
Experienced User Card
For Research Use Only. Not for use in diagnostic procedures.

Day 1
- Quantitate DNA
  - Hands-on: 30 min/plate
  - Fluorometer: 5 min/plate
- Reagents
  - Lambda DNA
  - PicoGreen dsDNA
  - 1X TE
- Output
  - Sample QDNA Plate with Quantitated DNA

Start Bisulfite Conversion
- Hands-on: ~1.5 hours
- Incubation: 17-18 hours
- Reagents
  - Zymo EZ DNA Methylation Kit
  - Genomic DNA
- Output
  - MSA4 Plate

Day 2
- Make BCD
  - Hands-on: ~2 hours
  - Reagents
    - Zymo EZ DNA Methylation Kit
    - Genomic DNA
  - Output
    - BCD Plate

Day 3
- Fragment MSA4
  - Hands-on: ~30 min
  - Incubation: 1 hour
  - Reagents
    - FMS
  - Output
    - MSA4 Plate

Precip MSA4
- Hands-on: ~30 min
- Incubation and Dry Time: 2 hours
- Reagents
  - 2-propanol
  - PM1
- Output
  - MSA4 Plate

Resuspend MSA4
- Hands-on: ~30 min
- Incubation: 1 hour
- Reagents
  - R1
- Output
  - MSA4 Plate

Day 4
Wash BeadChip
- Hands-on: ~20-30 min
- Reagents
  - PB1
- Output
  - BeadChip

XStain BeadChip
- Hands-on: ~3 hours
- Dry Time: 1 hour
- Reagents
  - RA1
  - Formamide / EDTA
  - PB1
  - XC1
  - XC2
  - XC3
  - XC4
  - TEM
  - STM
  - ATM
- Output
  - BeadChip

Image BeadChip
- iScan or HiScan System
- Scan Time: 50 to 60 min/BeadChip
- Output
  - Image and Data Files

Fill in the lab tracking form and the sample sheet as you perform the assay.
Make the BCD Plate (Pre-Amp)

Bisulfite convert the genomic DNA samples using the Zymo EZ DNA Methylation Kit. Transfer the bisulfite-converted samples to the BCD plate.

Estimated Time

Hands-on time:
- ~1.5 hours on Day 1
- ~2 hours on Day 2

Incubation time: 16–17 hours on Day 1 (Overnight)

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zymo EZ DNA Methylation kit</td>
<td>1 kit per 50 samples</td>
<td>Room temperature</td>
<td>User</td>
</tr>
<tr>
<td>(includes bisulfite conversion reagent, dilution buffer, desulphonation buffer, elution buffer)</td>
<td>1 kit per 200 samples or 1 kit per 2 plates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96-well 0.2 ml skirted microplate</td>
<td>1–3 plates</td>
<td>User</td>
<td></td>
</tr>
<tr>
<td>Genomic DNA (for HD Methylation)</td>
<td>≥ 250 ng for each bisulfite conversion reaction</td>
<td>User</td>
<td></td>
</tr>
<tr>
<td>FFPE DNA (for HD FFPE Methylation)</td>
<td>≥250 ng for each bisulfite conversion reaction for manual or automated process</td>
<td>User</td>
<td></td>
</tr>
</tbody>
</table>

CAUTION
Bisulfite conversion kits that are not specified in this guide are not supported for use with the Infinium HD Methylation Assay, Manual Protocol.

NOTE
Illumina supports down to 250 ng fresh DNA for this reaction. When possible, using more DNA, such as 500 ng or 1000 ng, results in higher reproducibility.

Preparation

- Prepare the conversion reagent according to the instructions from the manufacturer. For best results, use it immediately.
- Prepare the wash buffer according to the manufacturer instructions.
- Apply a BCD barcode label to each new 96-well 0.2 ml skirted microplate.
Steps to Make the BCD Plate

Day 1

[ ] 1 Follow the instructions in the Zymo EZ DNA Methylation Kit to denature the genomic DNA and add conversion reagent.

[ ] 2 Incubate in a thermal cycler using the following conditions for 16 cycles:
   • 95°C for 30 seconds
   • 50°C for 1 hour

[ ] 3 Hold DNA at 4°C for 10 minutes in the thermal cycler until you are ready to do the clean-up.

Day 2

[ ] 1 Follow the instructions in the Zymo EZ DNA Methylation Kit to do the following:
   [ ] a Wash off the conversion reagent.
   [ ] b Desulphonate the column or plate with desulphonation buffer. Incubate at room temperature for 15 minutes.
   [ ] c Wash off the desulphonation buffer.
   [ ] d Add elution buffer.
   [ ] e Centrifuge to elute.

[ ] 2 Transfer the bisulfite-converted DNA (BCD) samples to the BCD plate.

[ ] 3 Heat-seal the plate and store it at -25°C to -15°C.

[ ] 4 Proceed to Make MSA4 Plate (Pre-AMP).
Make MSA4 Plate (Pre-Amp)

Move BCD samples into the MSA4 plate. Denature and neutralize samples, and prepare them for amplification. Incubate overnight to amplify.

Estimated Time

Hands-on time:
- 45 minutes for 48 samples
- 60 minutes for 96 samples

Incubation time: 20–24 hours

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA1</td>
<td>2 tubes</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td>(per 96 samples)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPM</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>(per 96 samples)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>(per 96 samples)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1N NaOH</td>
<td>15 ml</td>
<td>2°C to 8°C</td>
<td>User</td>
</tr>
<tr>
<td>(per 96 samples)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96-well 0.8 ml microplate (MIDI)</td>
<td>1 plate</td>
<td></td>
<td>User</td>
</tr>
<tr>
<td>BCD plate with Bisulfite-converted DNA samples</td>
<td>1 plate</td>
<td>-25°C to -15°C</td>
<td>User</td>
</tr>
</tbody>
</table>

Preparation

1. Preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
2. Apply an MSA4 barcode label to a new 0.8 ml microplate (MIDI).
3. Thaw MA1, RPM, and MSM tubes to room temperature. Gently invert at least 10 times to mix contents.
4. Thaw BCD samples to room temperature.

Steps to Make MSA4 Plate

1. Dispense 20 µl MA1 into the MSA4 plate wells.
2. Transfer 4 µl of the DNA sample from the WG#-BCD plate to the corresponding wells in the MSA4 plate.
3. On the lab tracking form, record the original DNA sample ID for each well in the MSA4 plate.
4. Dispense 4 µl 0.1N NaOH into each well of the MSA4 plate that contains MA1 and sample.
5. Seal the MSA4 plate with the 96-well cap mat.

**CAUTION**

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.

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.
   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 RPM into each well of the MSA4 plate containing sample.

11. Dispense 75 µl MSM into each well of the MSA4 plate containing sample.

12. Seal MSA4 plate with cap mat.
   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 MSA4 plate at 1600 rpm for 1 minute.

14. Pulse centrifuge at 280 × g.

15. Incubate in the Illumina Hybridization Oven for 20–24 hours at 37°C.

16. Proceed to the next step.
Fragment DNA (Post-Amp)

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

Estimated Time

Hands-on time: ~30 minutes for 96 samples
Incubation time: 1 hour

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMS</td>
<td>2 tubes (per 96 samples)</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
</tbody>
</table>

Preparation

[ ] 1 Preheat the heat block with the midi plate insert to 37°C.
[ ] 2 Thaw FMS tubes to room temperature.
[ ] 3 Gently invert the FMS tubes at least 10 times to mix contents.
[ ] 4 Remove the MSA4 plate from the Illumina Hybridization Oven.

Steps to Fragment the MSA4 Plate

[ ] 1 Pulse centrifuge the plate at 280 × g.
[ ] 2 Carefully remove the cap mat.
[ ] 3 Add 50 µl FMS to each well containing sample.
[ ] 4 Seal the MSA4 plate with the 96-well cap mat.
[ ] 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 1 hour.
[ ] 8 Do either of the following:
  - Continue to the next step, Precipitate DNA (Post-Amp). Leave plate in 37°C heat block until setup is complete. Do not leave the plate in the 37°C heat block for longer than 2 hours.
  - If you do not plan to proceed to the next step within the next 4 hours, store the sealed MSA4 plate at -25°C to -15°C for no more than 24 hours.

SAFE STOPPING POINT
Now is a good stopping point in the process.
Precipitate DNA (Post-Amp)

Add PM1 and 2-propanol to the MSA4 plate to precipitate the DNA samples.

**Estimated Time**

- Hands-on time: ~30 minutes
- Incubation and dry time: 2 hours

**Consumables**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM1</td>
<td>2 tubes (per 96 samples)</td>
<td>2°C to 8°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>100% 2-propanol</td>
<td>40 ml (per 96 samples)</td>
<td>Room temperature</td>
<td>User</td>
</tr>
<tr>
<td>Adsorbent pad</td>
<td>1</td>
<td>Room temperature</td>
<td>User</td>
</tr>
</tbody>
</table>

**Preparation**

- [] 1 If frozen, thaw MSA4 plate to room temperature, then pulse centrifuge at 280 × g.
- [] 2 Preheat heat block to 37°C.
- [] 3 Thaw PM1 to room temperature. Gently invert at least 10 times to mix contents.
- [] 4 Remove the 96-well cap mat.

**Steps to Precipitate the MSA4 Plate**

- [] 1 Add 100 µl PM1 to each MSA4 plate well containing sample.
- [] 2 Seal the plate with the cap mat.
- [] 3 Vortex the plate at 1600 rpm for 1 minute.
- [] 4 Incubate at 37°C for 5 minutes.
- [] 5 Pulse centrifuge at 280 × g for 1 minute.

  **NOTE**

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

- [] 6 Carefully remove the cap mat and discard it.
- [] 7 Add 300 µl 100% 2-propanol to each well containing sample.
- [] 8 Carefully seal the MSA4 plate with a new, *dry* cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.
- [] 9 Invert the plate at least 10 times to mix contents thoroughly.
- [] 10 Incubate at 4°C for 30 minutes.
- [] 11 Centrifuge at 3000 × g at 4°C for 20 minutes. Immediately remove the MSA4 plate from centrifuge.
- [] 12 Remove the cap mat and discard it.
13. Quickly invert the MSA4 plate and drain the liquid onto an absorbent pad to decant the supernatant. Then smack the plate down on a dry area of the pad, avoiding the liquid that was drained onto the pad.

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

15. Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
   You can expect to see blue pellets at the bottoms of the wells.

16. Do either of the following:
   - Continue to the next step, Resuspend DNA (Post-Amp).
   - If you do not plan to proceed to the next step immediately, seal the MSA4 plate with a new cap mat and store it at -25°C to -15°C.

SAFE STOPPING POINT
Now is a good stopping point in the process.
Resuspend DNA (Post-Amp)

Add RA1 to the MSA4 plate to resuspend the precipitated DNA samples.

Estimated Time

Hands-on time: ~30 minutes
Incubation time: 1 hour

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA1</td>
<td>46 µl per sample well</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
</tbody>
</table>

**NOTE**

Pour out only the recommended volume of RA1 needed for the suggested number of samples listed in the consumables table. Additional RA1 can be stored at -20°C to be used later in the XStain BeadChip step.

Preparation

[.] 1 If you stored the MSA4 plate at -25°C to -15°C, thaw it to room temperature. Remove the cap mat and discard it.
[.] 2 Preheat the Illumina Hybridization Oven to 48°C.
[.] 3 Turn on the heat sealer to preheat. Allow 20 minutes.
[.] 4 Thaw RA1 to room temperature. Invert several times to redissolve the solution.

Steps to Resuspend the MSA4 Plate

[.] 1 Add 46 µl RA1 to each well of the MSA4 plate containing a DNA pellet. Reserve any leftover reagent for the Hybridization and XStain steps.
[.] 2 Apply a foil heat seal to the MSA4 plate by firmly and evenly holding the heat sealer sealing block down for 3 seconds.
[.] 3 Immediately remove the MSA4 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 1 hour at 48°C.
[.] 5 Vortex the plate at 1800 rpm for 1 minute.
[.] 6 Pulse centrifuge at 280 × g.
[.] 7 Do either of the following:
  - Continue to the next step, Hybridize to BeadChip (Post-Amp). If you plan to do so immediately, it is safe to leave the MSA4 plate at room temperature for up to 1 hour.
  - If you do not plan to proceed to the next step immediately, store the sealed MSA4 plate at -25°C to -15°C for no more than 24 hours. Store RA1 at -25°C to -15°C.
SAFE STOPPING POINT
Now is a good stopping point in the process.
Hybridize to BeadChip (Post-Amp)

Dispense the fragmented, resuspended DNA samples onto BeadChips. Incubate the BeadChips in the Illumina Hybridization Oven to hybridize the samples onto the BeadChips.

Estimated Time

Hands-on time:
- ~30 minutes for 48 samples
- ~40 minutes for 96 samples

Incubation time: 16–24 hours

Consumables for 8x1 HD BeadChip

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity (per 96 Samples)</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2</td>
<td>2 tubes</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td>BeadChips</td>
<td>12</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Hyb Chambers</td>
<td>3</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Hyb Chamber gaskets</td>
<td>3</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Hyb Chamber inserts</td>
<td>12</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>EtOH</td>
<td>330 ml</td>
<td></td>
<td>User</td>
</tr>
</tbody>
</table>

Consumables for 12x1 HD BeadChip

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity (per 96 Samples)</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2</td>
<td>2 tubes</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td>BeadChips</td>
<td>8</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Hyb Chambers</td>
<td>2</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Hyb Chamber gaskets</td>
<td>2</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Hyb Chamber inserts</td>
<td>8</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>EtOH</td>
<td>500 ml</td>
<td></td>
<td>User</td>
</tr>
</tbody>
</table>

Preparation

[1] 1 Preheat the heat block to 95°C.
[2] 2 Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.
Assemble the Hybridization Chambers

1. Place the resuspended MSA4 plate on the heat block to denature the samples at 95°C for 20 minutes.
2. Remove the BeadChips from 2°C to 8°C storage, leaving the BeadChips in their plastic bags and mylar packages until you are ready to begin hybridization.
3. During the 20-minute incubation, prepare the Hyb Chambers.
   a. Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers.
   b. Dispense 400 µl PB2 into the humidifying buffer reservoirs in the Hyb Chambers.
   c. After you fill the Hyb Chamber reservoirs with PB2, place the lid on the Hyb Chamber right away to prevent evaporation. It is not necessary to lock the lid.
   d. 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 1 hour.
   
   NOTE
   You can also prepare the Hyb Chambers later, during the 30-minute cool down.

4. After the 20-minute incubation, remove the MSA4 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.
5. After the 30-minute cool down, pulse centrifuge the MSA4 plate to 280 × g. Remove the foil seal.

Load BeadChip

1. Just before loading DNA samples, remove all BeadChips from their plastic bags and mylar packages.
   When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.
2. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the Hyb Chamber insert.
For the 8x1 BeadChip

1. Using a single-channel precision pipette, dispense 26 µl of each DNA sample onto the appropriate BeadChip section, according to the chart. Make sure that the pipette tip is in the sample inlet before dispensing.

   a. Load sample A1 from the MSA4 plate into sample inlet A1 of the BeadChip.

   Make sure that the pipette tip is in the sample inlet before dispensing.

   b. Load sample B1 from the MSA4 plate into sample inlet B1 of the BeadChip.

   c. Load sample C1 from the MSA4 plate into sample inlet C1 of the BeadChip.

   d. Load sample D1 from the MSA4 plate into sample inlet D1 of the BeadChip.

   e. Continue in this manner until all samples are loaded.
For the 12x1 BeadChip

[1] Using a multichannel precision pipette, dispense 15 µl of each DNA sample onto the appropriate BeadChip section, according to the chart. Make sure that the pipette tip is in the sample inlet before dispensing.

[1a] Load samples A1–F1 from the MSA4 plate into the left side of the BeadChip. Make sure that the pipette tip is in the sample inlet before dispensing.

[1b] Load samples in G1 and H1 from the MSA4 plate into the top 2 inlets of the right side of the BeadChip.

[1c] Load samples A2–D2 into the remaining 4 inlets on the right side of the BeadChip.

[1d] Continue in this manner, following the color-coded sections in the chart, until all samples are loaded.

On the lab tracking form, record the BeadChip barcode for each group of samples.

In the sample sheet Sentrix ID column, record the BeadChip sample ID and position. See the Sample Section Naming Diagram in the Lab Tracking Form.

Inspect all sections of the BeadChip to make sure that the DNA sample covers all areas of each bead stripe.

Record any sections that are not covered on the lab tracking form.

Set up BeadChip for Hybridization

[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.

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

[3] Close the clamps on both sides of the Hyb Chamber so that the lid is secure and even on the base (no gaps).

NOTE
Keep the Hyb Chamber steady and level when moving it or transferring it to the Illumina Hybridization Oven.
[.] 4 Place the Hyb Chamber in the 48°C Illumina Hybridization Oven with the clamps on the left and right sides of the oven and the Illumina logo facing you.

OVERNIGHT INCUBATION
Incubate at 48°C for at least 16 hours but no more than 24 hours.

[.] 5 Proceed to Wash BeadChips (Post-Amp) after the overnight incubation.

Resuspend XC4 Reagent for XStain BeadChip

[.] 1 Add 330 ml 100% EtOH to the XC4 bottle, for a final volume of 350 ml. Each XC4 bottle has enough solution to process up to 24 BeadChips.

[.] 2 Shake the XC4 bottle vigorously to ensure complete resuspension. After it is resuspended, use XC4 at room temperature. You can store it at 2°C to 8°C for 2 weeks if unused.
Wash BeadChips (Post-Amp)

Prepare the BeadChips for the staining process.

Estimated Time
- 20 minutes for 4 BeadChips
- 30 minutes for 8 BeadChips

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBI</td>
<td>550 ml for 1 alignment fixture</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td></td>
<td>700 ml for 2 alignment fixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>850 ml for 3 alignment fixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multisample BeadChip alignment fixture</td>
<td>1 (per 8 BeadChips)</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Te-Flow flow-through chambers, with black frames, spacers, glass back plates, and clamps</td>
<td>1 (per BeadChip)</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Wash dish</td>
<td>2 (up to 8 BeadChips)</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>Wash rack</td>
<td>1 (up to 8 BeadChips)</td>
<td></td>
<td>Illumina</td>
</tr>
</tbody>
</table>

Preparation

[  ] 1 Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 30 minutes before opening.

[  ] 2 While the Hyb Chamber is cooling:
   [  ] a Fill 2 wash dishes with PBI (200 ml per wash dish). Label each dish "PBI".
   [  ] b Fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PBI.
   [  ] c Separate the clear plastic spacers from the white backs.
   [  ] d Clean the glass back plates if necessary.

Steps to Wash BeadChips

[  ] 1 Attach the wire handle to the rack and submerge the wash rack in the wash dish containing 200 ml PBI.

[  ] 2 Remove the Hyb Chamber inserts from the Hyb Chambers.

[  ] 3 Remove each BeadChip from the Hyb Chamber insert.

[  ] 4 Remove the cover seal from each BeadChip.

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.
[.] 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 the barcode is facing up and 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. Do not touch the exposed arrays.

[.] 5 Immediately and carefully slide each BeadChip into the wash rack, making sure that the BeadChip is submerged in the PB1.

[.] 6 Repeat steps 4 through 5 until all BeadChips (a maximum of 8) are transferred to the submerged wash rack.

[.] 7 After all 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.

[.] 8 Move the wash rack to the other wash dish containing clean PB1. Make sure the BeadChips are submerged.

[.] 9 Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

[.] 10 When you remove the BeadChips from the wash rack, inspect them for remaining residue.

[.] 11 For each additional set of 8 BeadChips:

[.] a Assemble the flow-through chambers for the first 8 BeadChips, as described in Assemble Flow-Through Chambers on page 20.

[.] b Repeat the wash steps in this section to wash the next set of 8 BeadChips.

Assemble Flow-Through Chambers

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

![Correct Standard Back Plates and Spacers](image)

[.] 1 If you have not done so, fill the BeadChip alignment fixture with 150 ml PB1. If you plan to process more than 4 BeadChips, this 150 ml of PB1 can be reused for an additional set of 4 BeadChips. Use 150 ml of fresh PB1 for every additional set of 8 BeadChips.

[.] 2 For each BeadChip to be processed, place a black frame into the BeadChip alignment fixture prefilled with PB1.

[.] 3 Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges.
stamped onto the alignment fixture.

NOTE
Inspect the surface of each BeadChip for residue left by the seal. Use a pipette tip to remove any residue under buffer and be careful not to scratch the bead area.

[.] 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.

[.] 5 Place the alignment bar onto the alignment fixture. The groove in the alignment bar fits over the tab on the alignment fixture.

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

[.] 7 Attach the metal clamps to the flow-through chambers as follows:

[.] a Gently push the glass back plate against the alignment bar with one finger.

[.] b Place the first metal clamp around the flow-through chamber so that the clamp is approximately 5 mm from the top edge.

[.] c Place the second metal clamp around the flow-through chamber at the barcode end, approximately 5 mm from the reagent reservoir.

[.] 8 Using scissors, trim the ends of the clear plastic spacers from the flow-through chamber assembly. Slip scissors up over the barcode to trim the other end.

[.] 9 Immediately wash the Hyb Chamber reservoirs with DiH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains in the Hyb Chamber reservoir.

[.] 10 Proceed to the next step, Extend and Stain (XStain) BeadChip (Post-Amp).

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

Wash unhybridized and nonspecifically hybridized DNA sample 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.

Estimated Time

- Hands-on time: ~3 hours for 8 BeadChips
- Dry time: 1 hour

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity (Per 8 BeadChips)</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA1</td>
<td>10 ml</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>XC1</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>XC2</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>TEM</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>XC3</td>
<td>75 ml</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td>STM (Make sure that all STM tubes indicate the same stain temperature on the label)</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>ATM</td>
<td>2 tubes</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>PBI</td>
<td>310 ml</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td>XC4</td>
<td>310 ml</td>
<td>-25°C to -15°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>Alconox Powder Detergent</td>
<td>as needed</td>
<td></td>
<td>Illumina</td>
</tr>
<tr>
<td>EtOH</td>
<td>as needed</td>
<td>Room temperature</td>
<td>Illumina</td>
</tr>
<tr>
<td>95% formamide/1 mM EDTA</td>
<td>15 ml</td>
<td>-25°C to -15°C</td>
<td>User</td>
</tr>
</tbody>
</table>

Preparation

1. RA1 is shipped and stored at -25°C to -15°C. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that might be present.
2. Place all reagent tubes in a rack in the order in which they are used. If frozen, allow them to thaw to room temperature, and then gently invert the reagent tubes at least 10 times to mix contents.
3. Make sure that the water circulator is filled to the appropriate level.
4. 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 several locations on the Chamber Rack, using the Illumina Temperature Probe. All locations should be at 44°C ± 0.5°C.

**Single-Base Extension**

*CAUTION*
The remaining steps must be performed without interruption.

1. When the Chamber Rack reaches 44°C, quickly place each flow-through chamber assembly into the Chamber Rack. Pipette reagents into the reservoir of the glass back plate.

2. Into the reservoir of each flow-through chamber, dispense:
   - a 150 µl RA1. Incubate for 30 seconds. Repeat for a total of 5 times.
   - 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.
   - h 450 µl XC3. Incubate for 1 minute. Repeat one time.

3. Wait until the Chamber Rack reaches the correct temperature.

**Stain BeadChip**

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

2. Into the reservoir of each flow-through chamber, dispense:
   - a 250 µl 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.
450 µl XC3. Incubate for 1 minute. Repeat one time.

1)

Wait 5 minutes.

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

Wash and Coat 8 BeadChips

Pour 310 ml PB1 per 8 BeadChips into a wash dish.

Place the staining rack inside the wash dish.

For each BeadChip:

- Use the dismantling tool to remove the 2 metal clamps from the flow-through chamber.
- Remove the glass back plate, the spacer, and then the BeadChip.
- Immediately place each BeadChip into the staining rack that is in the wash dish with the barcode facing away from you. Make sure that all BeadChips are submerged.

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

Allow the BeadChips to soak for an additional 5 minutes.

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

Pour 310 ml XC4 into a wash dish.

**CAUTION**

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

Move the BeadChip staining rack into the XC4 dish.

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

Allow the BeadChips to soak for an additional 5 minutes.

Lift the staining rack out of the solution and place it on a tube rack with the staining rack and BeadChips horizontal, barcodes facing up.

Remove the BeadChips from the staining rack with locking tweezers, working from top to bottom. Place each BeadChip on a tube rack to dry. Remove the rack handle if it facilitates removal of the BeadChips.

Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 675 mm Hg (0.9 bar).

Make sure that the XC4 coating is dry before continuing to the next step.

Clean the underside of each BeadChip with a ProStat EtOH wipe or Kimwipe soaked in EtOH.

**CAUTION**

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

Clean and store the glass back plates and Hyb Chamber components.

Do either of the following:

- Proceed to Image BeadChip (Post-Amp).
- Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Image the BeadChips within 72 hours.
Image BeadChip (Post-Amp)

Follow the instructions in the iScan System User Guide or HiScan System User Guide to scan your BeadChips.

Use the **Methylation NXT** scan setting for your BeadChip.

**NOTE**
Follow the instructions in the Decode File Client User Guide (11337856) available on the Illumina support website to download your DMAPs.

**NOTE**
Use the GenomeStudio Methylation Module v1.8 User Guide (11319130) to analyze your data. Use the Infinium >> Infinium HD setting to create a GenomeStudio project for your BeadChip. Analysis requires a sample sheet that describes the location of each sample. See the support web page for your array for a sample sheet template.