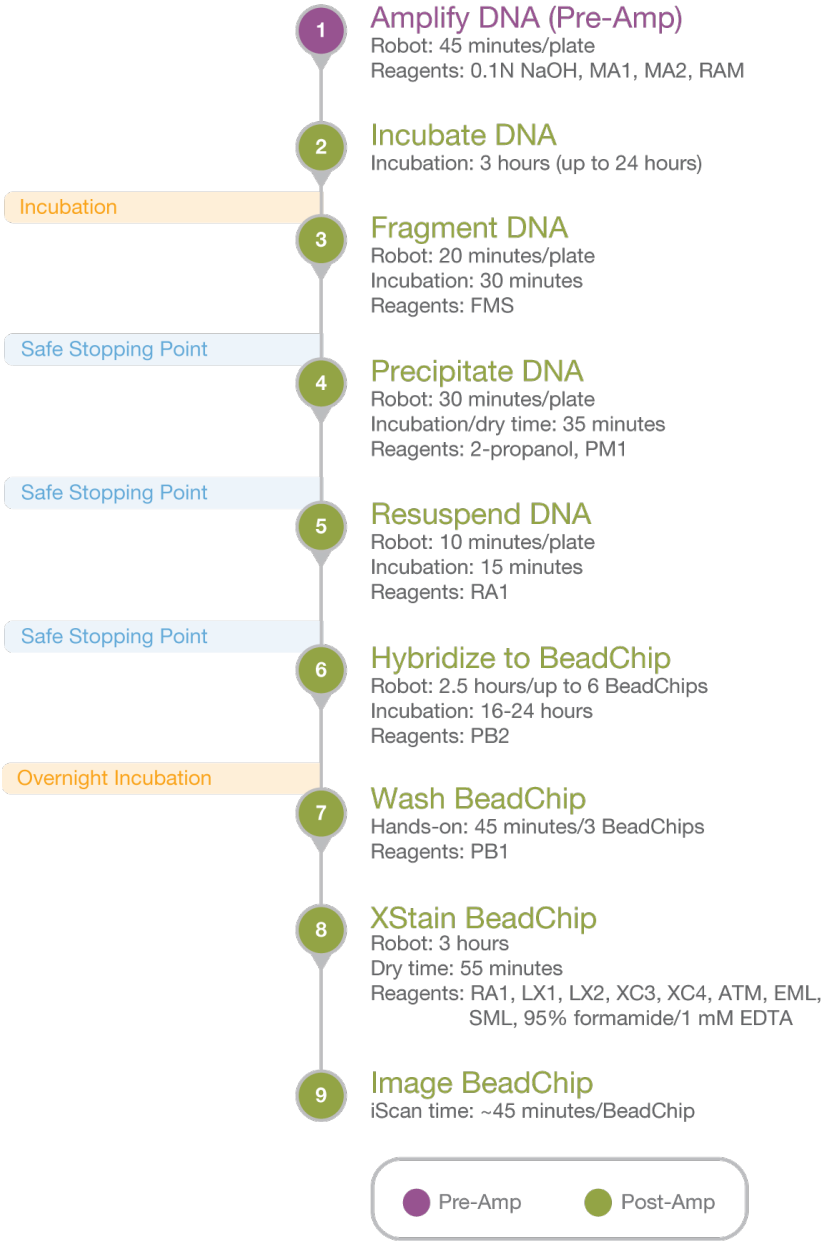
This section describes pre- and post-amplification automated laboratory protocols for the Infinium XT Assay. Follow the protocols in the order shown.

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 27 for protocol instructions.

Infinium XT ST Automated Workflow

The following figure graphically represents the Infinium XT Assay ST automated workflow for 4–12 BeadChips.

Figure 37 Infinium XT Protocol ST Automated Workflow



Amplify DNA (Pre-Amp)

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

If you are processing multiple plates, complete all amplification steps for one plate before starting another. You can then process plates in batches, starting with the incubation step. The recommended maximum batch size is 6 plates per user.

Consumables

- MA1 (1 tube/plate)
- MA2 (1 tube/plate)
- RAM (1 tube/plate)
- 0.1N NaOH (5 ml/plate)
- 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 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 ST Tasks | Make MSA7 ST**.

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 samples**.
The robot PC updates the Required Run Items and the bed map to show the correct position of items on the robot bed.

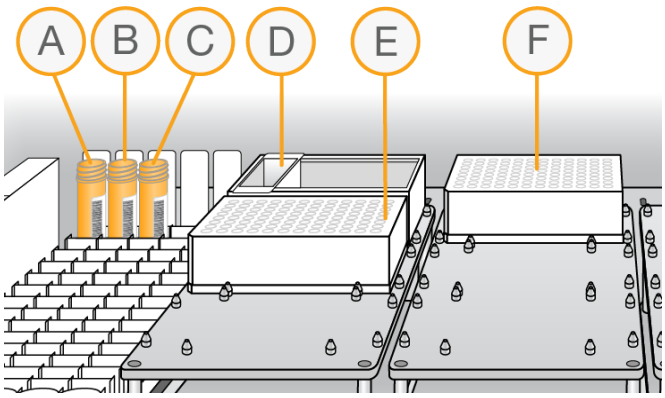
4 Place MA1, MA2, and RAM tubes into the tube rack according to the robot bed map. Remove the caps.

5 Add 0.1 N NaOH to a quarter reservoir (5 ml per plate), then place on the robot bed according to the bed map.

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 38 Robot Setup for Amplify DNA



A MA1 Tube
B MA2 Tube
C RAM Tube
D 0.1N NaOH Reservoir
E MSA7 Plate
F DNA Plate

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 × 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 (1 tube/plate)

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 × g at room temperature for 1 minute.

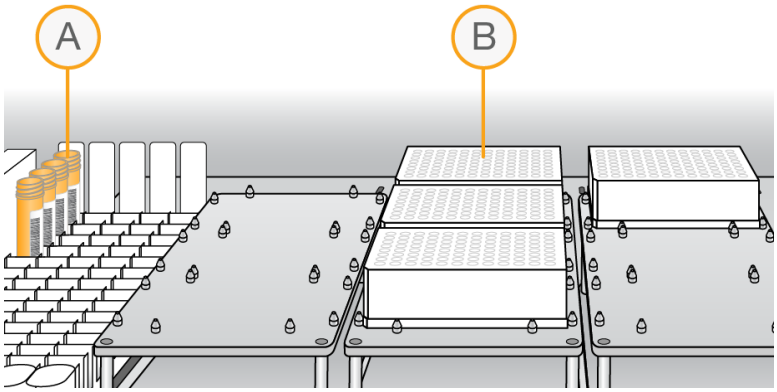
2 At the robot PC, select **MSA7 ST Tasks | Fragment MSA7 ST**.

3 In the Basic Run Parameters pane, enter the **Number of MSA7samples**.

4 Place the MSA7 plates on the robot bed according to the bed map. Remove the cap mats.

5 Place FMS tubes into the tube rack according to the robot bed map. Remove the caps.

Figure 39 Robot Setup for Fragment MSA7



A FMS Tubes
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 × g at room temperature for 1 minute.

Precipitate the MSA7 Plate

1 At the robot PC, select MSA7 ST Tasks | Precip MSA7 ST.

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.

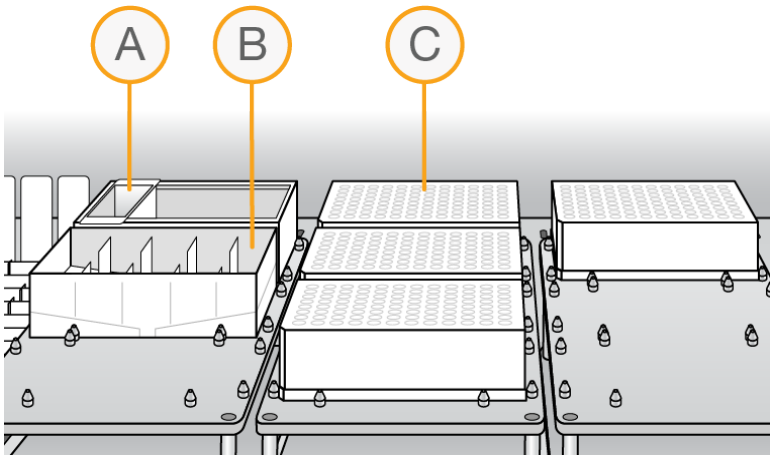
4 Place a quarter reservoir on the robot bed, according to the bed map, and add PM1:

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 40 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 × 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.

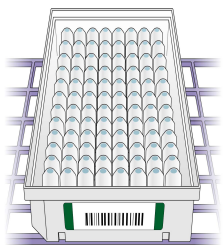
11 Quickly invert the plates and drain liquid to decant the supernatant. Then smack the plates down on a dry pad.

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 41 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 ST Tasks | Resuspend MSA7 ST**.

2 In the Basic Run Parameters pane, change the value for the **Number of MSA7 plates**.

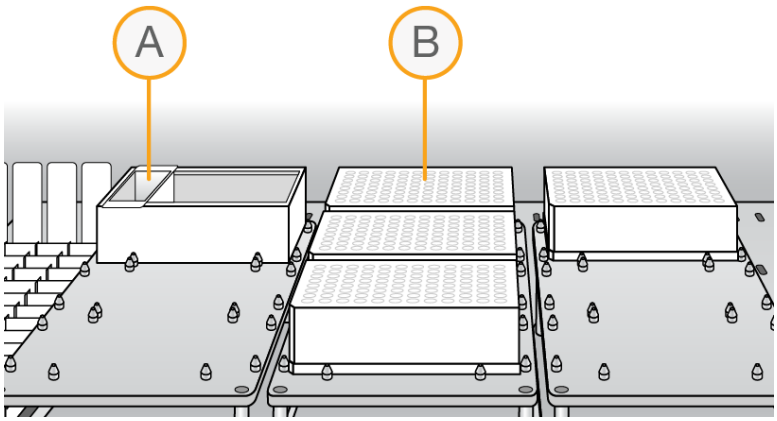
3 Place the MSA7 plates on the robot bed according to the bed map.

4 Place a quarter reservoir on the robot bed, according to the bed map, and add RA1:

Number of Plates	Volume
1	5 ml

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

Figure 42 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 H_2O

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.

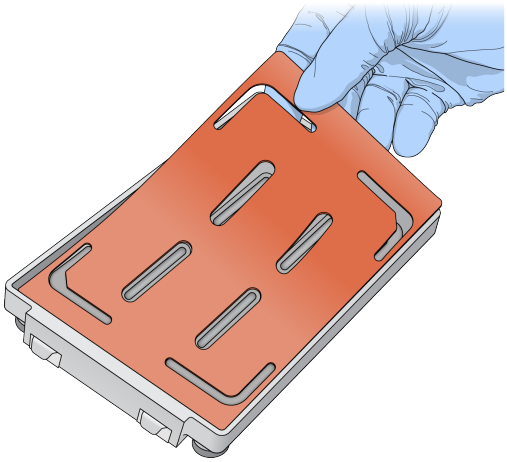
2 Cool the MSA7 plates on the benchtop at room temperature for 30 minutes.

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 43 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.

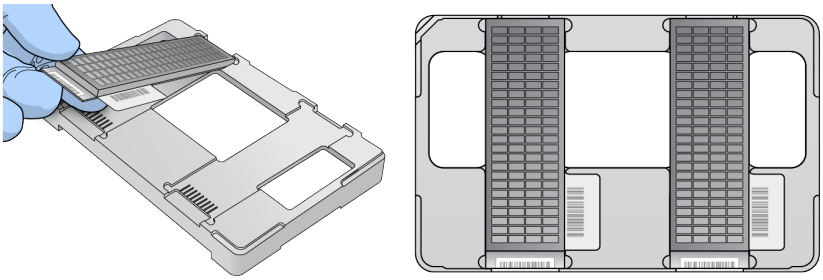
4 Leave closed XT Hyb chambers on the bench at room temperature until BeadChips are loaded with DNA sample.

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 44 Placing BeadChips on Baseplates



3 At the robot PC, select **MSA7 ST Tasks | Hyb Multi-BC2**.

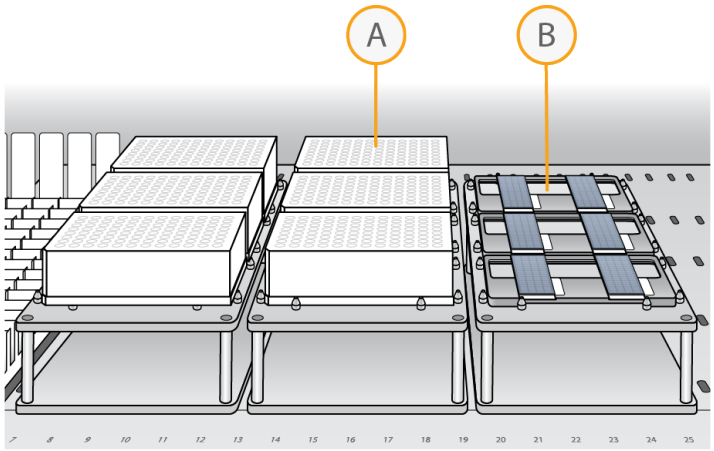
4 In the BeadChip Selection dialog box, select the 96-sample BeadChip.

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 45 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

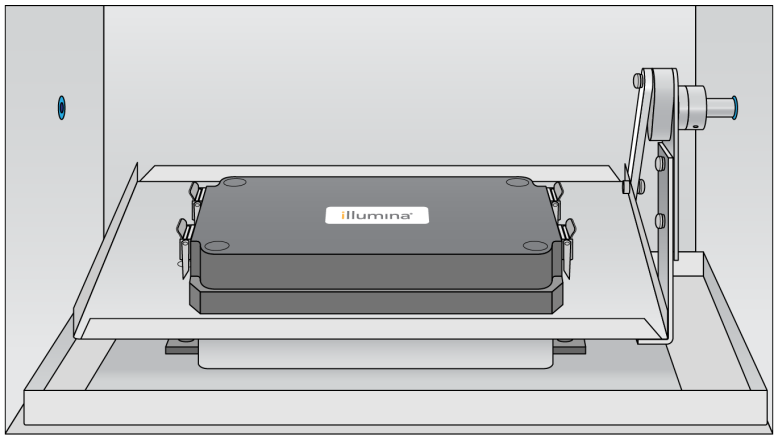
Keep Hyb chambers at room temperature when you load the BeadChips. Do not place the Hyb chambers in the Illumina Hybridization Oven before loading the BeadChips.

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.

4 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 46 XT Hyb Chamber 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 H_2O 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 contaminates 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

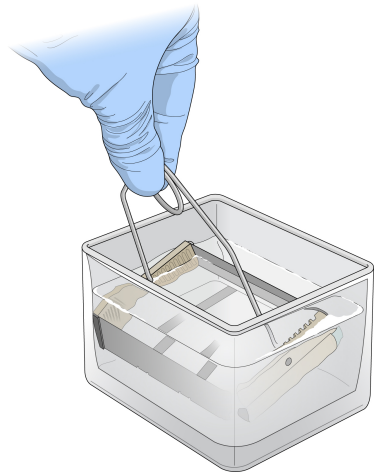
Wash BeadChips

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 47 Wash Rack in Wash Dish

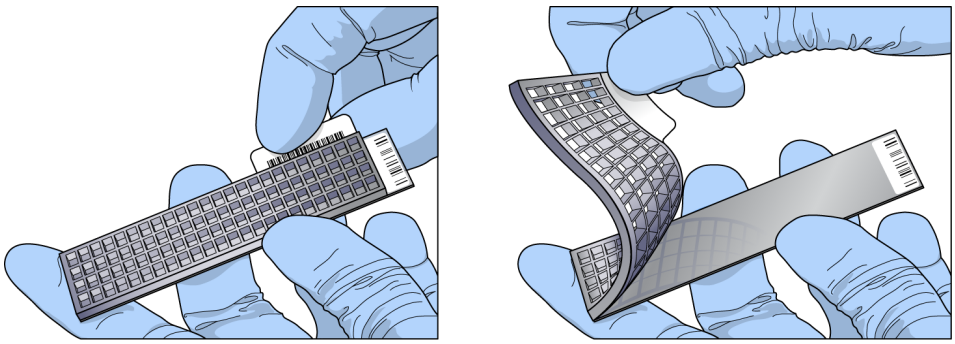


2 Remove the XT dual Hyb insert and baseplates from the XT Hyb chambers.

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 48 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.

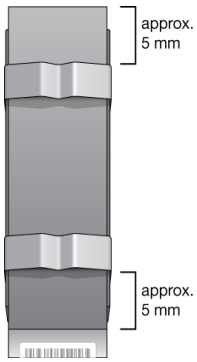
4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.

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 49 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
- LX1
- LX2
- EML
- XC3
- SML
- ATM
- PB1

- XC4
- 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 To prepare the following consumables, thaw to room temperature.
   - LX1
   - LX2
   - EML
   - XC3
   - SML
   - ATM

2 Thaw RA1 and 95% formamide/1 mM EDTA to room temperature, preferably in a 20°C to 25°C water bath. 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.

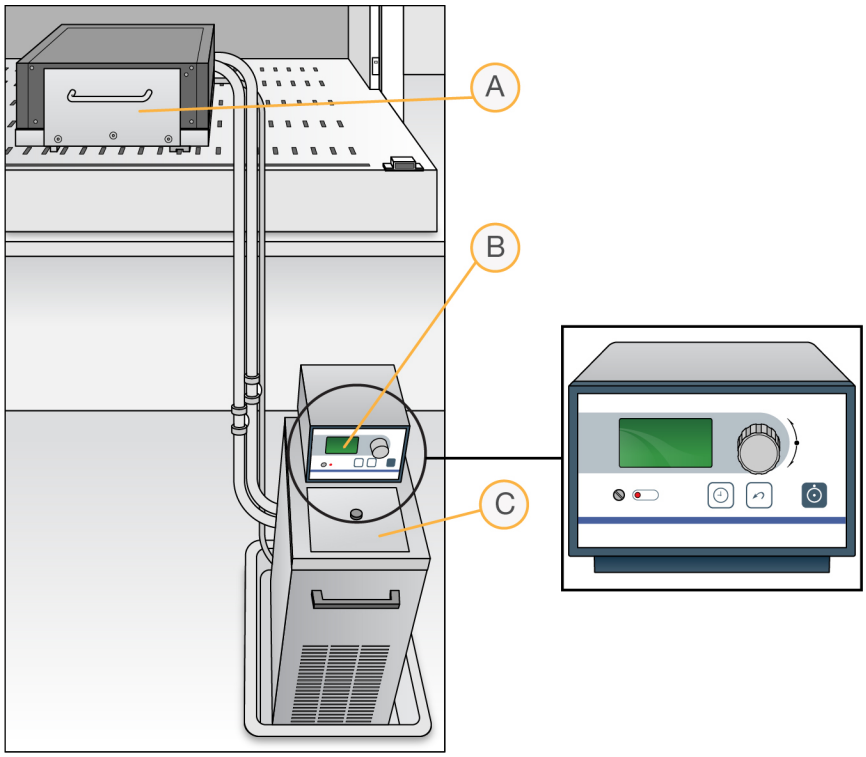
Procedure

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 50 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 ST**.

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	1–8	15 ml
	9–16	17 ml
RA1	1–8	10 ml

Reagent	# BeadChips	Volume
	9–16	20 ml
XC3	1–8	50 ml
	9–16	100 ml

4 Invert the LX1, LX2, EML, SML, and ATM tubes to mix. Remove the caps, and place on the robot bed, according to the bed map.

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

6 Click **Run**.

7 When prompted, enter the stain temperature listed on the XStain plate. Do not load the BeadChips yet.

8 When the chamber rack reaches 44°C, place the XCG Flow-Through Chamber assemblies into the chamber rack, according to the robot bed map.

9 At the robot PC, click **OK**.

10 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-loadingPB1 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 H_2O wash basin for later cleaning as detailed in the *Infinium Assay 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.

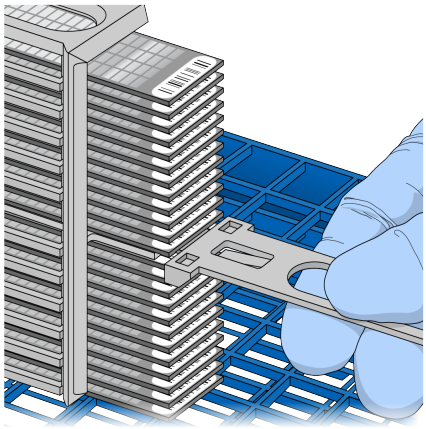
NOTE

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 51 Staining Rack in Correct Orientation



16 Remove the handle from the staining rack for easier access to the BeadChips.

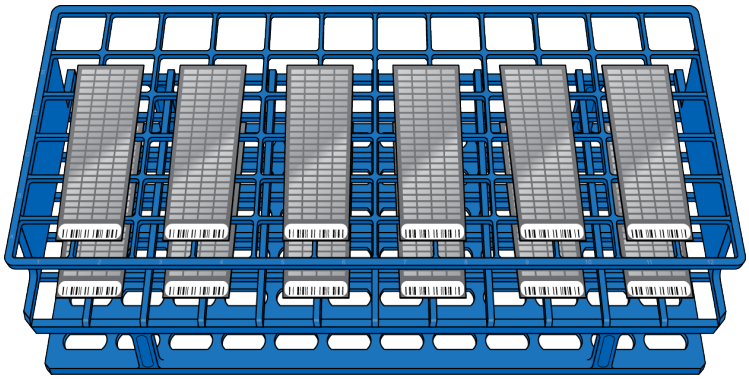
17 For each BeadChip, working top to bottom:

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 52 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

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_2O	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 (1152 samples)	20006613

Infinium XT iSelect-96 Kit (1152 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)	12	MA1	15°C to 30°C
	12	MA2	-25°C to -15°C
	12	RAM	-25°C to -15°C
Box 2	12	FMS	-25°C to -15°C
	3	LX1	-25°C to -15°C
	3	LX2	-25°C to -15°C
	3	EML	-25°C to -15°C
	3	SML	-25°C to -15°C
	3	ATM	-25°C to -15°C
Box 3	2	RA1	-25°C to -15°C
Box 4	2	PM1	2°C to 8°C
	1	PB2	15°C to 30°C
	1	XC3	15°C to 30°C
	1	XC4	15°C to 30°C
Box 5	3	Infinium XT iSelect Custom BeadChips (4)	2°C to 8°C

Infinium XT Starter Packages

Package	Description	Illumina Catalog #
Infinium XT ST starter kit (up to 12 BeadChip batches)	This kit includes accessory equipment required to process up to a 12 BeadChip XStain batch using the Infinium XT ST manual or automated protocol. Tip guides, AutoLoader 2.x, iScan, and automation robot sold separately.	20015525
Infinium XT ST upgrade kit (up to 12 BeadChip batches)	This kit supports existing Infinium customers looking to add the Infinium XT ST automated or manual workflow to scale their targeted genotyping applications. It includes Infinium XT specific accessories required to up to a 12 BeadChip XStain batch using the Infinium XT ST manual or automated protocol. Infinium XT tip guides are included. AutoLoader 2.x, iScan, and automation robot sold separately.	20015526
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 theInfinium 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
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
Pipette tips • 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 • 2 supplies: 1 for pre- and 1 for post-amplification processes	General lab supplier
ProStat EtOH presaturated wipes • 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 • 2 supplies: 1 for pre- and 1 for post-amplification processes	General lab supplier
Skirted microplates, 96-well, 0.2 ml • 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 • Midi plates, 1 per run	Thermo Fisher Scientific, catalog # AB0765
Tubes • 15 ml conical • 50 ml conical	General lab supplier

User-Supplied Reagents

Consumable	Supplier
Bleach	General lab supplier
Deionized water (DI H_2O)	General lab supplier
EDTA, 0.5 M	EMD Chemicals, catalog # 4056 Sigma-Aldrich, catalog # E7889

Consumable	Supplier
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_2O	Sigma-Aldrich, catalog # 221465
TE, 1X • 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

Equipment	Supplier
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 • Included with system, only needed if additional pairs are required	VWR, Int'l, 25601-008, www.vwr.com
Micropipettors • 2 separate sets: 1 for pre- and 1 for post-amplification processes • P-20 • P-200 • P-1000	General lab supplier
Multichannel precision pipettes • 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 • 2 separate sets: 1 for pre- and 1 for post-amplification processes	General lab supplier
Tube rack • 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

Equipment	Supplier
Tube vortexers • 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**.

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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

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