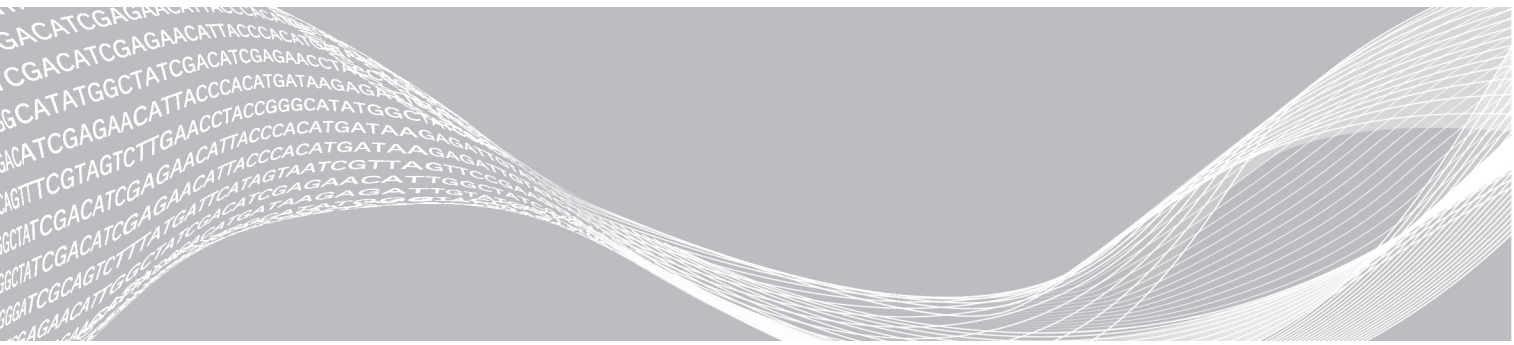
illumina®

Infinium CytoSNP-850K BeadChip

Reference Guide



Document # 15046990 v01

May 2019

Revision History

Document	Date	Description of Change
Document # 15046990 v01	May 2019	Document updated to reflect latest style and formatting standards. Removed Lab Setup and Maintenance chapter and added reference to *Infinium Assay Lab Setup and Procedures Guide (document # 11322460)* that consolidates all lab setup and maintenance requirements. Consumables and equipment information moved to *Infinium Assay Lab Setup and Procedures Guide (document # 11322460)*. Reference to Experienced User Card replaced with Infinium CytoSNP-850K BeadChip Checklist (document # 1000000069531) which provides current format of experienced user instructions.
Document # 15046990 Rev. C	June 2015	Removed references to BlueGnome and iScan System procedures. Referenced the *iScan System User Guide (part # 11313539)* and *NextSeq 550 System User Guide (part # 15069765)* for scanning instructions.
Document # 15046990 Rev. B	October 2013	Added incubation step to the resuspend DNA step. Corrections reagents in the MSA1 plate creation and incubation step.
Document # 15046990 Rev. A	August 2013	Initial release.

Table of Contents

Chapter 1 Overview

Introduction

The Infinium™ CytoSNP-850K BeadChip Kit provides comprehensive coverage of cytogenomic-relevant genes for constitutional and cancer applications. It provides robust performance across a range of sample types, including formalin-fixed paraffin-embedded (FFPE) tissue.

The CytoSNP-850K BeadChip uses an exon-centric design to target 3262 genes.

- The constitutional coverage includes updates and content evolution from the International Collaboration for Clinical Genomics (ICCG).
- The cancer coverage includes all Cancer Cytogenomics Microarray Consortium (CCMC) genes.

Important Note

Before using the procedures in this guide, read the *Infinium Lab Setup and Procedures Guide*. The guide explains how to equip and run an Infinium CytoSNP-850K v1.2 BeadChip laboratory, including information on the following topics:

- Prevention of amplification product contamination
- Safety precautions
- Consumables and equipment to purchase in advance
- Standard lab procedures
- Preparation for BeadChip imaging
- System maintenance
- Troubleshooting

The instructions apply equally to all Infinium BeadChips provided by Illumina. All CytoSNP-850K BeadChip documentation assumes that you have already set up the laboratory space and that you are familiar with the standard procedures and safety precautions.

Additional Resources

The Infinium CytoSNP-850K v1.2 BeadChip Kit support pages on the Illumina website provide additional kit resources. These resources include software downloads, training, product compatibility, and the following documentation. Always check support pages for the latest versions.

Resource	Description
Infinium CytoSNP-850K BeadChip Checklist (document # 1000000069531)	Provides a checklist of steps for experienced users of the CytoSNP-850K BeadChip.
Infinium Assay Lab Setup and Procedures Guide (document # 11322460)	Describes how to set up an Workflow lab including reagents, consumables, and equipment to purchase in advance, and best practices for lab operation.
Infinium Consumables and Equipment List (document # 1000000084294)	Provides an interactive checklist of Illumina-provided and user-provided consumables and equipment.

Using the CytoSNP-850K Lab Planner

Use the CytoSNP-850K Lab Planner to track samples from a 96-well plate to a BeadChip, and then in the BlueFuse Multi Software. The lab planner is a template in XLS format that includes instructions for using the template and the following worksheets:

- Samples and barcodes—Record a unique identifier and description for each sample, and the BeadChip barcode (one per eight samples).
- Lab plan—Automatically populates the sample layout in a 96-well plate and on the BeadChips. Can be printed for use as a reference when performing the assay.
- Lab tracking form—Print and use to record lot numbers, barcodes, and time metrics when performing the assay.
- Batch—Record the directory where the GTC files, manifest files, cluster files, and BlueFuse annotation database are saved. This information is included in the batch import file the software requires.

DMAP Files

Scanning requires decode files (DMAP), a manifest file, and a cluster file for the BeadChip.

- Use the Illumina DMAP Decode File Client to download the necessary DMAP files. For more information, see the *DMAP Decode File Client User Guide (document # 11337856)*.
- Download the manifest and cluster files from the Infinium CytoSNP-850K v1.2 BeadChip Kit support pages.

Preparation and Storage of User-Supplied Reagents

Maintain a first in, first out (FIFO) system for reagents. Rotate the stock of the remaining reagents to avoid using expired reagents.

To minimize errors when preparing user-supplied reagents, prepare large batches of 0.1 N NaOH and 95% Formamide/1 mM EDTA using the following guidance.

Preparing Batches of 0.1 N NaOH

Prepare fresh 0.1 N NaOH in large batches. Divide batches into 15ml or 50 ml sealed tubes.

You can store the sealed tubes for 6 months at 2°C to 8°C and use the stored 0.1 N NaOH as needed. Use the 0.1 N NaOH the same day you open the tube, and discard any unused amounts.

Preparing Batches of 95% Formamide/1 mM EDTA

Prepare the 95% formamide/1 mM EDTA mixture in large batches. Divide batches into 15 ml sealed tubes.

You can store the sealed tubes for up to 5 months at -25°C to -15°C and use the stored mixture as needed. Use the mixture the same day you open the tube, and discard any unused amounts.

Tips and Techniques

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

Avoiding Cross-Contamination

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

Reagent Reuse

- After each protocol step, discard unused reagents per facility standards.
- The volume of each wash buffer (PB1 and XC4) is sufficient for one use.
- XC4 is reusable up to six times during a two-week period (maximum 24 BeadChips).

Sealing the Plate

- Always seal plates before vortexing 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.

Pipetting

- Make sure that pipettes are properly calibrated, clean, 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.

Handling BeadChips

- Touch the BeadChip at the barcode or along the edges only. Avoid the beadstripe area and sample inlets.
- BeadChips are glass. Inspect them for broken edges before use and handle with care.

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, and 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, the appropriate hybridization chambers can be investigated.
- When the hybridization chamber inserts contain BeadChips, keep them steady and level when lifting or moving.
  - Avoid shaking, and always keep parallel to the lab bench.
  - Do not hold by the sides near the sample inlets.

Acronyms

Acronym	Definition
ATM	Anti-Stain Two-Color Master Mix
EtOH	Ethanol
FMS	Fragmentation Solution
MA1	Multi-Sample Amplification Mix 1
MA2	Multi- Sample Amplification Mix 2
MSM	Multi- Sample Master Mix
PB1	Prepare BeadChip Buffer 1
PB2	Humidifying Buffer
PM1	Precipitation Solution
RA1	Resuspension, Hybridization, and Wash Solution
STM	Superior Two-Color Master Mix
TEM	Two-Color Extension Master Mix
XC1	XStain BeadChip Solution 1
XC2	XStain BeadChip Solution 2
XC3	XStain BeadChip Solution 3
XC4	XStain BeadChip Solution 4

Chapter 2 Protocol

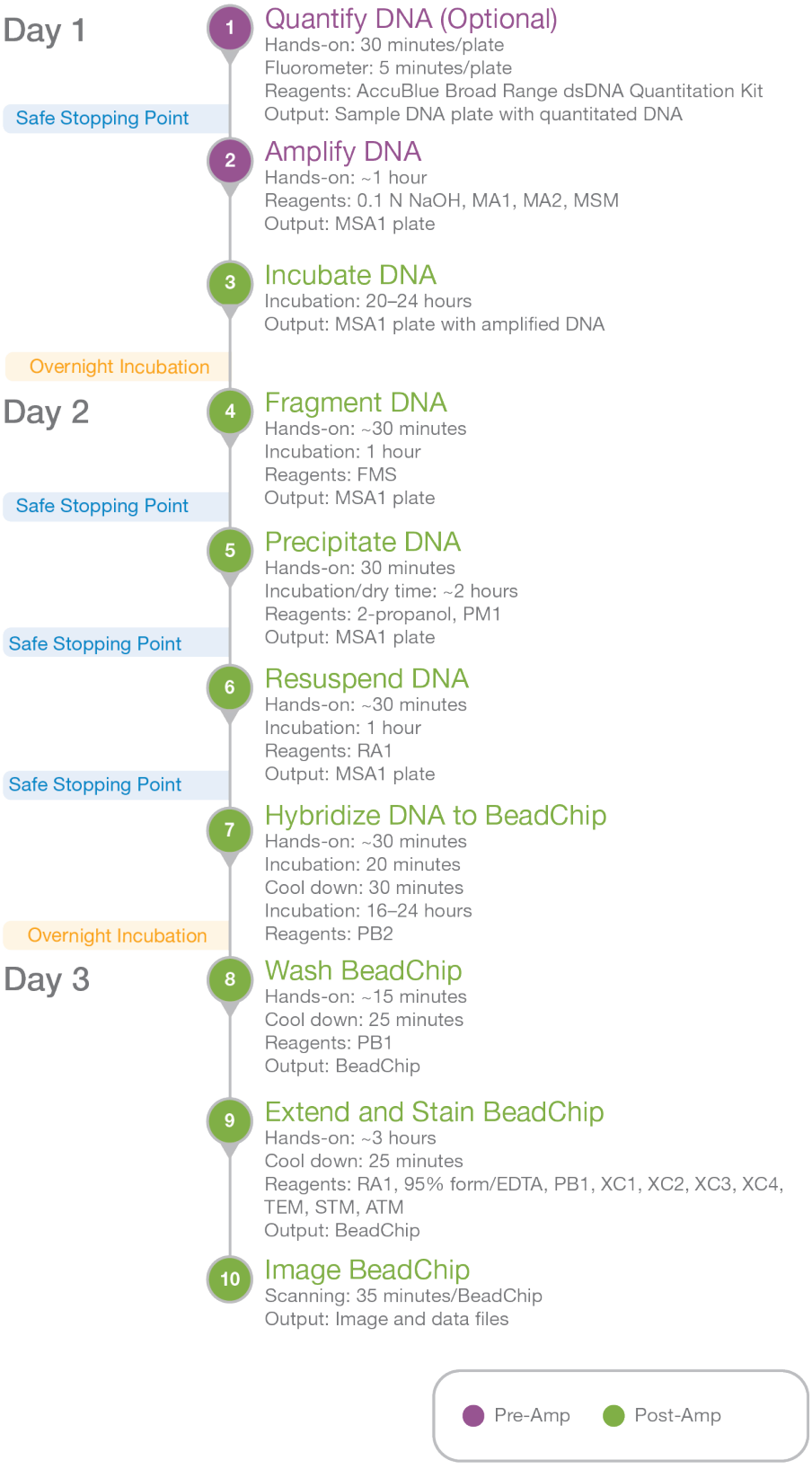
Introduction

This chapter describes the Infinium CytoSNP-850K v1.2 BeadChip Kit protocol.

- Follow the protocol in the order described using the specified volumes and other parameters.
- Confirm your kit contents and make sure that you have the required consumables and equipment for both pre-amp and post-amp areas.
- Use the CytoSNP-850K Lab Planner to record start and stop times and reagent and sample information throughout the assay.

Microarray Workflow

The following diagram illustrates the manual workflow for preparing DNA samples on the Infinium CytoSNP-850K v1.2 BeadChip Kit. Safe stopping points are marked between steps.


Day 1
1
Quantify DNA (Optional)
Hands-on: 30 minutes/plate
Fluorometer: 5 minutes/plate
Reagents: AccuBlue Broad Range dsDNA Quantitation Kit
Output: Sample DNA plate with quantitated DNA
Safe Stopping Point
2
Amplify DNA
Hands-on: ~1 hour
Reagents: 0.1 N NaOH, MA1, MA2, MSM
Output: MSA1 plate
3
Incubate DNA
Incubation: 20–24 hours
Output: MSA1 plate with amplified DNA
Overnight Incubation
Day 2
4
Fragment DNA
Hands-on: ~30 minutes
Incubation: 1 hour
Reagents: FMS
Output: MSA1 plate
Safe Stopping Point
5
Precipitate DNA
Hands-on: 30 minutes
Incubation/dry time: ~2 hours
Reagents: 2-propanol, PM1
Output: MSA1 plate
Safe Stopping Point
6
Resuspend DNA
Hands-on: ~30 minutes
Incubation: 1 hour
Reagents: RA1
Output: MSA1 plate
Safe Stopping Point
7
Hybridize DNA to BeadChip
Hands-on: ~30 minutes
Incubation: 20 minutes
Cool down: 30 minutes
Incubation: 16–24 hours
Reagents: PB2
Overnight Incubation
Day 3
8
Wash BeadChip
Hands-on: ~15 minutes
Cool down: 25 minutes
Reagents: PB1
Output: BeadChip
9
Extend and Stain BeadChip
Hands-on: ~3 hours
Cool down: 25 minutes
Reagents: RA1, 95% form/EDTA, PB1, XC1, XC2, XC3, XC4, TEM, STM, ATM
Output: BeadChip
10
Image BeadChip
Scanning: 35 minutes/BeadChip
Output: Image and data files
Pre-Amp
Post-Amp

Amplify DNA

This step adds the genomic DNA (gDNA) samples to the MSA1 plate. The samples are denatured and neutralized in the plate, then amplified during overnight incubation to produce sufficient input for the assay.

NOTE

The lab tracking form calls this step Make and Incubate DNA.

Consumables

- 0.1 N NaOH
- DNA samples (50 ng/µl)
- MA1
- MA2
- MSM
- 96-well 0.8 ml midi plate

Preparation

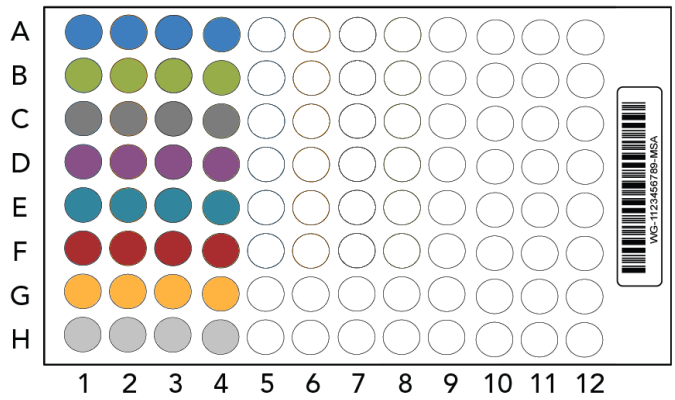
1 In the post-amp area, preheat the Illumina Hybridization Oven to 37°C.

2 Prepare the following consumables:

Item	Storage	Instructions
DNA	-25°C to -15°C	Thaw at room temperature.
MA1	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix, and then pulse centrifuge.
MA2	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix, and then pulse centrifuge.
MSM	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix, and then pulse centrifuge.

Procedure

1 Add 20 µl MA1 to the midi plate wells to prepare the MSA1 plate. Fill wells per the following diagram, where column 1 contains samples for one BeadChip. The following diagram depicts 4 BeadChips or 32 samples.



2 Transfer 4 µl DNA sample (50 ng/µl) from the DNA plate or tubes to the corresponding wells of the MSA1 plate.

3 Add 4 µl 0.1 N NaOH to each sample well of the MSA1 plate.
4 Seal the MSA1 plate with a 96-well cap mat.
5 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
6 Incubate for 10 minutes at room temperature.
7 Remove the sealing mat and set aside upside down in a safe location.
8 Add 68 µl MA2 to each sample well of the MSA1 plate.
9 Add 75 µl MSM to each sample well of the MSA1 plate.
10 Reseal with the cap mat using the original orientation.
11 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
12 Incubate in the preheated Illumina Hybridization Oven for 20–24 hours.

NOTE
Perform the remaining protocol steps in the post-amplification area.

Fragment DNA

This step enzymatically fragments the DNA, using endpoint fragmentation to avoid overfragmentation.

Consumable

- FMS (1 tube/96 samples)

Preparation

1 Preheat the heat block containing the midi plate insert to 37°C.
2 Prepare the following consumable:

Item	Storage	Instructions
FMS	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix, and then pulse centrifuge.

3 Remove the MSA1 plate from the hybridization oven.

Procedure

1 Pulse centrifuge the MSA1 plate to 280 × g.
2 Remove the sealing mat and set aside upside down in a safe location.
3 Add 50 µl FMS to each sample well.
4 Reseal with the cap mat using the original orientation.
5 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
6 Incubate on the preheated heat block for 1 hour. Do not incubate for longer then 2 hours.
7 Unless you are stopping, you can complete preparation for the next step while the plate incubates.

SAFE STOPPING POINT

If you are stopping, seal the plate, and store at -25°C to -15°C for up to 24 hours.

Precipitate DNA

This step uses 100% 2-propanol and PM1 to precipitate the DNA.

Consumables

- 100% 2-propanol
- PM1

Preparation

1 Leave the MSA1 plate on the heat block until preparation is complete. If frozen, thaw at room temperature then pulse centrifuge at 280 × g.

2 Preheat a heat block to 37°C.

3 Prepare the following consumable:

Item	Storage	Instructions
PM1	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.

Procedure

1 Add 100 µl PM1 to each sample well.

2 Reseal with the cap mat using the original orientation.

3 Vortex at 1600 rpm for 1 minute.

4 Incubate on the preheated heat block for 5 minutes.

5 Pulse centrifuge at 280 × g.

6 Set the centrifuge at 4°C to prepare for the next centrifuge step.

7 Add 300 µl 100% 2-propanol to each sample well.

8 Seal with a ***new, dry*** cap mat. Avoid shaking the plate until the cap mat is seated.

9 Invert 10 times to mix.

10 Incubate in a refrigerator set at 4°C for 30 minutes.

11 Place in the 4°C centrifuge opposite another plate of equal weight.

12 Centrifuge at 3000 × g for 20 minutes.
- When centrifuging is complete, proceed ***immediately*** to the next step to avoid dislodging the blue pellet.
- If a delay occurs, repeat the 20 minute centrifuge.

13 Remove the plate from centrifuge.

14 Remove and discard the cap mat.

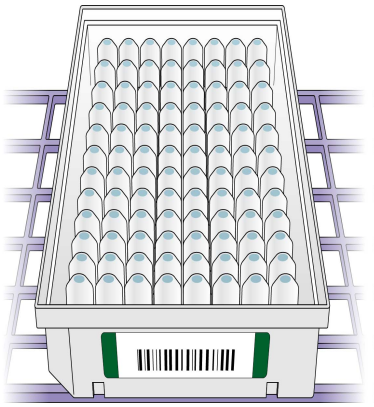
15 Hold the plate over an absorbent pad and do as follows.

a Quickly invert to decant the supernatant.

b Drain liquid onto the absorbent pad, and then smack the plate down. Avoid the liquid drained onto the pad.

16 Keeping the plate inverted, firmly tap until all wells are free of liquid (~1 minute). Do not allow supernatant to pour into other wells.

17 Place the uncovered, inverted plate on a tube rack for 1 hour at room temperature to air-dry the pellet.



18 Make sure that a blue pellet is present in the bottom of each sample well.

SAFE STOPPING POINT

If you are stopping, seal the MSA1 plate with a new sealing mat and store at -25°C to -15°C for up to 24 hours.

Resuspend DNA

The step uses RA1 to resuspend the precipitated DNA.

Consumable

- RA1

About Reagent

- Dispense only the volume necessary for each step. The *Extend and Stain BeadChips* procedure also requires RA1.
- Use fresh RA1 for each step that requires it. Properly stored RA1 that has not been dispensed for this resuspension step or the extend and stain step is considered fresh.
- RA1 might form visible precipitate or crystals. Before each use, hold in front of a light and inspect. Invert several times to redissolve the solution as needed.

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 If you plan to proceed immediately from this resuspension procedure to the subsequent *Hybridize DNA to the BeadChip* procedure, preheat the heat block to 95°C.

2 Prepare the following consumable:

Item	Storage	Instructions
RA1	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix. Alternatively, heat at 48°C for 15 minutes.

3 Preheat the Illumina Hybridization Oven to 48°C.

4 Preheat the heat sealer for a minimum of 10 minutes before use.

5 Invert thawed RA1 several times to dissolve.

Procedure

1 Add 46 µl RA1 to each pellet well of the MSA1 plate.

2 With the dull side facing down, apply a foil heat seal to the plate. Hold the heat sealer sealing block down firmly and evenly for 5 seconds.

3 Firmly roll the rubber plate sealer over the plate until all 96 well indentations are visible through the foil.

4 If all 96 wells are not defined, reapply the heat sealer.

5 Incubate in the preheated Illumina Hybridization Oven for 1 hour.

6 Vortex at 1800 rpm for 1 minute, and then pulse centrifuge at 280 × g. Repeat as needed to resuspend the pellets.

7 [Optional] Unless you are stopping, you can set aside the MSA1 plate for up to 1 hour before proceeding.

SAFE STOPPING POINT

If you are stopping, store the sealed MSA1 plate at -25°C to -15°C and RA1 at 2°C to 8°C for up to 24 hours.

Alternatively, store the MSA1 plate at -85°C to -65°C for up to one week. For more than 24 hours, store RA1 at -25°C to -15°C.

Hybridize DNA to the BeadChip

This step dispenses the fragmented, resuspended DNA onto BeadChips. Incubation then hybridizes each DNA sample to a section of the BeadChip.

Consumables

- 100% EtOH (Ethanol)
- PB2 (Humidifying Buffer)
- XC4 (XStain BeadChip Solution 4)

About Reagents

- Keep XC4 in the original bottle until you are ready to use it.
- Each XC4 bottle contains sufficient reagent to process up to 24 BeadChips.
- Use resuspended XC4 at room temperature.

Preparation

1 If frozen, thaw the MSA1 plate at room temperature, and then pulse centrifuge at 280 × g.

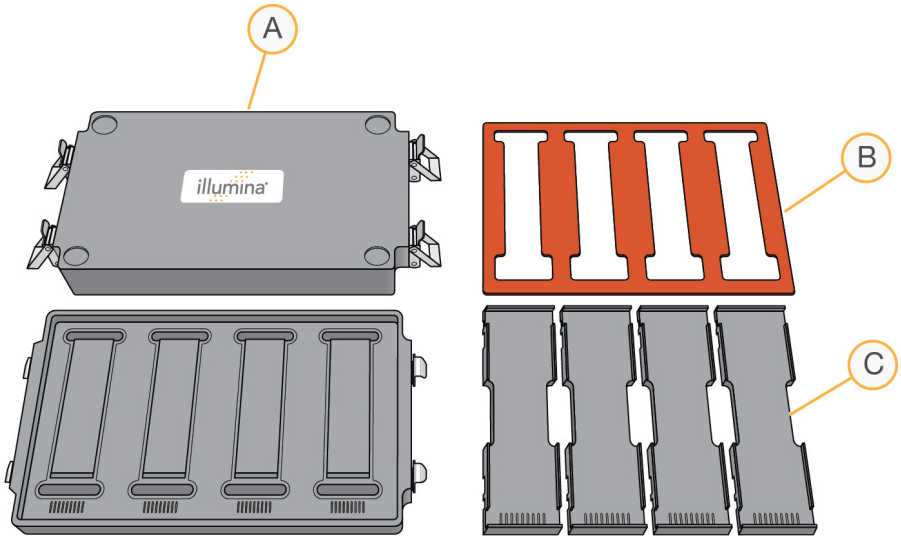
2 Preheat the heat block to 95°C.

3 Preheat the Illumina Hybridization Oven to 48°C.

Assemble the Hybridization Chambers

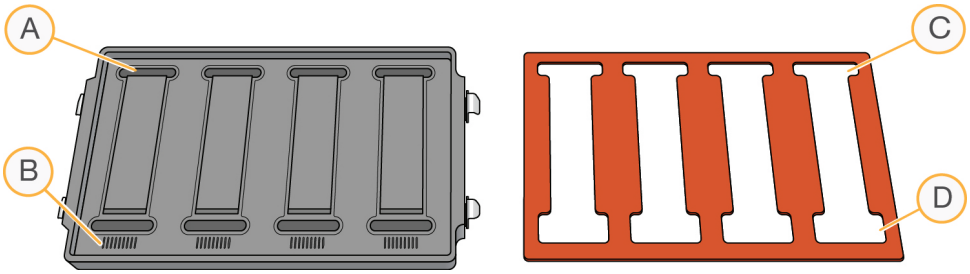
1 Place the MSA1 plate on the preheated heat block for 20 minutes to denature the DNA. During the incubation, proceed with the remaining assembly steps.

2 Place the hybridization chambers, hybridization chamber gaskets, and hybridization chambers inserts on the benchtop.



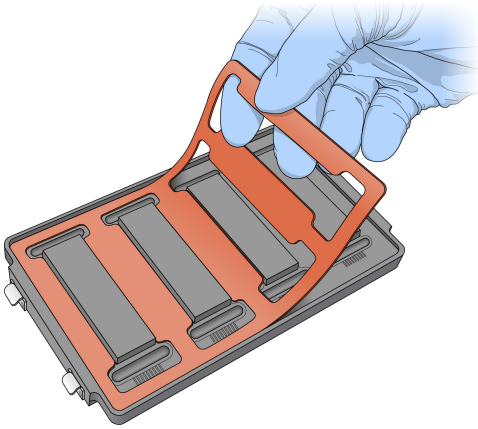
A Hybridization chambers
B Hybridization chamber gaskets
C Hybridization chamber inserts

3 Align the wider edge of the gasket to the barcode ridges.

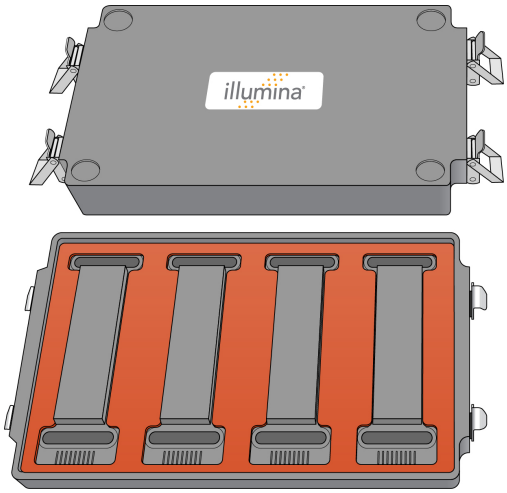


A Reagent reservoirs
B Barcode ridges
C Narrower gasket edges
D Wider gasket edges

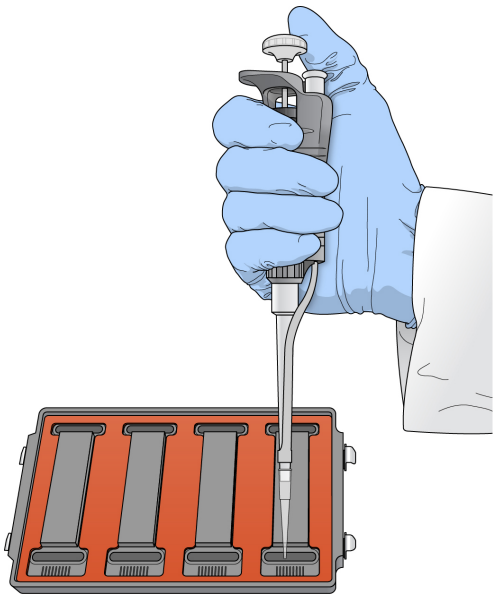
4 Place the gasket onto the chamber, and then press it into place.



5 Make sure that the gaskets are properly placed and seated, as shown in the following illustration.



6 Add 200 µl PB2 to the top and bottom reservoirs of each BeadChip.



7 Immediately cover the chamber with the lid to prevent evaporation.
Locking the lid is not necessary.

8 Leave the closed chambers on the benchtop at room temperature until the BeadChips are loaded with DNA (~1 hour).

9 When the 20 minute incubation is complete, transfer the MSA1 plate from the heat block to the benchtop and cool at room temperature for 30 minutes.

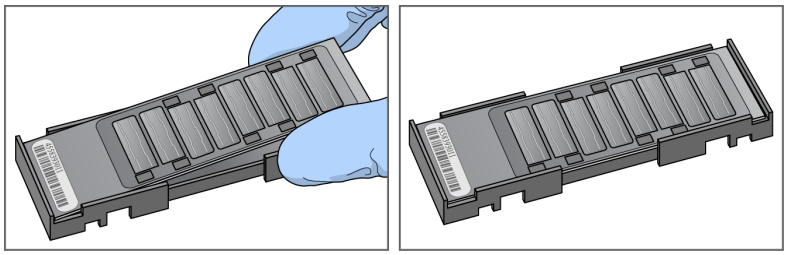
Procedure

Load DNA Onto BeadChips

1 Pulse centrifuge the MSA1 plate at 280 × g.

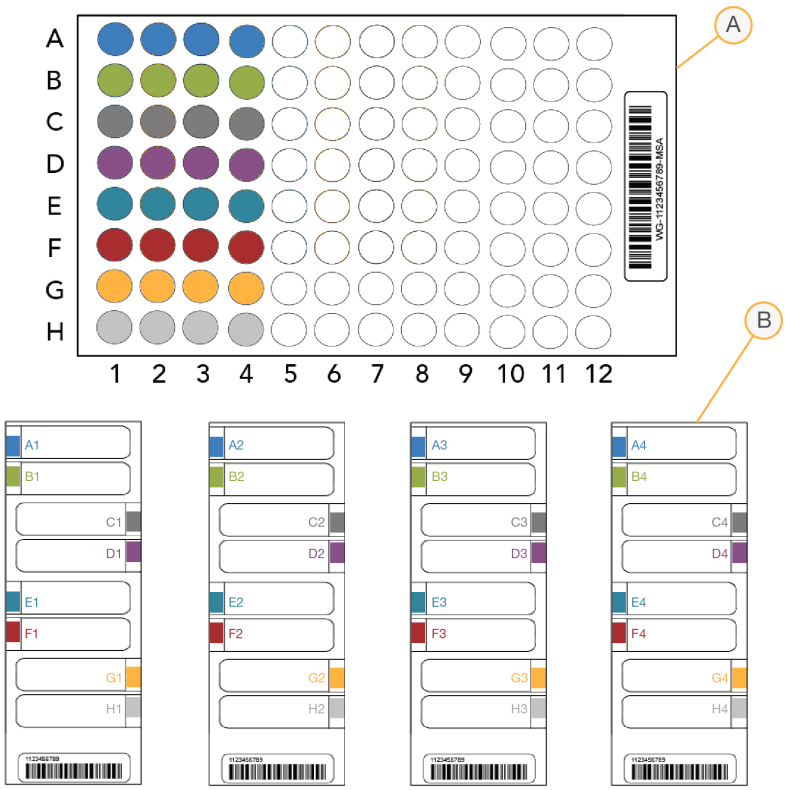
2 Remove the BeadChips from all packaging. Hold BeadChips by the ends, away from the sample inlets.

3 Place each BeadChip into an insert so that the barcode ends align.



4 Remove the foil seal from the MSA1 plate.

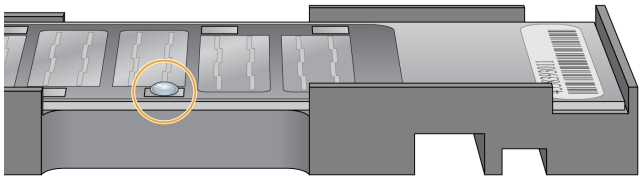
5 Transfer 26 µl each sample from the MSA1 plate to the appropriate section of the BeadChip.
- Insert the pipette into the sample inlet before dispensing.
- Load A1–H1, as shown in the following graphic.



A MSA1 plate
B BeadChips 1–4 (left to right)

6 Wait for the DNA to disperse over the entire surface.

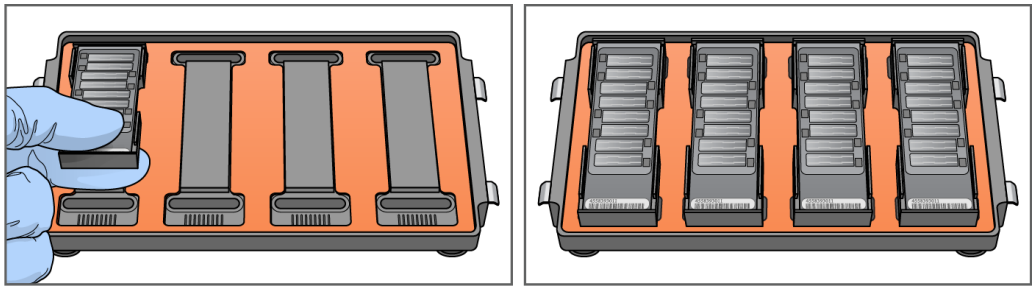
7 Inspect the loading port for excess liquid.



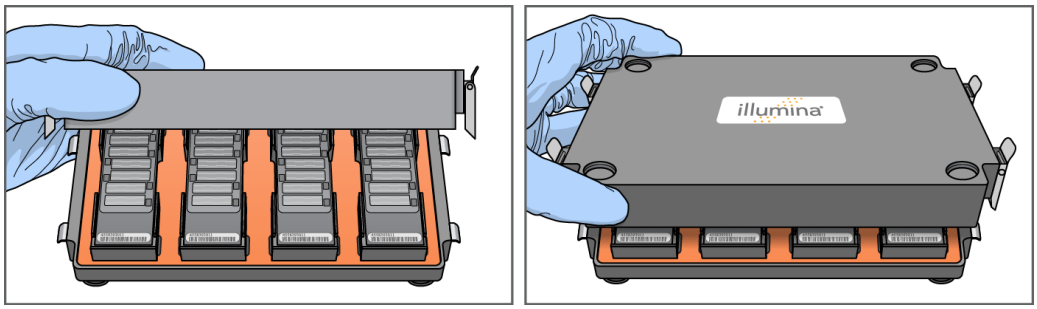
8 If excess liquid is not present, add leftover sample from the amplification plate to create a bolus around the loading port. Do not use RA1, which dilutes the sample.
Excess liquid is desired because it prevents evaporation and the creation of low-intensity areas.

Set Up BeadChips for Hybridization

1 Load the inserts containing BeadChips into the hybridization chamber.
- Position the barcode end over the ridges indicated on the chamber.
- Keep the inserts steady and level.

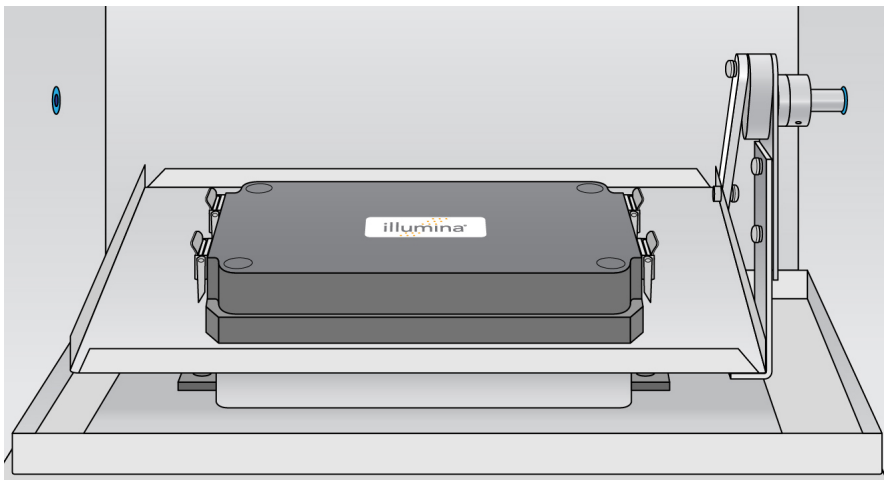


2 Place the back of the lid onto the chamber, and then slowly lower the front to avoid dislodging the inserts.



3 Close all four clamps so that the lid is secure and sits evenly on the base without any gaps.
Close the clamps in the following order: top-left, bottom-right, top-right, bottom-left.

4 Place the chamber into the preheated Illumina Hybridization Oven so that the top logo faces you.
You can stack up three chambers per row for a total of six chambers. Make sure that the feet of the top chamber fit into the indents on the bottom chamber.



5 Incubate at 48°C for 16–24 hours.

6 Store RA1 at 2°C to 8°C for use the next day.

7 Discard the MSA1 plate.

Resuspend XC4

Resuspend XC4 to prepare for the *Extend and Stain BeadChips* step.

1 Add 330 ml fresh 100% EtOH to the XC4 bottle.

The resulting volume is ~ 350 ml.

2 Vigorously shake XC4 to resuspend.

3 **[Optional]** Store at 2°C to 8°C and use up to six times over a period of two weeks.

Wash BeadChips

This step prepares the BeadChips for the staining process.

Consumables

- 95% Formamide/1 mM EDTA
- ATM (Anti-Stain Two-Color Master Mix)
- PB1 (Hybridization Buffer) (550 ml for up to four BeadChips)
- STM (Superior Two-Color Master Mix)
- TEM (Two-Color Extension Master Mix)
- XC1 (XStain BeadChip Solution 1)
- XC2 (XStain BeadChip Solution 2)
- XC4 (XStain BeadChip Solution 4)

About Reagents

- Decant only the reagent volume needed for each step. Some reagents are needed later in the protocol.
- Excepting PB1, all reagents are prepared in this step for use in a subsequent step.

WARNING

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

Preparation

1 To prepare for washing, remove each hybridization chamber from the hybridization oven. Allow to cool for 25 minutes before opening.

2 Prepare the following items:
- Fill two wash dishes with 200 ml PB1 each and label them accordingly.
- Using a graduated cylinder, fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PB1.

3 Remove the following Te-Flow flow-through chamber components from storage:
- Black frames
- Spacers (separated for ease of handling)
- Clean glass back plates
- Clamps

4 Prepare the following consumables for the subsequent *Extend and Stain BeadChips* step:

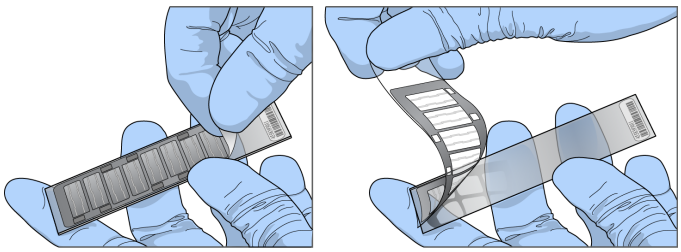
Item	Storage	Instructions
95% Formamide/1mM EDTA	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.
ATM	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.
STM	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.
TEM	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.
XC1	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.
XC2	-25°C to -15°C	Thaw at room temperature. Invert 10 times to mix.
XC4	Room temperature	Shake vigorously to resuspend. If necessary, vortex until dissolved.

Procedure

1 Attach the wire handle to the wash rack, and then submerge the rack into one of the wash dishes containing 200 ml PB1.

2 Remove a BeadChip from the hybridization chamber, and then remove the cover seal as follows.

a Wearing powder-free gloves, position the BeadChip over an absorbent cloth or paper towels to absorb splatter. A hood is recommended.
b In one hand, hold the BeadChip so the barcode side faces up and is closest to you, and the top side is slightly angled away. Hold securely by the edges.
c Starting with a corner near the barcode, remove the seal in one continuous motion away from you and toward the opposite corner of the BeadChip.
d Discard the seal.



3 Slide the BeadChip into the wash rack so that the BeadChip is submerged in PB1.

4 Repeat steps 2 and 3 until all BeadChips are transferred to the submerged wash rack.

5 Lift the wash rack up and down for 1 minute, breaking the PB1 surface, to create slow and gentle agitation.

6 Move the wash rack to the other wash dish containing 200 ml clean PB1, submerging the BeadChips.

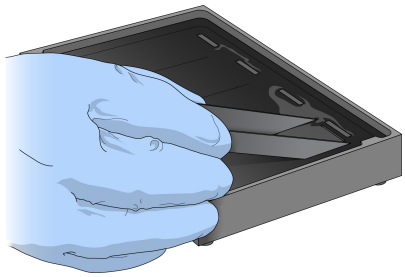
7 Repeat step 5 in the new wash dish.

8 Remove BeadChips from the wash rack and inspect them for residue.

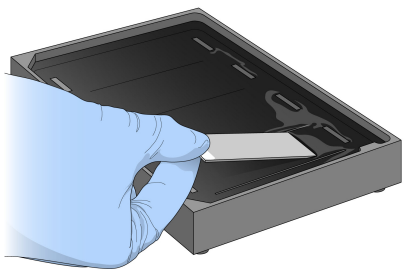
9 If residue is present, use a 200 µl pipette tip to scrape it away from the bead sections. Use a new pipette tip for each BeadChip.

Assemble Flow-Through Chambers

1 For each BeadChip, place one black frame into the BeadChip alignment fixture containing 150 ml PB1. For example, if you are processing four BeadChips, place four black frames into the fixture.

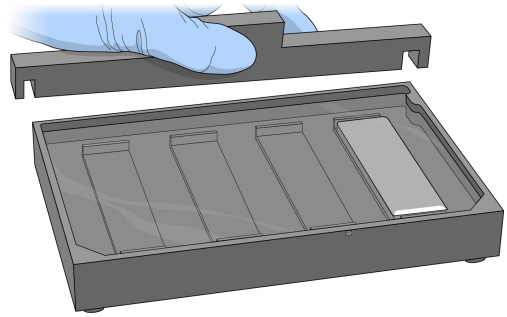


2 Place each BeadChip into a black frame, aligning the barcode with the Alignment Fixture ridges. Fully submerge each BeadChip.

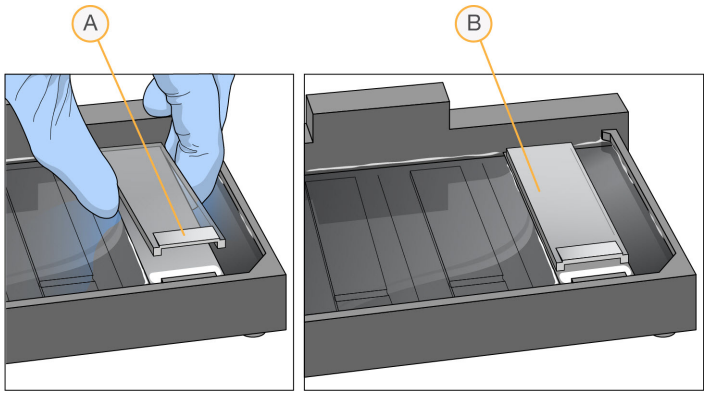


3 Place a *clear* spacer onto the top of each BeadChip. Use the alignment fixture grooves to guide the spacers into position.
The white spacers are not a substitute for the clear spacers.

4 Place the alignment bar onto the alignment fixture. Fit the groove on the alignment bar over the tab on the alignment fixture.



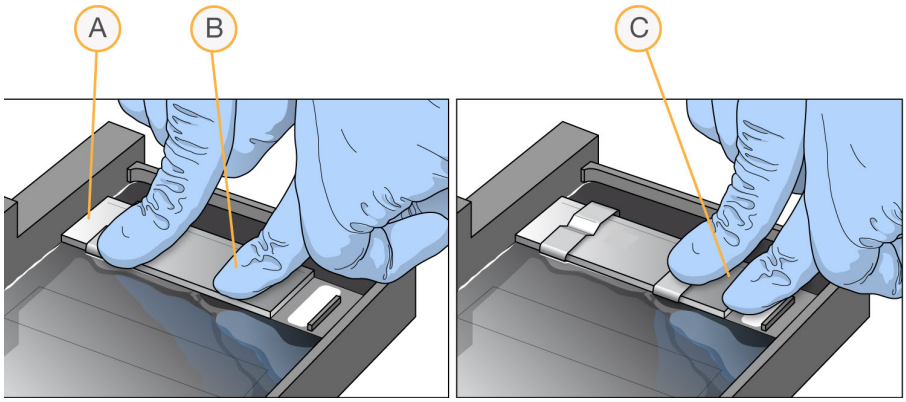
5 Place a clean glass back plate on top of each clear spacer. Position the plate reservoir at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface.



A Reservoir at the barcode end of the glass back plate.
B Glass back plate in position.

6 Secure each flow-through chamber assembly with metal clamps as follows.

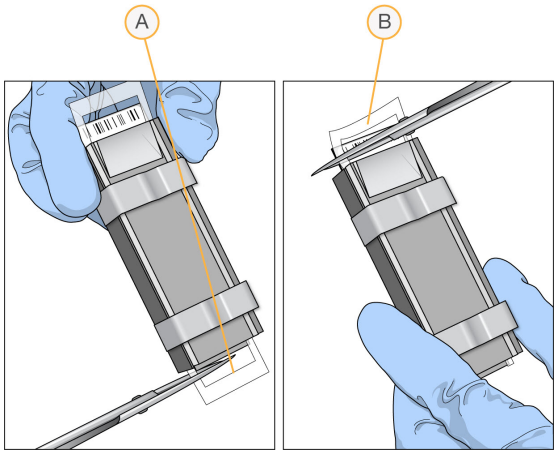
a Using one finger, gently push the glass back plate against the alignment bar.
b Place a metal clamp around the flow-through chamber 5 mm from the top edge.
c Place a second metal clamp around the flow-through chamber at the barcode end, 5 mm from the bottom of the reagent reservoir.



A One stripe is visible between the first clamp and the alignment bar.
B Glass back plate pressed against the alignment bar.
C Stripes are not visible between the second clamp and the barcode.

7 Remove the assembled flow-through chamber from the alignment fixture.

8 Starting at the nonbarcode end, trim the spacers from each end of the assembly using scissors.



A Trimming the spacer at the nonbarcode end.
B Trimming the spacer at the barcode end.

9 Leave the assembled flow-through chambers in PB1 in the alignment fixture until ready to load onto the chamber rack in *Extend and Stain BeadChips* on page 21.
- Do not place on absorbent paper.
- Do not place in the chamber rack until instructed to do so.

10 Wash the hybridization chamber reservoirs with deionized water.
Immediate and thorough washing ensures complete removal of PB2 from the wells.

Extend and Stain BeadChips

This step washes unhybridized and nonspecifically hybridized DNA samples from the BeadChips, adds labeled nucleotides to extend primers hybridized to the sample, and stains the primers. After the flow-through chambers are disassembled, the BeadChips are coated for protection.

Consumables

- 70 %EtOH (Ethanol)
- 95% formamide/1 mM EDTA (10 ml for up to 4 BeadChips)
- ATM (1 tube/4 BeadChips)
- PB1 (310 ml for up to 4 BeadChips)
- RA1 (5 ml/4 BeadChips)
- STM (1 tube/4 BeadChips)
- TEM (1 tube/4 BeadChips)
- XC1 (1 tube/4 BeadChips)
- XC2 (1 tube/4 BeadChips)
- XC3 (25 ml/4 BeadChips)
- XC4(310 ml for up to 4 BeadChips)

About Reagents

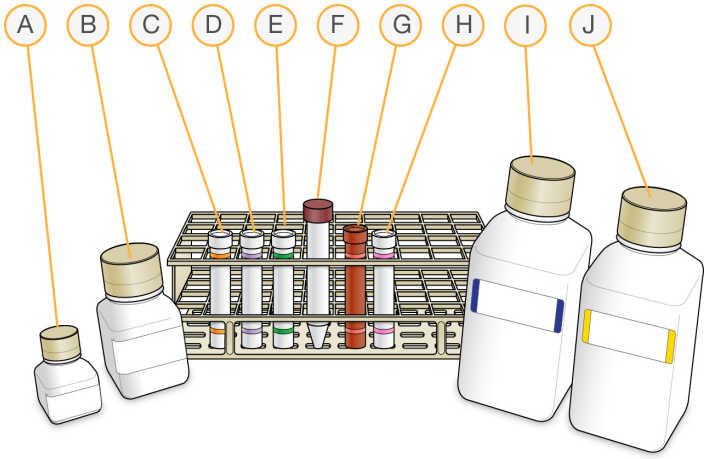
- Make sure that the label of each STM tube indicates the same stain temperature.
- Decant only the necessary volume of reagent.
- Use fresh RA1 for each step that requires it. Properly stored RA1 that has not been dispensed for the previous resuspension step or this extend and stain step is considered fresh.
- RA1 might form visible precipitate or crystals. Before each use, hold in front of a light and inspect. Invert several times to redissolve the solution as needed.
- The XC4 coat is slippery and makes the BeadChips difficult to hold. Self-locking tweezers grip the BeadChip firmly and help prevent damage.

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 Place reagent tubes in a rack in the order of use: RA1, XC1, XC2, TEM, 95% formamide/1 mM EDTA, STM, XC3, and ATM.



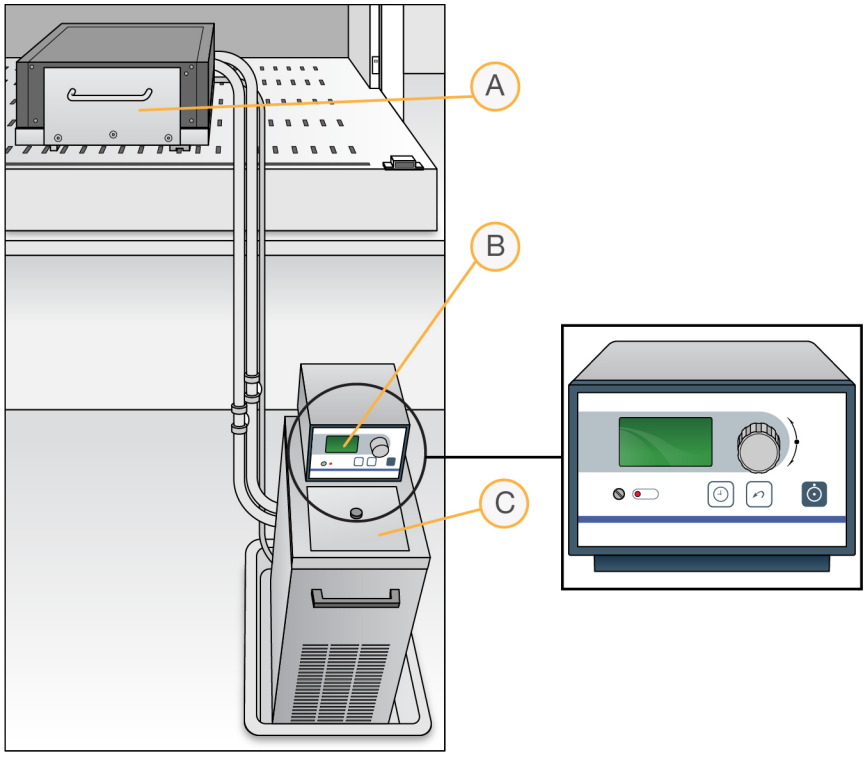
A RA1
B XC3
C XC1
D XC2
E TEM
F 95% formamide/1 mM EDTA
G STM
H ATM
I PB1
J XC4

Procedure

Set Up the Chamber Rack

1 Make sure that the water circulator is filled to the appropriate level.

2 Turn on the water circulator and set it to a temperature that brings the chamber rack to 44°C at equilibrium.
The temperature can vary depending on ambient conditions.



A Chamber rack
B Water circulator with programmable temperature controls
C Reservoir cover

3 Confirm the actual temperature using the chamber rack temperature probe.
The temperature displayed on the water circulator screen might not accurately reflect the chamber rack temperature.

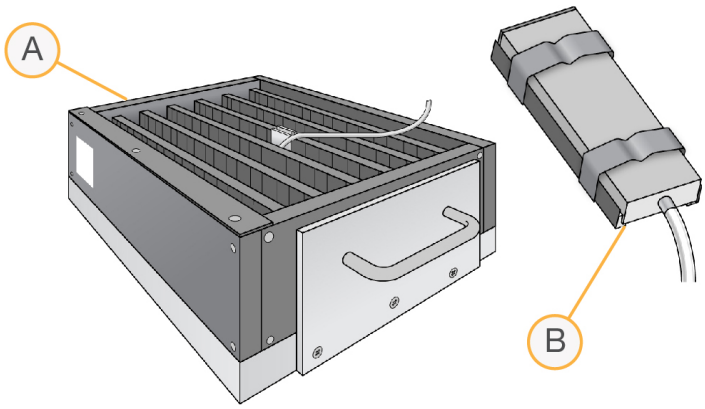
4 Remove bubbles trapped in the chamber rack.

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 pine in the reagent pan, make sure that the Te-Flow is flush.

5 Using the Illumina temperature probe, test at least three locations on the chamber rack.

a For accurate measurements, make sure that the temperature probe touches the base of the chamber rack.
b Make sure that all locations are at 44°C ± 0.5°C.
c If the temperature is not within ± 0.5°C, adjust the water circulator control knob to reach 44°C ± 0.5°C.

d Do not leave the temperature probe in the first three rows of the chamber rack. Reserve these rows for BeadChips.



A Chamber rack with temperature probe
B Temperature probe

Perform Single-Base Extension

1 When the chamber rack reaches 44°C ± 0.5°C, quickly place each flow-through chamber assembly into the chamber rack.

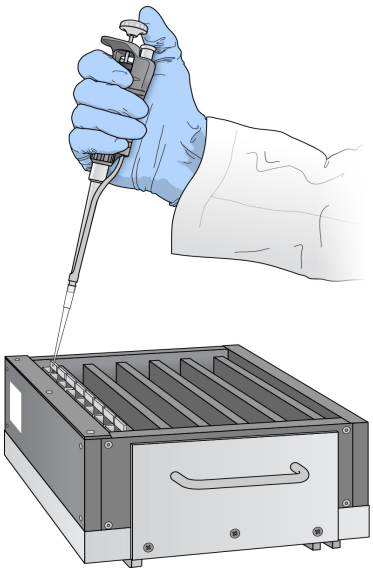
CAUTION

To avoid assay failure, complete this procedure without interruption.

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

3 Without allowing pipette tips to touch BeadChip surfaces, fill the reservoir of each flow-through chamber as follows.

a Add 150 µl RA1 and incubate for 30 seconds. Repeat five times.
b Add 450 µl XC1. Incubate for 10 minutes.
c Add 450 µl XC2. Incubate for 10 minutes.
d Add 200 µl TEM. Incubate for 15 minutes.
e Add 450 µl 95% formamide/1 mM EDTA and incubate for 1 minute. Repeat one time.
f Incubate for 5 minutes.
g Set the chamber rack temperature to the temperature indicated on the STM tube ± 0.5°C.
h Add 450 µl XC3 and incubate for 1 minute. Repeat one time.



4 Wait for the chamber rack to reach the appropriate temperature.

Stain BeadChips

1 Fill the reservoir of each flow-through chamber as follows.

a 250 µl STM. Incubate for 10 minutes.
b 450 µl XC3 and incubate for 1 minute. Repeat one time.
c Wait 5 minutes.
d 250 µl ATM. Incubate for 10 minutes.
e 450 µl XC3 and incubate for 1 minute. Repeat one time.
f Wait 5 minutes.
g 250 µl STM. Incubate for 10 minutes.
h 450 µl XC3 and incubate for 1 minute. Repeat one time.
i Wait 5 minutes.
j 250 µl ATM. Incubate for 10 minutes.
k 450 µl XC3 and incubate for 1 minute. Repeat one time.
l Wait 5 minutes.
m 250 µl STM. Incubate for 10 minutes.
n 450 µl XC3 and incubate for 1 minute. Repeat one time.
o Wait 5 minutes.

2 Immediately remove the flow-through chambers from the chamber rack and place in reserved alignment fixtures submerged in PB1 at room temperature on a lab bench.

Wash and Coat BeadChips

1 Gather the following equipment:
- Kimwipes, large
- Staining rack
- Self-locking tweezers
- Tube rack
- Vacuum desiccator

- Vacuum hose
- Wash dishes (2)

2 During the procedure, prevent dust or lint from entering the wash dishes.
   - Clean wash dishes with low-pressure air before use.
   - Cover wash dishes with wash dish covers when not in use.

3 Wash the tube racks and wash dishes thoroughly after each use.
   - Rinse with deionized water.
   - Dry racks and wash dishes upside down on a wash rack.

4 Place a clean tube rack on top of several layers of Kimwipes or an absorbent pad.
   After the staining rack containing BeadChips is removed from the XC4 wash dish, it is placed on this rack.

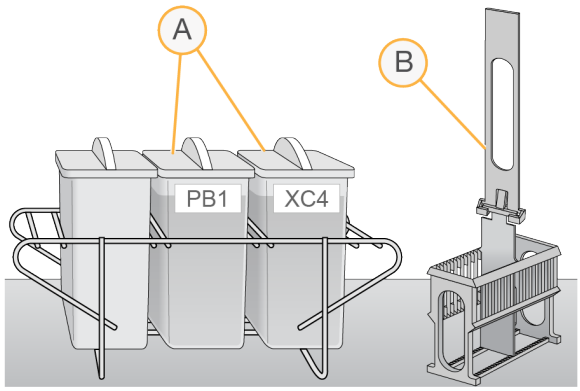
5 Prepare another clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per eight BeadChips.
   Kimwipes are not needed under this tube rack.

6 Set up two top-loading wash dishes labeled PB1 and XC4.

7 To indicate fill volume of each wash dish:

   a Add 310 ml water.
   b Mark the water level on the side.
   c Empty the water.

   Indicating fill volume before adding reagents allows you to add reagents directly from the bottles, minimizing contamination.



   A Labeled and filled wash dishes
   B Staining rack

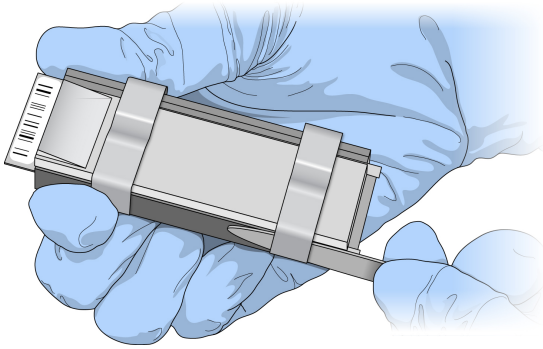
8 Add 310 ml PB1 to the PB1 wash dish.

9 Submerge the staining rack in the wash dish so that the locking arms and tab *face you*.
   This orientation ensures that you can safely remove the BeadChips.

   A Locking arms
   B Tab

10 Leave the staining rack in the wash dish for later use (carrying the BeadChips after disassembling the flow-through chambers).

11 *Using the dismantling tool*, remove the two metal clamps from a flow-through chamber.
The dismantling tool prevents chipping the glass back plates.



12 Lift the glass back plate straight up to remove. Set aside for cleaning after finishing this procedure.
Sliding the glass along the BeadChip can damage the BeadChip.

13 Remove the spacer, avoiding contact with the BeadChip stripes.

14 Remove the BeadChip from the black frame. Handle the BeadChip only by the barcode end or edges.

15 Repeat steps 11–14 to disassemble each flow-through chamber one at a time.

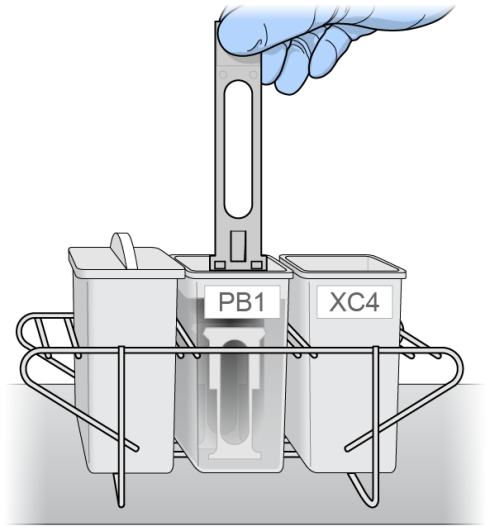
16 Place the BeadChips into the submerged staining rack. Make sure that the BeadChip barcodes face *away* from you and the locking arms face *toward* you.

CAUTION
Submerge each BeadChip as quickly as possible to prevent drying.

17 If necessary to seat a BeadChip, briefly lift the staining rack from the wash dish and seat the BeadChip.

18 Make sure that the BeadChips are submerged.

19 Slowly lift the staining rack up and down 10 times, breaking the PB1 surface. If the tops of the BeadChips touch, gently wiggle the staining rack to separate the slides.
Free circulation of PB1 between BeadChips is important.

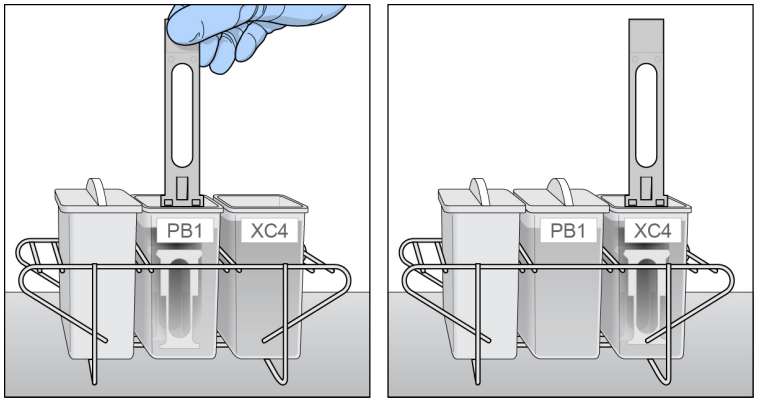


20 Soak for 5 minutes.

21 Vigorously shake the XC4 bottle to resuspend completely. If necessary, vortex until dissolved.

22 Add 310 ml to the XC4 wash dish.
- Cover to prevent lint or dust from entering.
- Do not let sit for more than 10 minutes.

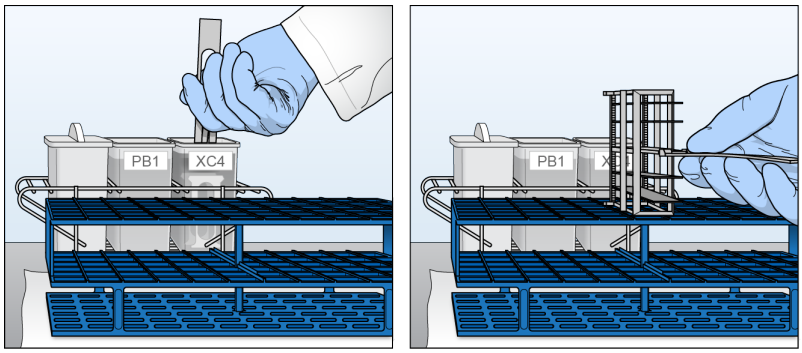
23 Transfer the staining rack from the PB1 wash dish to the XC4 wash dish.



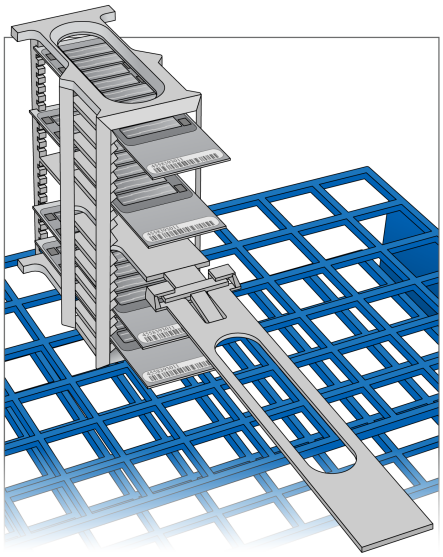
24 Slowly lift the staining rack up and down 10 times, breaking the XC4 surface. If the tops of the BeadChips touch, gently wiggle the staining rack to separate the slides.

25 Soak for 5 minutes.

26 Remove the staining rack in one quick motion and place it onto the prepared tube rack.



27 Make sure that the staining rack is in the center of the tube rack to ensure uniform coating. Avoid the raised edges.

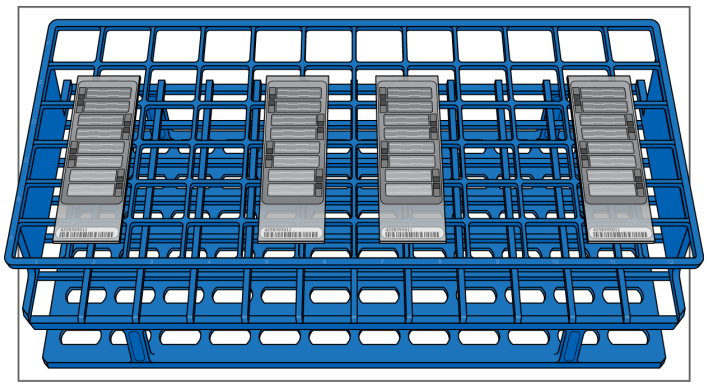


28 [Optional] Remove the staining rack handle to facilitate BeadChip removal:

a Holding the top of the staining rack in position, grasp the handle between your thumb and forefinger.
b Push up the tab with your thumb and push the handle away from you, unlocking the handle.
c Pull up the handle and remove.

29 Working top to bottom, dry each BeadChip as follows.

a Holding the staining rack handle (if present), use self-locking tweezers to grip the BeadChip by the barcode end.
b Place the BeadChip onto a tube rack with the barcode facing up and toward you. Do not place on the bottom rack or allow BeadChips to rest on the tube rack edge or touch each other.



Proper BeadChip placement prevents wicking, uneven drying, and pooled dye protectant.

30 Place the tube rack into the vacuum desiccator.
Each desiccator can hold one tube rack (eight BeadChips).

31 Make sure that the vacuum valve is seated tightly and securely.

32 Remove the red plug from the three-way valve.

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

34 Gently lift the vacuum desiccator lid to ensure proper sealing. Make sure that the lid does not lift off the desiccator base.

35 Dry for 50–55 minutes.
Room temperature and humidity can cause variable drying times.

36 Turn the handle slowly to release the vacuum.

CAUTION

Air must enter the desiccator slowly to avoid disturbing the contents. Improper use of the vacuum desiccator can damage the BeadChips, particularly if you remove the valve plug while a vacuum is applied. For detailed instructions, see the desiccator documentation.

37 Return the desiccator to storage. Store with the red valve plug in the three-way valve of the desiccator to prevent dust and lint from accumulating in the valve port.

38 Touch the BeadChip ***borders*** (not the stripes) to make sure that the barcode sides of the BeadChips are dry to the touch.

39 Clean the back of each BeadChip using a Kimwipe sprayed with 70% EtOH:

a Hold the BeadChip at a downward angle to prevent excess EtOH from dripping onto the stripes.
b Without touching the stripes, wipe the underside of the BeadChip until XC4 is removed (5–6 times).

40 Clean the glass back plates.
For instructions, see the *Infinium Assay Lab Setup and Procedures Guide (document # 11322460)*.

SAFE STOPPING POINT

Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Scan within 72 hours.

Scan and Analyze BeadChips

The next step is to scan the BeadChips on the NextSeq 550 System or iScan System. For instructions, see the guide for your system: *NextSeq 550 System Guide (document # 15069765)* or *iScan System Guide (document # 11313539)*.

Output files are generated during the scan and stored in the specified output folder. When scanning is complete, you are ready to perform analysis using BlueFuse Multi Software. The software requires scanning data in genotype call (GTC) file format.

- By default, the NextSeq 550 System generates normalized data and associated genotype calls in GTC file format.
- The iScan System can be configured to generate GTC files in addition to intensity data (IDAT) files and other standard file types. Alternatively, use Illumina Beeline Software to convert IDAT files to GTC files after scanning. For more information, see the *Beeline Software 2.0 User Guide (document # 1000000022181)*.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
South Korea	+82 80 234 5300	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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

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

Document # 15046990 v01

Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

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

illumina®