GoldenGate® Indexing
Assay Guide

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ILLUMINA PROPRIETARY
Catalog # GT-922-1002
Part # 15006937 Rev. A

illumina®

Notice

Revision History

Catalog # Part #, Revision Letter	Date
Cat # GT-922-1002 Part # 15006937, Rev. A	September 2010
Cat # GT-922-1002 Part # 15006937, Rev. A (Pre-Print)	March 2010

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Introduction

The Illumina® GoldenGate Indexing® Assay allows for a high degree of loci multiplexing (96 to 384 loci) during the extension and amplification steps, minimizing time, reagent volumes, and material requirements compared to standard GoldenGate throughput. The GoldenGate Indexing Assay combines a greatly multiplexed assay, high quality BeadChips, and a precise scanning system (the Illumina® BeadArray Reader or iScan Reader) to deliver unparalleled sample throughput, cost savings, and data quality. The GoldenGate Indexing Assay offers:

- Sample throughput of 128 to 512 samples on a single BeadChip
- Custom SNP assay design
- Low sample requirements (approximately 250 ng DNA per assay)
- High multiplex capability (up to 384 plex)
- Sample indexing up to 16 samples per well
- Illumina quality controlled reagent set for consistent assay performance
- Fully integrated LIMS and automation for positive sample tracking

Genotyping assays performed on the Universal BeadChips require extremely low sample and reagent volumes. The GoldenGate Indexing Assay is based on Illumina's Golden Gate Genotyping assay, which targets specific SNPs in genomic DNA samples. The genotyping application is based on sequence-specific extension and ligation of correctly hybridized query oligos, which are distinguished by their shared primer landing sites. The GoldenGate Indexing Assay protocol employs allele-specific extension methods and PCR amplification reactions conducted at high multiplex levels.

The Illumina GoldenGate Indexing Assay system is designed for processing using the Illumina Laboratory Information Management System (Illumina LIMS) tracking software with integrated data handling. Illumina LIMS assigns a unique identifier to every sample that links it with specific reagents, microtiter plates, and oligos, and populates the database for workflow management. The Illumina LIMS program controls real-time activity of every uniquely identified component, ensuring proper queuing and accurate sample processing.

Audience and Purpose

This guide is for laboratory technicians running the Illumina GoldenGate Indexing Assay with Universal-32 BeadChips. The guide documents the laboratory protocols associated with the assay. Follow all of the protocols in the order shown.

GoldenGate Indexing Assay System Workflow

The following sections describe the overall GoldenGate Indexing Assay system workflow. Figure 1 provides a high-level diagram of the workflow, showing the interaction between different components.

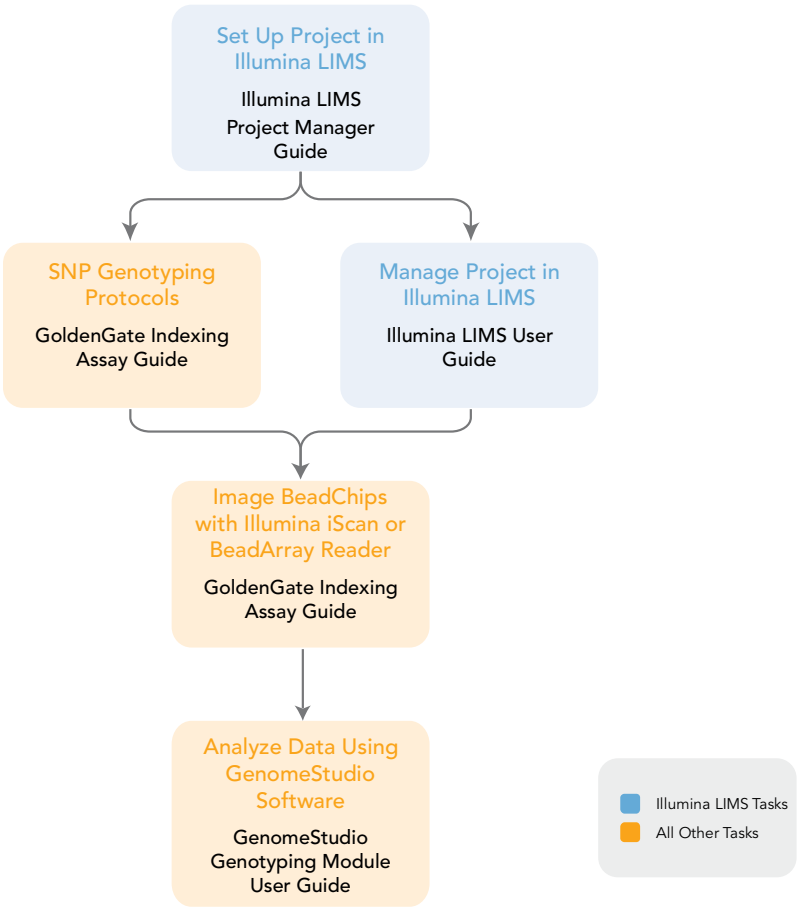


Figure 1 Illumina GoldenGate Indexing Assay System Flow

Set Up a Project in Illumina LIMS

Illumina LIMS uses the term Project to denote a group of DNA samples to be genotyped. Each Project is associated with a Principal Investigator, who is in turn associated with an Institute. The Project is also associated with a Universal-32 BeadChip version and one or more Oligo Pool All (OPA) tubes within a single Master Oligo Set. Once the lab or project manager sets these up in the system, DNA plates can be "accessioned" into Illumina LIMS and assigned to Projects. Illumina LIMS uses barcodes to move the samples from one task queue to the next, and track the progress of all samples, plates, and reagents. Reporting and history tools are also available.

For instructions on how to set up and track a Project in Illumina LIMS, see the *Illumina LIMS Project Manager Guide* and the *Illumina LIMS User Guide*.

The Universal BeadChip

The Universal BeadChip platform is composed of several individual arrays manufactured on a microscope slide-shaped substrate. Each individual array on the BeadChip contains 1536 illumicodes attached to 3-micron beads. Multiple beads of each bead type are assembled into microwells etched into the slide. The BeadChip manufacturing process includes hybridization-based quality control of array features, allowing consistent production of high-quality, reproducible arrays.

GoldenGate Indexing Assay Controls

Appendix A describes the GoldenGate Indexing Assay Guide control oligos, including the IllumiCode Sequence IDs used, their expected outcomes, and how to view them. The control oligos include:

- Second hybridization controls

BeadArray™ Reader, iScan, AutoLoader and AutoLoader2

BeadChips are imaged using either the Illumina iScan System or BeadArray Reader. Both of these are two-channel high-resolution laser imagers that scan BeadChips at two wavelengths simultaneously and create an image file for each channel (i.e., two per array). The iScan System incorporates advanced optics and sensors to support much higher throughput than the BeadArray Reader, while providing equally high data quality.

The iScan Control Software (or BeadScan, for BeadArray Reader), determines intensity values for each bead type and creates data files for each channel. GenomeStudio uses this data file in conjunction with the individual super manifest file (*.xtm) to analyze the data from the assay.

Loading and unloading of BeadChips into the iScan System or BeadArray Reader can be automated with the optional AutoLoader2 or AutoLoader, respectively. Both AutoLoaders support unattended processing by placing BeadChips carriers in the imaging system's tray, so that it can scan the BeadChips. Features include:

Table 1 *AutoLoader and AutoLoader2 Features*

	AutoLoader	AutoLoader2
Integrated with iScan Control Software		•
Integrated with BeadScan software	•	
Integrated with Illumina LIMS	•	•
Email alert system	•	•

Table 1 *AutoLoader and AutoLoader2 Features*

	AutoLoader	AutoLoader2
Single-reader or dual-reader configuration	•	•
Number of BeadChips supported per carrier	2	4
Number of carriers processed at a time	20	48

GenomeStudio Integrated Informatics Platform

GenomeStudio, Illumina's integrated data analysis software platform, provides a common environment for analyzing data obtained from microarray and sequencing technologies. Within this common environment, or framework, the GenomeStudio software modules allow you to perform application-specific analyses. The GenomeStudio Genotyping Module, included with your Illumina GoldenGate Indexing Assay Guide system, is an application for extracting genotyping data from intensity data files (*.idat files) collected from systems such as the Illumina iScan System or BeadArray Reader.

Data analysis features of the GenomeStudio Genotyping Module include:

- Choice of assay analysis within a single application
- Data tables for information management and manipulation
- Plotting and graphing tools
- Whole-genome display of sample data in the IGV (Illumina Genome Viewer)
- Data visualization of one or more samples in the ICB (Illumina Chromosome Browser)
- Data normalization
- Custom report file formats
- Genotype calling
- Clustering
- Detection of LOH (loss of heterozygosity)
- Analysis of structural variation including CNV (copy number variation)

The GenomeStudio Genotyping Module is fully integrated with the Illumina LIMS server.

For feature descriptions and instructions on using the GenomeStudio platform to visualize and analyze genotyping data, see the *GenomeStudio GT Module Workflow for the GoldenGate Indexing Assay, GenomeStudio Framework User Guide* and the *GenomeStudio Genotyping Module User Guide*.

Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 2 *Illumina Customer Support Contacts*

Contact	Number
Toll-free Customer Hotline	1-800-809-ILMN (1-800-809-4566)
International Customer Hotline	1-858-202-ILMN (1-858-202-4566)
Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at http://www.illumina.com/msds.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website. Go to http://www.illumina.com/documentation. When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF.

If you do not already have an iCom account, then click New User on the iCom login screen and fill in your contact information. Indicate whether you wish to receive the iCommunity newsletter (a quarterly newsletter with articles about, by, and for the Illumina Community), illumiNOTES (a monthly newsletter that provides important product updates), and announcements about upcoming user meetings. After you submit your registration information, an Illumina representative will create your account and email login instructions to you.

Chapter 2

GoldenGate Indexing Assay Automated Protocols

Topics

Introduction

The instructions in this chapter explain how to perform the GoldenGate Indexing Assay using the Tecan EVO 200 system hardware, database servers and database, and the LiHa 8-tip and MCA 96-tip robot. The instructions assume that you are preparing 96 DNA samples.

Workflow

Figure 2 graphically represents the workflow for the automated GoldenGate Indexing Assay utilizing the Universal-32 BeadChip.

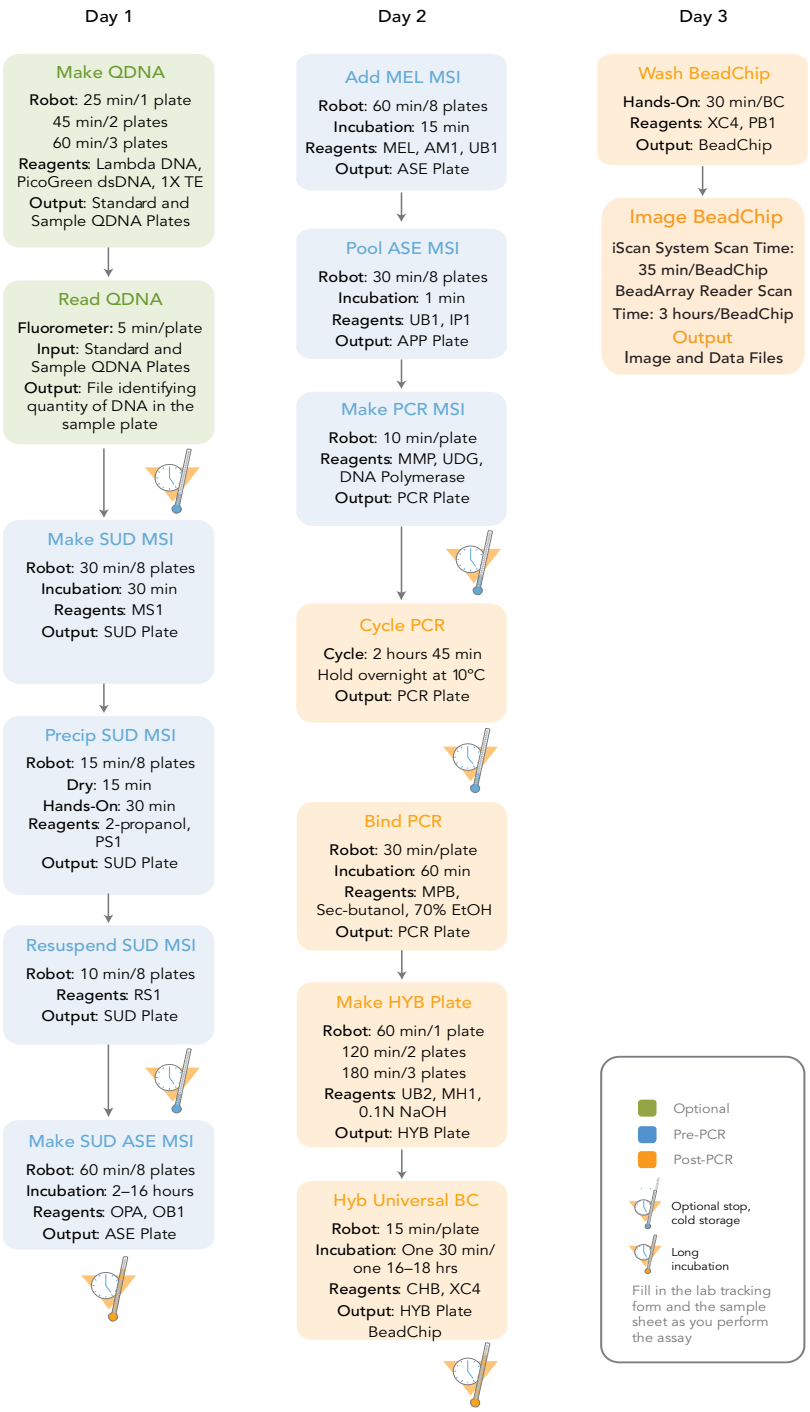


Figure 2 GoldenGate Indexing Assay Workflow

Quantitate DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples. Illumina recommends using a spectrofluorometer because fluorometry provides DNA-specific quantification. Spectrophotometry might also measure RNA and yield values that are too high.

Estimated Time

Hands-on time: ~20 minutes per plate

Robot: 20 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	-15°C to -25°C	User
1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA (TE))	See Instructions	Room temperature	User
Lambda DNA	See Instructions	2°C to 8°C	User
96-well 0.65 ml microtiter plate	1 per 96 samples		User
Fluotrac 200 96-well flat-bottom plate	1 per Std DNA plate 1 per Sample DNA plate		User

PicoGreen is susceptible to differential contaminants. False positives may occur for whole-genome amplification. Therefore, it is important to quantitate the input into the whole-genome amplification reaction.

Preparation

- Thaw PicoGreen to room temperature in a light-impermeable container.
- Thaw the sample DNA plates to room temperature.
- Apply a QDNA barcode label to a new Fluotrac plate for each GS#-DNA plate to be quantified.
- Hand-label the microtiter plate “Standard DNA.”
- Hand-label one of the Fluotrac plates “Standard QDNA.”
- In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Steps

In this section, you will perform the following steps:

- Make a Standard DNA plate with serial dilutions of stock Lambda DNA.
- Dilute PicoGreen with 1X TE.
- Create a Standard QDNA Fluotrac plate containing serial dilutions of DNA plus diluted PicoGreen.

- Create a QDNA plate by adding diluted PicoGreen to the sample DNA you plan to assay.

Make Standard DNA Plate

In this process, you create a Standard DNA plate with serial dilutions of stock Lambda DNA in the wells of column 1 (Figure 3).

1. Add stock Lambda DNA to well A1 in the plate labelled "Standard DNA" and dilute it to 75 ng/µl in a final volume of 233.3 µl. Pipette up and down several times.
   a. Use the following formula to calculate the amount of stock Lambda DNA to add to A1:

$$\frac{\text{(233.3 µl) X (75 ng/µl)}}{\text{(stock Lambda DNA concentration)}} = \text{µl of stock Lambda DNA to add to A1}$$

   b. Dilute the stock DNA in well A1 using the following formula:

$$\text{µl of 1X TE to add to A1} = \text{233.3 µl - µl of stock Lambda DNA in well A1}$$

2. Add 66.7 µl 1X TE to well B1.
3. Add 100 µl 1X TE to wells C, D, E, F, G, and H of column 1.

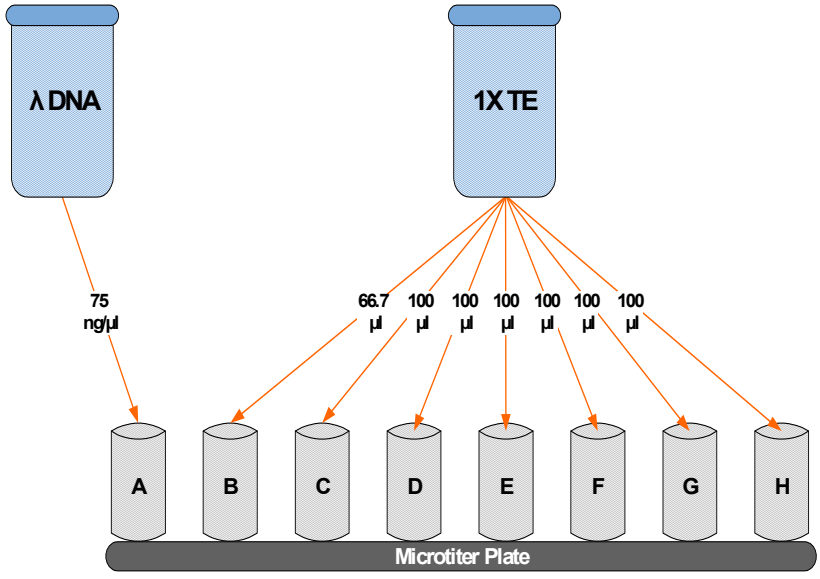


Figure 3 Dilution of Stock Lambda DNA Standard

4. Transfer 133.3 µl of Lambda DNA from well A1 into well B1. Pipette up and down several times.
5. Change tips. Transfer 100 µl from well B1 into well C1. Pipette up and down several times.

6. Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

Table 3 Concentrations of Lambda DNA

Row-Column	Concentration (ng/µl)	Final Volume in Well (µl)
A1	75	100
B1	50	100
C1	25	100
D1	12.5	100
E1	6.25	100
F1	3.125	100
G1	1.5262	200
H1	0	100

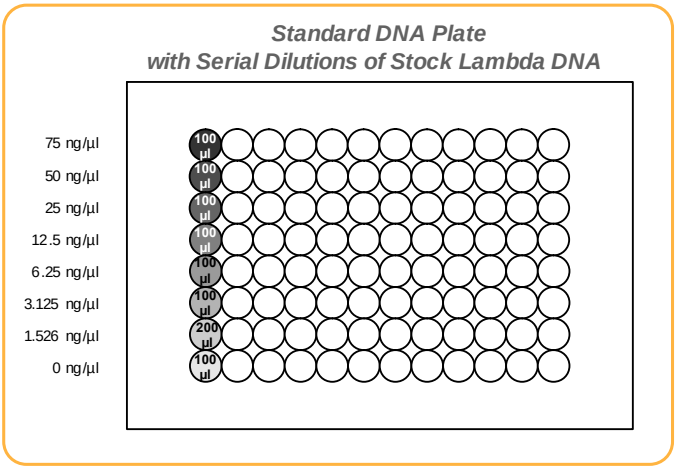


Figure 4 Serial Dilutions of Lambda DNA

7. Cover the Standard DNA plate with cap mat.
8. Proceed to *Dilute PicoGreen*.

Dilute PicoGreen

Diluted PicoGreen will be added to both the Standard QDNA and QDNA plates, intercalating into available dsDNA and fluorescing upon excitation in a spectrofluorometer.

CAUTION

PicoGreen reagent degrades quickly in the presence of light. Also, do not use glass containers for PicoGreen reagent, because it adheres to glass, thereby lowering its effective concentration in solution and effecting the upper response range accuracy.

1. Prepare a 1:200 dilution of PicoGreen into 1X TE, using a sealed 100 ml or 250 ml Nalgene bottle wrapped in aluminum foil.

   Refer to Table 4 to identify the volumes needed to produce diluted reagent for up to three 96-well QDNA plates. For fewer than 96 DNA samples, scale down the volumes.

Table 4 Volumes for PicoGreen Reagents

# QDNA Plates	PicoGreen Volume (µl)	1X TE Volume (ml)
1	125	25
2	230	45
3	325	65

2. Cap the foil-wrapped bottle and vortex to mix.

Create Standard QDNA Standard and Sample Plates

In this process, PicoGreen is distributed to Standard QDNA and Sample QDNA Fluotrac plates and mixed with aliquots of DNA from the respective DNA plates.

WARNING

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

1. At the robot PC, select **DNA Quant | Make Quant**.
2. In the DNA Plate Selection dialog box, select the plate type of the Standard DNA and Sample DNA plates. They should all be MIDI plates, TCY plates or ABGN plates. Roll the mouse pointer over each picture to see a description of the plate.

Figure 5 DNA Plate Selection Dialog Box

3. In the Basic Run Parameters pane, enter the **Number of DNA/QNT plates** (1, 2, or 3 pairs) and the **Total samples in DNA**.

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

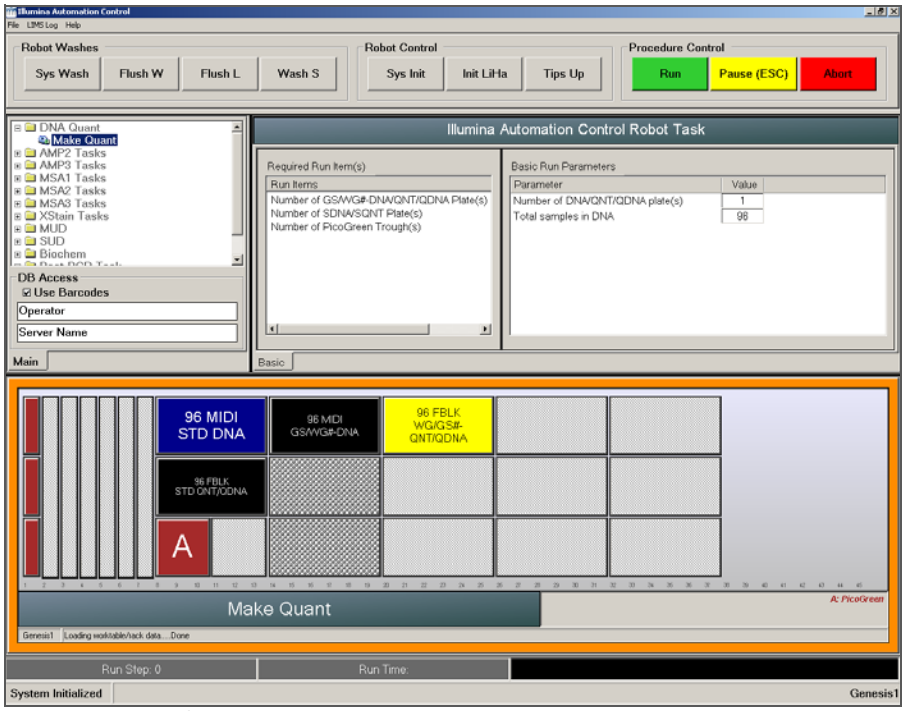


Figure 6 Make Quant Screen

4. Vortex the GS#-DNA Sample plate at 1450 rpm for 1 minute.
5. Centrifuge the GS#-DNA Sample plate to 280 xg for 1 minute.
6. Vortex the Standard DNA plate at 1450 rpm for 1 minute.
7. Centrifuge the Standard DNA plate to 280 xg for 1 minute.
8. Place the GS#-DNA Sample, Standard DNA, Standard QDNA, and QDNA Sample plates on the robot bed according to the robot bed map. Place well A1 at the top-left corner of its robot bed carrier. Remove any plate seals.
9. Pour the PicoGreen dilution into half reservoir A and place it on the robot bed.
10. Make sure that all items are placed properly on the robot bed, that all seals have been removed, and that all the barcodes face to the right.
11. On the lab tracking form, record the position of the plates on the robot bed.
12. If you are not using Illumina LIMS, clear the Use Barcodes checkbox. If you are using Illumina LIMS make sure the Use Barcodes checkbox is selected.
13. Click **Run**.

14. (Illumina LIMS only) When prompted, log in to the Illumina LIMS database.

    Observe the beginning of the robot run to ensure there are no problems.

    The robot transfers 195 µl of diluted PicoGreen to all Fluotrac plates, then transfers 2 µl aliquots of DNA from Standard DNA plate to Standard QDNA plate and from GS#-DNA plate to sample QDNA plates.

    The robot PC sounds an alert and displays a message when the process is complete.

15. Click **OK** in the message box.

16. On the lab tracking form, record:
    - Date/Time
    - Operator
    - Robot
    - The QDNA barcode that corresponds to each GS#-DNA barcode
    - The Standard QDNA plate that corresponds to each Standard DNA plate

17. After the robot finishes, immediately seal all plates:
    a. Place foil adhesive seals over Sample QDNA and Standard QDNA plates.
    b. Place cap mats on GS#-DNA Sample and Standard DNA plates.

18. Discard unused reagents in accordance with facility requirements.

19. Store the GS#-DNA and Standard DNA plates at 2°C to 8°C or -15°C to -25°C.

20. Centrifuge the Sample QDNA Plate and Standard QDNA plates to 280 xg for 1 minute.

21. Proceed to *Read QDNA Plate*.

Read QDNA Plate

In this process, you use the Gemini XS or XPS Spectrofluorometer along with the Illumina Fluorometry Control software to read the Standard QDNA and Sample QDNA plates. You use the software to create a standard curve based on the quantities of Standard DNA with PicoGreen. Then you read the Sample QDNA plates to compare their data against the standard curve to obtain the concentration of sample DNA. For the best genotyping performance, Illumina recommends a minimum concentration of 50 ng/µl.

1. Turn on the spectrofluorometer.

2. At the PC, open the Illumina Fluorometry Control program.

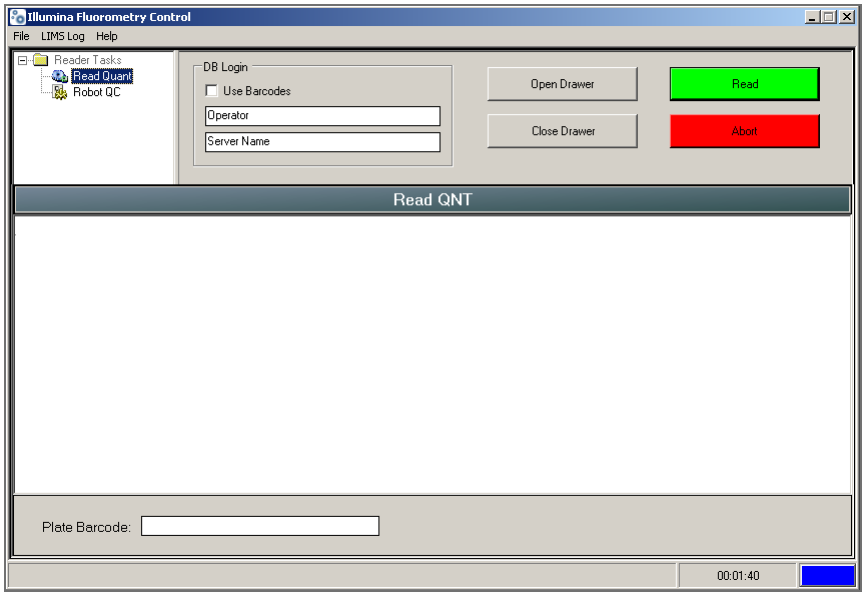


Figure 7 Illumina Fluorometry Analysis Main Screen

3. Select **Reader Tasks | Read QDNA**.
4. If you are not using Illumina LIMS, clear the Use Barcodes checkbox. If you are using Illumina LIMS make sure the Use Barcodes checkbox is selected.
5. Click **Read**.
6. When prompted, log in to the Illumina LIMS database.
7. When asked if you want to read a new Standard QDNA plate, click **Yes**. Remove the plate seal and load the Standard QDNA plate in the open fluorometer tray. Click **OK**. The spectrofluorometer reads the plate data.
8. Review the data from the Standard QDNA plate. Either accept it and go on to the next step, or reject it which will stop the Read Quant process.
9. Remove the Standard QDNA plate from the spectrofluorometer tray.
10. When prompted, enter the number of Sample QDNA plates you want to read (1, 2, or 3). Do not include the Standard QDNA plate in this number. Click **OK**.
11. When prompted, hand-scan or enter the Sample QDNA plate barcode. Click **OK**.
12. When prompted, remove the plate seal from the Sample QDNA plate and load it into the spectrofluorometer tray, with well A1 at the upper left corner. Click **OK**.

    The spectrofluorometer reads the Sample QDNA plate.
13. When prompted, click **Yes** to review the raw Sample QDNA plate data.

    The Illumina Fluorometry Control screen fills in with information about the fluorescence in the wells.

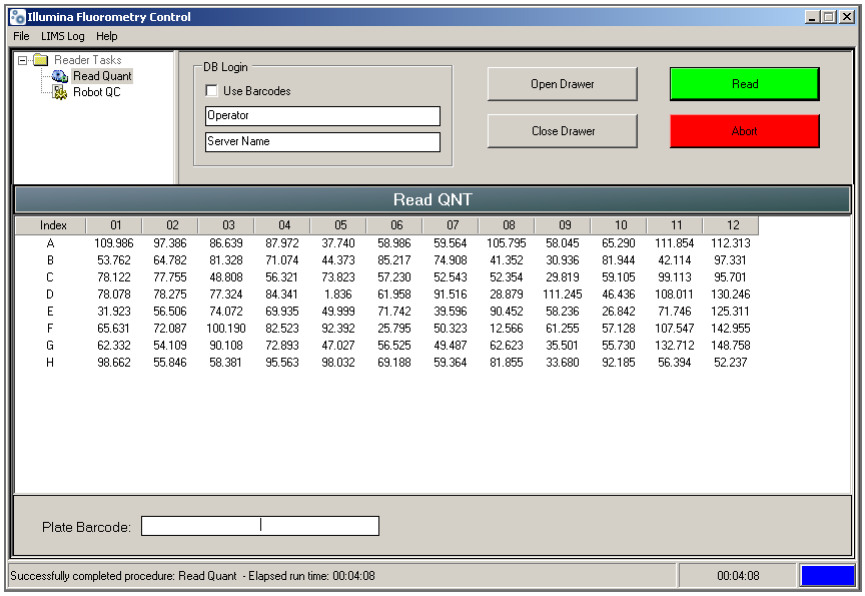


Figure 8 Sample QDNA Data

Microsoft Excel opens automatically at the same time and displays the quantitation data for the Sample QDNA plate. There are three tabs in the file:

- **SQDNA_STD**—Generates the standard curve by plotting the Relative Fluorescence (RF) values measured in the Standard QDNA plate against assumed concentrations in the Standard DNA Plate.
- **QDNA**—Plots the concentration (ng/µl) of each well of the Sample QDNA Plate as derived from the standard curve.
- **Data**—A readout of the raw data values for the Standard QDNA plate and the Sample QDNA Plate.

The Illumina Fluorometry Control software prompts you to indicate whether you wish to save the QDNA data shown in the Excel file.

14. Do one of the following:
    - Click **Yes** to send the data to Illumina LIMS. In Illumina LIMS, the QDNA plate moves into the *Make SUD MSI* queue.
    - Click **No** to delete the quant data. You can read the quant data again for the same plate.
15. If you entered more than one Sample QDNA plate to read, repeat 11 to 14 for each additional plate.
16. Discard the QDNA plates and reagents in accordance with facility requirements.
17. Do one of the following:
    - Proceed to *Make Single-Use DNA (SUD) MSI Plate* on page 18.
    - Store the Sample DNA plate at 2°C to 8°C for up to one month.

Make Single-Use DNA (SUD) MSI Plate

In this process, the robot transfers 5 µl of nucleic acid activator reagent (MS1) to the SUD plates, followed by 5 µl volume containing 250 ng sample DNA. Next, the plates are incubated on heat blocks at 95°C for 30 minutes to activate the genomic DNA. The DNA plates used to make the SUD plates must already be accessioned into Illumina LIMS and assigned to the current project. For instructions, see the *Illumina LIMS User Guide*. Fill in the lab tracking form as you work.

Estimated Time

Robot: ~30 minutes per 8 plates

Incubation: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
MS1 reagent	1 bottle	-20°C	Illumina
Genomic DNA Plate[a]	Up to 8 plates MIDI: ≥ 20 µl per sample well TCY: ≥ 10 µl per sample well	Room temperature	User
96-well 0.2 ml skirted microplate (TCY)	1 per 96 samples		User

a. Thawed, normalized to 50 ng/µl, diluted in 10 mM Tris pH 8.0/1 mM EDTA, and quantitated using the PicoGreen method. (For instructions, see *Quantitate DNA (Optional)* on page 10.)

Preparation

- Preheat the heat block to 95°C (one for each SUD plate). Allow 20 minutes for it to equilibrate.
- Turn on the heat sealer and push the **AIR ON/OFF** switch to the **ON** position.
- Prepare the heat sealer as follows:
  - **a.** Set up the heat sealer:
    - *Check the pressure gauge on the back of the heat sealer. If air is flowing into the device, the gauge should show a non-zero pressure value.*
    - *Check the touch screen* **Main Menu**. *The air pressure is displayed below the* **Status** *and should be >80 psi. If the air pressure falls below 80 psi turn the air pressure off and then back on to restore operating pressure.*
  - **b.** Set the heat sealer parameters:
    - *From the touch screen* **Main Menu**, *press* **Setup**.
    - *In the* **Set Parameters** *Menu that appears, set the Seal Time to three seconds and the Seal Temp to 150°C if you are using Agilent seals. If you are using E&K seals set the Seal Time to two and a half seconds and the Seal Temp to 150°C.*
- Thaw the MS1 bottle to room temperature.

NOTE

MS1 reagent is photosensitive. Store it and thaw it away from light.

- Prepare the robot for use:
  - Perform the *Prepare the Robot for the First Use of the Day* steps from the *System Maintenance* appendix on page 107.
  - Perform the *LiHa 8-tip Bleach Wash* steps from the *System Maintenance* appendix on page 107.
  - Perform the *MCA 96-tip Bleach Wash (Tecan 200 only)* steps from the *System Maintenance* appendix on page 108.
- Apply a GS#-DNA barcode label to each DNA plate.
- Accession the DNA plates into Illumina LIMS and assign them to the current project, if they are not already.
- Apply a GS#-SUD barcode label to each new 96-well TCY microplate.
- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Make SUD MSI** (Figure 10).
2. In the DNA Plate Selection dialog box (Figure 9), click on the plate type you wish to use. Roll the mouse pointer over each picture to see a description.

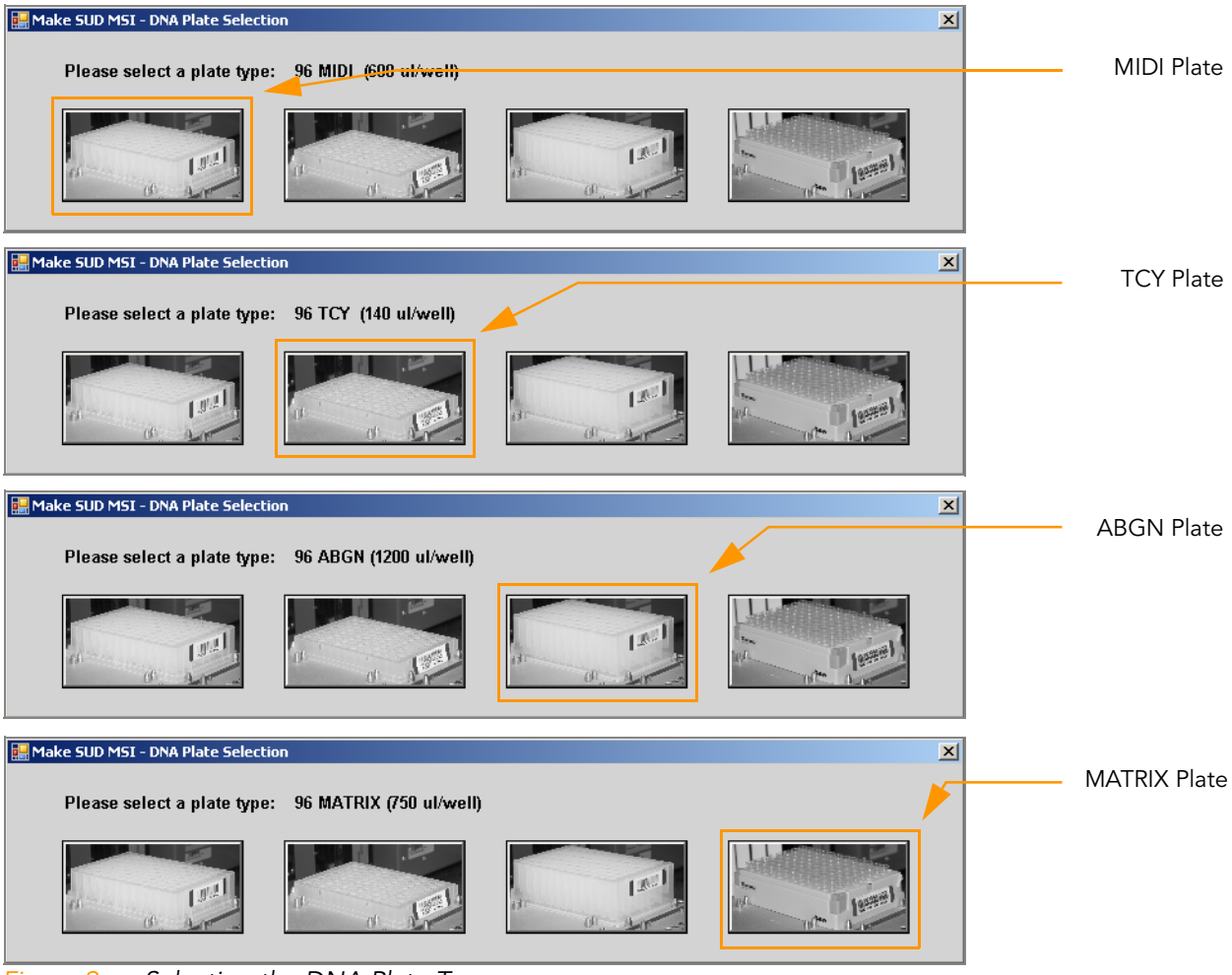


Figure 9 Selecting the DNA Plate Type

3. The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

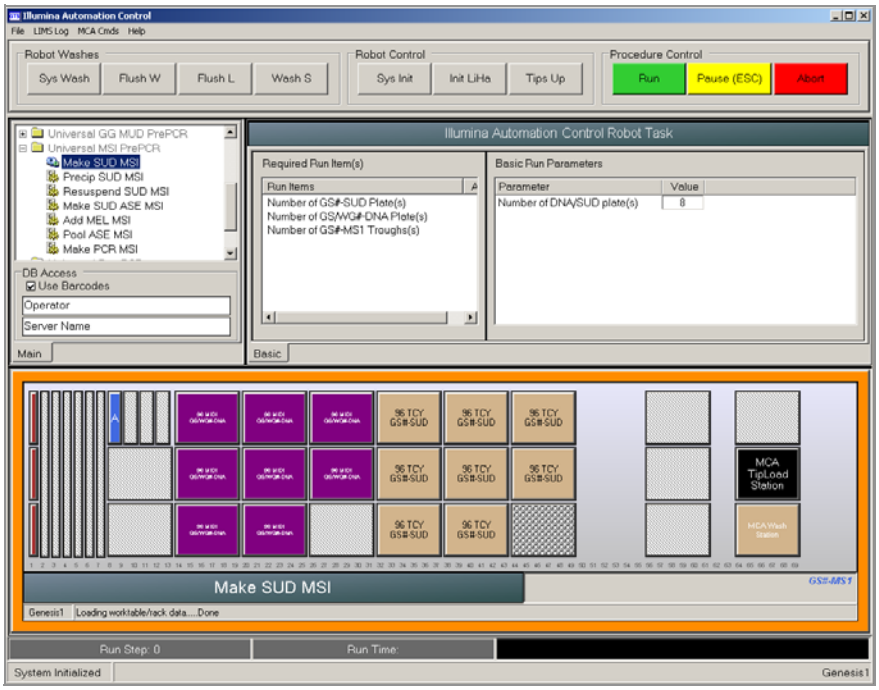


Figure 10 Universal MSI Pre-PCR | Make SUD MSI

4. Pour entire bottle of MS1 in to the quarter trough according to the robot bed map.
5. Vortex each GS#-DNA plate to 2500 rpm for 20 seconds.
6. Pulse centrifuge each GS#-DNA plate to 280 xg for 1 minute.
7. Place the SUD MSI plates on the robot bed according to the robot bed map. Make sure all barcodes face to the right.
8. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

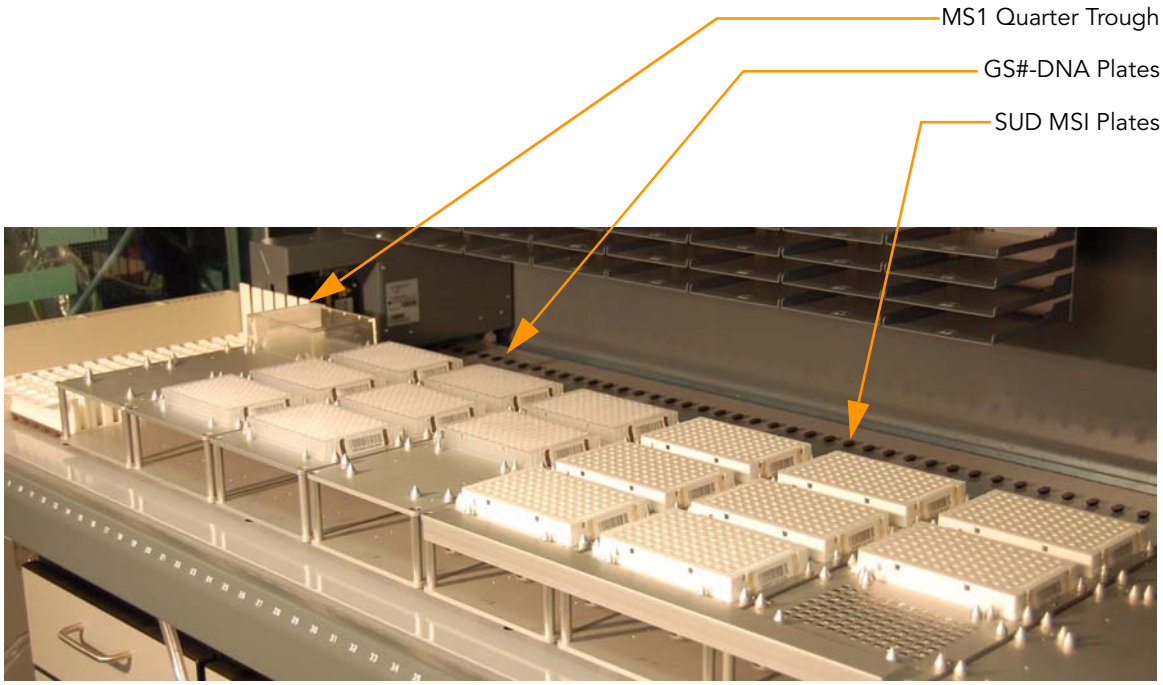


Figure 11 Robot Bed for Make SUD MSI

9. Make sure that all items are placed properly on the robot bed and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
      — *After the robot initializes, the Make SUD MSI screen appears after a moment showing the available projects (Figure 12).*

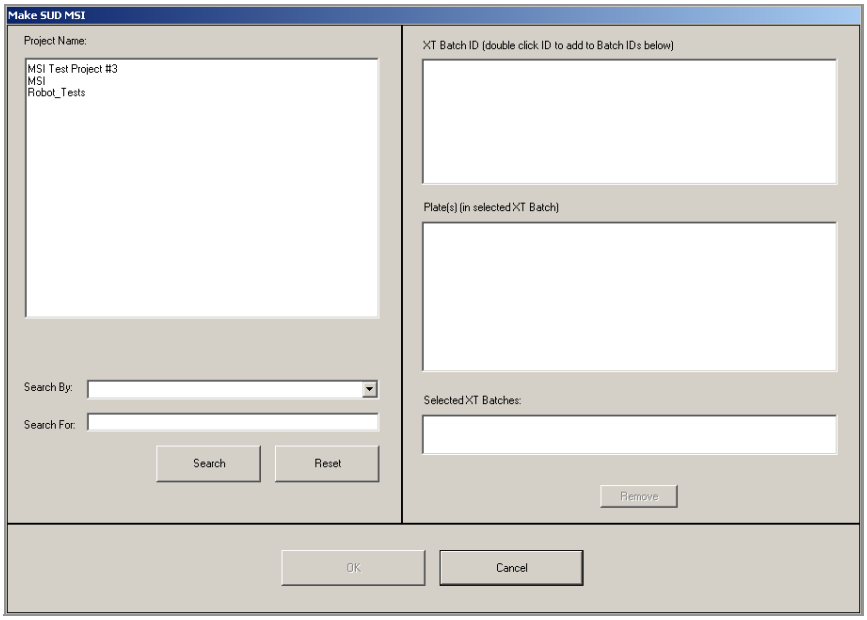


Figure 12 Selecting Project or Batch for Make SUD MSI

b. Select your current project by clicking on the name. The available batches appear in the XT Batch ID pane.

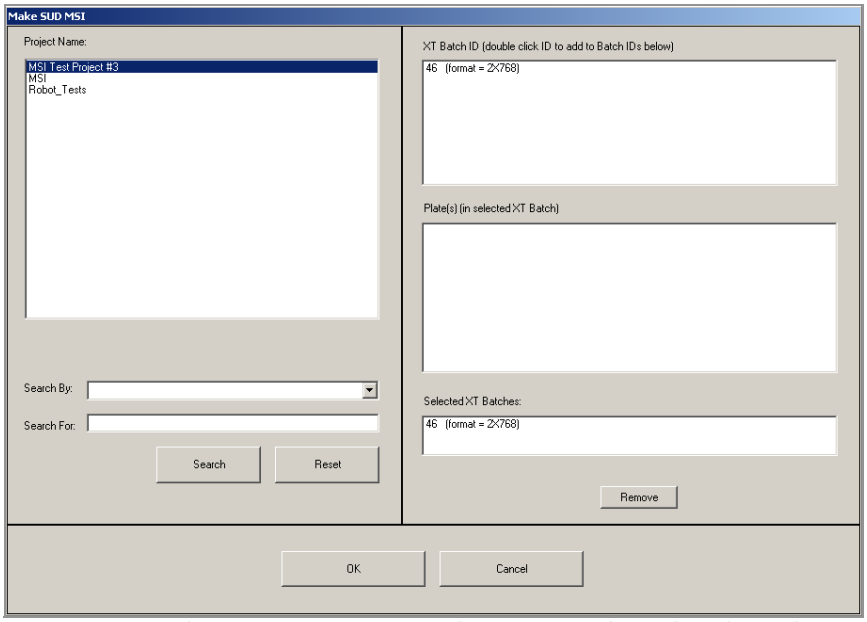


Figure 13 Make SUD MSI Screen with Project and Batch Selected

— *Use the* **Search** *box to search for a specific Batch ID or DNA Plate.*

c. In the XT Batch ID pane double-click the batch you want to run and click **OK**.

d. Place the GS#-DNA plate(s) on the robot bed according to the bed map and remove and plate seals. Click **OK**. The robot begins running when the plates are in place.

e. Click **OK** to confirm the required DNAs.

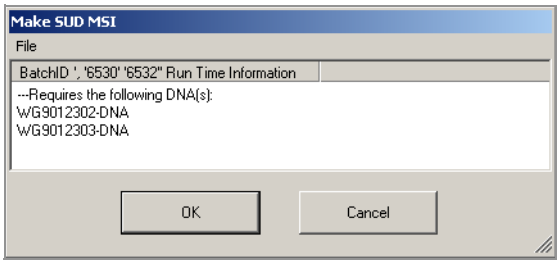


Figure 14 Confirm DNAs

f. When prompted, hand-scan the MS1 barcode.

2. Observe the robot start to run to ensure there are no problems.

The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. When the robot finishes, heat-seal each SUD plate with a peelable foil heat seal.

CAUTION Make sure that all wells are completely sealed, to prevent sample loss.

To heat-seal the SUD plate:
a. Ensure that the plate mat on the heat sealer is securely in place.
b. Place the plate on the plate mat.
c. Press **Run** on the touch screen.

2. Vortex each plate at max speed, 2500 rpm for 20 seconds.

3. Pulse centrifuge each plate to 280 xg for 1 minute.

4. Place each sealed SUD plate in a preheated 95°C heat block and close the lid. Incubate the plate for 30 minutes.

5. Log in to Illumina LIMS.
a. Select **Universal MSI PrePCR | Incubate SUD MSI** (Figure 15).

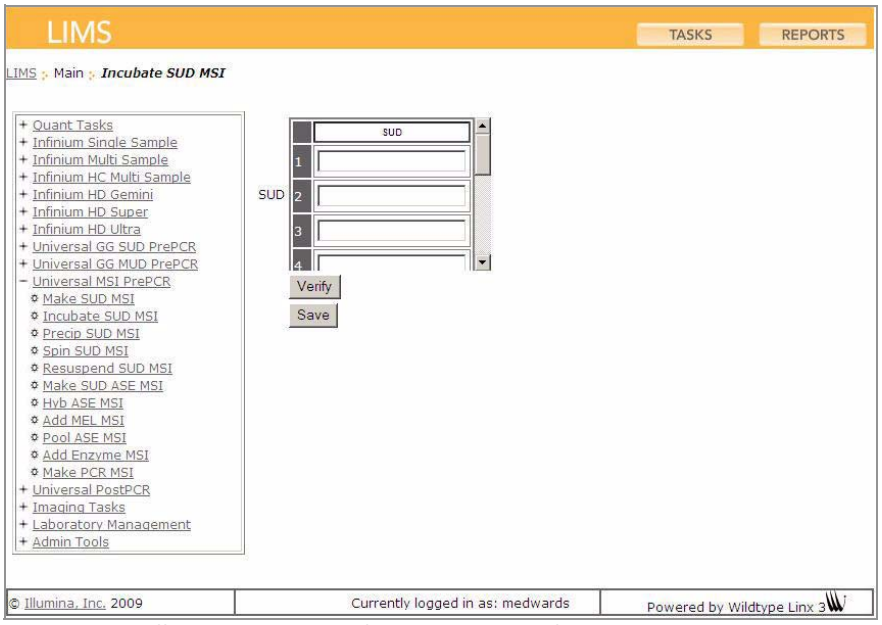


Figure 15 Illumina LIMS Incubate SUD MSI Plate

b. Using the hand-held scanner, scan each SUD plate barcode into one of the boxes.

c. Click **Verify**, then **Save**.

6. Pulse centrifuge each SUD plate to 280 xg for 1 minute.
7. If you plan to perform the Make ASE protocol today, preheat the Illumina Hybridization Oven to 70°C.
8. On the lab tracking form, check off **Incubate SUD MSI**.
9. Discard used and unused reagents in accordance with facility requirements.
10. Do one of the following:
    - Proceed to *Precipitate SUD MSI Plate* on page 26.
    - Store the sealed SUD MSI plate at 4°C for up to 12 hours.

Precipitate SUD MSI Plate

In this process, the robot adds 15 µl of 2-propanol and 5 µl of PS1 reagent to the SUD MSI plates to precipitate the DNA. Fill in the lab tracking form as you work.

Estimated Time

Robot: ~15 minutes per 8 plates

Hands-on: 30 minutes

Dry: 15 minutes

Consumables

Item	Quantity	Storage	Supplied By
PS1 reagent	Bottle	4°C	Illumina
2-propanol	Bottle	Room temperature	User

Preparation

- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps

Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Precip SUD MSI** (Figure 16).

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

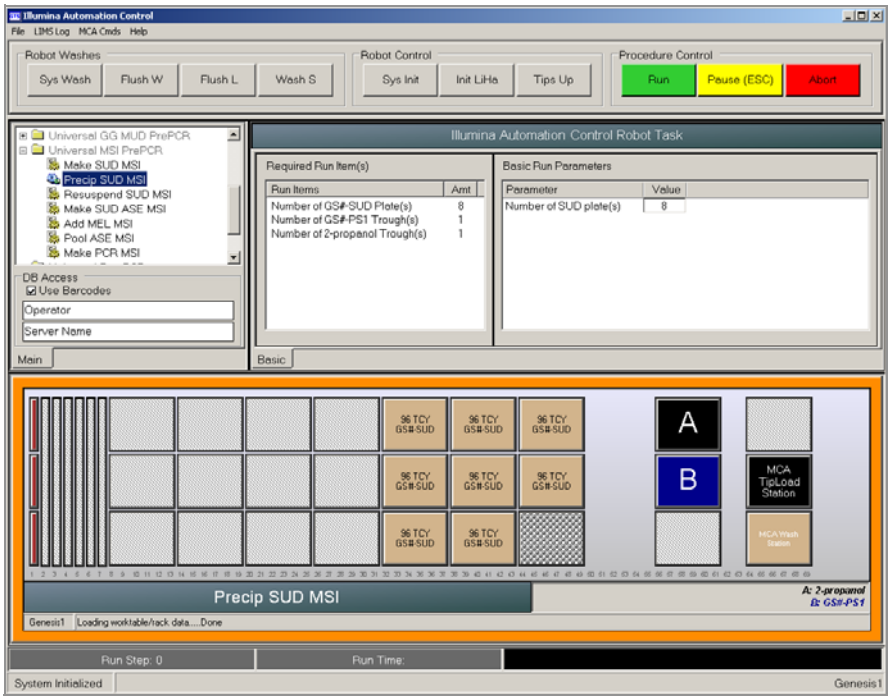


Figure 16 Universal MSI Pre-PCR | Precip SUD MSI

2. Place full trough A onto the robot bed according to the robot bed map. Dispense 50 ml of 2-propanol into the trough A.
3. Place full trough B onto the robot bed according to the robot bed map. Dispense 30 ml of PS1 into the trough B.
4. Place each SUD MSI plate on the robot bed according to the robot bed map. Remove any plate seals.
5. In the Basic Run Parameters pane, change the value for **Number of SUD plate(s)** to reflect the number of SUD plates being processed.
6. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

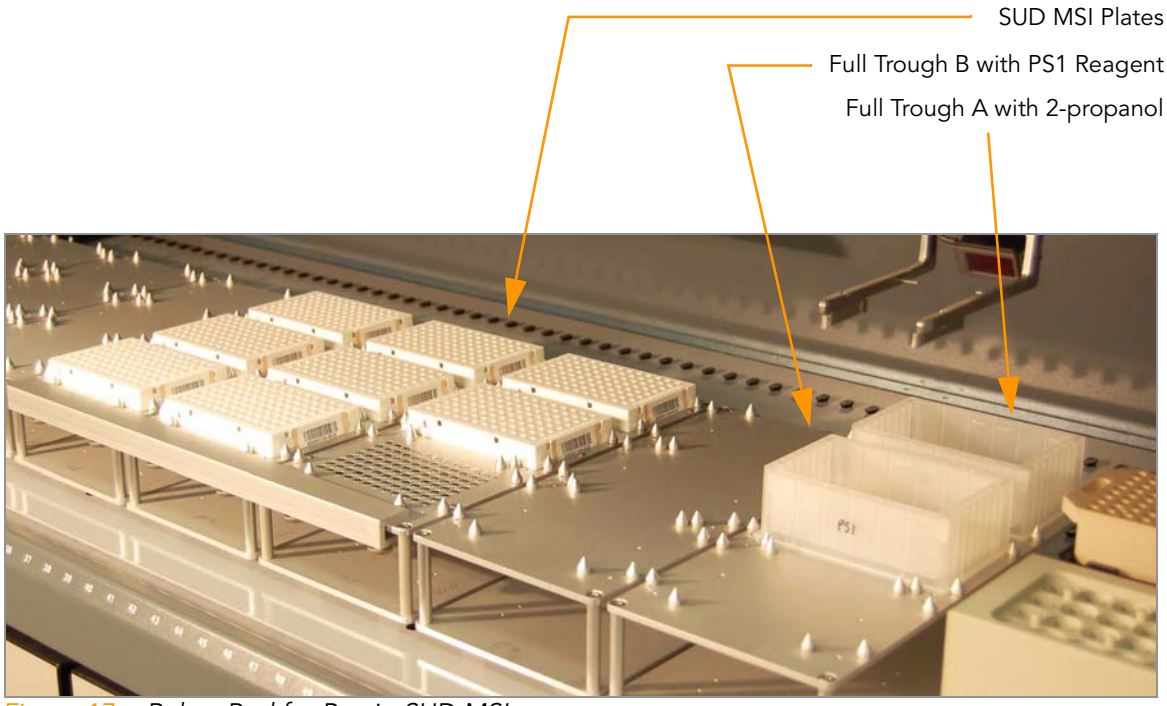


Figure 17 Robot Bed for Precip SUD MSI

7. Make sure that all items are placed properly on the robot bed and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
   b. When prompted, hand-scan the PS1 barcode.
2. Observe the robot start to run to ensure there are no problems.

   The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. When the robot finishes, seal each SUD MSI plate with adhesive film.
2. Vortex each plate at 2000 rpm for 2 minutes, or until all wells are uniformly blue.
3. Centrifuge each SUD MSI plate to 3000 xg for 20 minutes. A faint blue pellet should be at the bottom of each well.

NOTE

If you do not see a faint blue pellet at the bottom of each well, the DNA has not precipitated. In some cases, depending on DNA quality, the blue pellet may appear diffuse.

Perform the next step immediately to avoid dislodging the activated DNA pellets. If any delay occurs, recentrifuge to 3000 xg for 10 minutes before proceeding.

4. Remove each SUD plate seal and decant the supernatant by inverting the plate and smacking it down onto an absorbent pad.

Do not tilt the plate, as this can cause cross-contamination between wells. Tap the plate firmly enough to decant all the supernatant; tapping lightly will not work as well.

5. Tap the inverted plate onto the pad to blot excess supernatant. Keep the plates inverted until the the next step is completed.
6. Do one of the following:
   a. Place each inverted SUD MSI plate on an absorbent pad and centrifuge to 8 xg for 1 minute.

Do not spin the inverted plate to more than 8 xg, or the sample will be lost!

   b. Remove each SUD MSI plate from centrifuge and allow to dry at room temperature for 15 minutes.

   Or

   a. Set each plate upright and allow it to dry at room temperature for 1 hour.
7. Discard used and unused reagents in accordance with facility requirements.

8. Log in to Illumina LIMS.
   a. Select **Universal MSI PrePCR | Spin SUD MSI** (Figure 18).

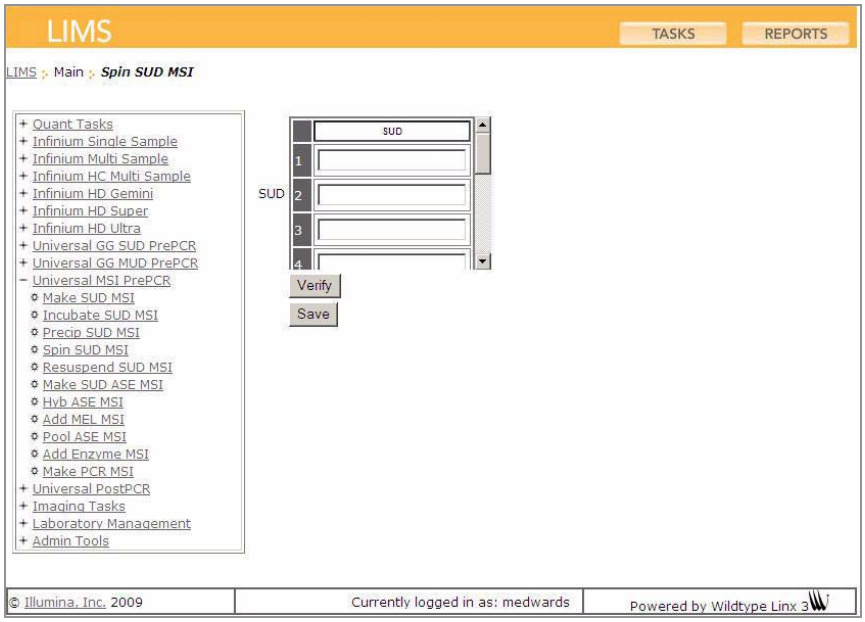


Figure 18 Illumina LIMS Spin SUD Plate

   b. Using the hand-held scanner, scan each SUD plate barcode into one of the boxes.
   c. Click **Verify**, then **Save**.
   d. On the lab tracking form, check off **Spin SUD MSI**.

9. Proceed to *Resuspend SUD MSI Plate* on page 31.

Resuspend SUD MSI Plate

In this process, the robot adds 10 µl of RS1 reagent to the SUD plates to resuspend the samples. Fill in the lab tracking form as you work.

Estimated Time

Robot: ~10 minutes per 8 plates

Consumables

Item	Quantity	Storage	Supplied By
RS1 reagent	Bottle	Room temperature	Illumina

Preparation

- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps

Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Resuspend SUD MSI** (Figure 19).

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

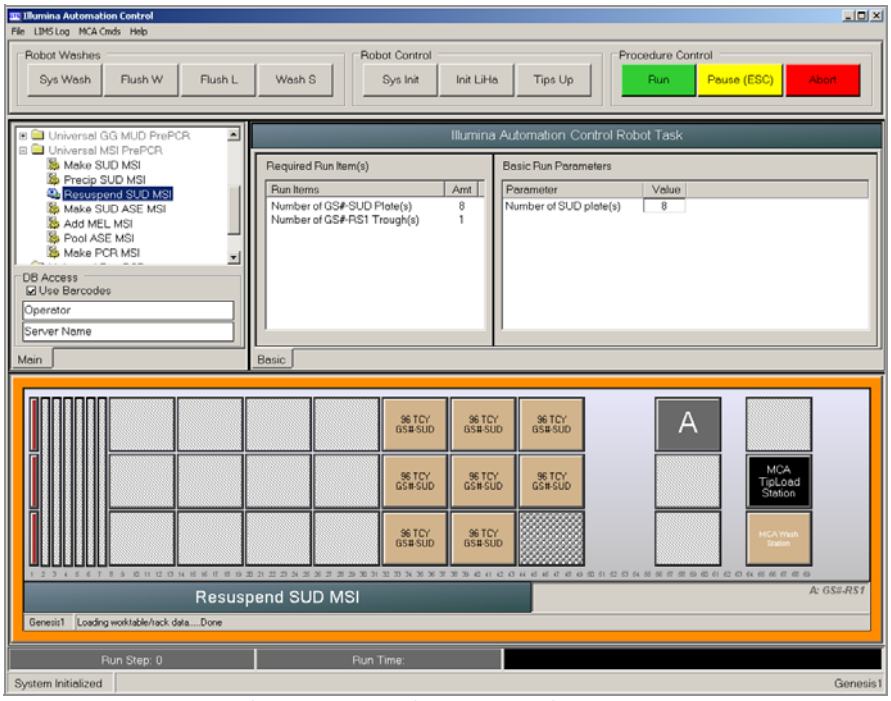


Figure 19 Universal MSI PrePCR | Resuspend SUD MSI

2. Place full trough A onto the robot bed according to the robot bed map. Dispense 50 ml of RS1 into trough A.
3. Place each SUD MSI plate on the robot bed according to the robot bed map. Remove any plate seals.
4. In the Basic Run Parameters pane, change the value for **Number of SUD plate(s)** to reflect the number of SUD plates being processed.
5. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

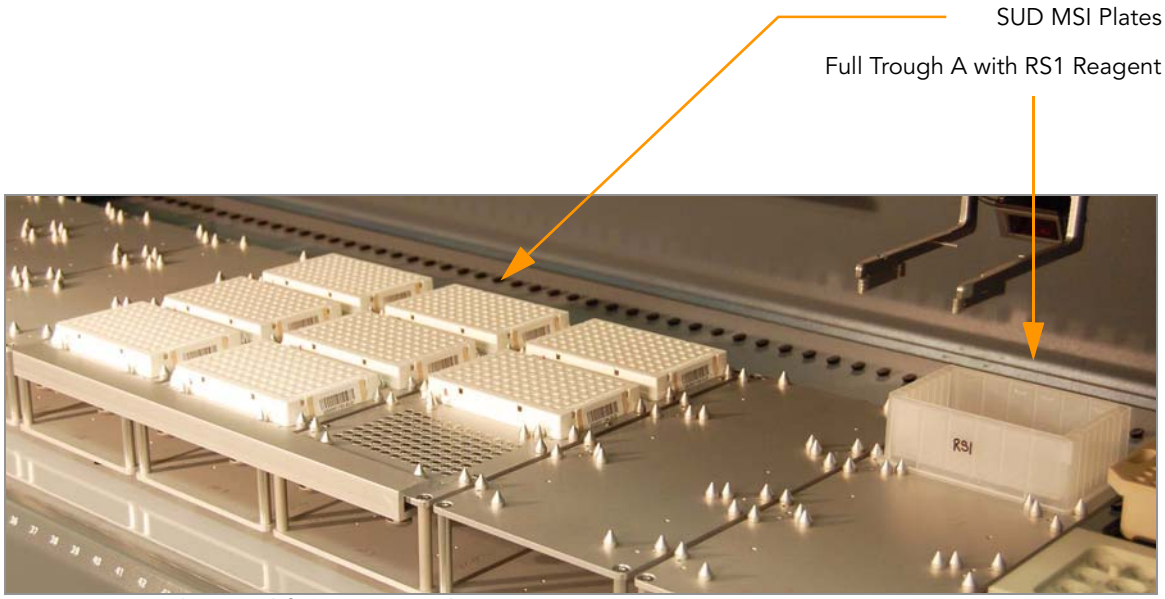


Figure 20 Robot Bed for Resuspend SUD MSI

6. Make sure that all items are placed properly on the robot bed and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
   b. When prompted, hand-scan the RS1 barcode.
2. Observe the robot start to run to ensure there are no problems.

   The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. When the robot finishes, seal each SUD MSI plate with adhesive film.
2. Vortex each plate at max speed, 2500 rpm for 2 minutes, or until all wells are completely mixed. Complete mixing is necessary to ensure quality data.
3. Pulse centrifuge each plate to 280 xg for 1 minute.
4. Discard used and unused reagents in accordance with facility requirements.
5. Do one of the following:
   - Proceed to *Make SUD Allele-Specific Extension (ASE) MSI Plate* on page 34.
   - Remove the adhesive seal from each SUD MSI plate and heat-seal the SUD MSI plate with a foil heat seal. Store it at 4°C for up to 24 hours or at -20°C for up to 2 months.

Make SUD Allele-Specific Extension (ASE) MSI Plate

In this process, the robot combines the hybridization reagents and buffers from the 30 µl of OB1 reagent and up to 8 OPAs into new 96-well ASE MSI plates. 10 µl of resuspended DNA samples are transferred from the SUD plate into the ASE plate. The ASE plate is subsequently incubated to allow the activated DNA to bind with the paramagnetic particles and anneal with the oligonucleotides. Fill in the lab tracking form as you work.

The OPA reagent contains a pool of oligonucleotide triplets designed to hybridize to the SNPs of interest: one triplet for each SNP being analyzed. The triplets consist of two allele-specific oligonucleotides (ASO1 and ASO2, one for each of two possible SNP variants) and a locus-specific oligonucleotide (LSO).

Estimated Time

Robot: 1 hour per 8 plates

Incubation: 2–16 hours (heat block) or 3.5–16 hours (Illumina Hybridization Oven)

Consumables

Item	Quantity	Storage	Supplied By
OPA reagent	1 tube per ASE plate	-20°C	Illumina
OB1 reagent	Bottle	-20°C	Illumina
96-well 0.2 ml microplate (TCY)	1 per SUD plate		User

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, contact Illumina Technical Support and ask for the MSDS for this assay.

Preparation

- Set the Illumina Hybridization Oven to 70°C.
- Thaw the OB1 bottle to room temperature. Vortex for at least 5 seconds to fully resuspend the beads.
- Thaw the OPA tubes to room temperature. Invert tubes 5 times to mix and pulse centrifuge to 280xg for 1 minute.
- Prepare the heat sealer for use if you have not done already.
- Apply a GS#-ASE barcode label to each new 96-well TCY microplate.
- On the lab tracking form, record:
  - Operator
  - Robot

- Date

NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Make SUD ASE MSI** (Figure 21).

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

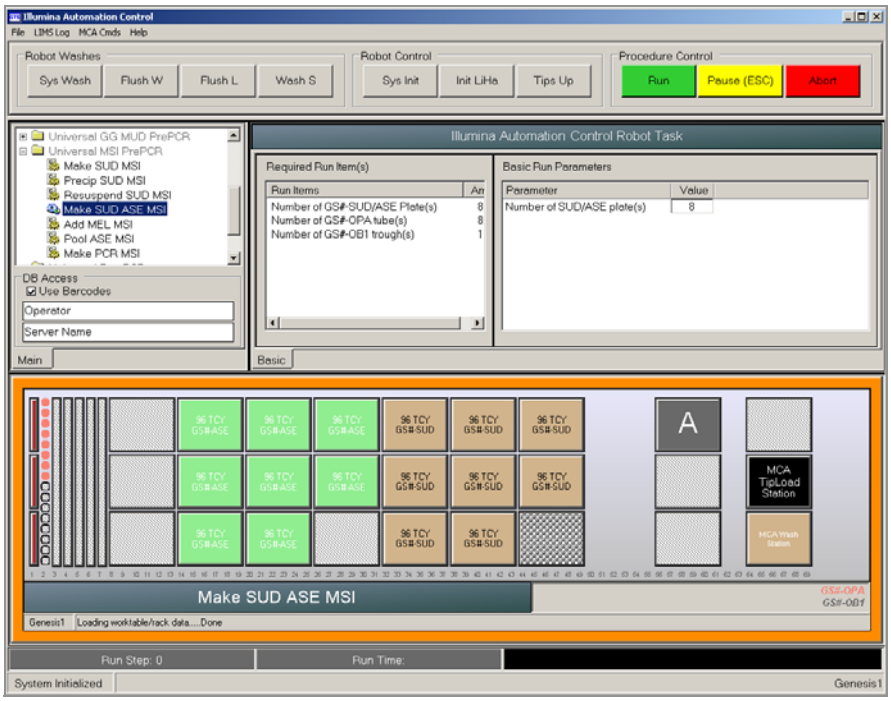


Figure 21 Universal MSI PrePCR | Make SUD ASE MSI

2. Place the OPA tubes in the robot tube rack according to the robot bed map. Remove the caps.
3. Place full trough A onto the robot bed according to the robot bed map. Dispense 40 ml of OB1 into trough A.
4. Pulse centrifuge the SUD MSI plate to 280 xg for 1 minute.
5. Place the SUD MSI and ASE MSI plates on the robot bed according to the robot bed map. Remove any plate seals.
6. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

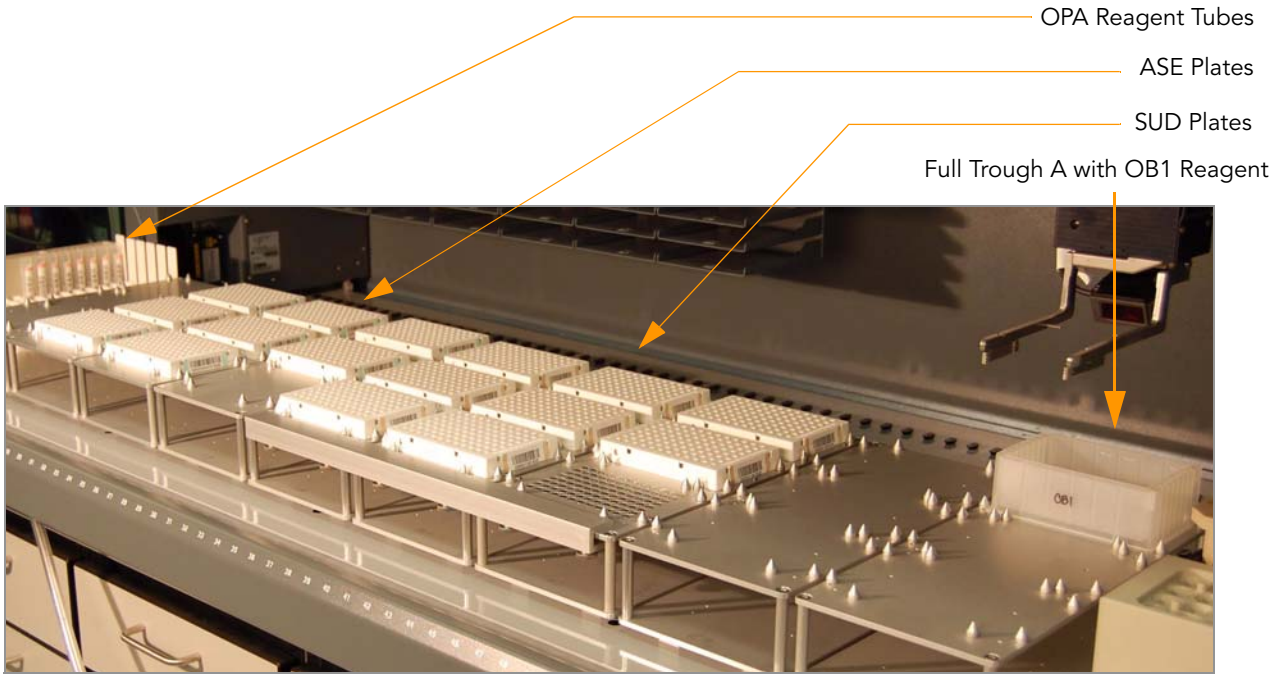


Figure 22 Robot Bed for Make SUD ASE MSI

7. Make sure that all items are placed properly on the robot bed and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
      — *After the robot initializes, the Make SUD ASE MSI screen appears after a moment.*

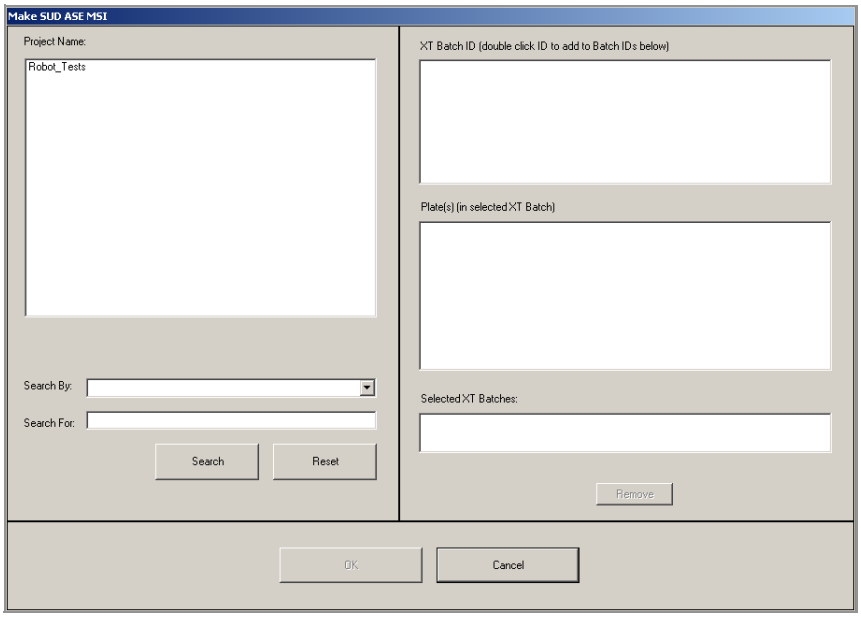


Figure 23 Selecting Project or Batch for Make SUD ASE MSI

b. Select your current project. The available batches appear in the XT Batch ID pane.

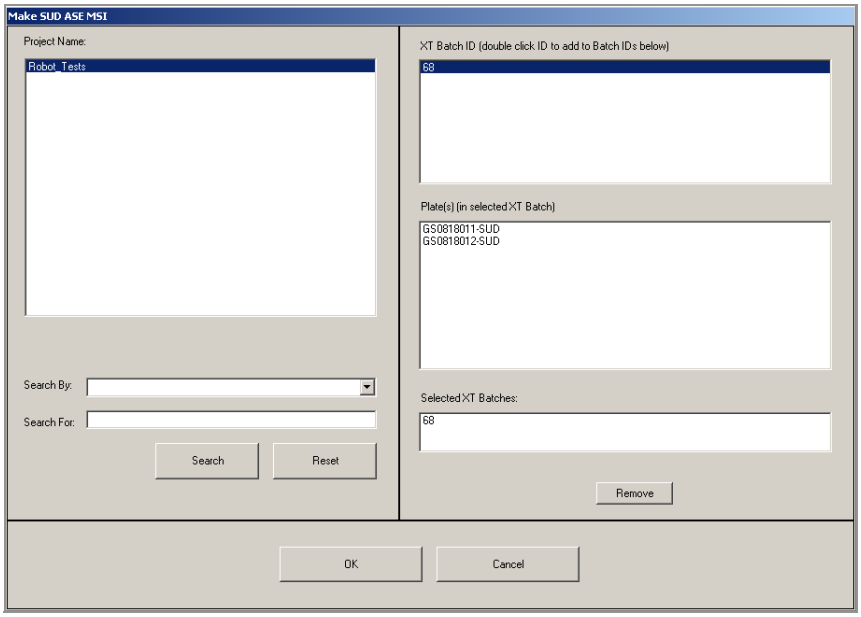


Figure 24 Make SUD ASE MSI Screen with Project and Batch Selected

— *Use the* **Search** *box to search for a specific Batch ID or Plate.*

c. Double-click to select the batch you want to run and click **OK**.

d. Click **OK** to confirm the OPA tubes and SUD plates.

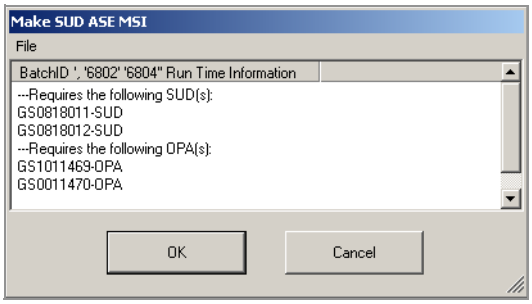


Figure 25 Confirm OPA and SUD plate(s)

e. When prompted, hand-scan the OB1 barcode.

2. Observe the robot start to run to ensure there are no problems.

   The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. When the robot finishes, heat-seal each ASE MSI plate with a foil heat seal.
2. Vortex each plate at 1800 rpm for 2 minutes or until all beads are completely resuspended.
3. Place the ASE plate(s) in the Illumina Hybridization Oven at 70°C for 30 minutes. After 30 minutes, set the temperature to 30°C. Leave the Hyb plates in the hyb oven up to 16 hours (minimum of 3 hours).

NOTE If you are using the Illumina Hybridization Oven for incubation do not place the plates on the top rack directly underneath the fan.

NOTE You can also use heat blocks for incubation. Set the heat block(s) to 70°C. When the heat block reaches 70°C, place the ASE plate(s) in the heat block(s) and immediately set the temperature to 30°C. You can leave the ASE plate(s) in the heat block for up to 16 hours (minimum of two hours).

4. Discard used and unused reagents in accordance with facility requirements. Discard the SUD plate.
5. Thaw the IP1 bottle at room temperature overnight for the *Pool Allele-Specific Extension (ASE) MSI* steps on page 46.

6. After the overnight hybridization and the plates have been removed from the oven, log in to Illumina LIMS.
   a. Select **Universal MSI PrePCR | Hyb ASE MSI** (Figure 26).

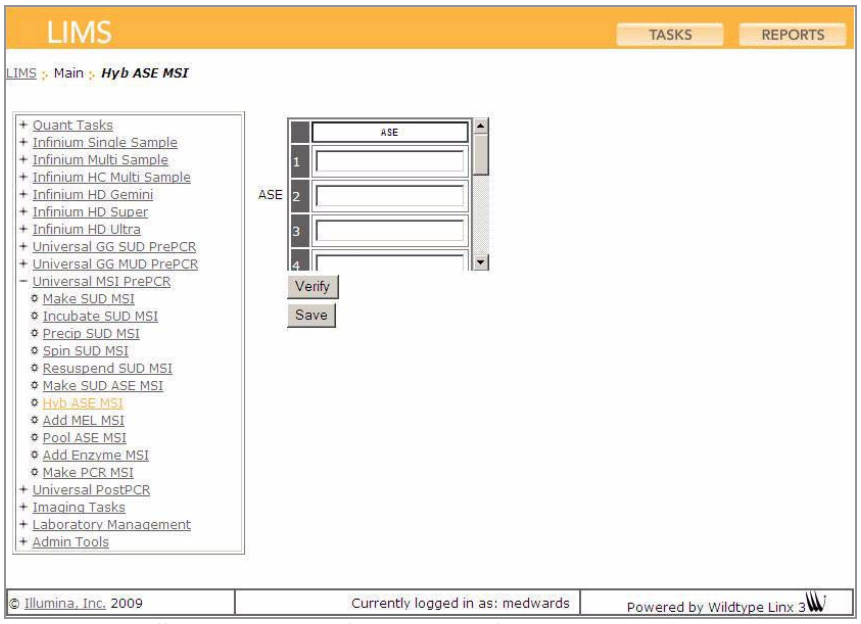


Figure 26 Illumina LIMS Hyb ASE MSI Plate

   b. Scan each ASE plate barcode into one of the boxes.
   c. Click **Verify**, then **Save**.

7. Proceed to *Make SUD Allele-Specific Extension (ASE) MSI Plate* on page 34.

Add Extension and Ligation Reagents MSI (MEL)

In this protocol, hybridized ASO-LSO pairs on the bound genomic DNA are extended and ligated. The robot washes the ASE MSI plates multiple times using the AM1 and UB1 reagents to remove non-specifically hybridized and excess oligos from the DNA. Four total washes are executed in this protocol. For the first two washes the robot transfers 50 µl of AMI to the ASE MSI plates. For the last two washes the robot transfers 50 µl of just the UB1 reagent to the ASE MSI plates. The ASE MSI plates are removed from the robot bed, sealed, and vortexed between each wash step. After the wash steps are completed the robot transfers 37 µl of MEL (extension and ligation enzymes) reagent to each sample in the ASE MSI plates.

Next, the ASE MSI plates are incubated at 45°C for 15 minutes to allow (1) the 3′ ends of the properly hybridized ASOs to extend downstream to the 5′ ends of their paired LSO, and (2) the extended 3′ ASO ends to ligate to the 5′ LSO ends. Fill in the lab tracking form as you work.

Estimated Time

Robot: ~1 hour per 8 plates

Incubation: 15 minutes

Consumables

Item	Quantity	Storage	Supplied By
MEL reagent	Bottle	-20°C	Illumina
AM1 reagent	Bottle	4°C	Illumina
UB1 reagent	Bottle	4°C	Illumina

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, contact Illumina Technical Support and ask for the MSDS for this assay.

Preparation

- Preheat the heat block to 45°C (one for each plate) and allow it to equilibrate.
- Thaw all reagents to room temperature. Invert 10 times to mix.
- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Add MEL MSI** (Figure 27).

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

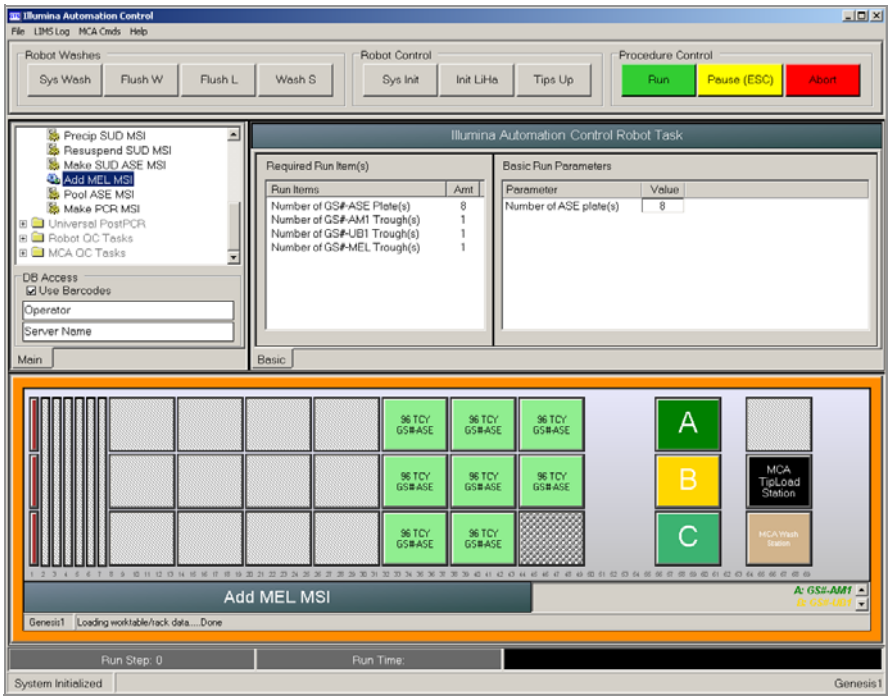


Figure 27 Universal MSI PrePCR | Add MEL MSI

2. Place full trough A onto the robot bed according to the robot bed map. Dispense 100 ml of AM1 into trough A.
3. Place full trough B onto the robot bed according to the robot bed map. Dispense 150 ml of UB1 into trough B.
4. Place full trough C onto the robot bed according to the robot bed map. Dispense the entire bottle of MEL into trough C.
5. Pulse centrifuge each ASE MSI plate to 280 xg for 1 minute.
6. Remove the heat seal foil before placing the ASE MSI plates on the robot bed.

7. Place each ASE MSI plate on the magnetic carrier on the robot bed according to the robot bed map.
8. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

CAUTION To avoid cross-contamination, do not remove the plate seal until the beads are fully captured.

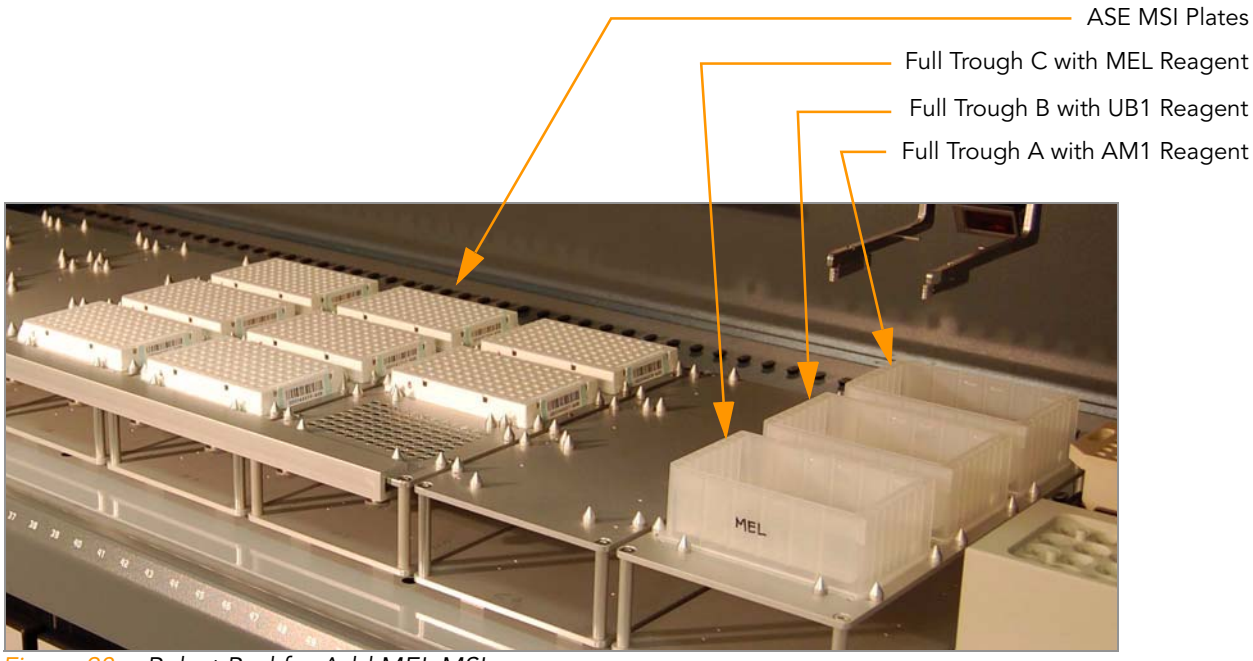


Figure 28 Robot Bed for Add MEL MSI

9. Make sure that all items are placed properly on the robot bed and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
   b. When prompted, hand-scan the AM1, UB1, and MEL barcodes.
2. Observe the robot start to run to ensure that there are no problems.
3. The robot pauses while the beads settle.
4. When prompted, remove each ASE MSI plate from the robot bed and seal it with an adhesive seal.

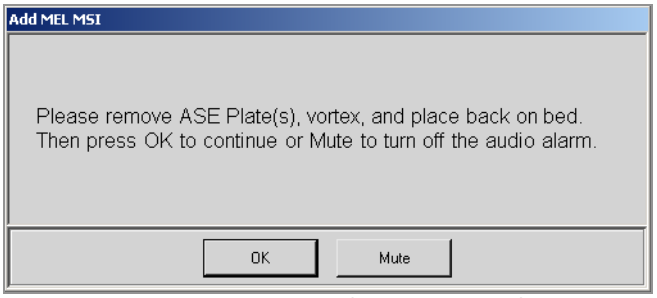


Figure 29 First Remove and Vortex ASE Plate Message

5. Secure each plate to the high-speed shaker with the clampshell lid. If you are vortexing multiple plates, position them so that the high-speed shaker is balanced.

CAUTION

Prevent splashing! Each vortexer in your lab should carry a unique label indicating its calibration variance for all recommended settings. Before vortexing, set the digital display value according to the appropriate values indicated on the label.

CAUTION

Illumina recommends using the raised bar magnetic plate to manually loosen the beads before vortexing.

6. Vortex each sealed plate at 1900 rpm for 1 minute, or until resuspended.

   If all wells are resuspended but there is splashing:

   a. Spin down the plate at 280 xg for one minute before removing the seal and putting it back on the robot deck.

   If there is splashing and all wells are not resuspended:

   a. Centrifuge the plate to 280 xg for one minute, use the bar magnet to loosen the beads and re-vortex until all wells are resuspended. If necessary, decrease the speed of the vortexer to minimize splashing in the wells.

7. Remove the seal and return each ASE MSI plate to the correct position on the robot bed according to the bed map. Click **OK** in the message box.

NOTE

Illumina LIMS requires that each plate be placed back into its original position on the robot bed.

8. When prompted, remove each ASE MSI plate from the robot bed and seal it with an adhesive seal.

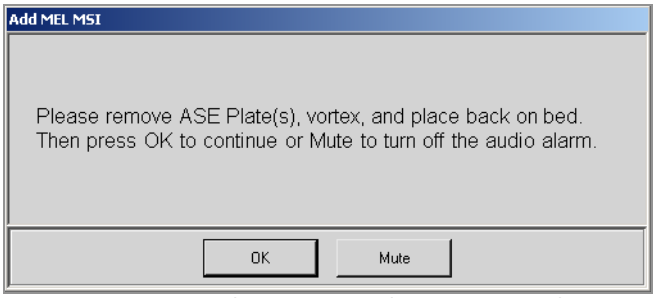


Figure 30 Second Remove and Vortex ASE Plate Message

CAUTION Illumina recommends using the raised bar magnetic plate to manually loosen the beads before vortexing.

9. Vortex each plate at 1900 rpm for 1 minute or until all beads are completely resuspended.

   If all wells are resuspended but there is splashing:

   a. Spin down the plate at 280 xg for one minute before removing the seal and putting it back on the robot deck.

   If there is splashing and all wells are not resuspended:

   a. Centrifuge the plate to 280 xg for one minute, use the bar magnet to loosen the beads and re-vortex until all wells are resuspended. If necessary, adjust the speed of the vortexer to minimize splashing in the wells.

10. Remove the seal and return each ASE MSI plate to the correct position on the robot bed according to the bed map. Click **OK** in the message box.

    The robot will perform two UB1 washes and add MEL to each plate.

NOTE Illumina LIMS requires that each plate be placed back into its original position on the robot bed.

11. When prompted, remove each ASE MSI plate from the robot bed.

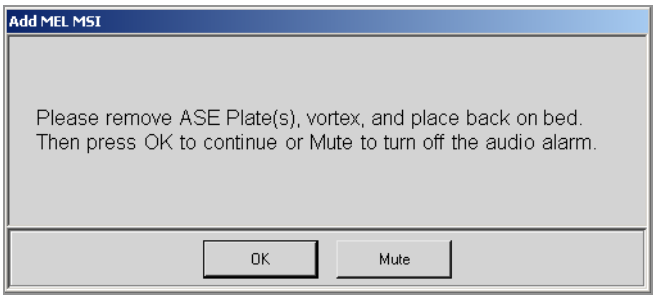


Figure 31 Final Vortex and Incubate Message

12. Seal each ASE MSI plate with an adhesive seal.

Illumina recommends using the raised bar magnetic plate to manually loosen the beads before vortexing.

13. Vortex each plate at 1900 rpm for 1 minute, or until the beads are resuspended. Click **OK** in the message box.

    If all wells are resuspended but there is splashing:

    a. Spin down the plate at 280 xg for one minute before removing the seal and putting it back on the robot deck.

    If there is splashing and all wells are not resuspended:

    a. Centrifuge the plate to 280 xg for one minute, use the bar magnet to loosen the beads and re-vortex until all wells are resuspended. If necessary, adjust the speed of the vortexer to minimize splashing in the wells.

Complete the Protocol

1. Place each sealed plate on the preheated 45°C heat block and close the lid. Incubate the plate for exactly 15 minutes.

Do not leave the ASE plate in the heat block for more than 15 minutes.

2. Discard all used and unused reagents in accordance with facility requirements except the UB1. The remaining UB1 reagent in trough B will be used in the next step, Pool ASE MSI.
3. Do one of the following:
   - Proceed to *Pool Allele-Specific Extension (ASE) MSI* on page 46.
   - Store the ASE plate(s) at 4°C for up to 1 hour.

Pool Allele-Specific Extension (ASE) MSI

The extended and ligated DNA strands from the Add MEL MSI step are systematically pooled together into a single ASE Pool Plate (APP) to take advantage of the indexing capabilities of this assay. The robot pools 20 µl of sample per well. Samples being pooled together have the same ASO primers but have unique LSO primers to be able to distinguish the individual samples from one another. Fill in the lab tracking form as you work.

Estimated Time

Robot: ~30 minutes per 8 plates

Incubation: 1 minute

Consumables

Item	Quantity	Storage	Supplied By
IP1 reagent	Bottle	-20°C	Illumina
UB1 reagent	Bottle	4°C	Illumina
MIDI plate	1 for each APP plate used		User
Cap mat	1 for each APP plate used		User

This protocol involves the use of an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, contact Illumina Technical Support and ask for the MSDS for this assay.

Preparation

- Preheat the heat block to 95°C (one for each ASE plate).
- Thaw the IP1 bottle to room temperature and invert to mix.
- Apply APP barcode to each 96-well MIDI plate.
- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Pool ASE MSI** (Figure 32).

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

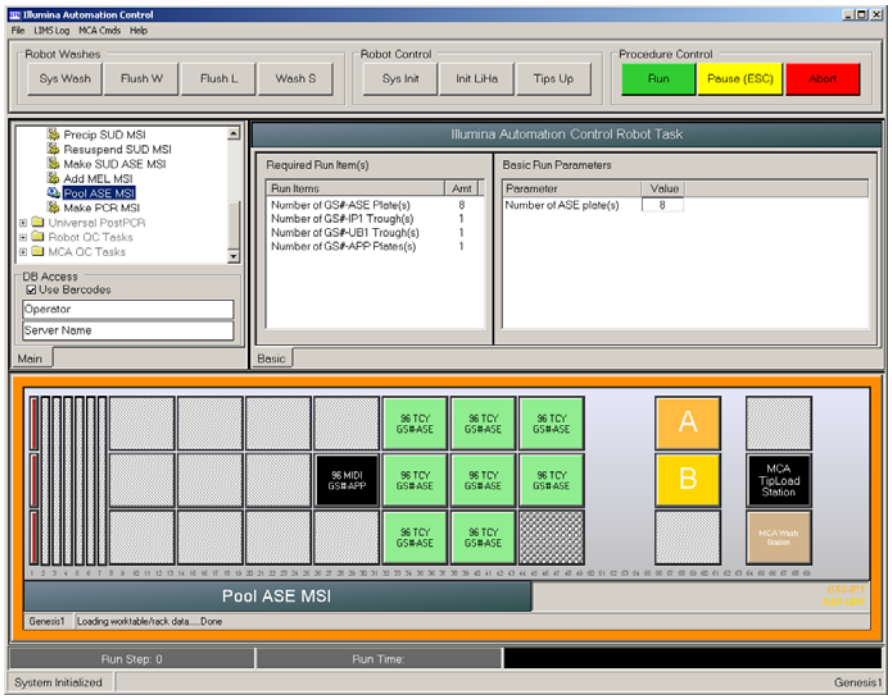


Figure 32 Universal MSI PrePCR | Pool ASE MSI

2. Place full trough A onto the robot bed according to the robot bed map. Dispense 50 ml of IP1 into trough A.
3. Place full trough B onto the robot bed according to the robot bed map. Use the remaining UB1 reagent in trough B from the Add MEL MSI steps and refill to 70 ml.
4. Pulse centrifuge each ASE plate to 280 xg for 1 minute.
5. Remove the adhesive seal before placing the ASE plate(s) on the robot bed.
6. Place each ASE plate on the magnetic carrier on the robot bed according to the robot bed map.
7. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

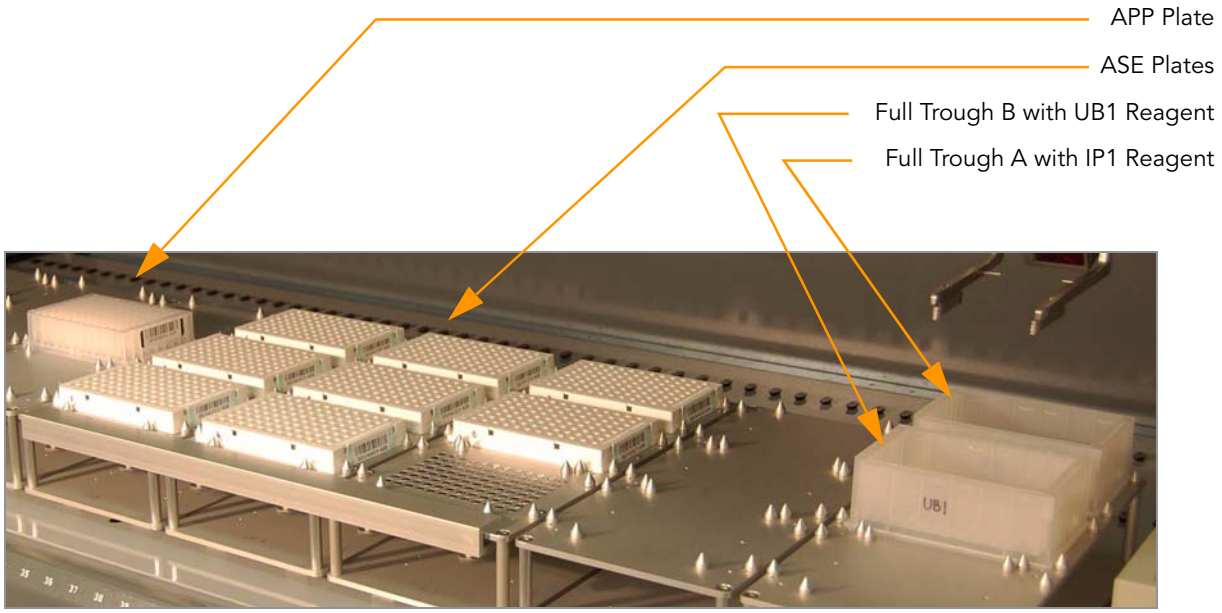


Figure 33 Robot Bed for Pool ASE MSI

8. Make sure that all items are placed properly on the robot bed and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
      — *After the robot initializes, the Pool ASE MSI screen appears after a moment.*

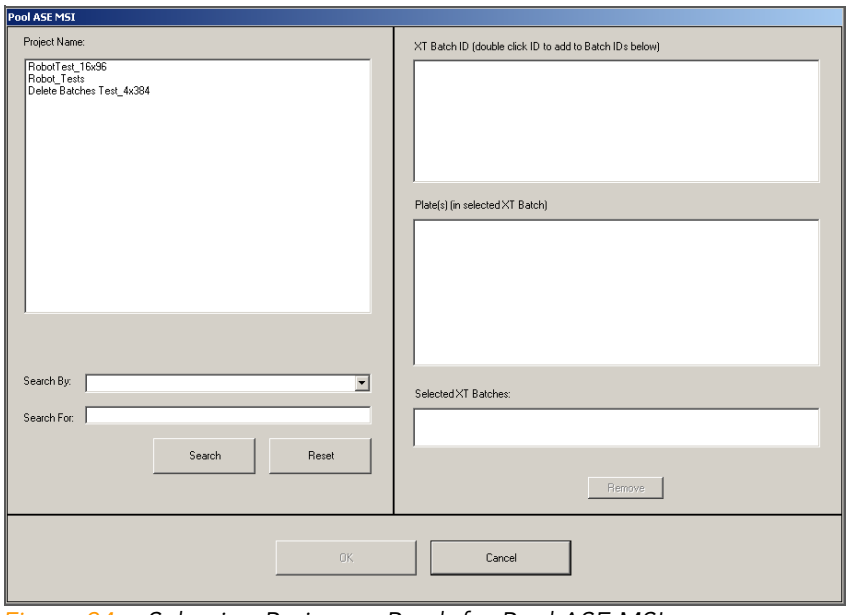


Figure 34 Selecting Project or Batch for Pool ASE MSI

b. Do one of the following:

— *Select your current project. The available batches appear in the XT Batch ID pane. Select a batch to see the associated ASE plate appear in the Plate(s) pane.*

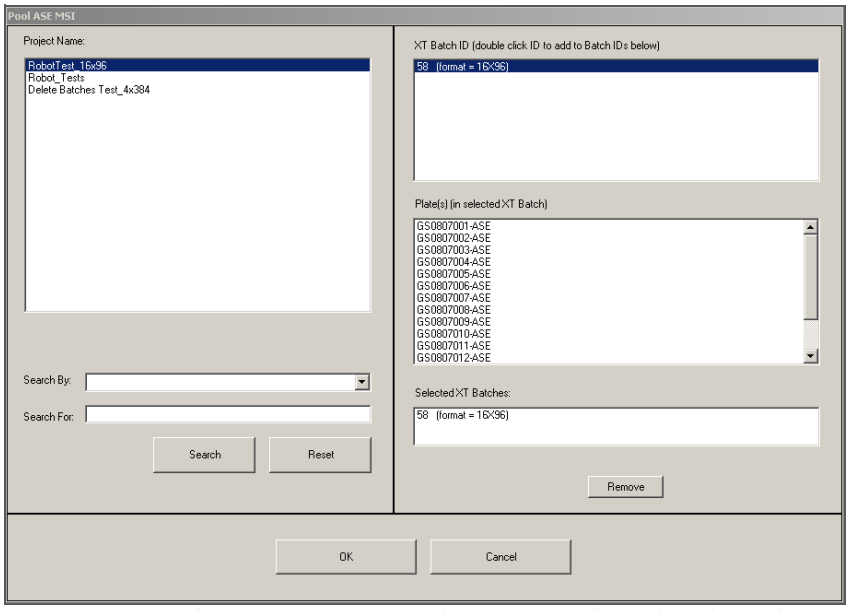


Figure 35 Pool ASE MSI Screen with Project and Batch Selected

— *Use the* **Search** *box to search for a specific Batch ID or Plate.*

c. Double-click to select the batch you want to run and click **OK**.

d. Click **OK** to confirm the ASE MSI and APP plate(s).

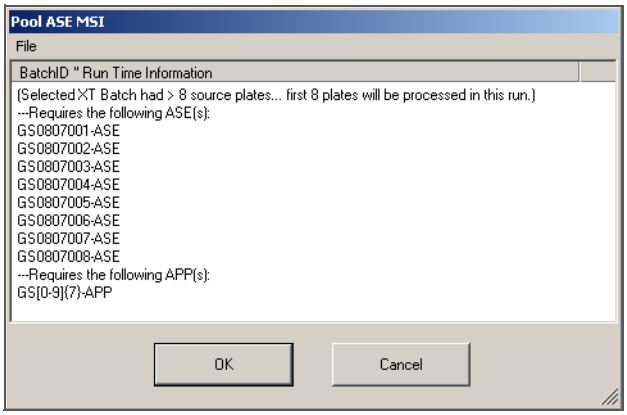


Figure 36 Confirm ASE and APP plate(s)

e. When prompted, hand-scan the IP1 and UB1 barcodes.

2. Observe the robot start to run to ensure there are no problems.

The robot PC sounds an alert and displays a message when the process is complete.

3. When prompted, remove the ASE MSI plates from the robot bed and seal it with an adhesive seal.

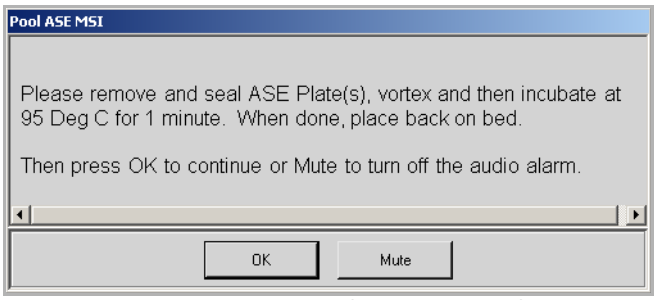


Figure 37 First Remove and Vortex ASE Plate Message

4. Secure each plate to the high-speed shaker with the clampshell lid. If you are vortexing multiple plates, position them so that the high-speed shaker is balanced.

CAUTION Prevent splashing! Each vortexer in your lab should carry a unique label indicating its calibration variance for all recommended settings. Before vortexing, set the digital display value according to the appropriate values indicated on the label.

CAUTION Illumina recommends using the raised bar magnetic plate to manually loosen the beads before vortexing.

5. Vortex each sealed plate at 2000 rpm for 1 minute, or until resuspended.
6. If the wells splashed do the following:
   a. Pulse centrifuge each ASE plate to 280 xg for 1 minute.
   b. Use the raised bar magnetic plate to manually loosen the beads.
   c. Vortex each sealed plate at 2000 rpm for 1 minute. If the wells splash again lower the rpm by 50 and vortex again.

   It is important that beads in all wells are resuspended prior to moving on to step 7. If you are having trouble with beads resuspending after trying step 6, use the following steps to resuspend the beads:
   a. Pulse centrifuge each ASE plate to 280 xg for one minute.
   b. Incubate each sealed plate at 95°C for 1 minute.
   c. Vortex each plate at 1700 rpm for 1 minute.
   d. Pulse centrifuge each ASE plate to 280 xg for one minute.
   e. Skip step 7 and immediately proceed to step 8.
7. Incubate each plate at 95°C for 1 minute. Pulse centrifuge each ASE plate to 280 xg for 1 minute.
8. Remove the seal and return each ASE plate to the correct position on the robot bed according to the bed map and then click **OK** in the message box.

   The Robot software will wait for all of the beads to settle. The robot will automatically continue when the beads have settled.

Illumina LIMS requires that each plate be placed back into its original position on the robot bed.

9. Click **OK** when the database successfully updates the barcode information.
10. Discard used and unused reagents in accordance with facility requirements.
11. Do one of the following:
    - Proceed to *Make PCR MSI Plate* on page 52.
    - Store the APP plate at -20°C.

Make PCR MSI Plate

In this process, the user adds 64 µl of an Illumina-recommended DNA polymerase and 50 µl of Uracil DNA Glycosylase (UDG) to each master mix for PCR (the MMP reagent) tube. The robot adds 30 µl of MMP reagent per well to the PCR plate and then transfers 30 µl of samples from each well of the APP plate to the PCR plate. The PCR master mix contains buffer components, dNTPs, and PCR primers. Fill in the lab tracking form as you work.

Estimated Time

Robot: ~10 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
MMP reagent	1 tube per PCR plate	-20°C	Illumina
Uracil DNA Glycosylase	50 µl per PCR plate	-20°C	Illumina
DNA Polymerase	64 µl per PCR plate	-20°C	User
96-well 0.2 ml microplate (TCY)	1 per APP plate		User

Preparation

- Thaw the MMP reagent to room temperature. Invert 10 times to mix.
- If the APP plate was frozen, thaw to room temperature for 30 minutes, vortex, and then pulse centrifuge to 280 xg for 1 minute.
- Make sure the heat sealer is preheated.
- Apply a PCR barcode label to each new 96-well TCY microplate (1 per APP plate).
- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps Set Up the Robot

1. From the robot PC, select **Universal MSI PrePCR | Make PCR MSI** (Figure 38).

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

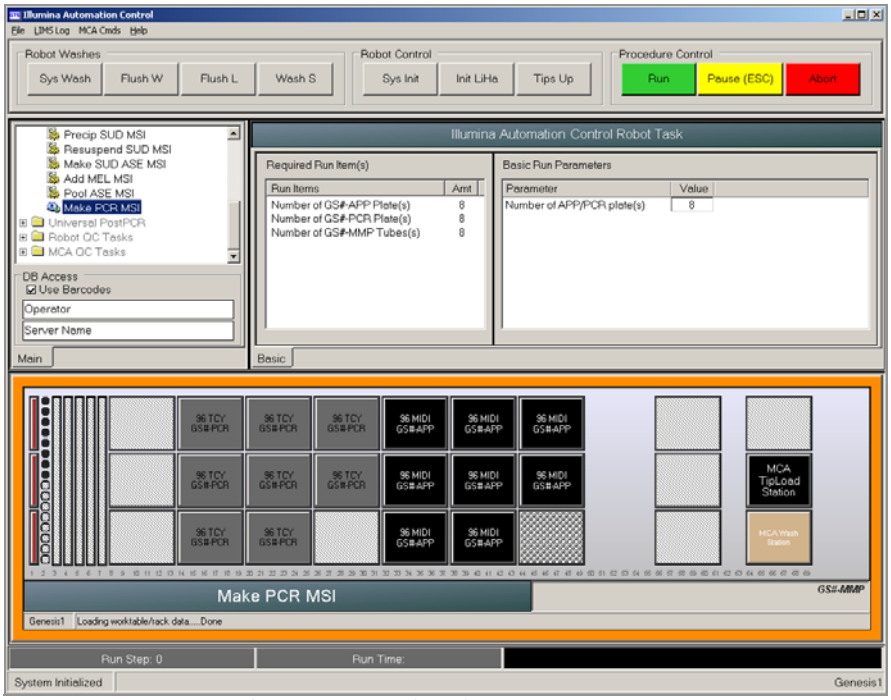


Figure 38 Universal MSI PrePCR | Make PCR MSI

2. On the lab tracking form, check off **Add Enzyme and UDG**.
3. Add 64 µl DNA polymerase to each MMP tube.
4. Add 50 µl Uracil DNA Glycosylase (UDG) to each MMP tube.
5. Cap each MMP tube, vortex, and then centrifuge to 280 xg for 1 minute to mix the contents.

Do not freeze the MMP tube after adding the DNA polymerase enzyme.

6. Log in to Illumina LIMS.
   a. Select **Universal MSI PrePCR | Add Enzyme MSI** (Figure 39).

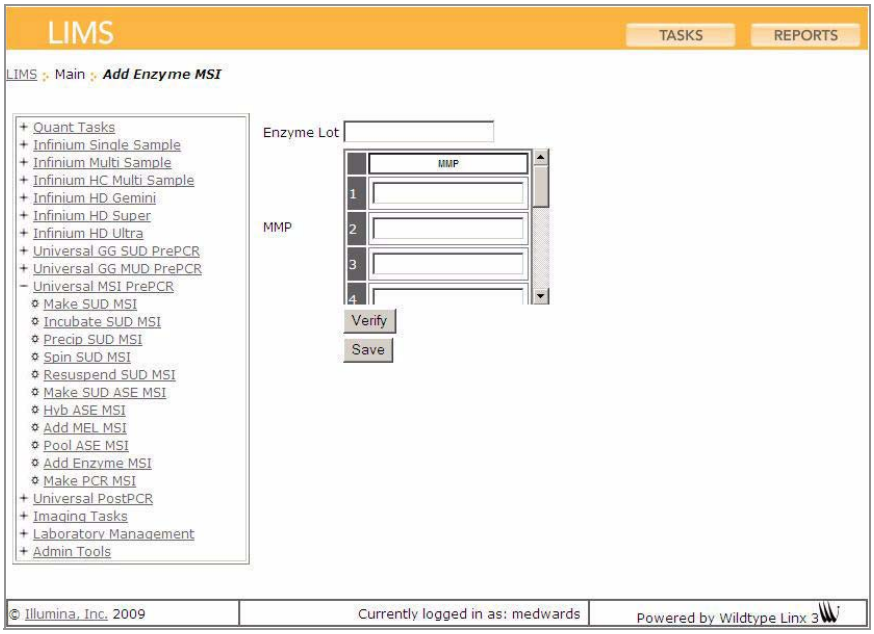


Figure 39 Illumina LIMS Add Enzyme MSI

   b. Enter the lot number of the DNA polymerase in the **Enzyme Lot** box.
   c. Scan each MMP barcode into one of the boxes.
   d. Click **Verify**, then **Save**.

7. Place each MMP tube in the robot tube rack according to the robot bed map. Remove the cap.

8. Place each PCR and APP plate on the robot bed according to the robot bed map. Remove any plate seals.

9. In the Basic Run Parameters pane, change the value for **Number of APP/PCR plate(s)** to reflect the number of plates being processed.

10. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

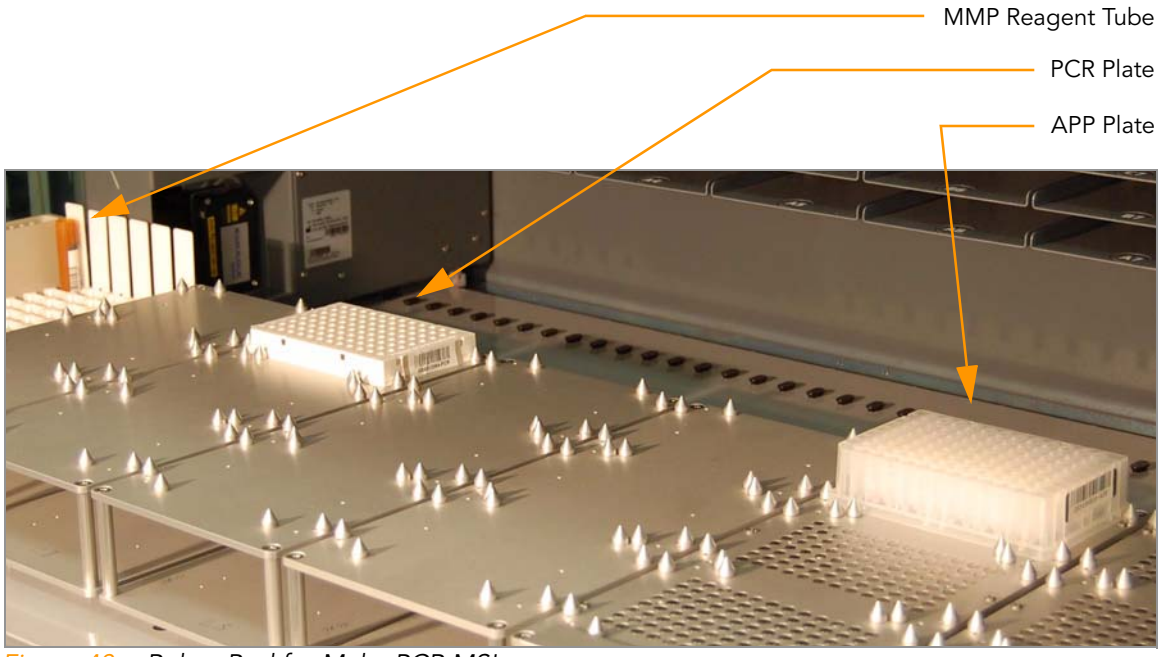


Figure 40 Robot Bed for Make PCR MSI

11. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
2. Observe the robot start to run to ensure that there are no problems.

   The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. Seal each PCR plate with peelable foil using a heat sealer or seal with a cap mat.
2. Discard used and unused reagents in accordance with facility requirements.
3. Do one of the following:
   - Proceed to *Cycle the PCR Plate* on page 56.
   - Place each sealed PCR plate in a vacuum-sealed autobag and store it at -20°C for up to 30 days.

Cycle the PCR Plate

This process thermal cycles the PCR plate to fluorescently label and amplify the templates generated in the pre-PCR process. Three universal primers are included in the reaction mix:

- Cy3-labelled complement to allele-specific oligo (ASO) 1
- Cy5-labelled complement to ASO 2
- Biotinylated complement to locus-specific oligo (LSO)

The fluor-labelled primers provide the signal for detection, while the biotinylated primer allows for immobilization of the PCR product. The result is highly amplified double-stranded PCR products containing a fluor-labelled strand (Cy3 or Cy5, depending on the genotype) and a biotinylated strand. If using the Thermocycler Control Software (TCCS) option package, refer to the Thermocycler Control Software User Guide for additional information on using the software with Illumina LIMS. Fill in the lab tracking form as you work.

Estimated Time

Thermolcycler: ~2 hours 45 minutes

Steps

1. Turn on the Thermocycler. (TCCS only) For Tetrad 2, make sure it is operating in auto-remote mode.
2. (TCCS only) Turn on the PC and start the Thermocycler Control Software.
3. (TCCS only) Make sure the LIMS Mode box is checked and log in to Illumina LIMS.
4. (TCCS only) Enter the name of the thermocycler, the block number, and the PCR plate barcode in the Barcode Entry fields. Click **OK**.

   The PCR plate is verified in LIMS and passed to the correct thermocycler block.
5. Place the sealed plate into the Thermolcycler and run the program shown in this table.

Illumina LIMS will already select the correct program from the Thermocycler when using TCCS.

Table 5 Thermocycler Program

	Temperature	Time
	37°C	10 minutes
	95°C	3 minutes

Table 5 Thermocycler Program

	Temperature	Time
X 34	95°C	35 seconds
	56°C	35 seconds
	72°C	2 minutes
	72°C	10 minutes
	4°C	5 minutes

6. (TCCS only) Press **Run**, or **Run All** if you are starting multiple plates in one transaction. The TCCS is now available to start other protocols in the remaining available blocks.
7. (TCCS only) When complete, the TCCS will automatically save the PCR info to Illumina LIMS. Click the **Stop** button to terminate the thermocycler program.
8. (Non-TCCS only) Log in to Illumina LIMS.
   a. Select **Universal PostPCR | Cycle PCR** (Figure 41).

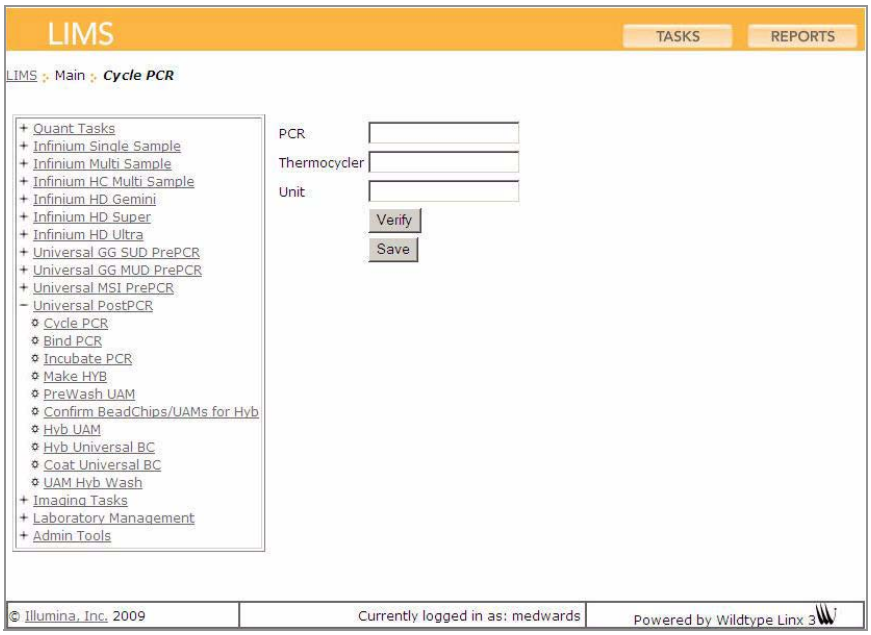


Figure 41 Illumina LIMS Cycle PCR

   b. Scan each PCR plate barcode into one of the boxes.
   c. Enter the Thermocycler and Unit numbers.
   d. Click **Verify**, then **Save**.
9. On the lab tracking form, check off **Cycle PCR**.
10. Do one of the following:
    - Proceed immediately to *Bind PCR Products* on page 59.

- Store the sealed plate at room temperature in a light-protected location for processing later that day.
- Store the sealed plate at -20°C for up to 24 hours.

Bind PCR Products

In this process, the robot first adds 30 µl of sec-butanol to the PCR plate. Next the robot adds 20 µl of the MPB reagent to the PCR plate. The plate is incubated for one hour at room temperature to bind the biotinylated strand to paramagnetic particles, thus immobilizing the double-stranded PCR products. Fill in the lab tracking form as you work.

Estimated Time

Robot: 30 minutes per plate

Incubation: 60 minutes

Consumables

Item	Quantity	Storage	Supplied By
MPB reagent	1 tube per PCR plate	4°C	Illumina
Sec-butanol	5 ml per PCR plate	Room temperature	User

This protocol involves the use of sec-butanol. Sec-butanol is flammable. Sec-butanol is an irritant to the eyes, respiratory system, and skin. Serious damage can occur with contact to the eyes. For more information, contact Illumina Technical Support and ask for the MSDS for this assay.

Preparation

- Bring the MPB reagent to room temperature.
- If frozen, thaw each PCR plate at room temperature for at least 15 minutes in a light-protected location.
- On the lab tracking form, record:
  - Operator
  - Robot
  - Date

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps

Set Up the Robot

1. From the robot PC, select **Universal PostPCR | Bind PCR** (Figure 42).
2. The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

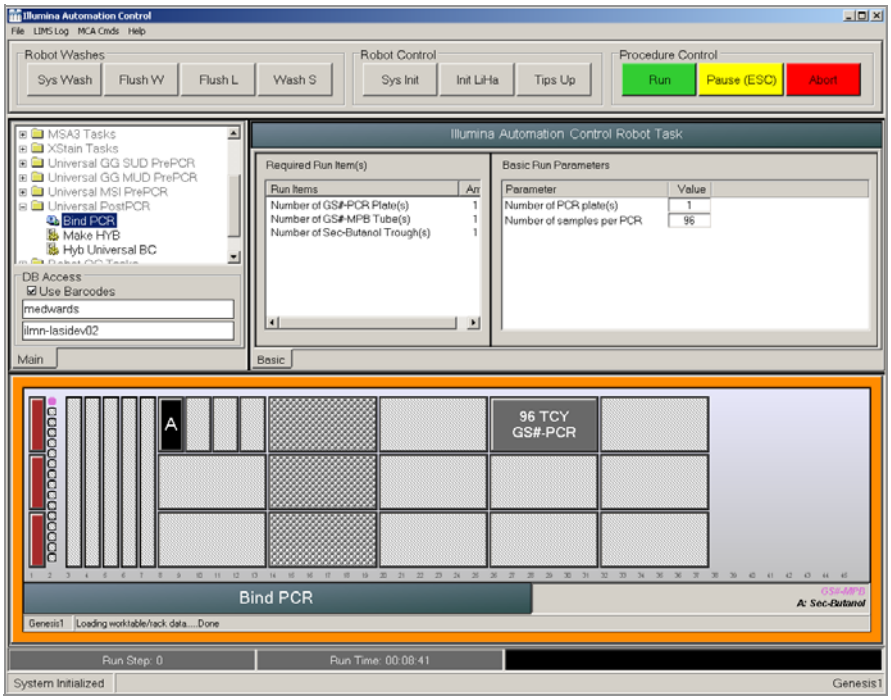


Figure 42 Universal PostPCR | Bind PCR

3. Vortex each MPB tube at tube vortexer for 30 seconds or until the beads are resuspended. Invert the tube to make sure that all of the beads are resuspended.
4. Place each MPB tube in the robot tube rack according to the robot bed map. Remove the cap.
5. Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense sec-butanol as follows:
   - 1 PCR plate: 5 ml
   - 2 PCR plates: 10 ml
   - 3 PCR plates: 15 ml
6. Centrifuge each PCR plate to 1000 xg for 30 seconds.
7. In the Basic Run Parameters pane, change the value for **Number of PCR plate(s)** to reflect the number of PCR plates being processed.
8. Place each PCR plate on the robot bed according to the robot bed map. Remove any plate seals.
9. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed. Check off **Cycle PCR**.

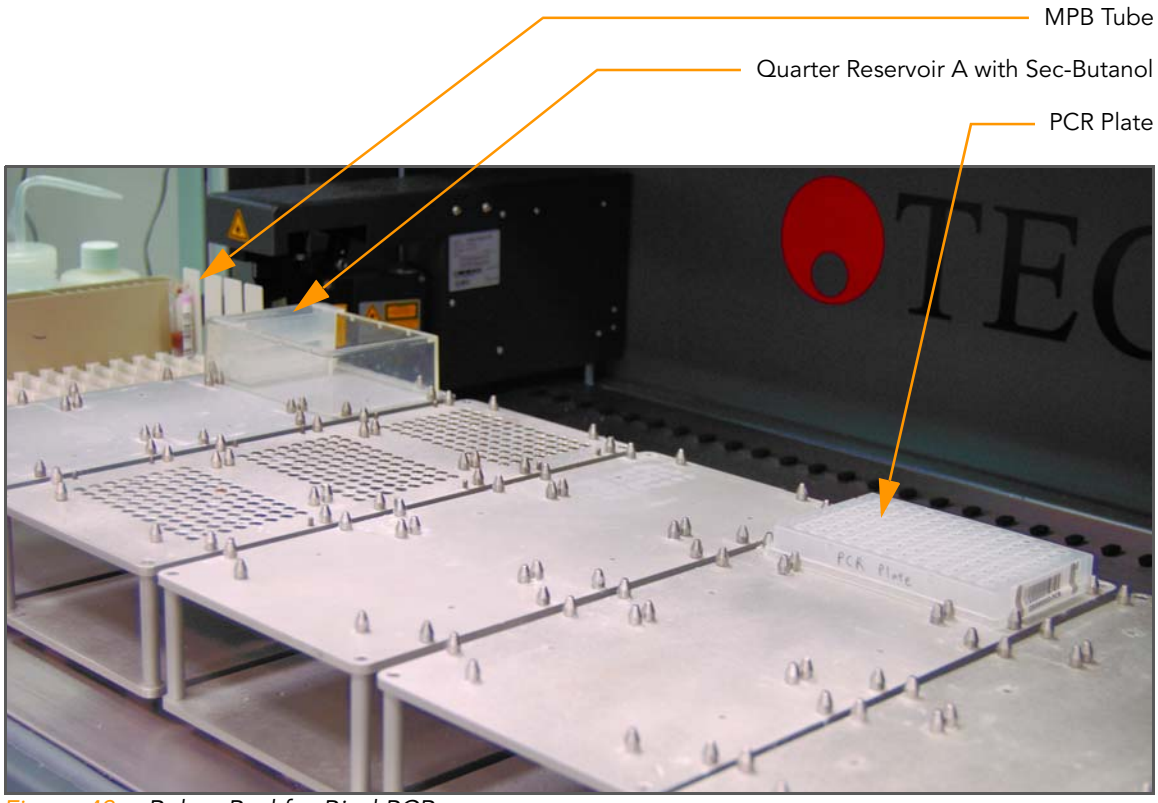


Figure 43 Robot Bed for Bind PCR

10. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.
2. Observe the robot start to run to ensure that there are no problems.

NOTE

If you pause the robot for any reason, remove the MPB tube from the rack, re-vortex it, and return it to the rack in its original position before restarting the robot.

The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. When the robot finishes, seal each PCR plate with adhesive film.
2. Incubate each PCR plate in a light-protected location for 60 minutes.

3. Log in to Illumina LIMS.
   a. Select **Universal PostPCR | Incubate PCR** (Figure 44).

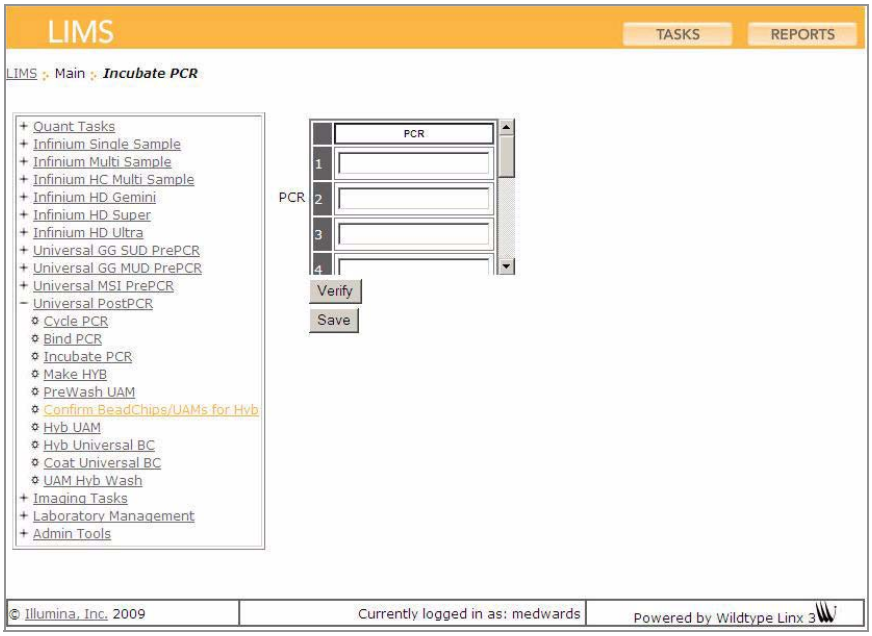


Figure 44 Illumina LIMS Incubate PCR

   b. Scan each PCR plate barcode into one of the boxes.
   c. Click **Verify**, then **Save**.

4. On the lab tracking form, check off **Incubate PCR**.

5. Discard used and unused reagents in accordance with facility requirements.

6. Do one of the following:
   - If you are processing the Universal BeadChip, proceed to *Make HYB Plate (Universal BeadChip)* on page 63.
   - Store each sealed PCR plate at 4°C for up to 4 hours.

Make HYB Plate (Universal BeadChip)

In this process, the robot transfers the single-stranded, fluor-labelled PCR product from the PCR plate to a new HYB plate that is suitable for pairing with a Universal BeadChip.

The PCR plate is placed on a magnetic carrier on the robot, where it is washed with 50 µl of UB2 reagent to remove all previous reagents (PCR master mix, sec-butanol, and unbound PCR products). Next, the fluor-labeled strands are denatured with 30 µl NaOH from the bound biotinylated strands and transferred to a clear Cliniplate 384-well microplate (the HYB plate).

Estimated Time

Robot:

- 1 HYB plate: 60 minutes
- 2 HYB plates: 120 minutes
- 3 HYB plates: 180 minutes

Consumables

Item	Quantity	Storage	Supplied By
MH1 reagent	1 tube per HYB plate	Room temperature	Illumina
UB2 reagent	Bottle	Room temperature	Illumina
0.1N NaOH	Bottle	4°C	User
Cliniplate 384-well microplate	1 per PCR plate		User
96-well 0.2 ml microplate (TCY)	1 per PCR plate		User

This protocol involves the use of NaOH. NaOH is corrosive and may be fatal if swallowed. NaOH is harmful if inhaled, and causes burns to any area of contact. NaOH reacts with water, acids, and other chemicals. For more information, contact Illumina Technical Support and ask for the MSDS for this assay.

Preparation

- If you plan to proceed immediately to Hyb Universal BC, then preheat the hybridization oven to 60°C.
- Make sure that all reagents are at room temperature.
- Prepare the robot for use.
- Apply a INT barcode label to each new 96-well TCY microplate.
- Apply a HYB barcode label to each Cliniplate 384-well microplate.
- On the lab tracking form, record:
  - Operator

- Robot
- Date

NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps Set Up the Robot

1. From the robot PC, select **Universal PostPCR | Make HYB** (Figure 45).
2. Click **OK** to confirm you are using the correct hyb plate type.
3. The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

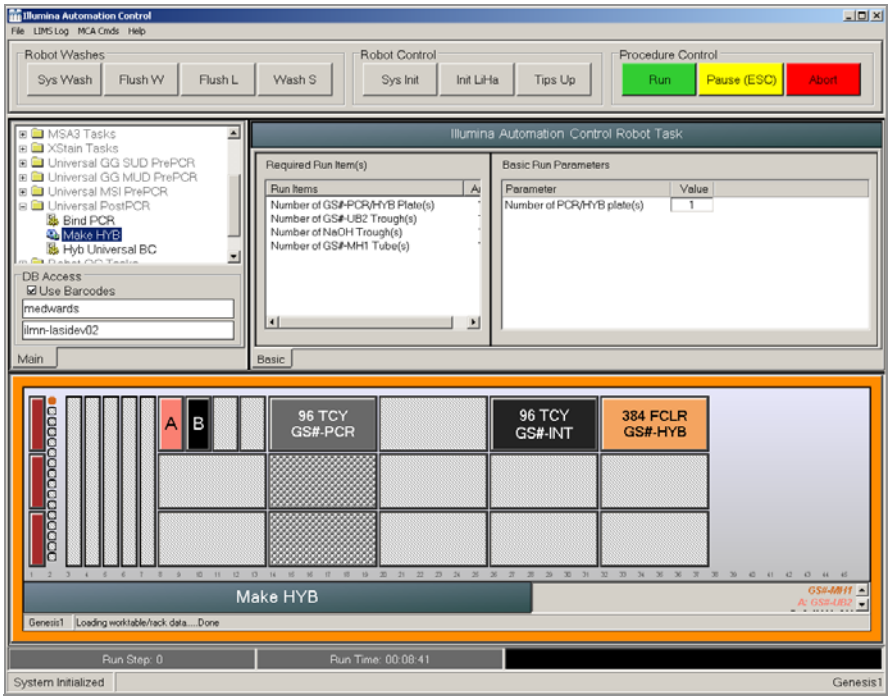


Figure 45 Universal PostPCR | Make HYB

4. Pulse centrifuge each MH1 tube to 280 xg.
5. Place quarter reservoir A and B onto the robot bed according to the robot bed map. Dispense UB2 into reservoir A as follows:
   - 1 HYB plate: 10 ml
   - 2 HYB plates: 15 ml
   - 3 HYB plates: 22 ml
6. Dispense 0.1N NaOH into reservoir B as follows:
   - 1 HYB plate: 10 ml

- 2 HYB plates: 15 ml
- 3 HYB plates: 20 ml

7. In the Basic Run Parameters pane, change the value for **Number of PCR/ HYB plate(s)** to reflect the number of plates being processed.
8. Place each MH1 tube in the robot tube rack according to the robot bed map. Remove the cap.
9. Place the PCR, INT, and HYB plates on the robot bed according to the robot bed map. Remove any plate seals.
10. On the lab tracking form, record the plate and reagent barcodes and their positions on the robot bed.

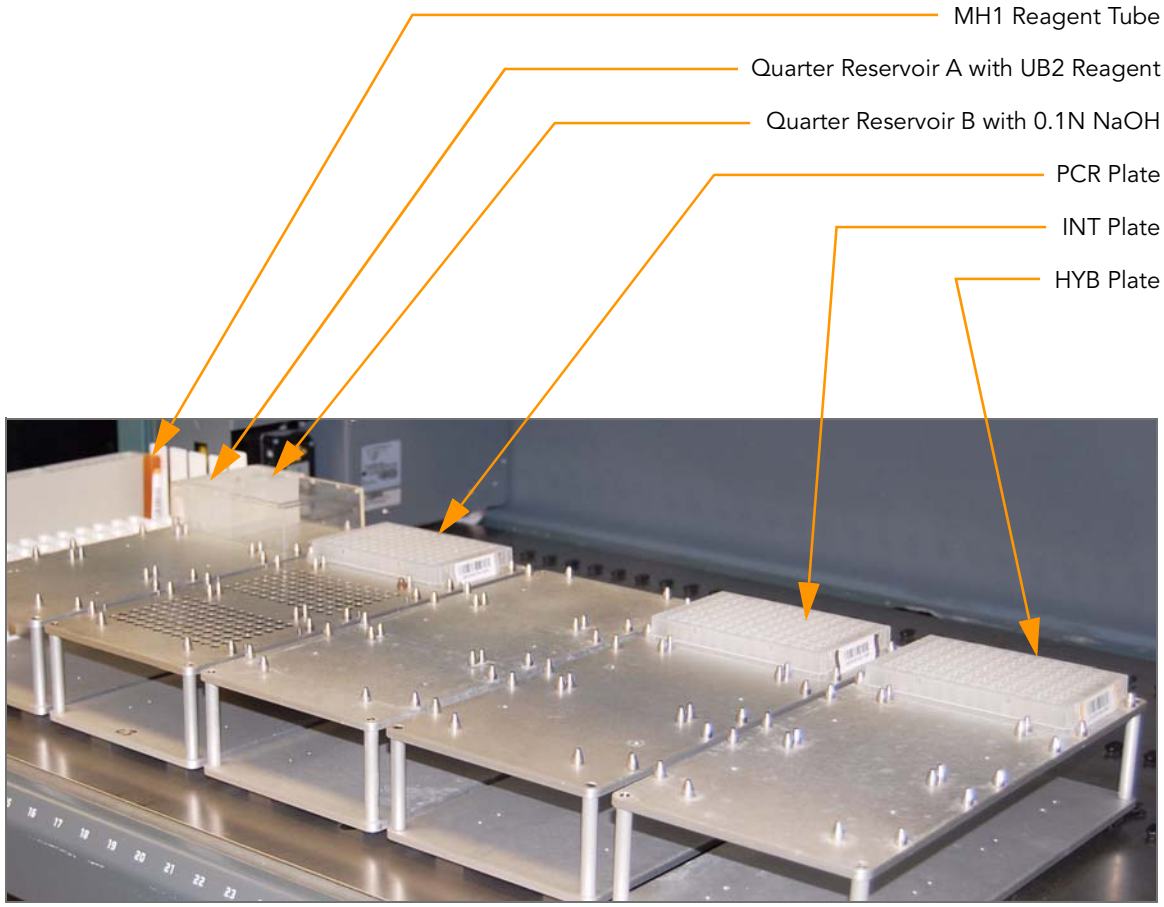


Figure 46 Robot Bed for Make Hyb

11. Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted.

b. When prompted, hand-scan the UB2 barcode at the robot PC (Figure 47).

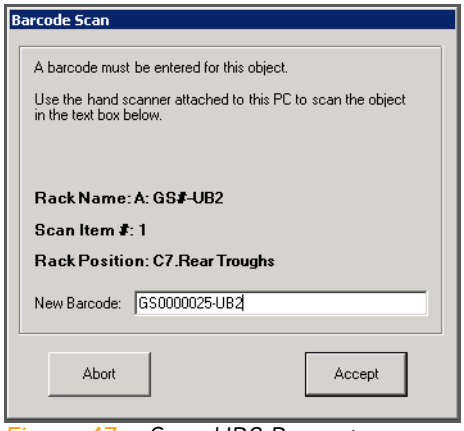


Figure 47 Scan UB2 Prompt

The robot PC sounds an alert and displays a message when the process is complete.

Complete the Protocol

1. Remove each HYB plate from the robot bed and seal with clear adhesive film. Protect the plates from light.
2. Centrifuge each HYB plate at 1000 xg for 4 minutes to remove bubbles.
3. Discard used and unused reagents in accordance with facility requirements.
4. Immediately proceed to *Hybridize Universal BeadChip* on page 67.

Hybridize Universal BeadChip

In this process, 12 µl of sample are hybridized to each sample section of the Universal-32 BeadChip using the Illumina Hyb Chamber and a hybridization oven. After the Hyb Chamber has been assembled, the samples are ready for hybridization. The BeadChip is hybridized overnight in the Illumina Hybridization Oven, with a temperature ramp from 60°C to 45°C. Fill in the lab tracking form as you work.

Estimated Time

Robot time: ~15 minutes per plate

Incubation time: One 30 minute incubation, one 16–18 hour incubation

Consumables and Equipment

Item	Quantity	Storage	Supplied By
CHB reagent	1 tube per Hyb Chamber	Room temperature	Illumina
XC4 reagent	Bottle	Room temperature	Illumina
100% EtOH	Bottle	Room temperature	User
Hyb Chamber	1 per 4 BeadChips		Illumina
Hyb Chamber inserts	1 per BeadChip		Illumina
Hyb Chamber gaskets	1 per Hyb Chamber		Illumina
Robot BeadChip Alignment Fixtures (three BeadChip version)	1 per 96 samples		Illumina
Robot Tip Alignment Guide (Guide-C)	1 per 96 samples		Illumina
BeadChips (32x1)	3 per 96 samples	2°C to 8°C	Illumina

Preparation

- If you plan to perform the Wash protocol the next day, begin thawing the XC4 reagent on a rocker. For instructions, see *Resuspend XC4 Reagent for Washing BeadChip* on page 78.
- Preheat the Illumina Hybridization Oven to 60°C. Allow 30 minutes for it to equilibrate.

For more information about the Illumina Hybridization Oven, see the *Hybridization Oven System Guide* provided with the instrument or go to http://www.illumina.com/documentation to download it.

- If the HYB plate has been frozen, thaw it completely at room temperature for at least four hours in a light-protected drawer, and then pulse centrifuge it to 250 xg for 1 minute.
- Remove the BeadChips from cold storage (2°–8°C) at least ten minutes before you begin the Hyb process but do not unpackage. Leave them on the benchtop (no longer than 24 hours) in their packages until you are ready to begin.
- Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
- Set the rocker speed to 5 (optional if available).
- For each BeadChip, download the decode content from iCom, from the DMAP download client, or copy the contents of the DVD provided with the BeadChip (if purchased) into the Illumina LIMS Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).

Verify PCR Plate and BeadChips for Hyb

1. In the Illumina LIMS left sidebar, click **Universal PostPCR | Confirm BeadChips for Hyb**.
2. Scan the barcode of the HYB plate.
3. Scan the barcodes of all the BeadChips you plan to hybridize with the plate. You can scan up to 24 BeadChips.

Only scan BeadChips that have been accessioned into the system. The BeadChip type must match the type associated with this batch in Illumina LIMS.

4. Click **Verify**.

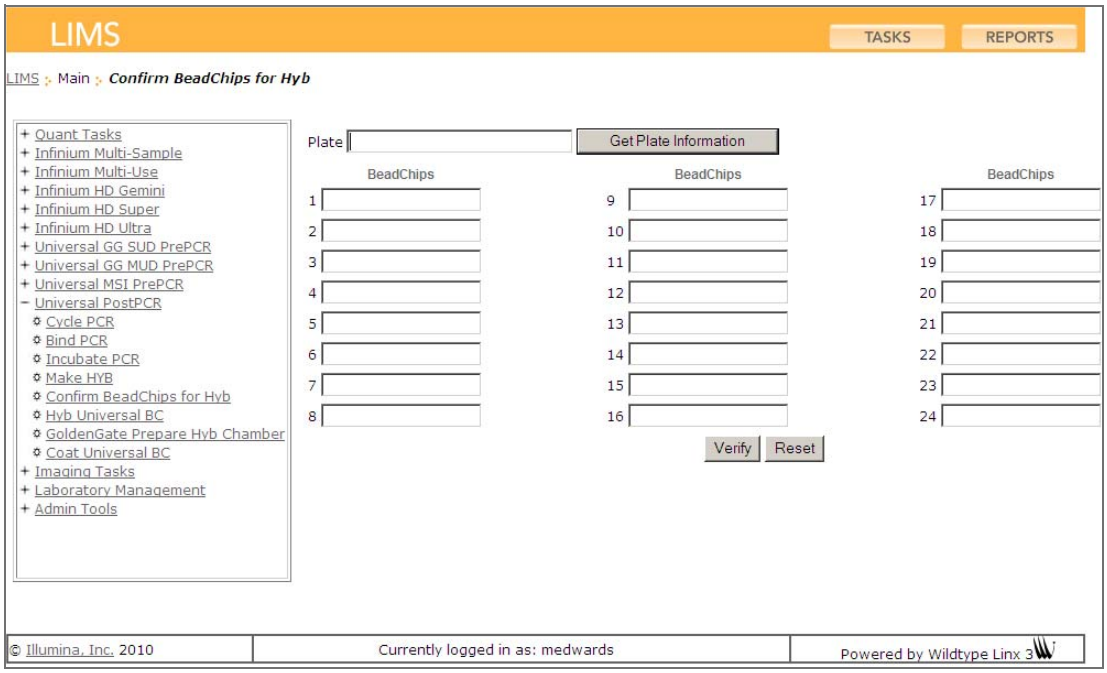


Figure 48 Verifying HYB Plate and BeadChips for Hyb

5. If the HYB plate and BeadChips are queued for hybridization, a blue confirmation message appears at the top of the window. Proceed to loading the BeadChips.

   If the HYB plate is not queued for hybridization, if any of the BeadChips have not been accessioned into the system, or if any of the BeadChips are the wrong type, a red error message appears at the top of the window. The error message indicates the first incorrect barcode it finds. Do *not* proceed with hybridization.

6. If the plate is not queued for hybridization:
   a. Click the Reports tab in the upper-right corner.
   b. In the left sidebar, click **Tracking | Get Queue Status**.
   c. Scan the plate barcode and click **Go**.
   d. If the plate is queued for another step, proceed with that step.

7. If one of the BeadChips is not accessioned into the system, accession it and then repeat the verification step.

8. If one of the BeadChips is not the right type for this batch, accession one that is the right type and repeat the verification step.

9. When the verification is successful, proceed to *Prepare Robot Tip Alignment Guides* on page 70.

Prepare Robot Tip Alignment Guides

1. Wash and dry the Robot Tip Alignment Guides prior to each use. See *Wash Robot Tip Alignment Guides* at the end of the Hyb Universal BC steps (page 79) for washing instructions.
2. Make sure you have the correct Robot Tip Alignment Guide for the GoldenGate assay. The guide barcode should say Guide-C on it (Figure 49).

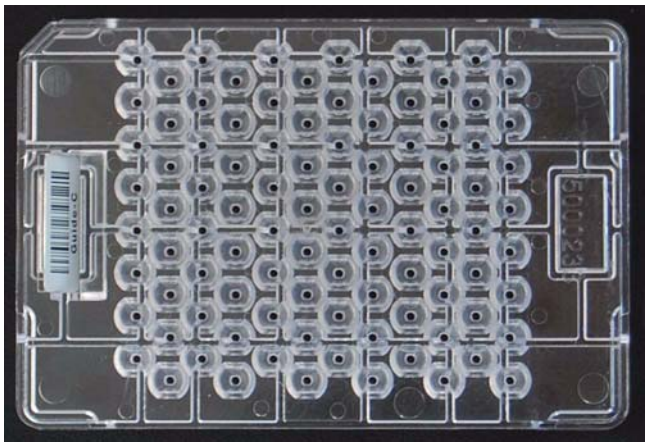


Figure 49 Guide-C Robot Tip Alignment Guide

Steps

This section involves the following procedures:

- *Assemble the Hyb Chambers* on page 70
- *Verify PCR Plate and BeadChips for Hyb* on page 68
- *Load BeadChips* on page 74
- *Start the Robot* on page 75
- *Hybridize BeadChips* on page 78

Assemble the Hyb Chambers

1. Place the following items on the bench top (Figure 50):
   - BeadChip Hyb Chamber (1 per 4 BeadChips)
   - BeadChip Hyb Chamber gasket (1 per Hyb Chamber)
   - BeadChip Hyb Chamber inserts (4 per Hyb Chamber)

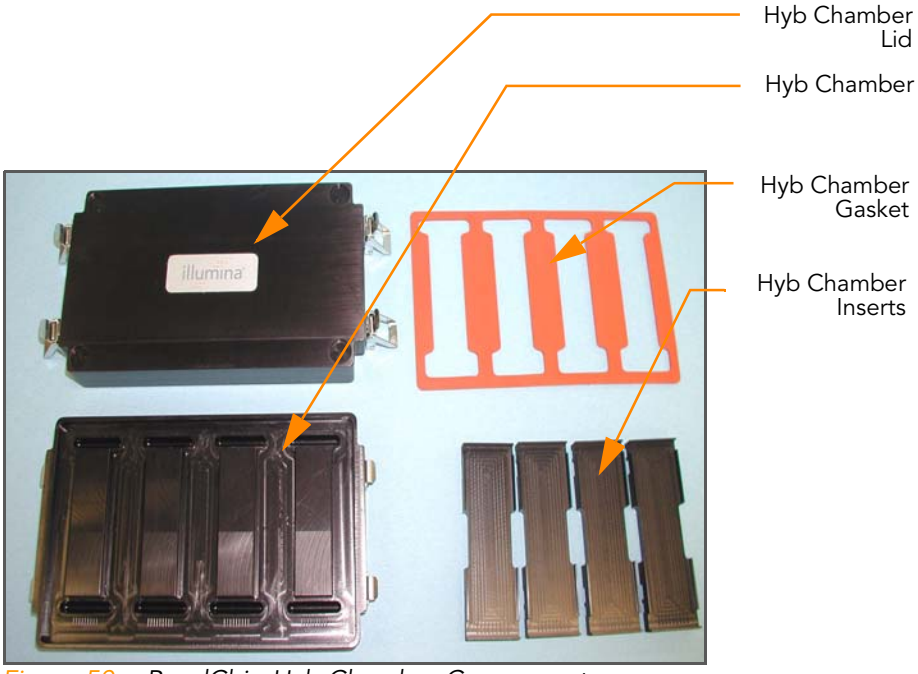


Figure 50 BeadChip Hyb Chamber Components

2. Place the Hyb Chamber Gasket into the Hyb Chamber.
   a. Match the wider edge of the Hyb Chamber gasket to the barcode-ridge side of the Hyb Chamber (Figure 51).

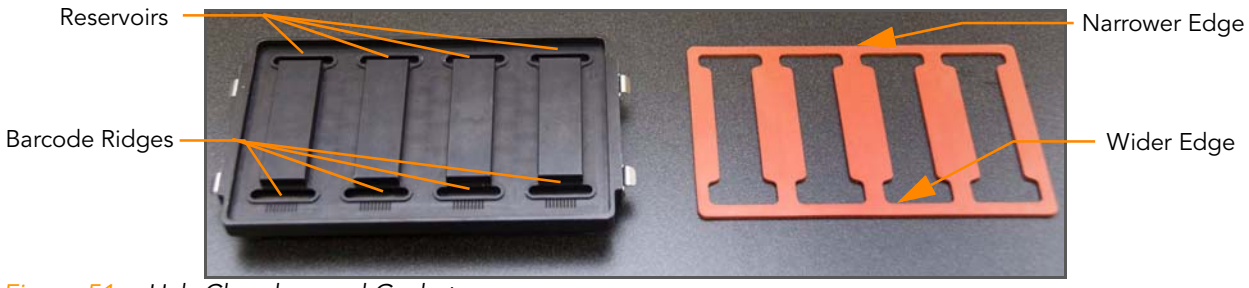


Figure 51 Hyb Chamber and Gasket

   b. Lay the gasket into the Hyb Chamber (Figure 52), and then press it down all around.

Figure 52 Place Gasket into Hyb Chamber

c. Make sure the Hyb Chamber gasket is properly seated (Figure 53).

Figure 53 BeadChip Hyb Chamber with Gasket in Place

3. Add 200 µl CHB into each of the eight humidifying buffer reservoirs in the Hyb Chamber (Figure 54). If you are hybridizing fewer than four BeadChips, only fill the reservoirs of sections that will contain BeadChips.

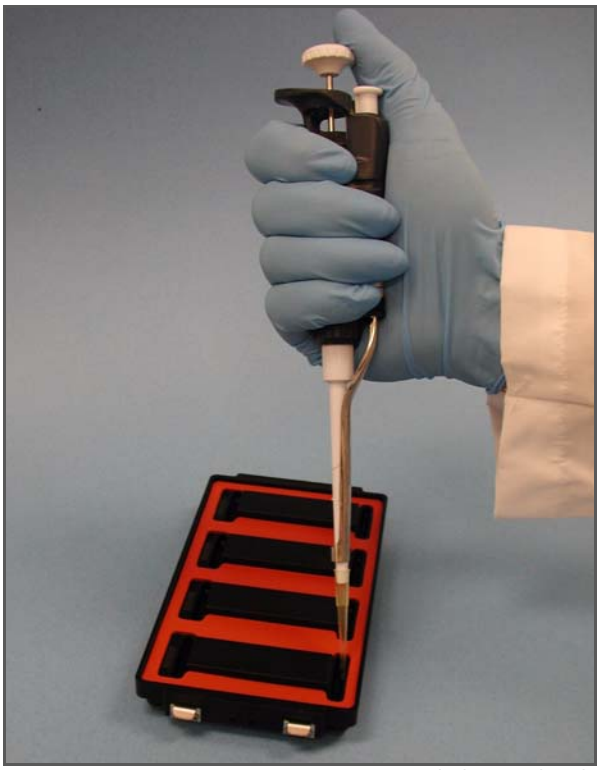


Figure 54 Dispense CHB into BeadChip Hyb Chamber Reservoir

4. Close and lock the BeadChip Hyb Chamber lid (Figure 55).
   a. Seat the lid securely on the bottom plate.
   b. Snap two clamps shut, diagonally across from each other.
   c. Snap the other two clamps.

Figure 55 Seal the Hyb Chamber

5. Leave the closed Hyb Chamber on the bench at room temperature until the BeadChips are loaded with DNA sample.

Steps Load BeadChips

Perform the BeadChip loading near the robot to minimize the distance you need to move the loaded Robot BeadChip Alignment Fixtures.

NOTE

Make sure the Robot Tip Alignment Guide inserts are washed and thoroughly dried before you begin the following steps. See *Wash Robot Tip Alignment Guides* at the end of the *Hyb Universal BC* section.

1. Remove all BeadChips from their packages.
2. Place up to three BeadChips into each vertical Robot BeadChip Alignment Fixture slot (three BeadChip version) with the barcode end aligned to the ridges on the fixture (Figure 56).

Figure 56 Placing Universal-32 BeadChips in Robot BeadChip Alignment Fixture

3. If you did not load the BeadChips near the robot, stack the Robot BeadChip Alignment Fixtures (Figure 57) and carry them to the robot.

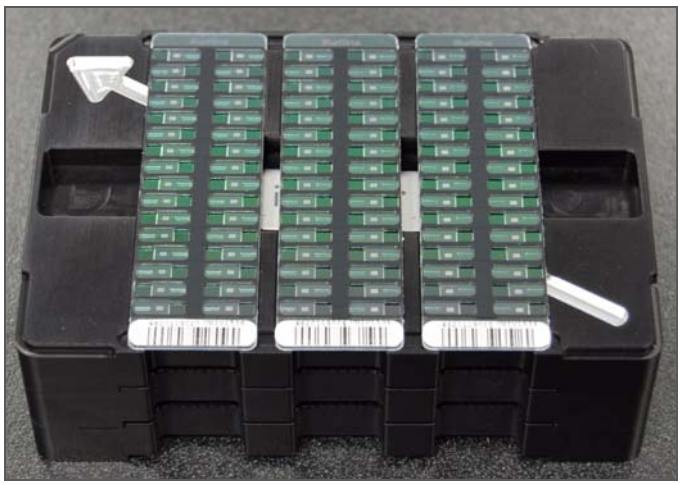


Figure 57 Stacked Robot BeadChip Alignment Fixtures

4. From the robot PC, select **Universal PostPCR | Hyb Universal BC** (Figure 58).

5. Choose **96 Plate** from the **Hyb Universal BC - Hyb Plate Selection** dialog box.
6. Choose the appropriate BeadChip type from the dialog box.
7. In the Basic Run Parameters pane, change the value for **Number of HYB plates** to reflect the number of DNA samples being processed per plate.

   The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

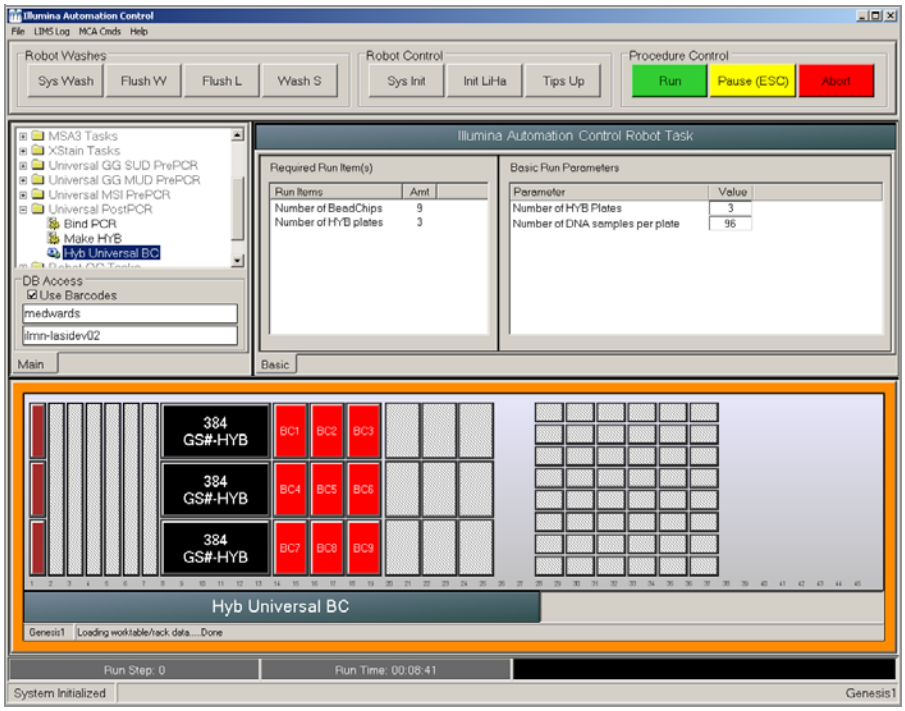


Figure 58 Universal PostPCR | Hyb Universal BC Screen

8. Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map (Figure 58). Push the Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed. Make sure the silver arrow is pointing to the top left when you place the Robot BeadChip Alignment Fixture on the Robot bed.
9. On the lab tracking form, record the plate position on the robot bed.

Start the Robot

1. Make sure the **Use Barcodes** check box is selected and click **Run**.
   a. Log in to Illumina LIMS when prompted. The robot scans the barcodes on the HYB plate(s) and BeadChips to confirm the correct BeadChips are loaded. Once the correct BeadChips are confirmed, the robot pauses.

b. Place the Guide-C Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture (Figure 59). The Guide-C barcode should be on the left side of the plate. Push both the Robot Tip Alignment Guide and Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed. See *Prepare Robot Tip Alignment Guides* on page 70 for the correct Robot Tip Alignment Guide preparation instructions.

c. At the robot PC, click **OK** to confirm you have placed the Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture.

Figure 59 Full Set of Robot Tip Alignment Guides on Robot Bed

d. The robot scans the barcode on the Robot Tip Alignment Guide to confirm the correct tip guide is being used.

NOTE

In the rare occasion the robot scanner fails to read a barcode, use the hand scanner attached to the PC to manually scan the barcode.

The robot dispenses the sample onto the BeadChips, following the lay-out shown in the lab tracking form. The robot PC sounds an alert and displays a message when the process is complete.

Click **OK** in the message box.

2. Carefully remove each Robot Tip Alignment Guide from the robot bed and set it to the side. Next, carefully remove the Robot BeadChip Alignment Fixtures from the robot bed and visually inspect all sections of the BeadChips. Ensure DNA sample covers all of the sections of each bead stripe. Record any sections that are not completely covered.

NOTE

For optimal performance, the Robot Tip Alignment Guide should be washed and dried after every run. See *Wash Robot Tip Alignment Guides* at the end of this section for wash instructions.

3. Seal and store the HYB plate at -20°C.

4. To ensure optimal performance, immediately continue on to the *Set Up Universal BeadChip for Hyb*.

Set Up Universal BeadChip for Hyb

1. Ensure the Illumina Hybridization Oven is set to 60°C.
2. Open the Hyb Chamber.
3. Load up to four Hyb Chamber Inserts containing sample-laden BeadChips into each Hyb Chamber (Figure 60).

When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.

Figure 60 Universal-32 BeadChips in BeadChip Hyb Chamber

4. Position the barcode end over the ridges indicated on the Hyb Chamber and ensure the inserts are securely seated.

For optimal performance, keep the BeadChips steady and level when lifting or moving. Avoid shaking and keep parallel to the lab bench at all times. Avoid contacting the sample inlets when handling the BeadChips.

5. In the Illumina LIMS left sidebar, click **Universal PostPCR | GoldenGate Prepare Hyb Chamber**.
   a. Scan the barcode(s) of the CHB tube(s) and scan the BeadChip barcodes. Click **Verify** and then **Save**.
6. Close and lock the BeadChip Hyb Chamber lid (Figure 61).
   a. Seat the lid securely on the bottom plate.
   b. Snap two clamps shut, diagonally across from each other.
   c. Snap the other two clamps.

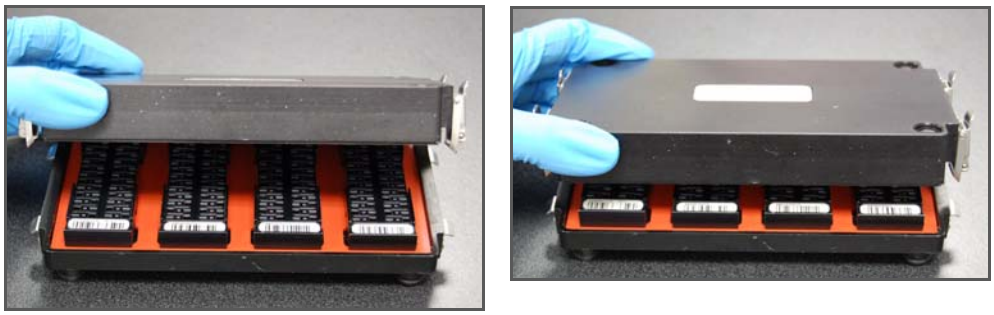


Figure 61 Secure Hyb Chamber Lid for Universal-32 BeadChip

CAUTION

For optimal performance, keep the Hyb Chamber steady and level when lifting or moving. Avoid shaking the Hyb Chamber, and keep the Hyb Chamber parallel to the lab bench while you transfer it to the Illumina Hybridization Oven.

Hybridize BeadChips

1. Place the Hyb Chamber into the 60°C Illumina Hybridization Oven so that the clamps face the left and right sides of the oven. The Illumina logo on top of the Hyb Chamber should face you.
2. (Optional) Start the rocker at speed 5.
3. Incubate for exactly 30 minutes at 60°C.
4. After 30 minutes, reset the temperature to 45°C.
5. Incubate for at least 16 hours but no more than 18 hours at 45°C.
6. Update the lab tracking form with the start and stop times.
7. Proceed to *Wash & Coat BeadChip* on page 80.

Resuspend XC4 Reagent for Washing BeadChip

The XC4 solution should be thawed and resuspended overnight. Keep it in the bottle in which it was shipped until ready for use. Each XC4 bottle (350 ml) has enough solution to process up to 8 BeadChips. In preparation for the Wash protocol, follow these steps to resuspend the XC4:

1. Add 335 ml 100% EtOH to the XC4 bottle. The final volume will be 350 ml.
2. Shake vigorously for 15 seconds.
3. Leave the bottle upright on the lab bench overnight.
4. Shake again to ensure that the pellet is completely resuspended. The solution should be clear and homogeneous, with no gelatinous or stringy remains. If any coating is visible, vortex at 1625 rpm until it is in complete suspension.

5. Once resuspended with 335 ml 100% EtOH, use XC4 at room temperature. You can store it at 2°C to 8°C overnight.

If the XC4 was not left to resuspend overnight, you can still proceed with the assay. Add the EtOH and put the XC4 on its side on a rocker to resuspend for 30 or 40 minutes. Leave it there until the BeadChips are ready for coating.

Wash Robot Tip Alignment Guides

1. Soak the Robot Tip Alignment Guides in a 1% aqueous Alconox solution (one part Alconox to 99 parts water) using a 400 ml Pyrex beaker for 5 minutes.

Do not use bleach or ethanol to clean the Robot Tip Alignment Guides.

2. After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the Robot Tip Alignment Guide(s) with DiH_2O at least three times to remove any residual detergent. Make sure the DiH_2O runs through all the tip guide channels.
3. Dry the Robot Tip Alignment Guides, especially the channels, using a Kimwipe or lint-free paper towels. Use a laboratory air gun to ensure they are dry. Be sure to inspect the channels, including the top and bottom of the insert. Robot Tip Alignment Guides should be completely dry and free of any residual contaminates before next use.

Wash & Coat BeadChip

In this process, the BeadChips are removed from the Hyb Chamber and washed three times with PB1 and XC4 reagents. There are two separate PB1 washes and one XC4 wash. To process multiple BeadChips in parallel, set up a group of wash stations for each wash rack filled with BeadChips. Start washing subsequent BeadChips while the previous ones are incubating in PB1 or XC4. Fill in the lab tracking form as you work.

Estimated Time

Hands-on time: ~1 hour

Consumables and Equipment

Item	Quantity	Storage	Supplied By
PB1 reagent	Bottle	Room temperature	Illumina
XC4 reagent	Bottle	Room temperature	Illumina
Wash Dish	12 BeadChips: 3 dishes		Illumina
Wash Rack	1 rack		Illumina
Vacuum desiccator	(1 per 12 BeadChips processed simultaneously)		Illumina
Self-locking tweezers	1		Illumina

Preparation

- If you are using the BeadArray Reader, turn it on 1–2 hours before imaging. If this is the first time the BeadArray Reader is being used today, Initialize the BeadArray Reader.
- If you are using the iScan Reader, turn it on five minutes before imaging.
- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates before use.
- In preparation for XC4 BeadChip coating, wash the tube racks and wash dishes thoroughly before and after use. Rinse with DI water. Immediately following wash, place racks and wash dishes upside down on a wash rack to dry.
- Place Kimwipes in three layers on the lab bench. Place a tube rack on top of these Kimwipe layers. Do not place on absorbent lab pads. You will place the wash rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

- If the XC4 has not already been prepared (for instructions, see *Resuspend XC4 Reagent for Washing BeadChip* on page 78), then add 335 ml 100% EtOH to the bottle and place it on a rocker for 30–40 minutes to resuspend. When it is resuspended, fill a wash dish with the XC4 and label the dish "XC4".
- Fill two wash dishes with PB1 (300 ml per wash dish). Label each dish "PB1".

Steps Wash and Coat BeadChips

1. Place the following equipment on the lab bench:
   - 1 wash rack
   - 1 vacuum desiccator
   - 2 tube racks
   - Self-locking tweezers
   - Large Kimwipes
   - Vacuum hose
2. Set up three top-loading wash dishes, labeled as shown (Figure 62).

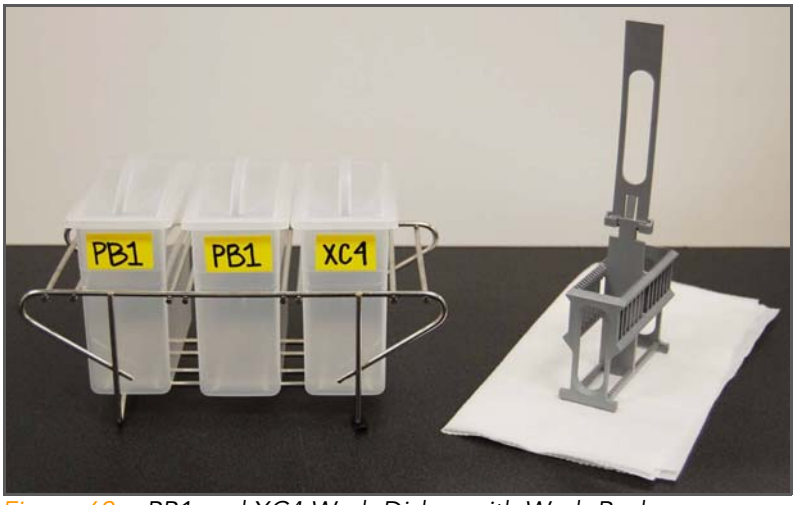


Figure 62 PB1 and XC4 Wash Dishes with Wash Rack

3. Submerge the unloaded wash rack into the first PB1 wash dish with the locking arms facing you. This orients the wash rack so that you can safely remove the BeadChips.

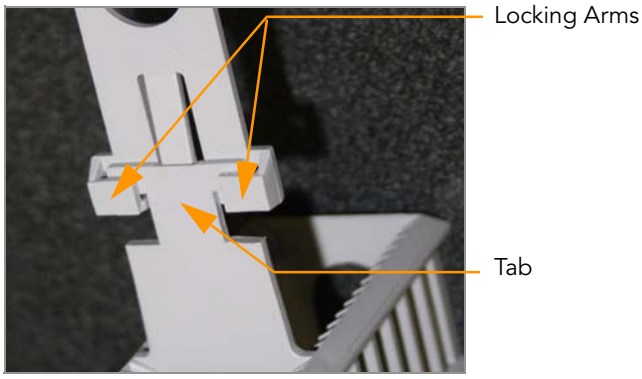


Figure 63 Wash Rack Locking Arms and Tab

CAUTION

If the wash rack handle is not correctly oriented, the BeadChips may be damaged when you remove the wash rack handle.

4. Remove up to 3 Hyb Chambers containing BeadChips from the Hyb oven at one time. Leave any remaining Hyb Chambers in the oven. Do not open all of the Hyb Chambers at once. Only open a Hyb Chamber when you are ready to remove the seals from the BeadChips. If you have more than one Hyb Chamber to process, leave the other Hyb Chambers closed on the bench while you process the first Hyb Chamber.

   No longer than five minutes should elapse from the time the first Hyb Chamber is opened until the last BeadChip is placed in the wash rack and submerged in the first PB1 wash.

   If there are more than 3 Hyb Chambers worth of BeadChips to process, complete the wash and coat step for the first 12 BeadChips, replace the PB1 in the wash dishes with fresh PB1, and follow this same step.

5. With a hand on top of the first Hyb Chamber, un-snap the four clips one at a time. Start with the first clip, then un-snap the clip that is diagonally across from it and so on. Lift the lid straight up and off. Set the lid to the side on the bench.

6. Remove the first BeadChip from a Hyb Chamber insert.

7. Remove the IntelliHyb Seal from the BeadChip (Figure 64):

CAUTION

To ensure no solution splatters on you, Illumina recommends removing the coverseal over an absorbent cloth or paper towels, preferably in a hood.

   a. Wearing powder-free gloves, hold the BeadChip in one hand with your thumb and forefinger on opposing edges of the BeadChip. Do not touch the sample inlets. The barcode should face up and be closest to you, and the top side of the BeadChip should be angled slightly away from you.

b. Remove the entire seal in a single, slow, consistent motion by pulling it off in a diagonal direction. 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 stop and start the pulling action. Do not touch the exposed active areas.

c. Discard the seal.

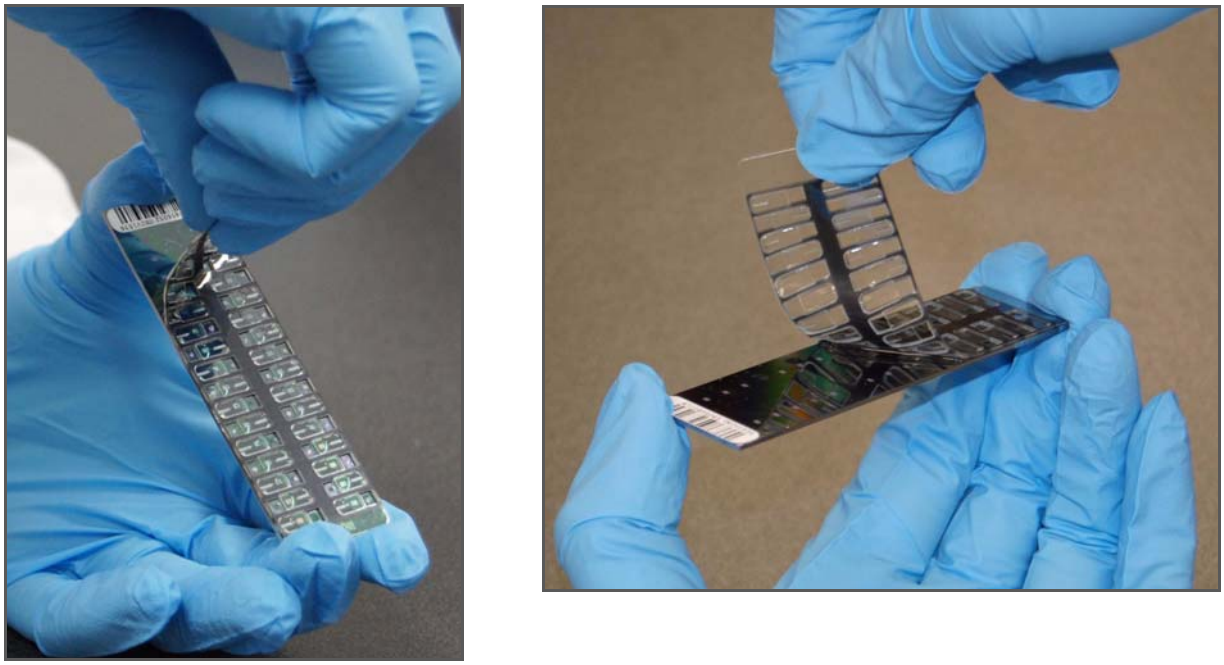


Figure 64 Remove the Coverseal from the BeadChip

8. Holding the BeadChip by the barcode end, immediately and carefully slide the BeadChip into the wash rack while it is submerged in PB1.

   If necessary, briefly lift the wash rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting the BeadChip. Place BeadChips in every other slot.

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

9. Ensure that the BeadChip barcodes are correctly positioned in the wash rack, with the labels facing up and away from you. This is essential for proper handling and coating.

10. Repeat steps 5–8 until all BeadChips are transferred to the submerged wash rack. Load the wash rack with up to 12 BeadChips. Put six BeadChips above the wash rack handle and six below. Try to evenly space the BeadChips in the rack.

11. Once all BeadChips are in the wash rack, move the wash rack up and down ten times, breaking the surface of the PB1 with gentle, slow agitation (Figure 65).

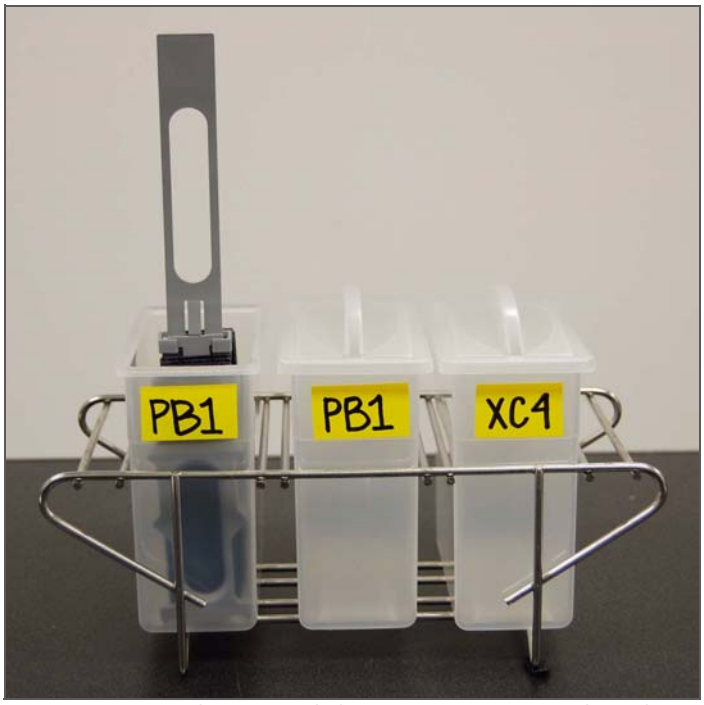


Figure 65 Washing BeadChips in First PB1 Wash Dish

12. Transfer the wash rack to the second PB1 wash dish and let it soak for five minutes (Figure 66).

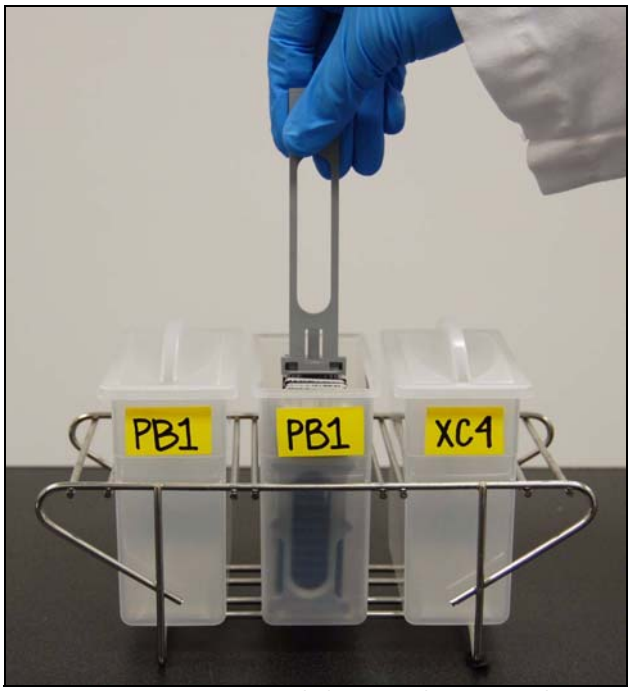


Figure 66 Moving BeadChips to the Second PB1 Wash Dish

13. Transfer the wash rack to the XC4 wash dish and slowly move the wash rack up and down ten times. Let it soak for five minutes. The barcode labels on the BeadChips must face away from you, while the locking arms on the handle face towards you, for proper handling and coating (Figure 67).

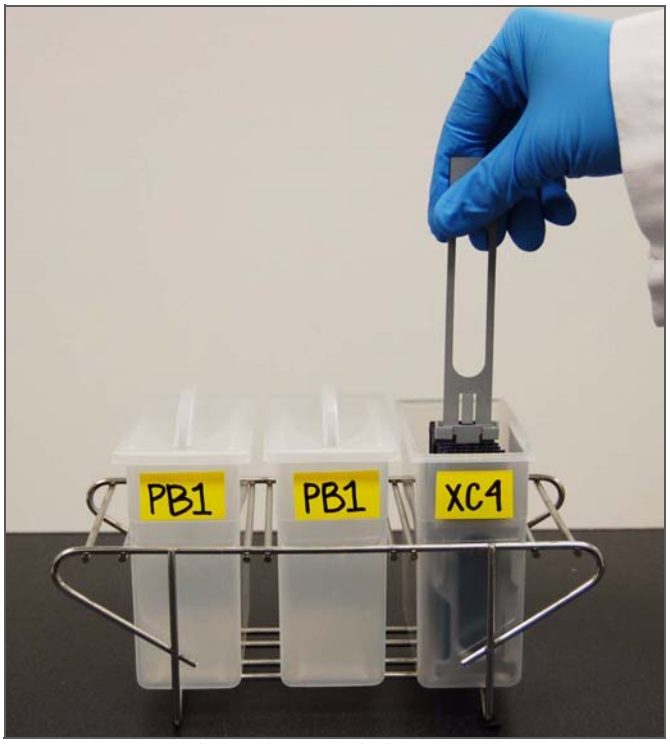


Figure 67 Washing the BeadChips in XC4

NOTE

You can use the XC4 for two sets of 12 BeadChips processed in succession. Use fresh XC4 if you plan to process more than 24 BeadChips in succession.

Dry BeadChips

1. Prepare a clean tube rack for the wash rack by placing two folded Kimwipes under the tube rack.
2. Prepare one additional tube rack per 8 BeadChips that fits the internal dimensions of the vacuum desiccator.

3. Remove the wash rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes face *up* and the locking arms and tab face *down* (Figure 68 and Figure 69).

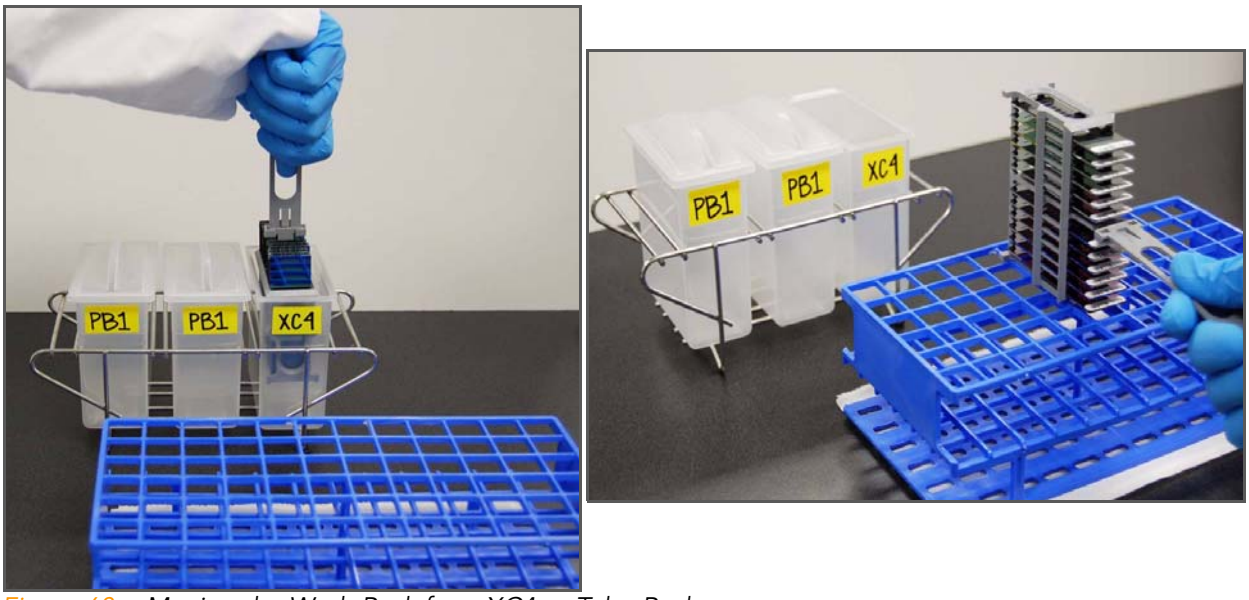


Figure 68 Moving the Wash Rack from XC4 to Tube Rack

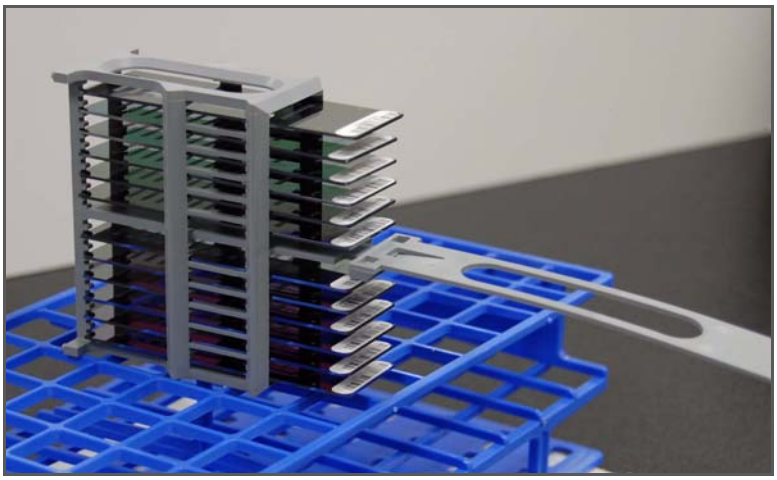


Figure 69 Wash Rack in Correct Orientation

4. For the top four BeadChips, working top to bottom:
   a. Continuing to hold the wash rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

NOTE

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

   b. Place the BeadChip on a tube rack with the barcode facing up and towards you (Figure 71).

5. Holding the top of the wash rack in position, gently remove the wash rack handle by grasping the handle between the thumb and forefinger. Push the tab up with your thumb and push the handle away from you (unlocking the handle), then pull up the handle and remove (Figure 70).

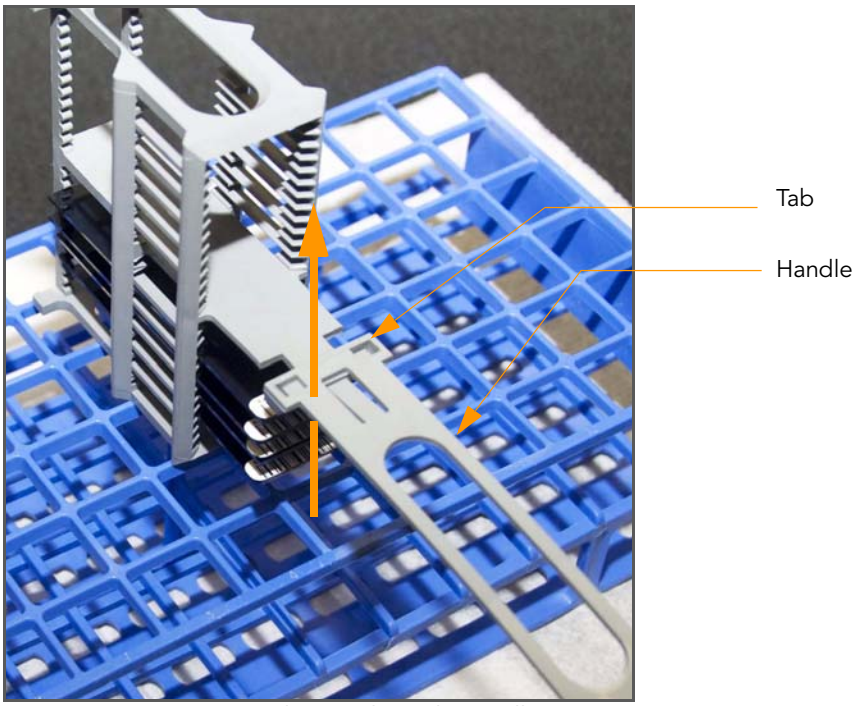


Figure 70 Removing the Wash Rack Handle

6. Place any remaining BeadChips on the tube rack (Figure 71), with six BeadChips on top of the rack and two BeadChips on the bottom. The barcode ends should be towards you, and the BeadChips should be completely horizontal.

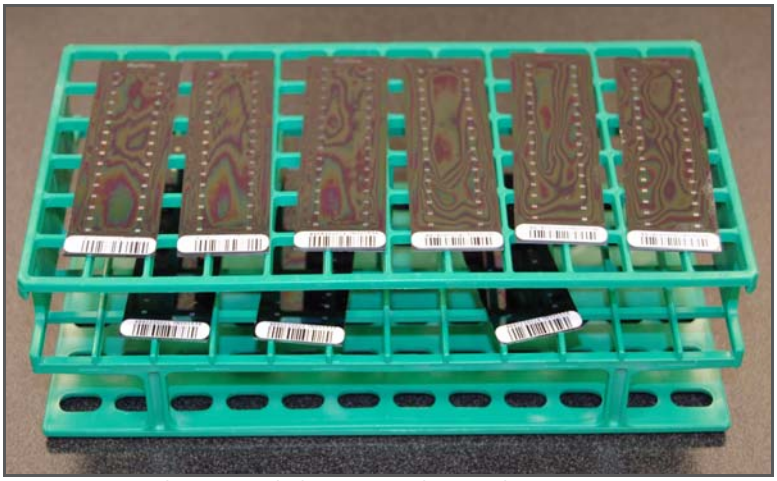


Figure 71 Place BeadChips on Tube Rack

To prevent wicking and uneven drying, do not allow the BeadChips to rest on the edge of the tube rack or to touch each other while drying.

7. Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack (8 BeadChips).
8. Ensure the vacuum valve is seated tightly and securely.
9. Start the vacuum, using at least 508 mm Hg (0.68 bar).
10. To ensure that the dessicator is properly sealed, gently lift the lid of the vacuum desiccator (Figure 72). It should not lift off the desiccator base.

Figure 72 Test Vacuum Seal

11. Dry under vacuum for 50–55 minutes.

    Drying times may vary according to room temperature and humidity.
12. Log in to Illumina LIMS.
    a. In the Illumina LIMS left sidebar, click **Universal PostPCR | Coat Universal BC**.
    b. Scan the barcode(s) of the PB1, XC4, and BeadChips and click **Verify** and then click **Save**.

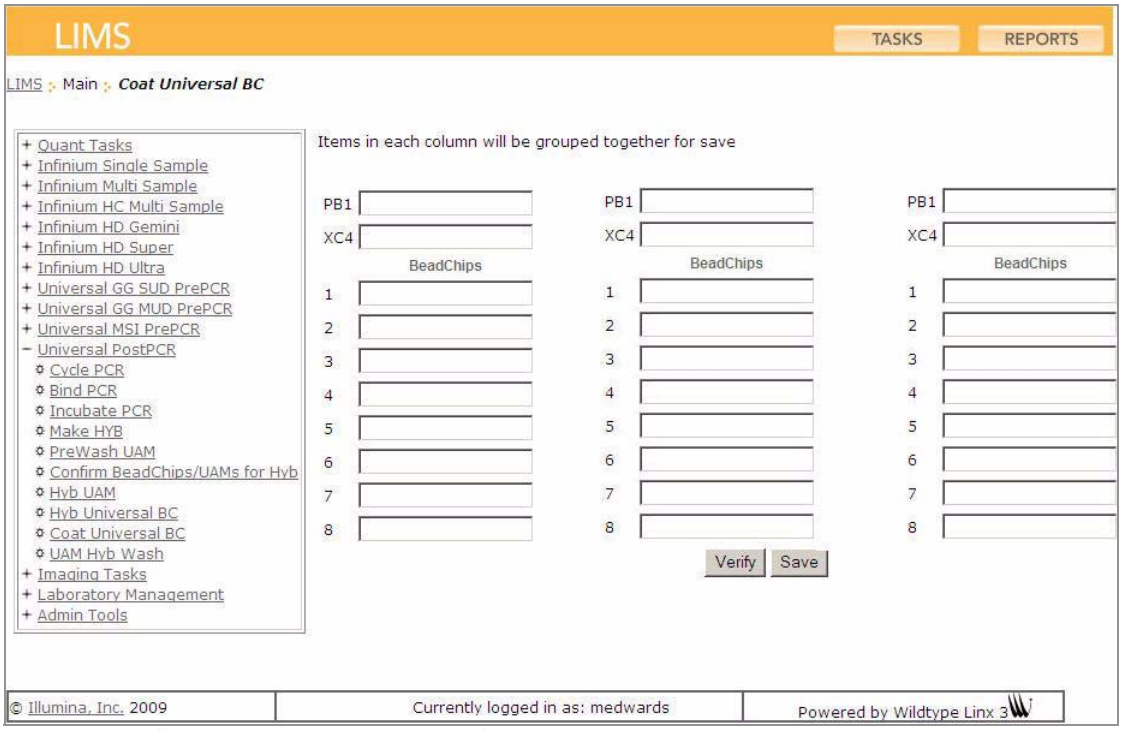


Figure 73 Illumina LIMS Coat Universal BC

13. Release the vacuum by turning the handle very slowly.

WARNING

Air should enter the desiccator very slowly to avoid disturbing the contents. Improper use of the vacuum desiccator can result in damage to the BeadChips. This is especially true if you remove the valve plug while a vacuum is applied. For detailed vacuum desiccator instructions, see the documentation included with the desiccator.

14. Touch the borders of the chips (*do not touch the stripes*) to ensure that the etched, bar-coded side of the BeadChips are dry to the touch.

15. If the underside feels tacky, manually clean the underside of the BeadChip to remove any excess XC4. The bottom two BeadChips are the most likely to have some excess.

a. Wrap a pre-saturated Prostat EtOH Wipe around your index finger.

b. Wipe along the underside of the BeadChip five or six times, until the surface is clean and smooth.

CAUTION

Do not touch the stripes.

16. Clean the Hyb Chambers:

a. Remove the rubber gaskets from the Hyb Chambers.

b. Rinse all Hyb Chamber components with DI water.
c. Thoroughly rinse the humidifying buffer reservoirs.

17. Clean and dry the wash dishes:
a. Rinse the PB1 wash dishes with DI water.
b. Rinse the XC4 wash dish with ethanol.
c. Air dry all wash dishes.

18. Discard unused reagents in accordance with facility standards.

19. Proceed to scanning the BeadChip(s). See the B*eadArray Reader User Guide*, *iScan System User Guide* or the *HiScanSQ System User Guide* for instructions on scanning your BeadChips.

Appendix A

GoldenGate Indexing Assay Controls

Topics

Introduction

This appendix describes the GoldenGate Indexing Assay Guide control oligos, including the IllumiCode Sequence IDs used, their expected outcomes, and how to view them. The control oligos include:

- Second hybridization controls

View the Control Graphs

To view control graphs using the GenomeStudio Genotyping Module, select **Analysis** | **View Controls Dashboard**. For more information about control graphs, see the *GenomeStudio Genotyping Module User Guide*.

Second Hybridization Controls

The second hybridization controls test the hybridization of single-stranded assay products to IllumiCode Sequences on the array beads. IllumiCode Sequence IDs 44 and 278 should result in Cy3 signal only, Sequence IDs 1112 and 1632 should result in only Cy5 signal, and Sequence IDs 501 and 1003 should have signal contributed by both Cy3 and Cy5.

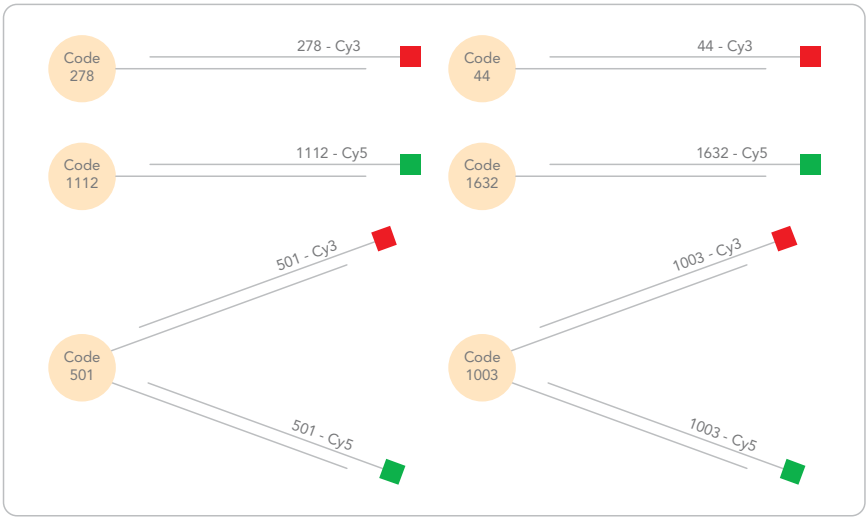


Figure 74 Second Hybridization Controls

Appendix B

GoldenGate Indexing Assay Automation Details

Topics

Introduction

This appendix describes the automation portion of the GoldenGate Indexing Assay with specific details of what and when each robot arm (MCA and LiHa) is aspirating and dispensing. This information is meant as a reference tool only; these details are not described within the main protocols for this assay. Only tasks performed by the LiHa and MCA robot arms are described in this appendix.

Make Single-Use DNA (SUD) MSI Plate

- The LiHa aspirates the MS1 reagent from the quarter trough and dispenses 5 µl of the MS1 reagent evenly to all wells of each SUD plate.
- The MCA aspirates 5 µl volume (containing 250 ng sample DNA) from each well of the first DNA plate and dispenses evenly to all wells of the corresponding SUD plate. The MCA head is washed after the DNA is transferred to the SUD plate. The MCA repeats these steps for each DNA/SUD plate being processed.

Precipitate SUD MSI Plate

- The MCA first aspirates 15 µl of 2-propanol from trough A and then aspirates 5 µl of the PS1 reagent from trough B and dispenses evenly to all wells of the first SUD plate. The MCA aspirates the 2-propanol and the PS1 reagent in one trip. The MCA repeats this step for each SUD plate being processed.

Resuspend SUD MSI Plate

- The MCA aspirates the RS1 reagent from trough A and dispenses 10 µl evenly to all wells of each SUD plate.

Make SUD Allele-Specific Extension (ASE) MSI Plate

- The MCA aspirates the OB1 reagent from trough A and dispenses 30 µl evenly to all wells of each ASE plate.
- The LiHa aspirates OPA from the Index 1 tube and dispenses 10 µl per well to the corresponding ASE plate. This step is repeated for each indexed OPA/ASE plate pair.
- The MCA then aspirates 10 µl of the resuspended DNA samples from the first SUD plate and dispenses 10 µl to each column of the corresponding ASE plate. This step is repeated for each SUD/ASE plate pairing.

Add Extension and Ligation Reagents MSI (MEL)

- The MCA aspirates all liquid from the ASE plates. The MCA then aspirates the AM1 reagent from trough A and distributes 50 µl evenly to each of the ASE plates being processed. The ASE plates are removed from the robot bed, sealed, and vortexed.
- The AM1 wash process above is repeated a second time.
- The MCA aspirates all liquid from the ASE plates. The MCA then aspirates the UB1 reagent from trough B and distributes 50 µl evenly to each of the ASE plates being processed.
- The UB1 wash process above is repeated a second time.
- The MCA aspirates all liquid from the ASE plates. The MCA then aspirates the MEL reagent from trough C and distributes 37 µl evenly to each of the ASE plates being processed.

Pool Allele-Specific Extension (ASE) MSI

- The MCA aspirates all liquid from the ASE plates. The MCA then aspirates the UB1 reagent from trough B and distributes 50 µl evenly to each of the ASE plates being processed.

- The MCA aspirates all liquid from the ASE plates. The MCA then aspirates the IP1 reagent from trough A and distributes 35 µl evenly to each of the ASE plates being processed. The ASE plates are removed from the robot bed, sealed, vortexed, heated to 95°C for one minute, and spun down.
- The MCA aspirates 20 µl from each sample well on the first ASE plate. This step is repeated for all remaining ASE plates.
- The MCA dispenses all samples into the corresponding APP plate.

Make PCR MSI Plate

- The LiHa aspirates the MMP reagent and dispenses 30 µl to each well of the PCR plate.
- Next the MCA aspirates 30 µl from the APP plate and dispenses them to the PCR plate.

Bind PCR Products

- The LiHa first aspirates Sec-butanol from the reagent trough and dispenses 30 µl to each well of the PCR plate.
- Next the LiHa aspirates the MPB reagent and dispenses 20 µl to each well of the PCR plate.
- The LiHa then blows air into each well of the PCR plate to bubble mix the reagents.

Make HYB Plate (Universal BeadChip)

- The LiHa first aspirates and discards the supernatant from the PCR plate sitting on the magnetic carrier.
- The LiHa then aspirates the UB2 reagent from quarter trough A and dispenses 30 µl to the PCR plate.
- The LiHa aspirates the MH1 reagent and dispenses 25 µl to the HYB plate.
- The LiHa aspirates the UB2 reagent from quarter trough A and dispenses 17 µl to the HYB plate.
- The LiHa aspirates and discards the all of the UB2 reagent from the PCR plate.
- The RoMa moves the PCR plate from the magnetic carrier and places the INT plate on the magnetic carrier.
- The LiHa aspirates 0.1N NaOH from quarter trough B and dispenses 30 µl to the first column of the PCR plate.
- The LiHa resuspends the reagents by aspirating and dispensing and then aspirates the entire volume and transfers it to the INT plate.
- Next the LiHa aspirates 25 µl of the single-stranded, fluor-labeled PCR product from the INT plate and dispenses it to the HYB plate that contains the 25 µl of the MH1 reagent.
- The last three steps are repeated by column until the Hyb plate is complete.

Hybridize Universal BeadChip

- The LiHa aspirates samples from the first column of the Hyb plate, and dispenses 12 µl of sample per inlet port to the first column of the Universal-32 BeadChip. This step is repeated for the rest of the sample columns of the HYB plate until three Universal-32 BeadChips have been loaded with samples for hybridization.

Appendix C
System Maintenance

Topics

Introduction

This chapter consists of recommendations for maintaining the Tecan EVO 150 (LiHa) and 200 (MCA) system hardware, database servers and database.

Use the Tecan Robot (Automated Customers Only)

Follow the instructions in this section to use the Tecan Robot:

Robot Control Software

This section explains how to use the Illumina Automation Control software. Figure 75 shows the main screen.

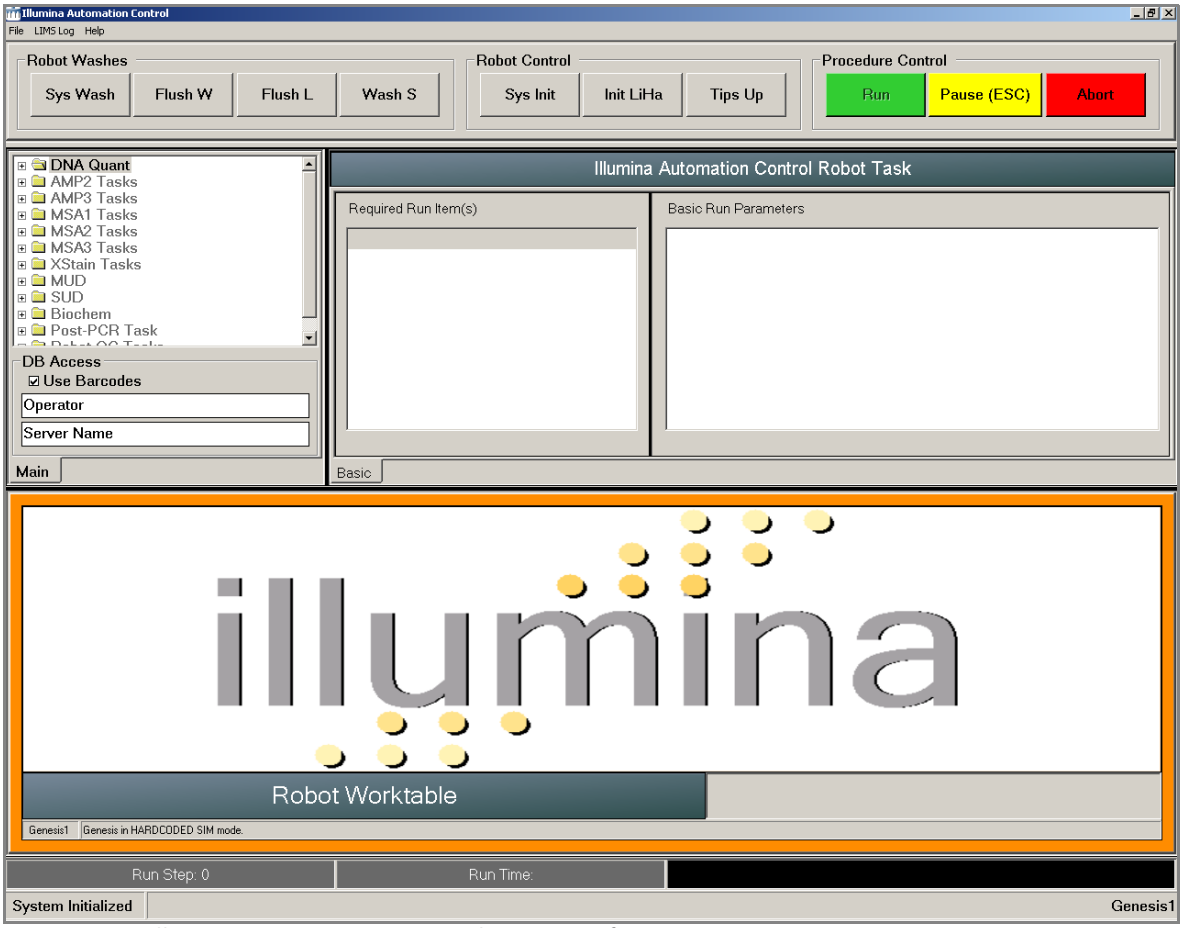


Figure 75 Illumina Automation Control User Interface

Table 6 lists each part of the Illumina Automation Control software main screen and explains how to use it.

Table 6 Illumina Automation Control Software User Interface

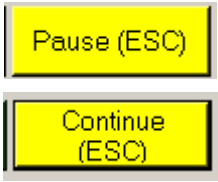
Screen Element	Function
Robot Washes Pane	
	Click to start a complete flush of system fluids: • Daily, per robot maintenance schedule (please refer to the documentation that came with the robot) • When changing system fluid • If bubbles are present in the lines • Per instructions in individual laboratory protocols
	Click to flush a large amount of water through the robot tips over the robot waste station: • If a robot process was stopped unexpectedly, and you want to flush the tips completely before proceeding • To validate the robot fast-wash pump operation
	Click to flush the robot tips in the "long wash" station (tips are deeply immersed): • If the robot tips were immersed in a deep receptacle (deep-well plate, tube, etc.) during a robot process that was stopped unexpectedly, and you want to flush the tips completely before proceeding
	Click to move the robot diluters up and down and wash the robot tips in the short-wash station (tips are immersed to a shallow depth): • To remove air bubbles that still remain following a flush wash • To validate the robot diluters' operation
Robot Control Pane	
	Click to initialize the system at the beginning of each day.
	Click to initialize the liquid handling system (moves to its home position): • If a robot process was stopped unexpectedly, and you want to re-initialize the LiHa robot arm
	Click to move the robot tips to their "up" position: • When you need to lift the robot tips to the maximum height without re-initializing the LiHa robot arm • During selected robot maintenance tasks
Procedure Control Pane	
	After selecting a robot procedure from the expandable list of options at the left, entering basic run parameters, and setting up the robot bed per the robot bed map, click **Run** to start the selected robot process. If you are *not* using Illumina LIMS, clear the Use Barcodes checkbox first.
	Should it become necessary to interrupt the robot run for any reason, click **Pause** on the robot PC (or press the **Esc** keyboard key). The button changes to **Continue**. When ready to resume the run, click **Continue**.

Table 6 Illumina Automation Control Software User Interface (Continued)

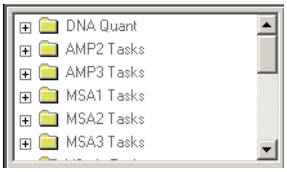
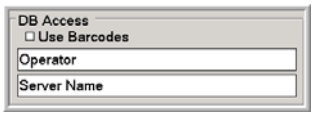
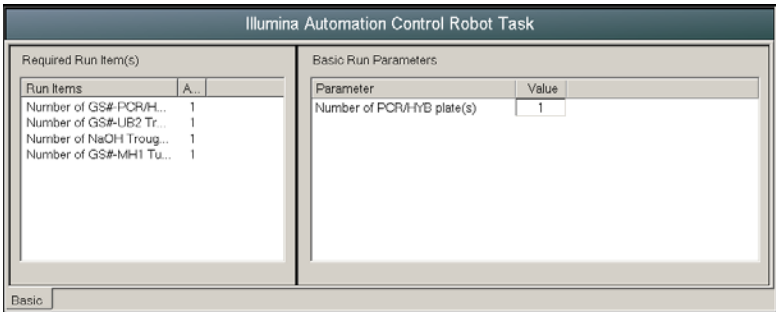
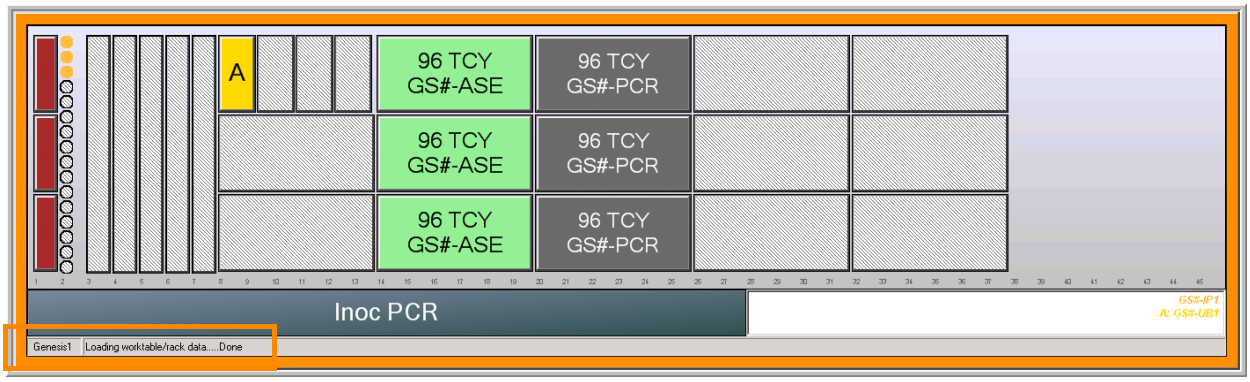
Screen Element	Function
	Should it become necessary to abort the robot run for any reason, click **Abort** on the robot PC. The button changes to **Restart**. To reset the instrument and make it available to run a selected task, click **Restart**.
Procedures Pane	
	To select a robot procedure, expand the appropriate task area in the pane on the left, and then select the procedure.
DB Access pane	
	Select the **Use Barcodes** checkbox before starting any procedure. This invokes the link to Illumina LIMS and ensures that items are tracked accurately throughout the assay.
Robot Task Pane	
The Required Run Items pane displays the items needed to run the selected procedure. The fields in the Basic Run Parameters pane are automatically filled with the default values for the selected process. You may change these values per individual laboratory protocols. 	

Table 6 Illumina Automation Control Software User Interface (Continued)

Screen Element	Function
Robot Bed Map	
After you have accepted or changed the basic run parameters for the selected procedure, the robot bed map shows where to place the various plates, tubes, and reservoirs on the robot bed. • **Reagent Tubes** appear as color-coded dots at the left, corresponding to their positions in the tube rack. The reagent associated with each color is listed in the lower right corner of the pane. • **Reagent Reservoirs** (quarter, half, or full) appear as lettered boxes to the right of the reagent tubes. The reagent for each reservoir is listed in the lower right corner of the pane or you can also scroll the mouse over the letter to see the reagent names. • **Plates** are located to the right of the reservoirs, and are color-coded to distinguish them from each other. Each plate shows the plate type (e.g., 96 TCY for a 96-well TCY plate, and 96 FBLK for a 96-well black Fluotrac plate) and the plate name (ASE, PCR, etc.). • **Numbers** along the bottom of the bed map show the positions of the items. For example, the green ASE plates in the image below are in position 14 and the grey PCR plates are in position 20. • The progress bar in the bottom left pane tells you what step of the protocol the robot is on. 	

Clean the Robot

Prepare the Robot for the First Use of the Day

Before using the robot for the first time each day, perform the procedures described in this section.

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

1. Reboot the robot PC.
2. From the robot PC desktop, open the Programs I Illumina folder.
3. Launch the Illumina Automation Control software.
   The robot takes a few moments to initialize.
4. Check the system fluid level and add fluid if necessary. Empty any system waste as necessary.

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

Robot Bleach Wash (Daily)

The following LiHa an d MCA robot bleach and system wash tasks should be performed daily. If you perform these tasks at the start of the day you do not need to repeat them if you are running samples later in the same day.

To prevent fluorescein from contaminating sample solutions, you must perform this bleach wash step after every robot QC test.

LiHa 8-tip Bleach Wash

On the Tecan 200, if the RoMA arm is next to the 8-tip LiHa arm perform a system initialization (Sys Init) to move the RoMA arm next to the MCA arm. If you start the LiHa Robot Bleach Wash task with the RoMA arm next to the LiHa arm they will crash into each other and cause a fatal software error.

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

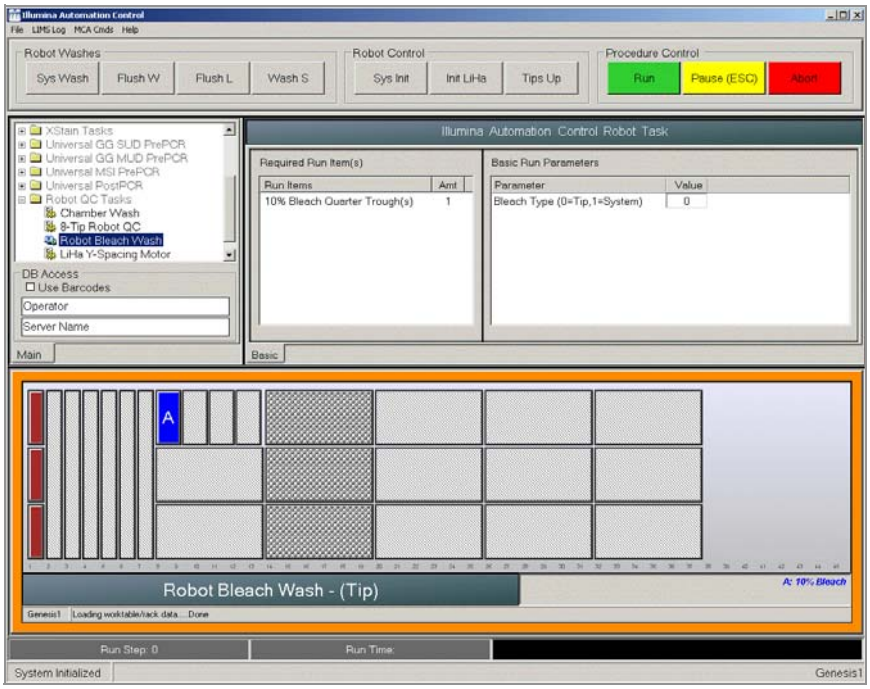


Figure 76 LiHa Robot Bleach Wash Task

2. Place a quarter reservoir A onto the robot bed according to the robot bed map. Dispense 5 ml of 10% bleach into the trough A.
3. Click **Run**.

   When the bleach procedure is complete, the robot returns to the main robot task screen.
4. Observe the lines for air bubbles.
5. For a Tecan 200 (MCA), immediately perform the MCA 96-tip bleach wash described in the next section, *MCA 96-tip Bleach Wash (Tecan 200 only)*.
6. For a Tecan 150 (LiHa), when the tip bleach process is complete, click **Sys Wash**.

   Observe the fluidic lines of the LiHa for air bubbles.
7. Perform the sys wash process at least **three times**, until the lines are completely free of air bubbles.

MCA 96-tip Bleach Wash (Tecan 200 only)

CAUTION

To prevent fluorescein from contaminating sample solutions, you must perform this bleach wash step after every robot QC test.

1. Select **MCA QC Tasks | MCA Bleach Wash**.

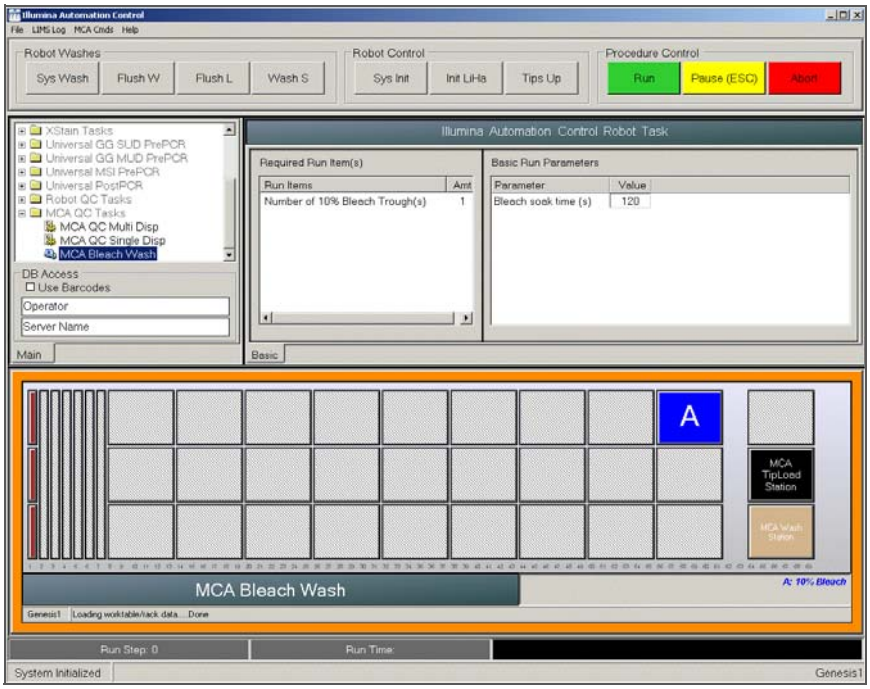


Figure 77 MCA Robot Bleach Wash Task

2. Place full trough A onto the robot bed according to the robot bed map. Dispense 150 ml of 10% bleach into the trough A.
3. Click **Run**.

   When the bleach procedure is complete, the robot returns to the main robot task screen.
4. When the tip bleach process is complete, click **Sys Wash**.

   Observe the fluidic lines of the LiHa for air bubbles.
5. Perform the sys wash process at least **three times**, until the LiHa lines are completely free of air bubbles.

Bleach-Bathe the Robot Carriers (Weekly)

Required Materials:

- Bleach
- DiH_20
- Two soaking trays or standard dishwashing tubs (24”L x 18”W x 6”D)
- Absorbent bench underpad

1. Prepare a 10% bleach bath (~500 ml concentrated bleach in 4500 ml DiH_2O) in one of the soaking trays.
2. Fill the other soaking tray with DiH_2O.
3. Lay out at least two rows of absorbent bench underpad on a benchtop.
4. Remove the robot carriers from the robot bed. Note the carriers’ original positions, 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 DiH_2O soaking tray for about 1 minute.
7. Remove the carriers from the DiH_2O soaking tray and rinse them under running water in the sink.
8. Dry the carriers on the absorbent bench underpad.
9. Allow carriers to dry on the underpad overnight before returning them to their proper positions on the robot bed.

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

Bleach-Bathe the Magnetic Carriers (Weekly)

While the Robot Carriers are drying on the absorbent bench underpad you should also bleach-bathe the magnetic carriers. On the Tecan 200 (MCA) do not remove the magnetic carriers from the robot bed.

Required Materials:

- Bleach
- DiH_20
- Kimwipes

1. Wipe the magnetic carriers down with 10% bleach.
2. Moisten a Kimwipe in DiH_2O water and wipe the magnetic carriers down again. Do not use the same DiH_2O water you used to bleach-bathe the robot carriers.
3. Use Kimwipes to dry off the magnetic carriers.

Clean the LiHa Robot Tip Surface (Weekly)

Required Materials:

- Kimwipes
- Ethanol

1. At the beginning of each day, check for any leaks.
2. In the robot software, click **Sys Init**.
3. Fold a Kimwipe in half lengthwise.
4. Using a squeeze bottle, soak the folded Kimwipe with 70% ethanol.
5. In the robot software, click **Tips Up**.
6. Fold the Kimwipe around the robot tip.
7. Starting from one end of the tip row, wipe each tip gently along the entire lower half of the tip.
8. Reverse the Kimwipe to use the other, fresh side.

9. Starting from the other end of the tip row, wipe each tip a second time along the entire lower half of the tip.
10. Clean the waste station with the Kimwipe.
11. If needed, clean the waste station a second time.

Perform Robot QC

Perform regular robot QC procedures to ensure that the liquid handling system causes minimal variation in the assay.

You should perform robot QC tasks:

- As part of a regular robot maintenance schedule (minimum of once every two week is recommended for the Tecan 150 but required for the Tecan 200)
- After any robot or robot tips mechanical problem has been resolved

This section explains how to perform the following robot QC procedures:

- *Test Individual- and Multi-Column Dispense Volume for LiHa Tips* on page 114
- *Test Volume Accuracy of Robot MCA Tips* on page 116
- *Create Standard Curve Plate* on page 119
- *Reading LiHa QC Data* on page 120
- *Measure Fluorescence in Ind-Col Dispense Plate (LiHa)* on page 126
- *Measure Fluorescence in Multi-Col Dispense Plate (LiHa)* on page 127
- *LiHa Robot QC Report* on page 128
- *Reading MCA QC Data* on page 134
- *MCA Robot QC Report* on page 136

Test Volume Accuracy of the Robot LiHa and MCA Tips

You should regularly test the volume accuracy of the robot's 8 LiHa tips and the MCA's 96 tip head according to Illumina's specifications. The Robot QC task performs two tests:

- **The individual-column dispense test** (Ind-Col Dispense Plate)

  LiHa: In this test, eight robot tips dispense reagent to the plate one column at a time. Ind-Col Dispense Plate tests for three volumes. The tips are washed after reagent has been dispensed to all columns.

  MCA (Tecan 200 only): In this test, the 96 tip head dispenses reagent into 9 different plates. Ind-Col Dispense Plate tests for three volumes. Each volume is tested in 3 positions across the robot deck. The tips are washed after reagent has been dispensed to all plates.

- **The multi-column dispense test** (Multi-Col Dispense Plate)

  LiHa: In this test, a large volume of reagent is dispensed multiple times over the entire plate (i.e., multiple times per six columns). Multi-Col Dispense Plate tests for two volumes. The tips are washed after reagent has been dispensed to six columns.

  MCA (Tecan 200 only): In this test the 96 tip head will aspirate a large volume of reagent and dispense smaller volumes to multiple plates on the robot deck. Multi-Col Dispense Plate tests for two volumes. Each volume is tested in 3 positions across the robot deck. The tips are washed after reagent has been dispensed.

Consumables

Item	Quantity	Storage	Supplied By
Fluotrac 200 QC plates	18 total (one standard curve, 15 for MCA, 2 for LiHa)		User
Plasma-treated reagent troughs	2 (MCA)		User
Quarter troughs	2 (LiHa)		User
Fluorescein Stock Solution			User
Fluorescein Dilution Buffer	457 ml (total for both LiHa and MCA robot QC tasks)		User
Low Concentration Fluorescein solution	88 ml (total for both LiHa and MCA robot QC tasks)		User
Aluminum foil or aluminum foil adhesive plate seals			User

Prepare Reagents

This section explains how to prepare reagents for cleaning and testing the robot.

Fluorescein Stock

Follow these steps to create fluorescein stock (1 mg/ml):

1. Weigh out 25 mg fluorescein into a 100 ml bottle.
2. Slowly add 25 ml DMSO.

Fluorescein Dilution Buffer

Follow these steps to prepare fluorescein dilution buffer:

1. Prepare 1X TE (Tris Ethylenediaminetetraacetic acid—EDTA):
   a. Add 10 ml 100X TE (1M Tris-HCL, 0.1M EDTA) to 990 ml purified water in a 1 L bottle.
   b. Mix thoroughly and filter.
2. Prepare 10% Tween 80:
   a. Weigh 107.4 grams Tween 80.

Tween 80 is extremely viscous, and so weight measurement is more accurate than liquid volume measurement.

   b. Add the Tween 80 to the 1 L bottle.
   c. Dissolve with purified water to 1000 ml.

d. Mix thoroughly and filter.

3. Prepare 1X TE with 0.01% Tween 80:
   a. Add 1 ml 10% Tween 80 to 1000 ml 1X TE buffer.
   b. Mix thoroughly.

High-Concentration Fluorescein

Follow these steps to prepare high-concentration fluorescein (0.25 mg/ml):

1. Add 25 ml fluorescein stock in DMSO (1.0 mg/ml) to 75 ml fluorescein dilution buffer (1X TE with 0.01% Tween 80) in a 100 ml bottle.
2. Mix thoroughly.

Low-Concentration Fluorescein

Follow these steps to prepare low-concentration fluorescein (0.025 mg/ml):

1. Add 50 ml newly prepared 0.25 mg/ml high-concentration fluorescein to 450 ml fluorescein dilution buffer in a 500 ml bottle.
2. Mix thoroughly.

Test Individual- and Multi-Column Dispense Volume for LiHa Tips

Before testing the volume accuracy make sure you have completed three system washes. These steps are described in *Clean the Robot* on page 107.

A bleach wash is not necessary at this step because a bleach wash will be performed once the all robot QC steps are completed.

For the Tecan 200, if the RoMA arm is next to the LiHa arm perform a system initialization to move the RoMA arm next to the MCA arm. If you start the LiHa Robot Bleach Wash task with the RoMA arm next to the LiHa arm they will crash into each other and cause a fatal software error.

1. Label a new Fluotrac 200 plate “LiHa Ind-Col Dispense.”
2. Label another new Fluotrac 200 plate “LiHa Multi-Col Dispense.”

3. Select **Robot QC Tasks | 8-Tip Robot QC** (Figure 78).

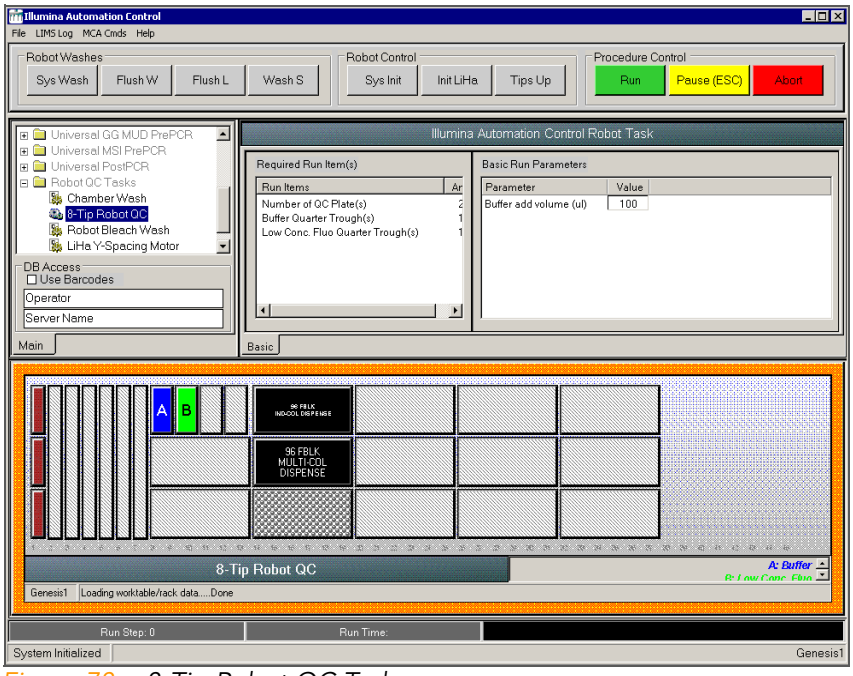


Figure 78 8-Tip Robot QC Task

4. Add 35 ml fluorescein dilution buffer to a quarter reservoir. Place the reservoir in position A of the reservoir frame, as shown on the robot bed map (Figure 78).
5. Add 6 ml low concentration fluorescein (0.025 mg/ml) to a quarter module reservoir and place in position B, as shown on the robot bed map.
6. Place the Fluotrac 200 plates on the robot bed according to the robot bed map.
7. Make sure that all items are placed properly on the robot bed.
8. Click **Run**. The robot conducts an internal QC process. A message in the lower status bar indicates when this is complete.

Illumina recommends you create the Standard Curve plate while one of the Robot QC tasks are being run on the robot (LiHa or MCA) to save time. This plate can be used for both the LiHa and MCA volume tests. See *Create Standard Curve Plate* on page 119.

9. Immediately cover the Fluotrac 200 plates with aluminum foil.
10. For a Tecan 200 (MCA) proceed immediately to the next section, *Test Volume Accuracy of Robot MCA Tips*.
11. If you are only testing the LiHa tips and plan to analyze the robot QC data in the post-PCR area, transfer the Fluotrac 200 plates to the post-PCR area or to the area where the fluorometer resides.

CAUTION

Take care not to splash any solution out of the wells during transport.

12. Once the robot QC tests have all been successfully completed, dispose of any remaining reagents in accordance with your facility requirements.
13. Perform the Robot Bleach wash (daily) steps for the LiHa once the QC results have been read. See *Robot Bleach Wash (Daily)* on page 107 for reference.

Test Volume Accuracy of Robot MCA Tips

There are two volume accuracy tests for the MCA 96-tip robot arm. There is a multi and single dispense test that should both be performed each time robot QC is done.

Test Multi-Plate Dispense Volume for 96-tip MCA

1. Label three new Fluotrac 200 plates "MD 5µL-1," "MD 5µL-2," and "MD 5µL-3."
2. Label three new Fluotrac 200 plates "MD 30µL-1," "MD 30µL-2," and "MD 30µL-3."
3. Select **MCA QC Tasks | MCA QC Multi Disp**.

MD 5µL-1, MD 5µL-2, and MD 5µL-3 Plates

MD 30µL-1, MD 30µL-2, and MD 30µL-3 Plates

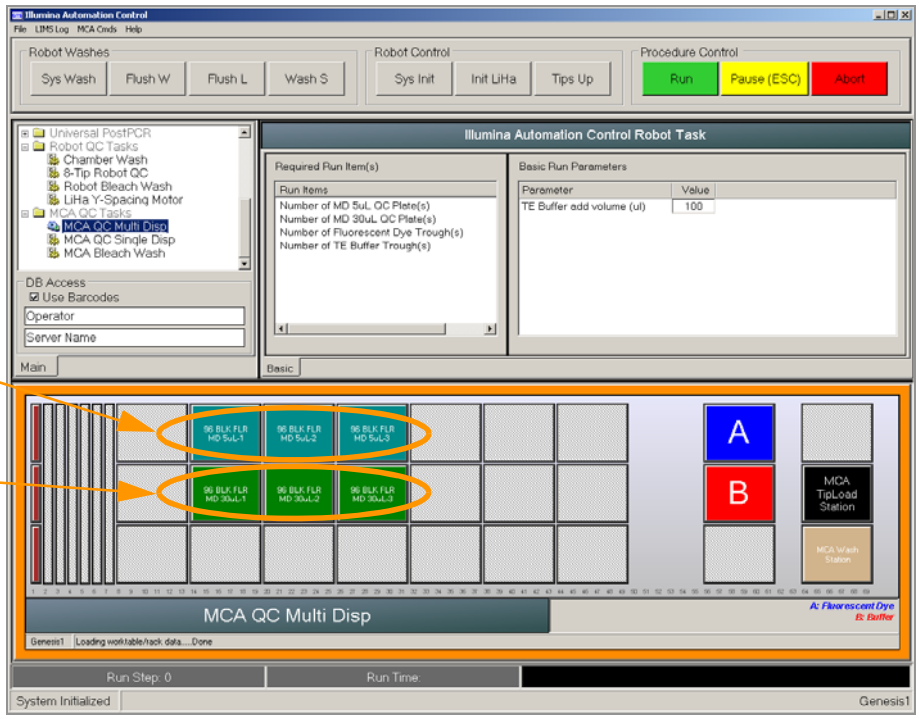


Figure 79 MCA QC Multi Disp Task

4. Add 150 ml Fluorescein Dilution Buffer to a full trough reservoir and place the reservoir in position B, as shown on the robot bed map.

5. Add 40 ml Low Concentration Fluorescein (0.025 mg/ml) to a full trough reservoir and place the reservoir in position A, as shown on the robot bed map.
6. Place the Fluotrac 200 plates on the robot bed according to the robot bed map. The MD 5µL-1, MD 5µL-2, and MD 5µL-3 plates should be on the back row, left to right, and the MD 30µL-1, MD 30µL-2, and MD 30µL-3 plates should be in the middle row, left to right. See Figure 79 for correct plate placement.
7. Click **Run**.

   The robot executes the pipetting required for the QC process. A message in the lower left status bar indicates when the test is complete.

If you did not create the Standard Curve plate while you tested the LiHa, Illumina recommends you create the plate while the MCA Robot QC tasks are being run on the robot to save time. This plate can be used for both the LiHa and MCA volume tests. See *Create Standard Curve Plate* on page 119.

8. Immediately after the QC procedure completes, cover the Fluotrac 200 plates with aluminum foil plate seals.
9. Proceed to the next section, *Test Individual Plate Dispense Volume for 96-tip MCA* on page 117.

Test Individual Plate Dispense Volume for 96-tip MCA

1. Label three new Fluotrac 200 plates "SD 2µL-1," "SD 2µL-2," and "SD 2µL-3."
2. Label three new Fluotrac 200 plates "SD 8µL-1," "SD 8µL-2," and "SD 8µL-3."
3. Label three new Fluotrac 200 plates "SD 32µL-1," "SD 32µL-2," and "SD 32µL-3."

4. Select **MCA QC Tasks | MCA QC Single Disp**.

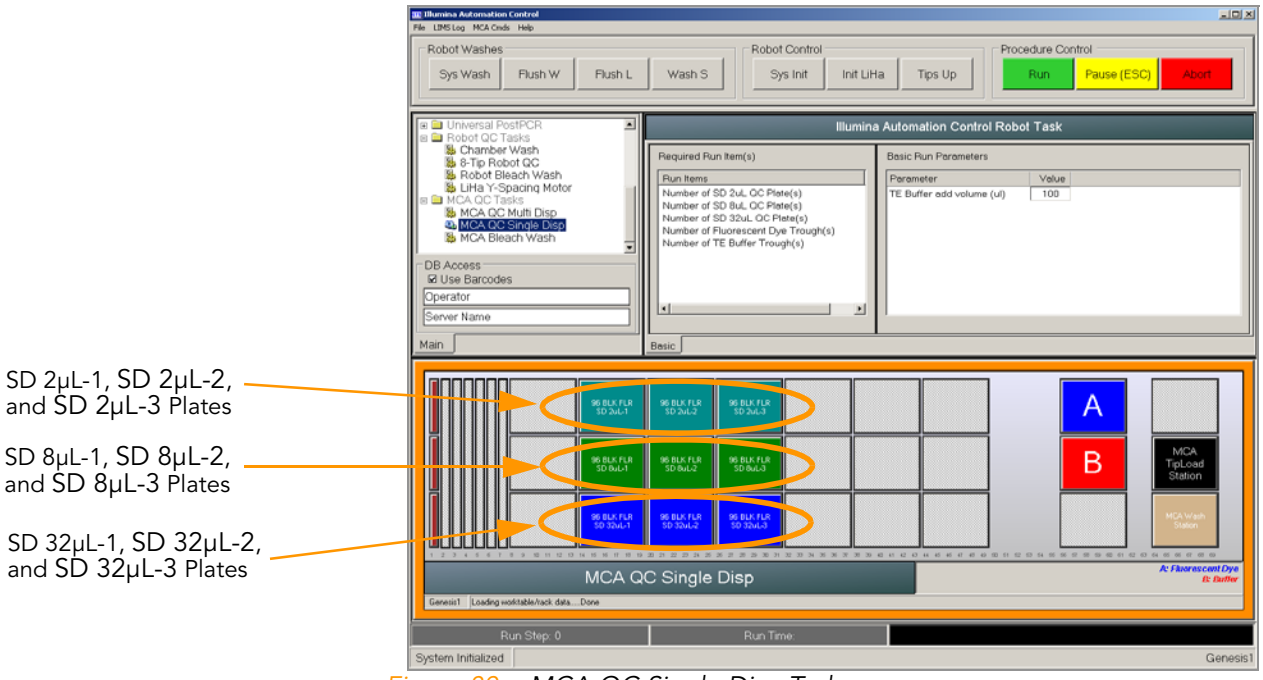


Figure 80 MCA QC Single Disp Task

5. Add 150 ml Fluorescein Dilution Buffer to a full trough reservoir, and place the reservoir in position B, as shown on the robot bed map.
6. Add 40 ml Low Concentration Fluorescein (0.025 mg/ml) to a full trough reservoir, and place the reservoir in position A, as shown on the robot bed map.
7. Place the Fluotrac 200 plates on the robot bed according to the robot bed map. SD 2µL-1, SD 2µL-2, and SD 2µL-3 plates should be on the back row, left to right, SD 8µL-1, SD 8µL-2, and SD 8µL-3 plates in the middle row, left to right, and SD 32µL-1, SD 32µL-2, and SD 32µL-3 plates in the front row, left to right. See Figure 80 for correct plate placement.
8. Click **Run**.

   The robot executes the pipetting required for the QC process. A message in the lower left status bar indicates when the test is complete.

NOTE

If you did not create the Standard Curve plate while you tested the LiHa, Illumina recommends you create the plate while the MCA Robot QC tasks are being run on the robot to save time. This plate can be used for both the LiHa and MCA volume tests. See *Create Standard Curve Plate* on page 119.

9. Immediately after the QC procedure completes, cover the Fluotrac 200 plates with aluminum foil plate seals.

Run all QC plates on the fluorometer as soon as possible after performing all Robot QC tests.

10. If you plan to analyze the 96-tip robot QC data in the post-PCR area, transfer all the plates tested, including the standard curve plate to the post-PCR area or to the area where the fluorometer resides. Take care to not splash the QC plates when transferring them to the fluorometer.
11. Once all Robot QC tests have been successfully completed, dispose of any remaining reagents in accordance with your facility requirements.
12. Perform the Robot Bleach wash (daily) steps for the LiHa and MCA once the QC results have been read. See *Robot Bleach Wash (Daily)* on page 107 for reference.
13. If you have not created the standard curve plate yet proceed to *Create Standard Curve Plate* on page 119. Otherwise, proceed to *Test Standard Curve Plate for LiHa 8-Tip* on page 120.

Create Standard Curve Plate

The Standard Curve Plate lets you generate a standard curve of fluorescent units versus volume dispensed. With the Standard Curve Plate, you can to calculate volumes dispensed by the robot into the Robot QC test plates. To make the Standard Curve Plate, you manually pipette fluorescein over a range of volumes (1–36 µl) into 100 µl fluorescein dilution buffer. Use of a properly calibrated pipette is recommended. Illumina recommends you create the Standard Curve plate while one of the Robot QC tasks are being run on the robot (LiHa or MCA). This plate can be used for both the LiHa and MCA volume tests.

1. Label a Fluotrac 200 plate "Standard Curve Plate."
2. Dispense 12 ml fluorescein dilution buffer into a disposable multichannel reservoir.
3. Add 100 µl fluorescein dilution buffer into each well of the standard FluoTrac 200 plate using a 12-channel pipette (size 50–300 µl).
4. Dispense 2 ml low fluorescein concentration standard (0.025 mg/ml) into a disposable multichannel reservoir.
5. Using an 8-channel pipette, add low fluorescein concentration standard (0.025 mg/ml) into the Standard Curve Plate according to the volumes shown in Table 7.

For dispensing fluorescein into column two you will need to use a 2 µl hand pipette to dispense the 1µl of fluorescein.

6. Cover the plate immediately after you complete the pipetting.
7. Use caution while transporting all QC & Std plates to the fluorometer.

Table 7 Fluorescein for the Standard Curve 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 LiHa QC Data

Test Standard Curve Plate for LiHa 8-Tip

After creating the Standard Curve plate, use the Illumina Fluorometry Analysis software to determine whether the plate passes or fails the robot QC test. The software employs a spectrofluorometer to read the fluorescein levels in the QC'ed Fluotrac 200 plates. If you're going to be performing the MCA 96-tip QC for the Tecan 200, retain the standard curve plate to be read again.

Set Up the Robot

1. At the computer associated with the spectrofluorometer, open the Illumina Fluorometry Control software.

   A drawer automatically opens on the reader.

2. In the Robot QC pane, select the Robot Name to specify which robot is being tested (Figure 81).

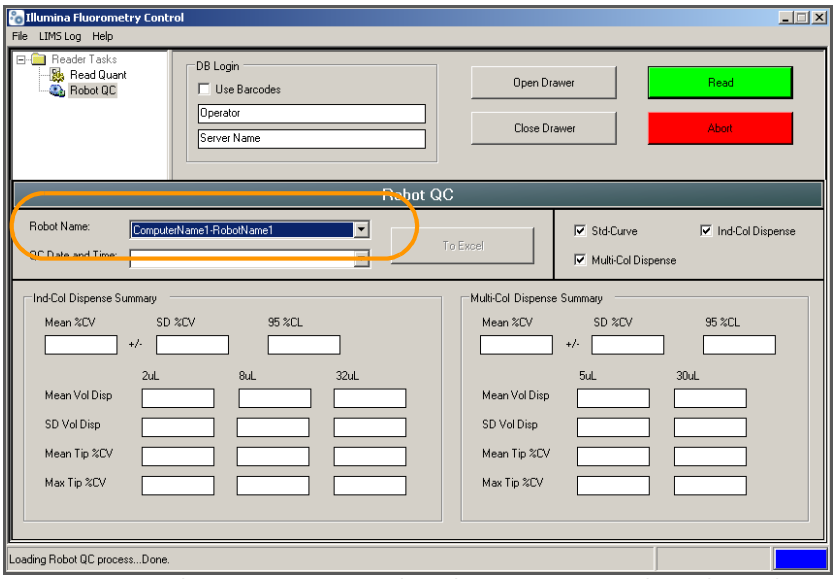


Figure 81 Robot QC Screen with Robot Name & Tasks Selected

3. In the adjacent pane, select the QC task checkboxes to indicate which QC plates you will be measuring (Figure 81). Uncheck the Std Curve checkbox if you want to use a previously read standard curve plate. Check the Std Curve checkbox if you want to read a new standard curve plate.

NOTE

Previously tested standard curve plates should only be used if re-reading a plate from that day's testing. Otherwise, a new standard plate should be read.

4. Do one of the following:
   - Proceed to *Read a New Standard Curve Plate* on page 121.
   - Proceed to *Perform Robot QC On a Previously Read Standard Curve Plate for LiHa 8-Tip* on page 125.

Read a New Standard Curve Plate

1. In the Illumina Fluorometry Control software, select the **Std Curve** checkbox (Figure 82).
2. Clear the **Use Barcodes** checkbox.

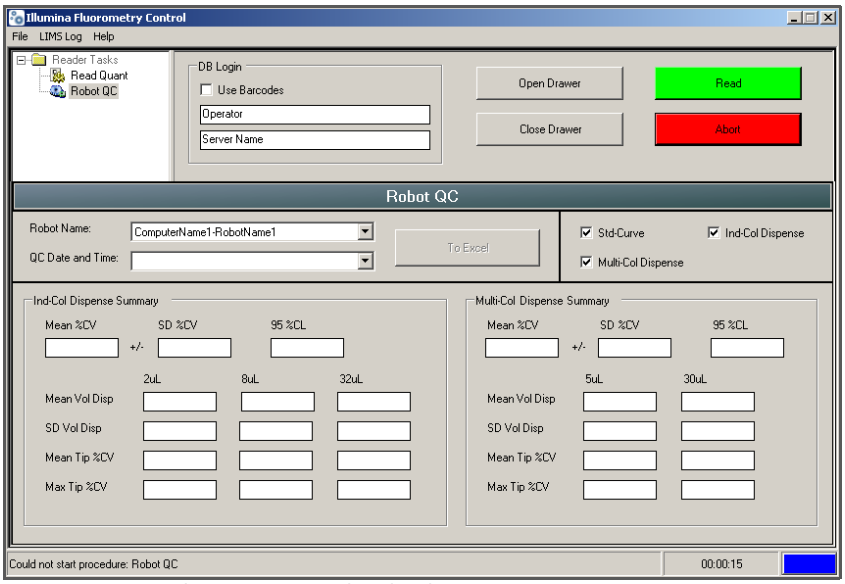


Figure 82 Std-Curve Box Checked

3. In the Action panel, click **Read**.
4. When prompted, load the Standard Curve plate into the reader tray (Figure 83).

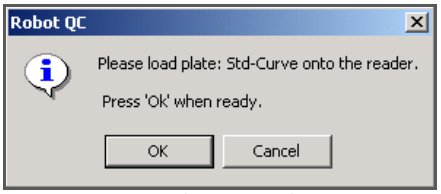


Figure 83 Std Curve Plate Prompt

5. Place the front edge of the plate (manufacturer's nameplate side) into the drawer first, with gentle pressure against both retaining clips (Figure 84).
6. Lower the back edge of the plate into the drawer and ensure proper seating.

CAUTION Take care not to splash any solution out of the wells.

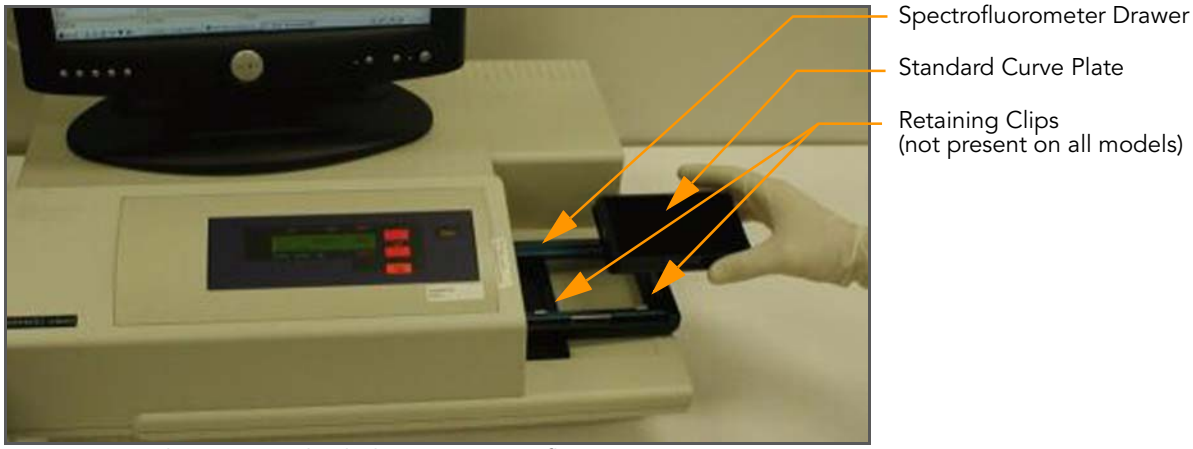


Figure 84 Placing Standard Plate in Spectrofluorometer Drawer

7. Click **OK** to close the drawer.

   The spectrofluorometer automatically begins reading the Standard Curve plate. This takes approximately two minutes. When the spectrofluorometer finishes reading the plate, the drawer opens automatically and the program prompts you to review the data (Figure 85).

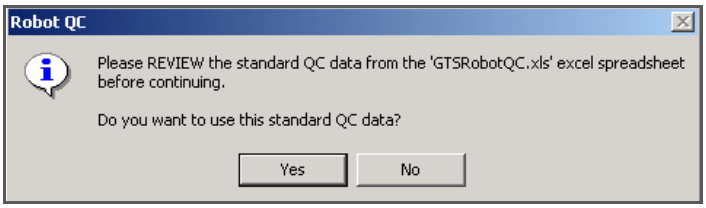


Figure 85 Use Standard Plate Data Prompt

8. Before clicking **Yes**, review the data (Figure 86).

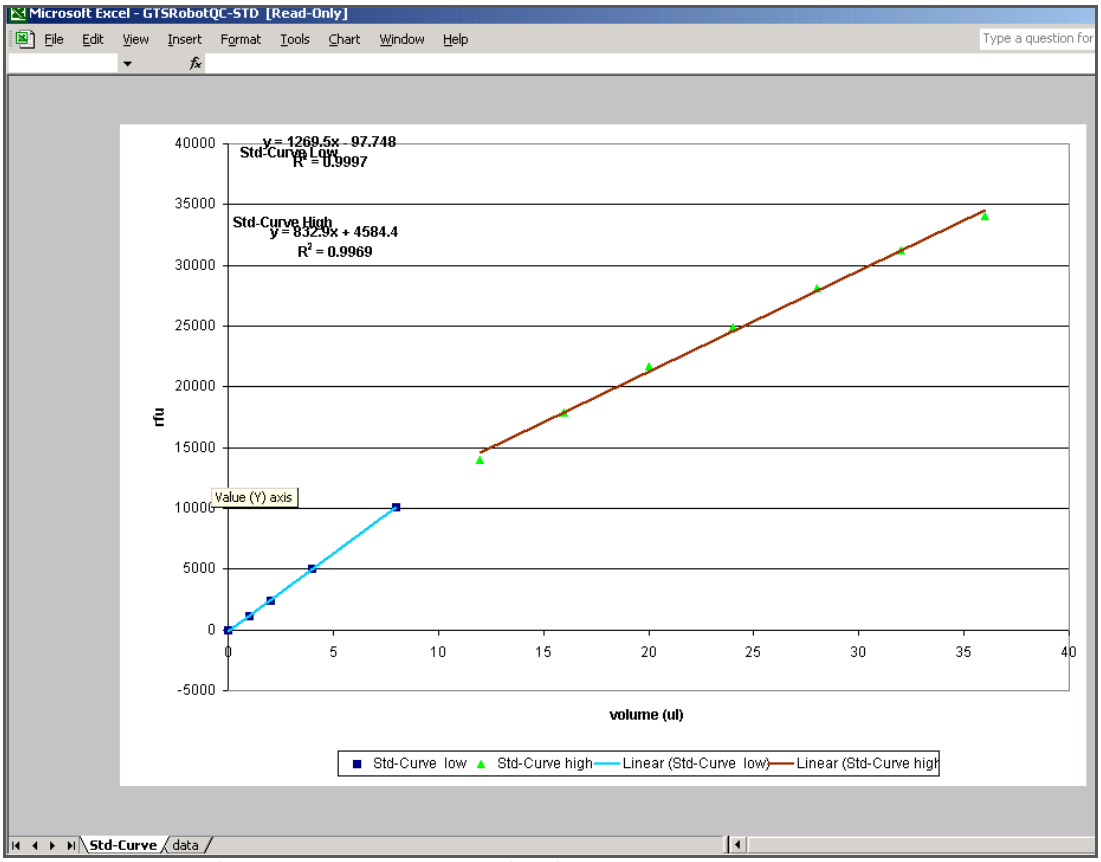


Figure 86 Sample GTSROBOTQC-STD.xls File

9. Do one of the following:
   - If you want to use this data, click **Yes**. When prompted, remove the Standard Curve plate from the reader (Figure 87). Otherwise, if you are performing the MCA Robot QC, save the standard plate for this analysis.

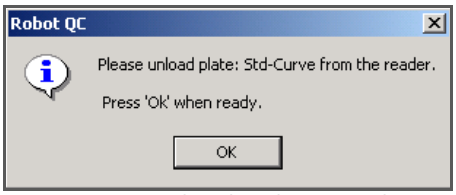


Figure 87 Unload Std Curve Plate Prompt

   - If the data are inaccurate, click **No**, and then repeat all steps, starting with *Create Standard Curve Plate* on page 119, up to this point.

10. Proceed to *Measure Fluorescence in Ind-Col Dispense Plate (LiHa)*, page 126.

Perform Robot QC On a Previously Read Standard Curve Plate for LiHa 8-Tip

In cases where one of the Robot QC plates failed and QC must be repeated, use a previously read Standard Curve plate.

CAUTION

Be sure to use the same stock of fluorescein.

1. In the Illumina Fluorometry Control software, clear the **Std Curve** checkbox in the Robot QC pane (Figure 88).

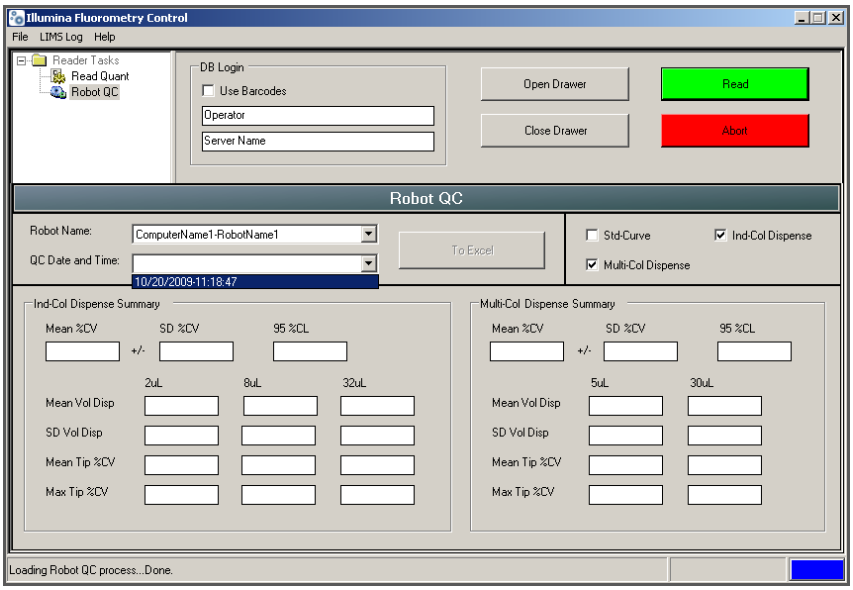


Figure 88 Using a Previously Read Standard Curve Plate

2. In the Action panel, click **Read**.
3. When prompted, accept the standard QC data read on the date and time displayed (Figure 89).

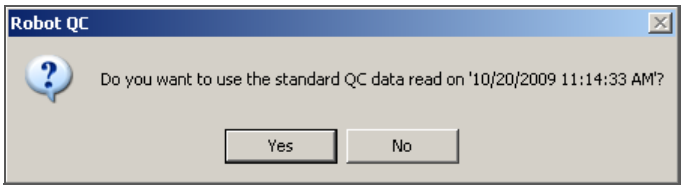


Figure 89 Use Standard QC Data Read Prompt

4. When prompted, accept the most recent Standard Curve data (Figure 90).

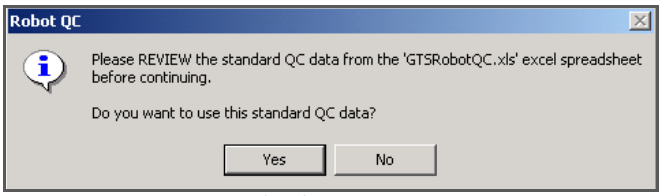


Figure 90 Use Standard Curve Data Prompt

5. When prompted, remove the Standard Curve plate from the reader (Figure 91).

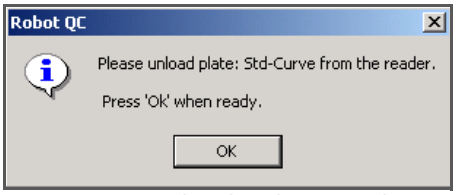


Figure 91 Unload Std Curve Plate Prompt

6. Discard the plate according to laboratory guidelines.
7. Proceed to *Measure Fluorescence in Ind-Col Dispense Plate (LiHa)*, page 126.

Measure Fluorescence in Ind-Col Dispense Plate (LiHa)

Only follow these steps if you selected Ind-Col Dispense as one of the plates to measure in *Set Up the Robot* on page 120. If you did not, then proceed to *LiHa Robot QC Report* on page 128.

1. When prompted, load the Ind-Col Dispense plate into the spectrofluorometer (Figure 92). Click **OK**.

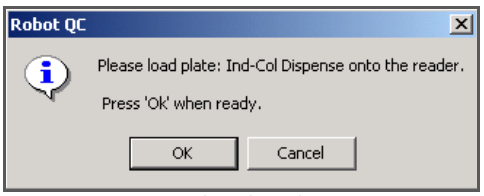


Figure 92 Load Ind-Col Dispense Prompt

CAUTION Take care not to splash any solution out of the wells.

The spectrofluorometer automatically begins reading the Ind-Col Dispense plate. This takes approximately two minutes. When the spectrofluorometer finishes reading the plate, the drawer opens.

2. When prompted (Figure 95), unload the Ind-Col Dispense plate, and then click **OK**. Discard the plate according to laboratory guidelines.

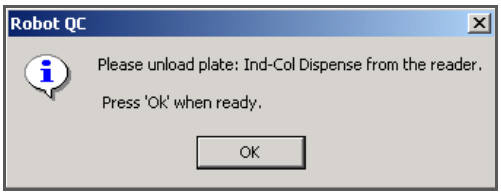


Figure 93 Unload Ind-Col Dispense Plate Prompt

The Ind-Col Dispense pane (Figure 94) updates to reflect summary statistics for the robot being tested.

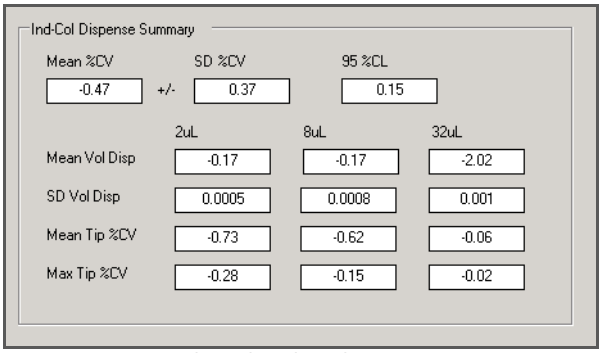


Figure 94 Updated Ind-Col Dispense Summary

3. Proceed to *Measure Fluorescence in Multi-Col Dispense Plate (LiHa)* on page 127.

Measure Fluorescence in Multi-Col Dispense Plate (LiHa)

Only follow these steps if you selected Multi-Col Dispense as one of the plates to measure in *Set Up the Robot* on page 120. If you did not, then proceed to *LiHa Robot QC Report* on page 128.

1. When prompted (Figure 95), load the Multi-Col Dispense plate into the spectrofluorometer drawer. Click **OK**.

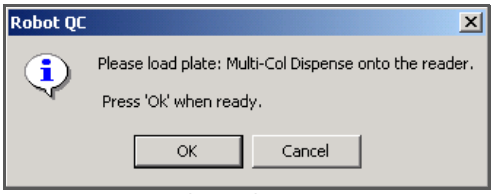


Figure 95 Multi-Col Dispense Prompt

CAUTION Take care not to splash any solution out of the wells.

The spectrofluorometer automatically begins reading the Multi-Col Dispense plate. This takes approximately two minutes. When the spectrofluorometer finishes reading the plate, the drawer opens.

2. When prompted (Figure 96), unload the Multi-Col Dispense plate. Click **OK**. Discard the plate according to laboratory guidelines.

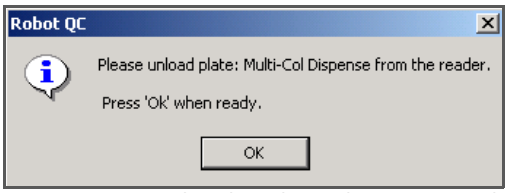


Figure 96 Unload Multi-Col Dispense Plate Prompt

3. Proceed to *LiHa Robot QC Report* on page 128

The Multi-Col Dispense pane (Figure 97) updates to reflect summary statistics for the robot being tested.

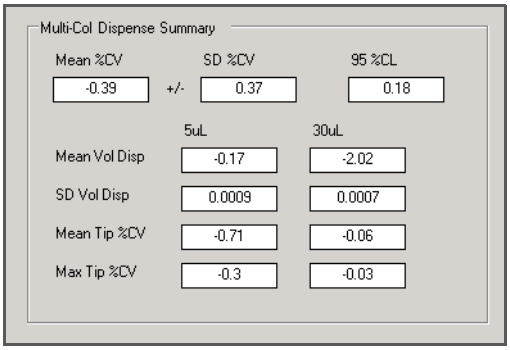


Figure 97 Updated Multi-Col Dispense Summary

LiHa Robot QC Report

After you have scanned all of the test plates and removed them from the spectrofluorometer, the software automatically generates the Robot QC report and opens it as a read-only file in Microsoft Excel. The Robot QC Report file contains six worksheets, described below.

Std Curve, Ind-Col Dispense Worksheet

The Std Curve, Ind-Col Dispense worksheet (Figure 98) contains a chart of the manually generated standard curve and the fluorescent readings from the Ind-Col Dispense plate.

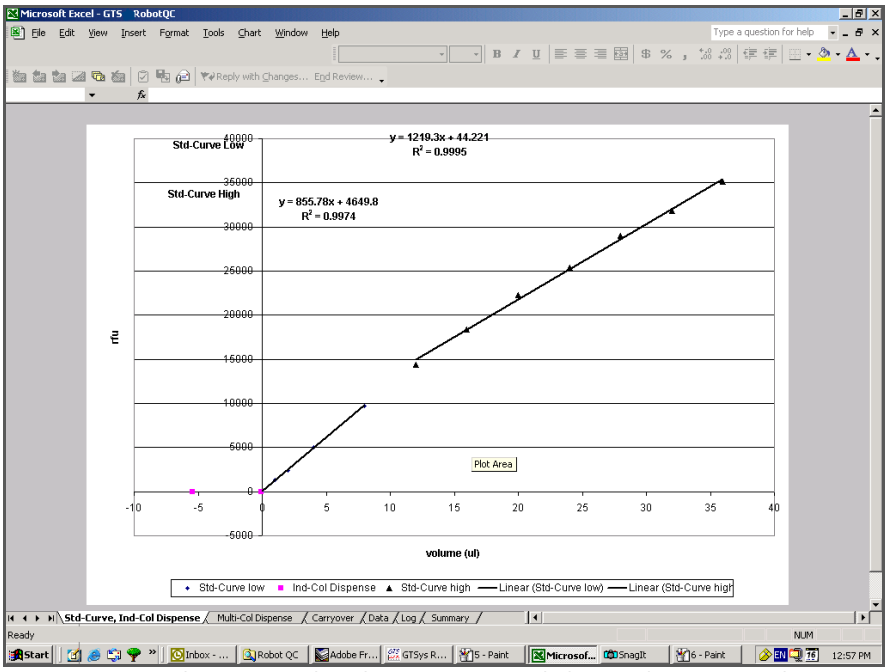


Figure 98 Std Curve, Ind-Col Dispense Worksheet Chart Example

Multi-Col Dispense Worksheet

The Multi-Col Dispense worksheet (Figure 99) contains a chart of the manually generated standard curve and the fluorescent readings from the Multi-Col Dispense plate.

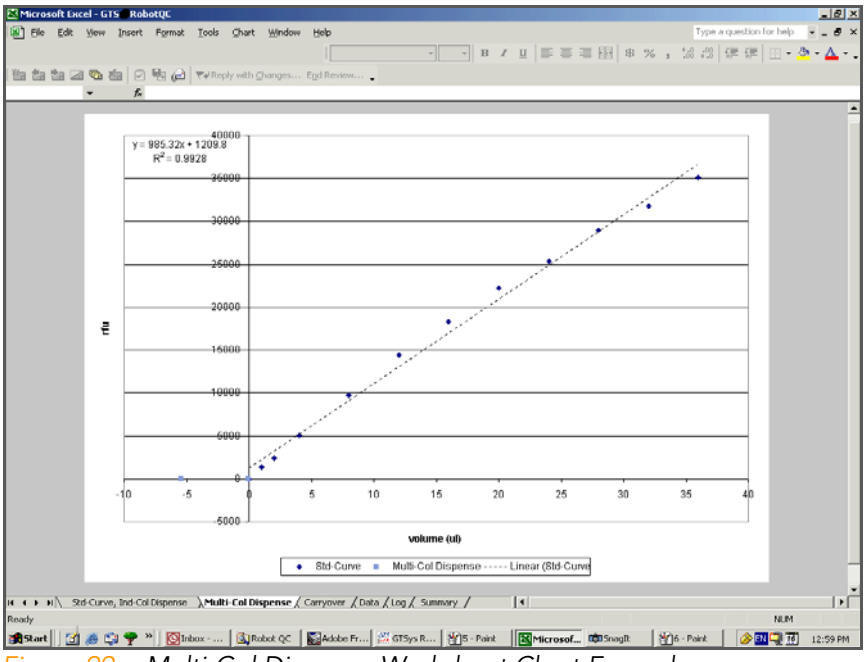


Figure 99 Multi-Col Dispense Worksheet Chart Example

Data Worksheet

The Data worksheet (Figure 100) contains the raw data generated by the reader for the Standard Curve plate and the test plates. This worksheet also contains the calculated volumes dispensed ("Result" columns) and the summary statistics for each robot tip.

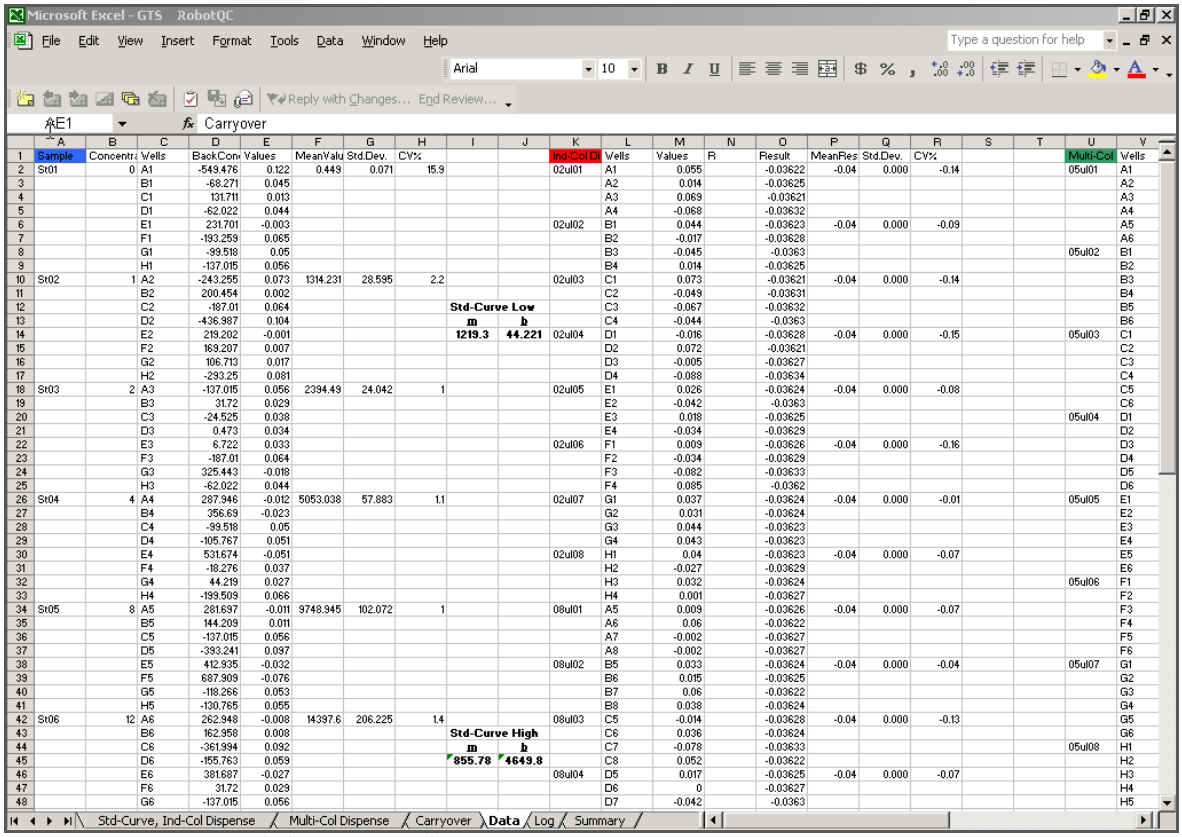


Figure 100 Data Worksheet Example

Log Worksheet

The Log worksheet (Figure 101) contains data tables and charts of the performance statistics for each robot tip. This worksheet is formatted to shade cells that perform outside of pre-set control limits.

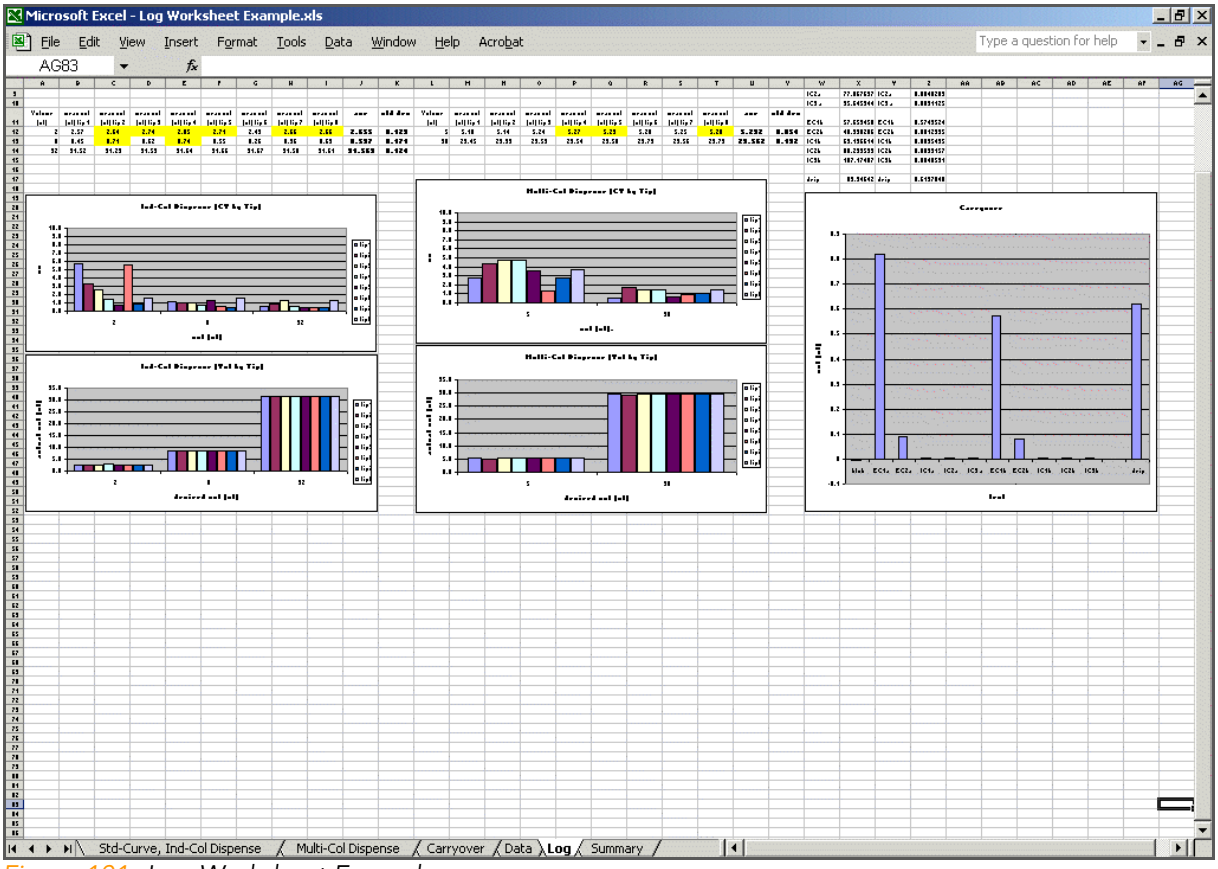
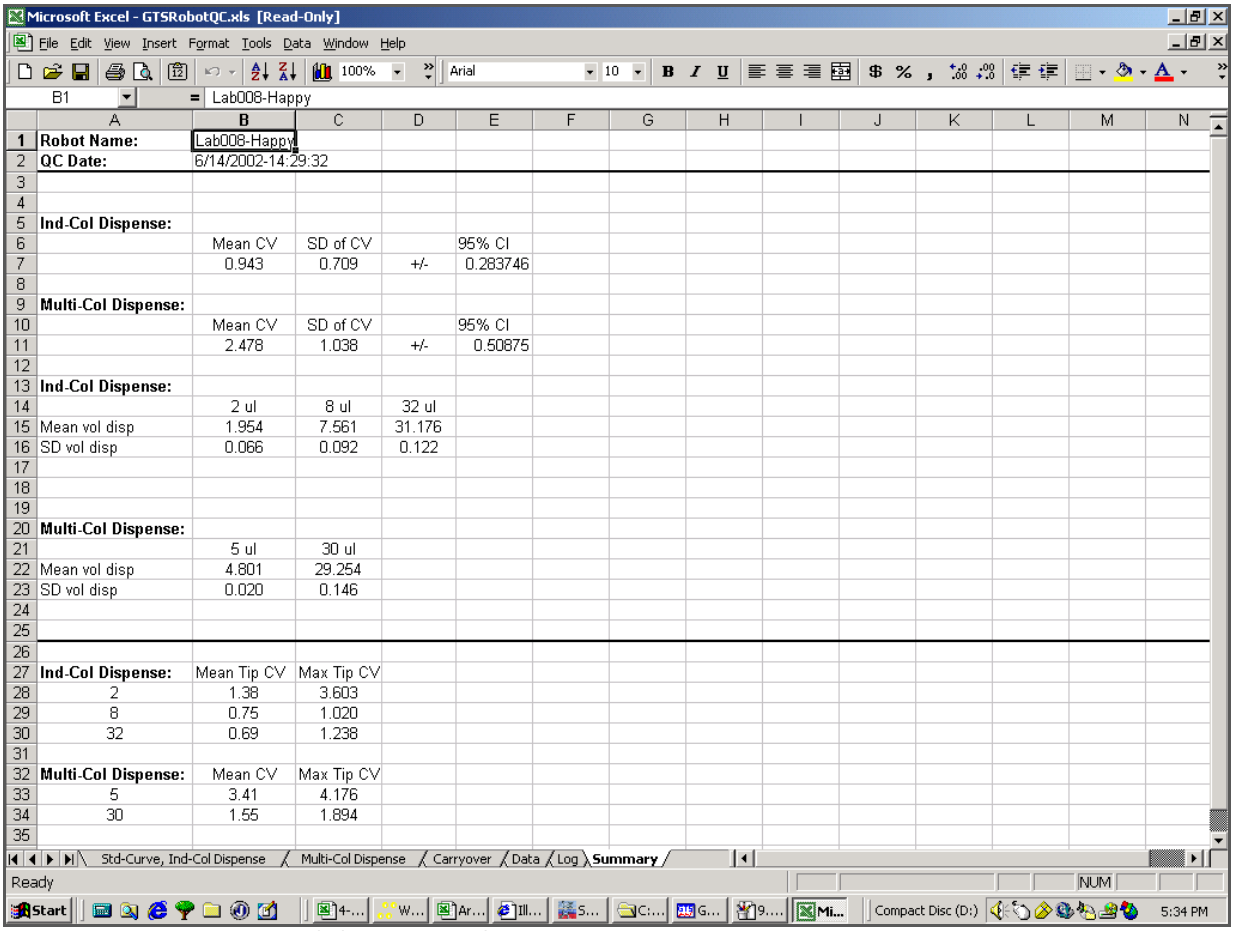


Figure 101 Log Worksheet Example

Summary Worksheet

The Summary worksheet (Figure 102) contains summary statistics for each test performed. These statistics are the same as those displayed in the GTS Robot QC software QC Summary panel (see Figure 97).



Microsoft Excel - GTSRobotQC.xls [Read-Only]

File Edit View Insert Format Tools Data Window Help

B1 = Lab008-Happy

	A	B	C	D	E
1	**Robot Name:**	Lab008-Happy			
2	**QC Date:**	6/14/2002-14:29:32			
3					
4					
5	**Ind-Col Dispense:**				
6		Mean CV	SD of CV		95% CI
7		0.943	0.709	+/-	0.283746
8					
9	**Multi-Col Dispense:**				
10		Mean CV	SD of CV		95% CI
11		2.478	1.038	+/-	0.50875
12					
13	**Ind-Col Dispense:**				
14		2 ul	8 ul	32 ul	
15	Mean vol disp	1.954	7.561	31.176	
16	SD vol disp	0.066	0.092	0.122	
17					
18					
19					
20	**Multi-Col Dispense:**				
21		5 ul	30 ul		
22	Mean vol disp	4.801	29.254		
23	SD vol disp	0.020	0.146		
24					
25					
26					
27	**Ind-Col Dispense:**	Mean Tip CV	Max Tip CV		
28	2	1.38	3.603		
29	8	0.75	1.020		
30	32	0.69	1.238		
31					
32	**Multi-Col Dispense:**	Mean CV	Max Tip CV		
33	5	3.41	4.176		
34	30	1.55	1.894		
35					

Std-Curve, Ind-Col Dispense / Multi-Col Dispense / Carryover / Data / Log / Summary

Ready NUM

Start Compact Disc (D:) 5:34 PM

Figure 102 Summary Worksheet Example

Performance Statistics

Use the summary statistics found in the Log and Summary worksheets to measure the performance of the each robot tip being tested, as well as overall robot performance.

The mean "CV" values of the Log worksheet are a measure of the variability in volumes dispensed across replicate dispenses, or tip precision in dispensing.

The "mean vol" values of the Log worksheet are used to measure accuracy in tip dispensing.

Pass/Fail Criteria for LiHa Robot QC Statistics

The LiHa QC Analysis template is used to analyze the data from the LiHa Robot QC tasks. The template calculates accuracy and precision based upon the manually generated standard cure. The individual and multi dispense volumes and CV values are computed and compared to historic QC data to determine if the robot QC tasks pass or fail. The values listed on the spreadsheet will either be highlighted in yellow, orange, or not highlighted at all. Refer to Table 8 below for a definition of each color and the action required.

Table 8 LiHa Robot QC Analysis Pass/Fail Criteria

Color	Meaning	Action
Non-colored	Values fall within an acceptable range and the robot QC passed.	No action required.
Yellow	Values fall within two out of three standard deviations of empirically measured values.	No action required. Continue to monitor statistics to make sure they are meeting acceptable passing criteria.
Orange	Values fall outside of the acceptable range and indicate failing criteria.	Run the Robot QC tasks again. If the values fail again do not run samples and contact Illumina Customer Support.

Illumina recommends focusing on the %CV values as the primary parameter for monitoring LiHa performance. The %CV is the best indicator of performance by each tip in relation to the others. A higher %CV indicates greater variance in both accuracy and precision for a given test volume.

The measured volumes for the LiHa are calculated from the standard curve and therefore directly proportional to the accuracy of the standard curve. Variation in creating the standard curve between personnel can result in variation of measured volumes between QC tests. For this reason, measured volumes that are less than or greater than the expected volume is common. It is important to use a calibrated multi channel pipette to reduce variation.

If the Robot QC is routinely failing the dispense volume tests then Illumina recommends you check your standard curve. Reanalyzing the data with an accurate standard curve could resolve the failing tests since errors with standard curves are the main cause for dispense volume issues. If the R^2 for either of the calculated standard curve lines is less than 0.98, Illumina recommends that the standard curve be regenerated.

Troubleshooting a Flagged %CV Value for the LiHa

Follow these steps to further evaluate a flagged %CV for one or more volumes:

1. Within the Log tab of the GTS Robot QC worksheet, locate the Individual Dispense CV Per Tip graph.
2. Evaluate the graph for each dispense volume and look for large spikes in the %CV's for all tips. Generally a spike would be a %CV value that was at least two or three times greater than the average for other tips.
3. Compare these values against the same tip position for other volumes.
   a. A problem with a tip will usually result in %CV spikes in the same position for more than dispense volume and type.
4. If a single or small number of spikes are apparent in only one volume/type (for example 8 µl Individual dispense), we suggest re-running that QC plate only and observing the process to look for any obvious signs of dripping or volume leakage during the run.
5. You may also refer to the Data tab within the GTS Robot QC worksheet for specific data per well and per dispense of each volume.
6. If a second re-run of the QC process fails, please contact Illumina Customer Support to schedule a service call.

Please have the QC Analysis Spreadsheet available and record any qualitative observations that are made during the QC process such as dripping tips, leaking, etc. This will be helpful to our field service personnel in troubleshooting and maintenance.

Reading MCA QC Data

Test Standard Curve and QC Plates for MCA 96-Tip

1. Launch the **SoftMax Pro** software.
2. Open the required template:
   a. Select **File | Open**.
   b. Change the Files of type to **Pro Protocol File (*.ppr)**.
   c. Select **MCAQC.ppr**.
   d. Click **Open**.
3. Each line item in the template represents a different QC plate. Place the plate to read into the loading rack and then highlight the corresponding plate in the SoftMax Pro software template as shown below for a standard curve plate.

The same standard curve plate used for the LiHa QC testing can be used for the MCA QC testing.

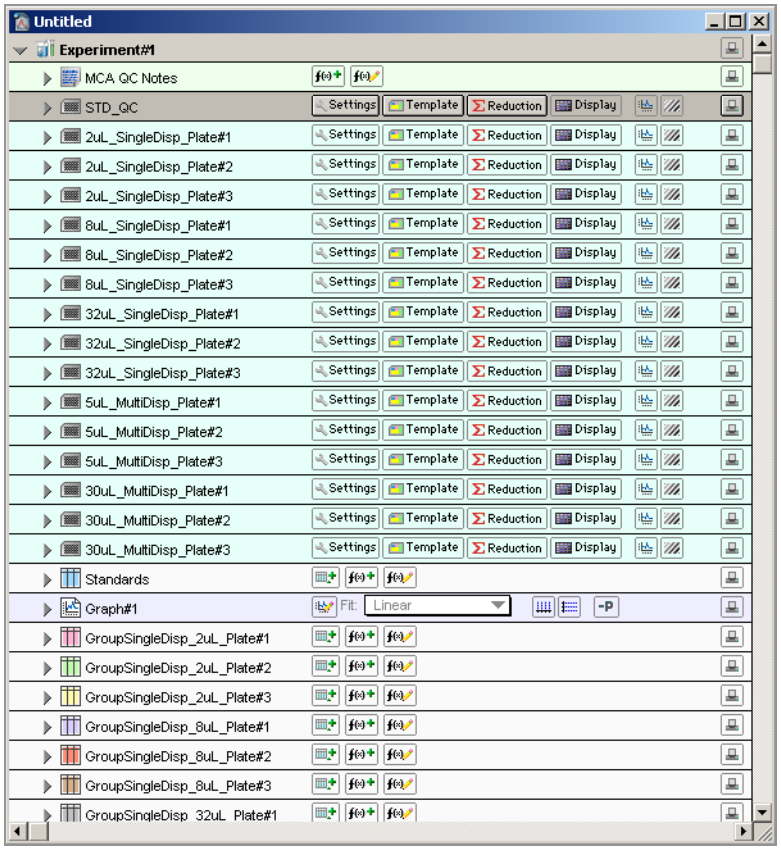


Figure 103 SoftMax Pro with Standard QC Plate Highlighted

4. Click **Read**.

   The plate will be drawn into the SpectroMax Plus reader, and the fluorescence will be measured.

5. When the software finishes reading the plate, remove the plate from the drawer.

6. Repeat Steps 3 through 5 for all remaining plates.

7. Once all plates have been read, click **File | Save As** and change the Save as type to **Pro Data Files (*.pda)**.

8. Assign a file name.

9. Recommendation: The file name should contain <Instrument Name>_MCA_<96-tip adaptor block serial number>_<date of QC run>; e.g., "Evo200#1_MCA_1321_22Oct2009."

10. Click **Save**.

11. When you have saved the *.pda file, click **File | Import/Export | Export** and export the file as a *.txt file. You can open the *.txt file in Microsoft Excel for data analysis.

12. In the Export Data to window, do the following:

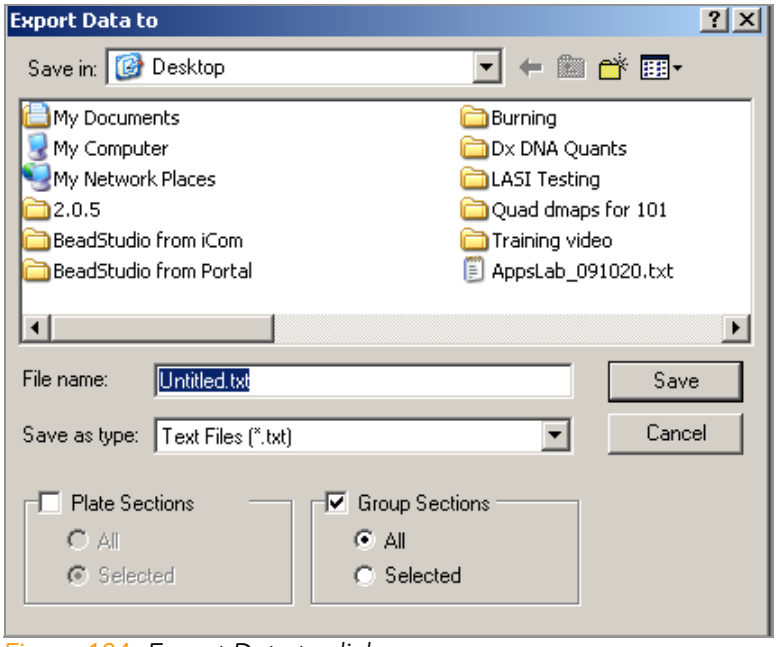


Figure 104 Export Data to dialog

a. Check the **Group Sections** box.
b. Within the Group Sections frame, select **All**.
c. Deselect the **Plate Sections** box.
d. Assign a file name.
e. Recommendation: The file name should contain <Instrument Name>_MCA_<96-tip adaptor block serial number>_date of QC run>; e.g., "Evo200#1_MCA_1321_22Oct2009."
f. Change the Save as type to **Text Files (*.txt)**.
g. Click **Save**.

MCA Robot QC Report

You must install the Illumina Add-In for Microsoft Excel before you can utilize the import data functionality within the MCA QC template. You only have to install the Illumina Add-In once per user account. There are separate steps to follow if you have Microsoft Office 2003 or 2007 installed on the PC you're using to analyze the robot QC data.

Install Illumina Add-In for Microsoft Excel 2003

To install the Illumina Add-In perform the following steps:

1. Save the MCAQC_v1_0_0.xla file to the Microsoft Add-Ins folder on the C: drive located at: C:\Documents and Settings\USERNAME\Application Data\Microsoft\AddIns.

   Save the MCA_QC_Analysis.xls file on the desktop or on the C: drive in the user defined location where the robot QC data will be saved.

NOTE

If you attempt to navigate to the Microsoft AddIns folder and it does not appear to exist you may need to show hidden files and folders. See *Display Hidden Files and Folders* on page 140.

2. Open Microsoft Excel. From the **Tools** drop down list select "Add-Ins".
3. Click **Browse** and navigate to the folder containing the "MCAQC_v1_0_0.xla" file that you saved to the Microsoft Add-Ins folder on the C: drive.
4. Select the "MCAQC_v1_0_0.xla" file and click **OK**.
5. "MCA QC" should now be listed in the **Add-Ins available** list.
6. Select "MCA QC" and click **OK**.
7. Close Excel and then open the MCA_QC_Analysis template file from the desktop or on the C: drive where the robot QC data is saved.
8. A new drop down list called "Illumina" should now be visible at the far right side of the toolbar (Figure 105).

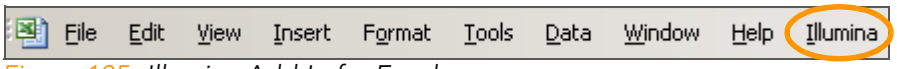


Figure 105 Illumina Add-In for Excel

Install Illumina Add-In for Microsoft Excel 2007

To install the Illumina Add-In perform the following steps:

1. Save the MCAQC_v1_0_0.xla file to the Microsoft Add-Ins folder on the C: drive located at: C:\Documents and Settings\USERNAME\Application Data\Microsoft\AddIns.

   Save the MCA_QC_Analysis.xls file on the desktop or on the C: drive in the user defined location where the robot QC data will be saved.

NOTE

If you attempt to navigate to the Microsoft AddIns folder and it does not appear to exist you may need to show hidden files and folders. See *Display Hidden Files and Folders* on page 140.

2. Open Microsoft Excel. Click the Microsoft Office Button, and then click Excel Options (Figure 106).

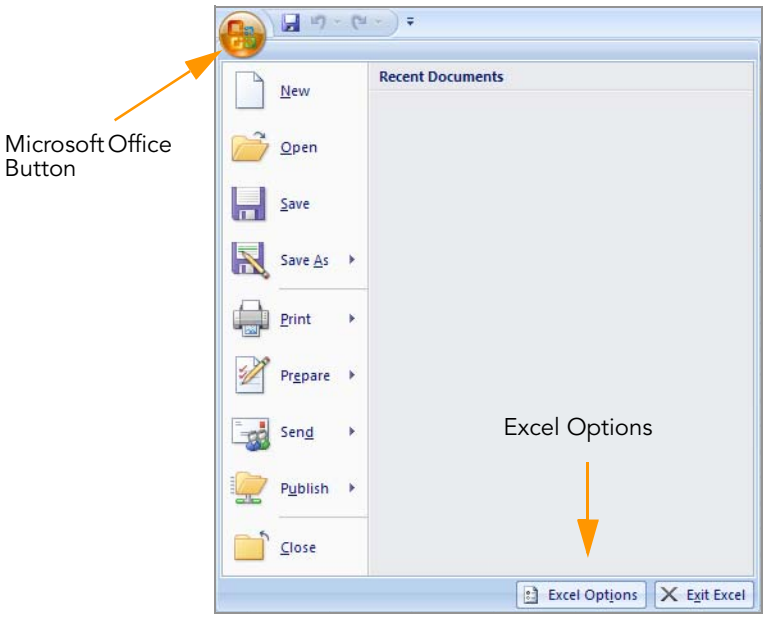


Figure 106 Excel Options

3. Click the **Add-Ins** category.
4. In the **Manage** box, click **Excel Add-ins**, and then click **Go** (Figure 107).

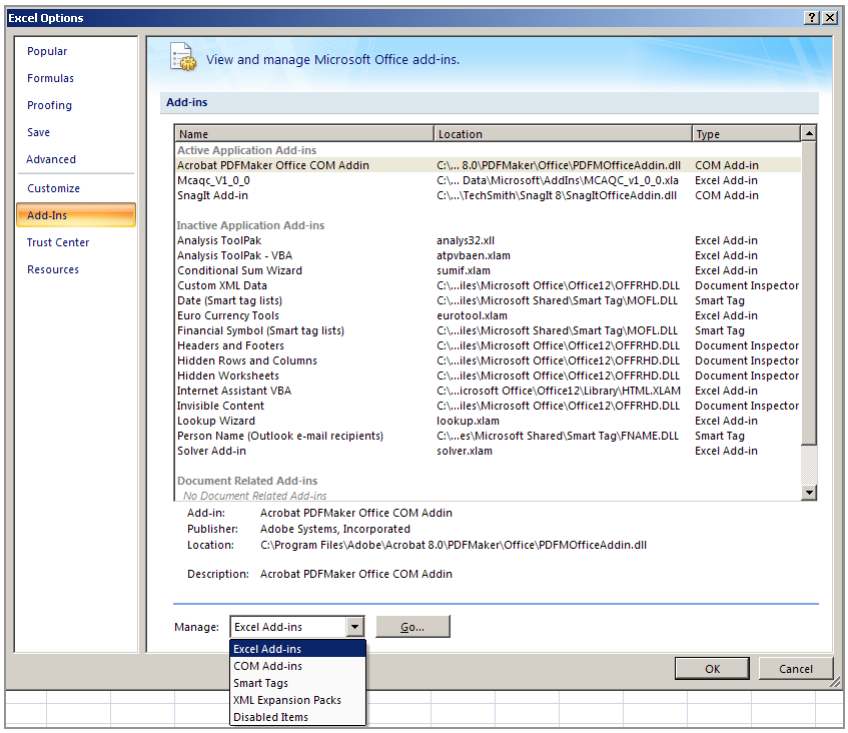


Figure 107 Selecting Excel Add-Ins

5. The **Add-ins** dialog displays (Figure 108). Click **Browse**.

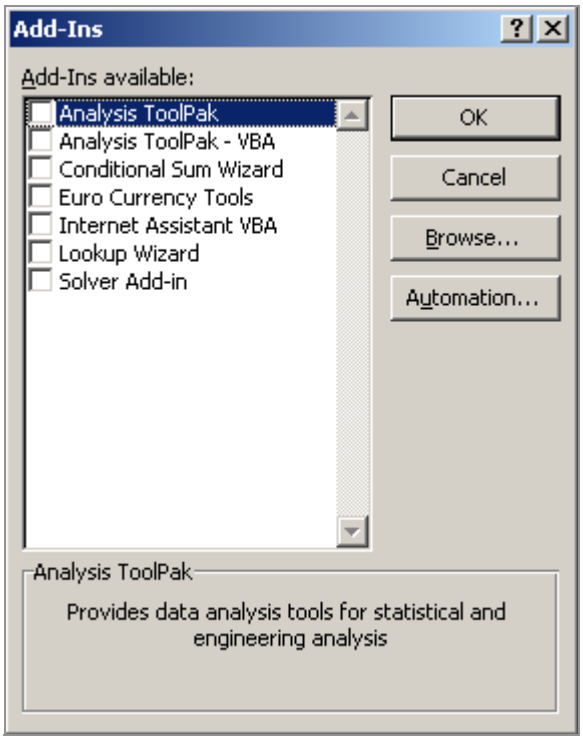


Figure 108 Excel Add-Ins Dialog

6. Excel opens the Add-Ins folder from the C: drive. The MCAQC_v1_0_0.xla file should be in the folder. Select the file and click **OK** (Figure 109).

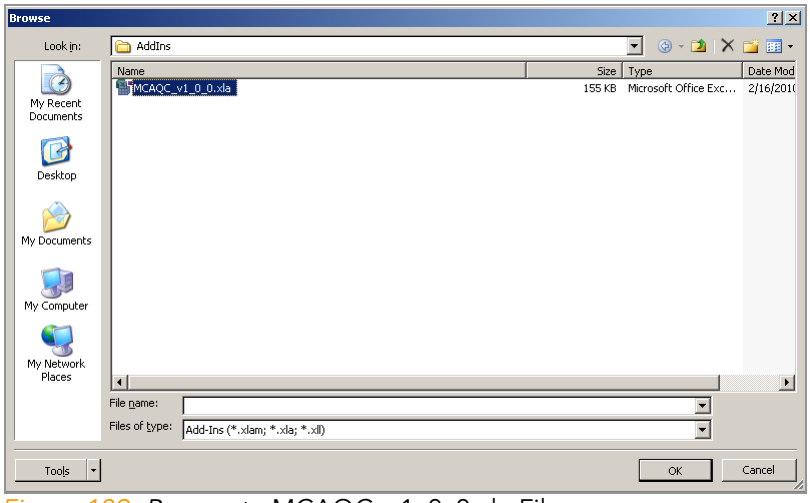


Figure 109 Browse to MCAQC_v1_0_0.xla File

7. The "MCA QC" Add-In should now be available in the **Add-Ins** list (Figure 110).

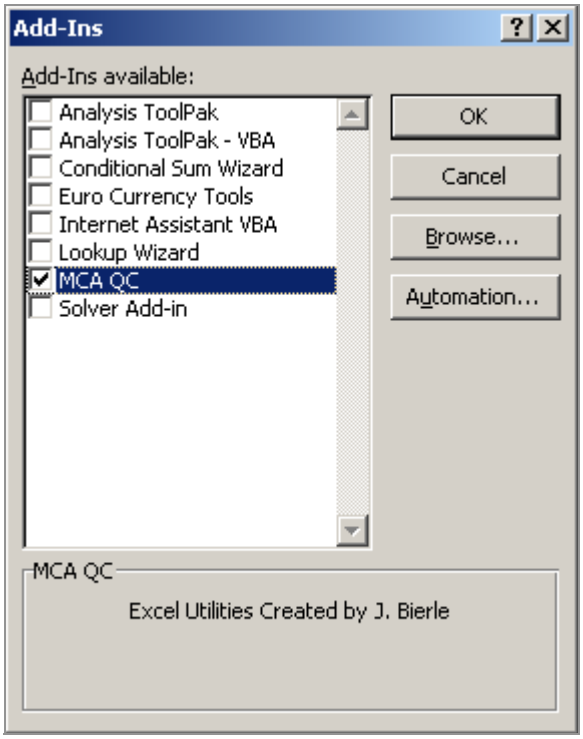


Figure 110 MCA QC File Add-In Now Available

8. Click **OK** again in the **Add-Ins** dialog box.
9. A new option called "Illumina" is now available under the **Add-Ins** tab of Excel (Figure 111).

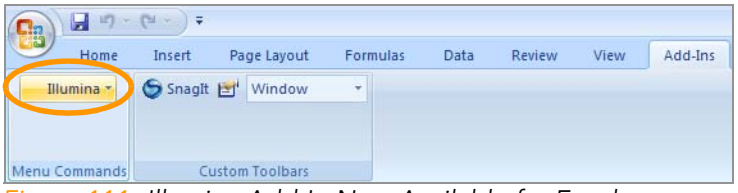


Figure 111 Illumina Add-In Now Available for Excel

Display Hidden Files and Folders

To display hidden files and folders:

1. Open Folder Options in Control Panel.
   - Click **Start**, point to **Settings**, and then click **Control Panel**.
   - Double-click **Folder Options**.

2. On the **View** tab, under **Hidden files and folders,** click **Show hidden files and folders**.

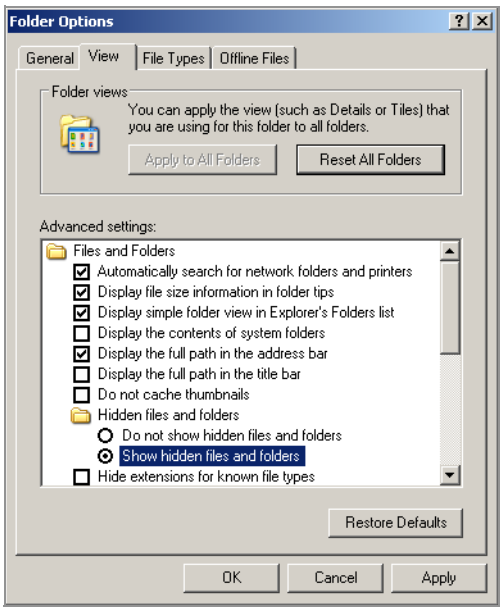


Figure 112 Folder Options: Show Hidden Files and Folders

3. You should now be able to navigate to the Microsoft Add-Ins folder on the C: drive.

Generate MCA Robot QC Report

To generate the MCA Robot QC Report:

1. Open the MCA_QC_Analysis template.
2. For Excel 2003 select "Import MCA QC Data" from the **Illumina** drop down list in the toolbar.

   For Excel 2007 select the **Add-Ins** tab and then "Import MCA QC Data" from the **Illumina** drop down list in the toolbar.

3. For a new MCA QC run check the **New Template File** Template Option and check the **All** Import Option. Click **OK** (Figure 113).

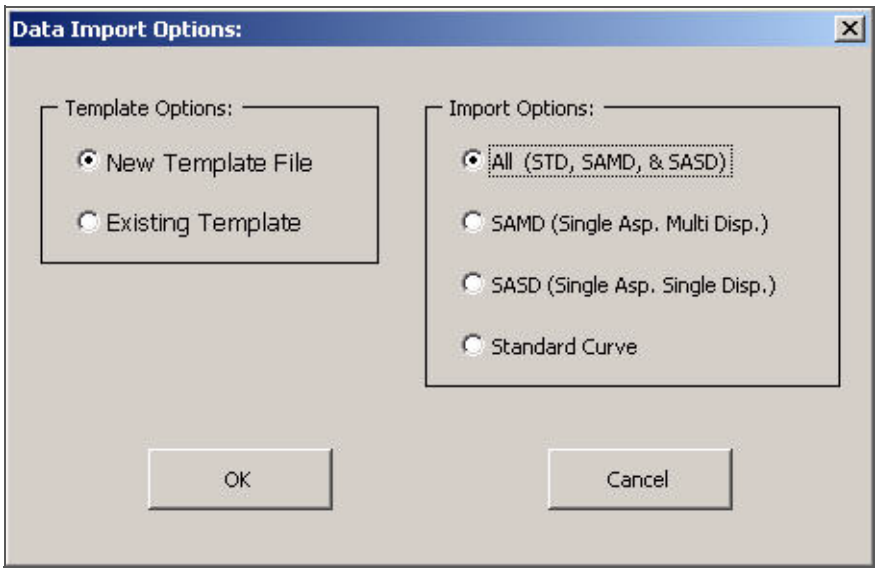


Figure 113 Data Import Options

4. The Robot QC Date field will appear and be automatically populated with the current date. Revise date as necessary. Click **OK** (Figure 114).

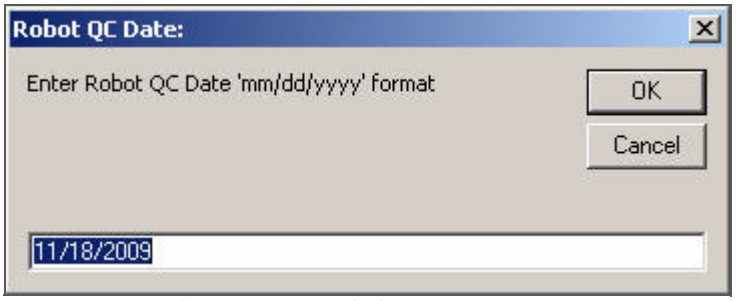


Figure 114 Robot QC Date dialog

5. Enter the Robot Name in the Robot Name field. Click **OK** (Figure 115).

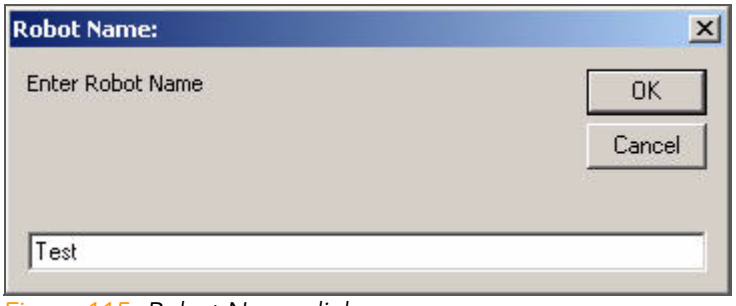


Figure 115 Robot Name dialog

6. When prompted, browse to the MCA_QC_Analysis template file to open it again and click **Open**.

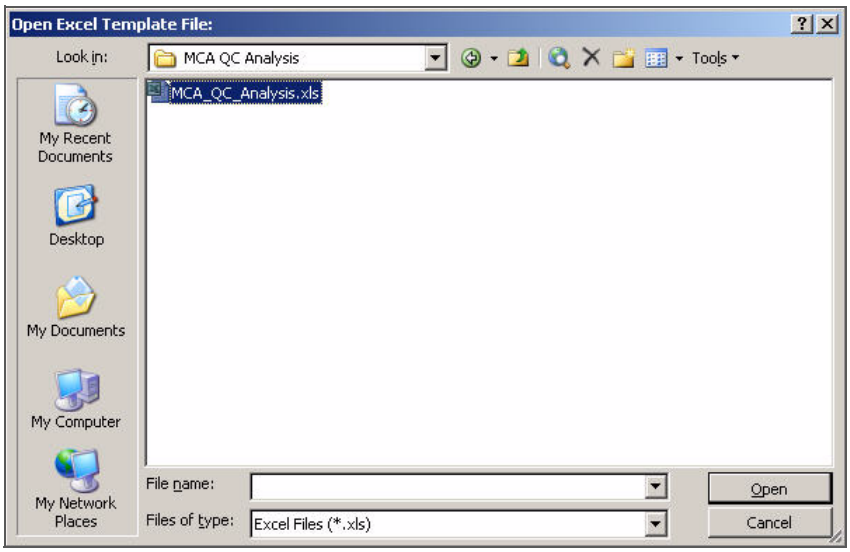


Figure 116 Opening the MCA QC Analysis Template Again

7. When prompted, browse to the desired raw data text file that was saved and exported from the Softmax Pro software and click **Open**.

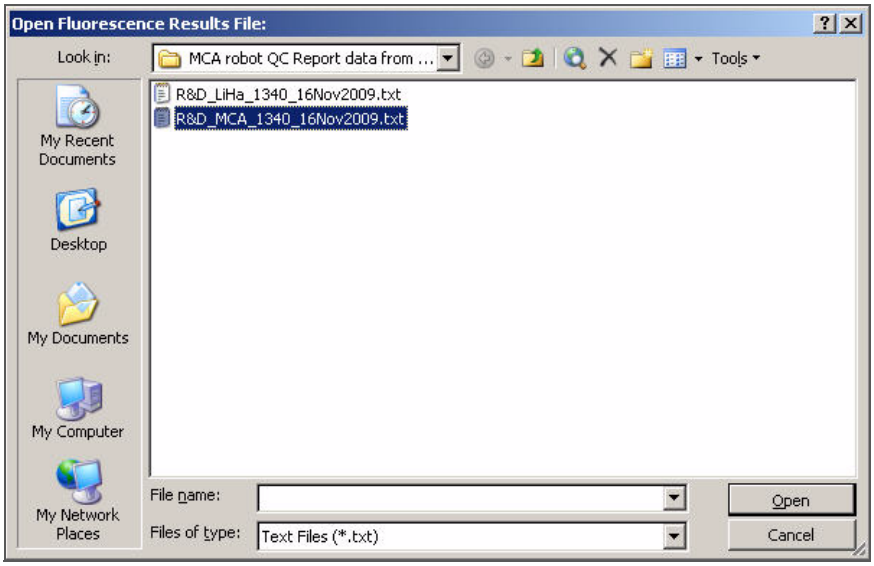


Figure 117 Opening the Fluorescence Results File

8. All fields, data tabs and plots will be generated automatically.
9. Select the "Save As" option from the Excel File drop down list and save the excel file with same name format given to the raw data and .pda files for QC data.
10. Proceed with data analysis. The template is read the same as the LiHa template, with the exception that both multi and single dispense data are now displayed on a single standard curve plot.

Repeat MCA QC Report for Existing Report

In the event that one task of the MCA QC protocol needs to be repeated, you may rerun and import a partial data set. For example, if you wanted to repeat the MCA Multi Dispense task only.

1. Open the MCA_QC_Analysis template.
2. For Excel 2003 select "Import MCA QC Data" from the **Illumina** drop down list in the toolbar.

   For Excel 2007 select the **Add-Ins** tab and then "Import MCA QC Data" from the **Illumina** drop down list in the toolbar.
3. When prompted check the **Existing Template** Template Option and check the desired Import Option:
   - SAMD (Single Asp. Multi Disp.)
   - SASD (Single Asp. Single Disp.)

   Or
   - Standard Curve.

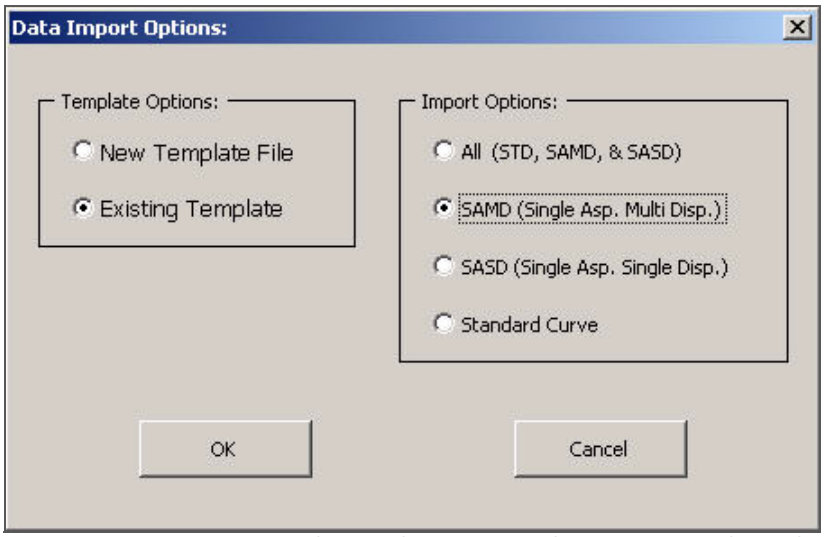


Figure 118 Existing Template with SAMD (Multi Dispense Selected)

4. Click **OK**.

5. When prompted, browse to the saved MCA_QC_Analysis file that you have already imported data into and click **Open**.

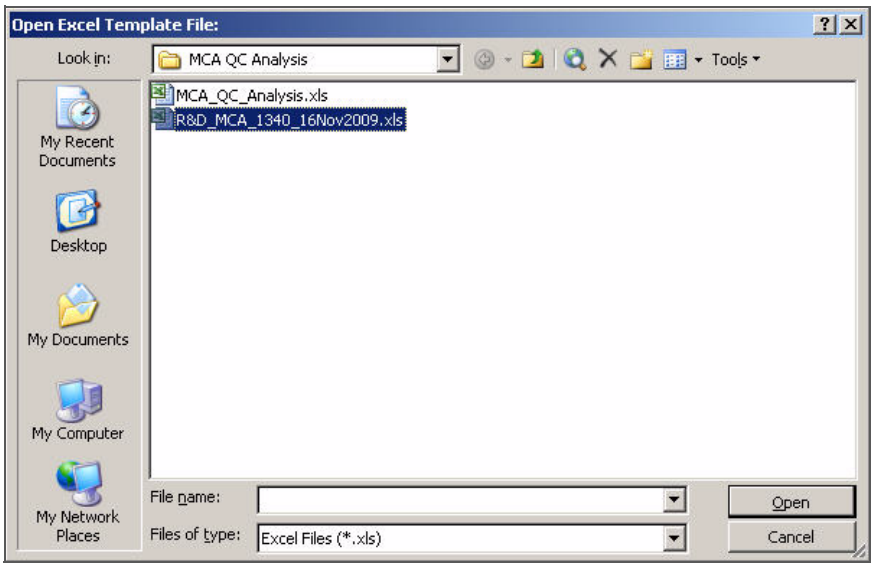


Figure 119 Opening a Saved MCA QC Analysis File

6. When prompted, browse to the desired raw data text file that was saved and exported from the Softmax Pro software and click **Open**.

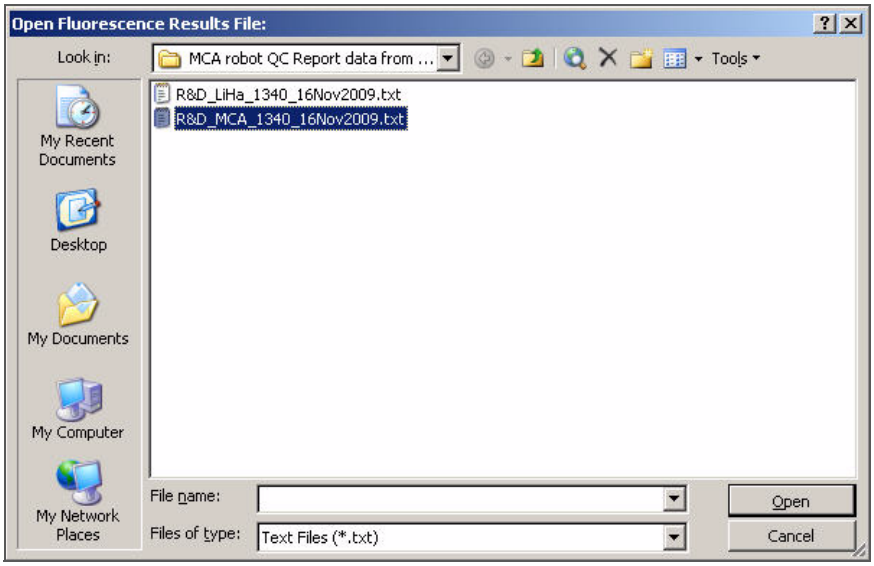


Figure 120 Opening the Fluorescence Results File

7. All fields, data tabs and plots will be generated automatically.
8. Select the "Save As" option from the Excel File drop down list and save the excel file with same name format given to the raw data and .pda files for QC data.

Robot MCA QC Report File

The Robot MCA QC Report file contains four worksheets.

Data Worksheet

The Data worksheet contains the data generated by the reader for the standard curve plate and the test plates. It also contains the calculated volumes dispensed (Result columns) and the summary statistics for each robot tip (Figure 121).

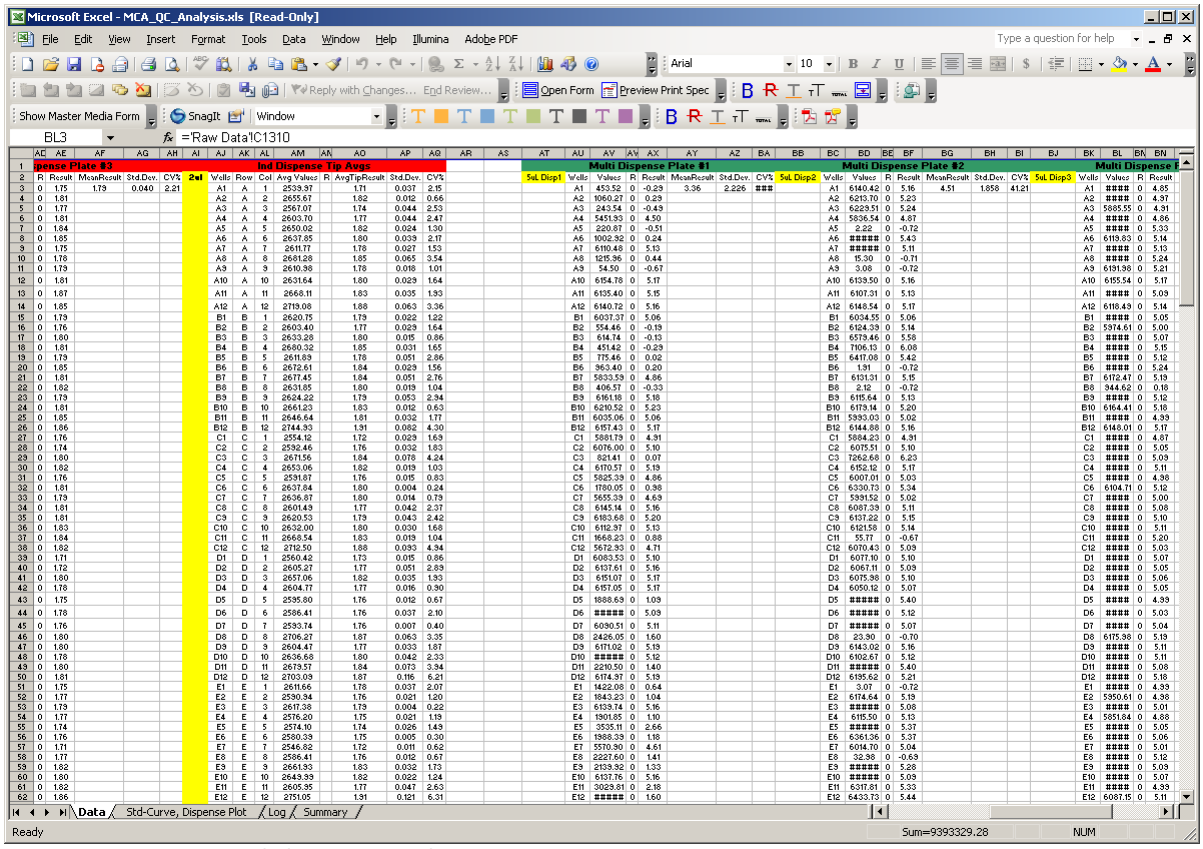


Figure 121 Data Worksheet Example

Std Curve, Dispense Plot Worksheet

The Std Curve, Dispense Plot worksheet contains a chart of the manually generated standard curve and the fluorescent readings from both the Individual and Multi Dispense plates generated by the MCA (Figure 122).

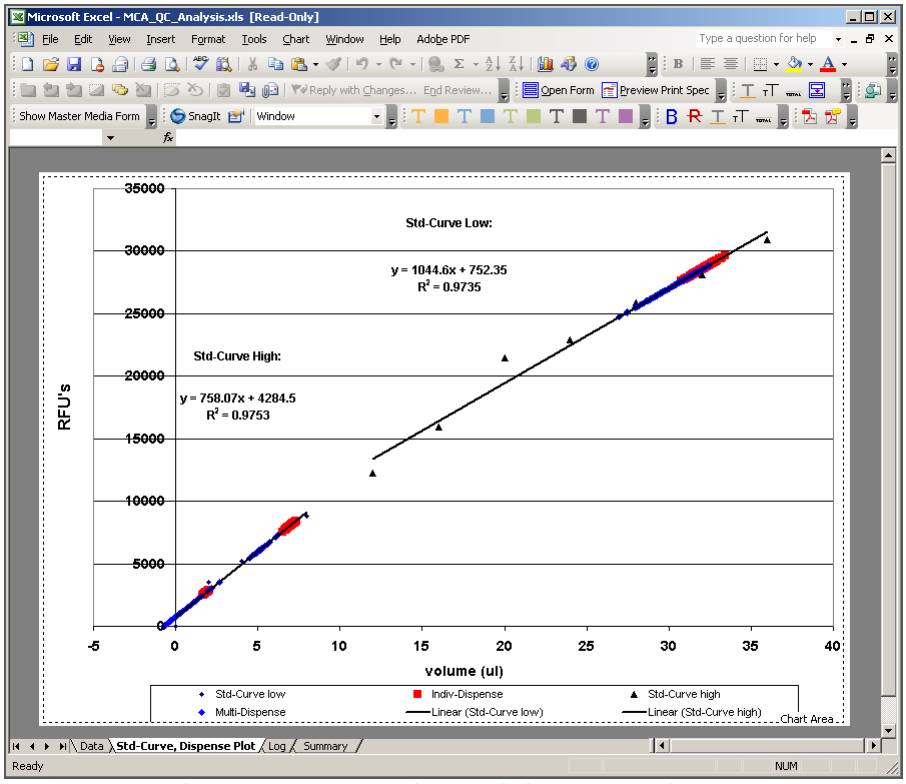


Figure 122 Std Curve, Dispense Plot Worksheet Example

Log Worksheet

The Log worksheet contains data tables and charts of the performance statistics for each robot tip. This worksheet is formatted to shade cells that are approaching or are currently performing outside of pre-set control limits (Figure 123).

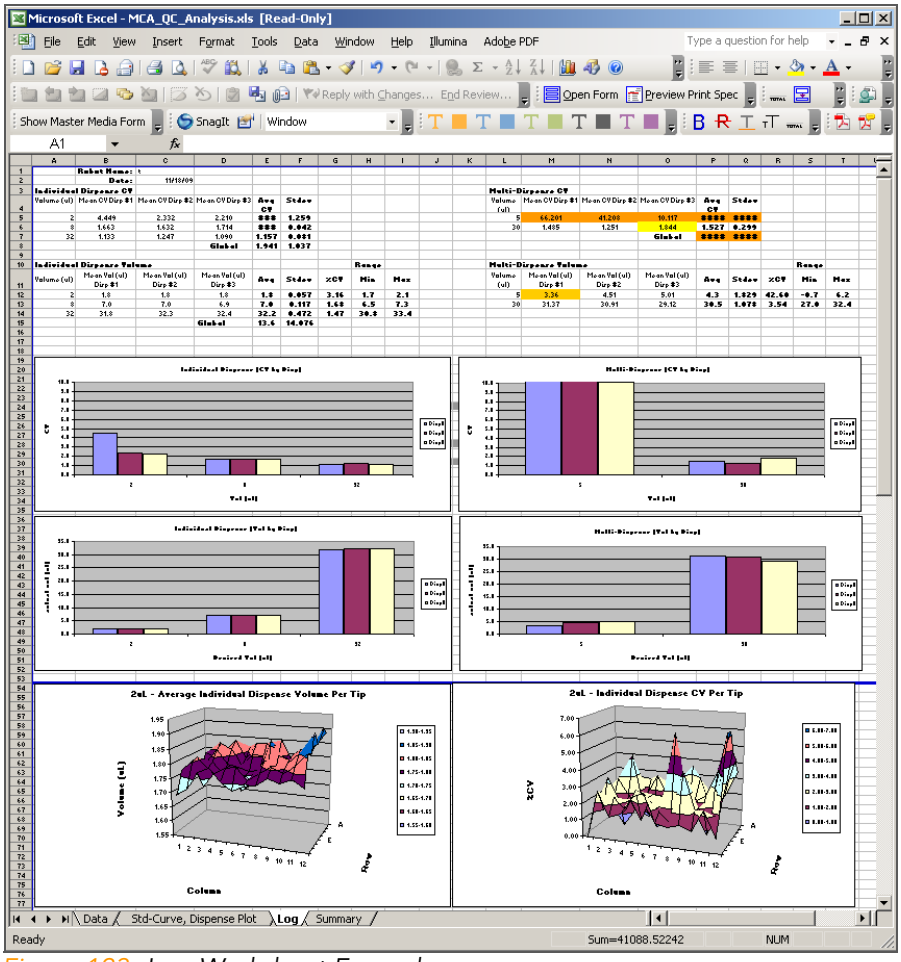


Figure 123 Log Worksheet Example

Summary Worksheet

The Summary worksheet contains summary statistics for each test performed (Figure 124).

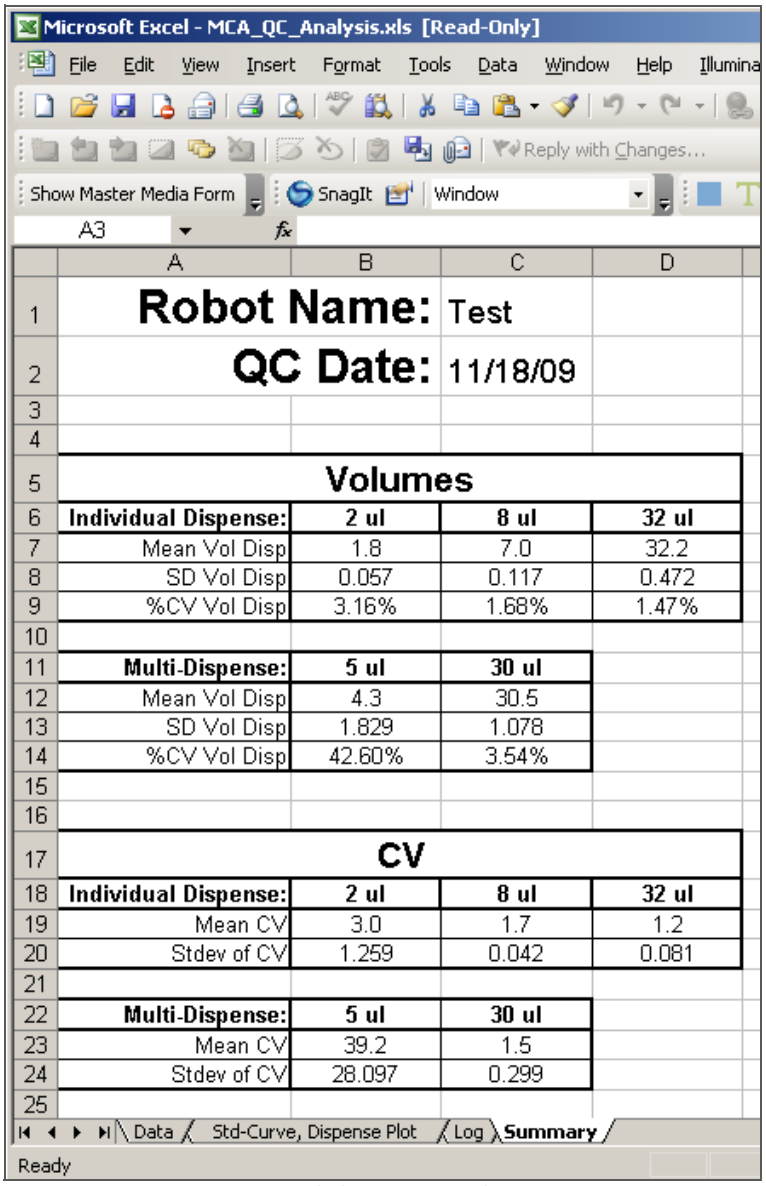


Figure 124 Summary Worksheet Example

Performance Statistics

Use the summary statistics found in the Log and Summary worksheets to measure the performance of each robot tip being tested, as well as an overall robot performance.

The mean "CV" values of the Log worksheet are a measure of the variability in volumes dispensed across replicate dispenses, or tip precision in dispensing.

The "mean vol" values of the Log worksheet are used to measure accuracy in tip dispensing.

Pass/Fail Criteria for MCA Robot QC Statistics

The MCA QC Analysis template is used to analyze the data from the MCA Robot QC tasks. The template calculates accuracy and precision based upon the manually generated standard cure. The individual and multi dispense volumes and CV values are computed and compared to historic QC data to determine if the robot QC tasks pass or fail. The values listed on the spreadsheet will either be highlighted in yellow, orange, or not highlighted at all. Refer to Table 9 below for a definition of each color and the action required.

Table 9 MCA Robot QC Analysis Pass/Fail Criteria

Color	Meaning	Action
Non-colored	Values fall within an acceptable range and the robot QC passed.	No action required.
Yellow	Values fall within two out of three standard deviations of empirically measured values.	No action required. Continue to monitor statistics to make sure they are meeting acceptable passing criteria.
Orange	Values fall outside of the acceptable range and indicate failing criteria.	Run the Robot QC tasks again. If the values fail again do not run samples and contact Illumina Customer Support.

Illumina recommends focusing on the %CV values as the primary parameter for monitoring MCA performance. The %CV is the best indicator of performance by each tip in relation to the others. A higher %CV indicates greater variance in both accuracy and precision for a given test volume.

The measured volumes for the MCA are calculated from the standard curve and therefore directly proportional to the accuracy of the standard curve. Variation in creating the standard curve between personnel has been observed and can result in variation of measured volumes between QC tests. For this reason, measured volumes that are less than or greater than the expected volume is common. It is important to use a calibrated multi channel pipette to reduce variation.

If the Robot QC is routinely failing the dispense volume tests then Illumina recommends you check your standard curve. Reanalyzing the data with an accurate standard curve could resolve the failing tests since errors with standard curves are the main cause for dispense volume issues. If the R^2 for either of the calculated standard curve lines is less than 0.98, Illumina recommends that the standard curve be regenerated.

Troubleshooting a Flagged %CV Value for the MCA

Follow these steps to further evaluate a flagged %CV for one or more volumes:

1. Within the Log tab of the MCA QC Analysis spreadsheet, locate the Individual Dispense CV Per Tip graph.
2. Evaluate the graph for each dispense volume and look for large spikes in the %CV's for all tips. Generally a spike would be a %CV value that was at least two or three times greater than the average for other tips.
3. Compare these values against the same tip position for other volumes.
   a. A problem with a tip will usually result in %CV spikes in the same position for more than dispense volume and type.
4. If a single or small number of spikes are apparent in only one volume/type (for example 8 µl Individual dispense), we suggest re-running that QC plate only and observing the process to look for any obvious signs of dripping or volume leakage during the run.
5. You may also refer to the Data tab within the MCA QC Analysis spreadsheet for specific data per well and per dispense of each volume.
6. If second a re-run of the QC process fails, please contact Illumina Customer Support to schedule a service call.

Please have the QC Analysis Spreadsheet available and record any qualitative observations that are made during the QC process such as dripping tips, leaking, etc. This will be helpful to our field service personnel in troubleshooting and maintenance.

Confirm Robot QC Pass/Fail in Illumina LIMS

After you have completed all robot QC analysis you need to confirm if they passed or failed in Illumina LIMS.

1. Log in to Illumina LIMS.
2. Select **Laboratory Management | Tecan Robot QC** (Figure 125).

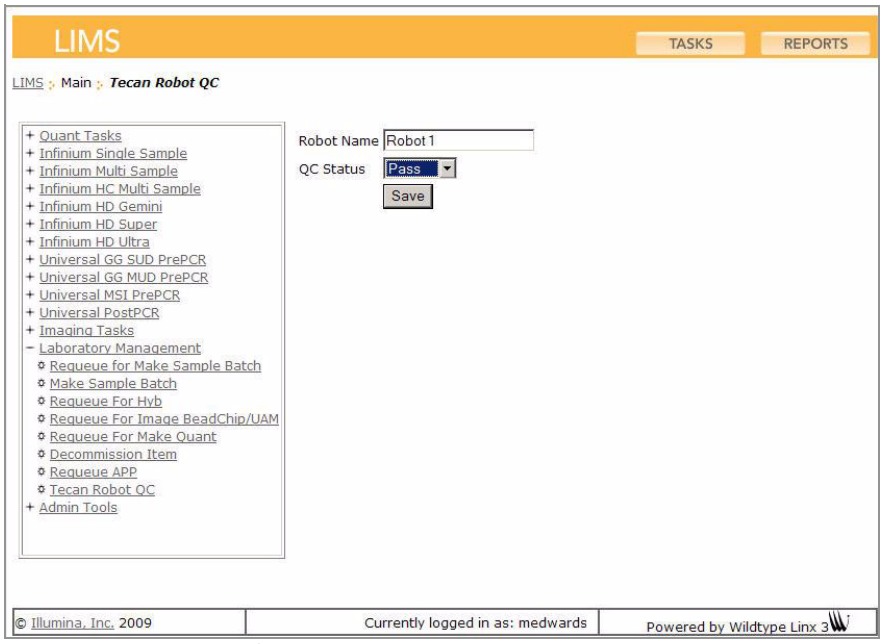


Figure 125 Tecan Robot QC

3. Enter the Robot name.
4. Select the appropriate QC status and click **Save**.

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