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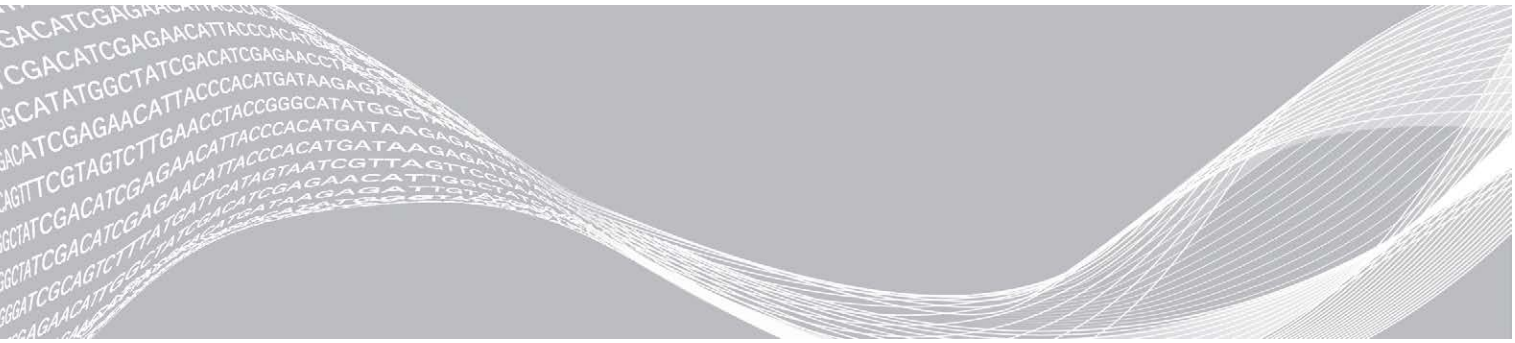
Infinium HTS Extra Assay

Reference Guide



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Revision History

Document	Date	Description of Change
Document # 1000000041072 v02	November 2019	Replaced the word Workflow with Infinium where appropriate.
Document # 1000000041072 v01	August 2019	Added Reagent Storage and Stability section to Prepare and Store Reagents.
Document # 1000000041072 v00	June 2018	Initial release.

Table of Contents

Chapter 1 Overview

Introduction to the Infinium™ HTS Extra Assay

The Infinium™ family of genotyping assays harness proven chemistry and a robust BeadChip platform to produce exceptional data quality, superior call rates, and high reproducibility. The Infinium HTS Extra Assay provides the highest throughput, most streamlined array workflow supporting mid-plexity (up to 700k beadtypes) 24-sample arrays to date from Illumina®. The new automation workflow and reagent configurations enable customers to increase their sample throughput significantly when running Infinium 24-sample HTS products (Infinium Global Screening Array, the Infinium Asia Screening Array, and the Infinium HTS iSelect™ Array, as examples). The Infinium HTS Extra kits are ideally suited for high-throughput screening applications, including healthy human screening and consumer genomic screening applications.

The Infinium HTS Extra solution optimizes the user experience, as labs take production-scale genotyping studies to the next level of throughput. It reduces overall hands-on time and enhances automation robot performance and utilization.

The Infinium HTS Extra solution is a high-throughput genotyping workflow designed to address the needs of laboratories running large scale genotyping studies. It expands the scalability and throughput of the existing Infinium HTS workflow to the next level. By maximizing process batching sizes and reducing the number of user interventions, the Infinium HTS Extra workflow provides increased throughput within the same laboratory infrastructure.

The Infinium HTS Extra Assay offers the following features:

- High-throughput genotyping
- High efficiency, streamlined workflow
- Bulk reagent kit packaging
- Robust, high-quality assay
- 24-sample HTS BeadChip processing

Important Note

Before using the procedures in this guide, read the *Infinium Assay Lab Setup and Procedures Guide (document # 11322460)*. The *Setup and Procedures* guide explains how to equip and run an Infinium HTS Extra 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 maintenance
- Troubleshooting

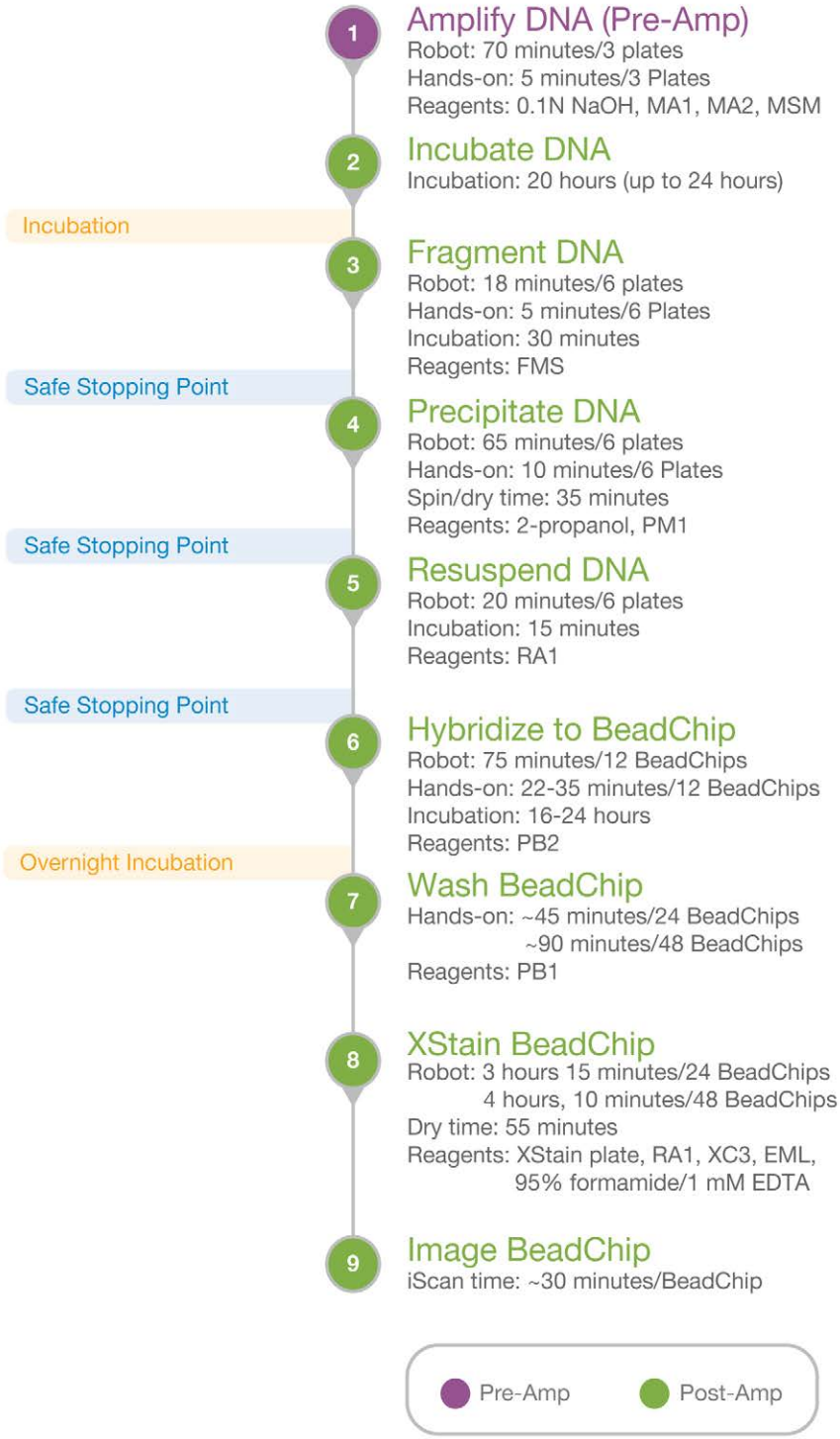
The instructions apply equally to all Infinium BeadChips provided by Illumina™. All Infinium HTS Extra documentation assumes that you have already set up the laboratory space and that you are familiar with the standard procedures and safety precautions.

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.

Infinium HTS Extra Workflow

Figure 1 Illumina Infinium HTS Extra Workflow



Tips and Techniques

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

Avoid Cross-Contamination

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

Preparing the Robot for First Use of the Day

Before using the robot each day, follow the *Tecan First-Use-of-the-Day Procedure* in the *Infinium Assay Lab Setup and Procedures Guide (Document # 11322460)*.

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.

Laboratory Cleaning Procedures

Wash Infinium hardware after use with 1% Alconox solution.

LCG Glass Back Plates

- For optimal performance, use LCG glass back plates that are free of chips and cracks along the beveled edge and the long edges. Chipped or cracked LCG glass back plates risk volume leakage and affect overall performance.
- Clean LCG glass back plates according to the procedures in the *Infinium Assay Lab Setup and Procedures Guide (Document # 11322460)*.
- A 10% bleach soak is required weekly, or every seven uses, to maintain glass performance.

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 HTS Extra robot tip alignment guide 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.

Rocker Platform [Optional]

If you are using an Illumina Hybridization Oven with a rocker platform, follow these guidelines:

- If 7 to12 hybridization chambers (Hyb Chambers) are placed in 1 hybridization oven, the rocker must be removed before beginning the *Hybridize to BeadChip* section.
- If 1 to 6 Hyb Chambers are placed in 1 hybridization oven, removing the rocker platform before beginning the *Hybridize to BeadChip* section is optional.
- Removing the rocker platform is optional for plate incubation in the *Amplify DNA, Incubate DNA, Fragment DNA, Precipitate DNA,* and *Resuspend DNA* sections.

Prepare and Store Reagents

Conserve Reagents

- Infinium HTS Extra kits contain reagents in exact quantities needed for the assay. Measure reagents carefully to avoid shortages.
- The Infinium HTS Extra Assay Reference Guide is written using the maximum number of MSA8 plates and BeadChips so that you can process the maximum number of samples (2,304 samples/24 plates). Using less than the specified number of plates may cause reagent shortages.
- This protocol and the supporting kits are designed for 24 or 48 BeadChips per XStain batch. If you process fewer than 24 BeadChips or between 25–47 BeadChips, surplus reagents cannot be reused.
- Use fresh reagents for each batch of plates, and empty reservoirs between batches.

Reagent Storage and Stability

Store the following bottled reagents at -25°C to -15°C until ready for use. These reagents are stable at 4°C for up to 6 days after being thawed.

- FMS
- MA2
- MSM
- RA1

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

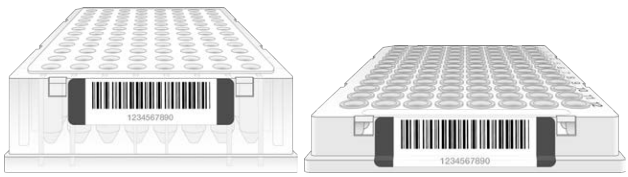
- Rinse the carboy with 10–20 L DI H_2O 3 times.
- Run 5 L DI H_2O through the spigot to flush it.

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 2 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 HTS Extra 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 HTS Extra Workflow Checklist with Illumina LIMS (document # 1000000048260)	Provides a checklist of steps for users who are experienced using the Infinium HTS Extra workflow, with IlluminaLIMS.
Infinium HTS Extra Workflow Checklist without Illumina LIMS (document # 1000000041073)	Provides a checklist of steps for users who are experienced at using the Infinium HTS Extra workflow, without IlluminaLIMS.
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 Assay Consumables and Equipment List (document # 1000000084294)	Provides an interactive checklist of Illumina-provided and user-provided consumables and equipment.

Chapter 2 Automated Protocol with Illumina LIMS

Introduction

This section describes pre- and post-amplification automated laboratory protocols for the Infinium HTS Extra 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 36 for protocol instructions. For information about how to use LIMS, see the *LIMS User Guide*.

Amplify DNA (Pre-Amp)

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

Consumables

- MA1
- MA2
- MSM
- 0.1 N NaOH
- 96-well 0.8 ml microplates (midi)
- DNA plates (TCY or midi) with DNA samples at 50 ng/µl.
  - Minimum of 10 µl for TCY plates.
  - Minimum of 20 µl for midi plates.
- Cap mats

Preparation

1 Thaw DNA plates to room temperature.

2 Prepare the following consumables.

Item	Storage	Instructions
MA2	-25°C to -15°C	Thaw at 4°C for 12 to 24 hours. Bring to room temperature before use. Invert to mix.
MSM	-25°C to -15°C	Thaw at 4°C for 12 to 24 hours. Bring to room temperature before use. Invert to mix.

- If necessary, use a room temperature water bath to complete thawing and bring the reagents to room temperature.

3 Preheat the Illumina hybridization oven in the post-amp area to 37°C, and allow the temperature to equilibrate.

4 Apply MSA8 barcode labels to new midi plates.

5 Vortex DNA plates at 1600 rpm for 1 minute.

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

7 Label four quarter reservoirs MA1, NaOH, MA2, and MSM.

Procedure

1 At the robot PC, select **MSA8 Tasks | Make MSA8**.

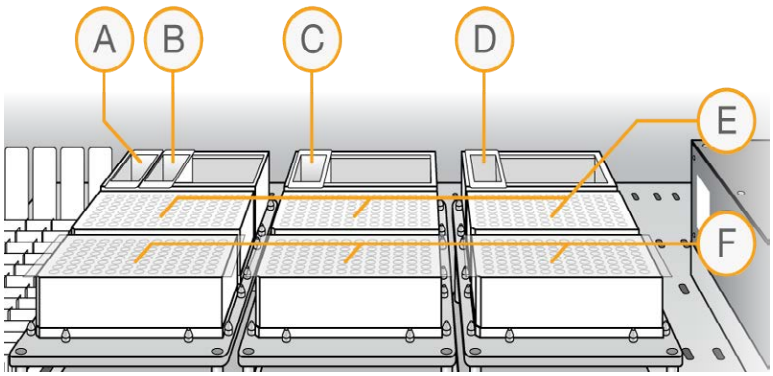
a Select the DNA plate type (midi or TCY). Do not mix plate types on the robot.

2 For every three MSA8 plates, perform the following steps:

a Place the MA1, NaOH , MA2, and MSM reservoirs on the robot deck according to the deck map in Figure 3.

b Use a serological pipette to add the reagents listed in Figure 3 to the reservoirs.

Figure 3 Robot Deck Setup for Amplify DNA



Legend	Consumable	Reagent	Volume
A	MA1 Reservoir	MA1	9 ml
B	0.1 N NaOH Reservoir	0.1 N NaOH	5 ml
C	MA2 Reservoir	MA2	13.5 ml
D	MSM Reservoir	MSM	15 ml
E	MSA8 Plates	N/A	N/A
F	DNA Plates	N/A	N/A

3 Place the DNA plates and MSA8 plates on the robot deck according to the deck map in Figure 3, and then remove the cap mats.

4 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.

5 When the robot finishes, apply cap mats to the MSA8 plates.

6 Vortex the MSA8 plates at 1600 rpm for 1 minute.

7 Centrifuge the MSA8 plates 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 HTS Extra Assay.

NOTE

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

1 In Illumina LIMS, select **Infinium HTS Extra | Incubate MSA8**.

   a Scan the barcode of each MSA8 plate, select **Verify**, and then select **Save**.

2 Incubate the MSA8 plates in the hybridization oven for 20–24 hours at 37°C.

Fragment DNA

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

Consumables

- FMS

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
FMS	-25°C to -15°C	Thaw at 4°C for 12 to 24 hours. Bring to room temperature before use. Invert to mix.

2 Preheat the hybridization oven to 37°C.

3 If you plan to resuspend the MSA8 plates today, remove RA1 from the freezer, and thaw at room temperature.

Procedure

1 Centrifuge the MSA8 plates at 280 × g at room temperature for 1 minute.

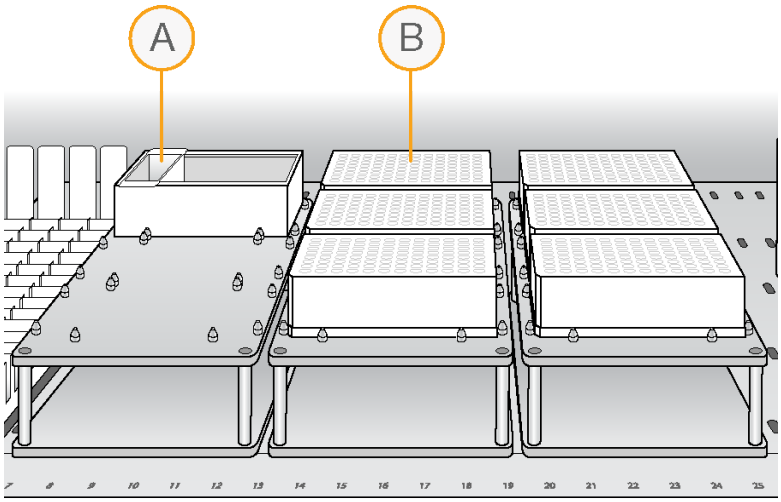
2 At the robot PC, select **MSA8 Tasks | Fragment MSA8**.

3 Place six MSA8 plates on the robot deck according to the deck map in Figure 4, and then remove the cap mats.

4 Place a quarter reservoir on the robot deck according to the deck map in Figure 4.

   a Add 20 ml FMS to the quarter reservoir.

Figure 4 Robot Deck Setup for Fragment MSA8

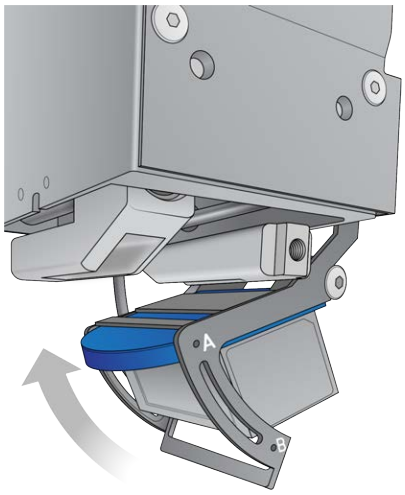


A FMS
B MSA8 Plates

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

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

Figure 5 Move Tecan Scanner Bracket to Position A



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 hybridization oven for 30 minutes.
If you are continuing, you can leave the plates in the 37°C hybridization oven until you have completed preparation for the next step. Do not leave the plates in the 37°C 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 MSA8 plates, thaw to room temperature.

4 Centrifuge the MSA8 plates at 280 × g for 1 minute.

5 Label one quarter reservoir as PM1.

6 Label one full reservoir as 2-propanol.

Precipitate the MSA8 Plate

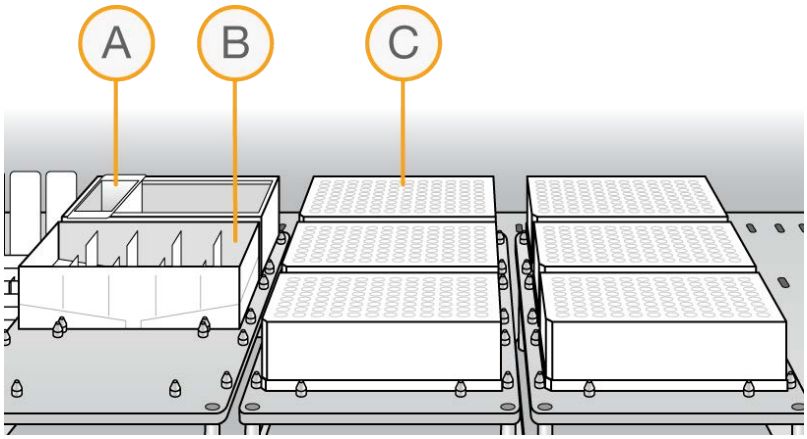
1 At the robot PC, select **MSA8 Tasks | Precip MSA8**.

2 Place six MSA8 plates on the robot deck according to the deck map in Figure 6, and then remove the cap mats.

3 Place the PM1 and 2-propanol reservoirs on the robot deck according to the deck map in Figure 6.

4 Add the reagents listed in Figure 6 to the reservoirs.

Figure 6 Robot Deck Setup for Precipitate MSA8



Legend	Consumable	Reagent	Volume
A	PM1 Reservoir	PM1	40 ml
B	2-propanol Reservoir	2-propanol	150 ml
C	MSA8 Plates	N/A	N/A

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 HTS Extra | Spin MSA8**.

   a Scan the barcode of each MSA8 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.

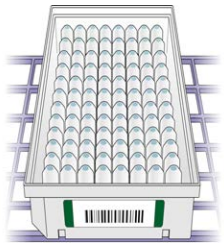
12 Tap the plates several times until all wells are devoid of liquid.

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 7 Uncovered MSA8 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 MSA8 plates at -25°C to -15°C, thaw to room temperature, centrifuge, and then remove the cap mats.
2. Preheat the hybridization oven to 48°C.
3. Preheat the heat sealer for 20 minutes before use.

Resuspend the MSA8 Plate

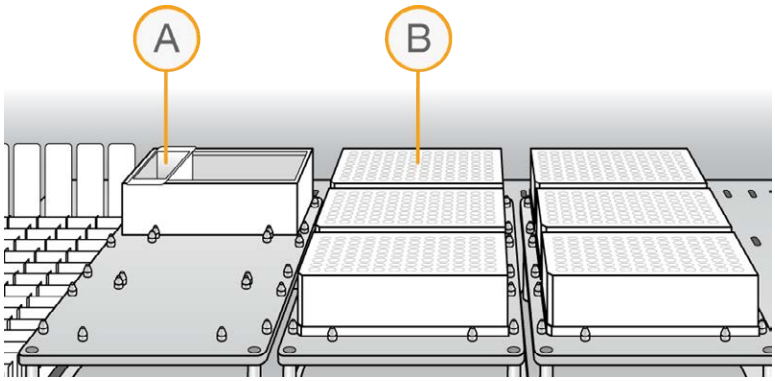
1. At the robot PC, select **MSA8 Tasks | Resuspend MSA8**.

2 Place six MSA8 plates on the robot deck according to the deck map in Figure 8, and then remove the cap mats.

3 Place a quarter reservoir on the robot deck according to the deck map in Figure 8.

   a Add 20 ml RA1 to the quarter reservoir.

Figure 8 Robot Deck Setup for Resuspend MSA8



A RA1 Reservoir
B MSA8 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 MSA8 plates from the robot deck.

6 Apply foil heat seals to the MSA8 plates using the heat sealer.

7 Incubate in the 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 for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA8 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 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 MSA8 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.

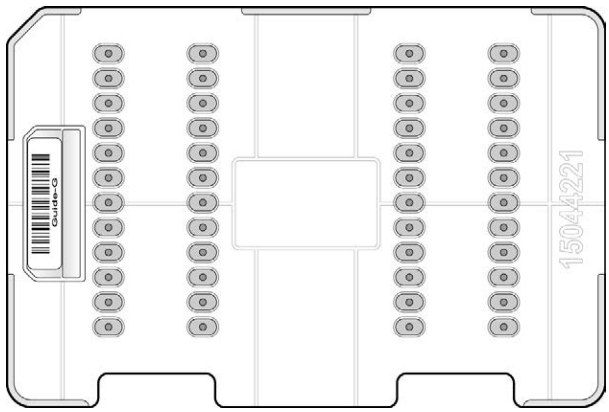
4 Preheat the hybridization oven to 48°C.

Procedure

Prepare Robot Tip Alignment Guide

1 Make sure that you have the correct robot tip alignment guide for the assay you are running. The barcode says **Guide-G**.

Figure 9 Guide-G Robot Tip Alignment Guide



2 Wash and dry the robot tip alignment guide. See *Wash Robot Tip Alignment Guide* at the end of the *Hybridize to BeadChip* steps for washing instructions.

Denature DNA

1 Place the MSA8 plates on the heat block at 95°C for 20 minutes to denature samples.

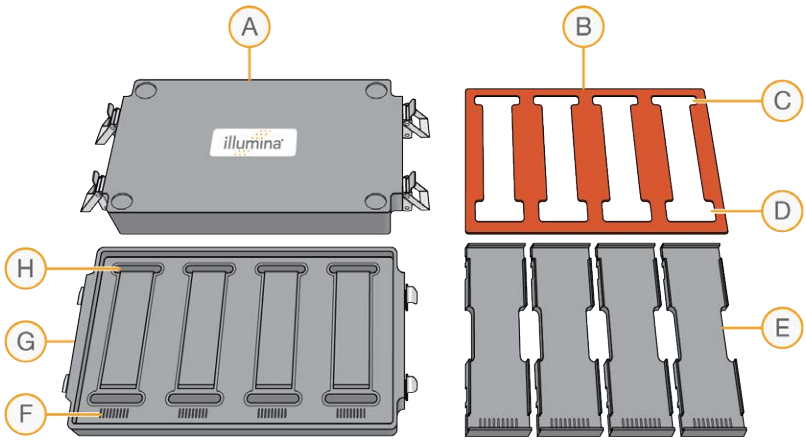
2 Cool the MSA8 plates on the benchtop at room temperature for 30 minutes.

3 Centrifuge at 1500 × g at room temperature for 1 minute.

Assemble Hybridization Chambers

Assemble one chamber for every four BeadChips by following the steps in this section. See Figure 10 for an illustration of the Hyb Chamber components.

Figure 10 BeadChip Hyb Chamber Components



A Top of BeadChip Hyb Chamber
B Hyb Chamber Gasket
C Narrower Edges
D Wider Edges
E Hyb Chamber Inserts
F Barcode Ridges
G Bottom of BeadChip Hyb Chamber
H Humidifying Buffer Reservoirs

1 Place the gasket into the Hyb Chamber according to Figure 11.
- Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber.
- Press down on the edges of the gasket to make sure it is properly seated.

Figure 11 Placing Gasket Into Hyb Chamber

2 Dispense 400 µl PB2 into each of the eight humidifying buffer reservoirs in the Hyb Chamber.

3 Place the Hyb Chamber insert into the Hyb Chamber. Position the barcode ridges on the Hyb Chamber insert over the barcode ridges on the Hyb Chamber.

4 Close the Hyb Chamber lid and clamps immediately to prevent evaporation.

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

6 In Illumina LIMS, select **Infinium HTS Extra | Confirm for Hyb**.

   a Scan the barcode of each MSA8 plate you plan to hybridize.
   b Scan the BeadChip barcode on the package of each BeadChip you plan to hybridize.
   c Select **Verify**.

Prepare the Robot

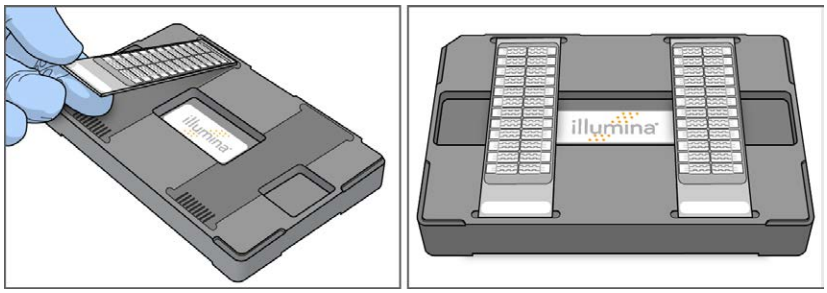
1 Remove all BeadChips from packaging.

CAUTION

Save the BeadChip packaging. You will need to scan the packaging barcodes throughout the HTS Extra Assay.

2 Place BeadChips into the robot BeadChip alignment fixtures. Align the barcode end with the ridges stamped into the robot BeadChip alignment fixture.

Figure 12 Placing BeadChips in the Robot BeadChip Alignment Fixture



3 Stack the robot BeadChip alignment fixtures (2–6 per robot), and carry them to the robot.

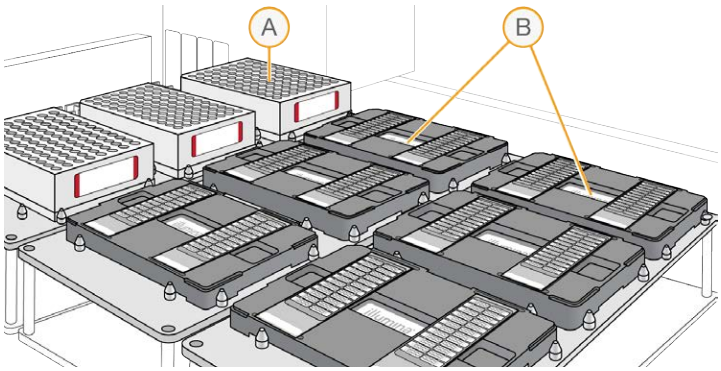
4 At the robot PC, select **MSA8 Tasks | Hyb**.

   a In the BeadChip Selection dialog box, select the 24-sample BeadChip.
   b In the Basic Run Parameters pane, enter the value for the **Number of MSA8 plates** (1–3 plates per robot).

5 Place the robot BeadChip alignment fixtures onto the robot deck according to the deck map in Figure 13.

6 Place the MSA8 plates onto the robot deck according to the deck map in Figure 13, and remove the heat seal.

Figure 13 Robot Deck Setup for Hybridization*



*The MSA8 plate and robot BeadChip alignment fixtures shown represent maximum throughput options.

A MSA8 Plate
B Robot BeadChip Alignment Fixtures

CAUTION

BeadChips must be transferred to Hyb Chambers immediately at the end of the next procedure *(Start the Robot)*. Do not begin *Start the Robot* if you cannot immediately transfer the BeadChips.

Start the Robot

1 At the robot PC, select **Run**.
   - The robot scans the barcode on the BeadChips to confirm the correct BeadChips are loaded.

2 Place each robot tip alignment guide on top of each robot BeadChip alignment fixture.

3 At the robot PC, select **OK**.
   - The robot scans the barcode on the robot tip alignment guide to confirm that the correct tip guide is being used.
   - The robot dispenses DNA sample to the BeadChips.
   - When the process is complete, the robot PC sounds an alert and opens a message box.

4 When the robot finishes, at the robot PC, select **OK** in the message box.

5 Remove the robot BeadChip alignment fixtures from the robot deck.

CAUTION

BeadChips must be removed from the robot deck immediately to prevent excess sample evaporation. Proceed to the next section *(Set Up and Incubate BeadChips)* immediately.

Set Up and Incubate BeadChips

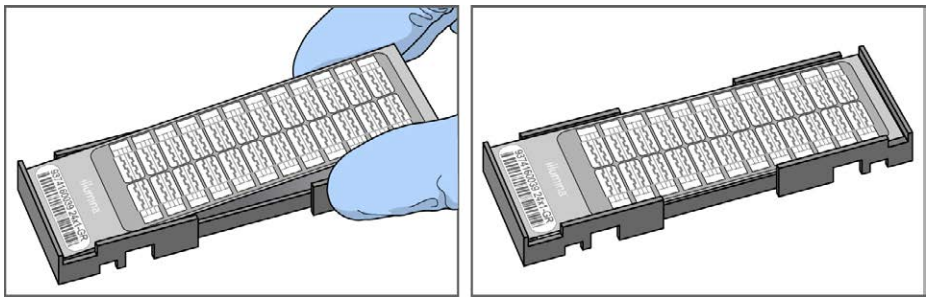
1 Make sure that the hybridization oven is set to 48°C.

WARNING

Keep Hyb Chambers at room temperature when you load the BeadChips. Do not place the Hyb Chambers in the hybridization oven when loading the BeadChips.

2 Open each Hyb Chamber, and then carefully place each BeadChip in a Hyb Chamber insert. Orient the barcode end so that it matches the barcode symbol on the insert.

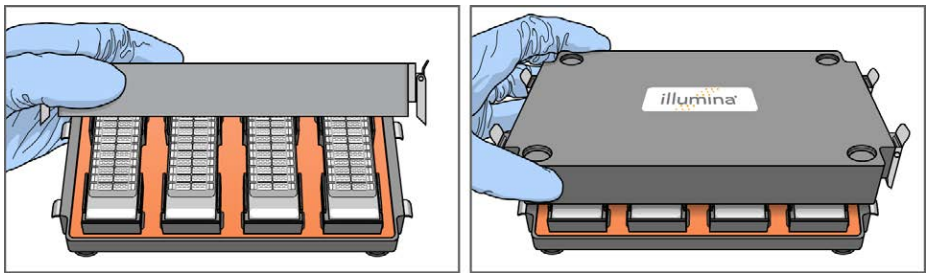
Figure 14 Matching the Barcode End to the Insert Fixture



3 Make sure that Hyb Chamber inserts are seated properly in the Hyb Chambers.

4 Apply the back side of the Hyb Chamber lid first, and then slowly bring down the front side to avoid dislodging the Hyb Chamber inserts.

Figure 15 Seating Lid onto Hyb Chamber



5 Close the Hyb Chamber clamps in this order: top-left, bottom-right, top-right, and then bottom-left. Make sure that the lid is secure and even on the base (no gaps).

CAUTION

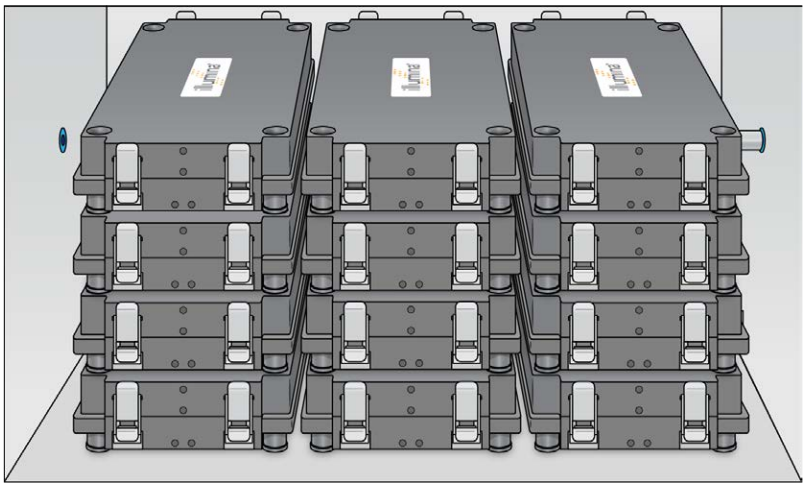
Keep the Hyb Chamber steady and level when moving it or transferring it to the hybridization oven.

6 In Illumina LIMS, select **Infinium HTS Extra** | **Prepare Hyb Chamber**.

a Scan the barcode of the PB2 bottles.
b Scan the BeadChip barcode on each BeadChip package.
c Select **Verify**, and then select **Save**.

7 Place the Hyb Chamber in the 48°C hybridization oven with the clamps of the Hyb Chamber facing the front and back of the oven.
If you are stacking multiple Hyb Chambers in the hybridization oven, fit the feet of each Hyb Chamber into the matching indents on the lid of the Hyb Chamber below it. You can stack up to four Hyb Chambers in three rows, for a maximum of 12 total Hyb Chambers in the hybridization oven.

Figure 16 HTS Extra Hyb Chambers Correctly Placed in hybridization oven



OVERNIGHT INCUBATION

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

TIP

While you wait for the Hyb Chamber to incubate overnight, you can also thaw the XStain plates by following the preparation steps in *Extend and Stain (XStain)*.

Wash Robot Tip Alignment Guide

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

1. Soak the robot tip alignment guides in 1% aqueous Alconox solution (1 part Alconox to 99 parts water) for 5 minutes. Do not use bleach or ethanol to clean HTS tip guide inserts.
2. Thoroughly rinse the robot tip alignment guides with DI H_2O at least 3 times to remove any residual detergent.
3. Dry the robot tip alignment guides. Make sure that the robot tip alignment guides are free of any residual contaminates before next use.

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.

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

- 1XPB1

Preparation

1 Make sure that you have diluted PB1.

2 Remove the Hyb Chambers from the hybridization oven. Cool for 30 minutes at room temperature before opening. While the Hyb Chambers are cooling, proceed to step 3.

3 While the Hyb Chambers are cooling:

   a Fill two wash dishes with PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
   b Fill a graduated cylinder with 150 ml PB1.
   c Make sure that the LCG glass back plates have no chips or cracks.
   d Clean the LCG glass back plates if necessary by following the cleaning procedure described in the *Infinium Assay Lab Setup and Procedures Guide (document # 11322460).*

4 Make sure that the multi-sample BeadChip alignment fixture, black frames, LCG spacers, and clamps are ready for use.

Procedure

Wash BeadChips

1 Attach the wire handle, and submerge the wash rack in Wash 1 (containing PB1).

CAUTION

Replace PB1 in Wash 1 after every eight BeadChips.

Figure 17 Wash Rack in Wash Dish

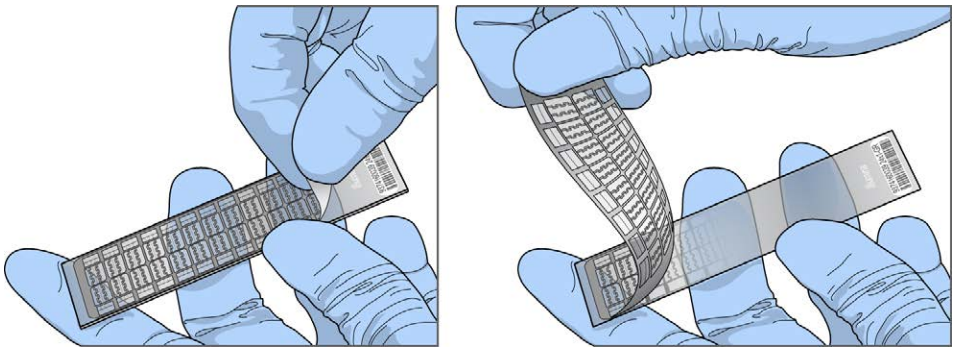
2 Remove the Hyb Chamber inserts from the Hyb Chambers.

3 Inspect the BeadChips. Note any sections that are not covered with DNA sample.

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

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

Figure 18 Removing the Cover Seal



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

7 Repeat these steps until all BeadChips (a maximum of eight) are transferred to the submerged wash rack in Wash 1.

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

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

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

11 If you are processing more than eight BeadChips, follow this procedure:

a Assemble the flow-through chambers for the first eight BeadChips, as described in the *Assemble*

Flow-Through Chambers section, and place them on the lab bench in a horizontal position.

CAUTION

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

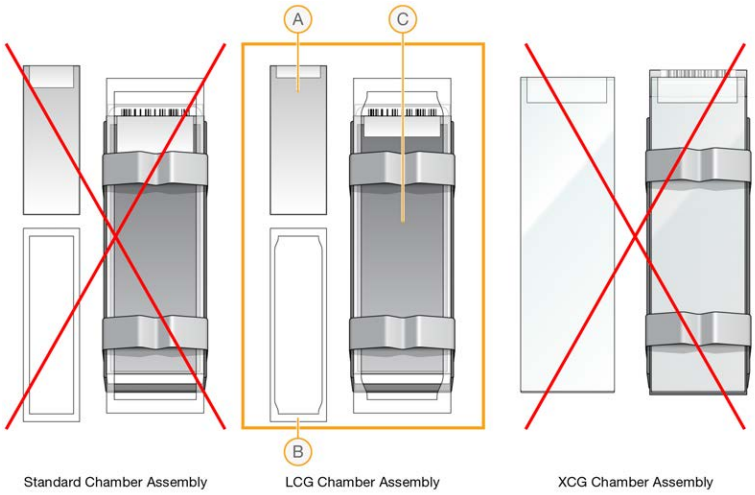
b Return to *Wash BeadChips* on page 23, and follow the steps to wash the next set of eight BeadChips.

c Repeat step 11 in *Wash BeadChips* for each remaining set of eight BeadChips.

Assemble Flow-Through Chambers

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

Figure 19 Correct LCG Back Plates and Spacers



A LCG Glass Back Plate
B LCG Spacer
C Assembled LCG Flow-Through Chamber

CAUTION

This protocol is not compatible with XCG-integrated spacer glass used for Infinium XT BeadChips.

2 Orient the stamped barcode ridges in the multi-sample BeadChip alignment fixture tray towards you.

3 Fill the multi-sample BeadChip alignment fixture with 150 ml PB1.

CAUTION

Replace the PB1 in the multi-sample BeadChip alignment fixture after every eight BeadChips.

4 For each BeadChip to be processed, place a black frame into the multi-sample BeadChip alignment fixture.

5 Place each BeadChip to be processed into a black frame. Align the BeadChip barcode with the ridges that are stamped into the multi-sample BeadChip alignment fixture. Make sure the barcode is facing you.

6 Inspect the surface of the BeadChip for residue left by the cover seal. Use a pipette tip to remove excess residue that was not removed by the PB1. Do not scratch the bead area.

CAUTION

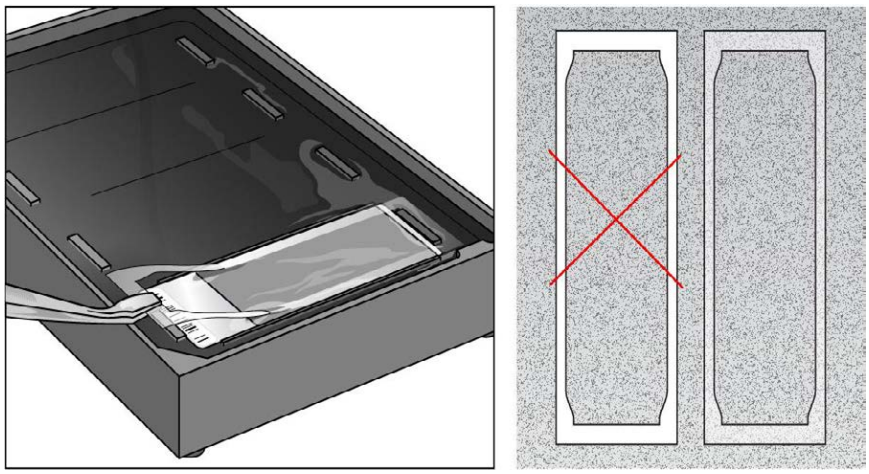
Make sure that each BeadChip remains submerged in PB1 throughout the flow-through chamber assembly process to prevent drying of the active area.

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

CAUTION

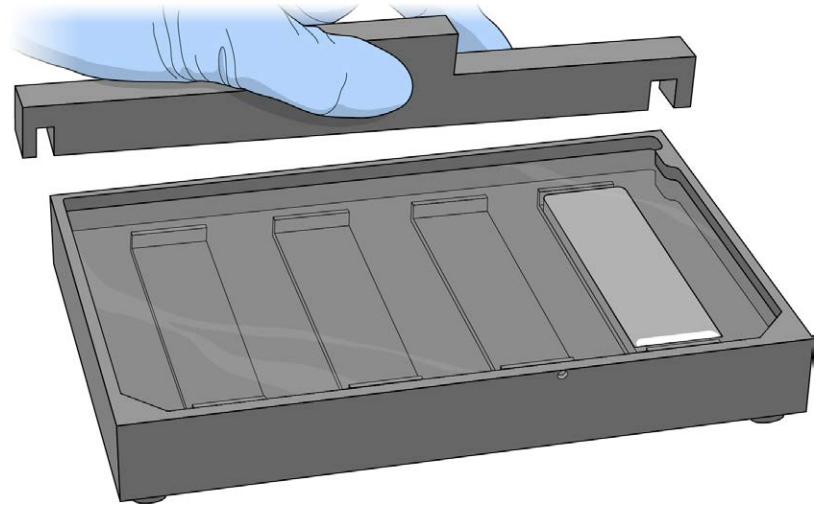
Make sure to use the clear plastic spacers, not the white spacers.

Figure 20 Placing Clear Plastic Spacer Onto BeadChip



8 Place the alignment bar onto the multi-sample BeadChip alignment fixture. The groove in the alignment bar fits over the tab on the alignment fixture.

Figure 21 Placing Alignment Bar Onto Multi-Sample BeadChip Alignment Fixture



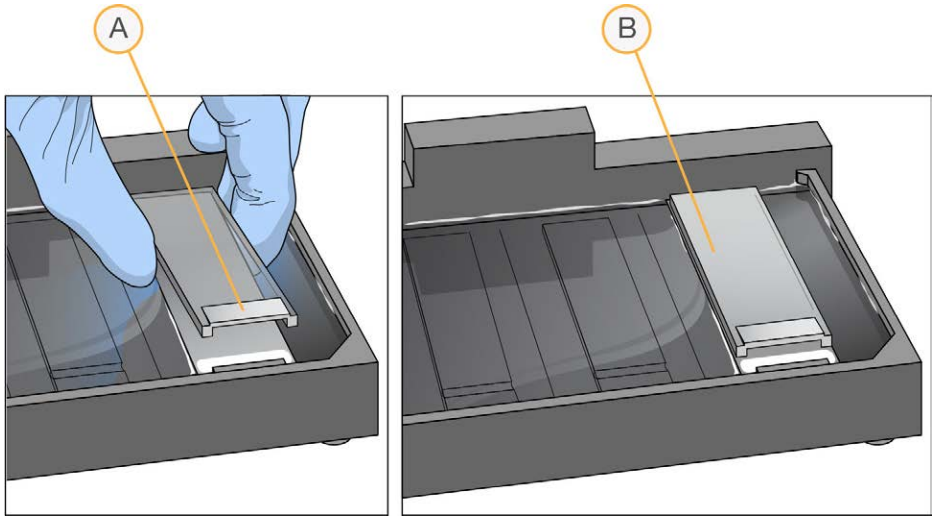
9 Place a clean LCG glass back plate on top of the clear spacer that is covering each BeadChip. Make sure the beveled edge of the LCG glass back plate is facing the barcode.

The beveled edge creates a reservoir against the BeadChip surface.

CAUTION

Make sure to keep BeadChips submerged to prevent drying.

Figure 22 Placing Glass Back Plate Onto BeadChip



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

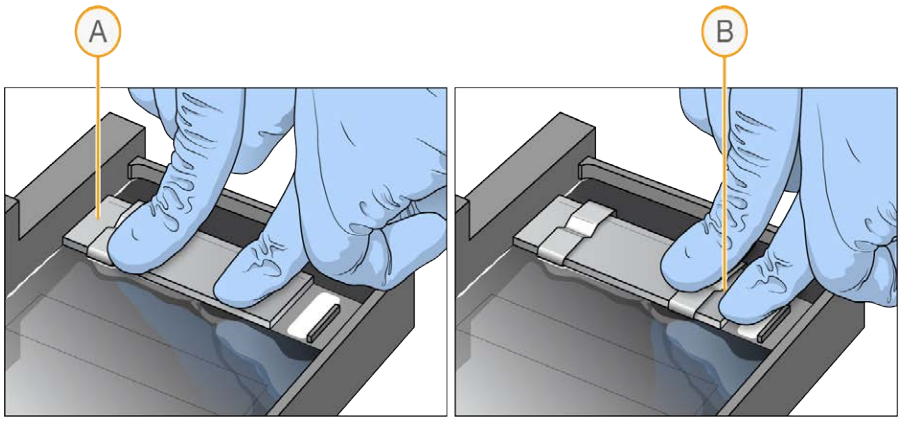
10 Attach the metal clamps to the flow-through chambers as follows:

a Gently push the glass back plate against the alignment bar with one finger.
b Place the first metal clamp around the flow-through chamber, approximately 5 mm from the edge closest to the alignment bar.
c Place the second metal clamp around the barcode end of the flow-through chamber, approximately 5 mm from the reagent reservoir.

CAUTION

Make sure spacers do not become misaligned or dislodged.

Figure 23 Securing Flow-Through Chamber With Metal Clamps



A Positioning First Clamp With Glass Back Plate Pressed Against Alignment Bar
B Positioning Second Clamp

11 Remove the flow-through chamber, and use scissors to trim the excess ends of the clear plastic spacers.

CAUTION
Do not trim the BeadChip.

12 Return the flow-through chamber to the multi-sample BeadChip alignment fixture.

13 Discard unused reagents in accordance with facility standards.

14 In Illumina LIMS, select **Infinium HTS Extra** | **Wash**.

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**.

CAUTION
Place all assembled flow-through chambers on the lab bench in a horizontal position while you perform the preparation steps for *Extend and Stain (XStain).* Do not place the flow-through chambers in the chamber rack until the *Extend and Stain (XStain)* preparation steps are complete.

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
- EML
- XC3
- PB1
- XC4
- XStain plates (1 XStain plate per 24 Beadchips.)

- Alconox powder detergent
- 95% formamide/1 mM EDTA

WARNING

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

Preparation

1. Thaw XStain plates for 24 hours at 4°C.
2. Bring XStain plates to room temperature for 1 hour before use.
   - The contents of the XStain plates are fully thawed when the bottom of the plate is no longer cool to the touch.
3. Invert the XStain plates 10 times to mix the reagents.
4. Centrifuge the XStain plates at 280 × g at room temperature for 1 minute.
5. Carefully remove the seal to avoid cross-contamination among wells.
6. Prepare the following consumables.

Item	Storage	Instructions
EML	-25°C to -15°C	Thaw at room temperature. Use one tube for every four BeadChips. Bring to room temperature 1 hour before use.
XC4	15°C to 30°C	Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.
95% formamide/1 mM EDTA	-25°C to -15°C	Bring to room temperature.
RA1	-25°C to -15°C or 4°C	Bring to room temperature.

CAUTION

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.

7. Label two half reservoirs Formamide/EDTA and RA1.
8. Label one full reservoir XC3.

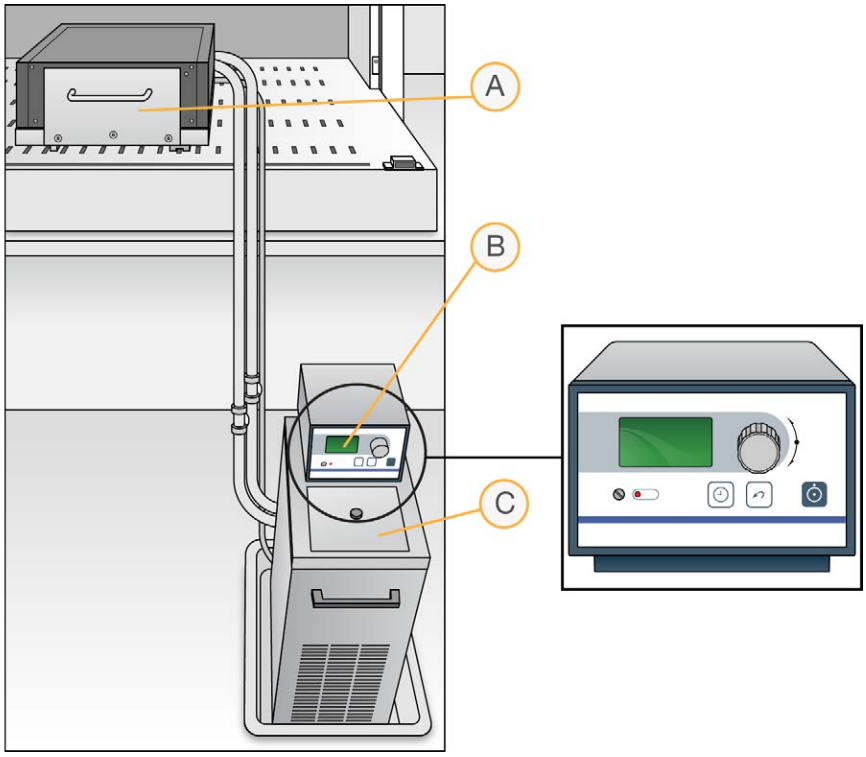
Procedure

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 24 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 LCG BeadChip HT**.

2 Select one BeadChip processing option:

- **[Option 1]** *If you are processing 24 BeadChips, perform the following steps:*

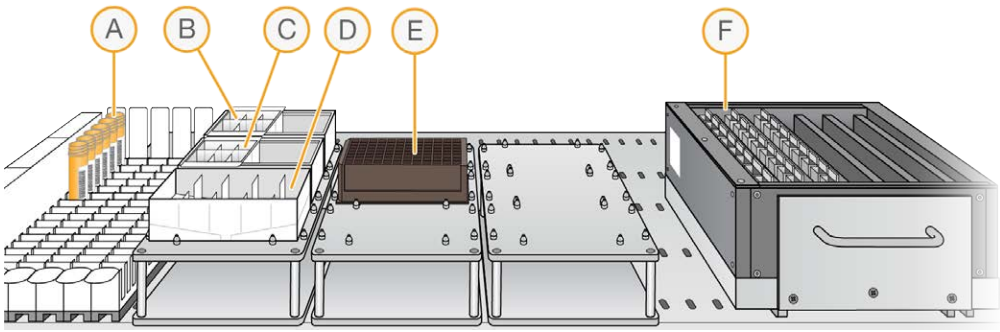
  a Place the Formamide/EDTA, RA1, and XC3 reservoirs on the robot deck according to the deck map in Figure 25.

  b Add the reagents listed in Figure 25 to the reservoirs.

  c Place the XStain plate on the robot deck according to the deck map in Figure 25. Remove the seals.

  d Invert the EML tubes to mix, remove the caps, and place the EML tubes on the robot deck according to the robot deck map in Figure 25.

Figure 25 XStain Robot Deck Setup for 24 BeadChips

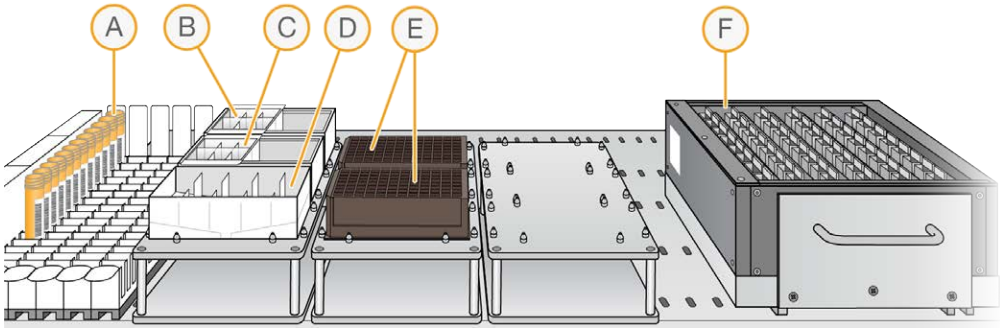


Legend	Consumable	Reagent	Volume
A	EML Tubes (6)	EML	(Predetermined)
B	95% Formamide/ 1 mM EDTA Reservoir	95% Formamide/ 1 mM EDTA	30 ml
C	RA1 Reservoir	RA1	30 ml
D	XC3 Reservoir	XC3	150 ml
E	XStain Plate	N/A	N/A
F	BeadChips in Chamber Rack	N/A	N/A

▶ [Option 2] *If you are processing 48 BeadChips, perform the following steps:*

a Place the Formamide/EDTA, RA1, and XC3 reservoirs on the robot deck according to the deck map in Figure 26.
b Add the reagents listed in Figure 26 to the reservoirs.
c Place the XStain plates on the robot deck according to the deck map in Figure 26. Remove the seals.
d Invert the EML tubes to mix, remove the caps, and place the EML tubes on the robot deck according to the robot deck map in Figure 26.

Figure 26 XStain Robot Deck Setup for 48 BeadChips



Legend	Consumable	Reagent	Volume
A	EML Tubes (12)	EML	(Predetermined)
B	95% Formamide/ 1 mM EDTA Reservoir	95% Formamide/ 1 mM EDTA	60 ml
C	RA1 Reservoir	RA1	60 ml
D	XC3 Reservoir	XC3	250 ml
E	XStain Plate	N/A	N/A
F	BeadChips in Chamber Rack	N/A	N/A

3 At the robot PC, in the Basic Run Parameters pane, enter the number of BeadChips.

a Select **Run**.
b When prompted, enter the stain temperature listed on the XStain plate. Do not load the BeadChips yet.
c Select **OK**.

4 When the chamber rack reaches 44°C, place the LCG flow-through chambers into the chamber rack according to the robot deck map in Figure 25 or Figure 26.

CAUTION
Start the robot immediately to prevent BeadChips from drying.

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

a When prompted, scan the barcode of the reagent bottle.

6 While the XStain task runs, wash the Hyb Chamber humidifying buffer reservoirs with DI H_2O, and scrub them with a small cleaning brush. Make sure that no PB2 remains in the Hyb Chamber humidifying buffer reservoir.

CAUTION
It is important to wash the Hyb Chamber humidifying buffer reservoirs thoroughly to make sure that no traces of PB2 remain in the wells.

7 When the robot finishes, remove the LCG flow-through chambers 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 LCG flow-through chamber:

   a Use the dismantling tool to remove the metal clamps. It is important to use the dismantling tool to avoid chipping LCG glass back plates.
   b Remove the LCG 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 LCG glass back plates in the DI H_2O wash basin for later cleaning, as detailed in the *Infinium Assay Lab Setup and Procedures Guide (document # 11322460) (document # 11322460)*.

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

NOTE

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

9 Soak the BeadChips for an additional 5 minutes.

CAUTION

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

10 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.

11 Pour 310 ml XC4 into a wash dish.

12 Move the staining rack from the PB1 dish to the XC4 wash dish.

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

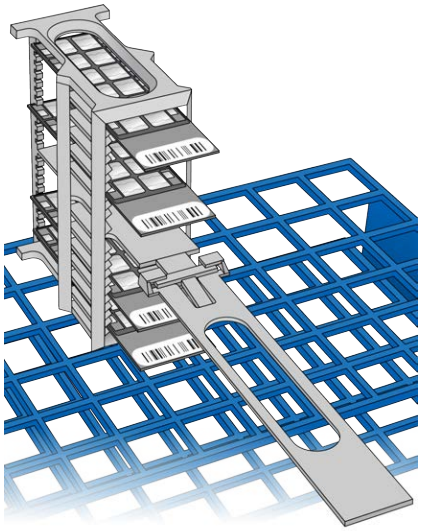
NOTE

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.

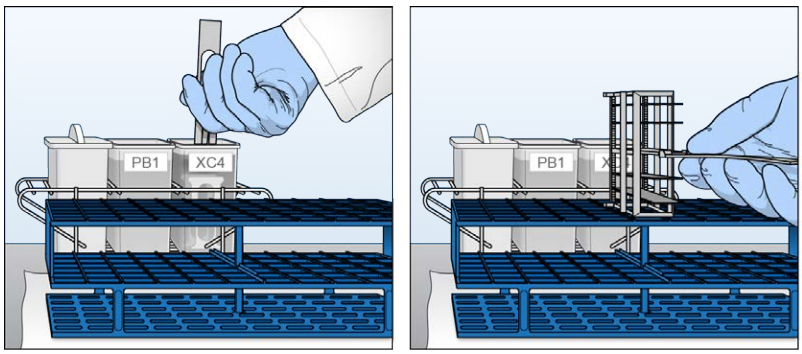
15 Remove the staining rack, and place it on the prepared tube rack with the barcode side of the BeadChips facing up.

Figure 27 Staining Rack in Correct Orientation



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

Figure 28 Moving the Staining Rack from XC4 to Tube Rack



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

17 For each BeadChip, working top to bottom:

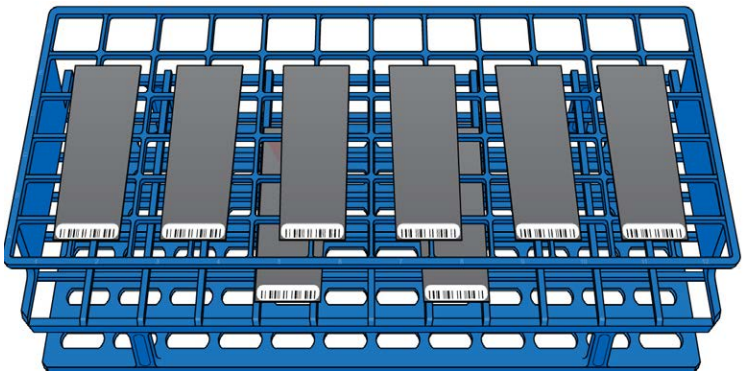
a Use self-locking tweezers to grip the BeadChip at its barcode end.

b Place the BeadChip on the tube rack with the 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 29 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 Turn on the iScan™ systems to allow the lasers to stabilize.

20 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.

21 Touch the edges of the BeadChips (**do not touch arrays**) to make sure etched, barcoded sides are dry.

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

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

24 When you are ready to image the BeadChips, in Illumina LIMS, select **Infinium HTS Extra** | **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 LCG** scan setting for your BeadChips.

Chapter 3 Automated Protocol without Illumina LIMS

Introduction

This section describes pre- and post-amplification automated laboratory protocols for the Infinium HTS Extra 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 9 for protocol instructions.

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.

Consumables

- MA1
- MA2
- MSM
- 0.1 N NaOH
- 96-well 0.8 ml microplates (midi)
- DNA plates (TCY or midi) with DNA samples at 50 ng/µl.
  - Minimum of 10 µl for TCY plates.
  - Minimum of 20 µl for midi plates.
- Cap mats

Preparation

1 Thaw DNA plates to room temperature.

2 Prepare the following consumables.

Item	Storage	Instructions
MA2	-25°C to -15°C	Thaw at 4°C for 12 to 24 hours. Bring to room temperature before use. Invert to mix.
MSM	-25°C to -15°C	Thaw at 4°C for 12 to 24 hours. Bring to room temperature before use. Invert to mix.

- If necessary, use a room temperature water bath to complete thawing and bring the reagents to room temperature.

3 Preheat the Illumina hybridization oven in the post-amp area to 37°C, and allow the temperature to equilibrate.

4 Apply MSA8 barcode labels to new midi plates.

5 Vortex DNA plates at 1600 rpm for 1 minute.

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

7 Label four quarter reservoirs MA1, NaOH, MA2, and MSM.

Procedure

1 At the robot PC, select **MSA8 Tasks | Make MSA8**.

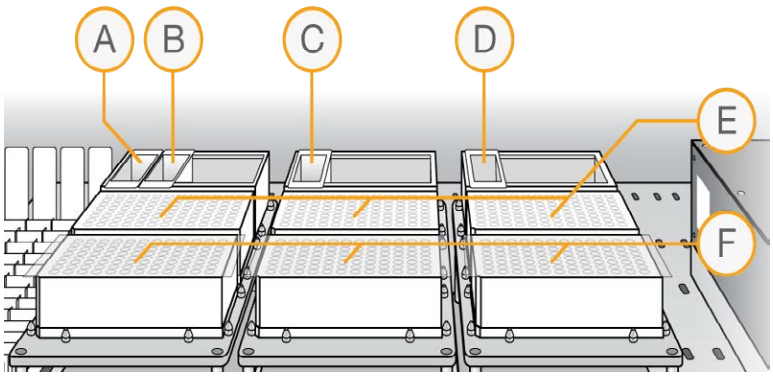
a Select the DNA plate type (midi or TCY). Do not mix plate types on the robot.

2 For every three MSA8 plates, perform the following steps:

a Place the MA1, NaOH , MA2, and MSM reservoirs on the robot deck according to the deck map in Figure 30.

b Use a serological pipette to add the reagents listed in Figure 30 to the reservoirs.

Figure 30 Robot Deck Setup for Amplify DNA



Legend	Consumable	Reagent	Volume
A	MA1 Reservoir	MA1	9 ml
B	0.1 N NaOH Reservoir	0.1 N NaOH	5 ml
C	MA2 Reservoir	MA2	13.5 ml
D	MSM Reservoir	MSM	15 ml
E	MSA8 Plates	N/A	N/A
F	DNA Plates	N/A	N/A

3 Place the DNA plates and MSA8 plates on the robot deck according to the deck map in Figure 30, and then remove the cap mats.

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

5 When the robot finishes, apply cap mats to the MSA8 plates.

6 Vortex the MSA8 plates at 1600 rpm for 1 minute.

7 Centrifuge the MSA8 plates 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 HTS Extra Assay.

NOTE

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

1 Incubate the MSA8 plates in the hybridization oven for 20–24 hours at 37°C.

Fragment DNA

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

Consumables

- FMS

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
FMS	-25°C to -15°C	Thaw at 4°C for 12 to 24 hours. Bring to room temperature before use. Invert to mix.

2 Preheat the hybridization oven to 37°C.

3 If you plan to resuspend the MSA8 plates today, remove RA1 from the freezer, and thaw at room temperature.

Procedure

1 Centrifuge the MSA8 plates at 280 × g at room temperature for 1 minute.

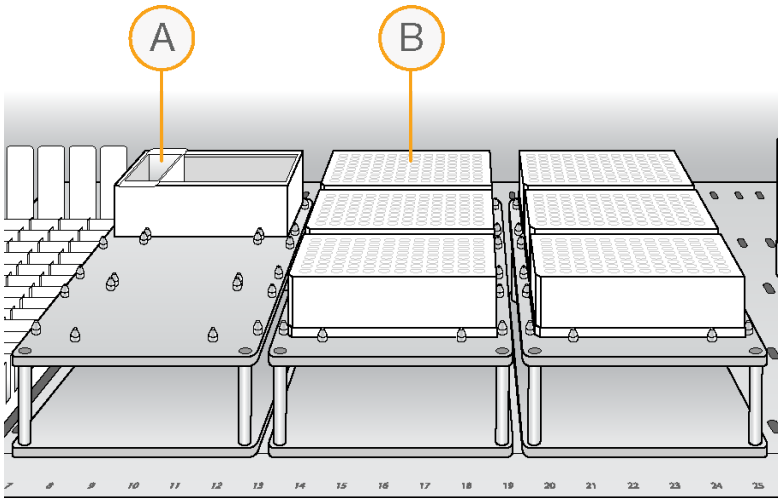
2 At the robot PC, select **MSA8 Tasks | Fragment MSA8**.

3 Place six MSA8 plates on the robot deck according to the deck map in Figure 31, and then remove the cap mats.

4 Place a quarter reservoir on the robot deck according to the deck map in Figure 31.

   a Add 20 ml FMS to the quarter reservoir.

Figure 31 Robot Deck Setup for Fragment MSA8



A FMS
B MSA8 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 hybridization oven for 30 minutes.
If you are continuing, you can leave the plates in the 37°C hybridization oven until you have completed preparation for the next step. Do not leave the plates in the 37°C 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 MSA8 plates, thaw to room temperature.

4 Centrifuge the MSA8 plates at 280 × g for 1 minute.

5 Label one quarter reservoir as PM1.

6 Label one full reservoir as 2-propanol.

Precipitate the MSA8 Plate

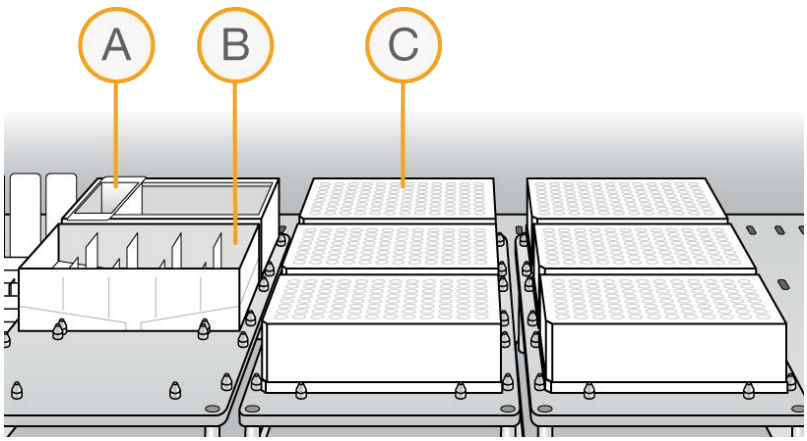
1 At the robot PC, select **MSA8 Tasks | Precip MSA8**.

2 Place six MSA8 plates on the robot deck according to the deck map in Figure 32, and then remove the cap mats.

3 Place the PM1 and 2-propanol reservoirs on the robot deck according to the deck map in Figure 32.

4 Add the reagents listed in Figure 32 to the reservoirs.

Figure 32 Robot Deck Setup for Precipitate MSA8



Legend	Consumable	Reagent	Volume
A	PM1 Reservoir	PM1	40 ml
B	2-propanol Reservoir	2-propanol	150 ml
C	MSA8 Plates	N/A	N/A

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.

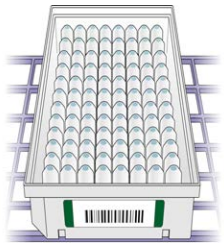
11 Tap the plates several times until all wells are devoid of liquid.

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 33 Uncovered MSA8 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 MSA8 plates at -25°C to -15°C, thaw to room temperature, centrifuge, and then remove the cap mats.

2 Preheat the hybridization oven to 48°C.

3 Preheat the heat sealer for 20 minutes before use.

Resuspend the MSA8 Plate

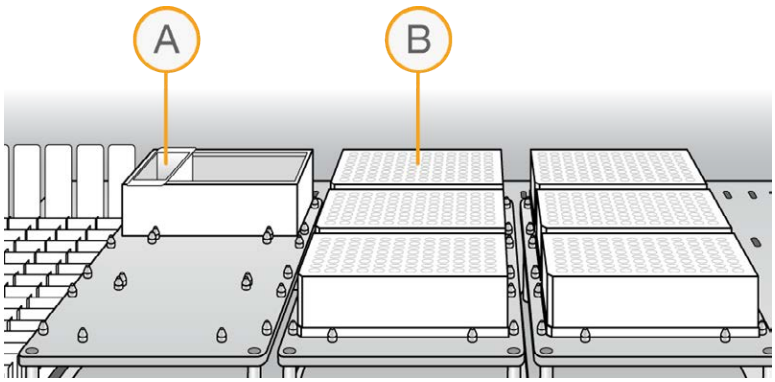
1 At the robot PC, select **MSA8 Tasks | Resuspend MSA8**.

2 Place six MSA8 plates on the robot deck according to the deck map in Figure 34, and then remove the cap mats.

3 Place a quarter reservoir on the robot deck according to the deck map in Figure 34.

   a Add 20 ml RA1 to the quarter reservoir.

Figure 34 Robot Deck Setup for Resuspend MSA8



A RA1 Reservoir
B MSA8 Plates

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

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

5 Remove the MSA8 plates from the robot deck.

6 Apply foil heat seals to the MSA8 plates using the heat sealer.

7 Incubate in the 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 for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA8 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 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

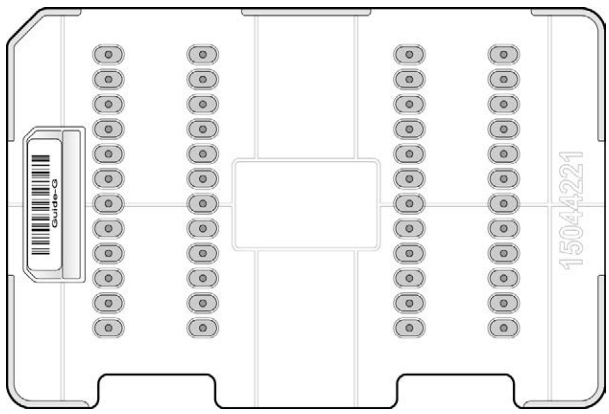
1. If you froze the MSA8 plates, thaw to room temperature, and then centrifuge at 280 × g at room temperature for 1 minute.
2. Remove BeadChips from storage, but do not unpackage.
3. Preheat the heat block to 95°C.
4. Preheat the hybridization oven to 48°C.

Procedure

Prepare Robot Tip Alignment Guide

1. Make sure that you have the correct robot tip alignment guide for the assay you are running. The barcode says **Guide-G**.

Figure 35 Guide-G Robot Tip Alignment Guide



2. Wash and dry the robot tip alignment guide. See *Wash Robot Tip Alignment Guide* at the end of the *Hybridize to BeadChip* steps for washing instructions.

Denature DNA

1 Place the MSA8 plates on the heat block at 95°C for 20 minutes to denature samples.

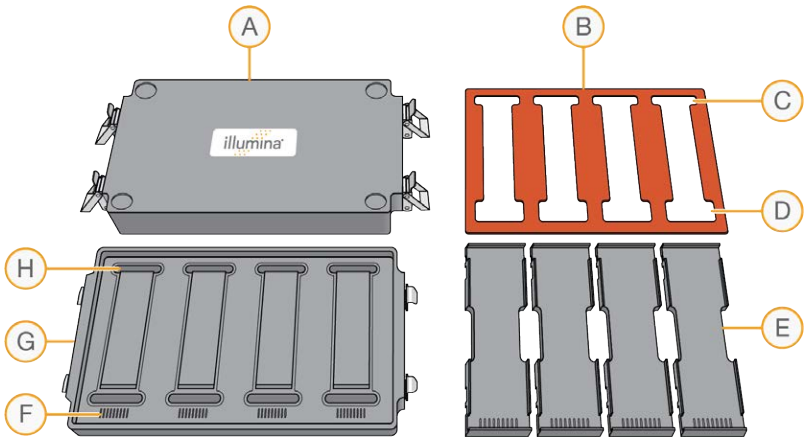
2 Cool the MSA8 plates on the benchtop at room temperature for 30 minutes.

3 Centrifuge at 1500 × g at room temperature for 1 minute.

Assemble Hybridization Chambers

Assemble one chamber for every four BeadChips by following the steps in this section. See Figure 36 for an illustration of the Hyb Chamber components.

Figure 36 BeadChip Hyb Chamber Components



A Top of BeadChip Hyb Chamber
B Hyb Chamber Gasket
C Narrower Edges
D Wider Edges
E Hyb Chamber Inserts
F Barcode Ridges
G Bottom of BeadChip Hyb Chamber
H Humidifying Buffer Reservoirs

1 Place the gasket into the Hyb Chamber according to Figure 37.
- Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber.
- Press down on the edges of the gasket to make sure it is properly seated.

Figure 37 Placing Gasket Into Hyb Chamber

2 Dispense 400 µl PB2 into each of the eight humidifying buffer reservoirs in the Hyb Chamber.

3 Place the Hyb Chamber insert into the Hyb Chamber. Position the barcode ridges on the Hyb Chamber insert over the barcode ridges on the Hyb Chamber.

4 Close the Hyb Chamber lid and clamps immediately to prevent evaporation.

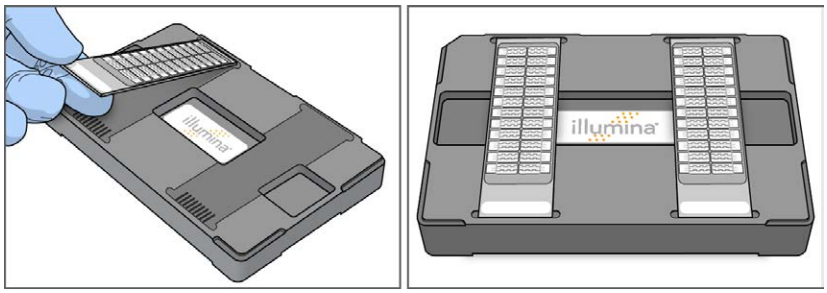
5 Leave the closed Hyb Chambers on the bench at room temperature until BeadChips are loaded with DNA sample.

Prepare the Robot

1 Remove all BeadChips from packaging.

2 Place BeadChips into the robot BeadChip alignment fixtures. Align the barcode end with the ridges stamped into the robot BeadChip alignment fixture.

Figure 38 Placing BeadChips in the Robot BeadChip Alignment Fixture



3 Stack the robot BeadChip alignment fixtures (2–6 per robot), and carry them to the robot.

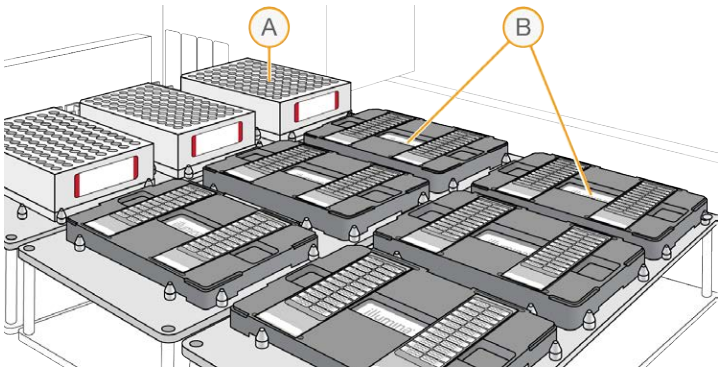
4 At the robot PC, select **MSA8 Tasks | Hyb**.

   a In the BeadChip Selection dialog box, select the 24-sample BeadChip.
   b In the Basic Run Parameters pane, enter the value for the **Number of MSA8 plates** (1–3 plates per robot).

5 Place the robot BeadChip alignment fixtures onto the robot deck according to the deck map in Figure 39.

6 Place the MSA8 plates onto the robot deck according to the deck map in Figure 39, and remove the heat seal.

Figure 39 Robot Deck Setup for Hybridization*



*The MSA8 plate and robot BeadChip alignment fixtures shown represent maximum throughput options.

A MSA8 Plate
B Robot BeadChip Alignment Fixtures

CAUTION

BeadChips must be transferred to Hyb Chambers immediately at the end of the next procedure *(Start the Robot)*. Do not begin *Start the Robot* if you cannot immediately transfer the BeadChips.

Start the Robot

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

2 Place each robot tip alignment guide on top of each robot BeadChip alignment fixture.

3 At the robot PC, select **OK**.
- The robot scans the barcode on the robot tip alignment guide to confirm that the correct tip guide is being used.
- The robot dispenses DNA sample to the BeadChips.
- When the process is complete, the robot PC sounds an alert and opens a message box.

4 When the robot finishes, at the robot PC, select **OK** in the message box.

5 Remove the robot BeadChip alignment fixtures from the robot deck.

CAUTION

BeadChips must be removed from the robot deck immediately to prevent excess sample evaporation. Proceed to the next section *(Set Up and Incubate BeadChips)* immediately.

Set Up and Incubate BeadChips

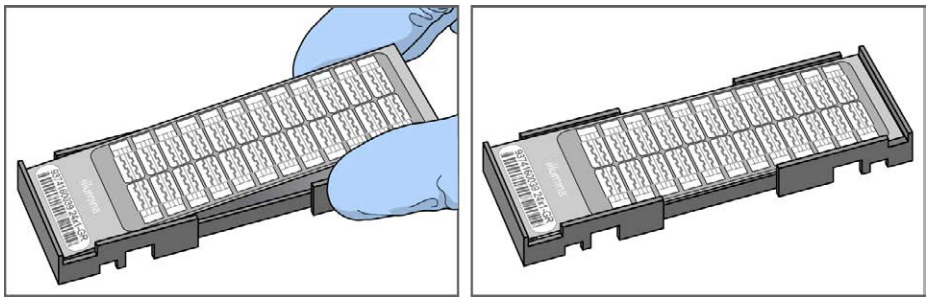
1 Make sure that the hybridization oven is set to 48°C.

WARNING

Keep Hyb Chambers at room temperature when you load the BeadChips. Do not place the Hyb Chambers in the hybridization oven when loading the BeadChips.

2 Open each Hyb Chamber, and then carefully place each BeadChip in a Hyb Chamber insert. Orient the barcode end so that it matches the barcode symbol on the insert.

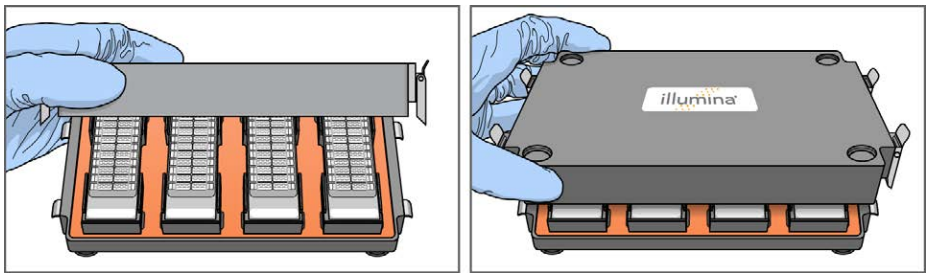
Figure 40 Matching the Barcode End to the Insert Fixture



3 Make sure that Hyb Chamber inserts are seated properly in the Hyb Chambers.

4 Apply the back side of the Hyb Chamber lid first, and then slowly bring down the front side to avoid dislodging the Hyb Chamber inserts.

Figure 41 Seating Lid onto Hyb Chamber



5 Close the Hyb Chamber clamps in this order: top-left, bottom-right, top-right, and then bottom-left. Make sure that the lid is secure and even on the base (no gaps).

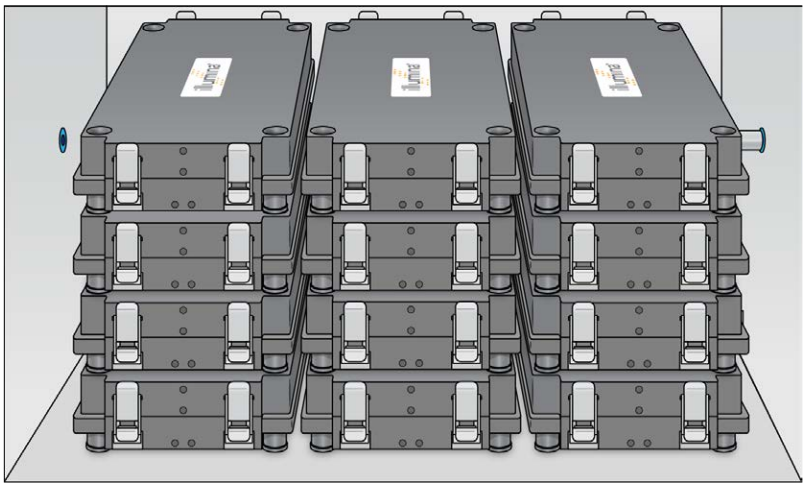
CAUTION

Keep the Hyb Chamber steady and level when moving it or transferring it to the hybridization oven.

6 Place the Hyb Chamber in the 48°C hybridization oven with the clamps of the Hyb Chamber facing the front and back of the oven.

If you are stacking multiple Hyb Chambers in the hybridization oven, fit the feet of each Hyb Chamber into the matching indents on the lid of the Hyb Chamber below it. You can stack up to four Hyb Chambers in three rows, for a maximum of 12 total Hyb Chambers in the hybridization oven.

Figure 42 HTS Extra Hyb Chambers Correctly Placed in hybridization oven



OVERNIGHT INCUBATION

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

TIP

While you wait for the Hyb Chamber to incubate overnight, you can also thaw the XStain plates by following the preparation steps in *Extend and Stain (XStain)*.

Wash Robot Tip Alignment Guide

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

1. Soak the robot tip alignment guides in 1% aqueous Alconox solution (1 part Alconox to 99 parts water) for 5 minutes. Do not use bleach or ethanol to clean HTS tip guide inserts.
2. Thoroughly rinse the robot tip alignment guides with DI H_2O at least 3 times to remove any residual detergent.
3. Dry the robot tip alignment guides. Make sure that the robot tip alignment guides are free of any residual contaminates before next use.

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.

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

- 1XPB1

Preparation

1 Make sure that you have diluted PB1.

2 Remove the Hyb Chambers from the hybridization oven. Cool for 30 minutes at room temperature before opening. While the Hyb Chambers are cooling, proceed to step 3.

3 While the Hyb Chambers are cooling:

   a Fill two wash dishes with PB1 (200 ml per dish), labeled Wash 1 and Wash 2.
   b Fill a graduated cylinder with 150 ml PB1.
   c Make sure that the LCG glass back plates have no chips or cracks.
   d Clean the LCG glass back plates if necessary by following the cleaning procedure described in the *Infinium Assay Lab Setup and Procedures Guide (document # 11322460).*

4 Make sure that the multi-sample BeadChip alignment fixture, black frames, LCG spacers, and clamps are ready for use.

Procedure

Wash BeadChips

1 Attach the wire handle, and submerge the wash rack in Wash 1 (containing PB1).

CAUTION

Replace PB1 in Wash 1 after every eight BeadChips.

Figure 43 Wash Rack in Wash Dish

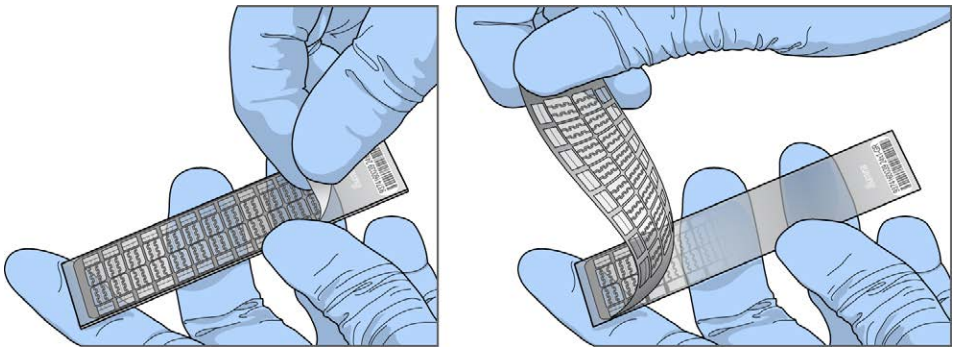
2 Remove the Hyb Chamber inserts from the Hyb Chambers.

3 Inspect the BeadChips. Note any sections that are not covered with DNA sample.

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

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

Figure 44 Removing the Cover Seal



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

7 Repeat these steps until all BeadChips (a maximum of eight) are transferred to the submerged wash rack in Wash 1.

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

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

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

11 If you are processing more than eight BeadChips, follow this procedure:

a Assemble the flow-through chambers for the first eight BeadChips, as described in the *Assemble*

Flow-Through Chambers section, and place them on the lab bench in a horizontal position.

CAUTION

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

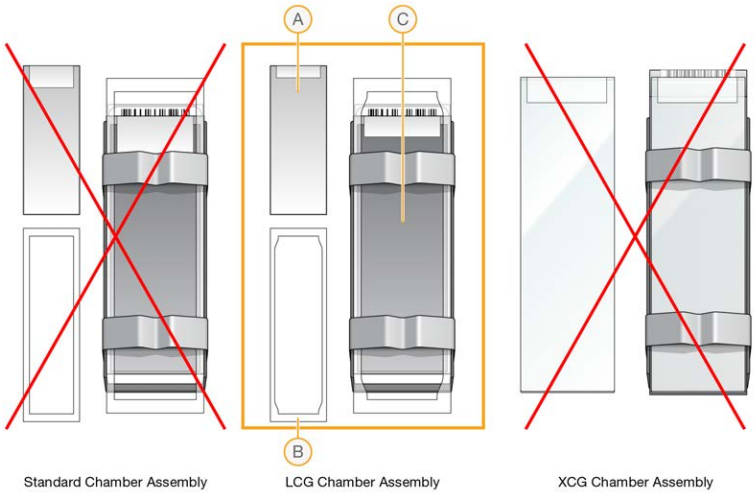
b Return to *Wash BeadChips* on page 49, and follow the steps to wash the next set of eight BeadChips.

c Repeat step 11 in *Wash BeadChips* for each remaining set of eight BeadChips.

Assemble Flow-Through Chambers

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

Figure 45 Correct LCG Back Plates and Spacers



A LCG Glass Back Plate
B LCG Spacer
C Assembled LCG Flow-Through Chamber

CAUTION

This protocol is not compatible with XCG-integrated spacer glass used for Infinium XT BeadChips.

2 Orient the stamped barcode ridges in the multi-sample BeadChip alignment fixture tray towards you.

3 Fill the multi-sample BeadChip alignment fixture with 150 ml PB1.

CAUTION

Replace the PB1 in the multi-sample BeadChip alignment fixture after every eight BeadChips.

4 For each BeadChip to be processed, place a black frame into the multi-sample BeadChip alignment fixture.

5 Place each BeadChip to be processed into a black frame. Align the BeadChip barcode with the ridges that are stamped into the multi-sample BeadChip alignment fixture. Make sure the barcode is facing you.

6 Inspect the surface of the BeadChip for residue left by the cover seal. Use a pipette tip to remove excess residue that was not removed by the PB1. Do not scratch the bead area.

CAUTION

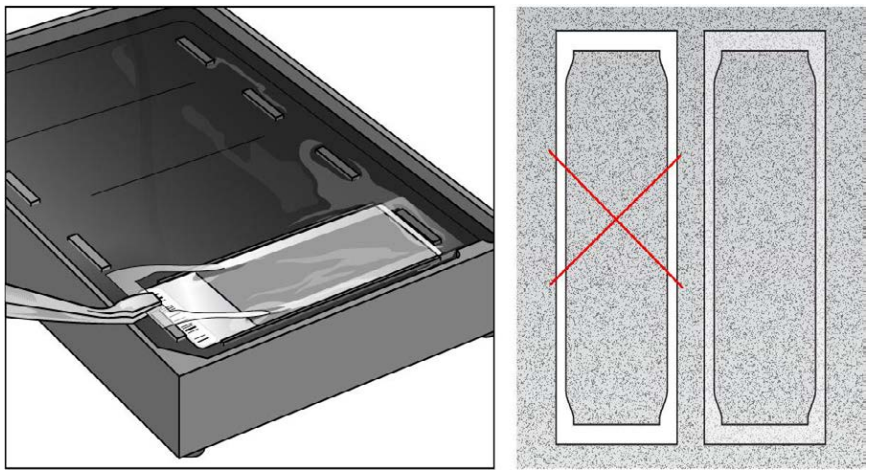
Make sure that each BeadChip remains submerged in PB1 throughout the flow-through chamber assembly process to prevent drying of the active area.

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

CAUTION

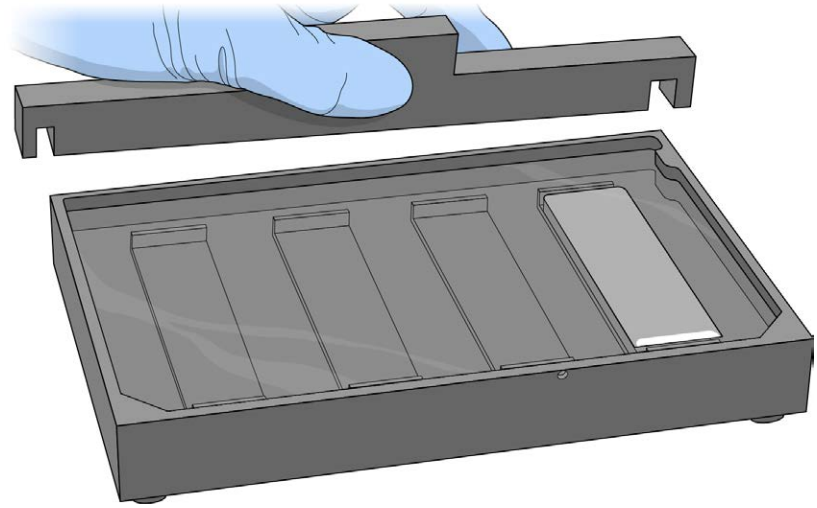
Make sure to use the clear plastic spacers, not the white spacers.

Figure 46 Placing Clear Plastic Spacer Onto BeadChip



8 Place the alignment bar onto the multi-sample BeadChip alignment fixture. The groove in the alignment bar fits over the tab on the alignment fixture.

Figure 47 Placing Alignment Bar Onto Multi-Sample BeadChip Alignment Fixture



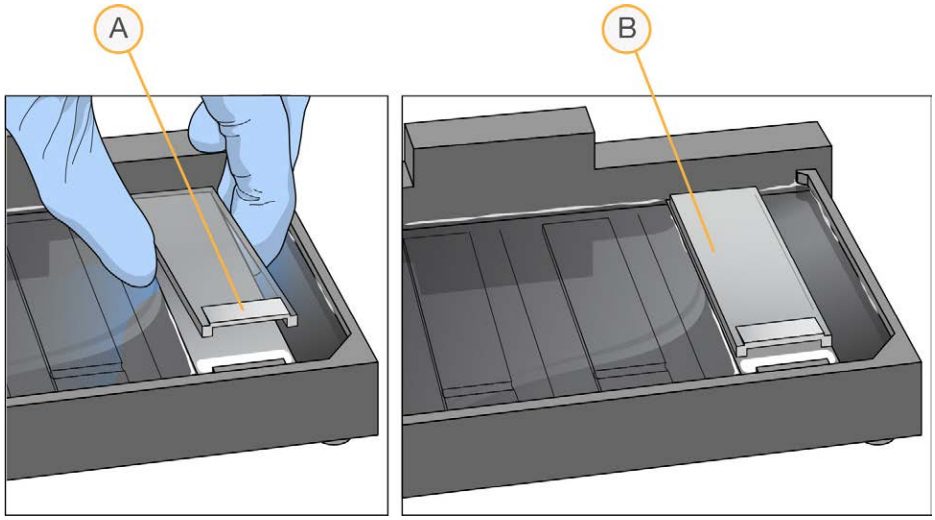
9 Place a clean LCG glass back plate on top of the clear spacer that is covering each BeadChip. Make sure the beveled edge of the LCG glass back plate is facing the barcode.

The beveled edge creates a reservoir against the BeadChip surface.

CAUTION

Make sure to keep BeadChips submerged to prevent drying.

Figure 48 Placing Glass Back Plate Onto BeadChip



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

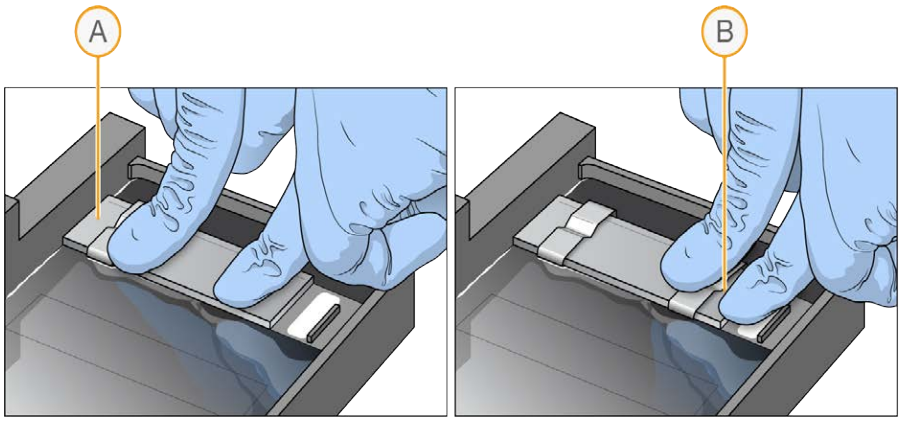
10 Attach the metal clamps to the flow-through chambers as follows:

a Gently push the glass back plate against the alignment bar with one finger.
b Place the first metal clamp around the flow-through chamber, approximately 5 mm from the edge closest to the alignment bar.
c Place the second metal clamp around the barcode end of the flow-through chamber, approximately 5 mm from the reagent reservoir.

CAUTION

Make sure spacers do not become misaligned or dislodged.

Figure 49 Securing Flow-Through Chamber With Metal Clamps



A Positioning First Clamp With Glass Back Plate Pressed Against Alignment Bar
B Positioning Second Clamp

11 Remove the flow-through chamber, and use scissors to trim the excess ends of the clear plastic spacers.

CAUTION
Do not trim the BeadChip.

12 Return the flow-through chamber to the multi-sample BeadChip alignment fixture.

13 Discard unused reagents in accordance with facility standards.

CAUTION
Place all assembled flow-through chambers on the lab bench in a horizontal position while you perform the preparation steps for *Extend and Stain (XStain).* Do not place the flow-through chambers in the chamber rack until the *Extend and Stain (XStain)* preparation steps are complete.

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
- EML
- XC3
- PB1
- XC4
- XStain plates (1 XStain plate per 24 Beadchips.)
- Alconox powder detergent
- 95% formamide/1 mM EDTA

WARNING

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

Preparation

1 Thaw XStain plates for 24 hours at 4°C.

2 Bring XStain plates to room temperature for 1 hour before use.
- The contents of the XStain plates are fully thawed when the bottom of the plate is no longer cool to the touch.

3 Invert the XStain plates 10 times to mix the reagents.

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

5 Carefully remove the seal to avoid cross-contamination among wells.

6 Prepare the following consumables.

Item	Storage	Instructions
EML	-25°C to -15°C	Thaw at room temperature. Use one tube for every four BeadChips. Bring to room temperature 1 hour before use.
XC4	15°C to 30°C	Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.
95% formamide/1 mM EDTA	-25°C to -15°C	Bring to room temperature.
RA1	-25°C to -15°C or 4°C	Bring to room temperature.

CAUTION

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.

7 Label two half reservoirs Formamide/EDTA and RA1.

8 Label one full reservoir XC3.

Procedure

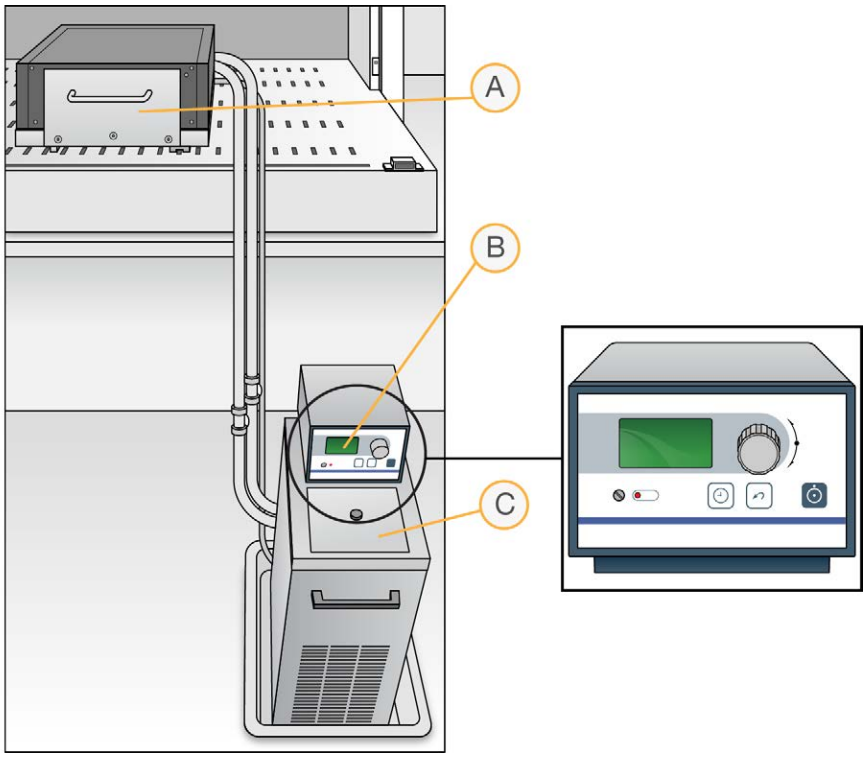
Set Up the Chamber Rack

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 50 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 LCG BeadChip HT**.

2 Select one BeadChip processing option:

- [Option 1] *If you are processing 24 BeadChips, perform the following steps:*

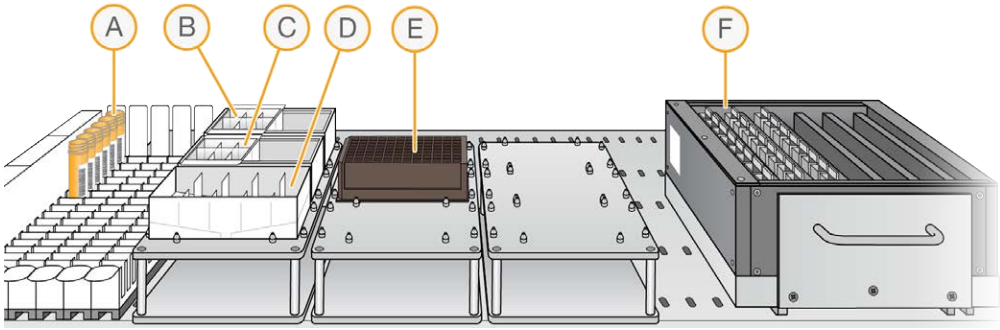
  a Place the Formamide/EDTA, RA1, and XC3 reservoirs on the robot deck according to the deck map in Figure 51.

  b Add the reagents listed in Figure 51 to the reservoirs.

  c Place the XStain plate on the robot deck according to the deck map in Figure 51. Remove the seals.

  d Invert the EML tubes to mix, remove the caps, and place the EML tubes on the robot deck according to the robot deck map in Figure 51.

Figure 51 XStain Robot Deck Setup for 24 BeadChips

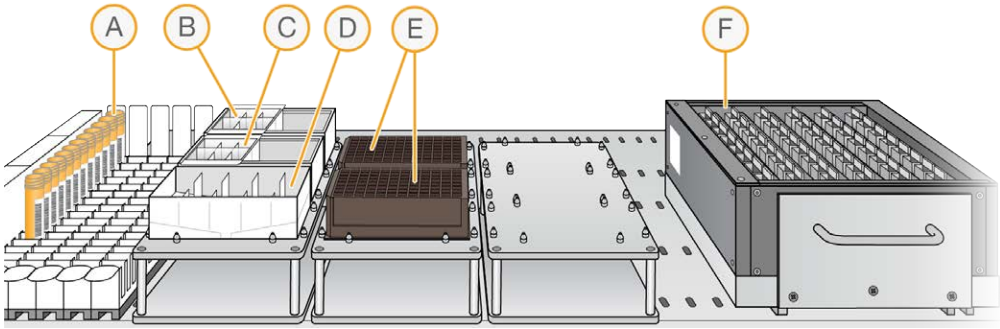


Legend	Consumable	Reagent	Volume
A	EML Tubes (6)	EML	(Predetermined)
B	95% Formamide/ 1 mM EDTA Reservoir	95% Formamide/ 1 mM EDTA	30 ml
C	RA1 Reservoir	RA1	30 ml
D	XC3 Reservoir	XC3	150 ml
E	XStain Plate	N/A	N/A
F	BeadChips in Chamber Rack	N/A	N/A

- [Option 2] *If you are processing 48 BeadChips, perform the following steps:*

  a Place the Formamide/EDTA, RA1, and XC3 reservoirs on the robot deck according to the deck map in Figure 52.
  b Add the reagents listed in Figure 52 to the reservoirs.
  c Place the XStain plates on the robot deck according to the deck map in Figure 52. Remove the seals.
  d Invert the EML tubes to mix, remove the caps, and place the EML tubes on the robot deck according to the robot deck map in Figure 52.

Figure 52 XStain Robot Deck Setup for 48 BeadChips



Legend	Consumable	Reagent	Volume
A	EML Tubes (12)	EML	(Predetermined)
B	95% Formamide/ 1 mM EDTA Reservoir	95% Formamide/ 1 mM EDTA	60 ml
C	RA1 Reservoir	RA1	60 ml
D	XC3 Reservoir	XC3	250 ml
E	XStain Plate	N/A	N/A
F	BeadChips in Chamber Rack	N/A	N/A

3 At the robot PC, in the Basic Run Parameters pane, enter the number of BeadChips.

  a Select **Run**.
  b When prompted, enter the stain temperature listed on the XStain plate. Do not load the BeadChips yet.
  c Select **OK**.

4 When the chamber rack reaches 44°C, place the LCG flow-through chambers into the chamber rack according to the robot deck map in Figure 51 or Figure 52.

CAUTION
Start the robot immediately to prevent BeadChips from drying.

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

6 While the XStain task runs, wash the Hyb Chamber humidifying buffer reservoirs with DI H_2O, and scrub them with a small cleaning brush. Make sure that no PB2 remains in the Hyb Chamber humidifying buffer reservoir.

CAUTION
It is important to wash the Hyb Chamber humidifying buffer reservoirs thoroughly to make sure that no traces of PB2 remain in the wells.

7 When the robot finishes, remove the LCG flow-through chambers 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 LCG flow-through chamber:

   a Use the dismantling tool to remove the metal clamps. It is important to use the dismantling tool to avoid chipping LCG glass back plates.
   b Remove the LCG 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 LCG glass back plates in the DI H_2O wash basin for later cleaning, as detailed in the *Infinium Assay Lab Setup and Procedures Guide (document # 11322460) (document # 11322460)*.

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

NOTE

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

9 Soak the BeadChips for an additional 5 minutes.

CAUTION

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

10 Shake the XC4 bottle vigorously to ensure complete resuspension. If any coating is visible, vortex at 1625 rpm until suspension is complete.

11 Pour 310 ml XC4 into a wash dish.

12 Move the staining rack from the PB1 dish to the XC4 wash dish.

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

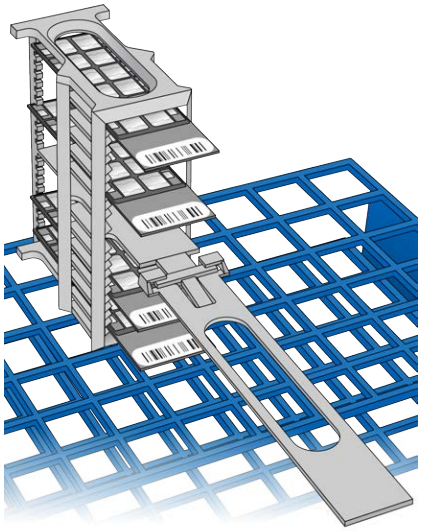
NOTE

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.

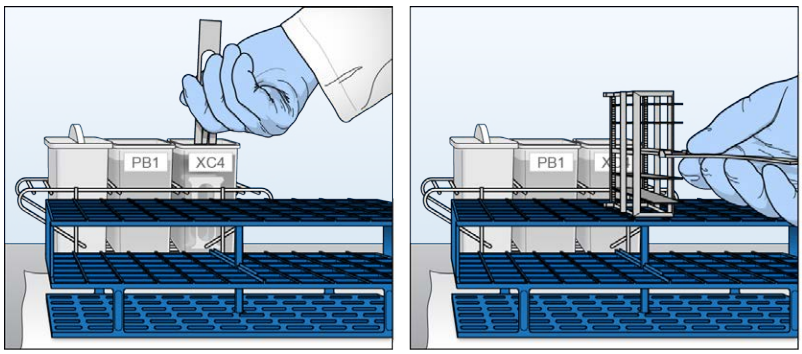
15 Remove the staining rack, and place it on the prepared tube rack with the barcode side of the BeadChips facing up.

Figure 53 Staining Rack in Correct Orientation



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

Figure 54 Moving the Staining Rack from XC4 to Tube Rack



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

17 For each BeadChip, working top to bottom:

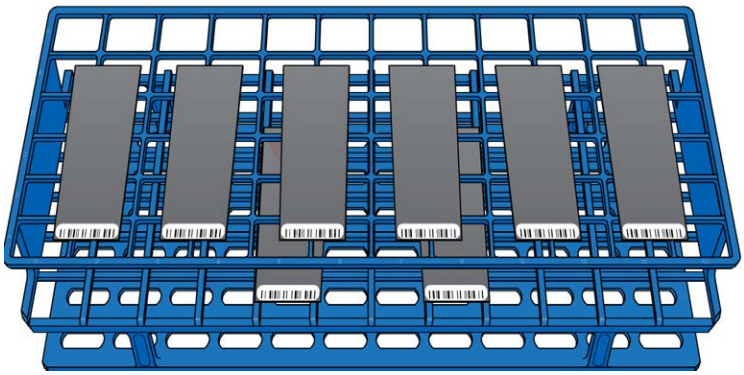
a Use self-locking tweezers to grip the BeadChip at its barcode end.

b Place the BeadChip on the tube rack with the 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 55 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 Turn on the iScan™ systems to allow the lasers to stabilize.

20 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.

21 Touch the edges of the BeadChips (**do not touch arrays**) to make sure etched, barcoded sides are dry.

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

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

Image BeadChip

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

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

Appendix A Supporting Information

Introduction

The protocol described in this guide assumes that you have reviewed the contents of this section, confirmed workflow contents, and obtained all required consumables and equipment.

Acronyms

Acronym	Definition
DI H_2O	Deionized water
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
EML	Extension mix long
FMS	Fragmentation solution
IPA	100% 2-propanol
MA1	Multi-Sample Amplification Mix 1
MA2	Multi-Sample Amplification Mix 2
MSA8 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
MSM	Multi-Sample Amplification Master Mix
TCY plate	Acceptable 96-well plate for DNA plate
XC3	XStain BeadChip solution 3
XC4	XStain BeadChip solution 4

Kit Contents

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

Infinium HTS Extra Kit (4608 Samples)

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

Box Number	Box Label	Quantity (Per Box)	Contents	Storage Temperature
Box 1(Pre-Amp)	Infinium Assay Kit Pre 1 MSM/MA2 4608	2	MA2	-25°C to -15°C
		2	MSM	-25°C to -15°C
Box 2 (Pre-Amp)	Infinium HTS Extra Assay Pre 2 4608	2	MA1	15°C to 30°C
		48	Labels for MSA8 plates	15°C to 30°C
		48	Labels for DNA plates	15°C to 30°C
Box 3	Infinium Assay Kit Post 1 4608	2	FMS	-25°C to -15°C
		2	RA1	-25°C to -15°C
		2	PM1	2°C to 8°C
Boxes 4–7	Infinium Assay Kit Post 2 4608	2	XStain (XP1) plate	-25°C to -15°C
Boxes 8–11	Infinium Assay Kit Post 3 4608	2	PB2	15°C to 30°C
		2	XC3	15°C to 30°C
		1	XC4	15°C to 30°C
Box 12	Infinium Assay Kit Post 4 EML Qty 48	48	EML	-25°C to -15°C
Box 13–20	N/A	24	Infinium 24-sample BeadChips	2°C to 8°C
Box 21	Infinium Assay PB20	1	PB20	15°C to 30°C

Infinium HTS Extra Kit (23,040 Samples)

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

Box Number	Box Label	Quantity (Per Box)	Contents	Storage Temperature
Box 1 (Pre-Amp)	Infinium Assay Kit MA2 23k	10	MA2	-25°C to -15°C
Box 2 (Pre-Amp)	Infinium Assay Kit MSM 23k	10	MSM	-25°C to -15°C
Box 3 (Pre-Amp)	Infinium Assay Kit MA1 23k	10	MA1	15°C to 30°C
Box 4	Infinium Assay Kit FMS 23k	10	FMS	-25°C to -15°C
Box 5	Infinium Assay Kit RA1 23k	10	RA1	-25°C to -15°C
Box 6	Infinium Assay Kit PM1 23k	10	PM1	2°C to 8°C
Box 7–8	Infinium Assay Kit XStain Qty 20	20	XStain (XP1) plate	-25°C to -15°C
Box 9–12	Infinium Assay Kit XC4PB2 23k	10	PB2	15°C to 30°C
	Infinium Assay Kit XC4 23k	5	XC4	15°C to 30°C
Box 13–16	Infinium Assay Kit XC3 23k	10	XC3	15°C to 30°C
Box 17–20	Infinium Assay Kit EML 23k	60	EML	-25°C to -15°C
Box 21–60	N/A	24	Infinium 24-sample BeadChips	2°C to 8°C
Box 61–63	Infinium Assay PB20 Kit	1	PB20	15°C to 30°C
Box 64	Infinium HTS Extra 23K Label Kit	240	Labels for MSA8 Plates and DNA	15°C to 30°C

Consumables and Equipment

Infinium protocols require the following Illumina-supplied and user-supplied consumables and equipment. Where applicable, items have been designated for pre- and post-amplification areas.

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

Consumables

Material or Equipment	Supplier
Absorbent pads/towels	General lab supplier
Heat sealer foil	Thermo Fisher Scientific, catalog # AB-0559
Cap mats, 96-well, pierceable, nonautoclavable	Thermo Fisher Scientific, catalog # AB-0566
Compressed air can	VWR, Int'l, catalog # 16650-027
Containers: • 1 L, for diluting NaOH • 4 L, for diluting PB20 reagent	General lab supplier
Heat sealing foil sheets, Thermo-Seal	Thermo Fisher Scientific, catalog # AB-0559
Kimwipes or any lint-free tissue	General lab supplier
Lab coats • 2 supplies: 1 for pre- and 1 for post-amplification processes	General lab supplier
Pipette tips • 20 µl aerosol filter • 200 µl aerosol filter • 1000 µl aerosol filter	General lab supplier
Pipettes, serological, 50 ml	General lab supplier
Pipetting troughs, disposable	VWR, Int'l, catalog # 21007-970
Powder-free gloves • 2 supplies: 1 for pre- and 1 for post-amplification processes	General lab supplier
ProStat EtOH presaturated wipes • Recommend 1 wipe per 2 chips; 30 wipes per package • Substitute with Kimwipes and 70% EtOH	Contec, catalog # PS-911EB/EtOH
Pyrex glass container, 9" x 13"	General lab supplier
Safety glasses • 2 supplies: 1 for pre- and 1 for post-amplification processes	General lab supplier
Skirted microplates, 96-well, 0.2 ml • Microseal 96-well skirted polypropylene microplates, 8x12 well array • TCY plates, 1 per run • Substitute with 0.8 ml storage plate (midi plate), conical well bottom, if desired	MJ Research, catalog # MSP-9601, www.mjr.com
Storage microplates, 96-well, 0.8 ml • Midi plates, 1 per run	Thermo Fisher Scientific, catalog # AB0765

Material or Equipment	Supplier
Tubes • 15 ml conical • 50 ml conical	General lab supplier
Adapters to centrifuge 96-well plates and tubes (2 sets)	General lab supplier
Cap mat sealer (recommended)	Corning, catalog # 3081
Foil adhesive seals (Microseal 'F')	MJ Research, catalog # MSF-1001
Forceps • Included with system, only needed if additional pairs are required	VWR, Int'l, catalog # 25601-008
Micropipettors • 2 separate sets: 1 for pre- and 1 for post-amplification processes • P-20 • P-200 • P-1000	General lab supplier
Multichannel precision pipettes • 2 separate sets: 1 for pre- and 1 for post-amplification processes • P-20 • P-200	General lab supplier
Reservoirs • Full, 150 ml • Half, 75 ml • Quarter, 40 ml	• Beckman Coulter, catalog # 372784 • Beckman Coulter, catalog # 372786 • Beckman Coulter, catalog # 372790
Reservoir Frame	Beckman Coulter, catalog # 372795
Serological pipette aid	General lab supplier
Stop watches/timers • 2 separate sets: 1 for pre- and 1 for post-amplification processes	General lab supplier
Bleach	General lab supplier
Deionized water (DI H_2O)	General lab supplier
EDTA, 0.5 M	EMD Chemicals, catalog # 4056 Sigma-Aldrich, catalog # E7889
Ethanol, 100%	General lab supplier
Formamide, OmniPur	VWR, Int'l, catalog # EM-4650
Isopropanol (2-propanol), 100%	General lab supplier
Mild detergent, such as Alconox® Powder Detergent	VWR, Int'l, catalog # 21835
Sodium hydroxide, purchase as solid and prepare a 0.1 N NaOH solution in DI H_2O	Sigma-Aldrich, catalog # 221465
TE, 1X • 10 mM Tris-HCl, pH 8.0, 1 mM EDTA • For diluting DNA	General lab supplier

Equipment

Equipment	Supplier
iScan System (110 V/220 V)	Illumina, catalog # SY-101-1001
Autoloaders: • Single-scanner configuration (110 V/220 V) • Dual-scanner configuration (110 V/220 V)	 • Illumina catalog # SY-201-1001 • Illumina catalog # SY-201-1002
Infinium Automation Kit: • 8 tip Tecan Non-Illumina LIMS (110 V) • 8 tip Tecan Non-Illumina LIMS (220 V) • 8 tip Tecan Illumina LIMS Ready (110 V) • 8 tip Tecan Illumina LIMS Ready (220 V)	 • Illumina catalog # SC-30-401 • Illumina catalog # SC-30-402 • Illumina catalog # SC-30-403 • Illumina catalog # SC-30-404
BeadChip wash dish	Illumina catalog # BD-60-460
BeadChip wash rack	Illumina catalog # BD-60-450
Heat sealer	Thermo Fisher, catalog # AB-1443A
Te-Flow chamber dismantling tool	Illumina, catalog # WG-10-204
LCG Te-Flow chambers (10)	Illumina, catalog # WG-100-1001
LCG Te-Flow Chamber Spacers (500)	Illumina, catalog # WG-100-1002
Hybridization oven	Illumina, catalog # SE-901-1001 (110 VAC) or # SE-901-1001 (220 VAC)
HYB Ex midi-plate insert	Illumina, catalog # BD-60-601
HYB EX heat block incubator	Illumina, catalog # SC-60-503 (110V) or SC-60-504 (220V)
Staining rack and wash dish	Illumina, catalog # WG-10-207
Robot BeadChip alignment fixture	Illumina, catalog # 222691
Vacuum desiccator	VWR, Int'l, catalog # 24988-197
Vortex mixer Signature™ High-Speed Microplate Shaker	Illumina, catalog #SC-201-1001 (110V) or 11140324 (220V)
Te-Flow rack, water circulator, tubing, and connections	Illumina, catalog # WG-10-211 (110V) and WG-10-212 (220V)
Glass back plate drying rack	Cole-Parmer. catalog # EW-06739-17
Multi-sample BeadChip alignment fixture	Illumina, catalog # WG-15-310
Hybridization chamber	Illumina, catalog # BD-60-402
Robot tip alignment guide-G	Illumina, catalog # SE-104-1015
Centrifuge, benchtop 120 V, for pre-amplification processes	General lab supplier
Centrifuge, benchtop refrigerated 120 V (8–3000 × g), for post-amplification processes	General lab supplier
Optical tachometer/stroboscope (recommended)	Cole-Parmer, catalog # A-87700-06
Tube rack • 2 separate sets: 1 for pre- and 1 for post-amplification processes • Any rack fitting the Infinium reagent 17 mm tube diameter	VWR, Int'l, catalog # 66023-540

Equipment	Supplier
Tube vortexers • 2 separate sets: 1 for pre- and 1 for post-amplification processes	General lab supplier
Vacuum source, hose, or pump capable of pulling greater than 508 mm Hg	General lab supplier
Vacuum desiccator tubing	VWR, Int'l , catalog # 62995-335
Vacuum desiccator racks	VWR, Int'l , catalog # 66023-526
[Optional] High-capacity desiccator	LabConco, catalog # 5530000

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
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Belgium	+32 80077160	+32 34002973
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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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