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Infinium® Karyomapping Assay Protocol Guide

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Revision History

Part #	Revision	Date	Description of Change
15052710	02	March 2018	• Removed extraneous volume numbers throughout protocol.
15052710	01	February 2018	• Removed references to SureMDA kit and replaced with recommendations to amplify gDNA from embryo biopsies for use as starting material in the Infinium Karyomapping Assay.
15052710	B	June 2015	• Removed references to BlueGnome. • Edited "Scan BeadChip" section. Removed iScan procedures and referenced iScan System User Guide (part # 11313539). Added reference to NextSeq 550 System User Guide (part # 15069765).
15052710	A	February 2014	Initial release.

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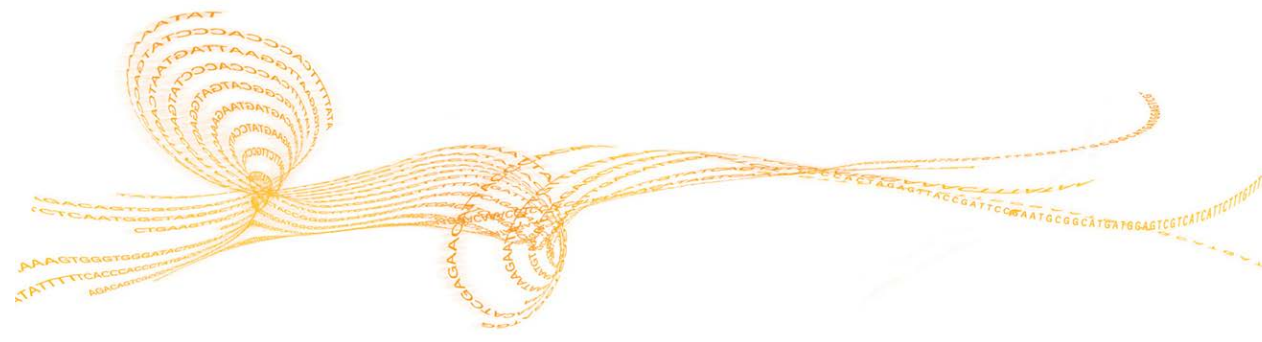

Introduction

Karyomapping is a molecular karyotyping method that can be used on a single cell or few cells from embryo biopsy samples. The method provides a comprehensive test for preimplantation genetic diagnosis (PGD) of single gene defects. It can be used where there is a risk of severe genetic disorders being inherited from parents. A couple could have a family history of a genetic disorder or have had a child with a genetic disease. PGD screening with karyomapping can be used to identify embryos that do not carry defective genes and can be safely transferred.

Infinium Karyomapping Assay Protocol

The Infinium Karyomapping Assay utilizes SNP array technology. It consists of BeadArray analysis using the HumanKaryomap-12 (v1.0) DNA Analysis Kit, scanning with the iScan System or NextSeq 550 System, and analysis/reporting using BlueFuse Multi software.

The assay requires single or multi-cell embryo biopsy samples amplified by Multiple Displacement Amplification (MDA) as starting material. The use of an MDA-based whole genome amplification (WGA) method is essential to amplify DNA from embryo biopsy samples (blastomere and trophectoderm biopsied cells) to quantities suitable for use in the Infinium Karyomapping Assay.

NOTE
Validate the suitability of your chosen MDA kit for use with the Infinium Karyomapping Assay. Illumina has found the REPLI-g SC kit (Qiagen Cat. No: 150343 or 150345) to be suitable for use with the Infinium Karyomapping Assay.

During the Infinium Karyomapping Assay, the MDA amplified products of the embryo biopsies, together with the parental and reference genomic DNA, are prepared and hybridized to the HumanKaryomap-12 BeadChip.

The Infinium protocol uses the HumanKaryomap-12 (v1.0) DNA Analysis Kit and features single-tube preparation and whole-genome amplification of samples, hybridization to the HumanKaryomap-12 BeadChip, and a two- step allele detection process using labeled nucleotides.

The Infinium Karyomapping Assay can be performed within 24 hours.

Audience and Purpose

This guide is for laboratory technicians running the Infinium Karyomapping Assay. The guide documents the laboratory protocols associated with the assay. It is important that all sections of this protocol are read before starting the assay. Follow all of the steps in the protocol in the order shown.

Tracking Tools

Illumina provides the following tools for sample tracking and guidance in the lab:

- The **Lab Tracking Form** guides you through the protocols with minimal illustrations and explanatory notes and includes user-entry fields for tracking progress and recording lot numbers, barcodes, and time metrics.

These documents and tools are available for download and reference at www.illumina.com/documentation.

Lab Setup and Maintenance

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Introduction

This chapter describes the essential equipment and operating procedures for an Infinium Karyomapping Assay laboratory. It explains how to equip a laboratory to perform the Infinium Karyomapping Assay, providing important information on the following topics:

- Safety precautions
- Preventing amplification product contamination
- Preparing and storing reagents
- Calibrating and using the vortexer
- Best practices
- Lab maintenance

The assay protocols assume that you are familiar with the contents of this chapter, have implemented all the recommendations, and have obtained all the requisite equipment, materials, and reagents.

Safety Precautions

CAUTION
Only qualified laboratory personnel can perform the protocols described in this guide. Exercise caution when handling biological samples to avoid cross-contamination among pre-amp and post-amp samples.

WARNING
This protocol uses an aliphatic amide (formamide) that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the safety data sheet for this assay at support.illumina.com/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

CAUTION
BeadChips are glass. Inspect for broken edges and handle with care.

Preventing Amplification Product Contamination

The Infinium Karyomapping Assay requires DNA generated from MDA-based WGA of embryo biopsies (blastomere or trophectoderm) as starting material. The first step of the Infinium Karyomapping Assay protocol is a further WGA of the MDA amplified embryo biopsies and genomic DNA from parents and reference samples to prepare the DNA for hybridization to the HumanKaryomap-12 BeadChip.

Unless sufficient caution is exercised, amplification products can contaminate reagents, instrumentation, and DNA samples, causing inaccurate and unreliable results. Amplification product contamination can shut down lab processes and significantly delay resumption of normal operations. For this reason, Illumina strongly encourages performing the pre-amplification (pre-amp) processes in a separate, dedicated laboratory space.

CAUTION
It is imperative to establish procedures for preventing amplification product contamination before working in the lab.

Separate Pre-Amplification and Post-Amplification Areas

The first step of the Infinium Karyomapping Assay protocol is the WGA of the DNA generated from MDA amplified embryo biopsies and the genomic DNA from parents and reference. The preparation for this amplification is included in *Prepare and Incubate the MSA3 Plate* on page 26. Ideally, this step should be performed in a pre-amp area specific for the WGA of the Infinium Karyomapping Assay.

The remaining Infinium Karyomapping Assay protocol steps can be performed in a post-amplification (post-amp) area that is a separate laboratory space from the amplification areas. The two laboratory areas are referred to as pre-amp and post-amp.

Dedicated Equipment and Supplies

Dedicate separate sets of instruments (pipettes, centrifuge, Illumina Hybridization Oven, heat block, etc) to the pre-amp and post-amp areas. Never share instruments between areas.

Follow these rules to avoid contaminating the pre-amp area:

- Never use the same sink to wash pre-amp and post-amp reservoirs.
- Never share the water purification system for pre-amp and post-amp processes.
- Store all supplies used in the Infinium Karyomapping Assay protocols in the pre-amp area and transfer to the post-amp area as needed.

Items Falling to Floor

The floor can be contaminated with amplification product transferred on the shoes of individuals coming from the post-amp area. Therefore, treat anything falling to the floor as if it were contaminated.

Throw away disposable items that have fallen to the ground at the completion of the assay. Disposable items can include empty tubes, pipette tips, gloves, or lab coat hangers. During the assay, never touch any items that have fallen to the ground.

Immediately and thoroughly clean nondisposable items that have fallen to the ground, such as pipettes or important sample containers. Use a 0.5% sodium hypochlorite (10% bleach) solution to remove product contamination.

Use a 0.5% sodium hypochlorite (10% bleach) solution to clean any lab surface that has contacted the contaminated item.

Individuals handling anything that has fallen to the floor, disposable or not, must throw away their lab gloves and put on a new pair.

Preparing and Storing Reagents

To minimize errors in preparing user-supplied reagents each day, prepare large batches of 0.1N NaOH and 95% Formamide/1 mM EDTA, then aliquot and store them as described in the following sections.

It is important to keep a first in, first out or FIFO system for reagents. Rotating the stock of the remaining reagents will help to avoid accidentally using expired reagents.

Prepare Batches of 0.1N NaOH

Prepare 0.1 N NaOH fresh on the day of use from a liquid 10 N NaOH stock. Alternatively, prepare it in large batches and aliquot it into 1 ml sealed tubes. These aliquots can be stored for up to 12 months at 2°C to 8°C and used in the protocol as needed. When you open an aliquot, use it on the same day that it was opened. Discard any reagent that is left at the completion of the assay.

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 sealed tubes. These aliquots can be stored up to 5 months at -25°C to -15°C and used in the protocol as needed. After you open an aliquot, use it on the same day that it was opened. Discard any reagent that is left at the end of the protocol.

WARNING

This protocol uses an aliphatic amide (formamide) that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the safety data sheet for this assay at support.illumina.com/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Best Practices

To optimize your data and minimize errors and waste, read and follow these best practices whenever performing the Infinium Karyomapping Assay protocols.

General Laboratory Practice

Perform the protocols described in this guide with care while wearing appropriate personal protective equipment, including laboratory coat, eye protection, and gloves.

Sample Mishandling

To reduce the risk of sample mishandling, it is important to witness and sign off critical steps when following the laboratory protocol.

Cleaning and Calibrating Pipettes

Make sure that pipettes are properly calibrated, clean, and decontaminated.

Pipette Carefully

Perform all pipette dispenses carefully and slowly to avoid creating turbulence within the plate wells and flow-through chambers.

Where possible, use a multichannel pipette to dispense reagents.

Handling Cap Mats

Orient the cap mat so that A1 on the cap matches A1 on the plate.

CAUTION
To prevent evaporation and spills, which could lead to assay variability and cross-contamination, make sure that all 96 caps are securely seated in the wells.

When you remove a cap mat, do so carefully and slowly to avoid splashing the contents. Set the cap mat aside, upside down, in a safe location for use later in the protocol. When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

Reagent Reuse

Never reuse excess reagents after dispensing. Discard according to your facility requirements.

Handling BeadChips

Avoid touching the BeadChip anywhere other than at the barcode end or on the edges.

Balancing the Centrifuge

Whenever you centrifuge plates or BeadChips, place a balance plate or rack with BeadChips opposite each plate or BeadChip rack being centrifuged. Make sure that the weights are as similar as possible.

Lab Maintenance

Perform the following standard lab maintenance procedures for Infinium Assay labs.

Daily and Weekly Bleaching

CAUTION
To prevent sample or reagent degradation, make sure that all bleach vapors have fully dissipated before starting any processes.

Post-amplification Area

Reducing the amount of product in the post-amp area reduces the risk of contamination in the pre-amp area.

Identify post-amp area hot spots that pose the highest risk of contamination, and clean these items daily with a 10% bleach solution.

Typical hot spots include:

- Bench space used to process amplified DNA
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards
- Centrifuges
- Vortexers
- Thermal cyclers

Once a week, thoroughly bleach the entire post-amp area, including benchtops and instruments that are not cleaned daily. Mop the floors with a 0.5% sodium hypochlorite (10% bleach) solution as well.

Provide training for personnel responsible for cleaning the lab areas so that they know how to prevent pre-amp product contamination.

Pre-amplification Area

Establish a daily and weekly bleaching schedule for the pre-amp area similar to the schedule for the post-amp area. Bleaching helps to eliminate product that could have entered the pre-amp area.

Identify high-risk pre-amp items such as the ones listed below, and clean them with a 0.5% sodium hypochlorite (10% bleach) solution each morning before beginning any pre-amp processes:

- Benchtops
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards

Once a week, thoroughly clean all laboratory surfaces and instruments, including all the benchtops and instruments that are not cleaned daily. Mop the floors with a 0.5% sodium hypochlorite (10% bleach) solution as well.

Provide training for personnel responsible for cleaning the lab areas so that they know how to prevent pre-amp product contamination.

Cleaning the Glass Back Plates

The glass back plates are used in the flow-through chambers to control the flow of sample and reagent onto the BeadChips. Clean the glass back plates when you open them for the first time and between uses. In addition, perform a more thorough cleaning after every 7 uses or weekly (depending on the throughput of your lab). Both procedures are described in the following sections.

Cleaning the Glass Back Plates after Every Use

When you open the glass back plates for the first time, and after every use, follow these instructions to clean them.

1. Prepare a 1% dilution (2.5 g per 250 ml) of Alconox Powder Detergent in water.
2. Dip each glass back plate into the solution.
3. Remove the glass back plates from the detergent solution and wipe them with a Kimwipe.
4. Hold the glass back plates under running DI water to remove any remaining detergent completely.
5. Allow the glass back plates to dry in the storage rack or by leaning the glass back plates at an angle against the black metal frames.
6. When the glass back plates are clean and dry, use a Kimwipe to wash with 70% EtOH.
7. Dry the glass back plates with a Whoosh duster or laboratory air gun.
8. To prevent surface damage to the glass back plates, store them in the Te-Flow Flow Cell storage box.

Cleaning the Glass Back Plates Weekly

In addition to the cleaning procedure in the previous section, clean the plates periodically after approximately every 7 uses and/or every week depending on individual lab throughput.

CAUTION
Wear a lab coat, safety goggles, and gloves during this cleaning process.

Consumables

Item	Quantity
Bleach (5.25% sodium hypochlorite, NaOCl)	
Deionized water	
9″ x 13″ Pyrex glass container	1
Fume hood	1
Backplate Tecan Storage rack	1
1L container for diluting bleach	1

CAUTION
Bleach is an irritant. Use caution when handling.

WARNING
Do not perform this cleaning protocol in the BeadChip production lab. Bleach fumes can degrade both the BeadChips and the fluorescent dyes used in the Infinium Assay.

Preparation

- Dilute bleach (5.25% sodium hypochlorite) with deionized water to 10% (0.525% hypochlorite) in a 1L container. Mix thoroughly.
  Example: Add 100 ml of bleach to 900 ml deionized water.

Cleaning Steps

1 Perform the following steps in the fume hood:

NOTE
Perform these steps in a location separate from the Infinium production lab.

a Place glass container in the fume hood.
b Place Pyrex glass back plates in the glass container with the plate reservoirs facing up. Do not stack glass back plates on top of each other.
c Add enough 10% bleach solution to cover the glass back plates (fill the glass container to approximately half full).

2 Let the glass back plates soak in bleach in the fume hood for 1 hour.

3 Fill the Backplate Tecan Storage Rack container with deionized water and transfer it to the fume hood.

4 After the 1-hour bleach soak, individually transfer the glass back plates to the Backplate Tecan Storage Rack that is submerged in deionized water. Be careful not to chip the glass back plates.

NOTE
The Backplate Tecan Storage Rack container with glass back plates can now be transferred from the fume hood to a nearby sink with deionized water.

5 Dip the rack containing the glass back plates up and down 20 times. Be careful not to chip the glass back plates.

6 Remove the Backplate Tecan Storage Rack containing the glass back plates and rinse with deionized water.
7 Dispose of the deionized water from the Backplate Tecan Storage Rack container; rinse and refill with fresh deionized water.
8 Return the rack containing the glass back plates to the Backplate Tecan Storage Rack container.
9 Dip rack containing glass back plates up and down 20 times and then let soak for 5 minutes.
10 Repeat 4 times—steps 7–9.
11 Dispose of the deionized water.
12 Allow the glass back plates to dry in the storage rack or by leaning the glass back plates at an angle against the black metal frames.
13 When the glass back plates are clean and dry, use a Kimwipe to wash with 70% EtOH.
14 Dry the glass back plates with a Whoosh duster or laboratory air gun.
15 To prevent surface damage to the glass back plates, store them in the Te-Flow Flow Cell storage box.

Maintaining and Calibrating Lab Equipment

Calibrate the following equipment on an annual basis, according to the specifications listed.

Table 1 Lab Equipment Maintenance

Equipment	Specification
High-speed microplate shaker	Determine offset at 1600 rpm, 1800 rpm, 2000 rpm, and 2300 rpm using a calibrated stroboscope
Hybridization oven	±2°C at 40°C and 60°C
Heated cover heat block	±1°C at 37°C and 95°C
Temperature probe for Te-Flow	±1°C

Calibrating the Vortexer

The displayed speed of the vortexer can vary from the actual vortex speed. Use a digital stroboscope to determine the actual vortex speed. When you have determined the actual vortex speed, record it along with the displayed speed and use these measurements for reference throughout the assay.

Follow the following instructions to calibrate the Signature* High-Speed Microplate Shaker (VWR International, catalog # 13500-890).

1 Set the digital stroboscope display speed to 1600 rpm.
2 Turn on the vortexer and adjust the vortexer speed until the actual vortex speed reaches 1600 rpm.
3 Record the displayed vortexer speed and note down that it represents an actual speed of 1600 rpm.

4 Use the same method described in steps 1–3 to determine the displayed speed for the actual vortex speed of 1800 rpm. These 2 vortex speeds are used in the Infinium Karyomapping Assay.

5 Place a label on the vortexer with the calibration information. The following table provides an example of a calibration label you can create and affix to your vortexer.

Table 2 Sample Vortexer Calibration Label

Display Speed	Actual Vortex Speed	Calibration Date
1450 rpm	1600 rpm	xx-xx-xx
1625 rpm	1800 rpm	xx-xx-xx

Calibrating the Hybridization Oven

Contact Illumina Technical Support to schedule yearly maintenance and calibration.

For more information, see the *Hybridization Oven System Guide*.

Infinium Karyomapping Assay Protocol

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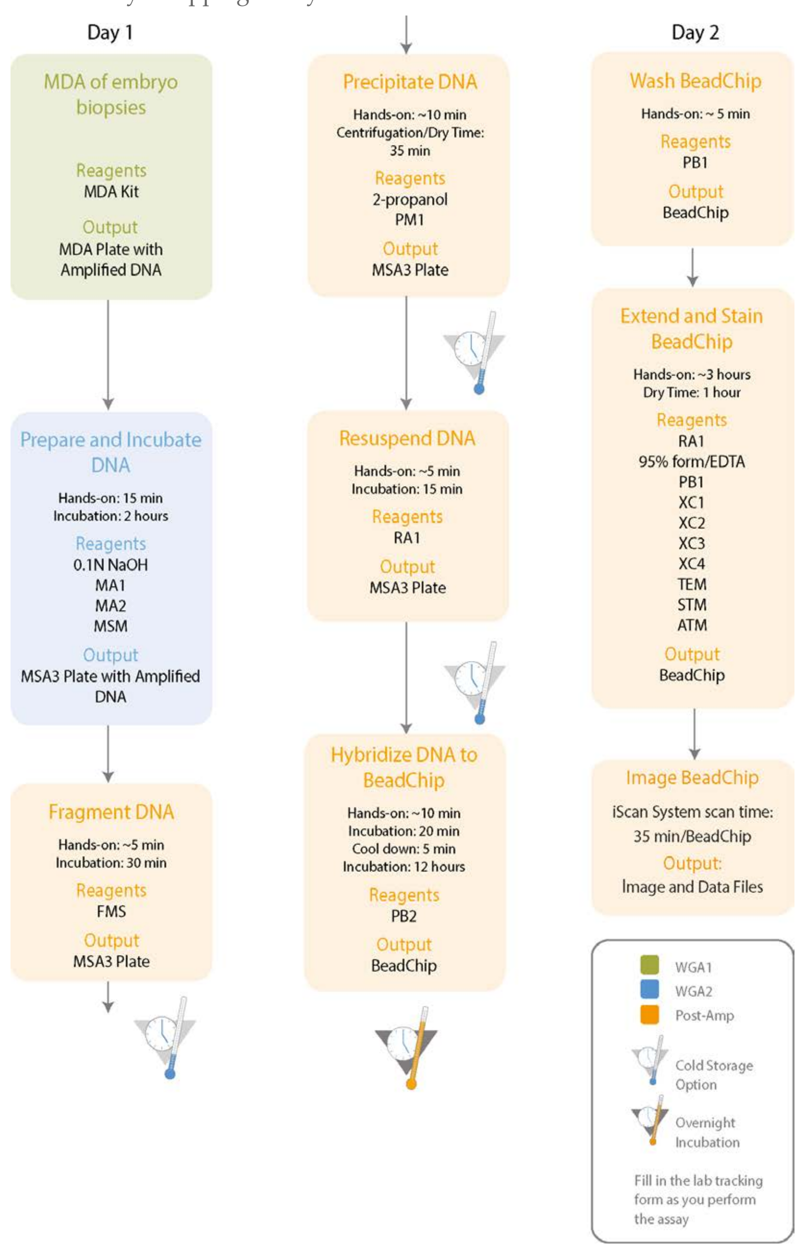
Infinium Karyomapping Assay Workflow

The following figure graphically represents the manual workflow for the Infinium Karyomapping Assay.

Figure 1 Infinium Karyomapping Assay Workflow



CAUTION
To avoid incidents of sample mishandling it is important to witness critical steps, such as tube transfers in the protocol. It is recommended a witness signature is included as part of the Lab Tracking Form.

Equipment, Materials, and Reagents

User-Supplied Equipment

Table 3 User-Supplied Equipment

Item	Catalog #
Air – clean, dry compressed source adjacent to iScan: Either: • Air tank with regulator and air hose, or • House air with constant >30 psi pressure *Check requirements with iScan engineer*	General lab supplier
Microplate centrifuge with g-force range 8 to 3000 × g *two, for pre-amp and post-amp use; post-amp centrifuge to include refrigeration*	General lab supplier
Centrifuge adapters for plates and tubes	General lab supplier
Agarose gel electrophoresis equipment	General lab supplier - see *Appendix I: Agarose Gel Electrophoresis* on page 65
Tube vortexer *For pre-amp and post-amp use*	General lab supplier
Pipettes *two separate sets, for pre-amp and post-amp use* • P-2, P-20, P-200, and P-1000	General lab supplier
8-channel precision pipettes *two separate sets, for pre-amp and post-amp use* • 50 µL to 300 µL	General lab supplier
Stop watch/timer *two, for pre-amp and post-amp use*	General lab supplier
Tube rack	VWR International catalog # 66023-526
Vacuum source *greater than 508 mm Hg (0.68 bar)*	General lab supplier
Vacuum gauge for vacuum desiccator [recommended]	General lab supplier

User-Supplied Materials

Table 4 User-Supplied Materials

Item	Catalog #
Powder-free gloves *two separate stocks, for pre-amp and post-amp use*	General lab supplier
Lab coats *two, for pre-amp and post-amp use*	General lab supplier

Item	Catalog #
Safety glasses *two, for pre-amp and post-amp use*	General lab supplier
15 ml conical tubes	General lab supplier
0.8 ml storage plate (MIDI plate), conical bottom	Abgene catalog # AB-0765
96-well PCR plate	Abgene catalog # AB-0600
96-well PCR plate seals	Abgene catalog # AB-0558
0.2 ml PCR tubes *Thin-walled, flip cap*	Abgene catalog # AB-0620
1.5 ml LoBind tubes *Safe-Lock lid cap, nuclease-free*	Eppendorf catalog # 0030108051
Heat Sealing foil sheets, Thermo-Seal	Abgene catalog # AB-0559
Rubber sealing roller	VWR International catalog # BRND701380 or equivalent
96-well cap mats – pierceable, non-autoclavable	Abgene catalog # AB-0566
Absorbent pads	General lab supplier
Kimwipes or any lint-free tissue	General lab supplier
Aerosol filter pipette tips *two separate stocks, for pre-amp and post-amp use* • 2 µL, 20 µL, 200 µL, 1000 µL	General lab supplier
Disposable pipetting troughs	VWR International catalog # 21007-970
Canned air, such as Aerosol Whoosh-Duster	VWR International catalog # 16650-027

User-Supplied Reagents

Table 5 User-Supplied Reagents

Item	Part #
MDA kit Recommended: REPLI-g Single Cell kit	Qiagen catalog # 150343 or 150345
Genomic DNA from Parents and Reference *Good quality genomic DNA - 50 ng/µl*	N/A
Deionized water	N/A
EDTA, 0.5 M	Sigma-Aldrich catalog # E7889
Ethanol, 100%	General lab supplier
Formamide, OmniPur	VWR International catalog # EM-4650

Item	Part #
Isopropanol (2-propanol) *Other alcohols cannot substitute*	General lab supplier
Mild detergent, such as Alconox Powder Detergent	VWR International catalog # 21835-032
Sodium Hydroxide solution NaOH, 10 M	Sigma-Aldrich catalog # T72068
PBS, 20X - dilute to 1x with nuclease-free water *For diluting DNA*	Cell Signaling Technologies 9808

Reagents

Table 6 Reagents

Item	Part #
HumanKaryomap-12 DNA Analysis BeadChip Kit (24 samples)	RH-103-1001

CAUTION
Download DMAP files before beginning the Infinium protocol. Scanning of BeadChips cannot occur without the relevant associated DMAP file.

Table 7 Reagents Supplied in the Infinium Analysis Kit

Item	Part #
HumanKaryomap-12 v1.0 (2x BeadChips)	15050055
MA1—Multi-Sample Amplification 1 Mix	11202880
MSM—Random Primer Mix	11203410
MA2—Multi-Sample Amplification Master Mix	11203401
FMS—Fragmentation solution	11203428
PM1—Precipitation solution	11203436
RA1—Resuspension, hybridization, and wash solution	11292441
PB2—Humidifying buffer used during hybridization	11191130
PB1—Reagent used to prepare BeadChips for hybridization. Also used to wash BeadChips after hybridization and in XStain reaction.	11291245
XC1—XStain BeadChip solution 1	11208288
XC2—XStain BeadChip solution 2	11208296
TEM—Two-Color Extension Master Mix	11208309
XC3—XStain BeadChip solution 3	11208392

Item	Part #
STM—Superior Two-Color Master Mix	11288046
ATM—Anti-Stain Two-Color Master Mix	11208317
XC4—XStain BeadChip solution 4	11208430

NOTE
A DNA Analysis Kit includes the BeadChips and reagents. Reagents are supplied in sufficient quantities to run all the BeadChips at the same time. It is important that the frozen components do not go through more than two freeze-thaw cycles from delivery.

The wash buffers (PB1 and XC4) are supplied in sufficient volume suitable for single use. XC4 reagent (resuspended) can be reused up to 6 times over a two-week period (maximum 24 BeadChips). PB1 and XC4 can be ordered separately as a buffer kit (GT-201-1005).

Table 8 Items from Illumina

Item	Part #
iScan System (110 V/220 V) or NextSeq 550 System	SY-101-1001
Infinium Option Starter Package (220 V)	WG-15-305
Infinium Option Starter Package (110 V)	WG-15-304

NOTE
New users not already running the Infinium Assay require the Infinium Option Starter Package. The Infinium Option Starter package (220 V and 110 V) includes: Hybridization Oven with rocker, high-speed microplate shaker, a Hybex heat block and accessories, heat sealer, vacuum desiccator, dessicator tube racks, BeadChip hybridization chamber and gaskets, BeadChip alignment tool, staining rack and wash dish, Tecan Te-Flow wash Chamber Rack and accessories, among other items.

Sample Input Requirements

Single and multi-cell biopsy samples do not have the required quantities of genomic DNA to be used as starting material in the Infinium Karyomapping Assay. To overcome this limitation, an initial WGA of the embryo biopsy samples using MDA is essential. In this process, genomic DNA starting from as low as 6.5 pg is amplified >1000-fold resulting in several micrograms of amplified DNA. A suitable MDA kit should be used for amplifying DNA from embryo biopsy samples. End products of MDA should meet the following criteria to be suitable for their use in the Infinium Karyomapping Assay:

- Product length after MDA should be in the range of 2 to 100 kb (this can be established using gel electrophoresis. See *Appendix I: Agarose Gel Electrophoresis* on page 65).
- Amplified product mass of 1600 to 6400 ng in a total of 8 µl (200 to 800 ng/µl of the completed MDA reaction).

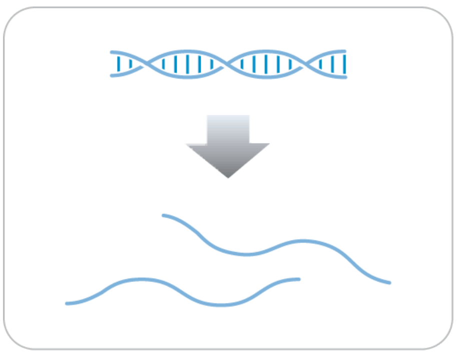
NOTE
Validate the suitability of your chosen MDA kit for use with the Infinium Karyomapping Assay. Illumina has found the REPLI-g SC kit (Qiagen Cat. No: 150343 or 150345) to be suitable for use with the Infinium Karyomapping Assay.

NOTE
Using the REPLI-g Single Cell kit (Qiagen Cat. No: 150343 or 150345), MDA input amounts between 1600 ng and 6400 ng in a total of 8 µl have been tested. A 2-hour MDA reaction was sufficient to generate the required concentration of DNA. However, you should validate the optimal MDA yield for use as input into the Infinium Karyomapping Assay in your own laboratory.

Prepare and Incubate the MSA3 Plate

These steps explain how to prepare a DNA plate (MSA3) for the Infinium Karyomapping Assay. Good quality parental and reference genomic DNA is required for karyomapping. The DNA is added to the MSA3 DNA plate at this stage together with the MDA amplified biopsy samples. This step denatures and neutralizes the samples, preparing them for WGA. During incubation, the genomic and amplified DNA is further amplified and prepared for downstream procedures.

Figure 2 Denaturing and Neutralizing DNA



Estimated Time

- Hands-on time: 15 minutes for 12 samples
- Incubation time: 2 hours

Consumables

Item	Quantity
MA1	1 tube
MSM	1 tube
MA2	1 tube
0.1 N NaOH	1 ml
96-well 0.8 ml storage plate (MIDI)	1 plate
DNA plate with genomic DNA samples (50 ng/µl)	1 plate or in tubes
DNA plate with MDA amplified products	1 plate or in tubes

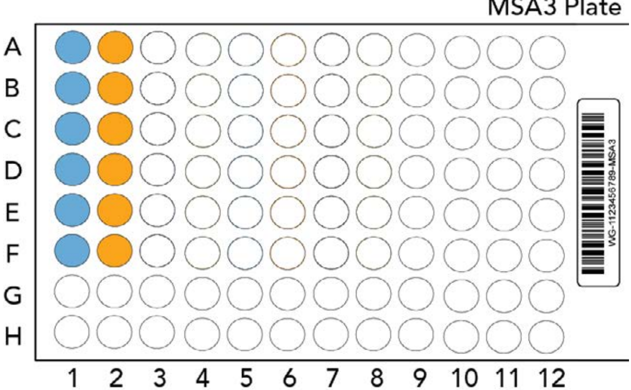
Preparation

- In preparation for the incubation process, preheat the heat block, with a midi plate insert, in the post-amp area to 37°C and allow the temperature to equilibrate.
- Thaw MA1, MSM, and MA2 tubes to room temperature. Gently invert at least 10 times to mix contents, pulse centrifuge 280 × g to gather contents.
- Thaw DNA samples to room temperature.
- On the Lab Tracking Form, record:
  - Date/Time
  - Operator

- DNA plate barcode (if necessary)
- MSA3 plate barcode (if necessary)
- MA1 tube lot number
- MA2 tube lot number
- MSM tube lot number

Steps

1. Dispense 40 µl MA1 into the MSA3 plate wells of the midi plate.
   Fill wells according to the plate layout diagram where columns 1 and 2 contain samples for a single BeadChip.



2. Transfer 8 µl (50 ng/µl) of gDNA from parents and reference, followed by 8 µl of amplified gDNA from the MDA plate, to the corresponding wells of the MSA3 plate.
3. In the Lab Tracking Form, record the original DNA sample ID for each well in the MSA3 plate.
4. Dispense 8 µl 0.1N NaOH into each well of the MSA3 plate that contains MA1 and sample.
5. Seal the MSA3 plate with a 96-well cap mat.

NOTE
Orient the cap mat so that A1 on the cap matches A1 on the plate. To prevent evaporation and spills, which could lead to assay variability and cross-contamination, make sure that all 96 caps are securely seated in the wells.

6. Vortex the plate at 1600 rpm for 1 minute.
7. Pulse centrifuge at 280 × g.
8. Incubate for 10 minutes at room temperature.
9. Carefully remove the cap mat.

NOTE
When you remove a cap mat, set it aside, upside down, in a safe location for use later in the protocol.

10. Dispense 68 µl MA2 into each well of the MSA3 plate containing sample.
11. Dispense 76 µl MSM into each well of the MSA3 plate containing sample.
12. Reseal the MSA3 plate with the cap mat.

NOTE
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

13 Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.

14 Pulse centrifuge at 280 × g.

15 Incubate in the heat block with midi insert for 2 hours at 37°C.
On the Lab Tracking Form, record the start and stop times.

16 Discard unused reagents in accordance with facility standards.

17 Proceed to *Fragment the DNA*.

NOTE
Perform the next step, *Fragment the DNA*, in a separate laboratory area - *Post-Amp*.

Fragment the DNA

These steps explain how to enzymatically fragment the DNA, using endpoint fragmentation to avoid overfragmentation.

Estimated Time

- Hands-on time: ~ 5 minutes for 12 samples
- Incubation time: 30 minutes

Consumables

Item	Quantity
FMS	1 tube

Preparation

- Keep the heat block with the midi plate insert at 37°C.
- Thaw FMS tube to room temperature. Gently invert at least 10 times to mix contents, pulse centrifuge 280 × g to gather contents.
- Remove the MSA3 plate from the heat block.
- Thaw RA1 to room temperature in preparation for the later step ***Resuspend the DNA***.
- On the Lab Tracking Form, record:
  - Date/Time
  - Operator
  - FMS tube lot number

Steps

1. Pulse centrifuge the MSA3 plate to 280 × g.
2. Carefully remove the cap mat.

NOTE
When you remove a cap mat, set it aside, upside down, in a safe location for use later in the protocol.

3. Add 50 µl FMS to each well containing sample.
4. Seal the MSA3 plate with the 96-well cap mat.

NOTE
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

5. Vortex the plate at 1600 rpm for 1 minute.
6. Pulse centrifuge the plate to 280 × g.
7. Place the sealed plate on the 37°C heat block for 30 minutes.
   On the Lab Tracking Form, record the start and stop times.
8. Discard unused reagents in accordance with facility standards.
9. Perform one of the following:
   - Continue to the next step *Precipitate DNA*, prepare for the next step during the 37°C heat block incubation.

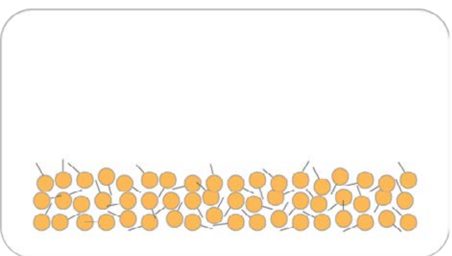
- If you do not plan to proceed to the next step within the next 2 hours, store the sealed MSA3 plate at -25°C to -15°C. Do not store for more than 24 hours.

SAFE STOPPING POINT
This is a good stopping point in the process.

Precipitate the DNA

These steps explain how to precipitate the DNA samples using 100% 2-propanol and PM1.

Figure 3 Precipitating DNA



Estimated Time

- Hands-on time: ~10 minutes for 12 samples
- Centrifugation: 20 minutes
- Dry time: 15 minutes

Consumables

Item	Quantity
PM1	1 tube
100% 2-propanol	15 ml

Preparation

- Preheat heat block to 37°C.
- Perform 1 of the following:
  - If you froze the MSA3 plate after fragmentation, thaw it to room temperature, and then pulse centrifuge the MSA3 plate to 280 × g.
  - If you proceeded immediately from *Fragment the MSA3 Plate*, leave the plate in the 37°C heat block until setup is complete.
- Bring PM1 to room temperature. Gently invert at least 10 times to mix contents.
- On the Lab Tracking Form, record:
  - Date/Time
  - Operator
  - PM1 tube lot number
  - 100% 2-propanol lot number and date opened

Steps

1. Remove the 96-well cap mat and add 100 µl PM1 to each MSA3 plate well containing sample.
2. Seal the plate with the cap mat.

NOTE
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

3 Vortex the sealed plate at 1600 rpm for 1 minute.

4 Place the sealed plate on the 37°C heat block for 5 minutes.

5 Pulse centrifuge at 280 × g.

NOTE
Set centrifuge at 4°C in preparation for the next centrifuge step.

6 Add 310 µl 100% 2-propanol to each well containing sample.

7 Carefully seal the MSA3 plate with a new, ***dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.

8 Invert the plate at least 10 times to mix contents thoroughly.

9 Place the sealed MSA3 plate in the centrifuge opposite another plate of equal weight.

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

CAUTION
Perform the next step immediately after the centrifuge stops to avoid dislodging the blue pellet. If any delay occurs, repeat the 20 minute centrifugation before proceeding.

11 Remove the MSA3 plate from centrifuge. Remove the cap mat and discard it.

12 Over an absorbent pad, decant the supernatant by quickly inverting the MSA3 plate. Drain liquid onto the absorbent pad and then smack the plate down, avoiding the liquid that was drained onto the pad.

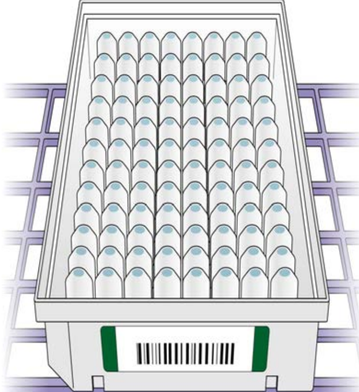
13 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

CAUTION
Keep plate inverted. To ensure optimal performance, do not allow supernatant in wells to pour into other wells.

14 Leave the uncovered, inverted plate on the tube rack for 15 minutes at room temperature to air dry the pellet.
After drying, make sure that blue pellets are present at the bottoms of the wells.

15 On the Lab Tracking Form, enter the start and stop times.

Figure 4 Uncovered MSA3 Plate Inverted for Air Drying



16 Discard unused reagents in accordance with facility standards.

17 Perform 1 of the following:
- Continue to the next step, *Resuspend the DNA*.

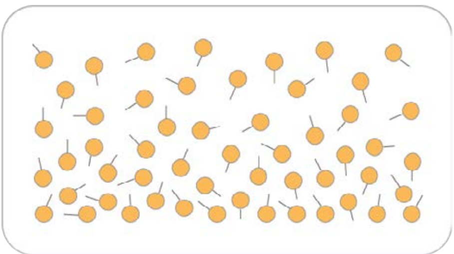
- If you do not plan to proceed to the next step immediately, seal the MSA3 plate with a new cap mat and store it at -25°C to -15°C. Do not store for more than 24 hours.

SAFE STOPPING POINT
This is a good stopping point in the process.

Resuspend the DNA

These steps explain how to resuspend the precipitated DNA using RA1.

Figure 5 Resuspending DNA



Estimated Time

- Hands-on time: ~5 minutes for 12 samples
- Incubation time: 15 minutes

Consumables

Item	Quantity
RA1	17 µl per sample well

NOTE
Pour out only the recommended volume of RA1 needed for the suggested number of samples listed in the consumables table. Additional RA1 is used later in *Extend and Stain (XStain) BeadChips* on page 54.

WARNING
This protocol uses an aliphatic amide (formamide) that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the safety data sheet for this assay at support.illumina.com/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

- If you plan to continue to the hybridization step immediately after resuspension, begin preheating the heat block to 95°C now.
- Preheat the Illumina Hybridization Oven to 48°C.
- Turn on the heat sealer to preheat. Allow 10 minutes.
- Invert the previously thawed RA1 several times to redissolve the solution.

NOTE
RA1 might form visible precipitates or crystals. Before use, hold the bottle in front of a light and visually inspect, invert several times to redissolve the solution. [Optional] Heat at 48°C for 15 minutes until RA1 is dissolved.

- On the Lab Tracking Form, record:
  - Date/Time
  - Operator
  - RA1 bottle lot number

NOTE
Use fresh RA1 for each protocol step where it is required. RA1 that has been stored properly and has not been dispensed for use in either the XStain or Resuspension step is considered fresh RA1.

Steps

1. Add 17 µl RA1 to each well of the MSA3 plate containing a DNA pellet.
   Reserve leftover reagent in the bottle for *Extend and Stain (XStain) BeadChips* on page 54.
2. Apply a foil heat seal (with the dull side facing down) to the MSA3 plate by firmly and evenly holding the heat sealer sealing block down for 5 seconds.
3. Immediately remove the MSA3 plate from the heat sealer and forcefully roll the rubber plate sealer over the plate until you can see all 96 well indentations through the foil. Repeat application of the heat sealer if all 96 wells are not defined.
4. Place the sealed plate in the Illumina Hybridization Oven and incubate for 15 minutes at 48°C.
5. Vortex the plate at 1800 rpm for 1 minute.
6. Pulse centrifuge to 280 × g.

NOTE
If you store the pellets at -15°C to -25°C for extended periods of time after the precipitate process, you might need to resuspend the pellets. Repeat the vortexing and centrifugation in the previous steps until pellets are resuspended.

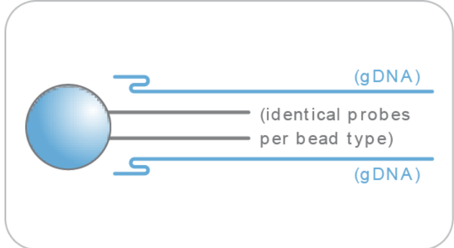
7. Discard unused reagents in accordance with facility standards.
8. Perform one of the following:
   - Continue to the next step, *Hybridize the DNA to the BeadChip*. If you plan to do so immediately, it is safe to leave the MSA3 plate at room temperature for up to 1 hour.
   - If you do not plan to proceed to the next step, immediately:
     - Store the sealed MSA3 plate at -15°C to -25°C for no more than 24 hours. Store at -80°C if storing for more than 24 hours. Do not store the MSA3 plate for longer than one week at -80°C.
     - Store RA1 at room temperature for no more than 24 hours. Store at -15°C to -25°C if storing for more than 24 hours.

SAFE STOPPING POINT
This is a good stopping point in the process.

Hybridize DNA to the BeadChip

These steps explain how to dispense the fragmented, resuspended DNA samples onto BeadChips and incubate the BeadChips in the Illumina Hybridization Oven to hybridize the samples onto the BeadChips.

Figure 6 Hybridize Multi BeadChip



Estimated Time

- Hands-on time: ~10 minutes for 1 BeadChip (12 samples)
- Incubation: 20 minutes
- Cool-down: 5 minutes
- Incubation: 12 hours

Consumables

Item	Quantity
PB2	1 tube
BeadChips	1
Hyb Chambers	1
Hyb Chamber gaskets	1
Hyb Chamber inserts	1
EtOH	330 ml

CAUTION
Inspect BeadChips for broken edges and handle with care.

Preparation

- Make sure that DMAP files for each BeadChip have been downloaded, scanning cannot proceed without a DMAP file.
- If frozen, thaw MSA3 plate to room temperature, and then pulse centrifuge the MSA3 plate to 280 × g.
- If the heat block is not already heated, preheat the heat block to 95°C.
- Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.
- Prepare the Illumina Hybridization Oven as follows:
  a Preheat the oven to 48°C:
    — Press the "F" button one time to change the display to **TSET**.

- Press the "S" button to enter the set-temperature mode, and then use the Increment/Decrement dial to set the oven to 48°C.
- Press the "S" button again to set 48°C as the temperature.

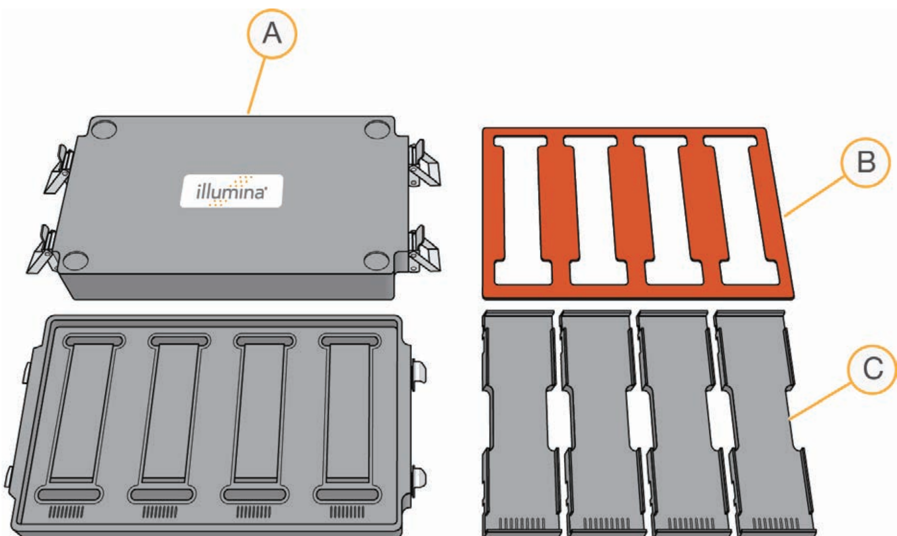
b Set the rocker speed to 5:
- Press the "F" button twice until **SPd** is indicated on the display.
- Press the "S" button to enter the rocker speed mode.
- Use the Increment/Decrement dial to set the rocker speed to "5".
- Press the "S" button again.

- On the Lab Tracking Form, record:
  - Date/Time
  - Operator
  - PB2 tube lot number

Prepare the Hybridization Chambers

1. Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.
   On the Lab Tracking Form, enter the start and stop times.
2. During the 20 minute incubation, prepare the Hyb Chambers.
   Place the following items on the benchtop for use in this procedure:

Figure 7 BeadChip Hyb Chamber Components



A Hyb Chambers
B Hyb Chamber Gaskets
C Hyb Chamber Inserts

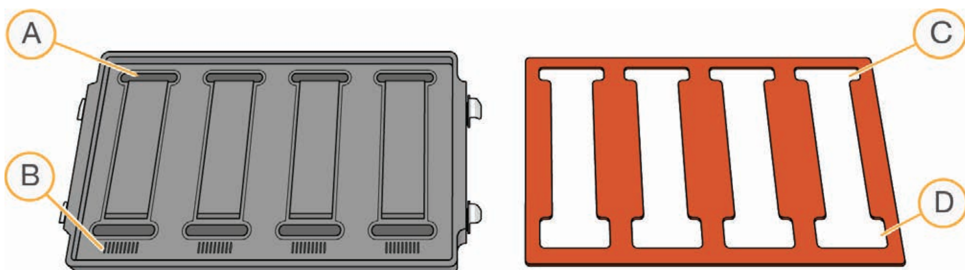
NOTE
To ensure optimal results from Hyb Chambers, keep the Hyb Chamber lids and bases together. Adopt a labeling convention that keeps each Hyb Chamber base paired with its original lid. Check Hyb Chamber lid-base pairs regularly to make sure that the fit remains secure. Check hinges regularly for any signs of abnormal wear or loose fittings. It is important that the hinges provide adequate clamping strength to ensure an airtight seal between the lid and the base. Record the Hyb Chamber that was used for each BeadChip, so that Hyb Chambers can be investigated and evaluated in the event of sample evaporation or other lab processing anomalies.

a Remove the BeadChips from 2°C to 8°C storage, leaving the BeadChips in their ziplock bags and mylar packages until you are ready to begin hybridization.

b Place the Hyb Chamber gaskets into the Hyb Chambers.

— Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber.

Figure 8 Hyb Chamber and Gasket



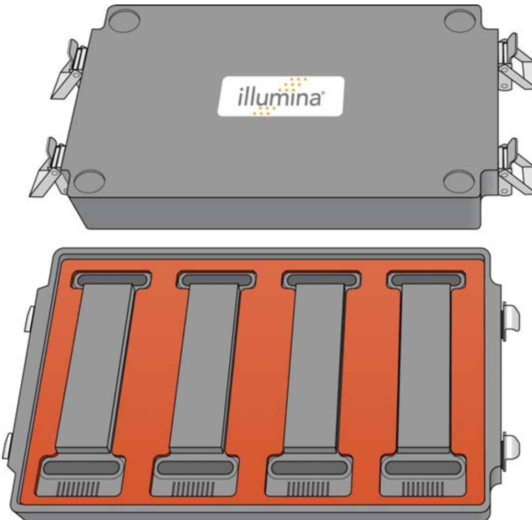
A Humidifying Buffer Reservoirs
B Barcode Ridges
C Narrower Edges
D Wider Edges

— Lay the gasket into the Hyb Chamber, and then press it down all around.

Figure 9 Placing Gasket into Hyb Chamber

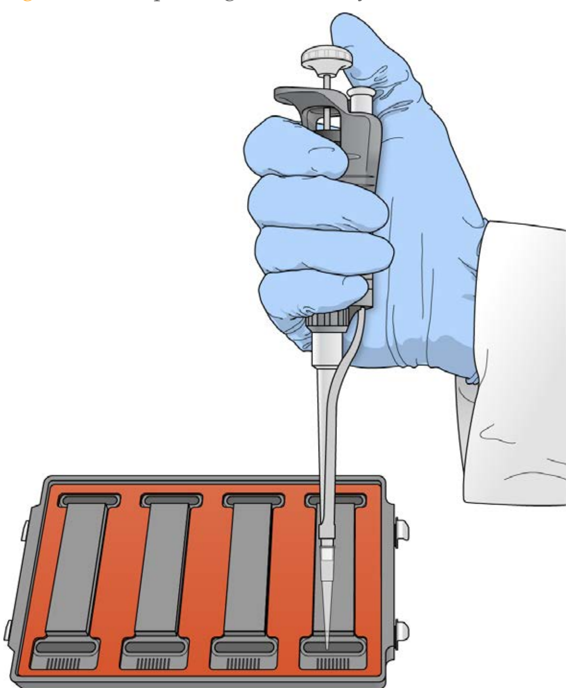
— Make sure that the Hyb Chamber gaskets are properly seated.

Figure 10 Hyb Chamber with Gasket in Place



c Dispense 400 µl PB2 into the required humidifying buffer reservoirs in the Hyb Chamber, two for each BeadChip.

Figure 11 Dispensing PB2 into Hyb Chamber Reservoir



d After filling the Hyb Chamber reservoirs with PB2, place the lid on the Hyb Chamber right away to prevent evaporation. You do not need to lock down the lid.

e Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample. Load BeadChips into the Hyb Chamber within one hour.

3 After the 20 minute incubation, remove the MSA3 plate from the heat block and place it on the benchtop at room temperature for 5 minutes to cool.

Load BeadChips

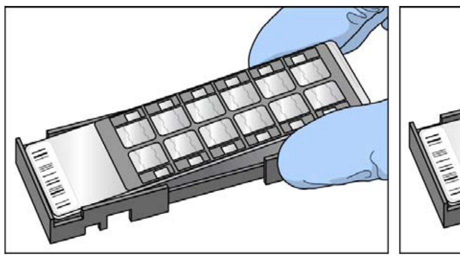
1. After the 5 minute cool down, pulse centrifuge the MSA3 plate to 280 × g.
2. Just before loading DNA samples, remove all BeadChips from their ziplock bags and mylar packages.

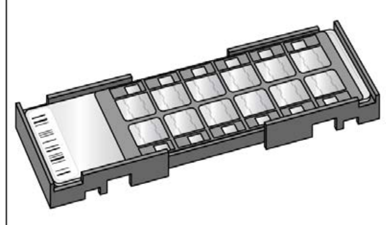
CAUTION
Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Do not hold the BeadChip by the sides near the sample inlets. Avoid contacting the beadstripe area and sample inlets.

CAUTION
Inspect BeadChips for broken edges and handle with care.

3. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the Hyb Chamber insert.

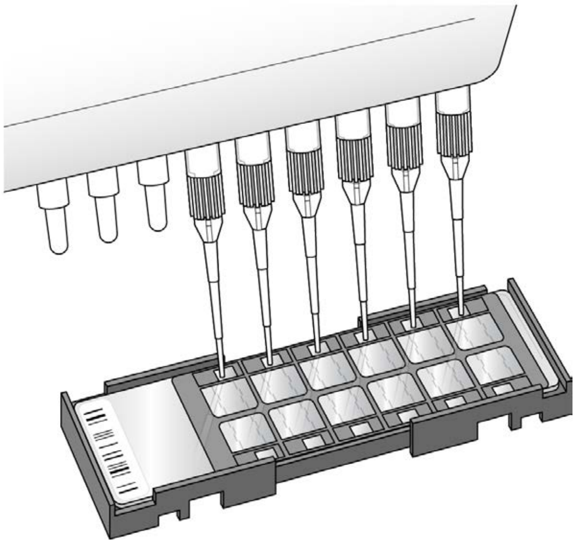
Figure 12 Placing BeadChips into Hyb Chamber Inserts





4. Remove the foil seal from the MSA3 plate, and then, using a multichannel precision pipette, dispense 15 µl of each DNA sample onto the appropriate BeadChip section.

Figure 13 Loading Samples



a. Load samples A1–F1 from the MSA3 plate into the left side of the BeadChip. Make sure that the pipette tip is in the sample inlet before dispensing.
b. Load samples A2–F2 from the MSA3 plate into the right side of the BeadChip.
c. Continue in this manner, following the color-coded sections in the figure, until all samples are loaded.

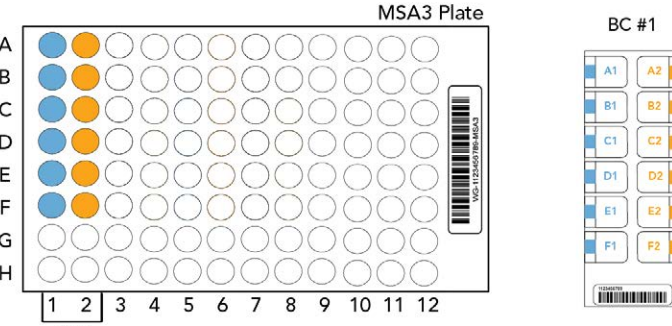


Figure 14 Distributing Sample in MSA3 Plate

5 On the Lab Tracking Form, record the BeadChip barcode for each group of samples
6 After loading all DNA onto the BeadChip, wait for the sample to disperse over the entire surface.
7 Inspect the loading port to see if a large bolus of liquid remains.
Excess sample volume in the BeadChip loading port helps prevent low-intensity areas resulting from evaporation.

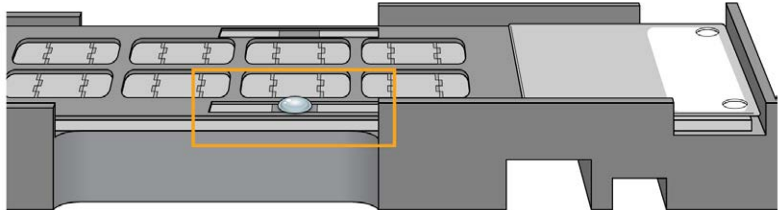


Figure 15 Bolus of Liquid at Loading Port

If no excess liquid is visible, it is acceptable to add additional sample from the leftover volume in the amplification plate until there is a large bolus around the loading port.

NOTE
Do not replenish with RA1 (sample Hyb buffer) as this will dilute the sample.

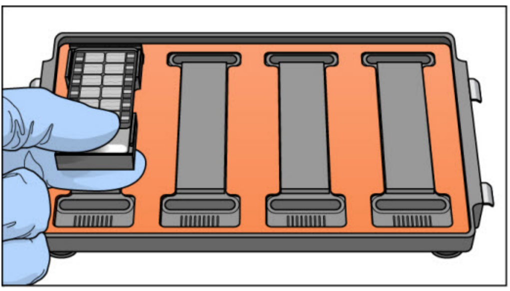
8 Record the amount of additional sample added on the Lab Tracking Form.
9 Proceed immediately to the next section, *Set up the BeadChips for Hybridization*.

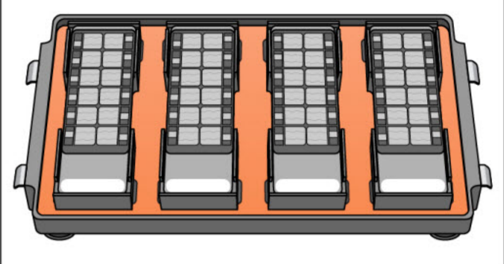
Set up the BeadChips for Hybridization

CAUTION
For optimal performance, take care to keep the Hyb Chamber inserts containing BeadChips 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.

1 Load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chamber.
Position the barcode end over the ridges indicated on the Hyb Chamber.

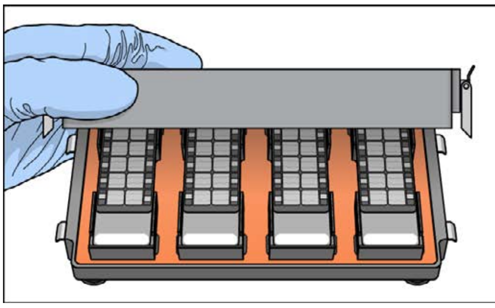
Figure 16 Placing Hyb Chamber Inserts into the Hyb Chamber

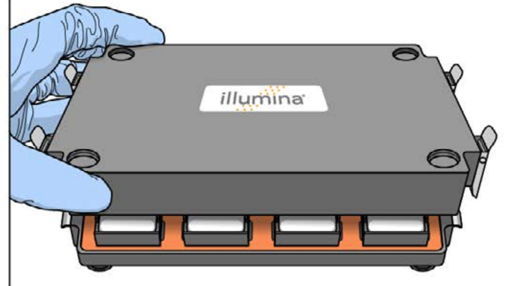




2 Place the back side of the lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts.

Figure 17 Seating Lid onto Hyb Chamber





3 Close the clamps on both sides of the Hyb Chamber so that the lid is secure and even on the base (no gaps). It is best to close the clamps in a kitty-corner fashion, closing first the top left clamp, then the bottom right, then the top right followed by the bottom left.

4 Place the Hyb Chamber in the 48°C Illumina Hybridization Oven so that the Illumina logo on top of the Hyb Chamber is facing you.

CAUTION

After loading the BeadChips into the Hyb Chambers, place the Hyb Chambers into the Illumina Hybridization Oven immediately. Do not modify the hybridization environment by adding additional fixtures or humidifying elements. Leave the Hyb Chambers in the oven at the correct orientation and temperature until hybridization is complete. Changes to the hybridization environment can have unexpected effects on data quality.

Figure 18 Hyb Chamber Correctly Placed in Hyb Oven

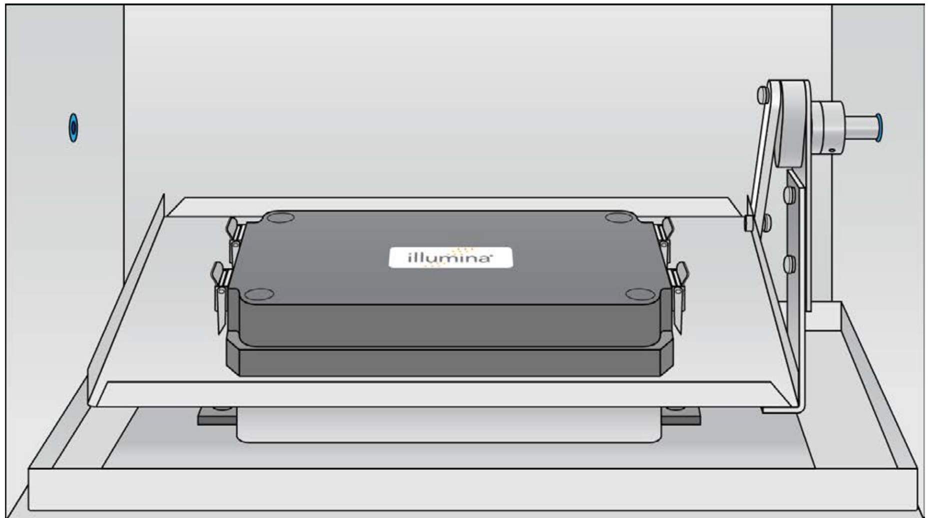
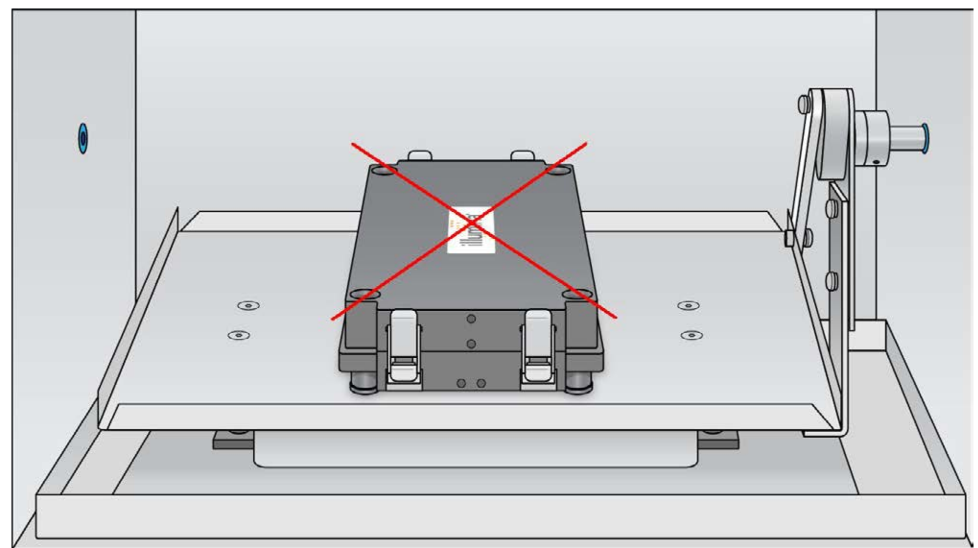


Figure 19 Incorrectly Placed Hyb Chamber



NOTE
If you are stacking multiple Hyb Chambers, make sure the feet of the top Hyb Chamber fit into the matching indents on top of the bottom Hyb Chamber. This will hold the Hyb Chambers in place while they are rocking. You can stack up to 3 Hyb Chambers per row for a maximum total of 6 Hyb Chambers in the Hyb Oven.

5 [Optional] Start the rocker, setting the speed to 5.
6 Incubate at 48°C for 12 hours or overnight but no more than 24 hours.
7 On the Lab Tracking Form, enter the start and stop times.
8 Place RA1 at 4°C for use the next day.
9 Discard the MSA3 plate.
10 After the overnight incubation, proceed to *Wash the BeadChip*.

Resuspend XC4 Reagent in Preparation for XStain

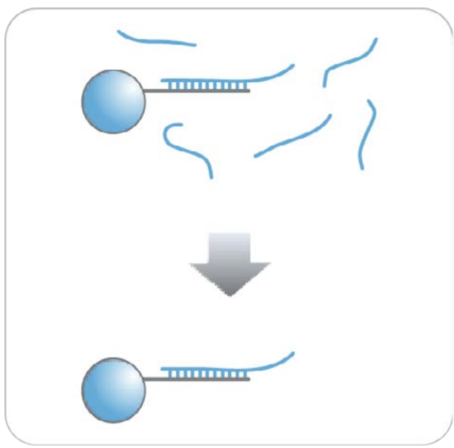
Keep the XC4 in the bottle in which it was shipped until you are ready to use it. Follow these steps to resuspend the XC4 reagent in preparation for the *Extend and Stain (XStain) BeadChips* on page 54.

1. Add 330 ml fresh 100% EtOH to the XC4 bottle. The final volume will be 350 ml.
   Each XC4 bottle has enough solution to process up to 24 BeadChips.
2. Shake the XC4 bottle vigorously to ensure complete resuspension.
   When it is resuspended, use XC4 at room temperature. You can store it at 2°C to 8°C and reuse up to 6 times over a two-week period for a maximum of 24 BeadChips.

Wash the BeadChip

These steps explain how to prepare the BeadChips for the staining process.

Figure 20 Washing BeadChip



Estimated Time

- Hands-on time: ~5 minutes for 1 BeadChip

Consumables

Item	Quantity
PB1	550 ml (up to 4 BeadChips)
Multi-Sample BeadChip Alignment Fixture	1 (per 4 BeadChips)
Te-Flow flow-through chambers (with Black Frames, Spacers, Glass Back Plates, and Clamps)	1 (per BeadChip)
Wash Dish	2
Wash Rack	1

CAUTION
Pour only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section. Some reagents are used later in the protocol.

WARNING
This protocol uses an aliphatic amide (formamide) that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the safety data sheet for this assay at support.illumina.com/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

- Make sure that the water circulator is filled to the appropriate level.
- Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.

- Remove bubbles trapped in the Chamber Rack.
- Test three or more locations on the Chamber Rack, using the Illumina Temperature Probe. Make sure that all locations are at 44°C ± 0.5°C. If the temperature on the probe is not within ± 0.5°C, adjust the water circulator control knob to obtain 44°C ± 0.5°C on the temperature probe.
- In preparation for the wash step, remove each Hyb Chamber from the Illumina Hybridization Oven.
- Have ready on the lab bench:
  - Two wash dishes:
    - Containing 200 ml PB1, and labeled as such
  - Multi-Sample BeadChip Alignment Fixture
    - Using a graduated cylinder, fill with 150 ml PB1
  - Te-Flow flow-through chamber components:
    - Black frames
    - Spacers (separated for ease of handling)
    - Clean glass back plates (see *Cleaning the Glass Back Plates* on page 14)
    - Clamps
- On the Lab Tracking Form, record:
  - Date/Time
  - Operator
  - PB1 bottle lot number

In preparation for *XStain BeadChip* step

- Thaw XC1, XC2, TEM, STM, ATM, and 95% Formamide / 1mM EDTA to room temperature. Gently invert the reagent tubes and bottles at least 10 times to mix contents.
- Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until dissolved.

Steps

1 Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1.

Figure 21 Wash Rack in Wash Dish Containing PB1

2 Remove a BeadChip from the Hyb Chamber and then remove its cover seal.

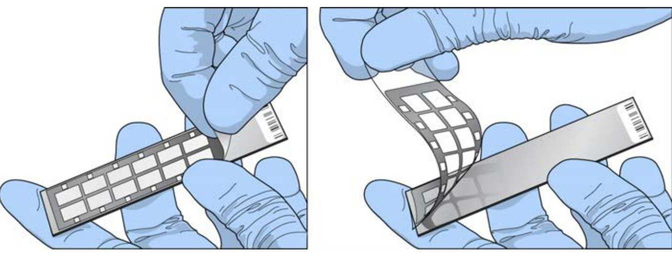
NOTE
To make sure that no solution splatters on you, Illumina recommends removing the cover seal over an absorbent cloth or paper towels, preferably in a hood.

CAUTION
Inspect BeadChips for broken edges and handle with care.

a Using powder-free gloved hands, hold the BeadChip securely and by the edges in one hand. Avoid contact with the sample inlets. Make sure that he barcode is facing up and is closest to you, and that the top side of the BeadChip is angled slightly away from you.

b Remove the entire seal in a single, continuous motion. Start with a corner on the barcode end and pull with a continuous upward motion away from you and towards the opposite corner on the top side of the BeadChip.

Figure 22 Removing the Cover Seal



c Discard the cover seal.

CAUTION
Do not touch the arrays!

3 Immediately and carefully slide the BeadChip into the wash rack, making sure that the BeadChip is submerged in the PB1.

Figure 23 Submerging BeadChips in Wash Dish Containing PB1

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

5. When all the BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.
6. Move the wash rack to the other wash dish containing clean PB1. Make sure the BeadChips are submerged.
7. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.
8. When you remove the BeadChips from the wash rack, inspect them for remaining residue.

NOTE
Residue that can adversely affect results is sometimes left on BeadChips after seals are removed. If there is residue left on the BeadChips after the second PB1 wash, use a 200 µl pipette tip for each BeadChip. Slowly and carefully scrape off the residues outward (away) from the bead-sections under PB1. Use a new pipette tip for each BeadChip. Then, continue with the protocol.

Assemble Flow-Through Chambers

1. If you have not done so, fill the BeadChip Alignment Fixture with 150 ml PB1.
2. For each BeadChip to be processed, place a black frame into the BeadChip Alignment Fixture.

Figure 24 Placing Black Frames into BeadChip Alignment Fixture, prefilled with PB1

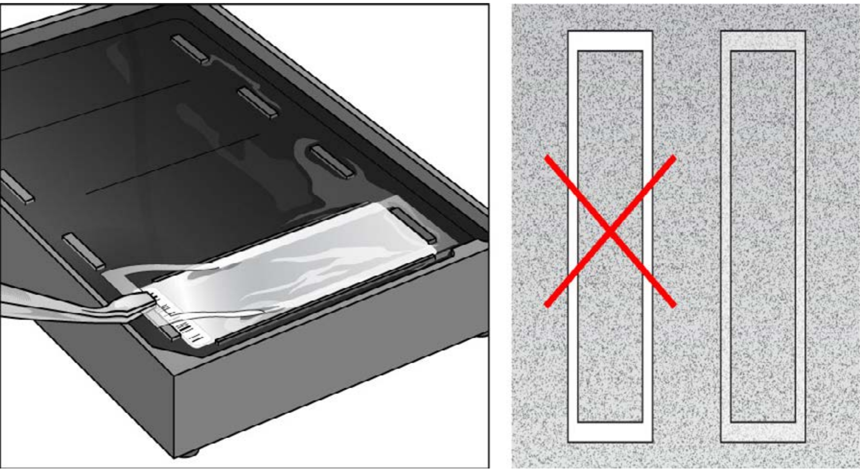
3. Place each BeadChip into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture. Make sure that each BeadChip is fully immersed in PB1.

Figure 25 Placing BeadChip into Black Frame on Alignment Fixture

4 Place a clear spacer onto the top of each BeadChip. Use the Alignment Fixture grooves to guide the spacers into proper position.

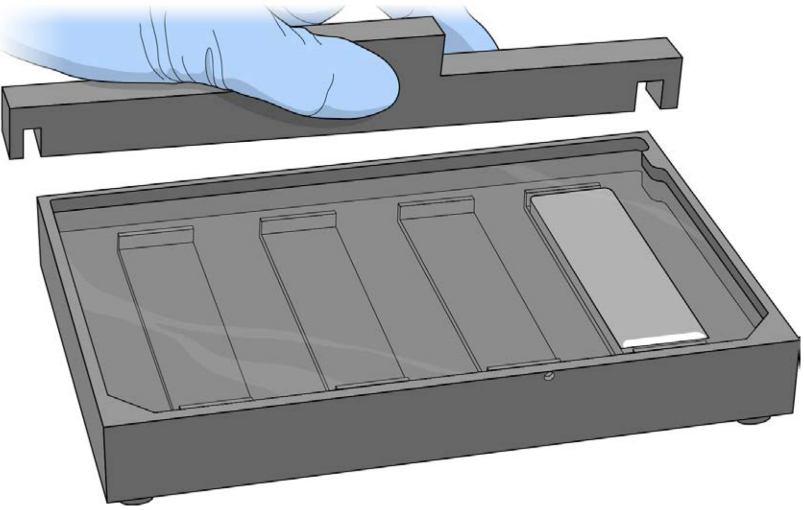
NOTE
Be sure to use the clear plastic spacers, not the white ones.

Figure 26 Placing Clear Plastic Spacer onto BeadChip



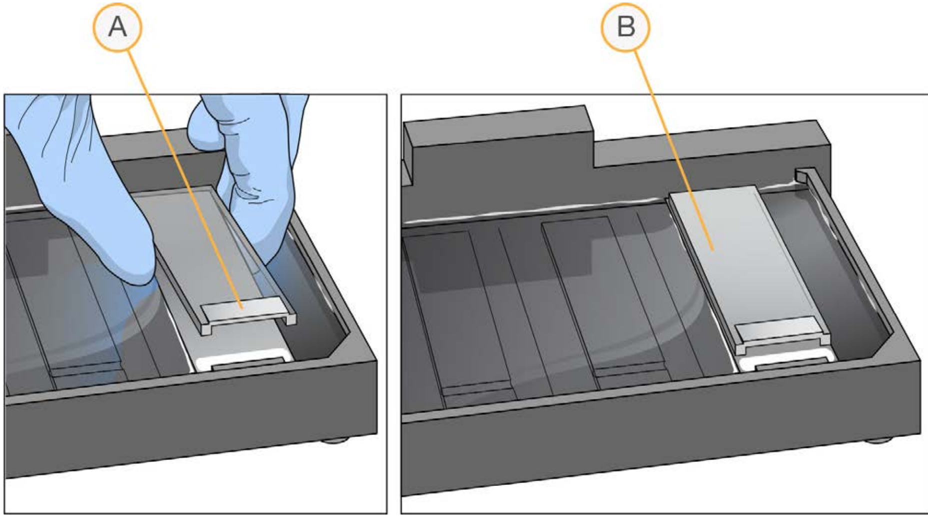
5 Place the Alignment Bar onto the Alignment Fixture.
Make sure that the groove in the Alignment Bar fits over the tab on the Alignment Fixture.

Figure 27 Placing Alignment Bar onto Alignment Fixture



6 Place a clean glass back plate on top of the clear spacer covering each BeadChip. Make sure that the plate reservoir is at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface.

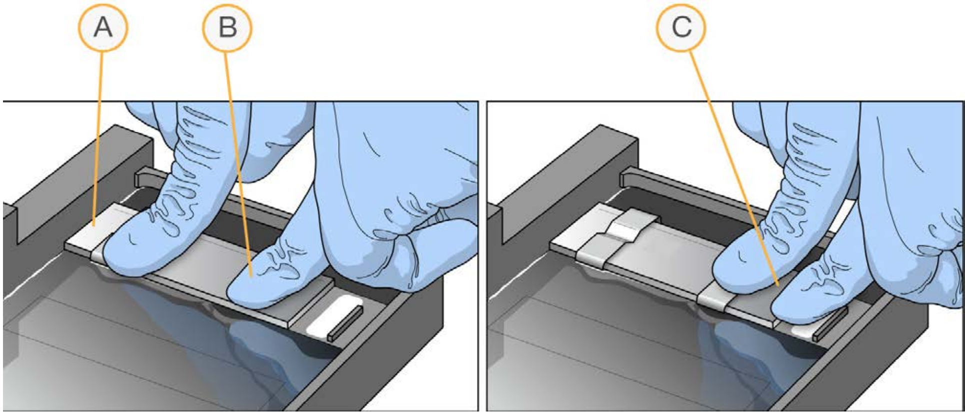
Figure 28 Placing Glass Back Plate onto BeadChip



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

7 Attach the metal clamps onto each flow-through chamber as follows:
   a Gently push up the glass back plate against the Alignment Bar with one finger.
   b Place the first metal clamp around the flow-through chamber so that the clamp is 5 mm from the top edge.
   c Place the second metal clamp around the flow-through chamber at the barcode end, 5 mm from the bottom of the reagent reservoir.

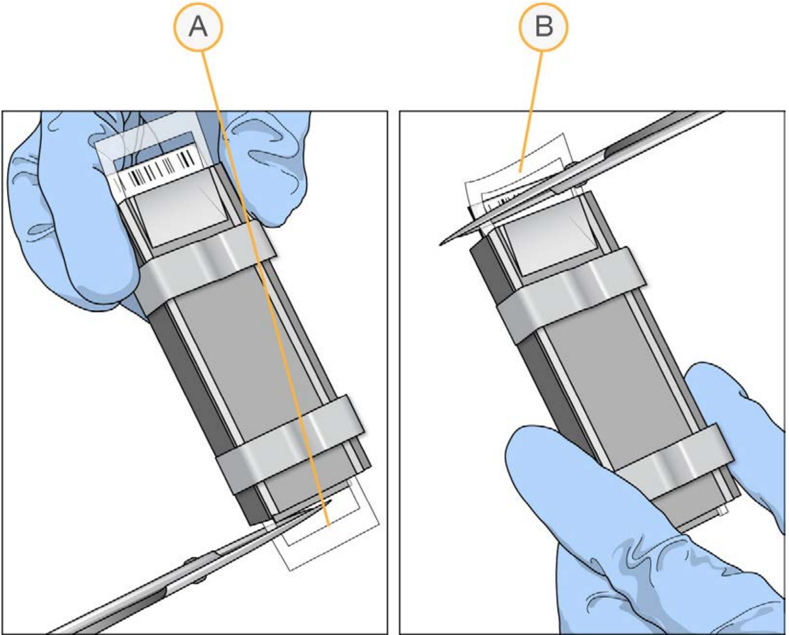
Figure 29 Securing Flow-Through Chamber Assembly with Metal Clamps



A One Stripe Shows Between First Clamp and Alignment Bar
B Glass Back Plate Pressed Against Alignment Bar
C No Stripes Show Between Second Clamp and Barcode

8 Remove the assembled flow-through chamber from the alignment fixture and, using scissors, trim the spacer at the nonbarcode end of the assembly. Slip the scissors up over the barcode to trim the other end.

Figure 30 Trimming Spacer Ends from Flow-Through Chamber Assembly



A Trim spacer at nonbarcode end of flow-through chamber
B Trim spacer at barcode end of flow-through chamber

9 Continue to the next step, *Extend and Stain (XStain) BeadChip*.

CAUTION
Keep assembled flow-through chambers on the lab bench in a horizontal position while you perform the preparation steps for *Extend and Stain (XStain) BeadChips* on page 54. Do not place the assembled chambers on absorbent paper. Do not place the flow-through chambers in the Chamber Rack until the preparation is complete.

10 Wash the Hyb Chamber reservoirs with DiH_2O.

CAUTION
It is important to wash the hybridization chamber reservoirs immediately and thoroughly to make sure that no traces of PB2 remain in the wells.

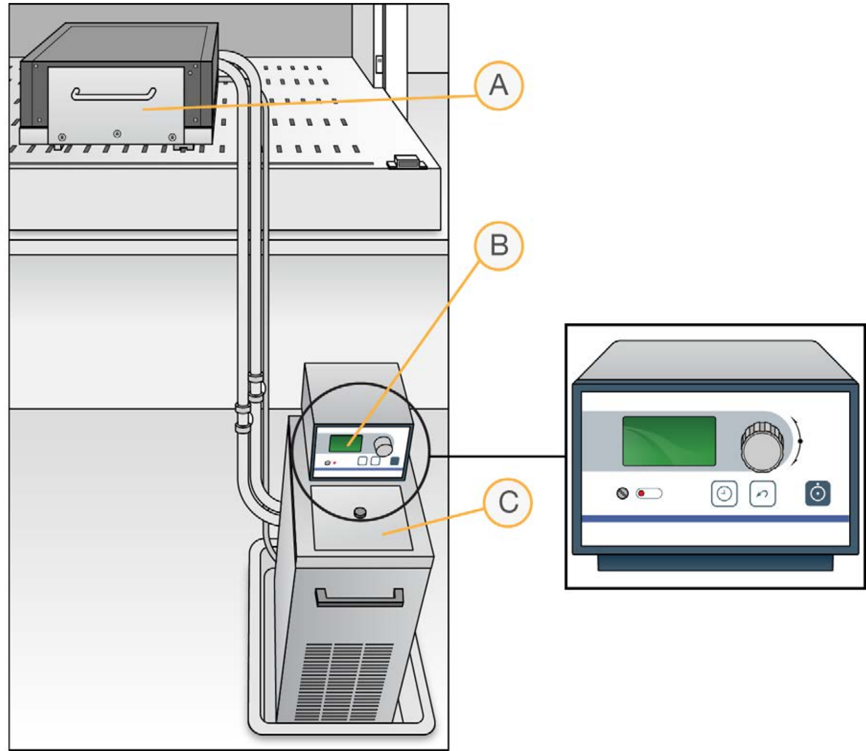
11 Discard unused reagents in accordance with facility standards.

Set Up the Chamber Rack

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

2 If not already done so, turn on the water circulator and set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.
This temperature can vary depending on facility ambient conditions.

Figure 31 Water Circulator Connected to Chamber Rack



A Chamber Rack
B Water Circulator with Programmable Temperature Controls
C Reservoir Cover

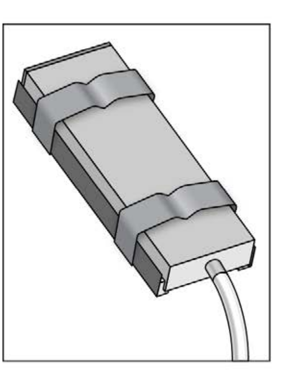
3 The temperature displayed on the water circulator LCD screen can differ from the actual temperature on the Chamber Rack. Confirm the actual temperature using the temperature probe for the Chamber Rack.

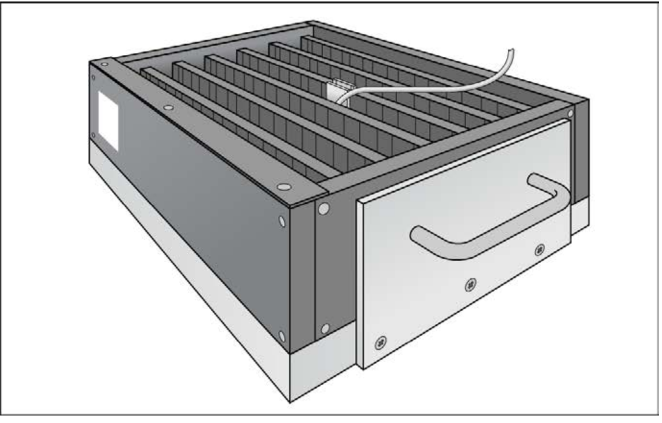
4 Remove bubbles trapped in the Chamber Rack ***each time*** you run this process.

5 Use the Illumina Temperature Probe in several locations to make sure that the Chamber Rack is at 44°C. Make sure that all locations are at 44°C ± 0.5°C.

NOTE
Do not leave the temperature probe in the first 3 rows of the Chamber Rack. Reserve this space for BeadChips.

Figure 32 Illumina Temperature Probe and Temperature Probe in Chamber Rack



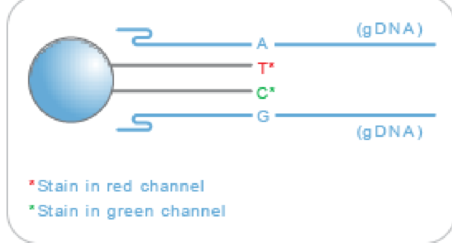


6 For accurate temperature measurement, make sure that the Temperature Probe is touching the base of the Chamber Rack.

Extend and Stain (XStain) BeadChips

These steps explain how to: wash unhybridized and nonspecifically hybridized DNA samples from the BeadChips, add labeled nucleotides to extend the primers hybridized to the DNA, stain the primers, disassemble the flow-through chambers, and coat the BeadChips for protection.

Figure 33 Extending and Staining BeadChip



Estimated Time

- Hands-on time: ~3 hours
- Dry time: 1 hour

Consumables

Item	Quantity
RA1	5 ml (per 4 BeadChips)
XC1	1 tube (per 4 BeadChips)
XC2	1 tube (per 4 BeadChips)
TEM	1 tube (per 4 BeadChips)
XC3	25 ml (per 4 BeadChips)
STM (make sure that all STM tubes indicate the same stain temperature on the label)	1 tube (per 4 BeadChips)
ATM	1 tube (per 4 BeadChips)
PB1	310 ml (up to 4 BeadChips)
XC4	310 ml (up to 4 BeadChips)
Alconox Powder Detergent	as needed
EtOH	as needed
95% formamide/1 mM EDTA	10 ml (up to 4 BeadChips)

CAUTION
Pour out only the recommended reagent volume needed for the suggested number of samples listed in the Consumables table of each section.

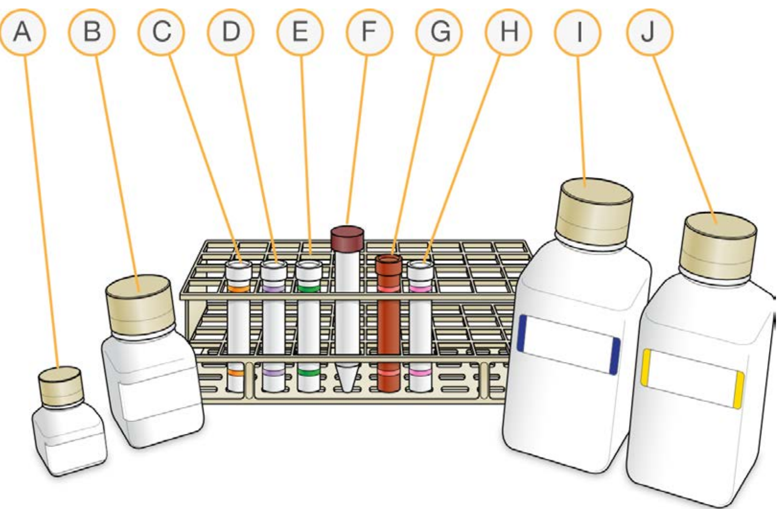
WARNING

This protocol uses an aliphatic amide (formamide) that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the safety data sheet for this assay at support.illumina.com/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

Preparation

- Place the reagent tubes in a rack in the order in which they will be used: RA1, XC1, XC2, TEM, 95% Formamide / 1mM EDTA, STM, XC3, ATM.

Figure 34 XStain BeadChip Reagent Tubes and Bottles



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

NOTE

It is important to use fresh RA1 for each protocol step in the assay where it is required. RA1 that has been stored properly and has not been dispensed previously is considered fresh RA1. After RA1 has been poured out into a reservoir and exposed to room temperature air for extended periods of time, it is no longer fresh.

NOTE

RA1 might form visible precipitates or crystals. Before use, hold the bottle in front of a light and visually inspect to make sure that all precipitates have dissolved. Heat to dissolve, if necessary.

- On the Lab Tracking Form, record:
  - Date/Time
  - Operator
  - RA1 lot number

- XC3 lot number
- XC1 lot number
- XC2 lot number
- TEM lot number
- STM lot number
- ATM lot number
- PB1 lot number
- XC4 lot number

Single-Base Extension

CAUTION
The remaining steps must be performed without interruption.

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

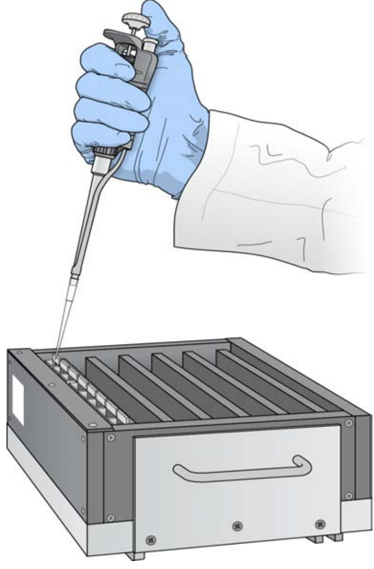
2 Make sure that each flow-through chamber is properly seated on its rack to allow adequate heat exchange between the rack and the chamber.

3 Into the reservoir of each flow-through chamber, dispense:

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

CAUTION
Do not allow pipette tips to contact BeadChip surface. Touch off in the reservoir of the glass back plate.

Figure 35 Dispensing RA1 into Each Flow-Through Chamber



b 450 µl XC1. Incubate for 10 minutes.
c 450 µl XC2. Incubate for 10 minutes.
d 200 µl TEM. Incubate for 15 minutes.
e 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat one time.
f Incubate 5 minutes.
g Begin ramping the Chamber Rack temperature to the temperature indicated on the STM tube ± 0.5°C.
h 450 µl XC3. Incubate for 1 minute. Repeat one time.

4 Wait until the Chamber Rack reaches the correct temperature.

Stain BeadChip

1 Into the reservoir of each flow-through chamber, dispense:
   a 250 µl STM. Incubate for 10 minutes.
   b 450 µl XC3. Incubate for 1 minute. Repeat one time.
   c Wait 5 minutes.
   d 250 µl ATM. Incubate for 10 minutes.
   e 450 µl XC3. Incubate for 1 minute. Repeat one time.
   f Wait 5 minutes.
   g 250 µl STM. Incubate for 10 minutes.
   h 450 µl XC3. Incubate for 1 minute. Repeat one time.
   i Wait 5 minutes.
   j 250 µl ATM. Incubate for 10 minutes.
   k 450 µl XC3. Incubate for 1 minute. Repeat one time.
   l Wait 5 minutes.
   m 250 µl STM. Incubate for 10 minutes.
   n 450 µl XC3. Incubate for 1 minute. Repeat one time.
   o Wait 5 minutes.

2 Immediately remove the flow-through chambers from the Chamber Rack and place horizontally on a lab bench at room temperature.

Wash and Coat BeadChips

Before starting the Wash and Coat process, read these important notes:

- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates before use.
- Place a clean tube rack on top of several layers of Kimwipes or on an absorbent pad. You will place the staining rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.
- Wash the tube racks and wash dishes thoroughly after use. Rinse with DI water. Immediately following wash, place racks and wash dishes upside down on a wash rack to dry.

Equipment Needed

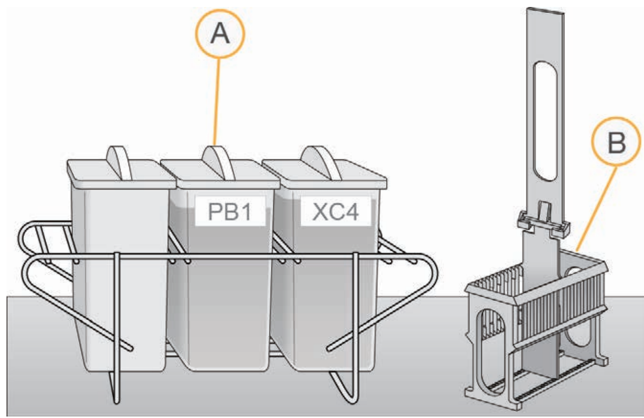
- Place the following items on the bench:
  - 1 staining rack and wash dishes
  - 1 vacuum desiccator
  - 1 tube rack
  - Self-locking tweezers
  - Large Kimwipes
  - Vacuum hose

Steps

1 Set up 2 top-loading wash dishes, labeled "PB1" and "XC4".

2 To indicate the fill volume before filling wash dishes with PB1 and XC4, pour 310 ml water into the wash dishes and mark the water level on the side. Empty the water from the wash dish. This enables you to pour reagent directly from the PB1 and XC4 bottles into the wash dishes, minimizing contaminant transfer from labware to wash dishes.

Figure 36 PB1 and XC4 Wash Dishes with Staining Rack

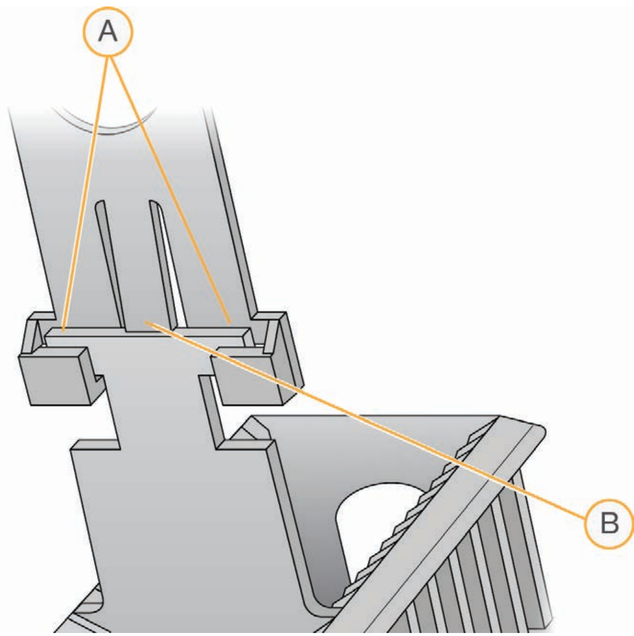


A Wash Dishes
B Staining Rack

3 Pour 310 ml PB1 into the wash dish labeled “PB1.”

4 Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing towards*** you. This orients the staining rack so that you can safely remove the BeadChips. Let the staining rack sit in the wash dish. You will use it to carry the BeadChips after disassembling the flow-through chambers.

Figure 37 Staining Rack Locking Arms and Tab

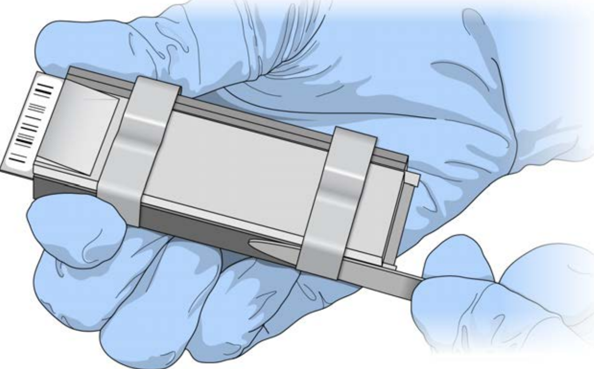


A Locking Arms
B Tab

5 One at a time, disassemble each flow-through chamber:
   a Use the dismantling tool to remove the 2 metal clamps.

CAUTION
It is important to use the dismantling tool to avoid chipping the glass back plates.

Figure 38 Removing the Metal Clamps from Flow-Through Chamber



   b Remove the glass back plate by lifting the glass straight up.

CAUTION
Do not slide the glass along the BeadChip. This can damage the BeadChip.

   c Set the glass back plate aside. When you finish the XStain protocol, clean the glass back plates. See *Cleaning the Glass Back Plates* on page 14 for instructions.
   d Remove the spacer. To avoid damaging the stripes on the BeadChip, pull the spacer out so that the long sides slide along the sides of the BeadChip.

CAUTION
Do not remove the spacer before removing the glass. This can cause the glass and BeadChip to come into contact and can damage the BeadChip.

e Remove the BeadChip from the black frame.

CAUTION
Do not touch the face of the BeadChips. Handle them by the barcode end or by the edges.

6 Place the BeadChips in the staining rack while it is submerged in PB1. Make sure that the BeadChip barcodes are ***facing away*** from you and that the locking arms on the handle are ***facing towards*** you.
If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting each BeadChip.

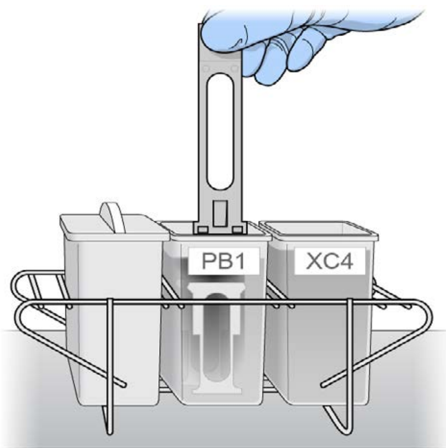
7 Make sure that the BeadChips are submerged.

CAUTION
Do not allow the BeadChips to dry. Submerge each BeadChip in the wash dish as soon as possible.

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

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, gently move the staining rack back and forth to separate the slides. It is important for the solution to circulate freely between all BeadChips.

Figure 39 Washing BeadChips in PB1



9 Soak for 5 minutes.

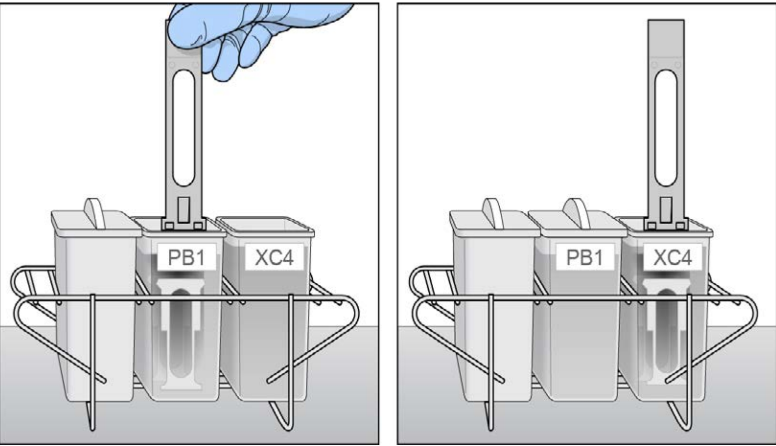
10 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until dissolved.

11 Pour 310 ml XC4 into the dish labeled "XC4," and cover the dish to prevent any lint or dust from falling into the solution.

CAUTION
Do not let the XC4 sit for longer than 10 minutes.

12 Remove the staining rack from the PB1 dish and place it directly into the wash dish containing XC4.

Figure 40 Moving BeadChips from PB1 to XC4



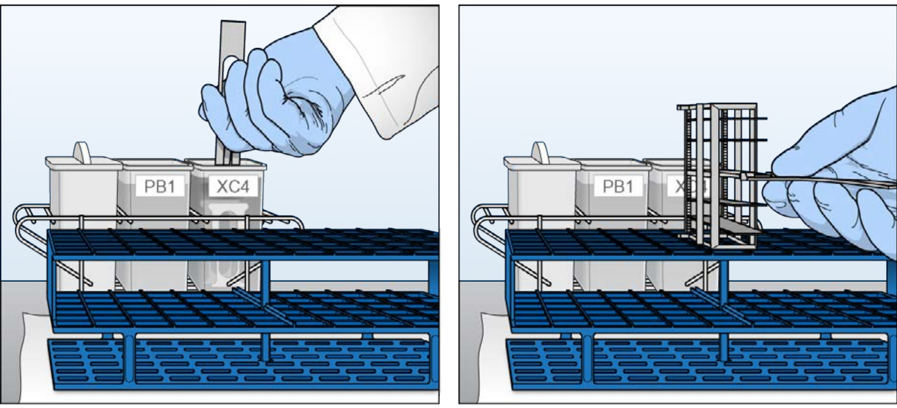
13 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

NOTE
If the top edges of the BeadChips begin to touch during either PB1 or XC4 washes, 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 for 5 minutes.

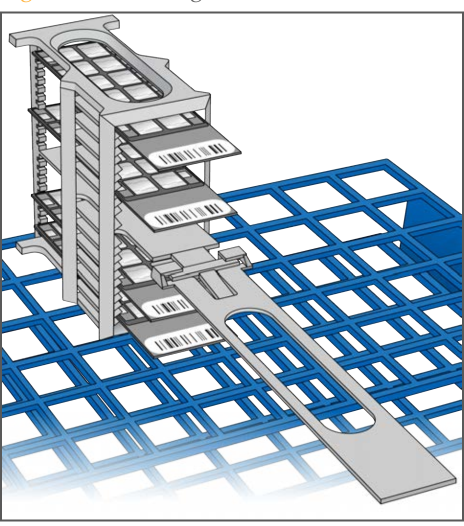
15 Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack.

Figure 41 Moving the Staining Rack from XC4 to Tube Rack



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

Figure 42 Staining Rack in Correct Orientation



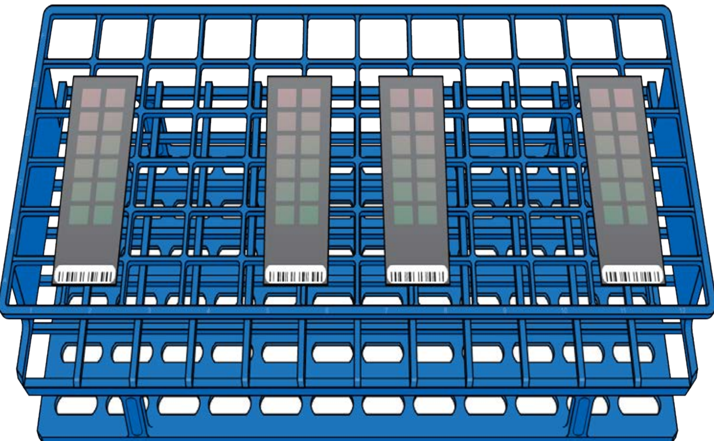
16 For each of the BeadChips, working top to bottom:

a Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

NOTE
The XC4 coat is slippery and makes the BeadChips difficult to hold. The self-locking tweezers grip the BeadChip firmly and help prevent damage.

b Place each BeadChip on a tube rack with the barcode ***facing up and towards*** you.

Figure 43 BeadChips on Tube Rack



TIP
Remove the staining rack handle if it facilitates removal of the BeadChips from the staining rack. Holding the top of the staining rack in position, gently remove the staining rack handle by grasping the handle between the thumb and forefinger. Push up the tab with your thumb and push the handle away from you (unlocking the handle), then pull up the handle and remove.

CAUTION
To prevent wicking and uneven drying, do not allow the BeadChips to rest on the edge of the tube rack or to touch each other while drying. Do not use the bottom level of the tube rack. The back of the BeadChips will not dry as effectively and dye protectant could pool.

17 Place the tube rack in the vacuum desiccator. Each desiccator can hold 1 tube rack (8 BeadChips).

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

18 Remove the red plug from the three-way valve before applying vacuum pressure.

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

20 To make sure that the desiccator is properly sealed, gently lift the lid of the vacuum desiccator. Make sure that the lid does not lift off the desiccator base.

21 Dry under vacuum for 50–55 minutes.
Drying times can vary according to room temperature and humidity.

22 Release the vacuum by turning the handle slowly.

WARNING
Make sure that air enters the desiccator slowly to avoid disturbing the contents. Improper use of the vacuum desiccator can result in damage to the BeadChips. This is especially true if you remove the valve plug while a vacuum is applied. For detailed vacuum desiccator instructions, see the documentation included with the desiccator.

23 Store the desiccator with the red valve plug in the three-way valve of the desiccator to stop accumulation of dust and lint within the valve port.

24 Touch the borders of the chips (**do not touch the stripes**) to make sure that the etched, barcoded sides of the BeadChips are dry to the touch.

25 If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4 using a Kimwipe sprayed with 70% EtOH. The bottom 2 BeadChips are most likely to have some excess.
   a Hold the BeadChip at a downward angle to prevent excess EtOH from dripping from the wipe onto the stripes.
   b Wipe along the underside of the BeadChip 5 or 6times, until excess XC4 is removed.

CAUTION
Do not touch the stripes with the wipe or allow EtOH to drip onto the stripes.

26 Perform 1 of the following:
- Proceed to *Scan the BeadChip*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Scan the BeadChips within 72 hours.

27 Clean the glass back plates. See *Cleaning the Glass Back Plates* on page 14.

28 Discard unused reagents in accordance with facility standards.

Scan BeadChip

Scan BeadChips using either the iScan system or the NextSeq 550 system. To perform a scan, you need Decode Map (DMAP) files, a manifest file, and a cluster file for the BeadChip you are using.

- Use the Decode File Client to download the required DMAP folder. DMAP folders are unique for each BeadChip type. For more information, see the *DMAP Decode File Client User Guide (part # 11337856)*.
- Download the manifest and cluster files from the Illumina support website. Each manifest and cluster file is unique for each BeadChip type. If you are using the NextSeq 550 system, make sure to use files that include NS550 in the file name.
- Scan your BeadChip using the iScan or the NextSeq system. For more information, see the *iScan System User Guide (part # 11313539)* or the *NextSeq 550 System User Guide (part # 15069765)*. In the Lab Tracking Form, record the Scanner ID and scan date for each BeadChip.
- Output files are generated during the scan and then queued for transfer to the specified output folder.

Perform analysis using BlueFuse Multi software, which requires that scanning data are available in a genotype call (GTC) file format. By default, the NextSeq 550 system generates normalized data and associated genotype calls in the format of a GTC file. The iScan can be configured to produce GTC files, in addition to the standard file types (such as IDAT files).

Alternatively, you can use the Illumina Beeline software to convert IDAT files to GTC files after scanning. For more information, see *Beeline Software User Guide (part # 15016340)*.

Appendix I: Agarose Gel Electrophoresis

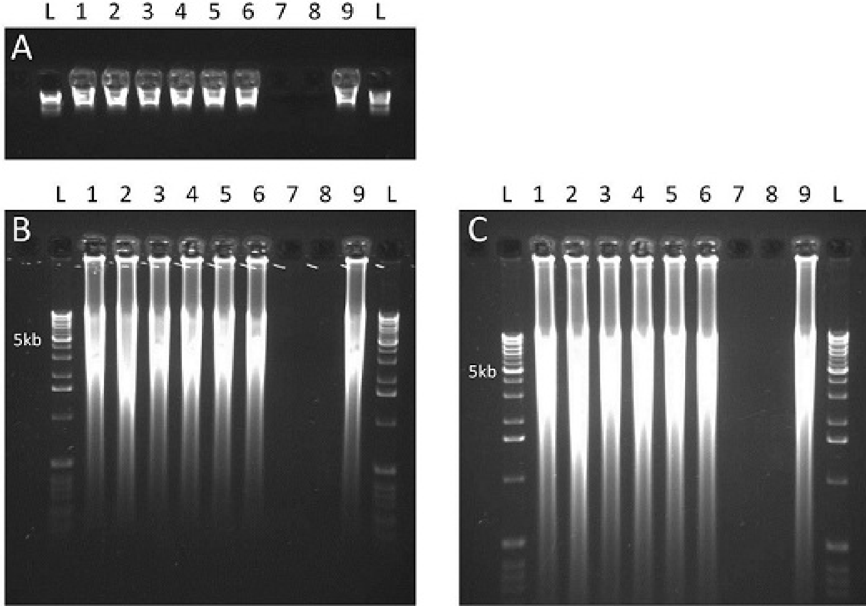


Figure 44 Single cells isolated from a cultured lymphoblastoid cell line were amplified in a 2-hour MDA reaction using the REPLI-g Single Cell kit (Qiagen). After MDA, 2 µl of MDA products, with 3 µl of water and 5 µl of 2x loading dye were electrophoresed for 5 (A), 40 (B), and 60 (C) minutes at 120 V in a 0.8% 1xTBE agarose gel. The lane contents are labeled as follows: 1–6: amplified DNA from a lymphoblastoid cell line in a 2-hour MDA reaction using the REPLI-g Single Cell kit (Qiagen). 7: BC (buffer control), cell collection negative control. 8: NTC (no-template control), amplification negative control. 9: 50pg male genomic DNA, amplification positive control. L: 1 kb DNA Extension Ladder.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 9 Illumina General Contact Information

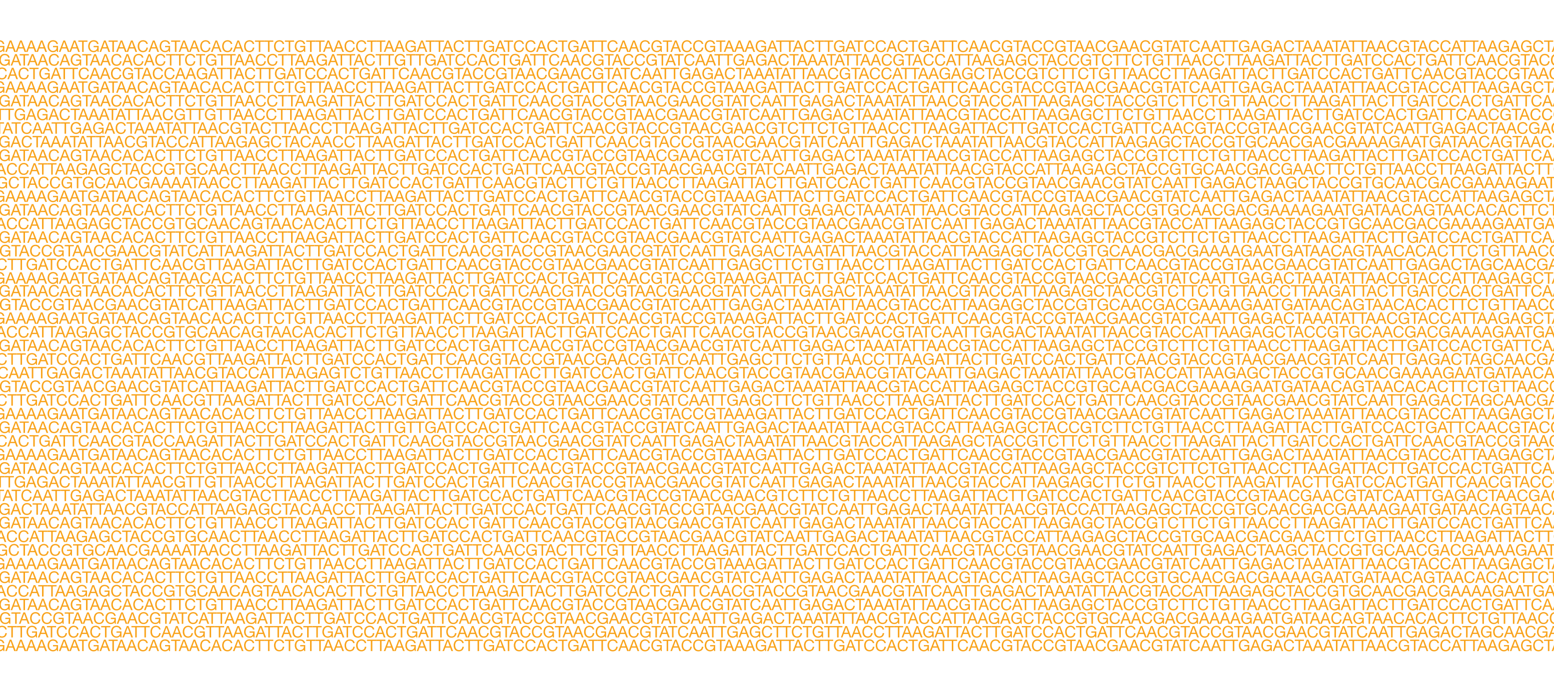
Website	www.illumina.com
Email	techsupport@illumina.com

Table 10 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

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

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



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