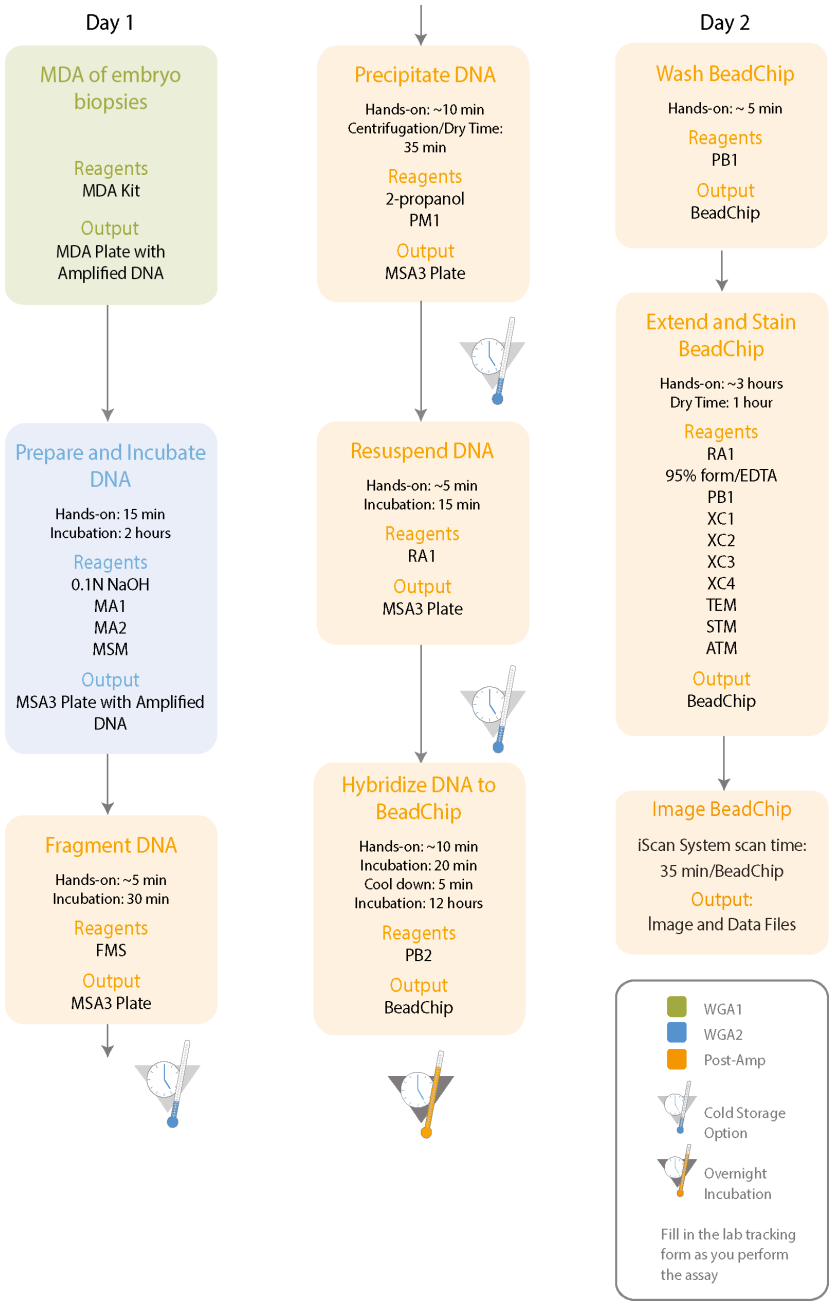
Lab Tracking Form

FOR RESEARCH USE ONLY



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.

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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 1).
- 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.

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Prepare and Incubate the MSA3 Plate (WGA)

Estimated Time

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

Consumables

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

Preparation

- 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.

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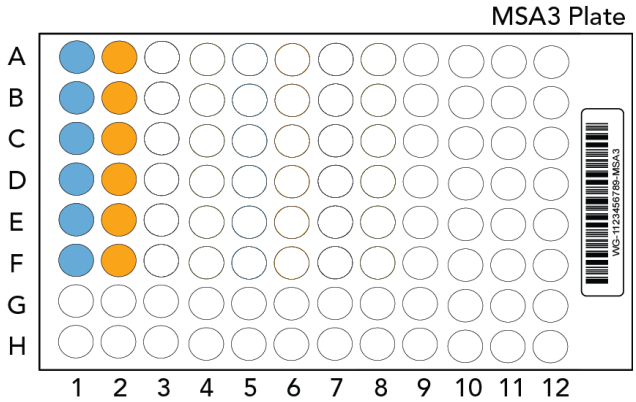
Steps

[_] 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 table, record the original DNA sample ID for each well in the MSA3 plate.

	1	2	3	4	5	6	7	8
A								
B								
C								
D								
E								
F								

[_] 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.

[_] 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.

[_] 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.

[_] 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.
Start time: _____ Stop time: _____

[_] 16 Proceed to *Fragment the DNA*.

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NOTE

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

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Date/Time: _____ Operator: _____

Fragment the DNA (Post-amp)

Estimated Time

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

Consumables

Item	Quantity
FMS Lot #: _____	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***.

Steps

[_] 1 Pulse centrifuge the MSA3 plate to 280 × g.

[_] 2 Carefully remove the cap mat.

[_] 3 Add 50 µl FMS to each well containing sample.

[_] 4 Seal the MSA3 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 30 minutes.

Start time: _____ Stop time: _____

[_] 8 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.

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Date/Time: _____ Operator: _____

Precipitate the DNA (Post-amp)

Estimated Time

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

Consumables

Item	Quantity
PM1 Lot #: _____	1 tube
100% 2-propanol Lot #: _____ Date Opened: _____	15 ml

Preparation

- Preheat heat block to 37°C.
- If frozen, thaw MSA3 plate to room temperature, and then pulse centrifuge the MSA3 plate to 280 × g.
- Bring PM1 to room temperature. Gently invert at least 10 times to mix contents.

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.

[_] 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 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.

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[_] 10 Remove the MSA3 plate from centrifuge. Remove the cap mat and discard it.

[_] 11 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.

[_] 12 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

[_] 13 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.

Start time: _____ Stop time: _____

[_] 14 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.

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Date/Time: _____ Operator: _____

Resuspend the DNA (Post-amp)

Estimated Time

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

Consumables

Item	Quantity
RA1 Lot #: _____	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 (Post-amp)* on page 17.

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.

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 (Post-amp)* on page 17.

[_] 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.

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[_] 4 Place the sealed plate in the Illumina Hybridization Oven and incubate for 15 minutes at 48°C.

Start time: _____ Stop time: _____

[_] 5 Vortex the plate at 1800 rpm for 1 minute.

[_] 6 Pulse centrifuge to 280 × g.

[_] 7 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.

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Hybridize DNA to the BeadChip (Post-amp)

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 Lot #: _____	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 Hybridization Chambers

[_] 1 Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.

Start time: _____ Stop time: _____

[_] 2 During the 20 minute incubation, prepare the Hyb Chambers.

[_] 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.

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

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[_] 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.

Start time: _____ Stop time: _____

Load BeadChips

[_] 1 After the 5 minute cool down, pulse centrifuge the MSA3 plate to 280 × g.

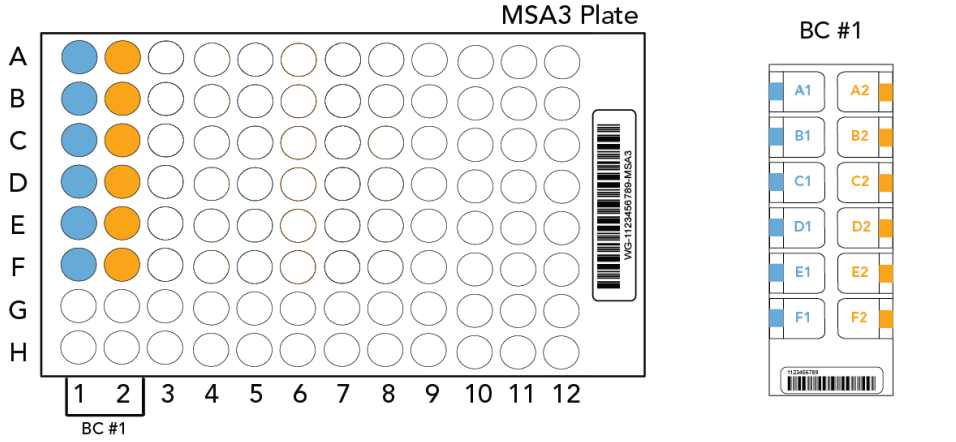
[_] 2 Just before loading DNA samples, remove all BeadChips from their ziplock bags and mylar packages.
When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.

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.

[_] 4 Remove the foil seal from the MSA3 plate. Then, 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.

Figure 1 Distributing Sample in MSA3 Plate



[_] 5 In the appropriate fields, record the BeadChip barcode for each group of samples.

[_] 6 Inspect the loading port to see if a large bolus of liquid remains.
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.

[_] 7 Record the amount of additional sample added in the fields above.

[_] 8 Proceed immediately to the next section, *Set up the BeadChips for Hybridization*.

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Set up the BeadChips 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 the 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).

[_] 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.

[_] 5 Incubate at 48°C for 12 hours or overnight but no more than 24 hours.

Start time: _____ Stop time: _____

[_] 6 Discard the MSA3 plate.

[_] 7 After the overnight incubation, proceed to *Wash the BeadChip*.

Resuspend XC4 Reagent in Preparation for XStain

[_] 1 Add 330 ml fresh 100% EtOH to the XC4 bottle.
Each XC4 bottle (350 ml) 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.

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Date/Time: _____ Operator: _____

Wash the BeadChip (Post-amp)

Estimated Time

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

Consumables

Item	Quantity
PB1 Lot #: _____	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

WARNING

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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.
  Temperature, Location 1: _____
  Temperature, Location 2: _____
  Temperature, Location 3: _____
- In preparation for the wash step, remove each Hyb Chamber from the Illumina Hybridization Oven.
- On the lab bench:
  - Fill two wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".
  - Fill the BeadChip Alignment Fixture with 150 ml PB1.
  - Separate the clear plastic spacers from the white backs.

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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.

Steps

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

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

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. Do not touch the exposed arrays.

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

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.

[_] 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.

[_] 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.
Make sure that the groove in the Alignment Bar fits over the tab on the Alignment Fixture.

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[_] 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.

[_] 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.

[_] 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.

[_] 9 Continue to the next step, *Extend and Stain (XStain) BeadChip (Post-AMP)*.

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 (Post-amp)* on page 17. 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.

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Extend and Stain (XStain) BeadChips (Post-amp)

Estimated Time

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

Consumables

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

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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.

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.

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.

[_] 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.

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

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.
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 5 times.
  [_] 1 [_] 2 [_] 3 [_] 4 [_] 5 [_] 6
- [_] 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.
  [_] 1 [_] 2
- [_] f Incubate 5 minutes.

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[_] 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.
[_] 1 [_] 2

[_] 3 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.
[_] 1 [_] 2

[_] c Wait 5 minutes.

[_] d 250 µl ATM. Incubate for 10 minutes.

[_] e 450 µl XC3. Incubate for 1 minute. Repeat one time.
[_] 1 [_] 2

[_] f Wait 5 minutes.

[_] g 250 µl STM. Incubate for 10 minutes.

[_] h 450 µl XC3. Incubate for 1 minute. Repeat one time.
[_] 1 [_] 2

[_] i Wait 5 minutes.

[_] j 250 µl ATM. Incubate for 10 minutes.

[_] k 450 µl XC3. Incubate for 1 minute. Repeat one time.
[_] 1 [_] 2

[_] l Wait 5 minutes.

[_] m 250 µl STM. Incubate for 10 minutes.

[_] n 450 µl XC3. Incubate for 1 minute. Repeat one time.
[_] 1 [_] 2

[_] 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

[_] 1 Pour 310 ml PB1 into a wash dish.

[_] 2 Place the staining rack inside the wash dish.

[_] 3 For each BeadChip:

[_] a Use the dismantling tool to remove the two metal clamps from the 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 Remove the spacer.

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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.

[_] d Remove the BeadChip from the black frame.

[_] e Immediately place each BeadChip into the staining rack that is in the wash dish with the barcode ***facing away*** from you. All chips should be completely submerged.

[_] 4 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

[_] 5 Soak for 5 minutes.

[_] 6 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until dissolved.

[_] 7 Pour 310 ml XC4 into a wash dish.

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

[_] 8 Move the BeadChip staining rack into the XC4 dish.

[_] 9 Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

[_] 10 Soak for 5 minutes.

[_] 11 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.

[_] 12 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 staining rack handle if it facilitates removal of the BeadChips.

[_] 13 Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 675 mm Hg (0.9 bar).

Start time: _____ Stop time: _____

[_] 14 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.

[_] 15 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.

[_] 16 Clean and store the glass back plates and Hyb Chamber components.

Lab Tracking Form

Date/Time: _____ Operator: _____

Scan the BeadChips

Follow the instructions in the *Infinium Karyomapping Assay Protocol Guide* to scan your BeadChips.

Record the scanner ID and scan date for each BeadChip

Scanner ID:	_____	BeadChip:	_____
Scan Date:	_____	BeadChip:	_____
		BeadChip:	_____
		BeadChip:	_____