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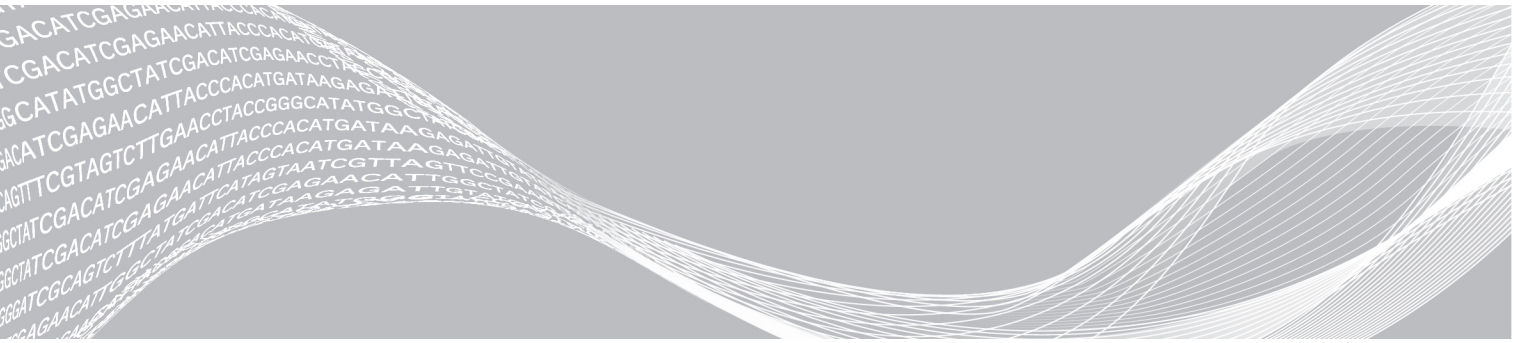
# Infinium Assay

## Lab Setup and Procedures Guide



Document # 11322460 v03

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# Revision History

| Document | Date | Description of Change |
|---|---|---|
| Document # 11322460 v03 | May 2019 | Consolidated consumables and equipment information for Infinium Assays to facilitate the creation of the *Infinium Consumables and Equipment Checklist (document # 1000000084294)*.<br>Added Preparation and Storage of Reagents section to consolidate preparation, handling, and storage of reagents information.<br>Consolidated pipetting information in the Best Practices section. |
| Document # 11322460 v02 | December 2017 | Updated instructions on glass back plate cleaning and handling. |
| Document # 11322460 v01 | December 2016 | Added information to support the Infinium XT Assay. |
| Document # 11322460 rev. A | October 2008 | Supported Infinium Assay setup and procedures. |

# Table of Contents

# Lab Setup and Maintenance



## Introduction

This section describes the essential equipment and operating procedures for an Infinium lab. It explains how to equip and run an Infinium laboratory, providing important information on the following topics:

- Safety precautions
- Consumables and equipment to purchase in advance
- Preventing amplification product contamination
- Preparing and storing reagents
- Best practices
- Lab maintenance

## Safety Precautions

Adhere to the following cautions and warnings while performing the protocols described in this guide.

**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 that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. For more information, consult the material data safety sheet for this assay at www.illumina.com/sds. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.**

## Consumables and Equipment

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

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

### Illumina Supplied Reagents

BeadChip Kits contain sufficient quantities of the reagents required to complete the workflow being performed. Contact your local Illumina representative if additional quantities of a reagent are needed.

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

## User-Supplied Materials

| Material | Supplier | Lab Designation |
|---|---|---|
| Absorbent pads/towels | General lab supplier | Pre-amp and Post-amp |
| Aluminum foil | General lab supplier | Pre-amp and Post-amp |
| Cap mats, 96-well, pierceable, nonautoclavable | Thermo Fisher Scientific, catalog # AB-0566 | Pre-amp |
| Compressed air can | VWR, Int'l, catalog # 16650-027 | Post-amp |
| Containers:<br>• 100 ml<br>• 200 ml<br>• 1 L, for diluting bleach<br>• 2 containers capable of holding polypropylene test tube racks | General lab supplier | Post-amp |
| Foil adhesive seals (Microseal 'F') | MJ Research, catalog # MSF-1001 | Pre-amp |
| Heat sealing foil sheets, Thermo-Seal | Thermo Fisher Scientific, catalog # AB-0559 | Pre-amp |
| Kimwipes or any lint-free tissue | General lab supplier | Pre-amp and Post-amp |
| Lab coats<br>• 2 supplies: 1 for pre- and 1 for post-amplification processes | General lab supplier | Pre-amp and Post-amp |
| Pipette tips<br>• 10 µl aerosol filter<br>• 20 µl aerosol filter<br>• 200 µl aerosol filter<br>• 1000 µl aerosol filter | General lab supplier | Pre-amp and Post-amp |
| Pipettes, serological, 10–50 ml | General lab supplier | Pre-amp and Post-amp |
| Pipetting troughs, disposable | VWR, Int'l, catalog # 21007-970 | Pre-amp and Post-amp |
| Polypropylene test tube racks (recommended) | Cole-Parmer, catalog # EW-06739-17 | Post-amp |
| Powder-free gloves<br>• 2 supplies: 1 for pre- and 1 for post-amplification processes | General lab supplier | Pre-amp and Post-amp |
| ProStat EtOH presaturated wipes<br>• Recommend 1 wipe per 2 chips; 30 wipes per package<br>• Substitute with Kimwipes and 70% EtOH | Contec, catalog # PS-911EB/EtOH | Pre-amp and Post-amp |
| Safety glasses<br>• 2 supplies: 1 for pre- and 1 for post-amplification processes | General lab supplier | Pre-amp and Post-amp |
| Skirted microplates, 96-well, 0.2 ml<br>• Microseal 96-well skirted polypropylene microplates, 8x12 well array<br>• TCY plates, 1 per run<br>• Substitute with 0.8 ml storage plate (midi plate), conical well bottom, if desired | MJ Research, catalog # MSP-9601, www.mjr.com | Pre-amp, automated protocol only |
| Storage microplates, 96-well, 0.8 ml<br>• Midi plates, 1 per run | Thermo Fisher Scientific, catalog # AB0765 | Pre-amp |

| Material | Supplier | Lab Designation |
|---|---|---|
| Tubes<br>• 15 ml conical<br>• 50 ml conical | General lab supplier | Pre-amp and Post-amp |

## User-Supplied Reagents

| Consumable | Supplier | Lab Designation |
|---|---|---|
| Bleach | General lab supplier | Pre-amp and Post-amp |
| Deionized water (DI $H_2O$) | General lab supplier | Pre-amp and Post-amp |
| EDTA, 0.5 M | EMD Chemicals, catalog # 4056<br>Sigma-Aldrich, catalog # E7889 | Post-amp |
| Ethanol, 100% | General lab supplier | Post-amp |
| Formamide, OmniPur | VWR, Int'l, catalog # EM-4650 | Post-amp |
| Isopropanol (2-propanol), 100% | General lab supplier | Post-amp |
| Mild detergent, such as Alconox® Powder Detergent | VWR, Int'l, catalog # 21835 | Post-amp |
| Sodium hydroxide, purchase as solid and prepare a 0.1 N NaOH solution in DI $H_2O$ | Sigma-Aldrich, catalog # 221465 | Post-amp |
| TE, 1X<br>• 10 mM Tris-HCl, pH 8.0, 1 mM EDTA<br>• For diluting DNA | General lab supplier | Post-amp |
| Algecide, such as Aqua-Clear | VWR, Int'l, catalog # 13197-144 | Post-amp |

## User-Supplied Reagents – Tecan Robot

These items are specific to the Tecan 8-tip robot.

| Consumable | Supplier | Lab Designation |
|---|---|---|
| Dimethylsulfoxide (DMSO) (for robot QC) | Sigma, catalog # D-8779 | Post-amp |
| Fluorescein (for robot QC) | Sigma-Aldrich, catalog # F-2456 | Post-amp |
| Fluorescein Dilution Buffer (1X TE, 0.01% Tween 80) (for robot QC) | General lab supplier | Post-amp |
| System Liquid, 1000X (100 mM Tris (pH 8.0), 100 mM EDTA) | General lab supplier | Post-amp |
| Tween 80 (for robot QC) | General lab supplier | Post-amp |

## User-Supplied Equipment

| Equipment | Supplier | Lab Desgination |
|---|---|---|
| Adapters to centrifuge 96-well plates and tubes (2 sets) | General lab supplier | Pre-amp and Post-amp |
| Autodesiccator cabinet | VWR, Int'l, catalog # 74950-342 | |

| Equipment | Supplier | Lab Desgination |
|---|---|---|
| Cap mat sealer (recommended) | Corning, catalog # 3081 | Pre-amp and Post-amp |
| Centrifuge, benchtop 120 V | General lab supplier | Pre-amp |
| Centrifuge, benchtop refrigerated 120 V (8–3000 × g) | General lab supplier | Post-amp |
| Forceps<br>• Included with system, only needed if additional pairs are required | VWR, Int'l, catalog # 25601-008 | Post-amp |
| Micropipettors<br>• 2 separate sets: 1 for pre- and 1 for post-amplification processes<br>• P-20<br>• P-200<br>• P-1000 | General lab supplier | Pre-amp and Post-amp |
| Multichannel precision pipettes<br>• 2 separate sets: 1 for pre- and 1 for post-amplification processes<br>• P-20<br>• P-200 | General lab supplier | Pre-amp and Post-amp |
| Nalgene™ rectangular autoclavable PPCO carboy with spigot (10 L) | Thermo Fisher Scientific, 2319-0020 | Post-amp |
| Optical tachometer/stroboscope (recommended) | Cole-Parmer, catalog # A-87700-06 | Post-amp |
| Plates, 96-well, black, flat bottom FLUOTRAC 200 | Greiner, catalog #655076 | Pre-amp |
| Reservoirs<br>• Full, 150 ml<br>• Half, 75 ml<br>• Quarter, 40 ml | • Beckman Coulter, catalog # 372784<br>• Beckman Coulter, catalog # 372786<br>• Beckman Coulter, catalog # 372790 | Post-amp, automated protocol only |
| Reservoir Frame | Beckman, Coulter, catalog #372795 | Post-amp, automated protocol only |
| Serological pipette aid, 2 | General lab supplier | Pre-amp and Post-amp |
| Stop watches/timers<br>• 2 separate sets: 1 for pre- and 1 for post-amplification processes | General lab supplier | Pre-amp and Post-amp |
| Tube rack<br>• 2 separate sets: 1 for pre- and 1 for post-amplification processes<br>• Any rack fitting the Infinium reagent 17 mm tube diameter | VWR, Int'l, catalog # 66023-540 | Pre-amp and Post-amp |
| Tube vortexers<br>• 2 separate sets: 1 for pre- and 1 for post-amplification processes | General lab supplier | Pre-amp and Post-amp |
| Vacuum source, hose, or pump capable of pulling greater than 508 mm Hg | General lab supplier | Post-amp |
| Vacuum desiccator tubing (included in Infinium Starter Kit, only needed if additional repairs are required) | VWR, Int'l , catalog # 62995-335 | Post-amp |
| Vacuum desiccator racks (included in Infinium Starter Kit, only needed if additional repairs are required) | VWR, Int'l , catalog # 66023-526 | Post-amp |
| [Optional] Vacuum gauge for vacuum desicacator | General lab supplier | Post-amp |

| Equipment | Supplier | Lab Desgination |
|---|---|---|
| [Optional] High-capacity desiccator | LabConco, catalog # 5530000 | Post-amp, automated protocol only |

## Illumina Supplied Equipment

| Equipment | Supplier | Lab Designation |
|---|---|---|
| Scanning system, one of the following:<br>• iScan System (110V/220V)<br>• NextSeq 550 System | Illumina catalog #:<br>SY-101-1001<br>SY-415-1002 | Post-amp |
| Autoloaders:<br>• Single-scanner configuration (110 V/220 V)<br>• Dual-scanner configuration (110 V/220 V) | • Illumina catalog # SY-201-1001<br>• Illumina catalog # SY-201-1002 | Post-amp |
| Infinium Automation Kit:<br>• 8 tip Tecan Non-Illumina LIMS (110 V)<br>• 8 tip Tecan Non-Illumina LIMS (220 V)<br>• 8 tip Tecan Illumina LIMS Ready (110 V)<br>• 8 tip Tecan Illumina LIMS Ready (220 V) | • Illumina catalog # SC-30-401<br>• Illumina catalog # SC-30-402<br>• Illumina catalog # SC-30-403<br>• Illumina catalog # SC-30-404 | Post-amp, optional in pre-amp |
| Robot tip alignment guide-F (LCG) | Illumina, catalog # SE-104-1013 | Post- amp |
| Robot tip alignment guide-G (HD FFPE, HTS, HD Methylation, HD Super) | Illumina, catalog # SE-104-1015 | Post- amp |

## Starter Kits

Equipment starter kits contain the Illumina supplied equipment for the designated workflow.

| Starter Kit | Supplier |
|---|---|
| Infinium HD Starter Kit (8 beadchip) 110V | Illumina catalog # 20028872 |
| Infinium HD Starter Kit (8 beadchip) 220V | Illumina catalog # 20028873 |
| Infinium HTS Starter Kit (8 beadchip) 110V | Illumina catalog # 20028874 |
| Infinium HTS Starter Kit (8 beadchip) 220V | Illumina catalog # 20028875 |
| Infinium HD Starter Kit (24 beadchip) 110V | Illumina catalog # 20028878 |
| Infinium HD Starter Kit (24 beadchip) 220V | Illumina catalog # 20028879 |
| Infinium HTS Starter Kit (24 beadchip) 110V | Illumina catalog # 20028876 |
| Infinium HTS Starter Kit (24 beadchip) 220V | Illumina catalog # 20028877 |
| Infinium XT Starter Kit (24 beadchip) 110V | Illumina catalog # 20031915 |
| Infinium XT Starter Kit (24 beadchip) 220V | Illumina catalog # 20031992 |
| Infinium XT Starter Kit (48 beadchip) 110V | Illumina catalog # 20031916 |
| Infinium XT Starter Kit (48 beadchip) 220V | Illumina catalog # 20031993 |
| Infinium XT Upgrade Kit (24 beadchip) | Illumina catalog # 20011101 |
| Infinium XT ST Upgrade Kit (12 beadchip) | Illumina catalog # 20015526 |

## Starter Kit Contents, All Workflows

The following components are contained in the starter kits for all workflows and are available for purchase separately.

| Equipment | Supplier | Lab Designation |
|---|---|---|
| BeadChip wash dish | Illumina catalog # BD-60-460 | Post-amp |
| BeadChip wash rack | Illumina catalog # BD-60-450 | Post-amp |
| Heat sealer | Thermo Fisher, catalog # AB-1443A | Post-amp |
| Te-Flow chamber dismantling tool | Illumina, catalog # WG-10-204 | Post-amp |
| Hybridization oven | Illumina, catalog # SE-901-1001 (110 VAC) or # SE-901-1001 (220 VAC) | Post-amp |
| HYBEx MIDI Heat Block Insert | Illumina, catalog # BD-60-601 | Post-amp |
| HYBEx heat block incubator | Illumina, catalog # SC-60-503 (110V) or SC-60-504 (220V) | Post-amp |
| Staining rack and wash dish | Illumina, catalog # WG-10-207 | Post-amp |
| Robot BeadChip alignment fixture | Illumina, catalog # 222691 | Post-amp |
| Vacuum desiccator | VWR, Int'l, catalog # 24988-197 | Post-amp |
| Vortex mixer Signature™ High-Speed Microplate Shaker | Illumina, catalog #SC-201-1001 (110V) or 11140324 (220V) | Pre-amp and Post-amp |
| Te-Flow rack, water circulator, tubing, and connections (110V/220v) | Illumina, catalog # 20028404 | Post-amp |
| Glass back plate drying rack, 2 | Cole-Parmer. catalog # EW-06739-17 | Post-amp |
| Multi-sample BeadChip alignment fixture | Illumina, catalog # WG-15-310 | Post-amp |
| Hybridization chamber with gasket | Illumina, catalog # BD-60-402 | Post-amp |

## Starter Kit Contents, Workflow Specific

The following workflow specific components are contained in the starter kits and are available for purchase separately.

| HTS Equipment | Supplier |
|---|---|
| LCG glass back plates | Illumina, catalog # 15019708 |
| LCG Te-Flow chambers (10) | Illumina, catalog # WG-100-1001 |
| LCG Te-Flow Chamber Spacers (500) | Illumina, catalog # WG-100-1002 |

| LCG Equipment | Supplier |
|---|---|
| LCG glass back plates | Illumina, catalog # 15019708 |
| LCG Te-Flow chambers (10) | Illumina, catalog # WG-100-1001 |
| LCG Te-Flow Chamber Spacers (500) | Illumina, catalog # WG-100-1002 |

| HD Equipment | Supplier |
|---|---|
| HD Chamber (1) | Illumina, catalog # WG-10-202 |
| HD Spacer (500) | Illumina, catalog # WG-10-203 |

| XT Equipment | Supplier |
|---|---|
| XT Te-Flow Chambers, quantity 8 | Illumina, catalog # 20012129 |
| XT Te-Flow Assembly Alignment Fixture | Illumina, catalog # 20011760 |
| XT chamber | Illumina, catalog # 20012129 |
| XT (XCG) glass | Illumina, catalog # 20011756 |
| XT Hybridization Chamber | Illumina, catalog # 20011755 |
| XT Dual Hyb Insert and Baseplate | Illumina, catalog #20011759 |
| Infinium 96-XT Tip Guide Set | Illumina, catalog # BD-60-500 |

| XT Upgrade Kit Equipment | Supplier |
|---|---|
| Tray Assembly | Illumina, catalog # 20006371 |
| Drying Rack | Illumina, catalog # 20007067 |
| Wash Rack | Illumina, catalog # 203676 |
| BeadChip Carrier | Illumina, catalog # 20004650 |
| XT Glass | Illumina, catalog # 20004180 |
| Chamber Frame | Illumina, catalog # 20007068 |
| Chamber Clamp | Illumina, catalog # 20007046 |
| XT Hybridization Chamber Replacement Gasket | Illumina, catalog # 20012127 |
| Infinium BeadChip Storage Boxes | Illumina, catalog # 20011102 |
| Infinium Staining Set (staining rack and wash dish) | Illumina, catalog # WG-10-207 |
| Infinium TeFlow thermometer assembly | Illumina, catalog # A1-99-109 |
| XCG Flow-Through Chamber Clips (16 pack) | Illumina, catalog # 20011758 |
| XCG Flow-Through Chamber Clips (8 pack) | Illumina, catalog # 20011757 |
| XCG glass drying rack | Illumina, catalog # 20011754 |
| Tip Guide No.1 | Illumina, catalog # 20004630 |
| Tip Guide No.2 | Illumina, catalog # 20004631 |
| Tip Guide No.3 | Illumina, catalog # 20004632 |

## Prevent Amplification Product Contamination

The Infinium protocol uses a linear amplification process to increase the quantity of input DNA samples to optimal levels. 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. The laboratory space where you perform pre-amplification processes, such as quantification and normalization, must be separate from the post-amplification laboratory space.

The *Infinium Assay Lab Setup and Procedures Guide* refers to these two laboratory spaces as pre-amp and post-amp areas.

To avoid contamination, follow these guidelines:

- Do not share equipment, such as lab coats, gloves, safety glasses, pipettes, centrifuges, heat blocks, and heal sealers, between pre-amp and post-amp areas.
- Do not use the same sink to wash pre-amp and post-amp reservoirs.
- Do not share a water purification system between the pre-amp and post-amp processes.
- Always store supplies for the Infinium protocols in the pre-amp area, and transfer supplies to the post-amp area as needed.
- Establish a daily and weekly decontamination schedule for both areas.

## Preparation and Storage of Reagents

- Reagent kits contain reagents in exact quantities needed for the assay. Measure reagents carefully to avoid shortages. Some kit components require a different temperature for storage than for shipping. On receipt of the kit, remove all components and store them at the specified temperature.
- Maintain a first in, first out (FIFO) system for reagents. Rotate the stock of the remaining reagents to avoid using expired reagents.
- Use fresh reagents for each batch of plates, and empty reservoirs between batches.
- To minimize errors when preparing user-supplied reagents, prepare large batches of 0.1 N NaOH and 95% Formamide/1 mM EDTA using the following guidance.

### Preparing Batches of 0.1 N NaOH

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

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

### Preparing Batches of 95% Formamide/1 mM EDTA

Prepare the 95% formamide/1 mM EDTA mixture in large batches. Divide batches into 15 ml or 50 ml sealed tubes.

Store the sealed tubes for up to 6 months at -25°C to -15°C and use the stored mixture as needed. Use the mixture the same day you open the tube, and discard any unused amounts.

### Prepare and Store PB20

For Infinium XT workflows do the following.

#### Store PB20

- Store PB20 at room temperature.

### Dilute PB20 to Make 1X PB1 (PB1) Solution

1 Add 10 L DI $H_2O$ to the 20 L carboy.

2 Pour the entire contents of PB20 (approximately 1 L) into the carboy.

3 Fill to the 20 L line with DI $H_2O$. Use a graduated cylinder or a gentle stream of DI $H_2O$ to avoid creating bubbles.

### Store PB1

- Store PB1 at room temperature.
- Keep PB1 for up to 3 months.

### Clean the Carboy

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

## Store PB1

For Infinium HD Super workflows do the following.

- Store PB1 at room temperature.
- Keep PB1 for up to 3 months.

# Best Practices

Adhere to the following best practices when performing the Infinium Assay protocols.

## Label the Plates

- Always use the provided barcodes to track plates throughout the Infinium process. As a convention, apply barcode labels to the right side of the plate (the column 12 end).

## Reagent Reuse

- After each protocol step, discard unused reagents per facility standards.
- Make sure the volume of each wash buffer is sufficient for one use.

## XC4 Storage and Use

- Store undiluted XC4 and XC4 that has been diluted with ethanol at room temperature.
- The XC4 reagent bottle displays the expiration date of the undiluted reagent. Illumina supports its products within the expiration date.
- Diluted XC4 can be reused up to six times over a two-week period for a maximum of 24 BeadChips.
- Clearly mark the XC4 bottle after ethanol has been added to avoid confusion with undiluted XC4 bottles.

## Handling Cap Mats

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

- Remove the cap mat carefully and slowly to avoid splashing the plate 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.

## Handling BeadChips

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

## Pipetting

- Make sure that pipettes are properly calibrated, clean, and decontaminated.
- Dispense slowly and carefully to prevent turbulence in the plate wells and flow-through chambers.
- Use a multichannel pipette whenever possible.

## Infinium Kit Configuration and Batching

- Infinium kits are configured to support various sample sizes with the assumption that the kit size corresponds to the batch size. For example, certain BeadChips are available in 48-sample, 288-sample, and 1152-sample kit configurations. When working with small batch sizes, order the smaller, 48-sample kits to make sure that the kit contains sufficient reagent volumes to process smaller batch sizes.

## Items Falling to the Floor

Follow these best practices for handling items that fall to the floor.

- Any items that fall to the floor are contaminated.
- Wear lab gloves to touch any items that fall to the floor.
- Immediately clean nondisposable items, such as pipettes or important sample containers, with a 10% bleach solution.
- Use a 10% bleach solution to clean any lab surface in contact with the contaminated item.
- Throw away your lab gloves and put on a new pair after handling items that have fallen to the floor.

## Balancing the Centrifuge

- When centrifuging 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.

## Calibrate the Microplate Shaker

Follow these 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 microplate shaker and adjust the speed until it reaches 1600 rpm.

3 Record the displayed speed and note that it represents an actual speed of 1600 rpm.

4 Use the same method to determine the displayed speed for the actual vortex speed of 1800 rpm.

5 Label the microplate shaker with the calibration information.

**Table 1 Sample Microplate Shaker Calibration Label**

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

# Lab Maintenance

The following standard lab maintenance procedures apply to all Infinium Assay labs.

## Daily and Weekly Bleaching

Establish a daily and weekly bleaching schedule for both the pre-amp and post-amp areas.

**CAUTION**

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

Keep both areas clean.

Identify “hot spots” in each area that pose the highest risk of contamination, and clean these areas daily with a 10% bleach solution.

Typical hot spots include:

- Bench space used to process DNA or amplified DNA
- Vortexers
- Centrifuges
- Thermal cyclers
- Instrument control panels
- Door handles
- Refrigerator/freezer door handles
- Computer mice
- Keyboards

Once a week, thoroughly clean all laboratory surfaces and instruments in both areas. Mop the floors with a 10% bleach solution.

You are responsible to train facility personnel to clean pre-amp and post-amp lab areas as described. Lab personnel should not move from the post-amp area to the pre-amp area.

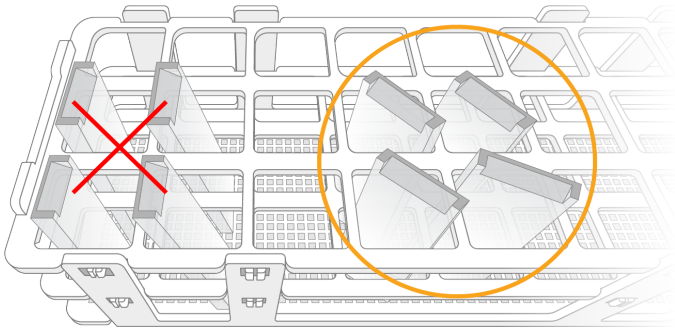
## Cleaning, Handling, and Inspecting the Glass Back Plates

The glass back plates are used in the Flow-Through Chambers during XStain to control the flow of reagent over the BeadChips. Clean the glass back plates when you open the package and after each use. In addition, perform a bleach cleaning after every seven uses, or more frequently, depending on individual lab throughput. Both cleaning procedures are described in the following sections. Inspect the glass back plates before each use.

## Recommended Racks for Glass Back Plate Cleaning, Bleaching, and Storage

Polypropylene test tube racks (ie plastic racks) are recommended for cleaning, bleaching, and storage of glass back plates. Place glass back plates diagonally in the racks to minimize contact between the glass and prevent damage.

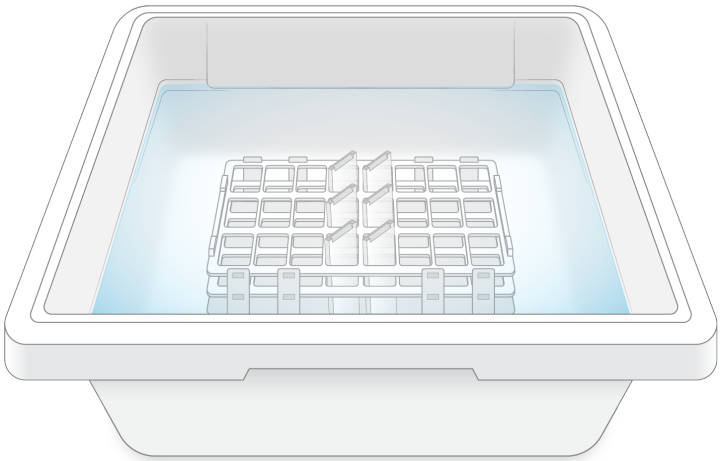
**Figure 1** Correct Placement of Glass Back Plates



## Disassembly of Flow-Through Chambers

Following XStain, the Flow-Through Chambers are disassembled. XStain reagents contain many components (eg proteins, enzymes, antibodies) and best practice is to prevent remaining reagent from drying on the glass back plates. Immediately after disassembly, place the glass back plate diagonally in a plastic rack that is submerged in a container of DI $H_2O$ to prevent drying. After all Flow-Through Chambers are disassembled and placed in the submerged rack, proceed to *Cleaning the Glass Back Plates after Every Use* on page 12.

**Figure 2** Plastic Rack Submerged in DI $H_2O$



## Cleaning the Glass Back Plates after Every Use

Clean the glass back plates when you open them for the first time and after every use.

1 Prepare a 1% Alconox solution.
Use 2.5 g Alconox powder per 250 ml DI $H_2O$.

2 Submerge the plastic rack with glass back plates in a container filled with 1% Alconox.

3 Wipe each glass back plate with a Kimwipe and return to rack.

4 Remove the rack from the 1% Alconox solution and thoroughly rinse the glass back plates with DI $H_2O$.

5 Allow the glass back plates to dry in the plastic rack.

6 After the glass back plates are clean and dry, wipe them with a Kimwipe soaked with 70% EtOH.

7 Store the glass back plates in the plastic rack in a dust-free area.

8 Use a can of compressed air or a laboratory air gun to remove any dust or lint on the glass back plates before use.

## Bleach Cleaning the Glass Back Plates

In addition to the cleaning procedure described previously, clean the glass back plates after approximately every seven uses, or more frequently, depending on individual lab throughput.

**CAUTION**

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

**CAUTION**

Bleach is an irritant. Use caution when handling.

### Preparation

- Make 10% bleach solution.
  Example: Add 100 ml of bleach to 900 ml DI $H_2O$ and mix thoroughly.

### Bleach Cleaning Steps

**WARNING**

**Perform this procedure in a fume hood or a lab space outside of the BeadChip production lab. Excessive bleach fumes can degrade the fluorescent dyes used in the Infinium Assay.**

1 Perform the following steps in the fume hood:
   - Fill a container with enough 10% bleach to cover both the rack and the glass back plates completely.
   - Place the plastic rack with the glass back plates into the container.
   - Soak for 1 hour.

2 Transfer the rack with glass back plates to a container filled with DI $H_2O$.

**TIP**

You can transfer the container and rack with glass back plates from the fume hood to a nearby sink with DI $H_2O$.

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

4 Remove the rack containing the glass back plates and rinse both with DI $H_2O$.

5 Dispose of the DI $H_2O$ from the container. Rinse and refill the container with fresh DI $H_2O$.

6 Return the rack containing the glass back plates to the container.

7 Dip the rack containing the glass back plates up and down 20 times and then soak for 5 min. Be careful not to chip the glass back plates.

8 Repeat steps 4–7 four times.

9 Dispose of the DI $H_2O$.

10 Remove the rack containing the glass back plates and rinse with DI $H_2O$.

11 Allow the glass back plates to dry in the plastic rack.

12 After the glass back plates are clean and dry, wipe them with a Kimwipe soaked with 70% EtOH.

13 Store the glass back plates in the plastic rack in a dust-free area.

14 Use a can of compressed air or a laboratory air gun to remove any dust or lint on the glass back plates before use.

## Inspecting the Glass Back Plates

Before each use, inspect the glass back plate to make sure it is in acceptable condition. If any of the following are observed, replace the glass back plate:

- Chip or damage in the reagent reservoir area
- Significant chips or damage on the surface facing the BeadChip in the flow-through chamber
- Cracks in the glass that may cause the glass to break during the assay
- Any chips or damage that may cause harm while handled, regardless of location

# Robot Usage and Maintenance



## Introduction

This section describes how to perform robot QC and maintain the Tecan 8-tip robot.

**WARNING**

**Do not run any other programs or applications while using the Tecan. Your computer and the Tecan may lock up and stop a run.**

## Preparing the Robot for Use

The preparation steps vary slightly depending on whether you are using the robot for the first time of the day. Follow one of the following procedures before every automated protocol.

### Tecan First-Use-of-the-Day Procedure

1 Reboot the Tecan control computer.

**CAUTION**

Do not place your hands on or near the Tecan bed while the Tecan is running.

2 Open the Illumina Automation Control Software.
The Tecan takes a few moments to initialize.

3 Check the system fluid level and add fluid if necessary.

**CAUTION**

If adding fluid, do so ***before*** the Bleach Wash step.

4 Select **Robot QC Tasks | Robot Bleach Wash**.

5 In the Basic Run Parameters pane, enter 0 for a tip wash.

6 Place a quarter reservoir with 5 ml 10% bleach solution in position A of the reservoir frame.

7 Select **Run**.

8 Select **OK** at the prompt.
The Tecan initiates the tip wash. When the bleach procedure is complete, the Tecan returns to the main task screen.

**CAUTION**

To prevent contamination, make sure that all bleach vapors have fully dissipated before starting any process involving samples.

### Subsequent Uses During the Day

Follow this procedure every time you are about to use the Tecan. If it is the first time the Tecan is being used for the day, follow the steps described under *Tecan First-Use-of-the-Day Procedure* on page 15 instead.

**CAUTION**

Do not place your hands on or near the Tecan bed while the Tecan is running.

1 Select Sys Wash.

2 Observe the lines for air bubbles.

3 Repeat the system flush until the lines are free of air bubbles. If bubbles persist in the lines, contact Illumina Technical Support.

4 Observe the Tecan tips for any dripping.

5 During the fast wash cycle, observe the Tecan tips as they dispense system liquid. Liquid should dispense in a straight stream. All tips should dispense liquid in equal volumes and at equal velocities.

## Testing Volume Accuracy of the Tecan Tips

This protocol uses fluorescein dye to measure quantities of liquid delivered by the Tecan compared to a manually derived standard curve.

It is critical that the fluorescein dyes used for the standard curve and the Tecan quantification are derived from the same starting dilution of stock. Failure can lead to erroneous assay results.

### Preparing Fluorescein Reagents

This section explains how to prepare reagents for testing the Tecan.

#### Fluorescein Stock

1 Weigh out 25 mg fluorescein into a 100 ml bottle.

2 Slowly add 25 ml DMSO.

3 Mix thoroughly.

4 Store at room temperature protected from light.

#### Fluorescein Dilution Buffer

1 Prepare 1X TE (Tris s acid—EDTA):
- Add 10 ml 100X TE (1M Tris-HCl, 0.1M EDTA) to 990 ml DI $H_2O$ in a 1 L bottle.
- Mix thoroughly.
- Store at room temperature.

2 Prepare 10% Tween 80:
- Weigh 107.4 grams Tween 80.

**NOTE**

Tween 80 is extremely viscous. Weight measurement is more accurate than liquid volume measurement.

- Add the Tween 80 to the 1 L bottle.
- Dissolve in DI $H_2O$ to 1000 ml.
- Mix thoroughly.
- Store at room temperature.

3 Prepare 1X TE with 0.01% Tween 80:

- Add 1 ml 10% Tween 80 to 1000 ml 1X TE buffer.
- Mix thoroughly.
- Store at room temperature.

### High-Concentration Fluorescein

1 In a 100 ml bottle, add 25 ml fluorescein stock in DMSO (1.0 mg/ml) to 75 ml fluorescein dilution buffer (1X TE with 0.01% Tween 80).

2 Mix thoroughly.

3 Discard after use.

### Low-Concentration Fluorescein

1 In a 500 ml bottle, add 50 ml newly prepared 0.25 mg/ml high-concentration fluorescein to 450 ml fluorescein dilution buffer (1X TE with 0.01% Tween 80).

2 Mix thoroughly.

3 Discard after use.

## Dispensing Reagents using the Tecan

1 Label a new FLUOTRAC 200 plate Ind-Col Dispense.

2 Label another new FLUOTRAC 200 plate Multi-Col Dispense.

3 From the Tecan control computer, open the Illumina Automation Control Software.

4 Select Robot QC Tasks | 8-Tip Robot QC.

5 Add 35 ml fluorescein dilution buffer to a quarter reservoir. Place the reservoir in position A of the reservoir frame, as shown on the Tecan bed map.

6 Add 6 ml low-concentration fluorescein (0.025 mg/ml) to a quarter module reservoir and place the reservoir in position B.

7 Place the FLUOTRAC 200 plates on the Tecan bed according to the Tecan bed map.

8 Make sure that:
- All items are placed properly on the Tecan bed
- All caps and seals have been removed
- For each plate, well A1 is in the upper left corner of the frame

9 Select Run.
The Tecan conducts an internal QC process. A message in the lower status bar indicates when the QC process is complete.

10 Remove the FLUOTRAC 200 plates from the Tecan bed and cover with aluminum foil.

11 Dispose of any remaining reagents in accordance with your facility requirements.

12 Select Robot QC Tasks | Robot Bleach Wash.

13 Add 5 ml 10% bleach to a quarter reservoir.

14 Place the reservoir in position A of the reservoir frame, as shown on the Tecan bed map.

15 Select Run.

16 When the tip bleach process is complete, select Sys Flush.

17 Observe the lines for air bubbles.

18 Repeat the system flush process at least *three times*, until the lines are free of air bubbles.
If bubbles persist in the lines, contact Illumina Technical Support.

## Creating a Standard Plate

The Standard plate is used to generate a standard curve of fluorescent units versus volume dispensed. You create the Standard plate by manually pipetting fluorescein over a range of volumes (1–36 µl) into 100 µl fluorescein dilution buffer. The Standard plate is then used to calculate volumes dispensed by the Tecan into the Tecan QC test plates.

**NOTE**

To ensure successful generation of the standard curve, use calibrated pipettes.

1 Label a FLUOTRAC 200 plate Standard Plate.

2 Dispense 12 ml fluorescein dilution buffer into a disposable multichannel trough.

3 Using a multichannel pipette, add 100 µl fluorescein dilution buffer into each well of the Standard plate.

4 Add 2 ml low-fluorescein concentration standard (0.025 mg/ml) to a disposable multichannel reservoir.

5 Using an 8-channel pipette, add low-fluorescein concentration standard (0.025 mg/ml) into each well of the Standard plate according to the volumes shown in Table 2.

6 Cover the plate and store it in the dark until reading on the fluorometer.

**Table 2 0.025 mg/ml Fluorescein volumes for the Standard plate, by well (in µl)**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| B | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| C | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| D | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| E | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| F | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| G | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| H | 0 | 1 | 2 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |

## Reading Fluorescence Intensities

1 Read the fluorescence intensities of the Standard and the Robot QC plates on a fluorometer at an excitation of 485 nm and 538 nm emission.

2 Set the fluorometer to Automix for 10 seconds before the first reading.

## Calculating Results

1 Open the Tecan QC Analysis Tool Excel workbook.

2 Select the Standard Data Input sheet. Enter the fluorescein intensities of the standard curve, following the template in the Excel workbook.
The standard curve statistics generate automatically.

3 Make sure that the standard curve R2 for the low and the high dispense volumes are ≥ 0.99.

**NOTE**

If the values are < 0.99, the standard curve must be repeated. If the standard curve is < 0.99, the cell for that curve is highlighted as failing.

4 Select the **Individual Tips Data Input** sheet. Enter the fluorescein intensities for the Ind-Col Dispense Robot QC plate, following the template in the Excel workbook.

5 Select the **Multi Tips Data Input** sheet. Enter the fluorescein intensities for the Multi-Col Dispense Robot QC plate, following the template in the Excel workbook.

6 Select the **Summary Analysis** sheet and review the pass/fail data.

7 Tips that do not meet the required QC specifications are flagged with red. If a tip is failing, perform a **Bleach Wash** followed by a **System Flush** on the Tecan, and then repeat the **Robot QC** procedure.

8 Evaluate the individual and multicolumn dispense results on the std curve.
The acceptable standard curve R2 for the low and the high dispense volumes is ≥ 0.99.

**Figure 3** Standard Curve with Individual Column Dispense

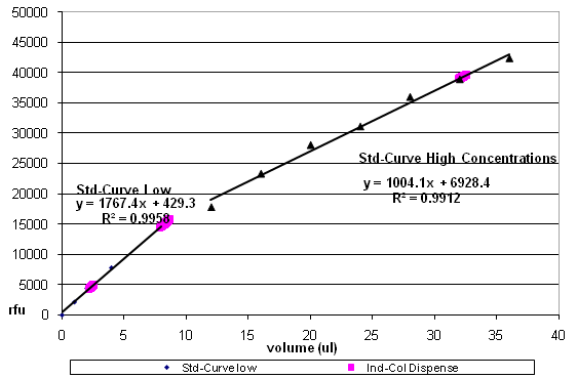
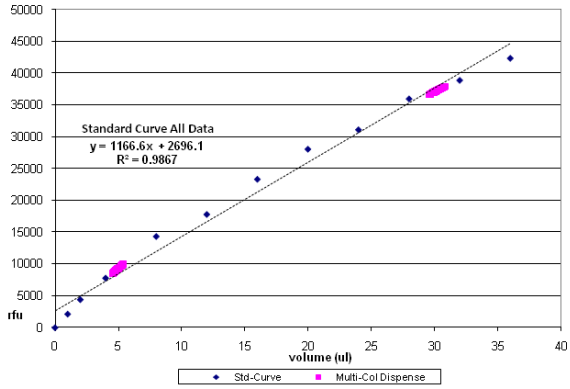



**Figure 4** Standard Curve with Multi-Tip Column Dispense




# Cleaning the Tecan

## Cleaning the Outer Surface of the Tecan Tips

Perform this activity once a week.

1 At the beginning of each day, check for any leaks.

2 In the Illumina Automation Control Software, select Sys Init.
3 Select Tips Up.
4 Fold a Kimwipe in half lengthwise and soak it with 70% EtOH.
5 Fold the Kimwipe around the Tecan tip.
6 Starting from one end of the tip row, wipe each tip gently along the entire lower half of the tip.
7 Reverse the Kimwipe to use the other, fresh side.
8 Starting from the opposite end of the tip row, wipe each tip a second time along the entire lower half.
9 Clean the waste station with the Kimwipe.

## Bleach-Bathing the Tecan Carriers

Perform this activity monthly.

1 Prepare a 10% bleach bath (~500 ml concentrated bleach in 4500 ml DI $H_2O$) in a soaking tray.
2 Fill the other soaking tray with DI $H_2O$.
3 Lay out at least two rows of absorbent bench underpad on a benchtop.
4 Remove the Tecan carriers from the Tecan bed. Note the original position of the carriers so that you can replace them correctly.
5 Submerge the carriers in the prepared bleach solution for about 1 minute.
6 Remove the carriers from the bleach solution and submerge them in the DI $H_2O$ soaking tray for about 1 minute.
7 Remove the carriers from the soaking tray and rinse them under running water.
8 Dry the carriers on the absorbent bench underpad.
9 Allow the carriers to dry completely before returning them to their proper positions on the Tecan bed.

**CAUTION**

To prevent contamination, make sure that all bleach vapors have fully dissipated before starting any process involving samples.

## Sterilize Fluidics

Perform this activity annually or whenever mold or bacterial growth is detected in the robot fluidics.

1 In the carboy, prepare a 1 L 10% bleach solution by adding 100 ml bleach to 900 ml DI $H_2O$.
2 Select Robot QC Tasks | Robot Bleach Wash.
3 In the Basic Run Parameters pane, enter 1 for a system wash.
4 Select Run.
5 When prompted, swap out the system liquid carboy for the carboy containing 10 % bleach solution.
6 Select OK.
7 When prompted, swap out the bleach solution with the system liquid carboy.
8 Select OK.
9 Observe lines for air bubbles.

10 Run additional washes (SysWash) until the lines are free of air bubbles. If bubbles persist in the lines, contact Illumina Technical Support.

**CAUTION**

To prevent contamination, make sure that all bleach vapors have fully dissipated before starting any process involving samples.

# System Controls



## Introduction

This section describes the controls used in the Infinium assay, their expected outcomes, and how to view them. Diagrams with descriptions are included for sample-independent and sample-dependent controls, and controls that are specific to the green or red channel. The controls are useful both by themselves and with each other.

### Sample-Independent Controls

The sample-independent controls are:

- Staining controls
- Extension controls
- Target removal controls
- Hybridization controls

### Sample-Dependent Controls

The sample-dependent controls are:

- Stringency controls
- Nonspecific binding controls
- Nonpolymorphic (NP) controls

Several key steps in the Infinium Assay require evaluation of both the red and green color channels. For these instances, both red and green channel controls are included. See Table 1 for a list of controls and the color channel they are intended to monitor (red, green, or both).

## The Control Dashboard

To view the controls, create a genotyping workspace using the GenomeStudio Wizard, as described in the *GenomeStudio Framework User Guide* and the *GenomeStudio Genotyping Module User Guide*. You can then view the controls performance from GenomeStudio's **Analysis | View Controls Dashboard** menu.

## Control Diagrams

The following diagrams illustrate control structure and function.

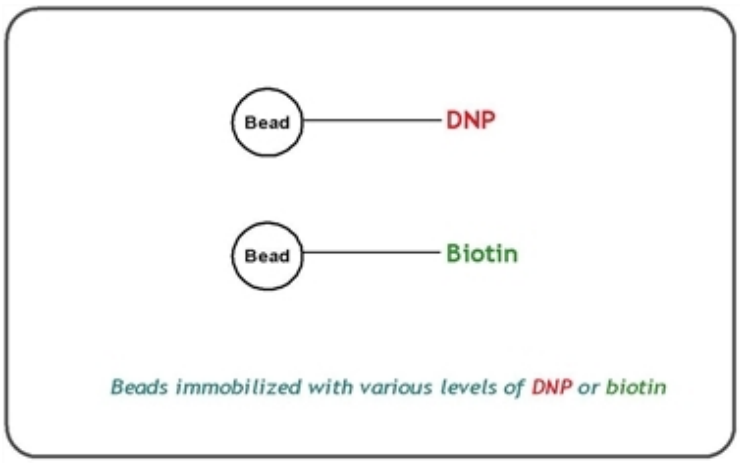
### Sample-Independent

Sample-independent controls evaluate the performance of specific steps in the process flow.

## Staining Controls

Staining controls are used to examine the efficiency of the staining step in both the red and green channels. Staining controls have various levels of dinitrophenyl (DNP) or biotin attached to the beads. See Table 1 for information about the bead type IDs, relevant color channel, and expected intensity of biotin and DNP labeling controls. These controls are independent of the hybridization and extension step. Various levels of DNP and biotin monitor the sensitivity and efficiency of the staining step. Both red and green channels can be evaluated using the Staining Controls.

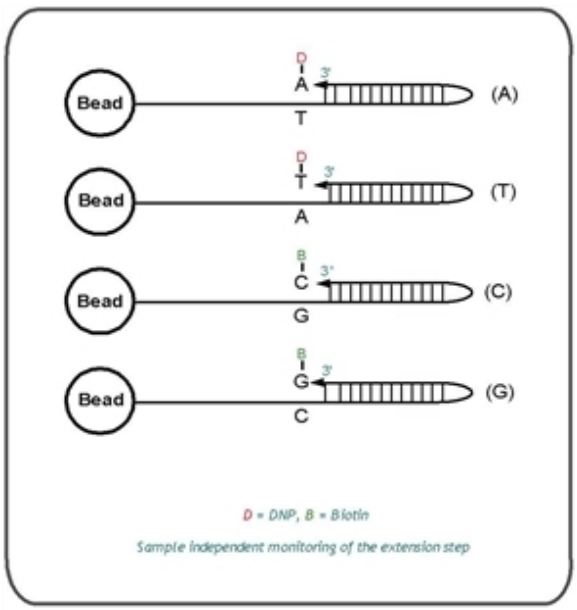
**Figure 5** Staining Controls (Sample-Independent)




## Extension Controls

Extension controls test the extension efficiency of A, T, C, and G nucleotides from a hairpin probe, and are, therefore, sample-independent. Both red (A, T) and green (C, G) channels are monitored. See Table 1 for information about the bead type IDs, relevant color channel, and expected intensity of the extension controls.

**Figure 6** Extension Controls (Sample-Independent)

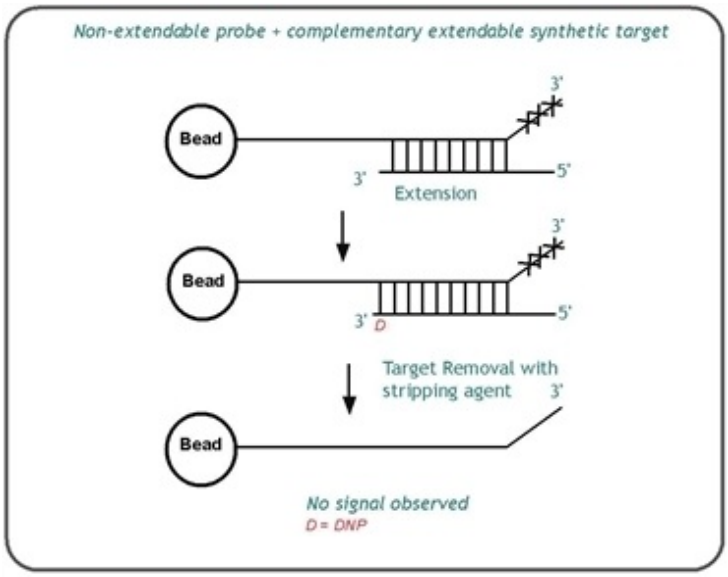


## Target Removal Controls

Target removal controls test the efficiency of the stripping step after the extension reaction. In contrast to allele-specific extension, the control oligos are extended using the probe sequence as template. This process generates labeled targets. The probe sequences are designed such that extension from the probe does not occur.

All target removal controls should result in low signal compared to the hybridization controls, indicating that the targets were removed efficiently after extension. The target removal controls are present in the Hybridization Buffer RA1. Performance of target removal controls should only be monitored in the red channel. See Table 1 for a list of the bead type IDs.

**Figure 7** Target Removal Controls (Sample-Independent)

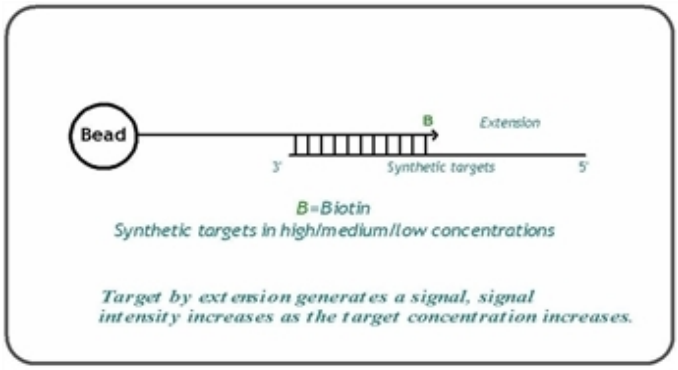



## Hybridization Controls

The hybridization controls test the overall performance of the entire assay using synthetic targets instead of amplified DNA. These synthetic targets complement the sequence on the array perfectly, allowing the probe to extend on the synthetic target as template.

The synthetic targets are present in the Hybridization Buffer (RA1) at three levels. They monitor the response from high-concentration (5 pM), medium-concentration (1 pM), and low-concentration (0.2 pM) targets. All bead type IDs should result in signal with various intensities, corresponding to the concentrations of the initial synthetic targets. Performance of hybridization controls should only be monitored in the green channel. See Table 1 for a list of the bead type IDs.

**Figure 8** Hybridization Controls (Sample-Independent)




## Sample-Dependent

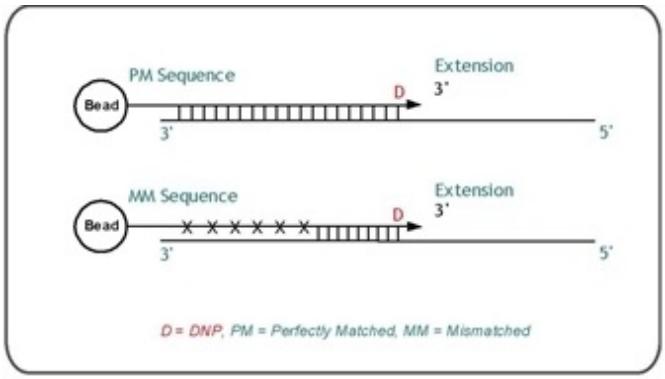
The sample-dependent controls evaluate performance across samples.

### Stringency Controls

These controls test the stringency of the hybridization process. The same target is used for each stringency control. The only difference between the stringency controls is the number of nucleotides hybridized between the target and the probe sequence on the bead.

The probes are designed such that the 3' end of the probe is available for extension. Mismatches are introduced into the body of the probe sequence to affect hybrid stability. The controls have 0 to 12 mismatched nucleotides between target and probe. Performance of stringency controls should only be monitored in the red channel. See Table 1 for a list of the bead type IDs.

**Figure 9** Stringency Controls (Sample-Dependent)




### Nonspecific Binding Controls

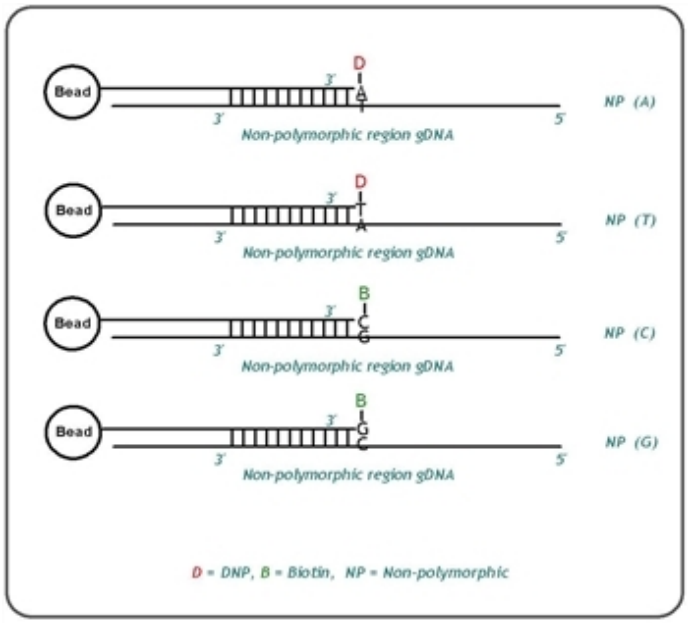
Nonspecific binding controls are included to monitor the specificity of the hybridization of the amplified DNA. The probe sequences for nonspecific binding controls are complementary to bacterial sequences and should not hybridize to human sequences under standard hybridization stringency conditions.

These controls should show low intensities, indicating there is minimal cross-hybridization of human sequences to bacterial sequences. Performance of nonspecific binding controls should be monitored in both green and red channels. See Table 1 for a list of the bead type IDs.

## Nonpolymorphic Controls

Nonpolymorphic controls test the overall performance of the assay from amplification to detection by querying a particular base in a nonpolymorphic region of the genome. They let you compare assay performance across different samples. One nonpolymorphic control has been designed for each of the four nucleotides (A, T, C, and G). See Table 1 for a list of the bead type IDs.

**Figure 10** Non-Polymorphic Controls (Sample-Dependent)



# Troubleshooting



## Introduction

Under certain circumstances, the Infinium Assay may operate in an unexpected manner. This section describes some of these circumstances and offers solutions and workarounds. If the issue cannot be resolved using this guide, contact Illumina Technical Support.

## Pre-Hybridization

| # | Symptom | Probable Cause | Resolution / Comment |
|---|---|---|---|
| 1 | No blue pellet observed in wells after 20 minute centrifugation. | • Original DNA sample degraded.<br>• Precipitation reaction solution was not mixed thoroughly before centrifugation. | Invert plate several times and centrifuge again.<br>If pellets appear, the solutions were not mixed before centrifuging. If pellets do not appear, the original DNA samples may be degraded.<br>Note: If samples were degraded, repeat the Amplify DNA (Make) step of the protocol you are performing.<br>Inspect wells for complete mixing before the 20 minute centrifugation. |
| | | Either PM1 or 2-propanol was not added. | Add missing reagent to wells.<br>Invert plate several times and centrifuge plate again.<br>Inspect wells for complete mixing before 20 minute centrifugation. |
| 2 | Blue color observed on absorbent pad after precipitation supernatant was decanted. | • Precipitation reaction solution was not thoroughly mixed before centrifugation.<br>• The plate was centrifuged at less than 3000 × g, or for less than the recommended time.<br>• Supernatant was not removed immediately after centrifugation. | The samples are lost. Repeat the Amplify DNA (Make) step of the protocol you are performing.<br>Check centrifuge program to make sure that correct speed was selected. |
| 3 | Blue pellet did not dissolve back into solution after vortexing. | An air bubble formed at the bottom of the well that prevented the pellet from mixing with RA1. | Pulse centrifuge plate to 280 × g to remove air bubble, then revortex plate at 1800 rpm for 1 minute. |
| | | Vortex speed is not fast enough. | Check the vortexer speed setting, as the speed setting may drift over time. Recalibrate if necessary.<br>Revortex plate at 1800 rpm for 1 minute. |
| | | The reaction plate did not incubate for long enough. | Incubate the plate for an additional 30 minutes.<br>Make sure that the cover is properly seated to prevent evaporation. |

## Hybridization to XStain

| # | Symptom | Probable Cause | Resolution / Comment |
|---|---|---|---|
| 1 | There was not enough reagent to dispense to all BeadChips. | Make sure to centrifuge the reagent tubes at 280 × g after thawing. | Check pipette calibration. A gravimetric test using water is a quick and easy way to check for accurate pipette dispensing volumes.<br>Recalibrate pipettes yearly. |
| | | Programmable pipettor may be set incorrectly. | Check to see if the programmable pipettors have an overasp volume that is discarded. |
| 2 | Cap mat deformed or melted while heat-denaturing DNA samples. | Foil heat sealer should have been used. | Samples have been ruined. Repeat experiment.<br>Use the foil heat sealer for all temperatures ≥ 45°C. |
| 3 | A small amount of precipitate was present in the hybridization solution. | A small amount of precipitate is normal, and does not affect data quality. | Continue with experiment. |
| 4 | BeadChips are still wet on the underside after 55 minutes in the vacuum desiccator. | The BeadChips must dry for a longer period.<br>XC4 may be old and must be replaced with fresh XC4.<br>An old bottle of ethanol may have absorbed atmospheric water. Replace with a fresh bottle of ethanol. | Dry BeadChips longer under vacuum desiccator.<br>Lab temperature and humidity affect drying time. |
| 5 | After coating the BeadChips with XC4, some uncoated areas remain. | During the coating process, a bubble formed between the BeadChips and prevented the XC4 solution from reaching the surface. | Briefly place the staining rack with BeadChips back into the wash dish containing XC4.<br>Gently move BeadChips back and forth while moving up and down, breaking the surface of the solution. The back-and-forth movement is especially important when processing 16 or 24 BeadChips. |
| 6 | During XStain, the liquid in the Flow-Through Chamber dropped below the bottom edge of the glass back plate reservoir. | Glass back plates may not have been completely clean, causing capillary gap failure.<br>Make sure that the correct spacer was used to assemble the Flow Through Chamber. | Before reassembling the Flow-Through Chambers, clean the glass back plates. |
| | | The Flow-Through Chambers may not be securely assembled. | Attach the metal clamps to the Flow-Through Chambers as described in the Reference Guide for the assay. |

## Imaging

| # | Symptom | Probable Cause | Resolution / Comment |
|---|---|---|---|
| 1 | The iScan System was unable to find all the fiducials during scanning. | XC4 coating was not properly removed from BeadChip edges. | Rewipe the edges of BeadChips with ProStat EtOH wipes and rescan. |
| | | BeadChips were not seated correctly in BeadChip carrier. | Reseat BeadChips in BeadChip carrier. |
| 2 | The scanning process did not generate any intensity signal. | The absence of an intensity image may be due to failures in any experimental steps upstream of scanning. | Repeat the experiment.<br>Check the staining controls. If the controls do not generate a signal either, then the staining reagents may be compromised. |
| 3 | The scanning process generated a low assay signal. The Hyb controls display expected intensities. | A low assay accompanied by normal performance from the sample-independent controls signal indicates a sample-dependent failure. The error may have occurred in any experimental steps between amplification and hybridization. | Repeat experiment.<br>Make sure that there is a DNA pellet after precipitation.<br>During resuspension, make sure that the DNA pellet dissolves properly. The blue color in the pellet should disappear. |
| 4 | The scanning process generated a low assay signal. The Hyb controls display low intensities. | A low assay signal accompanied by low sample-independent controls indicates a sample-independent failure related to assay processing. The error probably occurred after hybridization. | To eliminate the scanner as the problem, make sure that other chips that are scanned at the same time or in the same batch are yielding normal assay signals. If other chips show the same low signal, contact IlluminaTechnical Support to analyze the data in GenomeStudio to determine possible root causes. |
| 5 | Some spots on the BeadChip have low to 0 intensity. | Bubbles in the reagents can cause this phenomenon by preventing reagents from reaching the BeadChip surface. | Centrifuge all reagent tubes before using to prevent bubbles.<br>Always perform a system flush before running the experiment.<br>(Low to 0 intensity areas on the BeadChip may not have a negative effect on array data due to randomness and oversampling of BeadChips.) |
| 6 | The scanner returns red stripes indicating a failed scan. | • Assay process failure.<br>• Sample failure.<br>• Consumable failure.<br>• Instrument failure. | Move the chip to another position in the carrier and rescan. If this fails, contact Illumina Technical Support. |

## Miscellaneous

| # | Symptom | Probable Cause | Resolution / Comment |
|---|---|---|---|
| 1 | Genotyping results do not correlate with samples. | The samples may have been mixed up. | Check the sample sheet and lab tracking form to determine the location of DNA samples during amplification. |
| | | | Check to make sure that the correct sample was loaded onto the correct BeadChip or part of the BeadChip. |
| | | | Repeat the experiment. After resuspending the DNA pellet, make sure to consolidate the samples back to the original amplification position, if necessary. |
| 2 | Solution foams excessively when dispensed. | Pipetting may have been too vigorous. | Pipette gently without forming bubbles. Try centrifuging the plate to 280 × g to remove bubbles. |
| 3 | Heat block cooled to less than 37°C when it was set to 37°C after the 95°C incubation. | Heat block overshot set temperature. | Wait for heat block to reequilibrate to 37°C.<br>Many heat blocks will overshoot the bottom set temperature when cooling from a previously set higher temperature. |

# References



## Illumina BeadChips

Illumina Genotyping BeadChips are sophisticated silicon-based array devices composed of individual arrays on a slide. Each individual array in the matrix can hold over 30,000 different oligonucleotide probe sequences. This results in an overall capacity of up to millions of unique probe sequences represented on a single BeadChip. These unique probe sequences are in turn attached to 1–2-micron beads assembled into the microwells of the BeadChip substrate. Because the microwells outnumber the probe sequences, multiple copies of each bead type are present in the array. This built-in redundancy improves robustness and measurement precision. The BeadChip manufacturing process includes hybridization-based quality control of each array feature, allowing consistent production of high-quality, reproducible arrays.

## Tracking Tools

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

- Lab Tracking Form to map DNA samples to BeadChips and record the barcode of each reagent and plate used in the protocol.
- Sample sheet template to record information about your samples for later use in data analysis.

These documents are available at http://support.illumina.com/ for printing and reference.

## The iScan System

BeadChips are imaged using the iScan System.

The iScan System is a high-precision two-channel microarray scanner that scans BeadChips at two wavelengths simultaneously and creates an image file (.jpb) for each channel (2 per array). The iScan System incorporates advanced optics and sensors that support various throughput needs.

The iScan Control Software (iCS), determines intensity values for each bead type and creates data files for each channel. The GenomeStudio software uses these data intensity files with the individual bead pool map/manifest file (.bpm) to analyze assay data.

Loading and unloading the iScan System can be automated with the optional AutoLoader 2.x. The AutoLoader 2.x is fully integrated with the iScan Control Software and Illumina LIMS, and it contains an email alert system. The AutoLoader 2.x places carriers that contain up to 4 BeadChips in the iScan System tray for scanning. The AutoLoader 2.x supports unattended processing of up to 48 carriers at a time.

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

## Illumina Customer Support Telephone Numbers

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**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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