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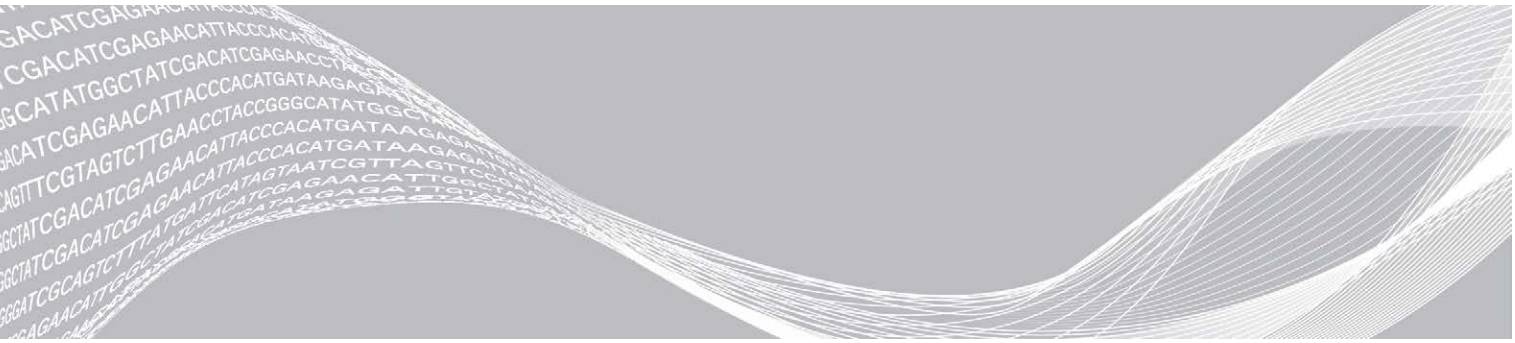
Infinium XT Assay

Reference Guide for the ST Workflow



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Revision History

Document	Date	Description of Change
Document # 1000000025687 v03	August 2019	Removed all references to WG# in barcode information in regards to DNA plates and MSA plates. Added Barcode Numbers section to Tips and Techniques to explain the new barcode numbering scheme.
Document # 1000000025687 v02	May 2019	Supporting Information Appendix removed. Consumables and equipment information moved to *Infinium Assay Lab Setup and Procedures Guide (document #11322460)*. Added *Infinium Consumables and Equipment Checklist (document #1000000084294)* to Additional Resources table. Acronym list moved to Overview section.
Document # 1000000025687 v01	June 2017	Added instructions to support the automated workflow with Illumina LIMS.
Document # 1000000025687 v00	December 2016	Initial release.

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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 *Setup and Procedures* 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
- Consumables and equipment to purchase in advance
- Standard lab procedures
- Robot use for automation
- Preparation for BeadChip imaging
- System Controls
- 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 that you 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. Use the Decode File Client to download the DMAP files.

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 that are 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.

Barcode Numbers

- Barcode serial numbers used for workflow enforcement and positive sample tracking on MSA plates, DNA plates, and reagents use a numbering pattern of two alphanumeric characters followed by seven random numbers.

Sealing the Plate

- Always seal plates before the vortex and centrifuge steps in the protocol.
- Orient sealing mats so that the A1 on a cap matches the A1 on the plate.
  - Make sure that all 96 caps are securely seated in the wells to prevent evaporation and spills, which introduce variability and cross-contamination.

- Remove sealing mats slowly and carefully to prevent splashing, and then set aside upside-down in a safe location.
- When returning a sealing mat to a plate, make sure that the orientation is correct.

Heat Sealer

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

Pipetting

- Make sure that pipettes are properly calibrated, cleaned, and decontaminated.
- Dispense slowly and carefully to prevent turbulence in the plate wells and flow-through chambers.
- Use a multichannel pipette whenever possible.

Centrifugation

- When centrifuging plates or BeadChips, place a balance plate or rack with BeadChips opposite each plate or rack being centrifuged. Make sure that the weights are as similar as possible.

Tip Alignment

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

Washing and Coating BeadChips

Perform the following steps 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 them upside down on wash rack to dry.
- Prepare an additional clean tube rack that fits the internal dimensions of the vacuum desiccator. Allow one rack per eight BeadChips.

Handling Hybridization Chambers

- Keep the chamber lids and bases together. Adopt a labeling convention that pairs each chamber base with the original lid.
- Regularly check lid-base pairs to make sure that they fit securely. Also check hinges for signs of abnormal wear or loose fittings. An airtight seal requires that the hinges have adequate clamping strength.
- Record which hybridization chamber was used for each BeadChip. If sample evaporation or other processing anomalies occur, investigate the appropriate hybridization chambers.
- When the hybridization chamber inserts contain BeadChips, keep them steady and level when lifting or moving.
  - Avoid shaking, and always keep parallel to the lab bench.
  - Do not hold by the sides near the sample inlets.

Acronyms

Acronym	Definition
DI H_2O	Deionized water
Purification reagent RG01	DNA purification beads
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
EML	Extension mix long
FMS	Fragmentation solution
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
PB1	Wash buffer
PB2	Humidifying buffer used during hybridization
PB20	Concentrated PB1
PM1	Precipitation solution
RA1	Resuspension, hybridization, and wash solution
RAM	Rapid Amplification MixAccelerated Amplification Mix
TCY plate	Acceptable 96-well plate for DNA plate
DNA	Whole genome-DNA plate
XC3	XStain BeadChip solution 3
XC4	XStain BeadChip solution 4

Prepare and Store Reagents

Conserve Reagents

- Infinium XT 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 is fresh when it meets the following criteria.

- Stored properly.
- Not exposed to room temperature air for extended periods of time.
- Not dispensed for use with XStain or Resuspension steps.

Additional RA1 Reagent Guidelines

For best use of RA1, follow these guidelines.

- Only pour the amount needed for the current step.
- If performing additional assay steps with RA1 on the same day, leave the remaining thawed reagent in the original, closed bottle. Store at room temperature until required.
- Follow 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 them in the protocol as needed. After you open an aliquot, use it on the same day. Discard leftover reagent.

Prepare Batches of 0.1 N NaOH

To minimize errors in preparing 0.1 N NaOH fresh each day, prepare it in large batches, and aliquot it into 15 ml or 50 ml sealed tubes. Store aliquots for 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

For Infinium XT workflows do the following.

Store PB20

- Store PB20 at room temperature.

Dilute PB20 to Make 1X PB1 (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

- Store PB1 at room temperature.
- Keep PB1 for up to 3 months.

Clean the Carboy

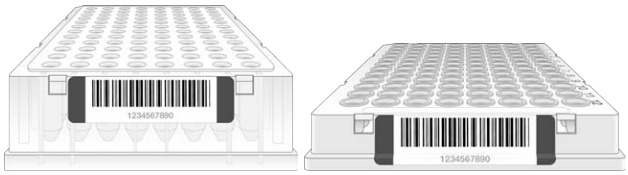
- Rinse the carboy with 10–20 L DI H_2O 3 times.
- Run 5 L DI H_2O through the spigot to flush it.

Illumina LIMS

If you are using the automated protocol with Illumina LIMS, follow these guidelines:

- At each step in the protocol, make sure that the **Use Barcodes** checkbox is selected.
- The barcode must be 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



- When prompted, enter the number of samples, plates, or BeadChips, and then select **OK**.
- Each time you select **Run** to start a new process, you are prompted to log in to LIMS.
- If prompted to select the project and the batch ID or DNA plate, do one of the following:
  - Select your current project. The available batches appear in the Sample Batch ID pane. Select a batch to see the associated DNA plate appear in the DNA Plates pane.
  - Use **Search** to search for a specific Batch ID or DNA plate.
- Some steps require verification in LIMS before you can start.
  - 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 Select the Reports tab in the upper-right corner.
    b In the left pane, select **Tracking Reports | Get Queue Status**.
    c Scan the plate barcode, and select **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 do the following:

- 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 select **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.

Resource	Description
Infinium Assay Lab Setup and Procedures Guide (document # 11322460)	Describes how to set up an Infinium lab including reagents, consumables, and equipment to purchase in advance, and best practices for lab operation.
Infinium Consumables and Equipment List (document # 1000000084294)	Provides an interactive checklist of Illumina-provided and user-provided consumables and equipment.

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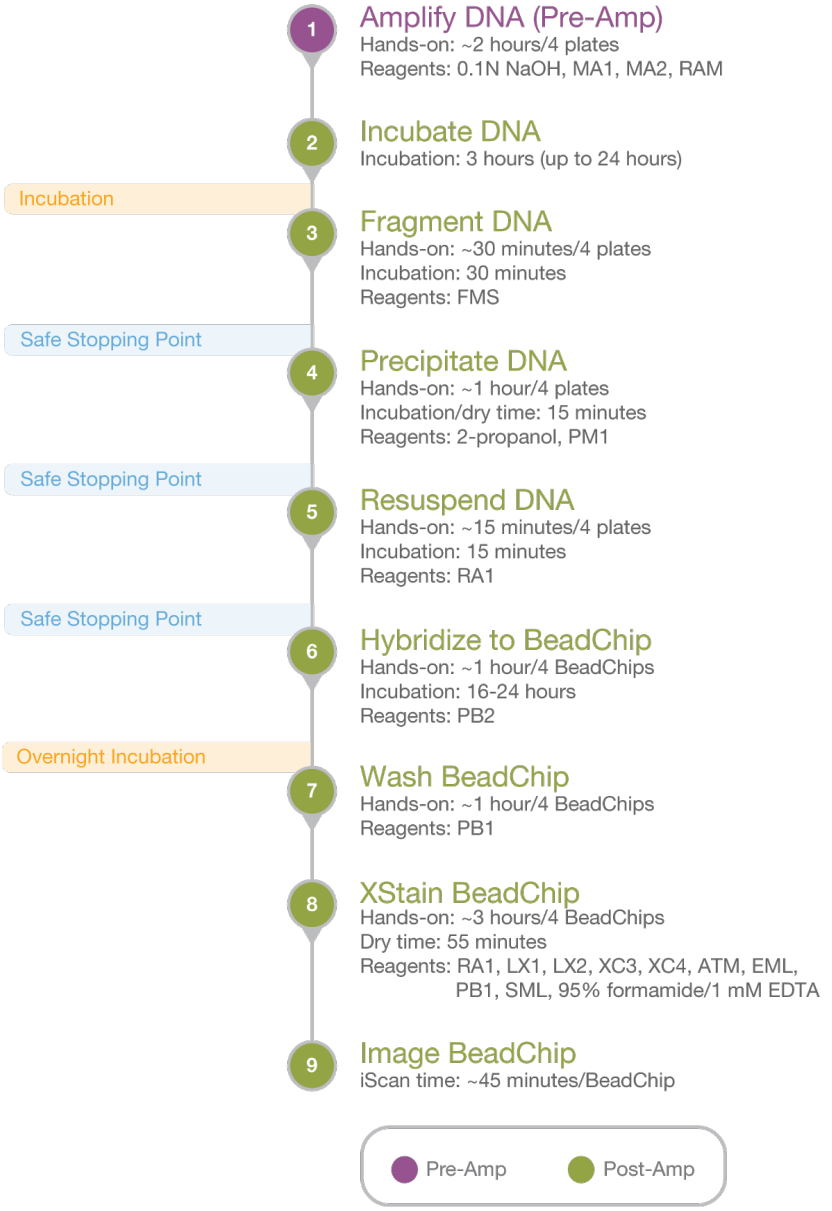
Introduction

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, and then it 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)
- 0.1 N NaOH (5 ml/plate)
- 96-well 0.8 ml microplates (midi)
- DNA plates with 96 DNA samples (10 µl at 50 ng/µl) (midi or TCY)
- Cap mats

Preparation

1 Thaw DNA plates 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 plates at 1600 rpm for 1 minute.

7 Centrifuge DNA plates at 280 × g at room temperature for 1 minute.

8 Label four 250 ml reservoirs NaOH, MA1, MA2, and RAM.

Procedure

1 Use a 200 µl pipette to add 20 µl MA1 to each well of the MSA7 plate.

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

3 Transfer 4 µl of the DNA sample from each well of the 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 step, 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 RA1 from the freezer, and thaw at room temperature.

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(s), and store at -25°C to -15°C.

Precipitate DNA

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

Consumables

- PM1
- 100% 2-propanol (IPA)
- Cap mats

Preparation

1 Prepare the following consumables.

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

2 Cool the refrigerated centrifuge to 4°C.

3 If you froze the MSA7 plates, thaw to room temperature.

4 Centrifuge the MSA7 plates 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.

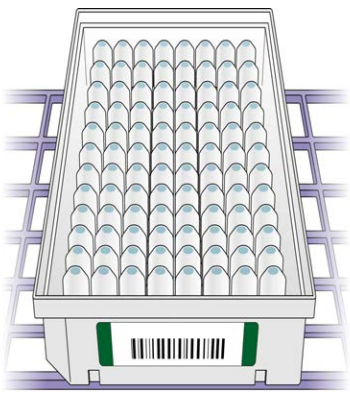
8 Quickly invert the plates and drain liquid to decant the supernatant. Then smack the plates down on a dry pad.

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(s), and store at -25°C to -15°C.

Resuspend DNA

This step uses RA1 to resuspend the precipitated DNA.

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.

Consumables

- RA1
- Foil heat seals

Preparation

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.

1. If you stored the MSA7 plates at -25°C to -15°C, thaw to room temperature, centrifuge, and then remove the cap mats.
2. Preheat the Illumina Hybridization Oven to 48°C.
3. 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 plate(s) at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C.

Store sealed RA1 at -25°C to -15°C. If RA1 will be used the next day, seal it, and 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, which enables 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, and then 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

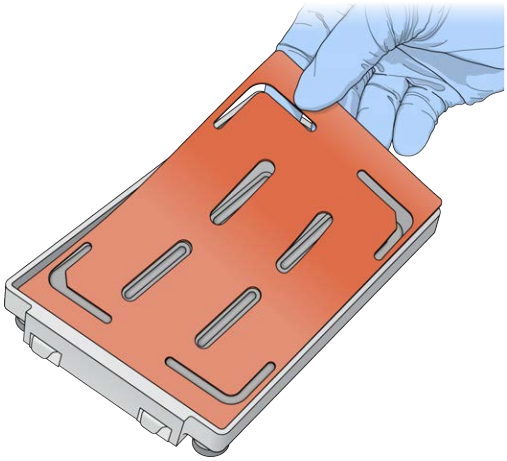
Denature DNA

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 15001000 × 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 four humidifying buffer reservoirs in the XT Hyb chambers.

3 Close the XT Hyb chamber immediately to prevent evaporation.

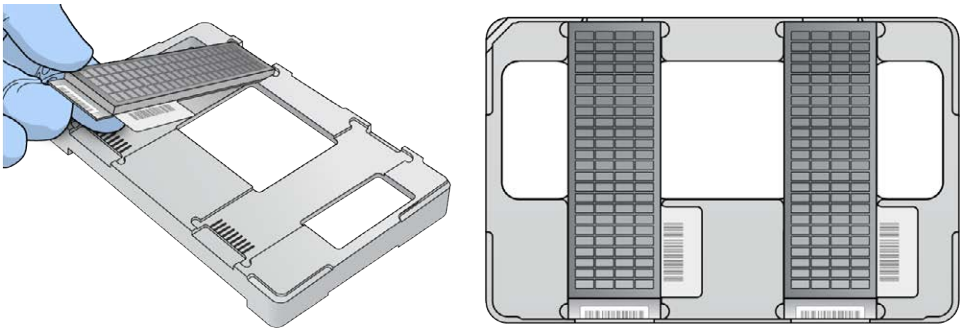
4 Leave the closed XT Hyb chambers on the bench at room temperature until BeadChips are loaded with DNA sample.

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

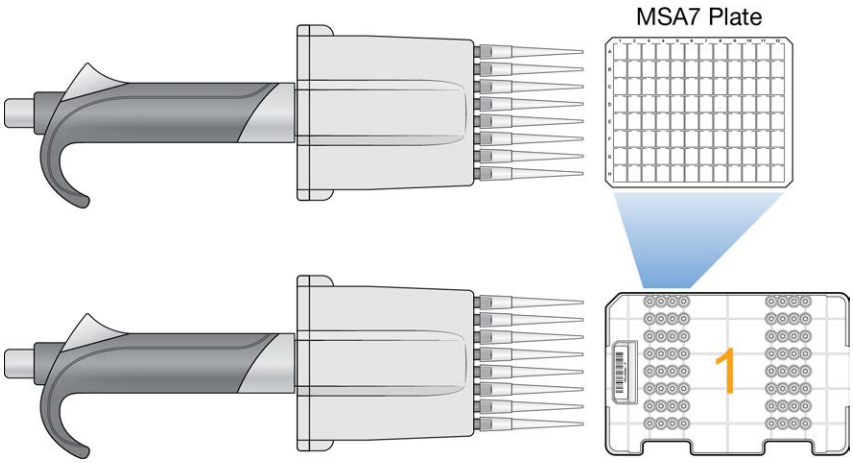
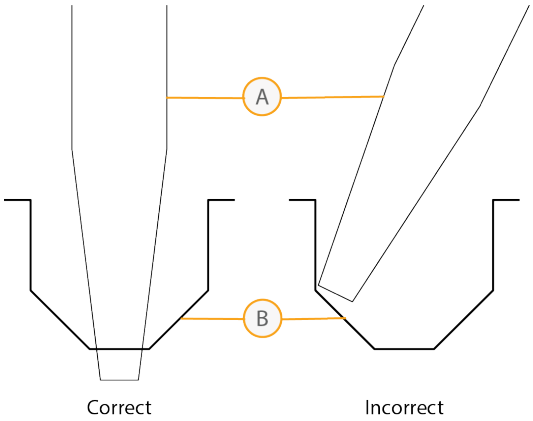


Figure 7 Correct and Incorrect Orientation of a Micropipette Tip in the XT Tip Guide



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

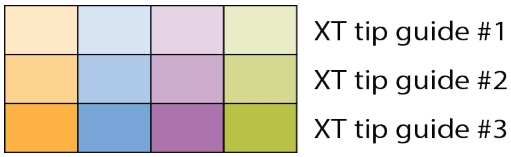
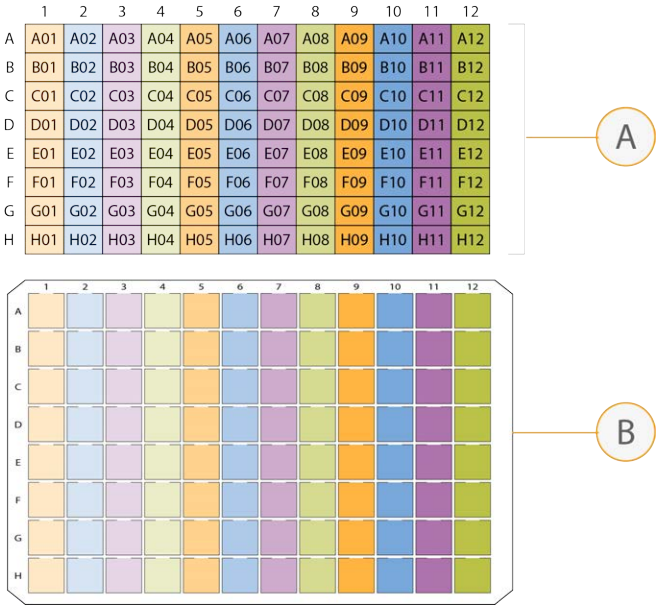
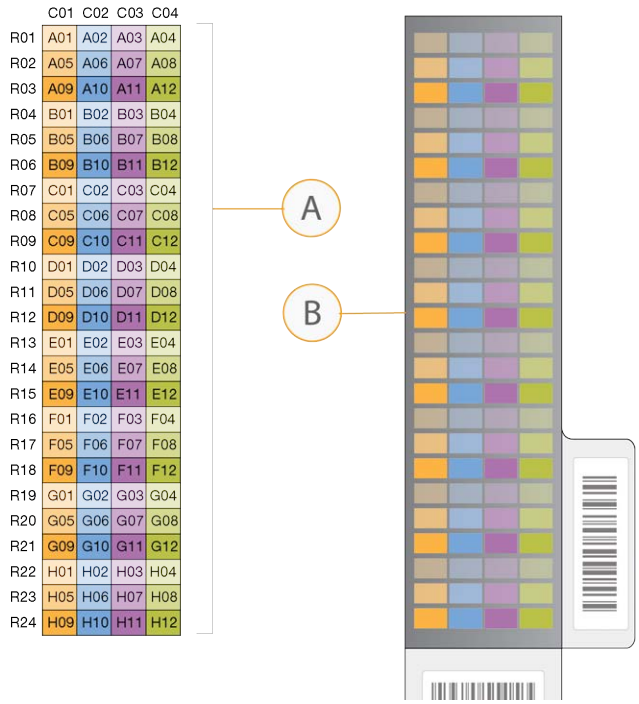


Figure 9 Color-Coded Transfer Matrix for 1 MSA7 Plate.



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.

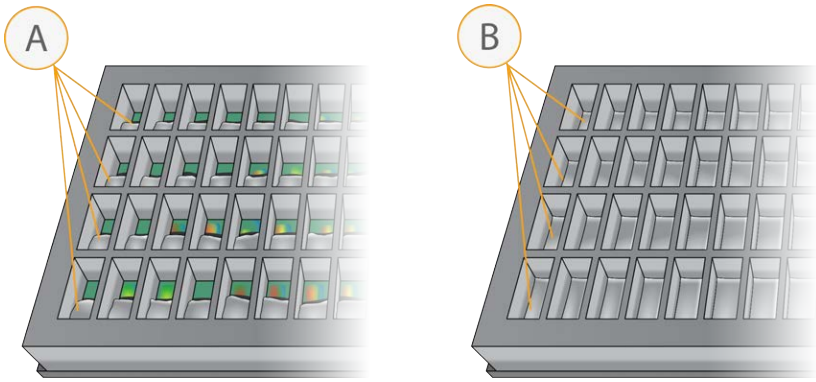
   a Load samples A05-H05 from the MSA7 plate into column C01 of tip guide #2.
   b Load samples A06-H06 from the MSA7 plate into column C02 of tip guide #2.
   c Load samples A07-H07 from the MSA7 plate into column C03 of tip guide #2.
   d Load samples A08-H08 from the MSA7 plate into column C04 of tip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

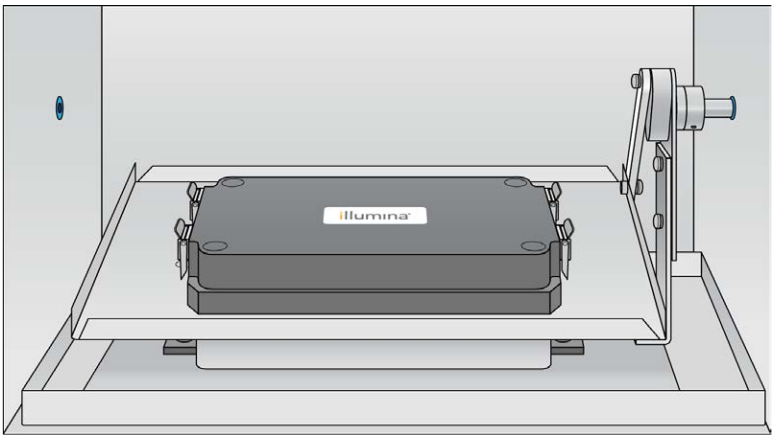
2 Load the XT dual Hyb insert and baseplates containing BeadChips inside the XT Hyb chambers. You can stack up to three XT dual Hyb insert and baseplates in each XT Hyb chamber.

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 three XT Hyb chambers for a maximum of six 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 is intended 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 EXXT Tip Guides

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

1 Soak the EXXT 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 EXXT tip guides.

2 Thoroughly rinse the EXXT tip guides with DI H_2O at least 3 times to remove any residual detergent.

3 Dry the EXXT 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 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.

Consumables

- PB1

Preparation

1 Dilute the PB1 to make it ready for use.

2 Remove the Hyb chambers from the Illumina Hybridization Oven. Cool for 30 minutes at room temperature before opening.

3 While the Hyb chambers are cooling do the following:

   a Fill two wash dishes with PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
   b Make sure that the XCG integrated spacer 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 200 ml PB1.

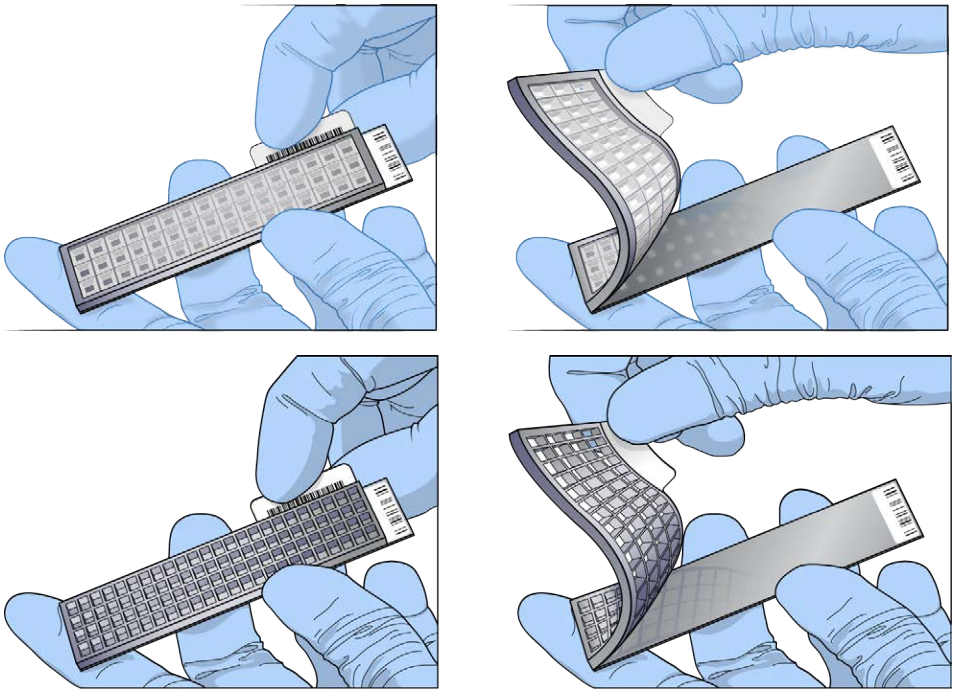
CAUTION

Replace PB1 in Wash 1 after every 12 BeadChips.

2 Remove the hybridization insert and baseplates from the hybridization chambers.

3 Remove the BeadChips from the hybridization 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.



5 Immediately and carefully slide each BeadChip into the wash rack in Wash 1. Make sure that the BeadChip is submerged in PB1.

6 Repeat steps 4–5 until all BeadChips are transferred to the submerged wash rack in Wash 1.

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

8 Move the wash rack to Wash 2 containing clean PB1. Make sure that the BeadChips are submerged.

9 Move the wash rack up and down for 1 minute. Break the surface of the 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 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 PB1.

3 For each BeadChip to be processed, place an XCG Flow-Through Chamber frame into the XT Flow-Through Chamber assembly tray.

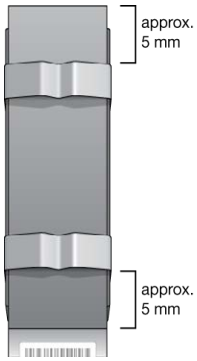
4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.

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 13 Assembled XCG Flow-Through Chamber



Extend and Stain (XStain)

Using the captured DNA as a template, the single-base extension of the oligos on the BeadChip incorporates detectable labels on the BeadChip to determine 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.

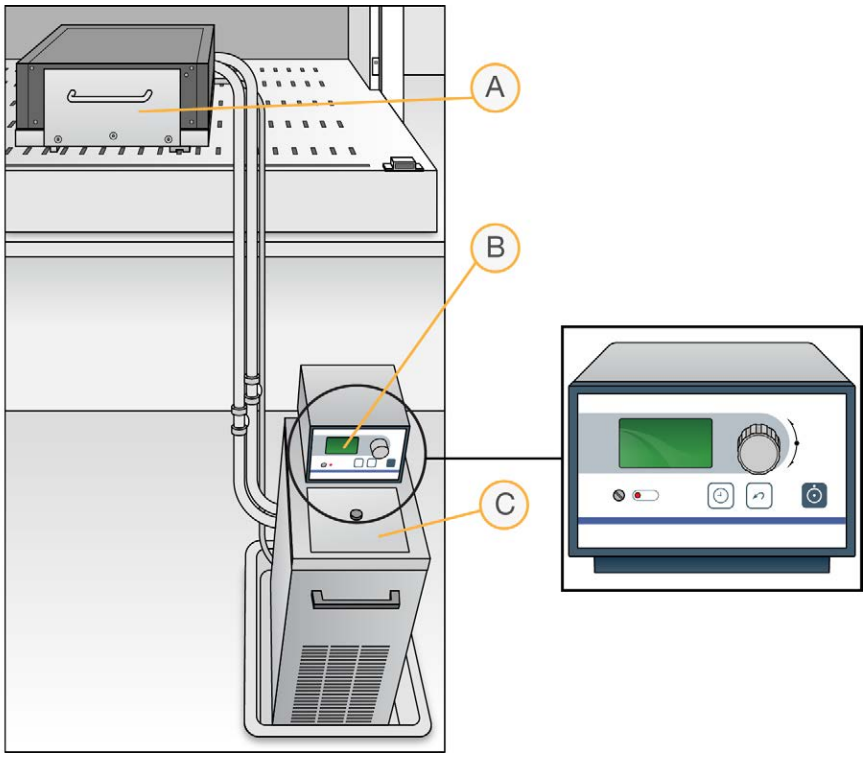
Procedure

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 14 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.

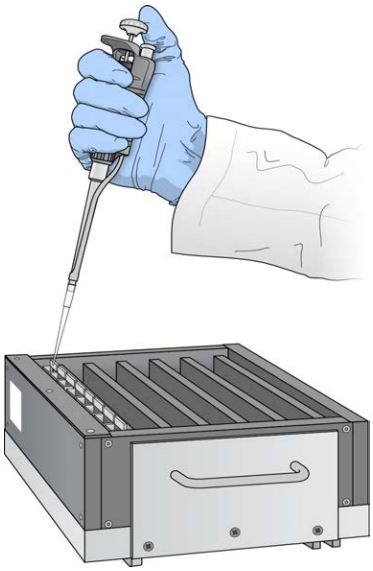
CAUTION

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 15 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 two top-loading PB1 and XC4 wash dishes, labeled PB1 and XC4.

2 To indicate fill volume, pour 310 ml water into the wash dishes, and mark the water level. Empty the water from the wash dish.

3 Pour 310 ml PB1 into a wash dish labeled PB1.

4 Place a staining rack inside the wash dish.

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 the 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 cleaning later, as detailed in the *Infinium Assay Lab Setup and Procedures Guide*.

8 Slowly move the staining rack up and down 10 times to break 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 to break the surface of the reagent.

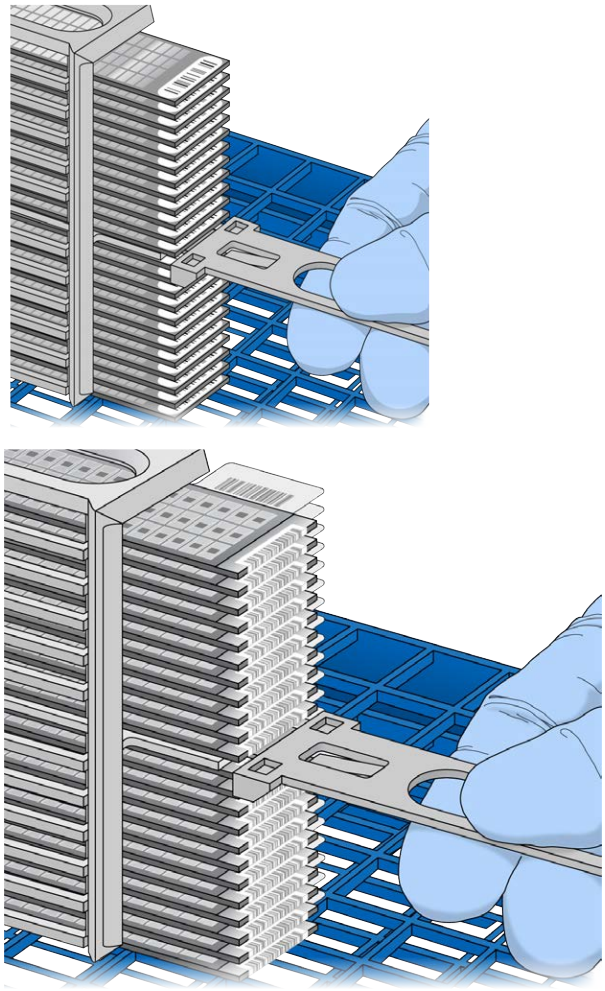
NOTE

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 barcode side of the BeadChips facing up.

Figure 16 Staining Rack in Correct Orientation



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

17 Work from the top to bottom of the staining rack, and perform the following steps for each BeadChip:

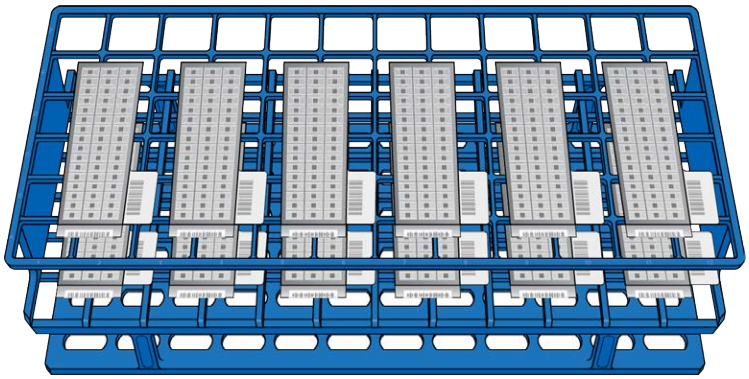
a Use self-locking tweezers to grip the BeadChip at its barcode end.

b Place the BeadChip on the tube rack with the barcode 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 17 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 the etched, barcoded sides are dry.

21 If the back of the BeadChip feels sticky, wipe it with a Prostat wipe or a Kimwipe wetted with 70% EtOH.

22 Image the BeadChips immediately, or store them, protected from light.

23 When you are ready to image the BeadChips, in Illumina LIMS, select **Infinium XT** | **Coat**.

a Scan the barcode of the reagent bottles.
b Scan each BeadChip barcode.
For this step, you can scan the BeadChip barcode on either the BeadChip or the BeadChip package.
c Select **Verify**, and then select **Save**.

Image BeadChip

Follow the instructions in the System Guide for your instrument to scan your BeadChips.

Use the **Infinium XTXCG** scan setting for your BeadChips.

Chapter 3 Automated Protocol with Illumina LIMS

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Introduction

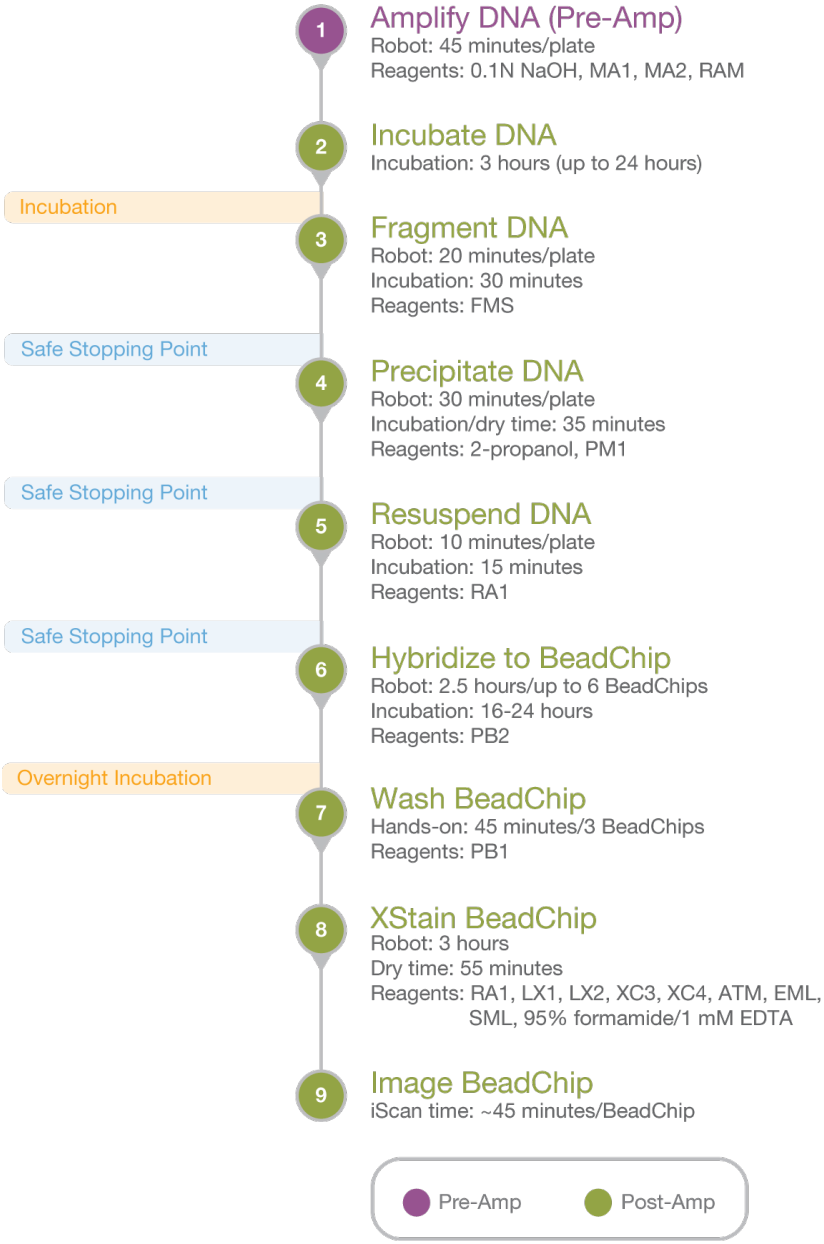
This section describes pre- and post-amplification automated laboratory protocols for the Infinium XT Assay. Follow the protocols in the order shown.

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 52 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 18 Infinium XT Protocol ST Automated Workflow



Amplify DNA (Pre-Amp)

This process adds the DNA samples to the plates, and then it 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)
- 0.1 N NaOH (5 ml/plate)
- 96-well 0.8 ml microplates (midi)
- DNA plates with 96 DNA samples (10 µl at 50 ng/µl) (midi or TCY)
- Cap mats

Preparation

1 Thaw DNA plates 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 plates at 1600 rpm for 1 minute.

7 Centrifuge DNA plates at 280 × g at room temperature for 1 minute.

8 Label four 250 ml reservoirs NaOH, MA1, MA2, and RAM.

Procedure

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

   a Select the DNA plate type (midi or TCY). Do not mix plate types on the robot.

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

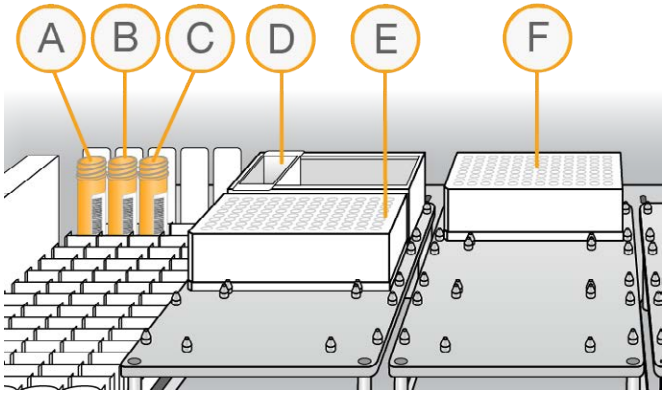
3 Add 0.1 N NaOH to a quarter reservoir (5 ml per plate), then place on the robot deck according to the deck map.

4 Place the DNA plates and MSA7 midi plates on the robot deck according to the deck map.

5 At the robot PC, select **Run**.

   a Select the project, and then select the batch ID.
   b Select **OK** to confirm the required DNA-plate barcodes.

Figure 19 Robot Setup for Amplify DNA



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

6 When the robot finishes, apply cap mats to the MSA7 plates, and then vortex at 1600 rpm for 1 minute.

7 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 step, and all remaining steps in the workflow, are performed in the post-amp lab.

1 In Illumina LIMS, select **Infinium XT | Incubate MSA7 ST**.

   a Scan the barcode of each MSA7 plate, select **Verify**, and then select **Save**.

2 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 RA1 from the freezer, and thaw at room temperature.

Procedure

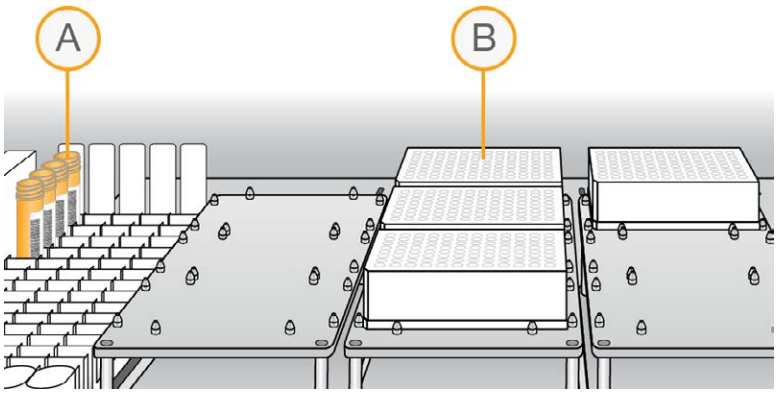
1 Centrifuge the MSA7 plates at 280 × g at room temperature for 1 minute.

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

3 Place the MSA7 plates on the robot deck according to the deck map in Figure 20. Remove the cap mats.

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

Figure 20 Robot Deck Setup for Fragment MSA7



A FMS Tubes
B MSA7 Plates

5 At the robot PC, select **Run**.

6 **[Optional]** If you are using a Tecan scanner bracket, adjust the Tecan scanner bracket to **Position B**. If you are not using a Tecan scanner bracket, you can skip this step.

Figure 21 Move Tecan Scanner Bracket to Position B

7 At the robot PC, when prompted, scan the barcode of the reagent bottle.

   a When the robot finishes, select **OK**.

8 Remove the plates from the robot deck, 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. Do not leave the plates in the 37°C Illumina Hybridization Oven longer than 2 hours.

SAFE STOPPING POINT

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

Precipitate DNA

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

Consumables

- PM1
- 100% 2-propanol (IPA)
- Cap mats

Preparation

1 Prepare the following consumables.

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

2 Cool the refrigerated centrifuge to 4°C.

3 If you froze the MSA7 plates, thaw to room temperature.

4 Centrifuge the MSA7 plates 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 Remove the cap mats, and place the MSA7 plates on the robot deck according to the deck map in Figure 22.

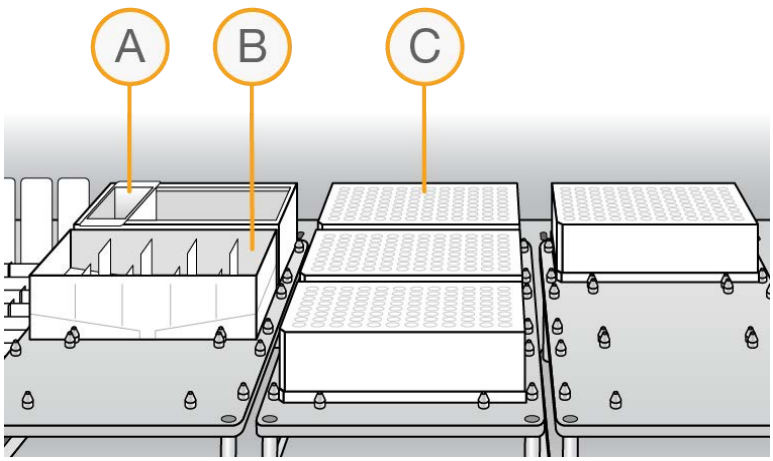
3 Place a quarter reservoir on the robot deck according to the deck map in Figure 22, and add PM1:

Reagent	Number of Plates	Volume
PM1	1	8 ml
	2	14 ml
	3	21 ml
	4	27 ml
	5	34 ml
	6	40 ml

4 Place a full reservoir on the robot deck, according to the deck map Figure 22, and add 2-propanol:

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

Figure 22 Robot Deck Setup for Precipitate MSA7



A PM1 Reservoir
B 2-Propanol Reservoir
C MSA7 Plates

5 At the robot PC, select **Run**.

a When prompted, scan the barcode of the reagent bottle.
b When the robot finishes, select **OK**.

6 Remove the plates from the robot deck, and apply fresh cap mats.

7 Invert the plates 10 times to mix.

8 In Illumina LIMS, select **Infinium XT ST| Spin MSA7 ST**.

a Scan the barcode of each MSA7 plate, select **Verify**, and then select **Save**.

9 Centrifuge at 3000 × g at 4°C for 20 minutes.

CAUTION
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.

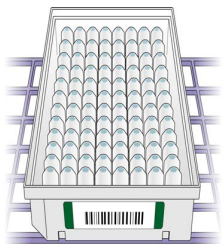
11 Quickly invert the plates, and drain liquid to decant the supernatant. Then, smack the plates down on a dry pad.

12 Tap the plates several times 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 the uncovered, inverted plates on the tube rack for 15 minutes at room temperature to air-dry pellets. Look for blue pellets at the bottom of the wells. Keep the plates inverted, and use a Kimwipe to remove any residual alcohol draining from the wells or remaining on the surface.

Figure 23 Uncovered MSA7 Plate Inverted for Air Drying



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

SAFE STOPPING POINT

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

Resuspend DNA

This step uses RA1 to resuspend the precipitated DNA.

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.

Consumables

- RA1
- Foil heat seals

Preparation

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.

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

Resuspend the MSA7 Plate

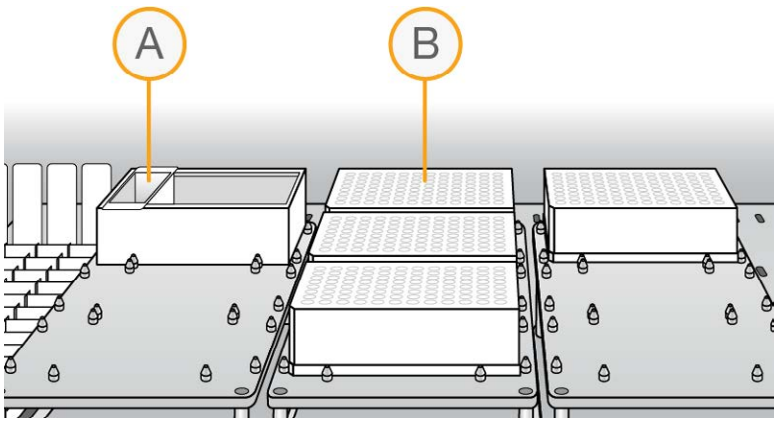
1. At the robot PC, select **MSA7 ST Tasks | Resuspend MSA7 ST**.

2 Place the MSA7 plates on the robot deck according to the deck map in Figure 24.

3 Place a quarter reservoir on the robot deck according to the deck map in Figure 24, and add RA1.

Reagent	Number of Plates	Volume
RA1	1	5 ml
	2	8 ml
	3	11 ml
	4	14 ml
	5	17 ml
	6	20 ml

Figure 24 Robot Deck Setup for Resuspend MSA7



A RA1 Reservoir
B MSA7 Plates

4 At the robot PC, select **Run**.

a When prompted, scan the barcode of the reagent bottle.
b When the robot finishes, select **OK**.

5 Remove the MSA7 plates from the robot deck.

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

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

8 Vortex at 1800 rpm for 1 minute.

9 Check to make sure that the pellets are resuspended. If the pellets are not resuspended, repeat steps 7 and 8.

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

SAFE STOPPING POINT

If you are stopping, store sealed MSA7 plate(s) at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C.

Store sealed RA1 at -25°C to -15°C. If RA1 will be used the next day, seal it, and 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, which enables 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, and then 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.

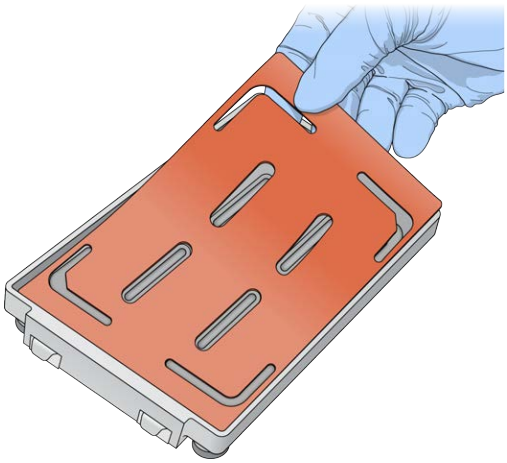
2 Cool the MSA7 plates on the benchtop at room temperature for 30 minutes.

3 Centrifuge at 15001000 × 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 25 XT Hyb Chamber and Gasket



2 Dispense 800 µl PB2 into each of the four humidifying buffer reservoirs in the XT Hyb chambers.

3 Close the XT Hyb chamber immediately to prevent evaporation.

4 Leave the 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**.

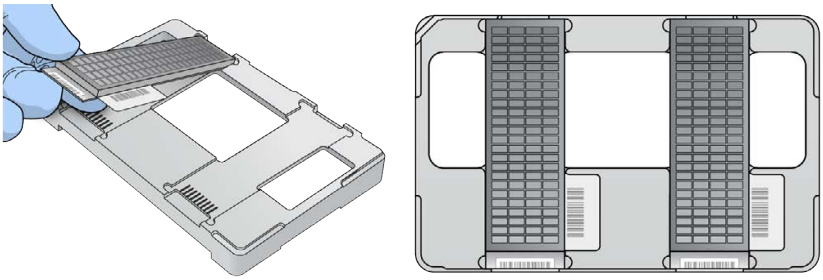
6 Scan the barcodes of the MSA7 plates, and all the BeadChips you plan to hybridize. Click **Verify**.

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 26 Placing BeadChips on Baseplates



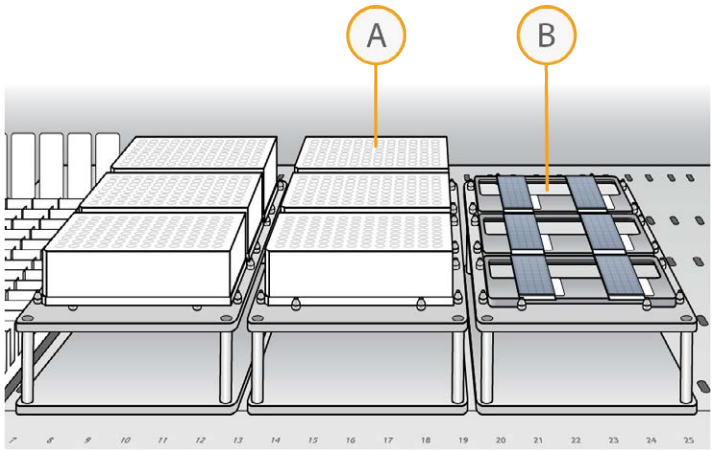
3 At the robot PC, select **MSA7 ST Tasks | Hyb Multi-BC2**.

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

5 Place the XT dual Hyb insert and baseplates onto the robot deck according to the deck map.

6 Place the MSA7 plates onto the robot deck according to the deck map, and remove the heat seals.

Figure 27 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 deck 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 three XT dual Hyb insert and baseplates in each XT Hyb chamber.

3 In Illumina LIMS, select **Infinium XT** | **Prepare Hyb Chamber**.

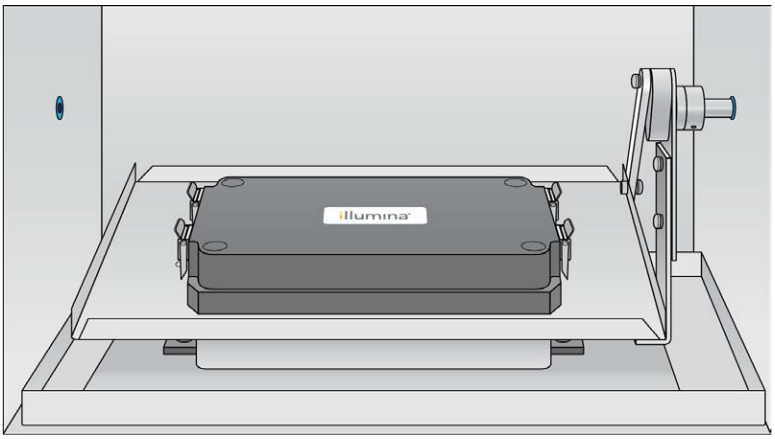
4 Scan the barcodes of the PB2 and BeadChips.

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 three XT Hyb chambers for a maximum of six total in the Illumina Hybridization Oven.

Figure 28 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 is intended 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 EXXT Tip Guides

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

1 Soak the EXXT 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 EXXT tip guides.

2 Thoroughly rinse the EXXT tip guides with DI H_2O at least 3 times to remove any residual detergent.

3 Dry the EXXT 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 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.

Consumables

- PB1

Preparation

1 Dilute the PB1 to make it ready for use.

2 Remove the Hyb chambers from the Illumina Hybridization Oven. Cool for 30 minutes at room temperature before opening.

3 While the Hyb chambers are cooling do the following:

   a Fill two wash dishes with PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
   b Make sure that the XCG integrated spacer 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 200 ml PB1.

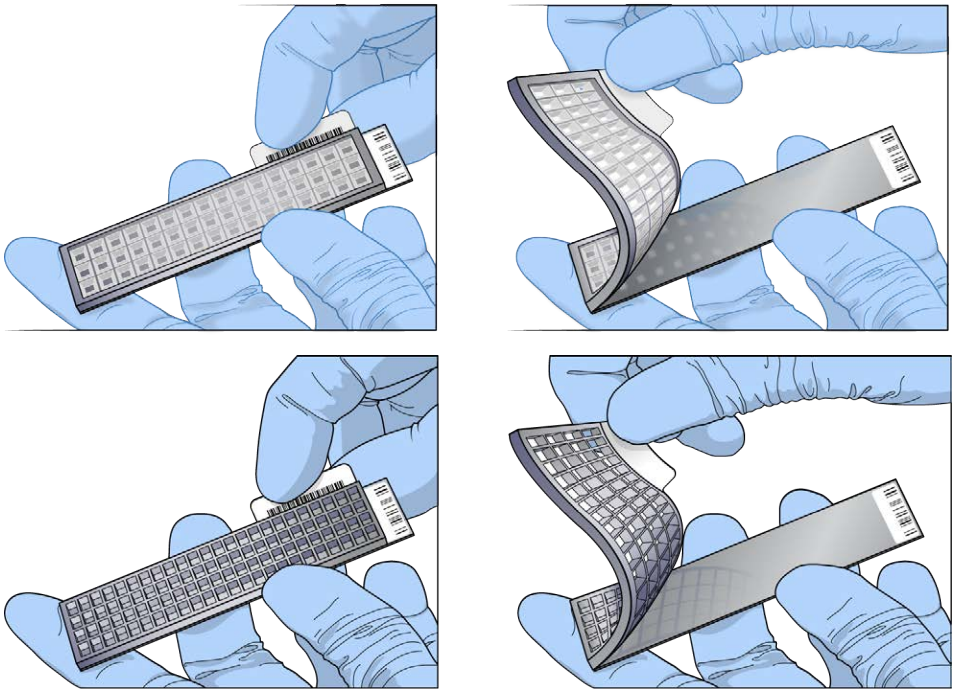
CAUTION

Replace PB1 in Wash 1 after every 12 BeadChips.

2 Remove the hybridization insert and baseplates from the hybridization chambers.

3 Remove the BeadChips from the hybridization 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.



5 Immediately and carefully slide each BeadChip into the wash rack in Wash 1. Make sure that the BeadChip is submerged in PB1.

6 Repeat steps 4–5 until all BeadChips are transferred to the submerged wash rack in Wash 1.

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

8 Move the wash rack to Wash 2 containing clean PB1. Make sure that the BeadChips are submerged.

9 Move the wash rack up and down for 1 minute. Break the surface of the 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 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 PB1.

3 For each BeadChip to be processed, place an XCG Flow-Through Chamber frame into the XT Flow-Through Chamber assembly tray.

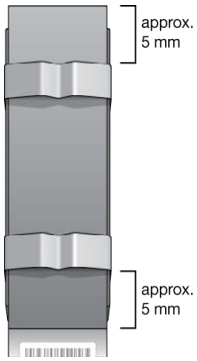
4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.

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 29 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)

Using the captured DNA as a template, the single-base extension of the oligos on the BeadChip incorporates detectable labels on the BeadChip to determine 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

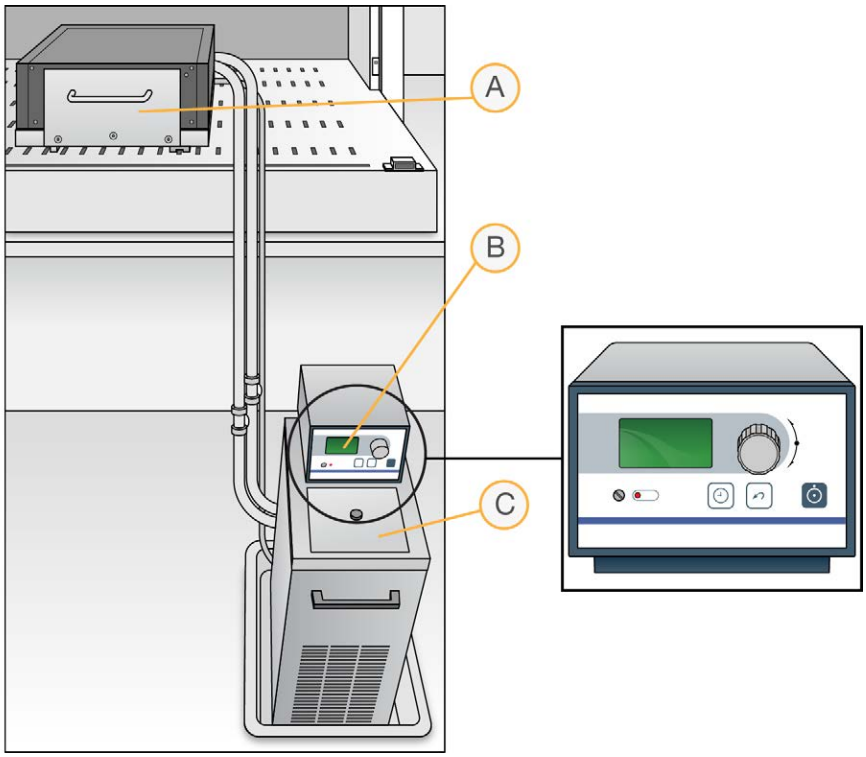
Set Up the Chamber Rack

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:

a In the Action section drop-down list, select **Set Target Temperature.**
b In the field below Set Target Temperature, enter **44.**
c Select the **Execute** button.

Figure 30 Water Circulator Connected to Chamber Rack



A Chamber Rack
B Water Circulator
C Reservoir Cover

3 Remove bubbles trapped in the chamber rack.

a Separate the heat exchanger from the reagent pan.
b Lift the heat exchanger upright and away from you with the tubing at the bottom, and turn 90° counter clockwise.
c Return the heat exchanger to a horizontal position.
d Repeat steps b and c 3 times for a total of 4 rotations or until all bubbles are removed.
e Using Kimwipes dampened with laboratory-grade water, clean all surfaces between the heat exchanger and reagent pan. Discard Kimwipes with formamide waste.
f Place the Te-Flow back on the reagent pan. Using the two guide pins in the reagent pan, make sure that the Te-Flow is flush.

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 deck, according to the deck 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
	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 deck, according to the deck map.

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

6 Select **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 deck map.

CAUTION
Start the robot immediately to prevent BeadChips from drying.

9 At the robot PC, select **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 two top-loading PB1 and XC4 wash dishes, labeled PB1 and XC4.

2 To indicate fill volume, pour 310 ml water into the wash dishes, and mark the water level. Empty the water from the wash dish.

3 Pour 310 ml PB1 into a wash dish labeled PB1.

4 Place a staining rack inside the wash dish.

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 the 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 cleaning later, as detailed in the *Infinium Assay Lab Setup and Procedures Guide*.

8 Slowly move the staining rack up and down 10 times to break 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 to break the surface of the reagent.

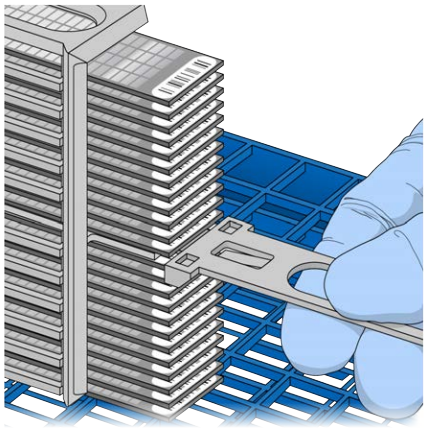
NOTE

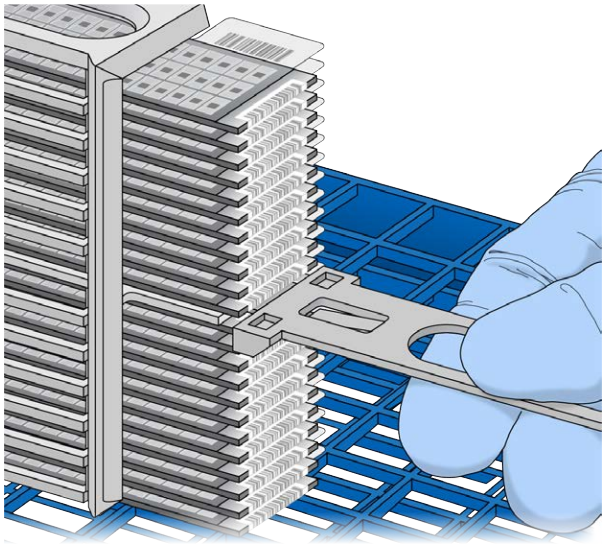
If BeadChip edges begin to touch during washing, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

14 Soak the BeadChips for an additional 5 minutes.

15 Remove the staining rack, and place it on the prepared tube rack with the barcode side of the BeadChips facing up.

Figure 31 Staining Rack in Correct Orientation





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

17 Work from the top to bottom of the staining rack, and perform the following steps for each BeadChip:

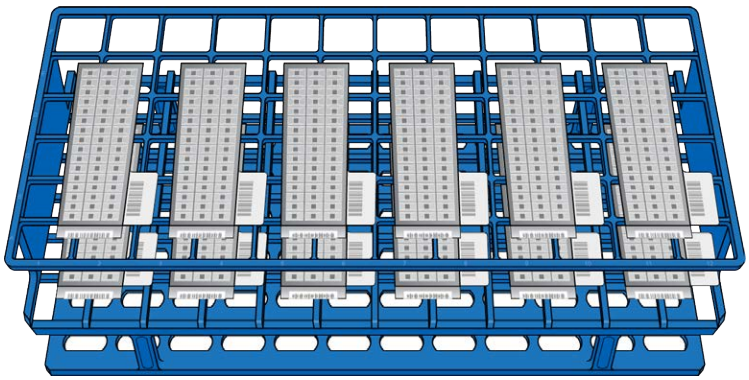
a Use self-locking tweezers to grip the BeadChip at its barcode end.

b Place the BeadChip on the tube rack with the barcode 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 32 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 the etched, barcoded sides are dry.

21 If the back of the BeadChip feels sticky, wipe it with a Prostat wipe or a Kimwipe wetted with 70% EtOH.

22 Image the BeadChips immediately, or store them, protected from light.

23 When you are ready to image the BeadChips, in Illumina LIMS, select **Infinium XT** | **Coat**.

   a Scan the barcode of the reagent bottles.
   b Scan each BeadChip barcode.
   For this step, you can scan the BeadChip barcode on either the BeadChip or the BeadChip package.
   c Select **Verify**, and then select **Save**.

Image BeadChip

Follow the instructions in the System Guide for your instrument to scan your BeadChips.

Use the **Infinium XTXCG** scan setting for your BeadChips.

Chapter 4 Automated Protocol without Illumina LIMS

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Introduction

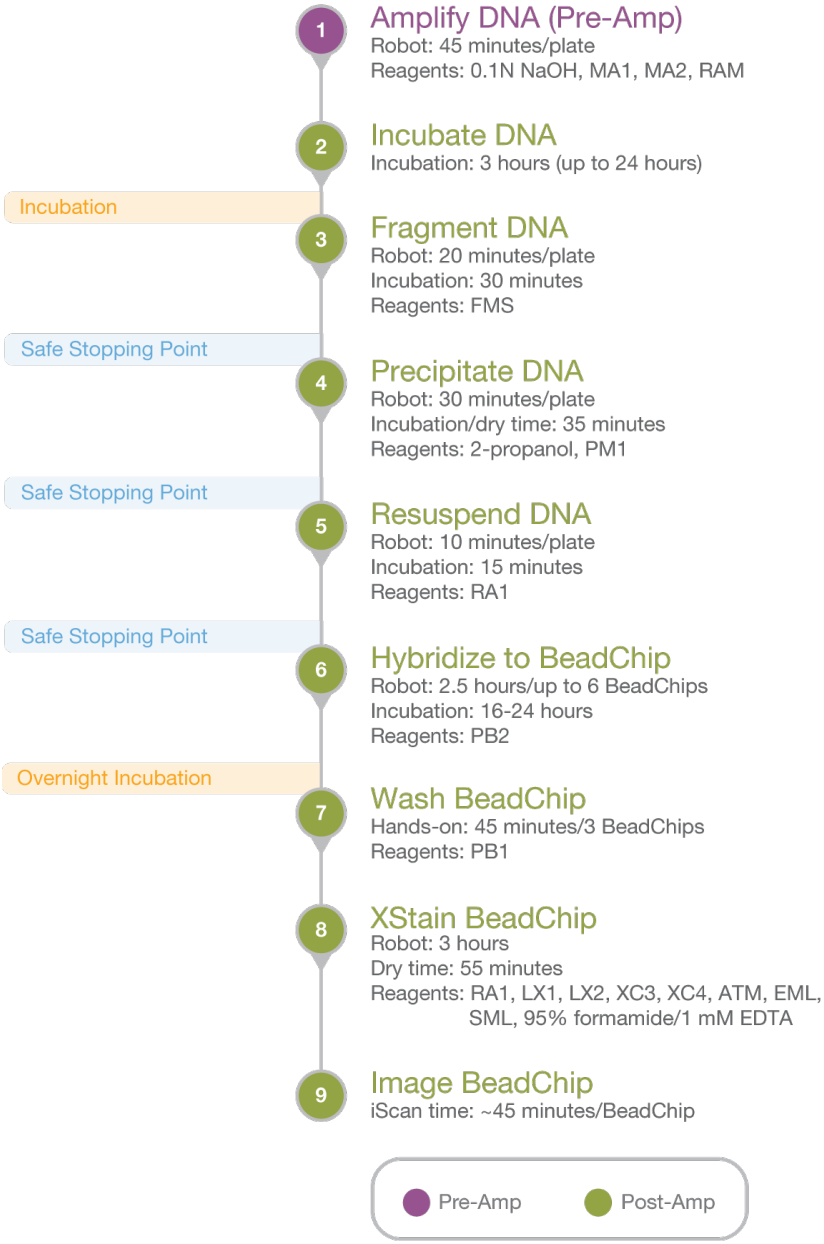
This section describes pre- and post-amplification automated laboratory protocols for the Infinium XT Assay. Follow the protocols in the order shown.

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 29 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 33 Infinium XT Protocol ST Automated Workflow



Amplify DNA (Pre-Amp)

This process adds the DNA samples to the plates, and then it 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)
- 0.1 N NaOH (5 ml/plate)
- 96-well 0.8 ml microplates (midi)
- DNA plates with 96 DNA samples (10 µl at 50 ng/µl) (midi or TCY)
- Cap mats

Preparation

1 Thaw DNA plates 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 plates at 1600 rpm for 1 minute.

7 Centrifuge DNA plates at 280 × g at room temperature for 1 minute.

8 Label four 250 ml reservoirs NaOH, MA1, MA2, and RAM.

Procedure

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

   a Select the DNA plate type (midi or TCY). Do not mix plate types on the robot.

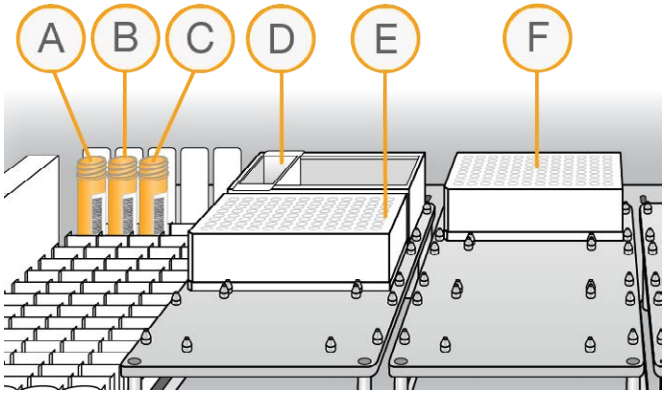
2 Place MA1, MA2, and RAM tubes into the tube rack according to the robot deck map. Remove the caps.

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

4 Place the DNA plates and MSA7 midi plates on the robot deck according to the deck map.

5 At the robot PC, select **Run**.

Figure 34 Robot Setup for Amplify DNA



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

6 When the robot finishes, apply cap mats to the MSA7 plates, and then vortex at 1600 rpm for 1 minute.

7 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 step, 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 RA1 from the freezer, and thaw at room temperature.

Procedure

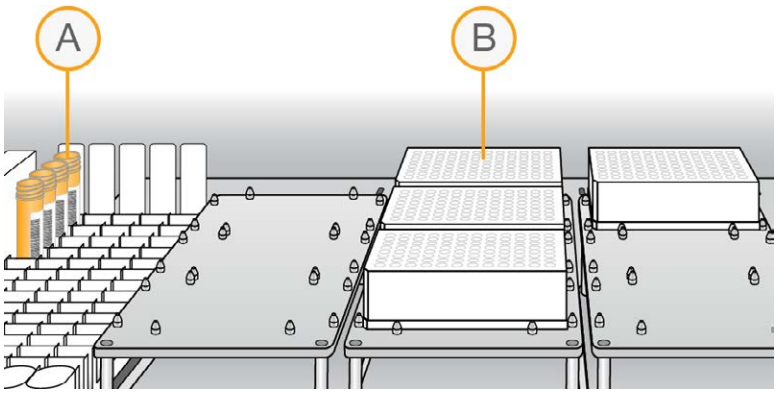
1 Centrifuge the MSA7 plates at 280 × g at room temperature for 1 minute.

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

3 Place the MSA7 plates on the robot deck according to the deck map in Figure 35. Remove the cap mats.

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

Figure 35 Robot Deck Setup for Fragment MSA7



A FMS Tubes
B MSA7 Plates

5 At the robot PC, select **Run**.

a When the robot finishes, select **OK**.

6 Remove the plates from the robot deck, and apply cap mats.

7 Vortex at 1600 rpm for 1 minute.

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

9 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. Do not leave the plates in the 37°C Illumina Hybridization Oven longer than 2 hours.

SAFE STOPPING POINT

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

Precipitate DNA

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

Consumables

- PM1

- 100% 2-propanol (IPA)
- Cap mats

Preparation

1 Prepare the following consumables.

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

2 Cool the refrigerated centrifuge to 4°C.

3 If you froze the MSA7 plates, thaw to room temperature.

4 Centrifuge the MSA7 plates 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 Remove the cap mats, and place the MSA7 plates on the robot deck according to the deck map in Figure 36.

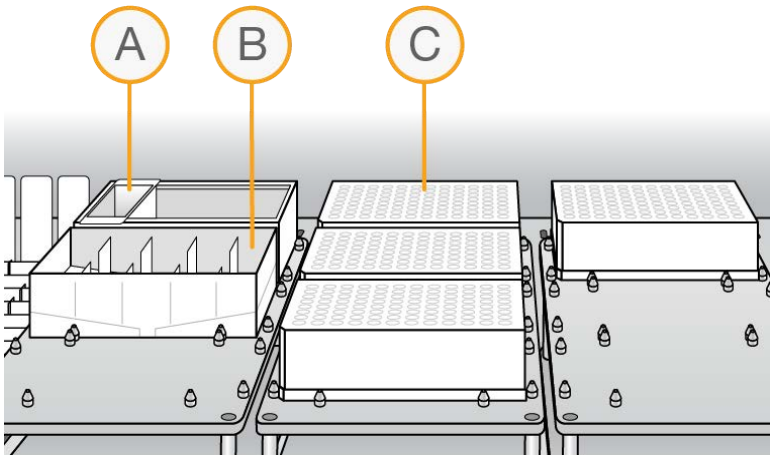
3 Place a quarter reservoir on the robot deck according to the deck map in Figure 36, and add PM1:

Reagent	Number of Plates	Volume
PM1	1	8 ml
	2	14 ml
	3	21 ml
	4	27 ml
	5	34 ml
	6	40 ml

4 Place a full reservoir on the robot deck, according to the deck map Figure 36, and add 2-propanol:

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

Figure 36 Robot Deck Setup for Precipitate MSA7



A PM1 Reservoir
B 2-Propanol Reservoir
C MSA7 Plates

5 At the robot PC, select **Run**.

a When the robot finishes, select **OK**.

6 Remove the plates from the robot deck, and apply fresh cap mats.

7 Invert the plates 10 times to mix.

8 Centrifuge at 3000 × g at 4°C for 20 minutes.

CAUTION
Perform the next steps immediately to avoid dislodging the blue pellets. If any delay occurs, repeat centrifugation before proceeding.

9 Remove the plates from the centrifuge, and remove the cap mats.

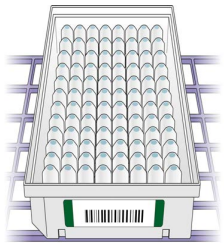
10 Quickly invert the plates, and drain liquid to decant the supernatant. Then, smack the plates down on a dry pad.

11 Tap the plates several times until all wells are devoid of liquid.

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

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

Figure 37 Uncovered MSA7 Plate Inverted for Air Drying



CAUTION

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

SAFE STOPPING POINT

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

Resuspend DNA

This step uses RA1 to resuspend the precipitated DNA.

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.

Consumables

- RA1
- Foil heat seals

Preparation

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.

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

Resuspend the MSA7 Plate

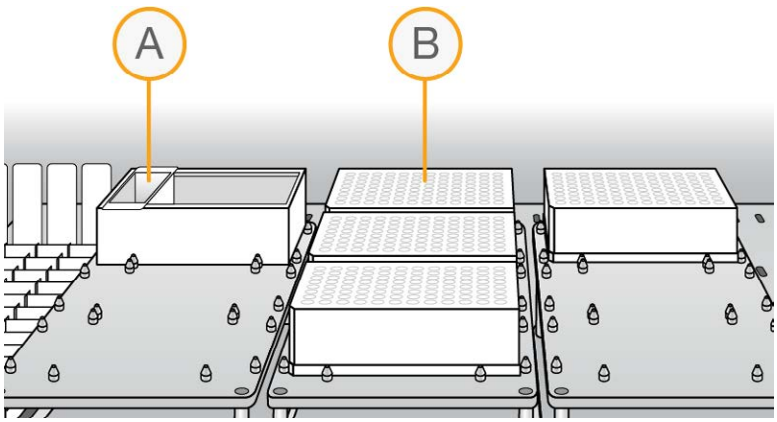
1. At the robot PC, select **MSA7 ST Tasks | Resuspend MSA7 ST**.

2 Place the MSA7 plates on the robot deck according to the deck map in Figure 38.

3 Place a quarter reservoir on the robot deck according to the deck map in Figure 38, and add RA1.

Reagent	Number of Plates	Volume
RA1	1	5 ml
	2	8 ml
	3	11 ml
	4	14 ml
	5	17 ml
	6	20 ml

Figure 38 Robot Deck Setup for Resuspend MSA7



A RA1 Reservoir
B MSA7 Plates

4 At the robot PC, select **Run**.

a When prompted, scan the barcode of the reagent bottle.
b When the robot finishes, select **OK**.

5 Remove the MSA7 plates from the robot deck.

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

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

8 Vortex at 1800 rpm for 1 minute.

9 Check to make sure that the pellets are resuspended. If the pellets are not resuspended, repeat steps 7 and 8.

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

SAFE STOPPING POINT

If you are stopping, store sealed MSA7 plate(s) at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C.

Store sealed RA1 at -25°C to -15°C. If RA1 will be used the next day, seal it, and 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, which enables 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, and then 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.

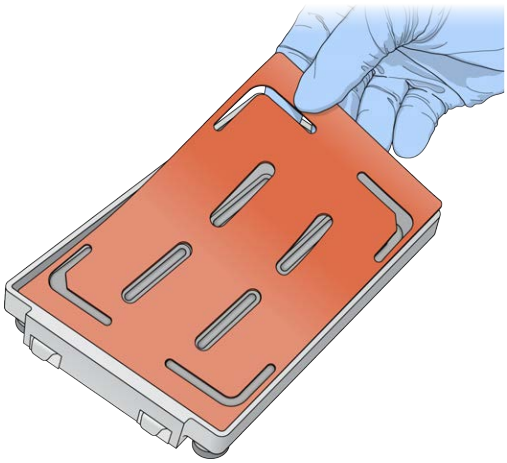
2 Cool the MSA7 plates on the benchtop at room temperature for 30 minutes.

3 Centrifuge at 15001000 × 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 39 XT Hyb Chamber and Gasket



2 Dispense 800 µl PB2 into each of the four humidifying buffer reservoirs in the XT Hyb chambers.

3 Close the XT Hyb chamber immediately to prevent evaporation.

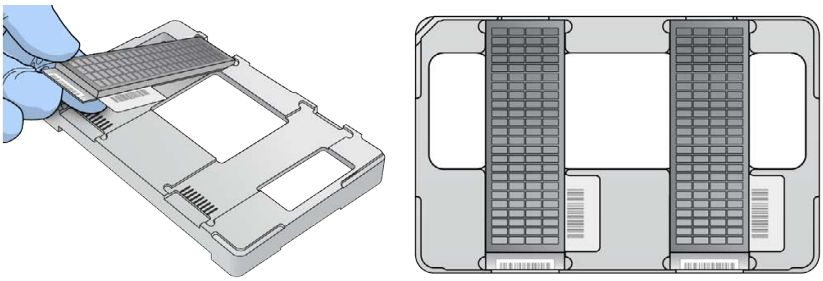
4 Leave the closed XT Hyb chambers on the bench at room temperature until BeadChips are loaded with DNA sample.

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 40 Placing BeadChips on Baseplates



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

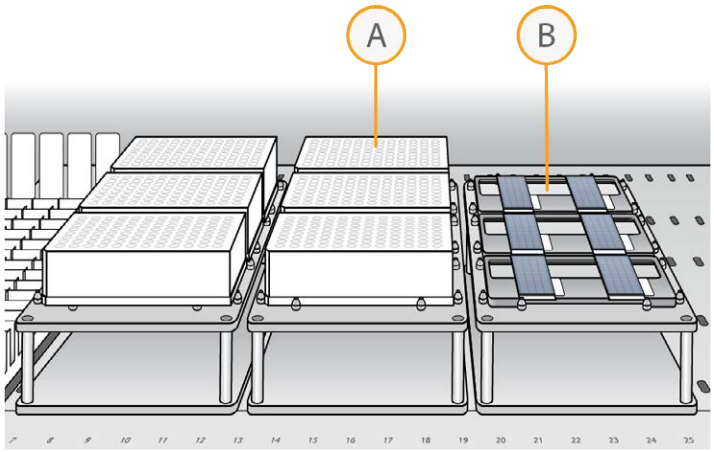
4 In the BeadChip Selection dialog box, select the 96-sample BeadChip.

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 deck according to the deck map.

7 Place the MSA7 plates onto the robot deck according to the deck map, and remove the heat seals.

Figure 41 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 deck 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

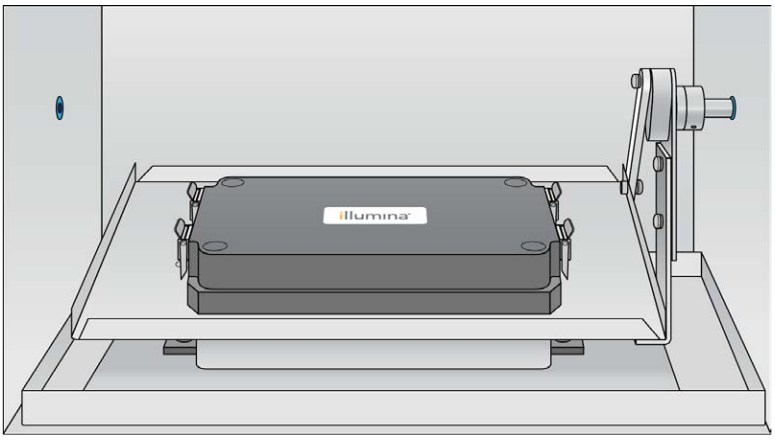
Keep Hyb chambers at room temperature when you load the BeadChips. Do not place the Hyb chambers in the Illumina Hybridization Oven before loading the BeadChips.

2 Load the XT dual Hyb insert and baseplates containing BeadChips inside the XT Hyb chambers.
You can stack up to three 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 three XT Hyb chambers for a maximum of six total in the Illumina Hybridization Oven.

Figure 42 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 is intended 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 EXXT Tip Guides

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

1 Soak the EXXT 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 EXXT tip guides.

2 Thoroughly rinse the EXXT tip guides with DI H_2O at least 3 times to remove any residual detergent.

3 Dry the EXXT 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 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.

Consumables

- PB1

Preparation

1 Dilute the PB1 to make it ready for use.

2 Remove the Hyb chambers from the Illumina Hybridization Oven. Cool for 30 minutes at room temperature before opening.

3 While the Hyb chambers are cooling do the following:

   a Fill two wash dishes with PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
   b Make sure that the XCG integrated spacer 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 200 ml PB1.

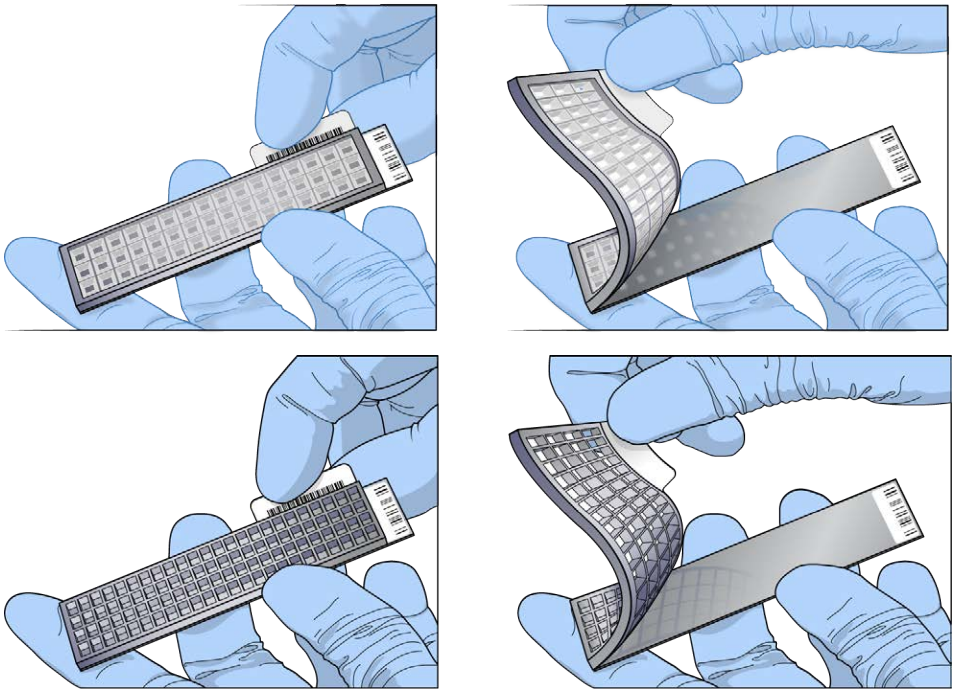
CAUTION

Replace PB1 in Wash 1 after every 12 BeadChips.

2 Remove the hybridization insert and baseplates from the hybridization chambers.

3 Remove the BeadChips from the hybridization 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.



5 Immediately and carefully slide each BeadChip into the wash rack in Wash 1. Make sure that the BeadChip is submerged in PB1.

6 Repeat steps 4–5 until all BeadChips are transferred to the submerged wash rack in Wash 1.

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

8 Move the wash rack to Wash 2 containing clean PB1. Make sure that the BeadChips are submerged.

9 Move the wash rack up and down for 1 minute. Break the surface of the 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 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 PB1.

3 For each BeadChip to be processed, place an XCG Flow-Through Chamber frame into the XT Flow-Through Chamber assembly tray.

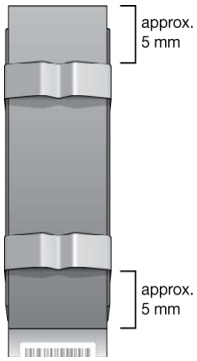
4 Place a BeadChip on a submerged XCG Flow-Through Chamber frame, aligning each BeadChip barcode with the ridges stamped into the frame, ensuring the array surface is facing you.

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 43 Assembled XCG Flow-Through Chamber



Extend and Stain (XStain)

Using the captured DNA as a template, the single-base extension of the oligos on the BeadChip incorporates detectable labels on the BeadChip to determine 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

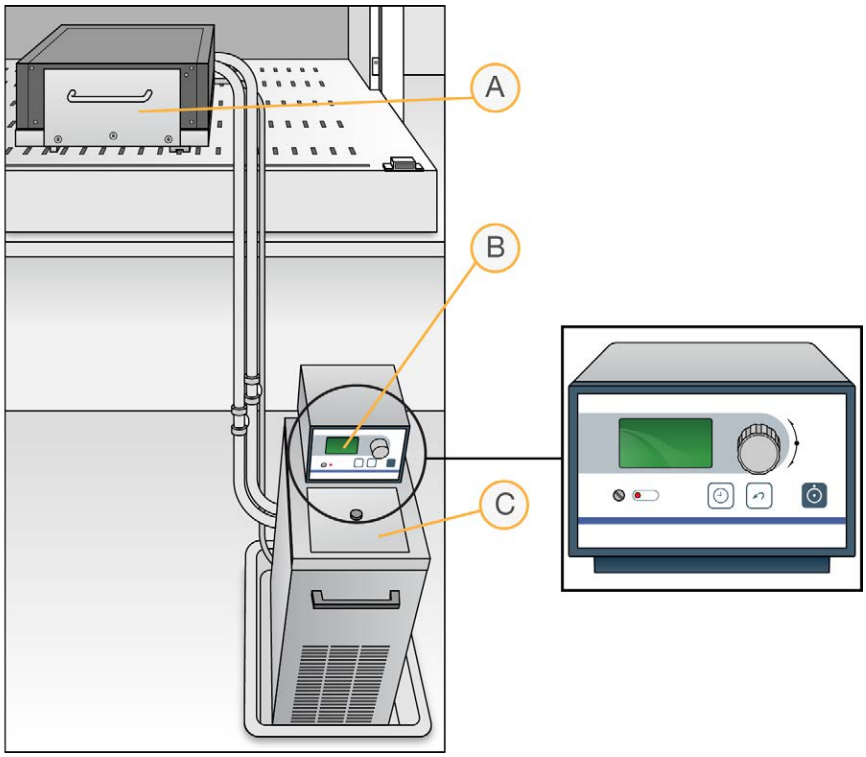
Set Up the Chamber Rack

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:

a In the Action section drop-down list, select **Set Target Temperature.**
b In the field below Set Target Temperature, enter **44.**
c Select the **Execute** button.

Figure 44 Water Circulator Connected to Chamber Rack



A Chamber Rack
B Water Circulator
C Reservoir Cover

3 Remove bubbles trapped in the chamber rack.

a Separate the heat exchanger from the reagent pan.
b Lift the heat exchanger upright and away from you with the tubing at the bottom, and turn 90° counter clockwise.
c Return the heat exchanger to a horizontal position.
d Repeat steps b and c 3 times for a total of 4 rotations or until all bubbles are removed.
e Using Kimwipes dampened with laboratory-grade water, clean all surfaces between the heat exchanger and reagent pan. Discard Kimwipes with formamide waste.
f Place the Te-Flow back on the reagent pan. Using the two guide pins in the reagent pan, make sure that the Te-Flow is flush.

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 deck, according to the deck 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
	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 deck, according to the deck map.

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

6 Select **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 deck map.

CAUTION
Start the robot immediately to prevent BeadChips from drying.

9 At the robot PC, select **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 two top-loading PB1 and XC4 wash dishes, labeled PB1 and XC4.

2 To indicate fill volume, pour 310 ml water into the wash dishes, and mark the water level. Empty the water from the wash dish.

3 Pour 310 ml PB1 into a wash dish labeled PB1.

4 Place a staining rack inside the wash dish.

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 the 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 cleaning later, as detailed in the *Infinium Assay Lab Setup and Procedures Guide*.

8 Slowly move the staining rack up and down 10 times to break 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 to break the surface of the reagent.

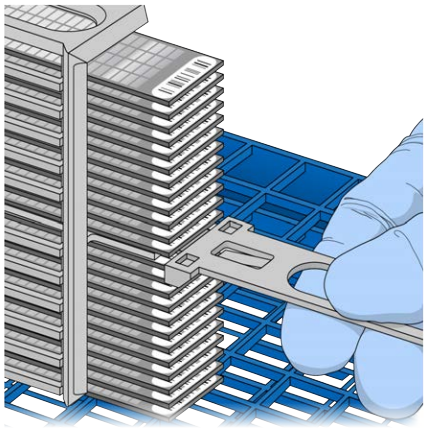
NOTE

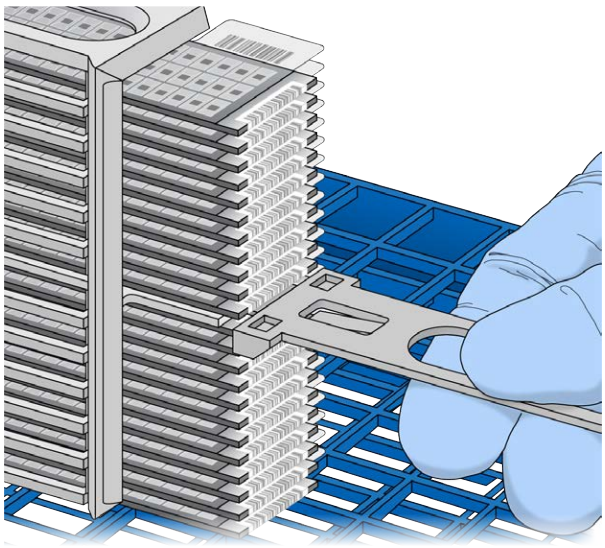
If BeadChip edges begin to touch during washing, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

14 Soak the BeadChips for an additional 5 minutes.

15 Remove the staining rack, and place it on the prepared tube rack with the barcode side of the BeadChips facing up.

Figure 45 Staining Rack in Correct Orientation





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

17 Work from the top to bottom of the staining rack, and perform the following steps for each BeadChip:

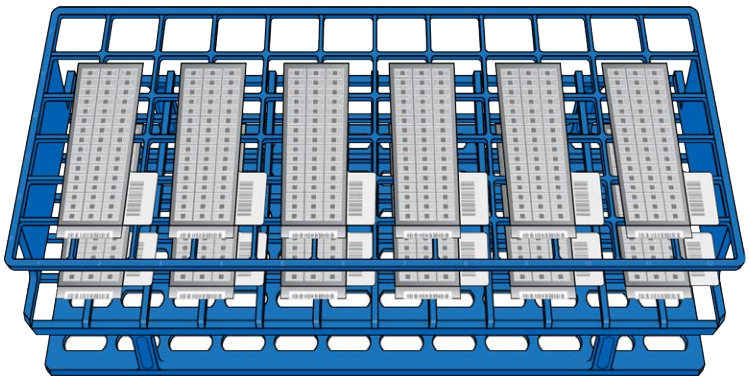
a Use self-locking tweezers to grip the BeadChip at its barcode end.

b Place the BeadChip on the tube rack with the barcode 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 46 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 the etched, barcoded sides are dry.

21 If the back of the BeadChip feels sticky, wipe it with a Prostat wipe or a Kimwipe wetted with 70% EtOH.

22 Image the BeadChips immediately, or store them, protected from light.

23 When you are ready to image the BeadChips, in Illumina LIMS, select **Infinium XT** | **Coat**.

   a Scan the barcode of the reagent bottles.
   b Scan each BeadChip barcode.
   For this step, you can scan the BeadChip barcode on either the BeadChip or the BeadChip package.
   c Select **Verify**, and then select **Save**.

Image BeadChip

Follow the instructions in the System Guide for your instrument to scan your BeadChips.

Use the **Infinium XTXCG** scan setting for your BeadChips.

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	400.066.5835	
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	
South Korea	+82 80 234 5300	
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 from support.illumina.com.

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