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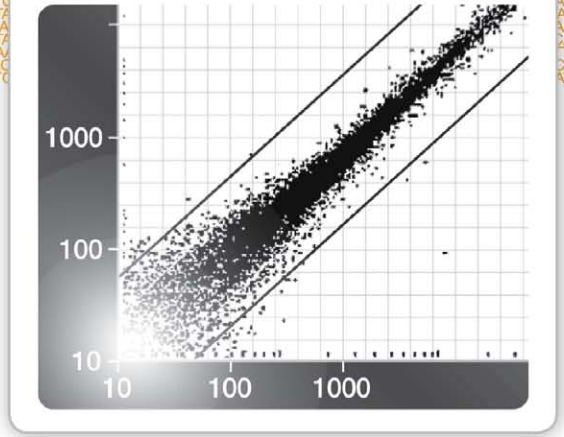
Whole-Genome Gene Expression DASL® HT Assay Guide



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Part # 15018210 Rev. D
April 2012

Revision History

Part #	Revision	Date	Description of Change
15018210	D	April 2012	• Corrected ratio of MCS4 and RTE reagent tubes per sample in the Pre-Qualify cDNA Samples procedure's consumables table. • Removed WG-DASL HT Assay Guide catalog number.
15018210	C	December 2011	• MCS3 reagent replaced with MCS4 and RTE and the protocol revised. • *DASL Single-Use cDNA Synthesis Kit* renamed to *cDNA Synth MCS4 Single-Use Kit*. • AM1 Washes - Added step to place ASE plate on magnetic plate.
15018210	B	December 2010	Specify Image Beadchip scan settings.
15018210	A	December 2010	Initial release

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Overview

Chapter 1

Introduction

The Illumina® BeadStation, equipped for the Illumina Whole-Genome Gene Expression DASL® (c**D**NA-mediated **A**nnealing, **S**election, Extension, and **L**igation) High Throughput (HT) Assay, is an efficient and cost-effective gene expression system. The system combines a novel, highly multiplexed expression assay, BeadChips, and a precise confocal scanning system (the Illumina HiScan® or iScan System or the Illumina BeadArray™ Reader) to deliver unparalleled data quality, sample throughput, and cost savings. The WG-DASL HT Assay incorporates unique methodologies enabling expression measurements in RNA samples that have undergone partial degradation, such as RNAs derived from formalin-fixed, paraffin-embedded tissues. The WG-DASL HT Assay offers parallel analysis of up to 12 samples at once, and is adaptable to high throughput automation.

The WG-DASL HT Assay offers:

- Sample throughput of 12 samples on a BeadChip
- Highest built-in feature redundancy of any currently available array platform
- Low sample requirements (approximately 100 ng intact RNA or 200 ng partially degraded RNA per multiplexed assay)
- Quality controlled reagent set for consistent assay performance

WG-DASL HT Assay

The WG-DASL HT Assay uses biochemistry similar to that in the Illumina DASL process. RNA is first converted to cDNA through a reverse transcription reaction with biotinylated primers. The biotinylated cDNA is then annealed to assay oligonucleotides (oligos), and bound to streptavidin conjugated paramagnetic particles (SA-PMPs). After the oligo hybridization, mis-hybridized and non-hybridized oligos are washed away. The hybridized oligos are then extended and ligated. These products form a synthetic template that is transferred to a PCR reaction containing a fluorescently labeled primer. The labeled PCR product strand is then isolated and the fluorescent products hybridized to a whole-genome expression BeadChip. The BeadChip is then washed and imaged on the HiScan or iScan System or BeadArray Reader.

RNA Samples

Because the WG-DASL HT Assay begins with a reverse transcription reaction, input RNA must be free of enzymatic inhibitors such as guanidine or formamide. The RNA from tissue or cell samples is purified using any standard method, followed by resuspension in DEPC-treated water. The WG-DASL HT Assay can accept intact total RNAs in concentrations ranging from 20–100 ng/µl. Lower levels of RNA input decrease reproducibility among replicates, and are not conducive to optimal assay performance.

Partially Degraded RNAs

The WG-DASL HT Assay can also be used to monitor gene expression in partially degraded RNAs, such as those derived from formalin-fixed, paraffin embedded (FFPE) tissues. For these RNAs, the WG-DASL HT Assay performs best with FFPE-derived RNAs ranging from 40 to 200 ng/µl, to compensate for the reduced abundance of intact target sequences.

Recommended Purification Kit

The method used for purification of RNA derived from FFPE tissues substantially impacts both the degree of RNA degradation and the performance of the RNA in the WG-DASL HT Assay. Illumina recommends the High Pure RNA Paraffin Kit from Roche Applied Science.

NOTE
In our experience, the Roche kit results in superior quality RNA as compared to other commercially available kits for RNA preparation from FFPE samples.

In brief, RNA purification using the High Pure RNA Paraffin Kit includes these steps:

1. FFPE tissues are cut into 5 µm sections, then deparaffinized.
2. Sections are extracted in ethanol, then homogenized by overnight incubation in Proteinase K.
3. Nucleic acids are bound to a glass fiber filter in the presence of guanidine salts.
4. Bound nucleic acids are washed, and RNA is preferentially eluted.

5 Residual DNA is digested using DNase I, followed by an additional Proteinase K digestion.

6 The RNA is bound to another glass fiber filter, washed, and eluted.

7 RNA yield is measured and the RNA is stored at -80°C.

Illumina recommends strict adherence to the High Pure RNA Paraffin Kit protocol. Based on our experience, important parameters include the following:

- Paraffin sections should not exceed 5 µm thickness, to allow adequate penetration by Proteinase K.
- Proteinase K should be freshly prepared. Frozen aliquots of Proteinase K are acceptable only if they have been stored for less than 1 month.
- Freshly cut sections are preferred, but sections may be stored for up to 1 week in xylene at room temperature. The xylene should be replaced before ethanol extraction.
- RNA yields are higher if 4 or 5 thin sections are pooled into one tube for RNA purification.
- Complete homogenization of the tissue during the first proteinase K digestion is a good predictor of adequate RNA yield.

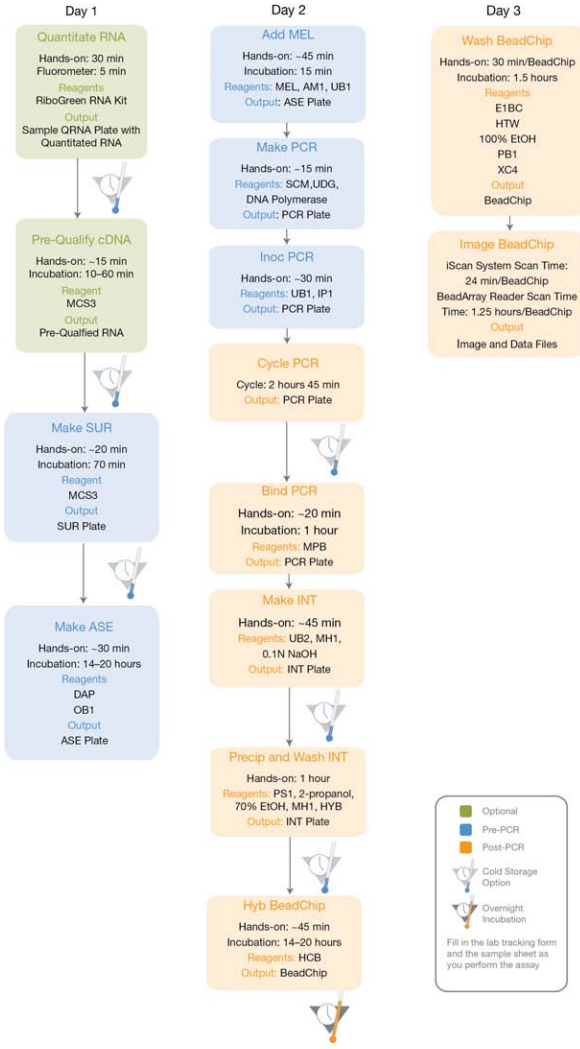
Storing Paraffin Sections

In accumulating paraffin sections for batch RNA extraction, store sections in xylene, rather than exposed to air. Sections may be stored in xylene for up to 1 week, then washed in fresh xylene to begin the RNA extraction with the High Pure RNA Paraffin Kit.

WG-DASL HT Assay Workflow

This section describes the overall Illumina WG-DASL HT Assay workflow.

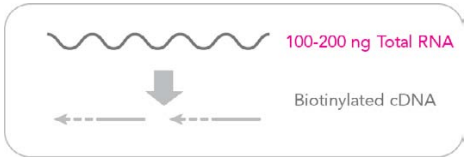
Figure 1 WG-DASL HT Assay Workflow



Reverse Transcribe RNA (Make SUR)

The assay starts by reverse transcribing sufficient RNA with a SUR enzyme (Master Mix cDNA Synthesis for Single Use 3) from each individual sample to be used in the WG-DASL HT Assay.

Figure 2 Reverse Transcribe RNA with SUR enzyme



See *Make Single-Use RNA (SUR) Plate* on page 60.

Hybridize cDNA to Oligos (Make ASE)

The WG-DASL HT Assay monitors gene expression by targeting sequences with chimeric-oligos containing universal PCR amplification primer sites. Two assay-specific oligos (ASOs) are used to assay each transcript: an upstream-specific oligo (USO) and a downstream-specific oligo (DSO), each of which contains a universal PCR priming site and a gene-specific sequence that complements a corresponding capture sequence on the BeadChip.

29,285 unique assay probes are used, corresponding to up-to-date content derived from the National Center for Biotechnology Information Reference Sequence (NCBI RefSeq) database (Release 38 (Nov 7, 2009).

In this process, the biotinylated cDNA is annealed to the query ASOs. This mixture is bound to streptavidin-conjugated paramagnetic particles (SA-PMPs) to select the cDNA/oligo complexes.

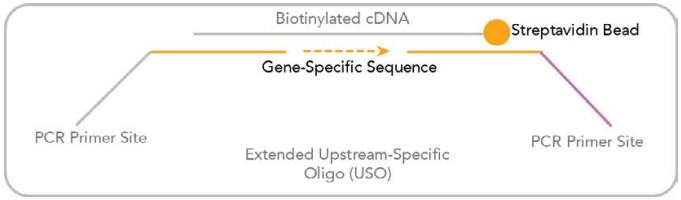
Figure 3 Hybridize cDNA to Assay Oligonucleotides

See *Make Assay-Specific Extension (ASE) Plate* on page 63.

Assay-Specific Primer Extension (Add MEL)

cDNA templates are hybridized with the set of ASOs that corresponds to all the targeted transcripts. After the oligo annealing, mis-hybridized and non-hybridized oligos are washed away. A polymerase is then added, causing the USOs to undergo extension (i.e., second-strand cDNA synthesis) and ligation to its corresponding DSO, thereby creating a template for PCR. The USOs are extended only if their 3′ bases are complementary to the cognate sequence in the cDNA template.

Figure 4 Extend Assay-Specific Primers

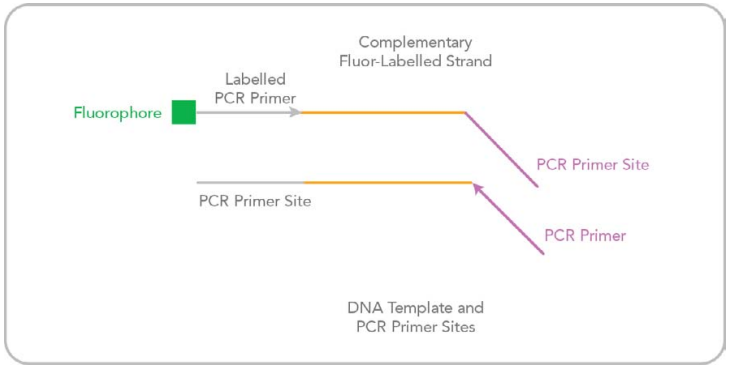


See *Add MEL* on page 69.

Universal PCR (Make, Inoc, Cycle PCR)

Because all query oligos share the same universal primer landing sites, the cDNA templates are all amplified with a pair of common PCR primers. The primer on the strand that is complementary to the array is fluorescently labelled.

Figure 5 Universal PCR



See:

- *Make PCR Plate* on page 70
- *Inoculate PCR Plate* on page 72
- *Thermal Cycle PCR Plate* on page 76

Bind PCR Product

After PCR amplification, the labelled, single-stranded product is prepared for hybridization to the BeadChip.

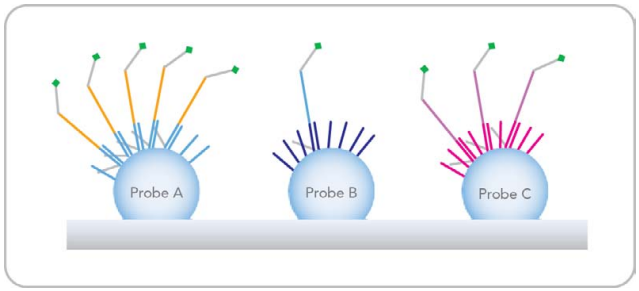
Figure 6 Bind PCR Product

See *Bind PCR Products* on page 77.

Hybridize Dye-Labelled Strand (Make, Precip and Wash INT, Hyb BeadChip)

The labelled strand is hybridized to the bead on the BeadChip containing the complementary gene-specific sequence.

Figure 7 Hybridize Strand to BeadChip



See:

- *Make Intermediate (INT) Plate* on page 80
- *Hybridize BeadChip* on page 87

Wash, Image BeadChip

The Illumina iScan System or BeadArray Reader measures fluorescence intensity at each addressed bead location. The intensity of the signal corresponds to the quantity of the respective transcript in the original sample.

See:

- *Wash BeadChip* on page 95
- *Image BeadChip* on page 111

BeadChips

The 12-sample BeadChip platform is composed of individual arrays manufactured on a microscope slide-shaped substrate. Each individual array on the BeadChip holds thousands to tens of thousands of different oligonucleotide probe sequences that are in turn attached to 3-micron beads assembled into the micro-wells of the BeadChip substrate. Because the micro-wells outnumber probe sequences, multiple copies of each bead type are present in the array. This built-in redundancy improves robustness and measurement precision. The BeadChip manufacturing process includes hybridization-based quality control of each array feature, allowing consistent production of high-quality, reproducible arrays.

HiScan, iScan, BeadArray Reader, AutoLoader and AutoLoader2

BeadChips are imaged using the Illumina HiScan or iScan System or BeadArray Reader. Each of these is a high-resolution laser imager that scans BeadChips and creates an image file for each channel. The HiScan or iScan System incorporate advanced optics and sensors to support higher throughput than the BeadArray Reader, while providing equally high data quality.

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

Loading and unloading of BeadChips into the HiScan or 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		X
Integrated with BeadScan software	X	
Integrated with Illumina LIMS	X	X
Email alert system	X	X
Single-reader or dual-reader configuration	X	X
Number of BeadChips supported per carrier	2	4
Number of carriers processed at a time	20	48

GenomeStudio

GenomeStudio, Illumina's new 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 Gene Expression Module is an application for analyzing mRNA expression data from scanned microarray images collected from systems such as the Illumina HiScan or iScan System or BeadArray Reader. Experiment performance is based on built-in controls that accompany each experiment. GenomeStudio Gene Expression Module results can be exported and analyzed by most standard gene expression analysis programs. You can perform these analyses on individual arrays or on groups of arrays treated as replicates.

Data analysis features of the GenomeStudio Gene Expression 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
- Gene expression and differential expression analysis
- Outlier removal for negative controls
- Ability to combine/merge methylation data into a gene expression project
- Ability to combine/merge mRNA data into a miRNA project
- Data imputation for missing probes on an array
- Assay-specific controls dashboards

For feature descriptions and instructions on using the GenomeStudio platform to visualize and analyze miRNA data, see the *GenomeStudio Framework User Guide* and the *GenomeStudio Gene Expression Module User Guide*.

Illumina Lab Protocols

Illumina lab protocols are designed to promote efficiency and minimize the risk of contamination. Chapter 2 Standard Operating Procedures describes the standard operating procedures and tools for an Illumina assay lab and explains how to set up and maintain separate pre- and post-PCR areas.

For instructions on how to perform the WG-DASL HT Assay protocol on BeadChips, see Chapter 3 WG-DASL HT Assay Lab Protocols.

Chapter 2

Standard Operating Procedures

Introduction

This chapter explains standard operating procedures and precautions for operating an Illumina assay lab. You will also find lists of standard equipment, materials, and reagents.

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

Acronyms

Table 2 WG-DASL HT Assay Acronyms

Acronym	Definition
AM1	Add MEL 1 Reagent
ASE	Assay-Specific Extension Plate
ASO	Assay Specific Oligo
cDNA	Complementary DNA
DAP	DASL Assay Pool
DASL	cDNA Mediated Annealing, Selection, Extension, and Ligation
DEPC	Diethyl Pyrocarbonate
DNA	Deoxyribonucleic Acid
DSO	Downstream-Specific Oligo
E1BC	Wash Buffer
EtOH	Ethanol
HCB	Humidity Control Buffer
HT	High Throughput
HTW	High Temperature Wash Buffer
Hyb	Hybridize or Hybridization
HYB	Hybridization Buffer
INT	Intermediate Plate

Acronym	Definition
IP1	Inoc PCR 1 Reagent
MCS4	Master Mix cDNA Synthesis for Single Use 4
MEL	Master Mix for Extension/Ligation Reagent
MH1	Make Hyb 1 Reagent
MPB	Magnetic Particle B Reagent
NaOH	Sodium Hydroxide
OB1	Oligo Hybridization & DNA Binding Buffer 1 Reagent
PB1	Reagent used to prepare BeadChips for hybridization
PCR	Polymerase Chain Reaction Plate
PMPs	Paramagnetic Particles
PS1	Precipitation Solution Reagent
QRNA	RNA Quantification
RNA	Ribonucleic Acid
RNase	Ribonuclease
RTE	Reverse Transcriptase Enzyme
SCM	Single Color Master Mix
SDS	Sodium Dodecyl Sulfate
SUR	Single-Use RNA Plate
UB1	Universal Buffer 1 Reagent
UB2	Universal Buffer 2 Reagent

Acronym	Definition
UDG	Uracil DNA Glycosylase
USO	Upstream-Specific Oligo
WG-DASL	Whole-Genome Gene Expression DASL
XC4	XStain BeadChip Solution 4
xg	Multiple of Gravitational Acceleration

Lab Setup

Lab setup procedures should be performed for WG-DASL HT Assay labs.

Separate Pre- and Post-PCR Areas

The WG-DASL HT Assay uses a PCR process to amplify specific sample sequences. The laboratory space where pre-PCR processes (including sample extraction, quantification and normalization) are performed should be physically separated from the laboratory space where amplified products are made and processed (post-PCR processes).

Prevent PCR Product Contamination

Unless sufficient caution is exercised, PCR products may contaminate reagents, instrumentation, and samples, causing inaccurate and unreliable results. PCR product contamination can shut down lab processes and significantly delay resumption of normal operations.

If possible, perform the pre-PCR processes in a separate, dedicated laboratory space.

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

Dedicated Equipment and Supplies

Dedicate separate sets of instruments (pipettes, centrifuges, oven, heat block, etc.) to the pre-PCR and post-PCR areas. Never share the instruments between areas.

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

- Never use the same sink to wash pre-PCR and post-PCR reservoirs.
- Never share the same water purification system for pre-PCR and post-PCR processes.
- Store all assay protocol supplies in the pre-PCR area, and transfer to the post-PCR area as needed.

Prepare Batches of 0.1N NaOH

To minimize errors in preparing 0.1N NaOH fresh each day, prepare it in large batches and aliquot it into 50 ml sealed tubes. These aliquots may be stored up to 6 months at 2° to 8°C and used in the protocol as needed. Once you open an aliquot, use it on the same day that it was opened. Discard any reagent that is left at the end of the day.

FIFO

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

Lab Maintenance

Standard lab maintenance procedures should be performed for WG-DASL HT Assay labs.

Daily and Weekly Cleaning

CAUTION

To prevent sample or reagent degradation, ensure all sodium hypochlorite (bleach) vapors have fully dissipated before starting any processes.

Post-PCR Area

Reducing the amount of product in the post-PCR area helps reduce the risk of contamination in the pre-PCR area.

Identify post-PCR area "hot spots" that pose the highest risk of contamination, and clean these items daily with a solution of 0.5% sodium hypochlorite (10% bleach). Typical hot spots include:

- Bench space
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards
- Centrifuges
- Vortexers
- Thermal cyclers

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

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

Pre-PCR Area

Establish a daily and weekly cleaning schedule for the pre-PCR area similar to the one in post-PCR. This will help to eliminate product that may have entered the pre-PCR area.

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

- Bench tops
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards

Once a week, thoroughly clean the entire pre-PCR area, including all of the bench tops and instruments that are not cleaned daily. Mop the floors with a 0.5% sodium hypochlorite (10% bleach) solution as well.

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

Safety Precautions

CAUTION

The protocols described in this guide should be performed by qualified laboratory personnel only. Exercise caution when handling biological samples to avoid cross-contamination among pre-amp and post-amp samples.

WARNING

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, see the MSDS for this kit, which is available at http://www.illumina.com/msds.

References

Please visit http://www.illumina.com/msds to see the latest material data safety sheets.

CAUTION

Please refer to governmental and facility safety standards applicable to your site.

Best Practices

To optimize your data and minimize errors and waste, read and follow best practices whenever performing the WG-DASL HT Assay protocols.

Items Falling to the Floor

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

Disposable items falling to the ground, such as empty tubes, pipette tips, gloves, or lab coat hangers should be thrown away at the end of the day or at the completion of the assay. During the assay, never touch any items that have fallen to the ground.

Non-disposable items falling to the ground, such as pipettes or important sample containers, should be immediately and thoroughly cleaned with a 0.5% sodium hypochlorite (10% bleach) solution to remove product contamination.

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

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

Applying Barcode Labels to Plates

As a convention, apply barcode labels to the right side of plate (column #12 end).

Reagent Reuse

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

Handling Cap Mats

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

CAUTION

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

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

Pipette Carefully

Perform all pipette dispenses carefully and slowly to avoid creating turbulence within the plate wells and Flow-Through Chambers.

BeadChip Handling

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

Preparing Fewer than 96 Samples

Each reagent tube supplied for your WG-DASL HT Assay contains enough volume to process 96 samples at once, using a multichannel pipette and a reservoir.

When processing fewer than 96 samples using a reagent reservoir, dead volume and pipetting error losses can increase. To ensure accurate reagent volume for all samples, single-pipette reagent into each well.

When using reagents to process fewer than 96 samples, it is best practice to aliquot the frozen reagents to minimize the number of freeze/thaw cycles. Multiple freeze/thaw cycles could potentially impact the quality of data generated by your assay.

Uracil DNA Glycosylase & dUTP

You can add Uracil DNA Glycosylase (UDG) to the PCR master mix to help prevent PCR product contamination.

You can purchase the WG-DASL HT Assay ***with*** or ***without*** UDG.

The PCR master mix contains a balanced mixture of the following items:

- Universal PCR primers
- PCR buffer
- dUTP
- dATP
- dGTP

- dCTP

The dUTP is incorporated into the post-PCR products. UDG targets dUTP for specific degradation in subsequent PCR reactions, thus reducing the chance for PCR products to contaminate the Pre-PCR products.

The kit does ***not*** contain a thermostable DNA polymerase. We recommend that you add an Illumina-recommended DNA polymerase (Titanium Taq DNA polymerase) to the PCR master mix before using the master mix in the WG-DASL HT Assay.

RNase-Free Techniques

Take the following precautions while working with RNA:

- Wear gloves throughout experiments to prevent contamination from the RNases found on most human hands.
- Use a solution of 0.1% SDS and 0.1N NaOH to decontaminate surfaces that are potentially contaminated with RNase.
- Change gloves after touching skin (e.g., your face or hair), door knobs, common surfaces, or other surfaces that have not been decontaminated.
- Use a dedicated set of pipettes for RNA work.
- Use freshly opened aerosol filter tips and tubes that are tested and guaranteed to be RNase-free.
- Use RNase-free chemicals and reagents, and DEPC-treated water.
- Designate a “low-traffic” area of the lab that is away or shielded from air vents or open windows.
- Do not leave RNase-free containers open when engaged in conversation.

Standard Lab Procedures

Running the WG-DASL HT Assay protocols requires that you perform some basic setup and familiarize yourself with standard procedures.

Calibrating and Using the Vortexer

Calibration

The vortexer's displayed speed may vary from the actual vortex speed. Illumina recommends using a digital stroboscope to determine the actual vortex speed. Once you have determined the actual vortex speed, record it along with the displayed speed and use these measurements for reference throughout the assay.

1. Set the digital stroboscope display speed to 1600 rpm.
2. Turn the vortexer on and adjust the vortexer speed until the actual speed reaches 1600 rpm.
3. Record the displayed vortexer speed and note down that it represents an actual speed of 1600 rpm.
4. Use the same method described above to determine the displayed speed for the actual vortex speeds of 1,800, 2,000, and 2,200 rpm. These vortex speeds are used in the WG-DASL HT Assay.
5. Place a label on the vortexer with the calibration information. The following is an example of a vortexer calibration label you can create and affix to your vortexer.

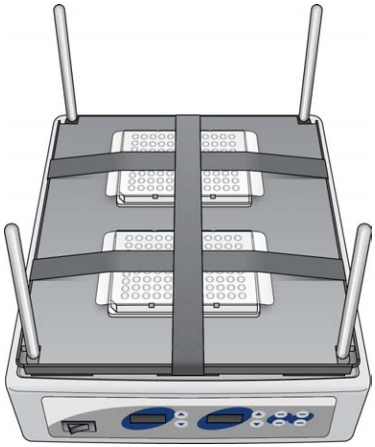
Table 3 Vortexer Calibration Speeds

Display Speed	Actual Vortex Speed	Calibration Date
1450 rpm	1600 rpm	xx-xx-xx
1625 rpm	1800 rpm	xx-xx-xx
1800 rpm	2000 rpm	xx-xx-xx
1975 rpm	2200 rpm	xx-xx-xx

Using Velcro Straps for Security

1 Replace the vortexer's top tray, which is used to secure the plate, with three Velcro straps for securing 96-well plates, as follows:
   a Cut six 2-inch lengths of adhesive backed Velcro hooks. Attach these hooks to the underside of the shaker platform bottom tray.
   b Cut three 20-inch lengths of Velcro loops. Use these as straps to secure plates onto the vortexer platform.

Figure 8 Velcro Straps on Vortexer Platform



CAUTION
Whenever you use the vortexer, secure the plates with the Velcro straps.

Balancing the Centrifuge

Whenever you centrifuge plates, place a balance plate opposite each plate being centrifuged. The weights should be as similar as possible.

Cleaning and Calibrating Pipettes

Ensure that pipettes are properly calibrated, clean, and decontaminated. Where possible, use a multi-channel pipette to dispense reagents.

Tracking Tools

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

- **Experienced User Card** – guides you through the protocols.
- **Lab Tracking Form** – allows you to map RNA samples from plate to plate, or from plate to BeadChip. Record the position of DAPs in the plate wells, and the barcode of each reagent used in the protocol.
- **Sample Sheet template** – used to record information about your samples for later use in data analysis.

Lab Tracking Form

Create a copy of the lab tracking form for each run. Use it to track information such as operator ID, start and stop times, reagent lot numbers and barcodes, and to record which samples are placed on which arrays. This form can be filled out and saved online or printed and filled in by hand.

NOTE
Lab Tracking Forms can be downloaded via http://www.illumina.com/documentation.

Sample Sheet

To effectively track your samples and assay, Illumina recommends you create a sample sheet. The sample sheet will later be used by the GenomeStudio application for data analysis. For instructions on data analysis, see the *GenomeStudio Gene Expression Module User Guide*. Create your sample sheet according to the following guidelines:

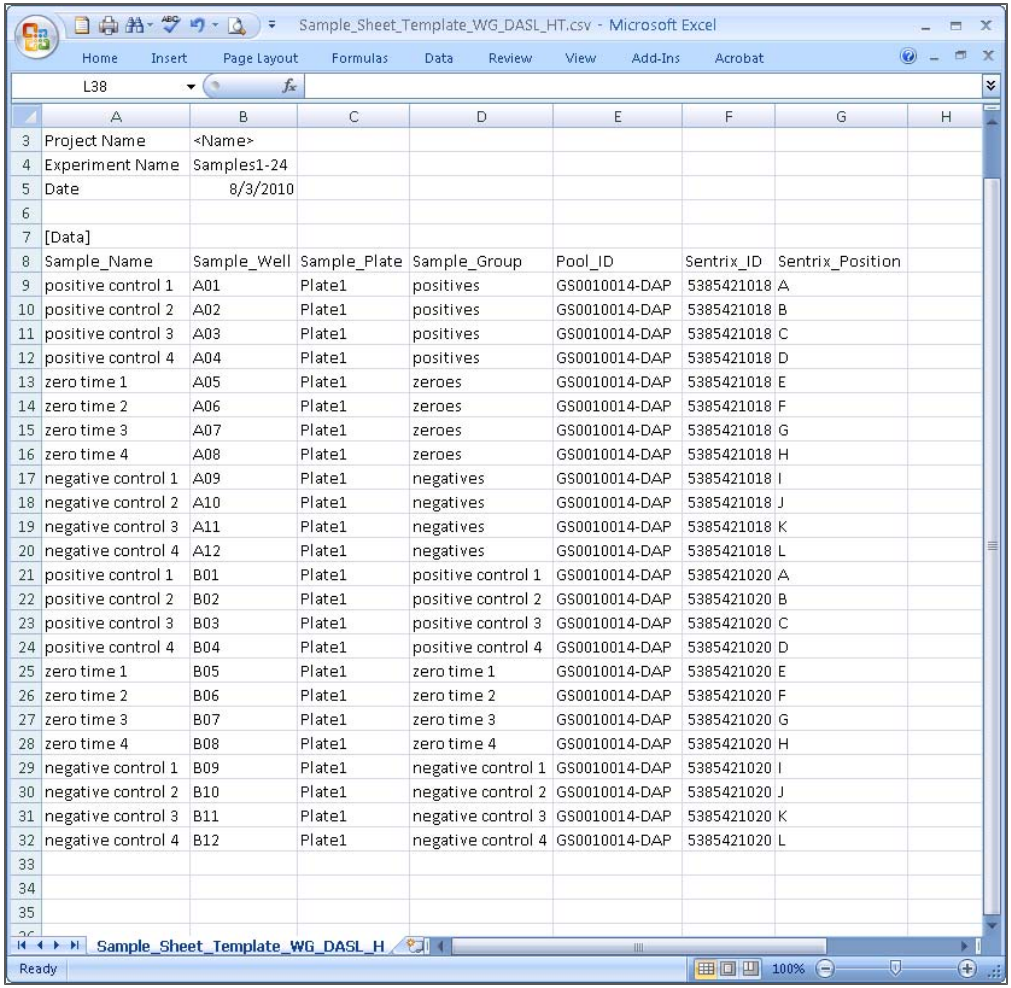
Table 4 Sample Sheet Guidelines

Column Heading	Description	Optional (O) or Required (R)
Sample_Name	Name of the sample. Used only for display in the table. If not user-specified, the GenomeStudio application will assign a default sample name, concatenating the sample plate and sample well names. Example: S12345	O

Column Heading	Description	Optional (O) or Required (R)
Sample_Well	The well containing the specific sample in the 96-well RNA plate. Example: A01	O
Sample_Plate	User-specified name for the plate containing RNA samples. Used only for display in the table. Example: XS0005623-SUR	O
Sample_Group	User-specified name of the sample group. If the Sample_Group is missing, GenomeStudio creates one group and assigns it a default name. Example: Group 1	O
Pool_ID	Name of the DAP. Example: XS0007005-DAP	O
Sentrix_ID	BeadChip ID. Example: 1529221001	R
Sentrix_Position	The BeadChip section to which the sample is hybridized. Example: A1	R
Notes	Your sample sheet header may contain whatever information you choose. Your sample sheet may contain any number of columns you choose. Your sample sheet must be in a comma-delimited (.csv) file format. Save the sample sheet under any name you wish; for example, the user-defined experiment name.	

The following is an example of the Sample Sheet format. The electronic sample sheet template can be downloaded via http://www.illumina.com/documentation.

Figure 9 Sample Sheet



Sample_Sheet_Template_WG_DASL_HT.csv - Microsoft Excel

Home Insert Page Layout Formulas Data Review View Add-Ins Acrobat

L38

	A	B	C	D	E	F	G	H
3	Project Name	<Name>						
4	Experiment Name	Samples1-24						
5	Date	8/3/2010						
6								
7	[Data]							
8	Sample_Name	Sample_Well	Sample_Plate	Sample_Group	Pool_ID	Sentrix_ID	Sentrix_Position	
9	positive control 1	A01	Plate1	positives	GS0010014-DAP	5385421018	A	
10	positive control 2	A02	Plate1	positives	GS0010014-DAP	5385421018	B	
11	positive control 3	A03	Plate1	positives	GS0010014-DAP	5385421018	C	
12	positive control 4	A04	Plate1	positives	GS0010014-DAP	5385421018	D	
13	zero time 1	A05	Plate1	zeroes	GS0010014-DAP	5385421018	E	
14	zero time 2	A06	Plate1	zeroes	GS0010014-DAP	5385421018	F	
15	zero time 3	A07	Plate1	zeroes	GS0010014-DAP	5385421018	G	
16	zero time 4	A08	Plate1	zeroes	GS0010014-DAP	5385421018	H	
17	negative control 1	A09	Plate1	negatives	GS0010014-DAP	5385421018	I	
18	negative control 2	A10	Plate1	negatives	GS0010014-DAP	5385421018	J	
19	negative control 3	A11	Plate1	negatives	GS0010014-DAP	5385421018	K	
20	negative control 4	A12	Plate1	negatives	GS0010014-DAP	5385421018	L	
21	positive control 1	B01	Plate1	positive control 1	GS0010014-DAP	5385421020	A	
22	positive control 2	B02	Plate1	positive control 2	GS0010014-DAP	5385421020	B	
23	positive control 3	B03	Plate1	positive control 3	GS0010014-DAP	5385421020	C	
24	positive control 4	B04	Plate1	positive control 4	GS0010014-DAP	5385421020	D	
25	zero time 1	B05	Plate1	zero time 1	GS0010014-DAP	5385421020	E	
26	zero time 2	B06	Plate1	zero time 2	GS0010014-DAP	5385421020	F	
27	zero time 3	B07	Plate1	zero time 3	GS0010014-DAP	5385421020	G	
28	zero time 4	B08	Plate1	zero time 4	GS0010014-DAP	5385421020	H	
29	negative control 1	B09	Plate1	negative control 1	GS0010014-DAP	5385421020	I	
30	negative control 2	B10	Plate1	negative control 2	GS0010014-DAP	5385421020	J	
31	negative control 3	B11	Plate1	negative control 3	GS0010014-DAP	5385421020	K	
32	negative control 4	B12	Plate1	negative control 4	GS0010014-DAP	5385421020	L	
33								
34								
35								

Sample_Sheet_Template_WG_DASL_H

Ready 100%

The Sentrix_Position name corresponds to the *.idat file naming conventions for the format being analyzed (BeadChip).

Equipment, Materials, and Reagents

The equipment, materials, and reagents listed in this section are all required for the WG-DASL HT Assay. Remember to maintain separate stocks, as necessary, for pre- and post-PCR areas.

Equipment

Illumina-Supplied Equipment

To perform the WG-DASL HT Assay, you need either a HiScan, iScan or BeadArray Reader System and either the Universal Starter Kit or both the GoldenGate and Gene Expression Option kits. For details on current configuration and kit options, consult your Illumina account representative or the latest Illumina product guide (available at http://www.illumina.com/literature).

Table 5 Illumina Equipment Options for the WG-DASL HT Assay

Item	Illumina Catalog #
HiScan System or iScan System	SY-103-1001 SY-101-1001
AutoLoader2 (optional)	Single-Scanner, SY-201-1001 Dual-Scanner, SY-201-1002
Universal Starter Kit or, both of the following: GoldenGate Product Option Kit and Gene Expression (IVT) Product Option Kit	SE-101-1006 (110V) SE-101-1007 (220V) SE-101-1001 (110V) SE-101-1002 (220V) SE-101-1003 (110V) SE-101-1004 (220V)

User-Supplied Equipment

The following user-supplied equipment is required.

Table 6 User-Supplied Equipment

Item	Source
8-channel precision pipettes (5 µl to 200 µl)	General lab supplier
96-well thermal cycler with heated lid	General lab supplier
Aerosol filter pipette tips	General lab supplier
[Optional] Fluorometer)	Molecular Devices, Gemini XS or XPS, www.moleculardevices.com
Heat sealer (Combi Heat Sealing Unit)	Matrix Tech Corp., catalog # ab-0384/110, www.matrixtechcorp.com
Heat sealer adapter plate (Combi Heat Sealing Unit adapter plate)	ABgene, catalog # 0563, www.abgene.com
Lab coats	General lab supplier
Microtiter plate centrifuges (2, capable of 8–3000 xg, 4°C) **Note**: Make sure this is 8-3000 xg, not 8-3000 rpm	General lab supplier
Orbital shaker	General lab supplier
Protective gloves	General lab supplier
Safety glasses	General lab supplier
Serological pipettes (50 ml)	General lab supplier
Stopwatch/timer	General lab supplier
Stroboscope	Cole-Parmer, A-87700-06,

Item	Source
	www.coleparmer.com
Tachometer/stroboscope, combination optical	Cole-Parmer, catalog # A-87700-06, www.coleparmer.com
Thermal Cycler	General lab supplier
Tube vortexer	General lab supplier

Materials

The following user-supplied materials are required.

Table 7 User-Supplied Materials

Item	Source
96-well skirted microplates, 8x12 well array	MJ Research, catalog # MSP-9601, www.mjr.com ABgene, catalog # AB-0800, www.abgene.com
96-well V-bottom plates	VWR International, catalog # 29444-102, www.vwr.com
96-well black, flat-bottom Fluotrac 200 plates	Greiner, catalog # 655076, www.gbo.com
Absorbent pads	General lab supplier
Aluminum foil	General lab supplier
Canned air (such as Aerosol Whoosh-Duster)	VWR International, catalog # 16650-027, www.vwr.com

Item	Source
Cap mats (for deep-well plates, polypropylene, pierceable, non-autoclavable)	ABgene, catalog # AB-0566, www.abgene.com
Costar* 96-well plates, polypropylene, non-sterile, without lids, V-bottom	VWR International, catalog # 29444-102, www.vwr.com
Filter plate adaptor	Centrifuge Alignment Frames, Millipore, catalog # MACF09604, www.millipore.com
Filter plates	Millipore, catalog # MAHV-N45 10/50, www.millipore.com
[Optional] Foil stripper	ABgene, catalog # AB-0592, www.abgene.com
Microplate clear adhesive film (2 mil sealplate adhesive film, non-sterile)	Phenix Research Products, catalog # LMT-SEAL-EX, www.phenix.com
Microplate heat seals (heat sealing, EZ peel, clear for polypropylene & polystyrene plates)	ABgene, catalog # AB-0812, www.abgene.com
Microseal "A" PCR plate-sealing film	MJ Research, catalog # MSA-5001, www.mjr.com
Microseal "F" film	MJ Research, catalog # MSF-1001, www.mjr.com
Non-sterile solution basins (55 ml)	Labcor Products, Inc., catalog # 730-001, www.labcorproducts.com VWA International, catalog # 21007-970, www.vwa.com

Item	Source
Sterile plastic containers (100 ml capacity)	General lab supplier
Sterile reservoirs (quarter reservoir)	Beckman Coulter, catalog # 372790, www.beckmancoulter.com
Thermo-Seal Heat-sealing foil sheets	ABgene, catalog # AB-0559, www.abgene.com
Tweezers	General lab supplier

Reagents

Illumina-Supplied Reagents

This section describes the consumables in the WG-DASL HT Assay kits. For exact details on current configuration and kit options, consult your Illumina account representative or the latest Illumina product catalog. For ordering information, see the appropriate data sheet at http://www.illumina.com/literature.

Table 8 WG-DASL HT Assay Kits

Kit Name	Catalog #	With UDG	Samples	Sets of Beadchips
WG-DASL Pre 1 MCS4	DA-905-0024		24	2
WG-DASL Pre 1 MCS4 w/UDG	DA-905-1024	X	24	2
WG-DASL Pre 1 MCS4	DA-905-0096		96	8
WG-DASL Pre 1 MCS4 w/UDG	DA-905-1096	X	96	8

Table 9 WG-DASL HT Assay Kit Contents

Item	Quantity
WG-DASL HT Assay Pool (DAP) (Based on up-to-date content derived from the NCBI RefSeq Database (Release 38 (Nov 7, 2009))	—
Add MEL 1 Reagent (AM1)	15 ml
Barcode labels for the QRNA, SUR, ASE, PCR, and INT plates	3 X each
BeadChip Solution (E1BC)	5 ml
High Temperature Wash Buffer (HTW)	500 ml
HumanHT-12 v4 BeadChips	12 samples each with 29,285 assays per sample
Humidity Control Buffer (HCB)	2.8 ml
Hybridization Buffer Reagent (HYB)	1.7 ml
Inoc PCR Reagent (IP1)	3.8 ml
Magnetic Particle B Reagent (MPB)	2.2 ml
Make Hyb 1 Reagent (MH1)	3.5 ml
Master Mix for Extension Ligation Reagent (MEL)	4.0 ml
Master Mix cDNA Synthesis for Single Use 4 (MCS4)	288 µl
Oligo Hybridization & DNA Binding Buffer 1 Reagent (OB1)	3.5 ml
Precipitation Solution Reagent (PS1)	30 ml
Reagent used to prepare BeadChips for hybridization (PB1)	950 ml
Reverse Transcriptase Enzyme (RTE)	32 µl
Single Color Master Mix Reagent (SCM)	3.2 ml

Item	Quantity
Universal Buffer 1 Reagent (UB1)	25 ml
Universal Buffer 2 Reagent (UB2)	300 ml
Uracil DNA Glycosylase (UDG)	not included in all kits
XStain BeadChip Solution 4 (XC4)	350 ml when reconstituted with EtOH

User-Supplied Reagents

The following user-supplied reagents are required.

Table 10 User-Supplied Reagents

Item	Source
0.1N NaOH (sodium hydroxide)	Sigma-Aldrich, catalog # S0899, www.sigmaaldrich.com
2-propanol	General lab supplier
70% and100% EtOH (Ethanol)	General lab supplier
0.5% sodium hypochlorite (10% bleach)	General lab supplier
cDNA Synth MCS4 Single-Use Kit (for *Pre-Qualify cDNA Samples (Optional)*: 96 samples, or 576 samples	Illumina, catalog # DA-950-6010 Illumina, catalog # DA-950-6020
Illumina-recommended DNA Polymerase (Titanium Taq DNA polymerase)	Clontech, catalog # 639220, www.clontech.com

Item	Source
Illumina-recommended High Pure RNA Paraffin Kit	Roche Applied Science, catalog # 03 270 289 001
Quant-iT RiboGreen RNA Assay Kit	Invitrogen, catalog # R-11490, www.invitrogen.com

WG-DASL HT Assay Lab Protocols

Chapter 3

Introduction

This chapter provides detailed pre- and post-PCR laboratory protocols for preparing 24 samples for BeadChips. If you are preparing fewer samples, scale down the protocols accordingly. Perform each protocol in the order shown.

The instructions in this chapter assume that you have already familiarized yourself with Chapter 2 Standard Operating Procedures and have set up the lab area appropriately.

NOTE

These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Quantitate RNA (Optional)

This process uses the RiboGreen RNA quantitation kit to quantitate RNA samples for the WG-DASL HT Assay. You can quantitate up to six plates, each containing up to 96 samples. If you already know the concentration, proceed to *Make Single-Use RNA (SUR) Plate* on page 60.

Illumina recommends the Quant-iT RiboGreen RNA Assay Kit to quantitate RNA samples. The RiboGreen assay can quantitate small RNA volumes, and measures RNA directly. Other techniques may pick up contamination such as small molecules and proteins. Illumina recommends using a fluorometer because fluorometry provides RNA-specific quantification. Spectrophotometry might also measure DNA and yield values that are too high.

CAUTION

RiboGreen is susceptible to chemical contaminants. For more information, see the Invitrogen website (www.invitrogen.com).

Estimated Time

Hands-on time: ~30 minutes

Fluorometer read time: ~5 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
Quant-iT RiboGreen RNA Assay Kit, containing RiboGreen quantitation reagent, 20X TE, and Ribosomal RNA Standard	1	2° to 8°C	User
RNA sample plate	Up to 96 samples	-80°C	User

Item	Quantity	Storage	Supplied By
96-well 0.65 ml microtiter plate	1 per 96 samples	See manufacturer's instructions	User
Fluotrac 200 96-well flat-bottom plate	1 per Std RNA plate 1 per Sample RNA plate		User
100 ml or 250 ml Nalgene bottle	1 per RiboGreen kit		User

Preparation

- Thaw all reagents to room temperature and then vortex to mix.
- Hand-label the microtiter plate "Standard RNA."
- Hand-label one of the Fluotrac plates "Standard QRNA."
- Hand-label the other Fluotrac plate "Sample QRNA." This plate will contain the quantitated RNA.
- In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

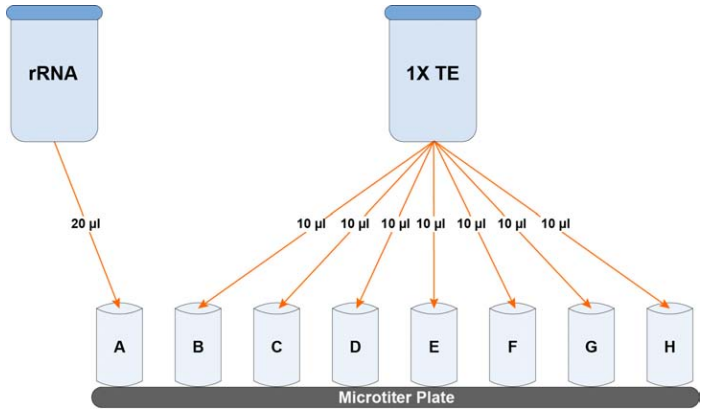
Make Standard RNA Plate

In this process, you create a Standard RNA plate with serial dilutions of standard ribosomal RNA in the wells of column 1.

1 Add 10 µl 1X TE (supplied in RiboGreen kit at 20X) to B1–H1 in the plate labeled "Standard RNA".

2 Add 20 µl ribosomal RNA to well A1.

Figure 10 Dilution of Ribosomal RNA Standard



3 Transfer 10 µl from well A1 to well B1. Pipette up and down several times.

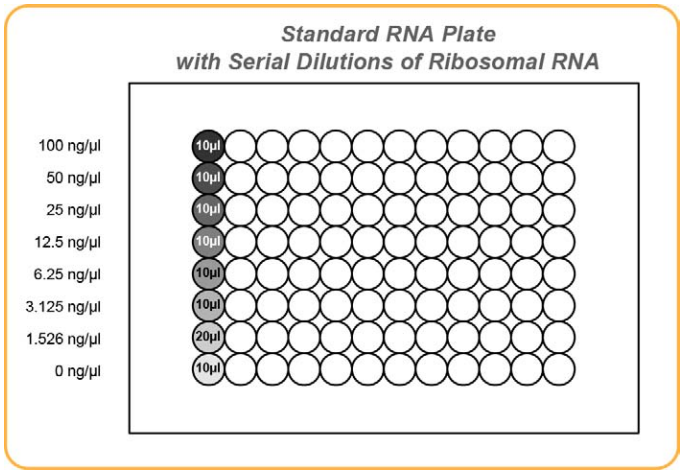
4 Change tips. Transfer 10 µl from well B1 to well C1. Pipette up and down several times.

5 Repeat for wells C1, D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.**

Table 11 Concentrations of Standard Ribosomal RNA

Row-Column	Concentration (ng/µl)	Final Volume in Well (µl)
A1	100	10
B1	50	10
C1	25	10
D1	12.5	10
E1	6.25	10
F1	3.125	10
G1	1.5262	20
H1	0	10

Figure 11 Serial Dilutions of Ribosomal RNA



6 Cover the Standard RNA plate with an adhesive seal.

7 Proceed to *Dilute RiboGreen* on page 48.

Dilute RiboGreen

The diluted RiboGreen will be added to both the Standard QRNA and Sample QRNA plates, to make the RNA fluoresce when read with the fluorometer.

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

Use 115 µl RiboGreen and 23 ml 1X TE for 1 plate, 215 µl RiboGreen and 43 ml 1X TE for 2 plates, and so on up to 6 plates.

The following table identifies the volumes needed to produce diluted reagent for multiple 96-well QRNA plates. For fewer than 96 RNA samples, scale down the volumes.

Table 12 Volumes for RiboGreen Reagents

# QRNA Plates	RiboGreen Volume (µl)	1X TE Volume (ml)
1	115	23
2	215	43
3	315	63
4	415	83
6	615	123

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

Create Standard QRNA Plate with Diluted RiboGreen

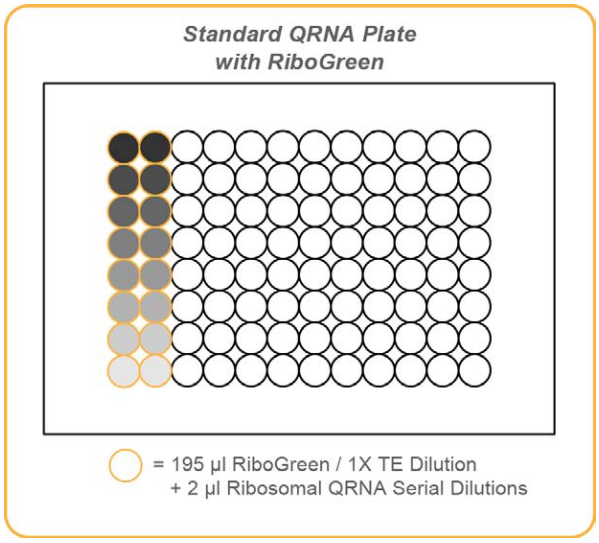
In this process you transfer the serial dilutions from the Standard RNA plate into the Standard QRNA Fluotrac plate and add diluted RiboGreen.

1 Pour the RiboGreen/1X TE dilution into a clean reagent reservoir.

2 Using a multichannel pipette, transfer 195 µl RiboGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled “Standard QRNA“.

3 Add 2 µl of each standard ribosomal RNA dilution from the Standard RNA plate to columns 1 and 2 of the Standard QRNA Fluotrac plate.

Figure 12 Standard QRNA Plate with RiboGreen



4 Immediately cover the plate with an adhesive aluminum seal.

5 Proceed to *Prepare Sample QRNA Plate with RiboGreen and RNA* on page 50.

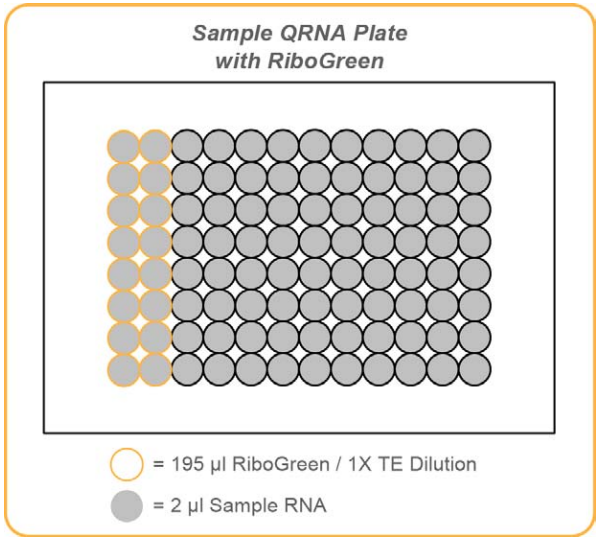
Prepare Sample QRNA Plate with RiboGreen and RNA

In this process, you create a new Sample QRNA plate that contains RNA sample and RiboGreen.

1 Using a multichannel pipette, transfer 195 µl RiboGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled “Sample QRNA“.

2 Add 2 µl of RNA sample to all 96 wells of the Sample QRNA plate. Only the first two columns will also contain RiboGreen/1X TE dilution.

Figure 13 Sample QRNA Plate with RiboGreen



NOTE
For fewer than 96 RNA samples, add the diluted RiboGreen reagent into the number of wells needed.

3 Immediately cover the plate with an adhesive aluminum seal.

4 Proceed to *Read QRNA Plate* on page 51.

Read QRNA Plate

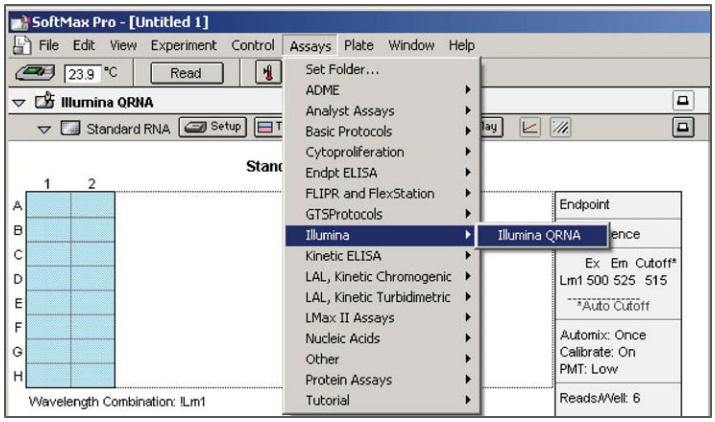
In this process, you use the Gemini XS or XPS Spectrofluorometer to read the Standard QRNA and Sample QRNA plates. The spectrofluorometer creates a standard curve from the known concentrations in the Standard QRNA plate, which you use to determine the concentration of RNA in the Sample QRNA plates.

1 Turn on the fluorometer. At the PC, open the SoftMax Pro program.

2 Load the Illumina QRNA.ppr file from the installation CD that came with your system.

3 Select **Assays** | **Illumina** | **Illumina QRNA**.

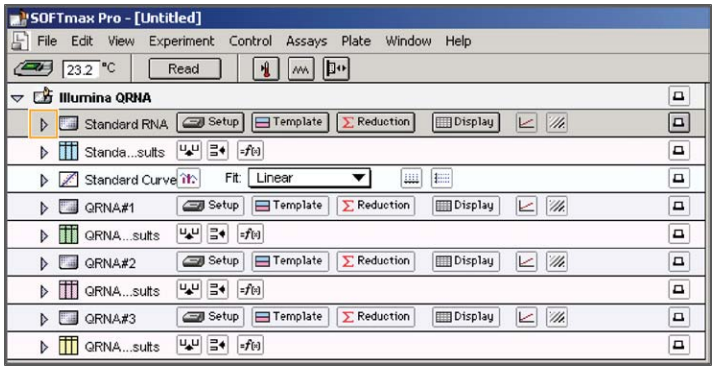
Figure 14 Load the Illumina QRNA Protocol in SoftMax Pro



4 Place the Standard QRNA Fluotrac Plate into the fluorometer loading rack with well A1 in the upper left corner.

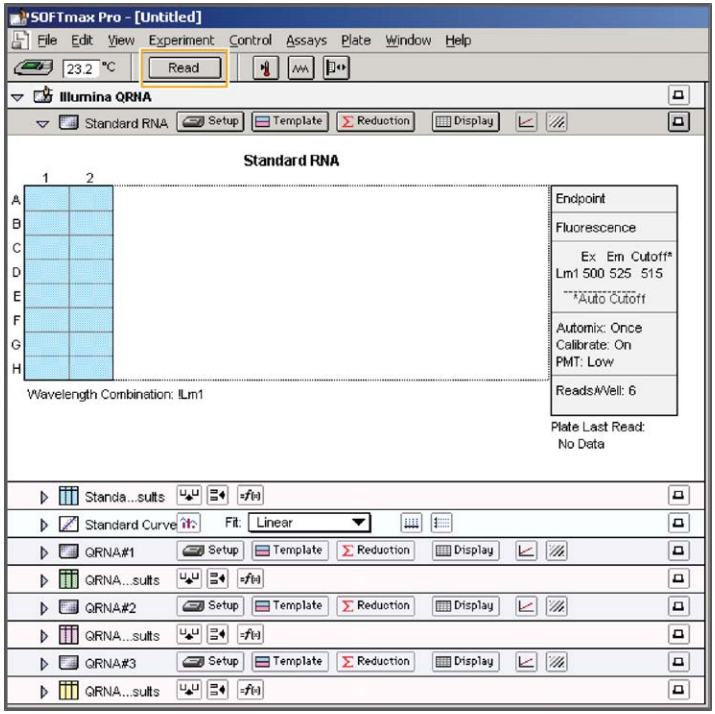
5 Click the blue arrow next to **Standard RNA**.

Figure 15 Select the Standard RNA Screen



6 Click **Read** in the SoftMax Pro interface to begin reading the Standard QRNA Plate.

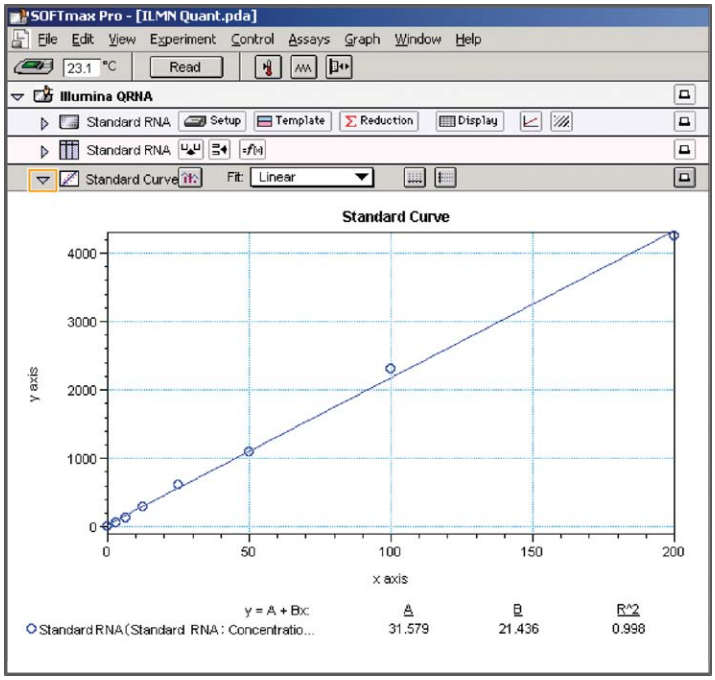
Figure 16 Read the Standard QRNA Plate



7 When the software finishes reading the data, remove the plate from the drawer.

8 Click the blue arrow next to **Standard Curve** to view the standard curve graph.

Figure 17 View Standard Curve

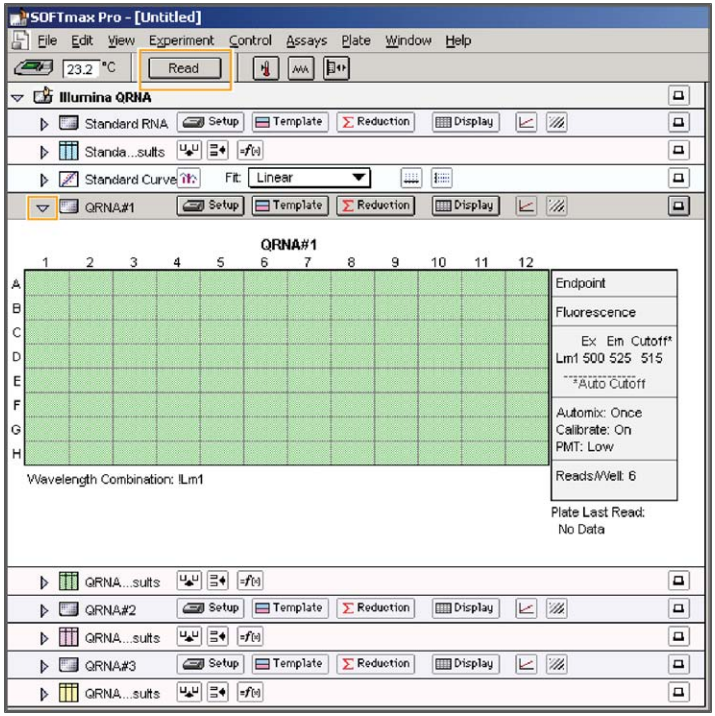


9 If the standard curve is acceptable, continue with the sample plate. Otherwise, click **Standard Curve** again.

10 Place the first Sample QRNA plate in the fluorometer with well A1 in the upper left corner.

11 Click the blue arrow next to **QRNA#1** and click **Read**.

Figure 18 Read the Sample QRNA Plate



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

13 Repeat steps 9 through 12 to quantitate all Sample QRNA plates.

14 Once all plates have been read, click **File** | **Save** to save the output data file (*.pda).

15 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.

16 Do one of the following:

- Proceed to *Pre-Qualify cDNA Samples (Optional)* on page 56
- Proceed to *Make Single-Use RNA (SUR) Plate* on page 60
- Store the quantitated RNA at 2° to 8°C for up to one month.

Pre-Qualify cDNA Samples (Optional)

This process guides you to determine if it is necessary to run replicates of poor quality RNA samples. RNAs derived from formalin-fixed, paraffin-embedded (FFPE) tissues are commonly degraded. The WG-DASL HT Assay can be used for partially degraded RNAs, but not for entirely degraded RNAs. To obtain a relative measure of RNA quality prior to WG-DASL HT Assay analysis, samples may be analyzed by qPCR. If your cDNA does not require pre-qualification, proceed to *Make Single-Use RNA (SUR) Plate* on page 60.

The process is based on amplification of a fragment of the highly expressed RPL13A ribosomal protein gene with detection by SYBR Green. The RPL13A primers amplify a 90 base pair fragment (GenBank accession # NM_012423.2). These primers are designed to span an intron and should produce a correctly amplified product only from cDNA, not genomic DNA.

Table 13 Primers for qPCR using SYBR Green Detection

Primer	Sequence
Forward	5' GTACGCTGTGAAGGCATCAA 3'
Reverse	5' GTTGGTGTTCATCCGCTTG 3'

Estimated Time

Hands-on time: ~15 minutes

Incubation time: 10–60 minutes

Consumables

Item	Quantity	Storage	Supplied By
MCS4 reagent from cDNA Synth MCS4 Single-Use Kit	1 tube per 24 samples	-15° to -25°C	User
RTE reagent from cDNA Synth MCS4 Single-Use Kit	1 tube per 24 samples	-15° to -25°C	User
Total RNA	200 ng	See manufacturer's instructions	User
96-well 0.2 ml skirted microplate	1 plate per 24 samples	See manufacturer's instructions	User

NOTE
In the Pre-Qualify cDNA protocol, you may use three 8-well strip tubes instead of a 96-well microplate.

Preparation

- Preheat the heat sealer.
- Preheat a heat block to 42°C and allow the temperature to stabilize.
- Thaw the MCS4 tube to room temperature.

NOTE
Be sure to use RNase-free materials and techniques and change pipette tips between RNA sample dispenses.

Steps

1 Normalize intact RNA samples to 20–100 ng/µl (or partially degraded RNA samples to 40-200 ng/µl) with DEPC-treated H_2O.

2 Add 32 µl RTE to 288 µl MCS4 and mix well.

3 Pour the entire contents of the MCS4 and RTE tube mixture into a new, nonsterile, disposable reservoir.

NOTE
Due to the small volume, you may wish to single-pipette directly out of the tube.

4 Add 5 µl MCS4 and RTE mixture to each well of the SUR plate that will contain a normalized RNA sample.

5 Quickly add 5 µl of normalized RNA sample to each well of the SUR plate containing 5 µl of the MCS4 and RTE mixture. Change tips between RNA sample dispenses.

6 Seal the SUR plate with a microplate heat seal. Ensure that all wells are completely sealed.

7 Vortex the sealed plate at 2,300 rpm for 20 seconds.

8 Pulse centrifuge the samples to 250 xg for 1 minute.

CAUTION
It is important to centrifuge the SUR plate to 250 xg ***before*** the 42°C incubation to prevent the wells from drying out.

9 Incubate the SUR plate at room temperature at least 10 minutes (up to 1 hour).

10 Place the SUR plate on the preheated heat block and close the lid to reduce condensation on the plate seal. Incubate at 42°C for 1 hour.

11 Pulse centrifuge the SUR plate to 250 xg for 1 minute to remove condensation from the walls of each well.

NOTE
If you are not proceeding to the qPCR step on the same day, cDNA samples can be stored after the 42°C incubation for up to four hours at 2° to 8°C or overnight at -15° to -25°C.

12 Assemble the duplicate PCR reactions appropriate for your qPCR instrument using SYBR Green detection and 1 µl of a 1:10 dilution of the cDNA product as a template.
For example, if you are using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems), the reaction should contain:
- 5 µl SYBR Green PCR Master Mix -
- 1 µl diluted cDNA product
- 250 nM each forward and reserve primers

The total reaction volume should be 10 µl.

13 Cycle according to the manufacturer's instructions for your instrument, and include a dissociation curve, if available, to assess the uniformity of the PCR product. For example, place the sealed plate into the thermal cycler and run the thermal cycler program as follows:

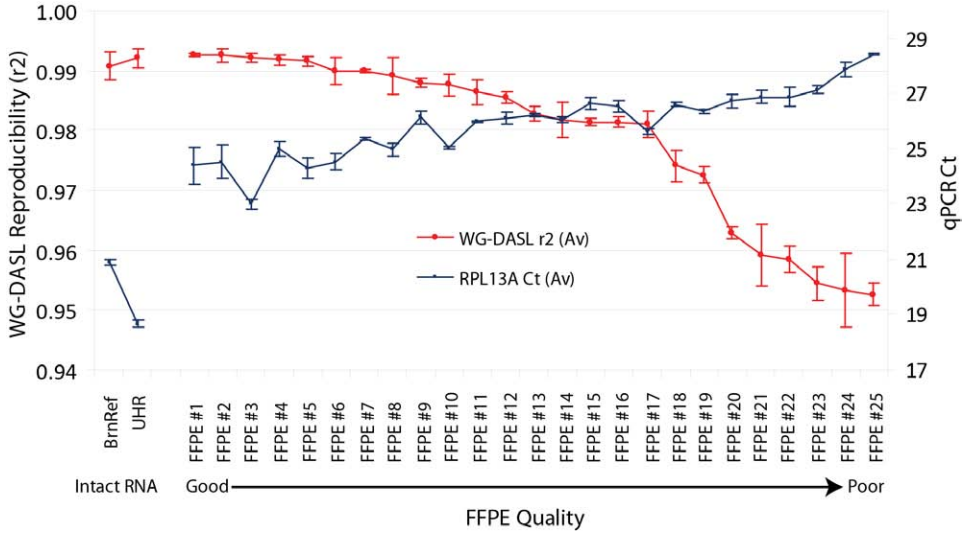
	Temperature	Time
	95°C	12 minutes
X 40	94°C	20 seconds
	54°C	20 seconds
	72°C	1 minute

Illumina routinely measures a crossover threshold (Ct) value of about 19 cycles for Universal Human Reference Total RNA (Stratagene, catalog # 740000) using RPL13A primers and the reagents listed above with a detection threshold set at 0.2

for the ABI Prism. We then compare the WG-DASL HT Assay self-reproducibility (r^2), and qPCR Ct values.

When the difference in Ct value between the UHR intact RNA sample and FFPE samples increases beyond seven, reproducibility for the WG-DASL HT Assay declines. As a result, Illumina recommends that you perform a pre-qualification qPCR screen with RPL13A for all FFPE samples along with an intact RNA sample as a reference. If ΔCt > 7 between the reference and any of the FFPE samples, then running technical replicates for those samples is recommended to ensure the WG-DASL HT Assay results will be reliable.

Figure 19 qPCR vs. WG-DASL: RPL13A 90nt

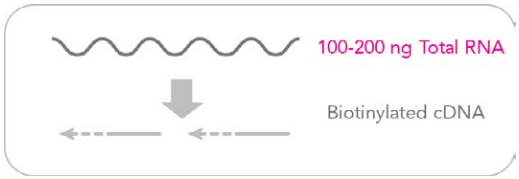


If you use other qPCR systems, you may expect a variation from these values, but in general, if the difference in cycle number at detection between cryo-preserved and paraffin-derived RNAs exceeds 7 cycles, then running technical replicates for those samples is recommended.

Make Single-Use RNA (SUR) Plate

This process reverse transcribes sufficient RNA from each individual sample to be used *once* in the WG-DASL HT Assay.

Figure 20 Make SUR



NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on time: ~15 minutes

Incubation time: One 10-minute incubation, one 1-hour incubation

Consumables

Item	Quantity	Storage	Supplied By
MCS4 reagent	1 tube per 24 samples	-15° to -25°C	Illumina
RTE reagent	1 tube per 24 samples	-15° to -25°C	Illumina
RNA samples	24	-80°C	User
96-well 0.2 ml skirted microplate	1 plate per 24 samples	See manufacturer's instructions	User

NOTE
In the Make SUR Plate protocol, you may use three 8-well strip tubes instead of a 96-well microplate.

NOTE
Be sure to use RNase-free materials and techniques throughout the Make SUR process.

Preparation

- Preheat the heat sealer.
- Preheat a heat block to 42°C and allow the temperature to stabilize.
- Thaw the MCS4 tube to room temperature.
- Apply a SUR barcode label to a new 96-well microplate.

Steps

1 Normalize intact RNA samples to 20–100 ng/µl (or partially degraded RNA samples to 40-200 ng/µl) with DEPC-treated H_2O.

2 Add 32 µl RTE to the MCS4 tube (288 µl) and mix well.

3 Pour the entire contents of the MCS4 and RTE tube mixture into a new, nonsterile, disposable reservoir.

NOTE
Due to the small volume, you may wish to single-pipette directly out of the tube.

4 Add 5 µl MCS4 and RTE mixture to each well of columns 1, 2, and 3 of the SUR plate.

5 Quickly add 5 µl of normalized RNA sample to each well of columns 1, 2, and 3 of the SUR plate. Change tips between RNA sample dispenses.

6 Seal the SUR plate with a microplate heat seal. Ensure that all wells are completely sealed.

7 Vortex the sealed plate at 2,300 rpm for 20 seconds.

8 Pulse centrifuge to 250 xg for 1 minute.

CAUTION
It is important to centrifuge the SUR plate to 250 xg ***before*** the 42°C incubation to prevent the wells from drying out.

9 Incubate the SUR plate at room temperature at least 10 minutes (up to 1 hour).

10 Place the SUR plate on the preheated heat block and close the lid to reduce condensation on the plate seal. Incubate at 42°C for 1 hour.

11 Pulse centrifuge the SUR plate to 250 xg for 1 minute to remove condensation from the walls of each well.

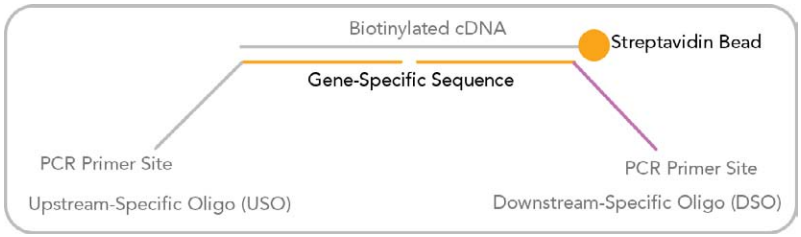
12 Do one of the following:

- Immediately set a heat block to 70°C and proceed to *Make Assay-Specific Extension (ASE) Plate* on page 63. Start thawing the DAP and OB1 reagents.
- If you do not plan to proceed immediately to *Make Assay-Specific Extension (ASE) Plate* on page 63, then store the sealed SUR plate after the 42°C incubation up to four hours at 2° to 8°C or up to 24 hours at -15° to -25°C.

Make Assay-Specific Extension (ASE) Plate

This process combines the biotinylated cDNAs with Assay-specific oligos (ASOs), hybridization reagents, and paramagnetic particles in an Assay Specific Extension (ASE) plate. The plate is then placed in a heat block and the ASOs for each sequence target of interest are allowed to anneal to the biotinylated cDNA samples. The cDNA is simultaneously captured by paramagnetic particles. The resulting ASE plate is ready for the extension and ligation of the hybridized oligos on the bound cDNAs.

Figure 21 Make ASE



NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on time: ~30 minutes

Incubation time: 14–20 hours

Consumables

Item	Quantity (per SUR plate)	Storage	Supplied By
OB1 reagent	1 tube	-15° to -25°C	Illumina
DAP reagent	1 tube	2° to 8°C or -15° to -25°C	Illumina
96-well 0.2 ml skirted microplate	1 plate	See manufacturer's instructions	User

Preparation

- In the Pool_ID column of the Sample Sheet, enter the DAP for each Sample_Well. For more information, see *Sample Sheet* on page 31.
- Preheat the heat sealer.
- Preheat the heat block to 70°C and allow the temperature to stabilize.
- If the SUR plate was stored at -15° to -25°C overnight, thaw it to room temperature and then pulse-centrifuge to 250 xg for 1 minute.
- Thaw the DAP reagent tube to room temperature and vortex the contents to mix completely, then pulse centrifuge to collect the contents at the bottom of the tube. Pour the entire contents of the tube into a sterile reservoir.
- Thaw the OB1 tube to room temperature. Vortex to completely resuspend the solution. Invert the tube to verify that all paramagnetic particles are evenly suspended in solution. Pour the entire contents of the OB1 tube into a sterile reservoir.

  CAUTION
  Do not centrifuge the OB1 tube.

- Apply an ASE barcode label to a new 96-well microplate.

Steps

1. Add 10 µl DAP to each well of columns 1, 2, and 3 of the ASE plate.
2. Add 30 µl OB1 to each well of columns 1, 2, and 3 of the ASE plate.
3. Carefully remove the heat seal from the SUR plate. Take care to avoid splashing from the wells.
4. Transfer 10 µl biotinylated cDNA from each occupied well of the SUR plate to the corresponding well of the ASE plate.

   NOTE
   Transfer the entire contents of each well from the SUR plate to the ASE plate.

5. Heat-seal the ASE plate with a microplate heat sealer. Ensure that all wells are completely sealed.
6. Pulse centrifuge the ASE plate to 250 xg for 1 minute.

7 Vortex the ASE plate at 1,600 rpm for 1 minute or until all beads are completely resuspended.

8 Place the sealed ASE plate on the preheated 70°C heat block and close the lid.

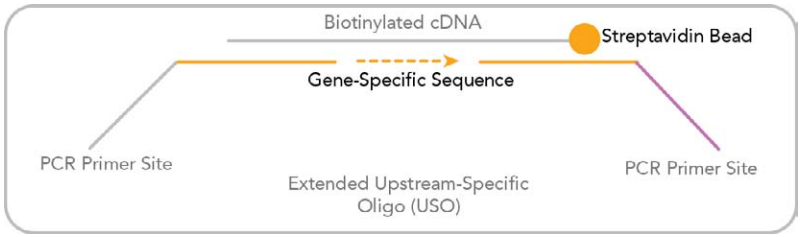
9 Immediately change the set temperature of the heat block to 30°C. Leave the ASE plate in the heat block for 14–20 hours while it cools to 30°C.

10 Proceed to *Add Master Mix for Extension & Ligation (MEL)* on page 66.

Add Master Mix for Extension & Ligation (MEL)

After the oligos are hybridized to the cDNA, mis-hybridized and excess oligos are washed away. Next, an extension and ligation master mix (MEL) (consisting of extension and ligation enzymes) is added to each cDNA sample. The extension and ligation reaction occurs at 45°C.

Figure 22 Add MEL



NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

CAUTION
In this process, the bead pellet may be difficult to resuspend. Follow the vortexing instructions to break up the pellet. If necessary, you can also shuttle the plate rapidly back and forth over the magnetic bars, so that the pellet is pulled first to one side and then to the other.

Estimated Time

Hands-on time: ~45 minutes

Incubation time: 15 minutes

Consumables

Item	Quantity	Storage	Supplied By
AM1 reagent	Bottle	2° to 8°C	Illumina
UB1 reagent	Bottle	2° to 8°C[a]	Illumina
MEL reagent	1 tube per ASE plate	-15° to -25°C	Illumina

Preparation

- Thaw the MEL tube to room temperature. Pour the entire contents of the tube into a sterile reservoir right before using it.
- Remove the AM1 bottle from the refrigerator and leave it at room temperature for 10 minutes. Pour 11 ml AM1 into a second sterile reservoir. Add 10 ml for each additional plate.
- Remove the UB1 bottle from the refrigerator. Pour 11 ml UB1 into a third sterile reservoir.
- Remove the IP1 and SCM tubes from the freezer and let them thaw.

AM1 Washes

1. Remove the ASE plate from the heat block and reset the heat block to 45°C.
2. Centrifuge the ASE plate to 250 xg.

   NOTE
   To avoid tip contamination and sample loss during this procedure, slant the pipette tips so that they draw liquid from the side of the well opposite the beads.

3. Immediately place the ASE plate on the raised-bar magnetic plate for 2 minutes or until the beads are completely captured.

[a] -15° to -25°C for long-term storage

4 Carefully remove the heat seal from the ASE plate. Take care not to splash sample out of the wells.

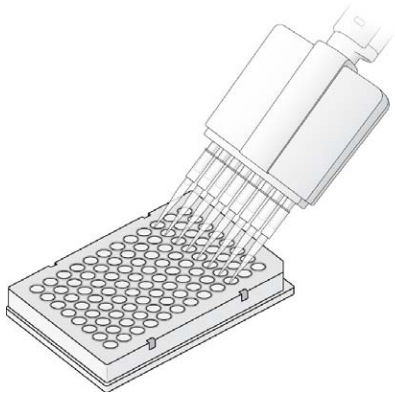
5 Using an 8-channel pipette with new tips, remove all the liquid (~50 µl) from the occupied wells and discard it. Leave the beads in the wells.

NOTE
To avoid disturbing the pellet or contaminating the tips, place the tips against the top edge of the well.

Visually inspect the pipette tips after removing liquid from each column to ensure no beads have been removed. If beads are visible in pipette tips, return the solution to the same wells, allow the magnet to re-collect beads, and change the pipette tips. You do not need to change pipette tips again until you have removed the liquid from all 3 columns.

6 With the ASE plate on the raised-bar magnetic plate, use an 8-channel pipette with new tips to add 50 µl AM1 to each occupied well of the ASE plate.

Figure 23 Avoid Tip Contamination



7 Seal the ASE plate with clear adhesive film.

8 Vortex the ASE plate at 1,600 rpm for 20 seconds or until all beads are resuspended.

9 Place the ASE plate on the raised-bar magnetic plate for approximately 2 minutes or until the beads are completely captured.

10 Remove the seal from the ASE plate, taking care to avoid splashing from the wells.

11 Using the same 8-channel pipette with the same tips, remove all AM1 reagent from each occupied well. Leave the beads in the wells.
You do not need to change pipette tips again until you have removed the liquid from all 3 columns.

12 Repeat steps 6 through 11 once.

UB1 Washes

1 Remove the ASE plate from the raised-bar magnetic plate.

2 Using an 8-channel pipette with new tips, add 50 µl UB1 to each occupied well of the ASE plate.

3 Place the ASE plate onto the raised-bar magnetic plate for approximately 2 minutes or until the beads are completely captured.

4 Using the same 8-channel pipette with the same tips, remove all UB1 reagent from each occupied well. Leave the beads in the wells.
You do not need to change pipette tips again until you have removed the liquid from all 3 columns.

5 Repeat steps 1 through 4 once.

Add MEL

1 Using an 8-channel pipette with new tips, add 37 µl MEL to each occupied well of the ASE plate.

2 Seal the plate with clear adhesive film.

3 Vortex the plate at 1,600 rpm for 1 minute to resuspend the beads.

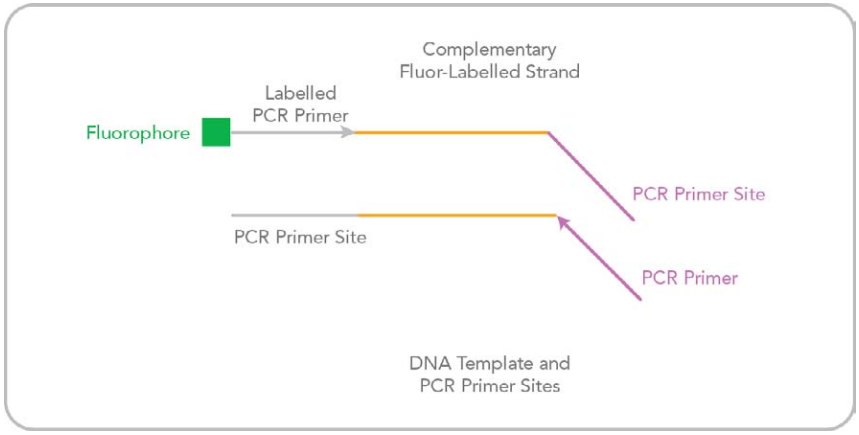
4 Incubate the ASE plate on the preheated 45°C heat block for exactly 15 minutes. During the incubation, perform *Make PCR Plate* on page 70.

CAUTION
Do not allow the ASE plate to incubate at 45°C for any longer than 15 minutes.

Make PCR Plate

This process adds the Illumina-recommended DNA Polymerase and the optional Uracil DNA Glycosylase to the SCM master mix for PCR. It creates a 24-sample plate for the Inoc PCR process.

Figure 24 Make, Inoculate, and Cycle PCR



NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on time: ~15 minutes

Consumables

Item	Quantity	Storage	Supplied By
Illumina-recommended DNA Polymerase	Tube	-15° to -25°C	User
Uracil DNA Glycosylase (Optional)	Tube	-15° to -25°C	User
SCM reagent	1 tube per PCR plate	-15° to -25°C	Illumina
96-well 0.2 ml skirted microplate	1 per ASE plate	See manufacturer's instructions	User

Preparation

- Apply a PCR barcode label to a new 96-well 0.2 ml skirted microplate.
- Invert the thawed SCM tube 10 times to mix.

Steps

1. Add 800 µl SCM and 16 µl Illumina-recommended DNA Polymerase to a clean 1.5 ml tube.
2. Add 12.5 µl Uracil DNA glycosylase to the SCM/Polymerase mixture.
3. Invert the tube several times to mix the contents and pipette the contents into a sterile reservoir.
4. Using an 8-channel pipette, add 30 µl of the SCM mixture to each well of columns 1, 2, and 3 of the PCR plate.
5. Seal the PCR plate with clear adhesive film.
6. As soon as the 15 minute ASE plate incubation is complete, proceed immediately to *Inoculate PCR Plate* on page 72.

Inoculate PCR Plate

This process uses the template formed during the extension and ligation process in a PCR reaction. This PCR reaction uses two universal primers. One is labeled with a fluorescent dye and the other is biotinylated. The biotinylated primer captures the PCR product and allows the strand containing the fluorescent signal to be eluted.

NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

CAUTION
In this process, the bead pellet may be difficult to resuspend. Follow the vortexing instructions to break up the pellet. If necessary, you can also shuttle the plate rapidly back and forth over the magnetic bars, so that the pellet is pulled first to one side and then to the other.

Estimated Time

Hands-on time: ~30 minutes

Consumables

Item	Quantity	Storage	Supplied By
UB1 reagent	Bottle	2° to 8°C[a]	Illumina
IP1 reagent	1 tube per PCR plate	-15° to -25°C	Illumina

Preparation

- Pour 6 ml UB1 into a sterile reservoir.
- Pour the entire contents of the IP1 tube into a second sterile reservoir.

[a] -15° to -25°C for long-term storage

Remove Supernatant

1. Remove the ASE plate from the heat block.
2. Reset the heat block to 95°C.
3. Place the ASE plate on the raised-bar magnetic plate for 2 minutes or until the beads are completely captured.
4. Remove the clear adhesive film from the plate.
5. Using an 8-channel pipette, remove and discard the supernatant (~50 µl) from all occupied wells of the ASE plate. Leave the beads in the wells.

NOTE
The amount of supernatant in this step is less than 50 µl. However, setting the pipette to that volume ensures that it is set correctly for the later washes, which require the full 50 µl.

NOTE
To avoid tip contamination and sample loss during this procedure, slant the pipette tips so that they draw liquid from the side of the well opposite the beads. If you suspect that the tips are contaminated with the contents of the well, discard the tips and use new ones.

Visually inspect the pipette tips after removing liquid from each column to ensure no beads have been removed. If beads are visible in pipette tips, return the solution to the same wells, allow the magnet to re-collect beads, and change the pipette tips. You do not need to change pipette tips again until you have removed the liquid from all 3 columns.

UB1 Wash

1. Leaving the plate on the magnet and using an 8-channel pipette with new tips, add 50 µl UB1 to each occupied well of the ASE plate.
2. Leave the ASE plate on the raised-bar magnetic plate for 2 minutes or until the beads are completely captured.
3. Remove and discard the supernatant (~50 µl) from all occupied wells of the ASE plate. Leave the beads in the wells.

You do not need to change pipette tips until you have removed the liquid from all 3 columns.

Add IP1

1. Using an 8-channel pipette with new tips, add 35 µl IP1 to each occupied well of the ASE plate.
2. Seal the plate with clear adhesive film.
3. Vortex at 1,800 rpm for 1 minute or until all beads are resuspended.
4. Place the plate on the preheated 95°C heat block for 1 minute.
5. Place the ASE plate on the raised-bar magnetic plate for 2 minutes or until the beads are completely captured.

CAUTION
Remove the adhesive seal very carefully so that the evaporation on the seal does not drip and cause cross-contamination.

Add Supernatant to PCR Plate

1. Remove the seal from the PCR plate.
2. Using an 8-channel pipette with new tips, transfer 30 µl supernatant from each occupied well of the ASE plate to the corresponding well of the PCR plate. Pipette the contents of the PCR plate wells up and down 3–4 times.
   Change tips between column dispenses.

CAUTION
Take special care not to disturb or transfer the beads when aspirating the eluted product.

3. Seal the PCR plate with the appropriate PCR plate-sealing film for your thermal cycler.
4. Pulse centrifuge the plate to 250 xg for 1 minute.
5. Immediately transfer the PCR plate to the thermal cycler. Discard the ASE plate.
6. Proceed to *Thermal Cycle PCR Plate* on page 76.

This concludes the Pre-PCR processes for the Whole-Genome Gene Expression DASL Assay. If you remove materials such as experienced user cards from the Pre-PCR lab, do not return with them in to the Pre-PCR lab at any time.

Thermal Cycle PCR Plate

This process thermal cycles the PCR plate to fluorescently label and amplify the templates generated in the pre-PCR process.

Estimated Time

Cycle time: ~2 hours 45 minutes

Steps

1 Place the sealed plate into the thermal cycler and run the thermal cycler program shown in this table.

Table 14 Thermal Cycler Program

	Temperature	Time
	37°C	10 minutes
	95°C	3 minutes
X 34	95°C	35 seconds
	56°C	35 seconds
	72°C	2 minutes
	72°C	10 minutes
	4°C	5 minutes

2 Do one of the following:

- Proceed immediately to *Bind PCR Products* on page 77.
- Seal and store the PCR plate at -15° to -25°C.

Bind PCR Products

In this step, the double-stranded PCR products are immobilized by binding the biotinylated strand to paramagnetic particles. The solution is transferred to a filter plate and incubated at room temperature so that the PCR product may bind to the paramagnetic particles.

Figure 25 Bind PCR Products

NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on time: ~20 minutes

Incubation time: 1 hour

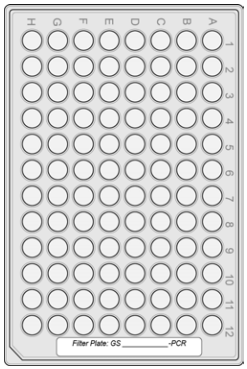
Consumables

Item	Quantity	Storage	Supplied By
MPB reagent	1 tube per PCR plate	2° to 8°C	Illumina
Filter plate with lid	1 per PCR plate	See manufacturer's instructions	User

Preparation

- Vortex the MPB tube several times or until the beads are completely resuspended. Pour the entire contents of the MPB tube into a sterile reservoir.
- Write the PCR plate barcode number in the space provided on the filter plate label. Apply the filter plate label to the top of the filter plate next to column 12.

Figure 26 Apply Label to Filter Plate



Steps

1 Pulse centrifuge the PCR plate to 250 xg for 1 minute.

2 Place new tips onto a 5–50 µl multichannel pipette and transfer 20 µl resuspended MPB from the reservoir into each occupied well of the PCR plate.
It is not necessary to change pipette tips until liquid has been transferred to all 3 columns.

NOTE
To avoid tip contamination and sample loss during this procedure, slant the pipette tips so that they draw liquid from the side of the well opposite the beads. If you suspect that the tips are contaminated with the contents of the well, discard the tips and use new ones.

3 Place new tips on an 8-channel pipette and set it to 85 µl. Place the PCR and filter plates next to each other with the A1 wells in the upper left corner.

4 Pipette the solution in the PCR plate up and down several times to mix the beads with the PCR product. Transfer the mixed solution from each occupied well of the PCR plate into the corresponding well of the filter plate.
Change pipette tips between column dispenses.

5 Discard the empty PCR plate.

6 Cover the filter plate with the filter plate lid.

7 Store at room temperature, protected from light, for 1 hour.

8 Proceed to *Make Intermediate (INT) Plate* on page 80.

Make Intermediate (INT) Plate

In this step, the single-stranded fluor-labeled PCR product from the filter plate is washed and then eluted into an intermediate (INT) plate.

NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on time: ~20 minutes

Consumables

Item	Quantity	Storage	Supplied By
0.1N NaOH	Bottle	2° to 8°C	User
UB2 reagent	Bottle	Room temperature	Illumina
MH1 reagent	1 tube per INT plate	Room temperature	Illumina
96-well V-bottom plate	1 per filter plate	See manufacturer's instructions	User
96-well 0.2 ml skirted microplate	1 per filter plate		User
Filter plate adapter	1 per filter plate		User

Preparation

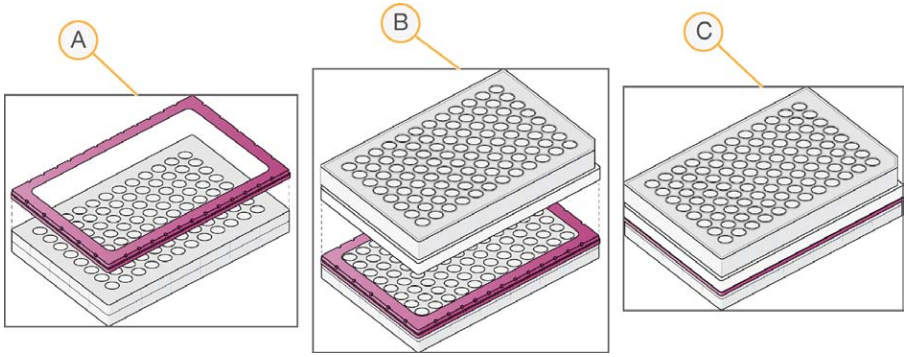
- Apply a INT barcode label to a new 96-well 0.2 ml skirted microplate.
- Using a serological pipette, transfer 10 ml UB2 into a sterile reservoir.
- Pour 5 ml 0.1N NaOH into a second sterile reservoir.
- Pour the contents of an MH1 tube into a third sterile reservoir.

Steps

1 Place the filter plate adapter on an empty, unlabeled 96-well V-bottom plate (waste plate).

2 Place the filter plate containing the bound PCR products onto the filter plate adapter.

Figure 27 Assemble Filter Plate



A Place adapter on waste plate
B Place filter plate on adapter
C Completed Assembly

3 Centrifuge to 1000 xg for 5 minutes at 25°C.

4 Remove the filter plate lid.

5 Using an 8-channel pipette with new tips, add 50 µl UB2 to each well of columns 1, 2, and 3 of the filter plate. Dispense slowly to avoid disturbing the beads.

CAUTION
To avoid disturbing the pellet or contaminating the tips, place the tips against the top edge of the well.

6 Replace the filter plate lid.

7 Centrifuge to 1000 xg for 5 minutes at 25°C.

8 Using an 8-channel pipette with new tips, add 30 µl MH1 to each well of columns 1, 2, and 3 of the INT plate.

9 Replace the waste plate with the INT plate. Orient the INT plate so that well A1 of the filter plate matches well A1 of the INT plate.

CAUTION
Be sure to replace the waste plate with the INT plate. Failure to replace the waste plate will result in loss of samples.

10 Discard the waste plate.

11 Using an 8-channel pipette with new tips, add 30 µl 0.1N NaOH to each occupied well of the filter plate.

12 Replace the filter plate lid.

CAUTION

Due to the sensitivity of the dyes to 0.1N NaOH, proceed quickly. Prolonged incubation with NaOH is unnecessary; less than 5 minutes is sufficient. The DNA is denatured almost instantly.)

13 Centrifuge immediately to 1000 xg for 5 minutes at 25°C. At the end, no beads should be visible in the wells of the INT plate.

14 Discard the filter plate. Save the adapter for later use in other protocols.

15 Gently mix the contents of the INT plate by moving it from side to side without splashing.

16 Seal the INT plate with a 96-well cap mat. Store the plate in the dark until ready to proceed with precipitation of samples.

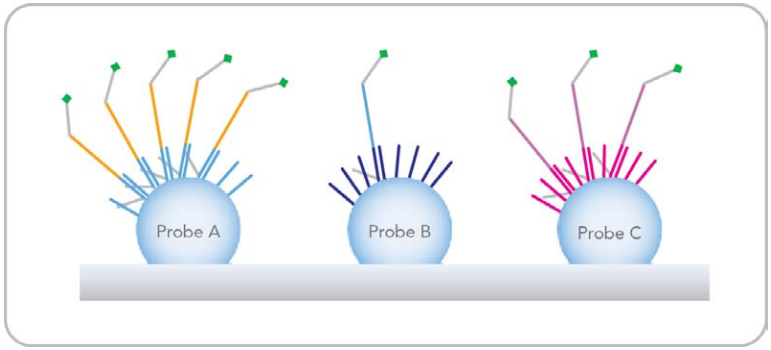
17 Do one of the following:

- Proceed to *Precipitate and Wash INT Plate* on page 83.
- If you do not plan to use the INT plate immediately in the protocol, store it at -15° to -25°C for up to 24 hours.

Precipitate and Wash INT Plate

In this step the single-stranded product from the INT plate is precipitated, washed and resuspended. The product from this plate is hybridized to the BeadChip.

Figure 28 Hybridize to BeadChip



NOTE
These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on: ~1 hour

Consumables

Item	Quantity	Storage	Supplied By
PS1 reagent	3.2 ml per INT plate	2° to 8°C	Illumina
2-propanol	Bottle	Room temperature	User

Item	Quantity	Storage	Supplied By
70% EtOH	Bottle	Room temperature	User
MH1	300 µl per INT plate	Room temperature	Illumina
HYB reagent	1.2 ml per INT plate	-15° to -25°C	Illumina

WARNING

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, see the MSDS for this kit, which is available at http://www.illumina.com/msds.

Preparation

- Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
- Preheat the Illumina Hybridization Oven to 58°C. Allow 30 minutes for it to equilibrate.
- Place the HYB tube in the 58°C oven for 10 minutes to dissolve any salts that may have precipitated in storage. If any salts remain undissolved, incubate at 58°C for another 10 minutes. Cool to room temperature and mix thoroughly before using.
- Preheat a heat block to 65°C and allow the temperature to stabilize.
- Vortex PS1 bottle then transfer 3.2 ml into a sterile reservoir.
- Pour 10 ml 2-propanol into a second sterile reservoir.
- Pour 20 ml 70% EtOH into a third sterile reservoir.
- In a sterile 15 ml centrifuge tube combine 300 µl MH1, 300 µl nuclease-free water and 1.2 ml HYB. Mix well by vortexing, followed by pulse centrifugation.
- Pour the MH1/water/HYB mix into a fourth sterile reservoir.

Steps

1 Remove the seal from the INT plate, taking care to avoid splashing from the wells.

2 Add 30 µl PS1 reagent to each well of the INT plate.

3 Using a multichannel pipette, thoroughly mix the contents by pipetting the solution up and down several times until the solution is uniformly blue.

4 Add 90 µl 2-propanol to each well of the INT plate.

5 Using a multichannel pipette, thoroughly mix the contents by pipetting the solution up and down several times until the solution is uniformly blue.

CAUTION
It is important to mix the contents thoroughly to ensure efficient precipitation of the DNA pellet.

6 Seal the INT plate with clear adhesive film.

7 Centrifuge the plate to 3000 xg at 2° to 8°C for 20 minutes.

8 Remove the INT plate from the centrifuge.

9 Remove the INT plate seal and decant the supernatant by inverting the INT plate and smacking it down onto an absorbent pad.

CAUTION
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 does not work as well.

10 Tap the inverted plate onto the pad to blot excess supernatant.

11 Add 150 µl 70% EtOH to each well of the INT plate.

12 Using a multichannel pipette, thoroughly wash the blue pellet in 70% EtOH by pipetting up and down several times.

13 Seal the INT plate with clear adhesive film.

14 Centrifuge the plate to 3000 xg at 2° to 8°C for 10 minutes.

15 Remove the INT plate from the centrifuge.

16 Remove the INT plate seal and decant the supernatant by inverting the INT plate and smacking it down onto an absorbent pad.

CAUTION
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 does not work as well.

17 Tap the inverted plate onto the pad to blot excess supernatant.

18 Place the INT plate in the preheated heat block and close the lid.

19 Incubate the INT plate at 65°C for 5 minutes or until the residual EtOH has evaporated.

20 Add 15 µl of the MH1/water/HYB mix to each well of the INT plate.

21 Seal the INT plate with clear adhesive film.

22 Pulse centrifuge the plate to 250 xg.

23 Remove the INT plate seal.

24 Using a multichannel pipet, thoroughly dissolve the pellets by pipetting the solution up and down several times.

25 Seal the INT plate with a 96-well cap mat. Store the plate in the dark until ready to dispense sample onto a BeadChip.

26 Do one of the following:
- Proceed to *Hybridize BeadChip* on page 87.
- If you do not plan to use the INT plate immediately in the protocol, store it at -15° to -25°C for up to 24 hours.

Hybridize BeadChip

In this process the BeadChips are hybridized using the Hyb Chamber. After the Hyb Chamber has been assembled, the samples are ready for hybridization. The BeadChip is hybridized overnight in the Illumina Hybridization Oven at 58°C.

NOTE

These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on time: ~30 minutes

Incubation time: 14–20 hours

Consumables and Equipment

Item	Quantity	Storage	Supplied By
HCB reagent	Tube	-15° to -25°C	Illumina
Hyb Chamber	1 per 4 BeadChips	Room temperature	Illumina
BeadChips (12x1)	2 per 24 samples	2° to 8°C	Illumina

Preparation

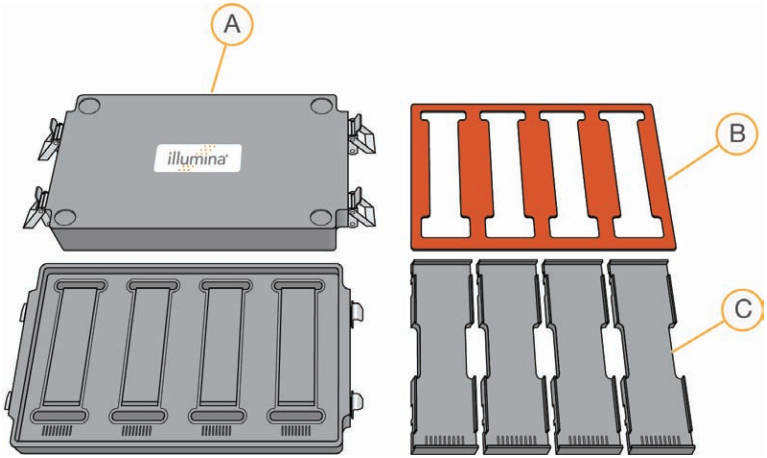
- In the Sentrix_ID column of the Sample Sheet, enter the BeadChip ID for each BeadChip section. For more information, see *Sample Sheet* on page 31.
- Calibrate the Illumina Hybridization Oven with the Full-Scale Plus digital thermometer supplied with your system.
- Preheat the Illumina Hybridization Oven to 58°C. Allow 30 minutes for it to equilibrate.
- Place the HCB tube in the 58°C oven for 10 minutes to dissolve any salts that may have precipitated in storage. If any salts remain undissolved, incubate at 58°C for another 10 minutes. Cool to room temperature and mix thoroughly before using.

- If the INT plate has been frozen, thaw it completely at room temperature in a light-protected drawer, and then pulse centrifuge it to 250 xg for 1 minute.

Assemble Hybridization Chambers

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

Figure 29 BeadChip Hyb Chamber Components



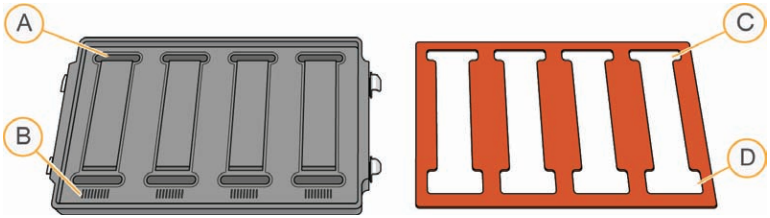
A Hyb Chamber
B Hyb Chamber Gasket
C Hyb Chamber Inserts

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

2 Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers as shown.

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

Figure 30 Hyb Chamber and Gasket



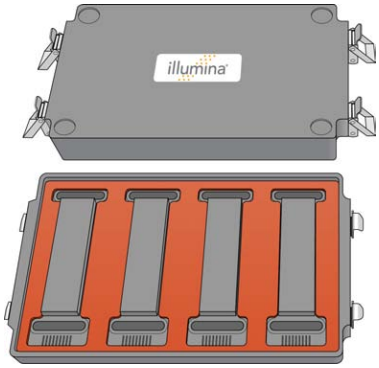
A Reservoirs
B Barcode Ridges
C Narrower Edges
D Wider Edges

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

Figure 31 Placing Gasket into Hyb Chamber

c Make sure the Hyb Chamber gaskets are properly seated.

Figure 32 Hyb Chamber with Gasket in Place



3 Add 200 µl HCB into the eight humidifying buffer reservoirs in the Hyb Chamber. Only fill the reservoirs of sections that will contain BeadChips.

Figure 33 Dispensing HCB into Hyb Chamber Reservoir

4 Close and lock the BeadChip Hyb Chamber lid.

a Seat the lid securely on the bottom plate.

b Snap two clamps shut, kitty-corner across from each other.

c Snap the other two clamps.

Figure 34 Sealing the Hyb Chamber

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

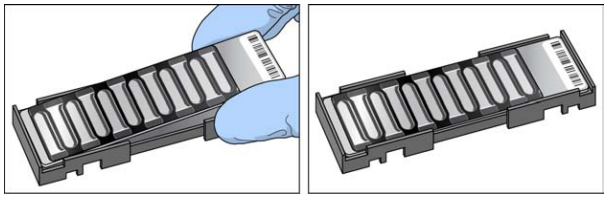
Prepare BeadChip for Hybridization

CAUTION
Do not unpackage BeadChips unless you are ready to begin hybridization.

1 Remove all the BeadChips from their packages.

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

Figure 35 Place BeadChips into Hyb Chamber Inserts

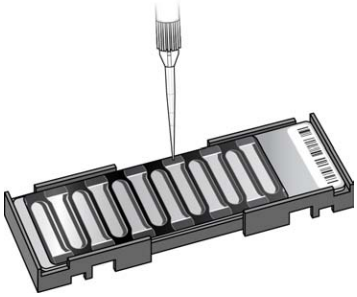


Load Sample

1 Using a single-channel precision pipette, add 15 µl sample onto the center of each inlet port.

NOTE
Load samples by directly placing pipette tips to the array surface. To avoid contamination/evaporation, proceed immediately to the next step as soon as all arrays have received sample.

Figure 36 Dispense Sample onto BeadChip

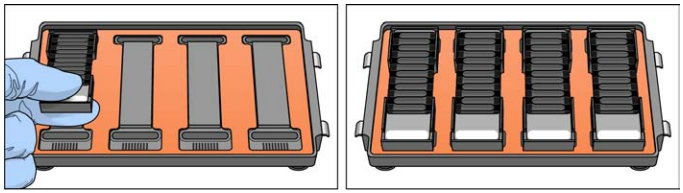


2 Visually inspect all sections. Ensure sample covers all of the sections of the stripe. Record any sections that are not covered.
Some residual sample may still remain in the inlet port. This is normal.

3 Open the Hyb Chamber.

4 Load 4 Hyb Chamber Inserts containing sample-laden BeadChips into each Hyb Chamber.

Figure 37 BeadChips in BeadChip Hyb Chamber

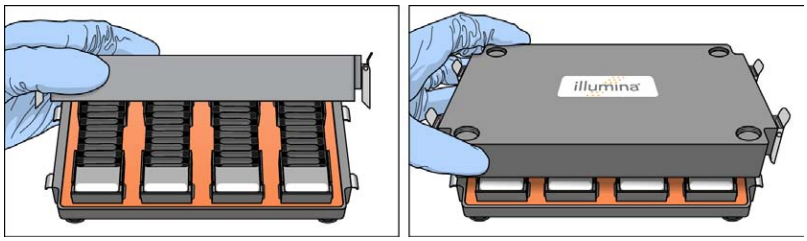


5 Position the barcode end over the ridges indicated on the Hyb Chamber and ensure the inserts are securely seated.

Hybridize BeadChips

1 Close and lock the BeadChip Hyb Chamber lid.
   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 38 Secure Lid



2 Place the Hyb Chamber into the 58°C Illumina Hybridization Oven.

3 Start the rocker by turning on the switch just above the power switch (optional).

4 Incubate for 16 hours at 58°C.

5 In preparation for the next day's washes, prepare 1X High-Temp Wash buffer from the 10X stock by adding 50 ml 10x High-Temp Wash buffer to 450 ml nuclease-free water.

6 Place the Hybex Waterbath insert into the Hybex Heating Base.

7 Add 500 ml prepared 1X High-Temp Wash buffer to the Hybex Waterbath insert.

Figure 39 Adding High-Temp Buffer to Hybex Waterbath Insert

8 Set the Hybex Heating Base temperature to 55°C.

9 Close the Hybex Heating Base lid and leave the High Temp Wash buffer to warm overnight.

10 Proceed to *Wash BeadChip* on page 95 the next day.

Wash BeadChip

In this process, prepare for the wash steps by removing the BeadChips from the overnight hybridization. Remove the BeadChip coverseals and then wash the BeadChips.

NOTE

These procedures describe preparing 24 samples using a 24 sample WG-DASL HT Assay Profiling Reagent Kit. If you are using a 96 sample WG-DASL HT Assay Profiling Reagent Kit, the kit provides enough reagent to prepare 96 samples at once.

Estimated Time

Hands-on: 30 minutes

Incubation: Two 5 minute washes, one 10 minute wash, one 1 hour incubation

Consumables

Item	Quantity	Storage	Supplied By
100% EtOH	Bottle	Room temperature	User
High Temperature Wash Buffer	Bottle	Room temperature	Illumina
PB1	Bottle	Room temperature	Illumina
Wash E1BC Buffer	Tube	Room temperature	Illumina
XC4	Bottle	Room temperature	Illumina

Preparation

- In preparation for the Coat BeadChip protocol, follow these steps to resuspend the XC4 reagent:
  - Add 335 ml 100% EtOH to the XC4 bottle. The final volume is 350 ml.
    - Each XC4 bottle contains enough to process up to 24 BeadChips.

- Re-cap the bottle, shake vigorously for 15 seconds, and place on a rocker for 30–40 minutes to resuspend. Place the bottle on the side opposite to the frozen pellet if possible.
- After 30–40 minutes, shake the bottle vigorously by hand to ensure all XC4 is in suspension and none is still coating the container. If coating is visible, vortex at 1,625 rpm until the XC4 is in complete suspension.
- Once resuspended, use XC4 at room temperature. You can store it at 2° to 8°C overnight. Keep the XC4 in the bottle in which it was shipped until ready for use.

▸ Add 6 ml E1BC buffer to 2 L RNase-free water to make the Wash E1BC solution.

▸ Place 1 L of diluted Wash E1BC buffer in a Pyrex No. 3140 beaker.

NOTE
A Pyrex No. 3140 beaker comes with the purchase of a Gene Expression (IVT) Product Option Kit or Universal Starter Kit. If you have not purchased one recently, please contact Illumina Customer Service to obtain a beaker.

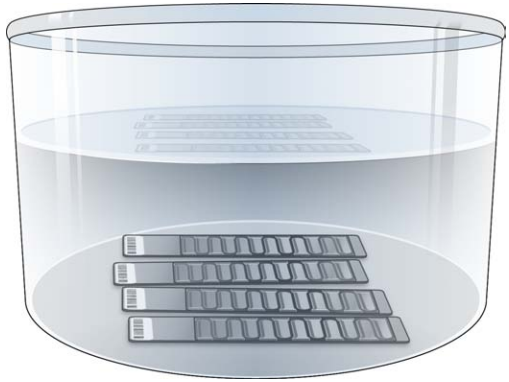
Remove Seal

1 Remove the Hyb Chamber from the oven and place it on the lab bench. Disassemble the chamber.

NOTE
If you are processing multiple chambers, remove them from the oven and process the BeadChips one at a time. Process all BeadChips in the first chamber as described in steps 2–5 below, then remove second chamber from the oven, process all of its BeadChips, and so on until all chambers are processed.

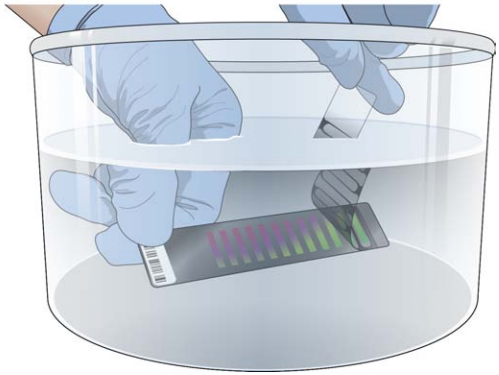
2 Using powder-free gloved hands, remove all BeadChips from the Hyb Chamber and submerge them face up at the bottom of the beaker.

Figure 40 BeadChips Submerged Face Up in Beaker



3 Using powder-free gloved hands, remove the coverseal from the first BeadChip. This may require significant force, due to the strength of the adhesive. Ensure that the entire BeadChip remains submerged during removal.

Figure 41 Removing the Coverseal



4 Using tweezers or powder-free gloved hands, transfer the BeadChip to the slide rack submerged in the dish containing 250 ml Wash E1BC solution.

Figure 42 Submerging BeadChips in Wash Dish Containing E1BC Buffer

5 Repeat steps 3 and 4 for all BeadChips from the same Hyb Chamber.

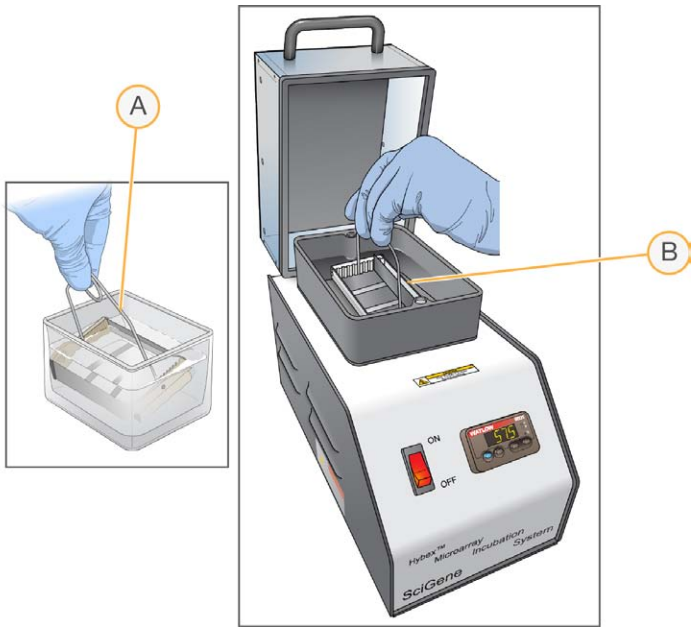
NOTE
Ensure the BeadChip is completely submerged in the diluted Wash E1BC buffer. When processing multiple BeadChips, submerge each BeadChip in Wash E1BC buffer before removing the next BeadChip from its Hyb Chamber Insert.

High Temp Wash

1 Using the slide rack handle, transfer the rack into the Hybex Waterbath insert containing High-Temp Wash buffer.

2 Close the Hybex lid.

Figure 43 Transfer Wash Rack to Waterbath Insert



A Slide rack handle attached
B Transfer to Hybex Waterbath insert

3 Incubate static for 10 minutes with the Hybex lid closed.

Figure 44 Static Incubation in High-Temp Wash Buffer

First Room-Temp Wash

1 After the 10 minute incubation in High-Temp Wash buffer is complete, immediately transfer the slide rack back into a dish containing 250 ml fresh Wash E1BC buffer.

Figure 45 Washing BeadChip in Diluted Wash E1BC Buffer

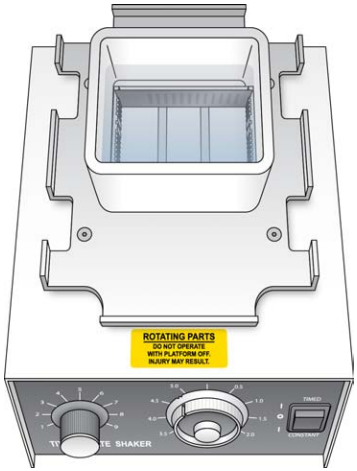
NOTE
When processing multiple BeadChips, submerge each in the Wash E1BC buffer before removing the next BeadChip from its Hyb Chamber.

2 Using the slide rack handle, plunge the rack in and out of the solution 5–10 times.

3 Set the orbital shaker to medium-low.

4 Place the dish on the orbital shaker and shake at room temperature for 5 minutes. Shake at as high a speed as possible without allowing the solution to splash out of the dish.

Figure 46 Washing Dish/BeadChip on Orbital Shaker



Ethanol Wash

1 Transfer the rack to a clean dish containing 250 ml fresh 100% Ethanol.

2 Using the slide rack handle, plunge the rack in and out of the solution 5–10 times.

3 Place the dish on the orbital shaker and shake at room temperature for 10 minutes.

Coat BeadChip

Prepare Wash Dishes and Tube Racks

Before starting the Coat BeadChip process, please read these important notes:

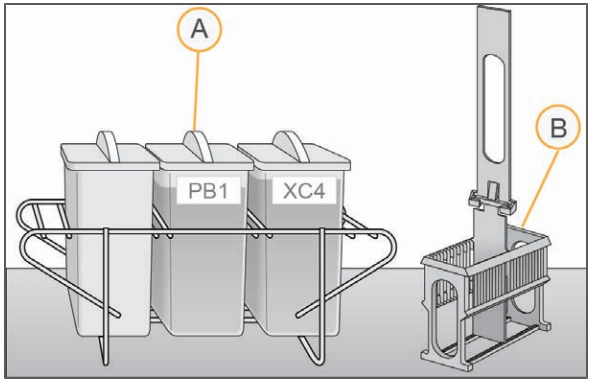
- Take the utmost care to minimize the chance of lint or dust entering the wash dishes, which could transfer to the BeadChips. Place wash dish covers on wash dishes when stored or not in use. Clean wash dishes with low-pressure air to remove particulates prior to use.
- In preparation for XC4 BeadChip coating, wash 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 the Kimwipe layers. Do not place on absorbent lab diapers. You will place the staining rack containing BeadChips on this tube rack after removing it from the XC4 wash dish.
- Prepare an additional clean tube rack that fits the internal dimensions of the vacuum desiccator for removal of the BeadChips. Allow one rack per 8 BeadChips. No Kimwipes are required under this tube rack.

1 Lay out the following equipment on the lab bench:
- 1 staining rack
- 1 vacuum desiccator
- 1 tube rack
- Self-locking tweezers
- Large Kimwipes
- Vacuum hose

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

3 Set up two top-loading wash dishes, labeled as PB1 and XC4.

Figure 47 PB1 and XC4 Wash Dishes with Staining Rack



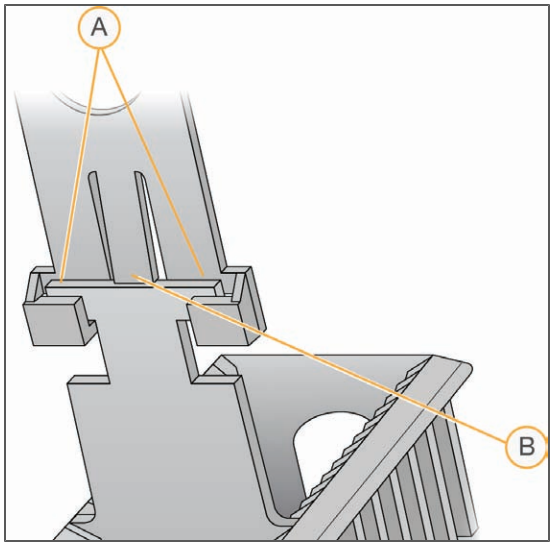
A Wash Dishes
B Staining Rack

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

5 Pour 310 ml PB1 into the wash dish labeled "PB1."

6 Submerge the unloaded staining rack into the wash dish with the locking arms and tab ***facing you***. This orients the staining rack so that you can safely remove the BeadChips.

Figure 48 Staining Rack Locking Arms and Tab



A Locking Arms
B Tab

CAUTION
If the staining rack handle is not correctly oriented, the BeadChips may be damaged when you remove the staining rack handle before removing the BeadChips.

Let the staining rack sit in the wash dish. You will use it to carry the BeadChips.

7 Quickly transfer each BeadChip from the EtOH wash to the staining rack while it is submerged in PB1.

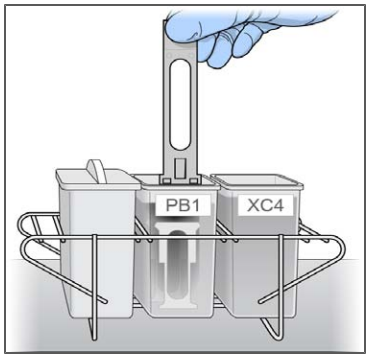
CAUTION
Do not touch the face of the BeadChips. Handle them by the barcode end or by the edges.

8 Place the BeadChips in the staining rack while it is submerged in PB1. Put four BeadChips above the staining rack handle and four below. The BeadChip barcodes should ***face away*** from you, while the locking arms on the handle ***face towards*** you. If necessary, briefly lift the staining rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting the BeadChip.

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

9 Move the staining rack up and down 10 times, breaking the surface of the PB1.

Figure 49 Washing BeadChips in PB1



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

10 Allow the BeadChips to soak for an additional 5 minutes.

CAUTION
Do not leave the BeadChips submerged in PB1 for longer than 30 minutes.

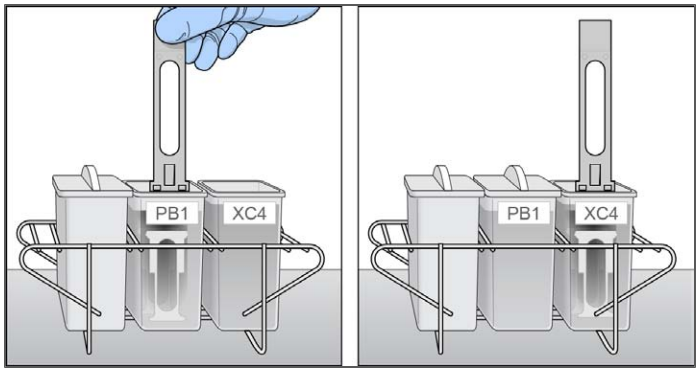
11 Pour 310 ml XC4 into the dish labeled "XC4," and cover the dish to prevent any lint or dust from falling into the solution.

NOTE
Use the XC4 within 10 minutes after filling the wash dish.

12 Place the bottle with excess XC4 in a readily available location for topping off the XC4 wash dish during the coating procedure.

13 Remove the staining rack from the dish containing PB1 and place it directly into the wash dish containing XC4. 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 50 Moving BeadChips from PB1 to XC4



14 Move the staining rack up and down 10 times, breaking the surface of the XC4.

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

15 Allow the BeadChips to soak for an additional 5 minutes.

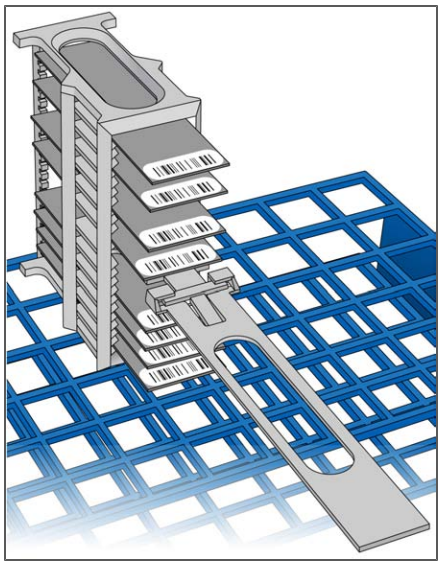
CAUTION
Use XC4 only once. To process subsequent BeadChips, use a new, clean wash dish with fresh XC4.

16 Prepare a clean tube rack for the staining rack by placing two folded Kimwipes under the tube rack.

17 Prepare one additional tube rack per 8 BeadChips that fits the internal dimensions of the vacuum desiccator.

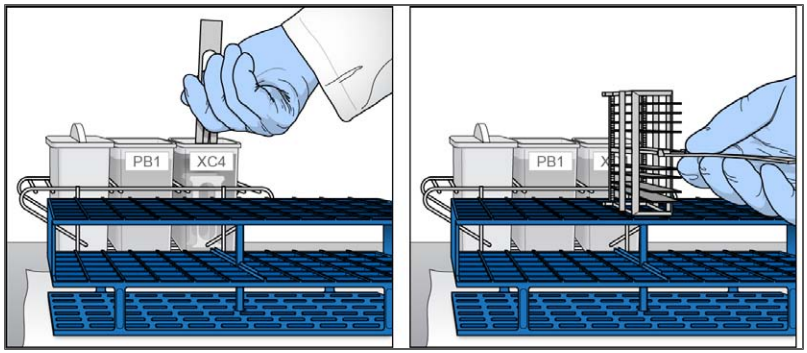
18 Remove the staining rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes ***face up*** and the locking arms and tab ***face down***.

Figure 51 Staining Rack in Correct Orientation



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

Figure 52 Moving the Staining Rack from XC4 to Tube Rack



20 For the top four BeadChips, working top to bottom:

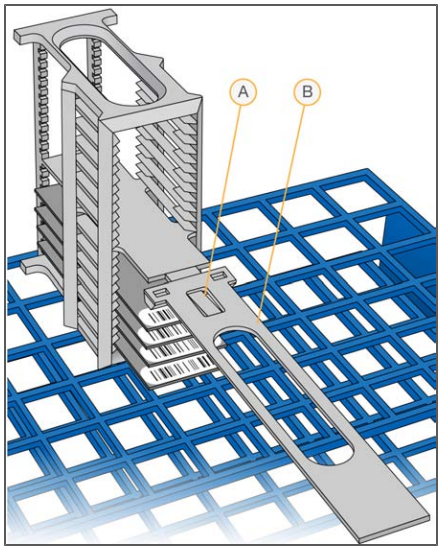
a Continuing to hold the staining rack handle, carefully grip each BeadChip at its barcode end with self-locking tweezers.

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

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

21 Holding the top of the staining rack in position, grasp the handle between your thumb and forefinger. Push the tab up with your thumb and push the handle away from you to unlock it. Pull up the handle and remove.

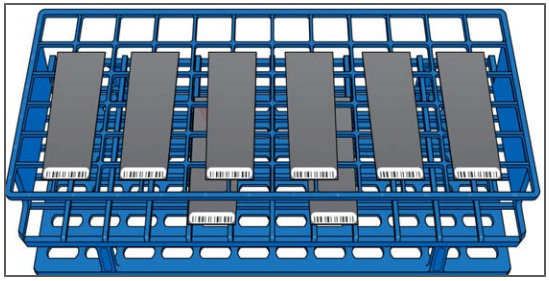
Figure 53 Removing Staining Rack Handle



A Tab
B Handle

22 Remove the remaining BeadChips to the tube rack 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 54 Placing 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.

23 Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack (8 BeadChips).

24 Ensure the vacuum valve is seated tightly and securely.

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

26 Start the vacuum, using at least 508 mm Hg (0.68 bar).

27 To ensure that the dessicator is properly sealed, gently lift the lid of the vacuum desiccator. It should not lift off the desiccator base.

Figure 55 Testing Vacuum Seal

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

29 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.

30 Store the desiccator with the red valve plug in the desiccator's three-way valve to stop accumulation of dust and lint within the valve port. Remove the red plug from the three-way valve before applying vacuum pressure.

31 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.

32 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 Hold the BeadChip at a downward angle to prevent excess EtOH from dripping onto the stripes.

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

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

CAUTION

Do not touch the stripes.

33 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 eight humidifying buffer reservoirs.

34 Discard unused reagents in accordance with facility standards.

35 Proceed to *Image BeadChip* on page 111.

Image BeadChip

Proceed to scanning the BeadChips. See the *BeadArray Reader User Guide*, *iScan System User Guide*, or the *HiScanSQ System User Guide* for general instructions on scanning your BeadChips. For specific scan settings refer to the following table:

Table 15 WG-DASL HT Assay Scan Settings

Scanner	Scan Setting
iScan or HiScan	Direct Hyb
BeadArray Reader	DirectHyb Gene Expression

GenomeStudio

The GenomeStudioGene Expression Module is an application for analyzing gene expression data from scanned microarray images collected from systems, such as the Illumina HiScan or iScan System or BeadArray Reader.

For feature descriptions and instructions on using the GenomeStudio platform to visualize and analyze gene expression data, see the GenomeStudio Framework User Guide and the GenomeStudio Gene Expression Module User Guide.

Appendix A

WG-DASL HT AssayControls

Introduction

This appendix describes the control oligos for the WG-DASL HT Assay and how to view them. The control oligos include:

View the Control Report

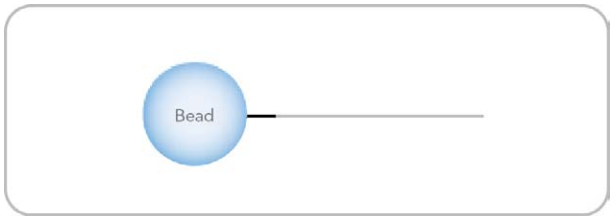
The GenomeStudio software platform tracks the performance of these controls and generates a report across all arrays on the BeadChip.

For more information, see the *GenomeStudio Framework User Guide and the GenomeStudio Gene Expression Module User Guide.*

Negative Controls

This category consists of query oligos targeting ~300 random sequences that do not appear in the human genome. The mean signal of these probes defines the system background. This background is represented by both the imaging system background and by any signal resulting from cross-hybridization or non-specific binding of dye. The GenomeStudio software platform uses the signals and signal standard deviation of these probes to establish gene expression detection limits.

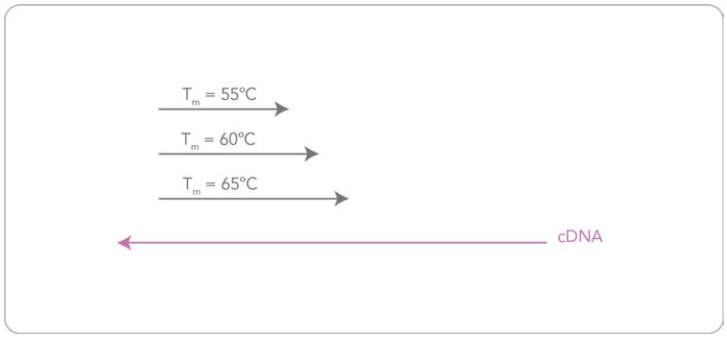
Figure 56 Negative Control Diagram



Oligo Annealing Controls

The oligo annealing controls test the efficiency of annealing ASOs with different T_ms to the same cDNA target. In each case, the higher T_m ASO should give higher signals than the lower T_m ASO.

Figure 57 Oligo Annealing Controls



Array Hybridization Controls

The array hybridization controls test the hybridization of single-stranded assay products to the array beads. The controls consist of 50-mer oligos labeled with Cy3 dye included in the HYB reagent.

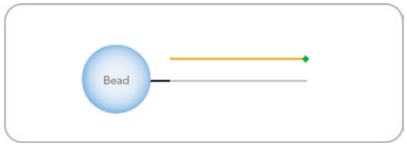
Two types of controls comprise this category:

- Cy3-Labeled Hyb Control
- Low Stringency Hyb Control

Cy3-Labeled Hyb Controls

These controls consist of six probes with corresponding Cy3-labeled oligonucleotides present in the HYB. Following successful hybridization, they produce a signal independent of both the cellular RNA quality and success of the sample prep reactions. Target oligonucleotides for the Cy3 Hyb controls are present at three concentrations (low, medium, and high), yielding gradient hybridization responses.

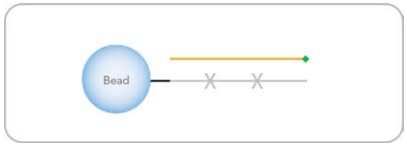
Figure 58 Cy3-Labeled Hyb Control Diagram



Low Stringency Hyb Control

This control category contains four probes, corresponding to the medium- and high-concentration Cy3 Hyb control targets. In this case, each probe has two mismatch bases distributed in its sequence. If stringency is adequate, these controls yield very low signal. If stringency is too low, they yield signal approaching that of their perfect match counterparts in the Cy3 Hyb control category.

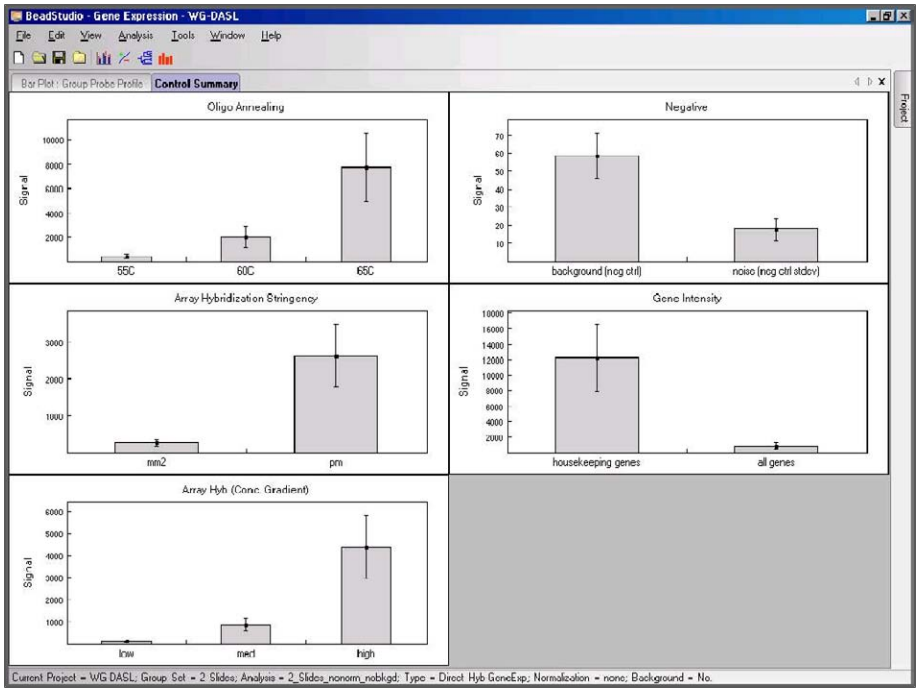
Figure 59 Low Stringency Hyb Control Diagram



Gene Intensity (Housekeeping and All Genes)

The intactness of the biological specimen can be monitored by housekeeping gene controls. These controls consist of probes to housekeeping genes that typically are expressed in most samples.

Figure 60 WG-DASL HT Control Summary



Assay Qualification Using gDNA

Introduction

One unique feature of the WG-DASL HT Assay is that the performance of each target site can be tested using genomic DNA as a sample. This is possible because the oligos used in the WG-DASL HT Assay are designed to fall within exon boundaries.

As an option, the WG-DASL HT Assay user can use the Make SUD (Make Single-Use DNA) process to prepare activated gDNA to include as a positive control sample. The data derived from the gDNA samples can then be used to qualify individual target assays during data analysis in the GenomeStudio software platform.

Make Single-Use DNA (SUD) Plate

This process activates sufficient DNA of each individual sample to be used ***once*** in the WG-DASL HT Assay.

Estimated Time

Hands-on: ~15 minutes

Incubation: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE)	See instructions	Room temperature	User
MS1 reagent	1 tube per SUD plate	-15° to -25°C	Illumina
Human genomic DNA	50 ng/µl	-15° to -25°C	User
96-well 0.2 ml skirted microplate	1 per sample plate	See manufacturer's instructions	User

Preparation

- In the appropriate columns of the Sample Sheet, enter the Sample_Name and Sample_Plate for each Sample_Well defined in the Sample Sheet. See *Sample Sheet* on page 31.
- Preheat the heat block to 95°C and allow the temperature to stabilize.
- Turn on the heat sealer to preheat it. Allow 15 minutes.
- Thaw the MS1 reagent tube to room temperature. Vortex to mix the contents, and pour the entire tube into a new, non-sterile reservoir.
- Thaw the DNA samples and controls to room temperature and vortex to mix the contents.
- Apply a SUD barcode label to a new 96-well microplate.
- On the lab tracking form, record:
  - Date and time
  - Operator

- SUD plate barcode
- MS1 reagent barcode

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.

Make SUD

1. Normalize DNA samples to 50 ng/µl with 10 mM Tris-HCl pH 8.0, 1 mM EDTA.
2. Add 5 µl MS1 reagent to each well of the SUD plate.
3. Using an 8-channel pipette, transfer 5 µl normalized DNA sample to each well of the SUD plate. Change tips between column dispenses.
4. Apply a microplate foil heat seal to the SUD plate and seal it with the heat sealer (3 seconds). Ensure that all wells are completely sealed.
5. Pulse centrifuge the SUD plate to 250 xg.
6. Vortex at 2300 rpm for 20 seconds, making sure the plate is firmly strapped to the vortexer platform to prevent plate movement.
7. Pulse centrifuge to 250 xg.

NOTE
It is important to centrifuge the SUD plate to 250 xg before the 95°C incubation to prevent the wells from drying out during incubation.

8. Place the SUD plate in the preheated heat block and close the lid.
9. Incubate the SUD plate at 95°C for exactly 30 minutes. Using the heat block cover, cover the SUD plate to reduce condensation on the plate seal.

CAUTION
Do not allow the 95°C incubation period to exceed 30 minutes.

10. Record the start and stop times on the lab tracking form.
11. Pulse centrifuge the plate to 250 xg.

12 If you plan to perform the Make ASE protocol today, then immediately set the heat block to 70°C.

13 To use the activated gDNA in the Make ASE protocol, substitute 10 µl of the Make SUD product in place of the usual 10 µl of the Make SUR product. Each well of the ASE plate will contain 10 µl DAP, 30 µl OB1, and 10 µl of either SUR or SUD.

Data Analysis

The GenomeStudio software platform will extract array intensity data from the gDNA samples in the same way as for the cDNA samples. A description of how this data is used to eliminate suboptimal probes is provided in the *GenomeStudio Framework User Guide*.

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Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 16 Illumina General Contact Information

Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

Table 17 Illumina Customer Support Telephone Numbers

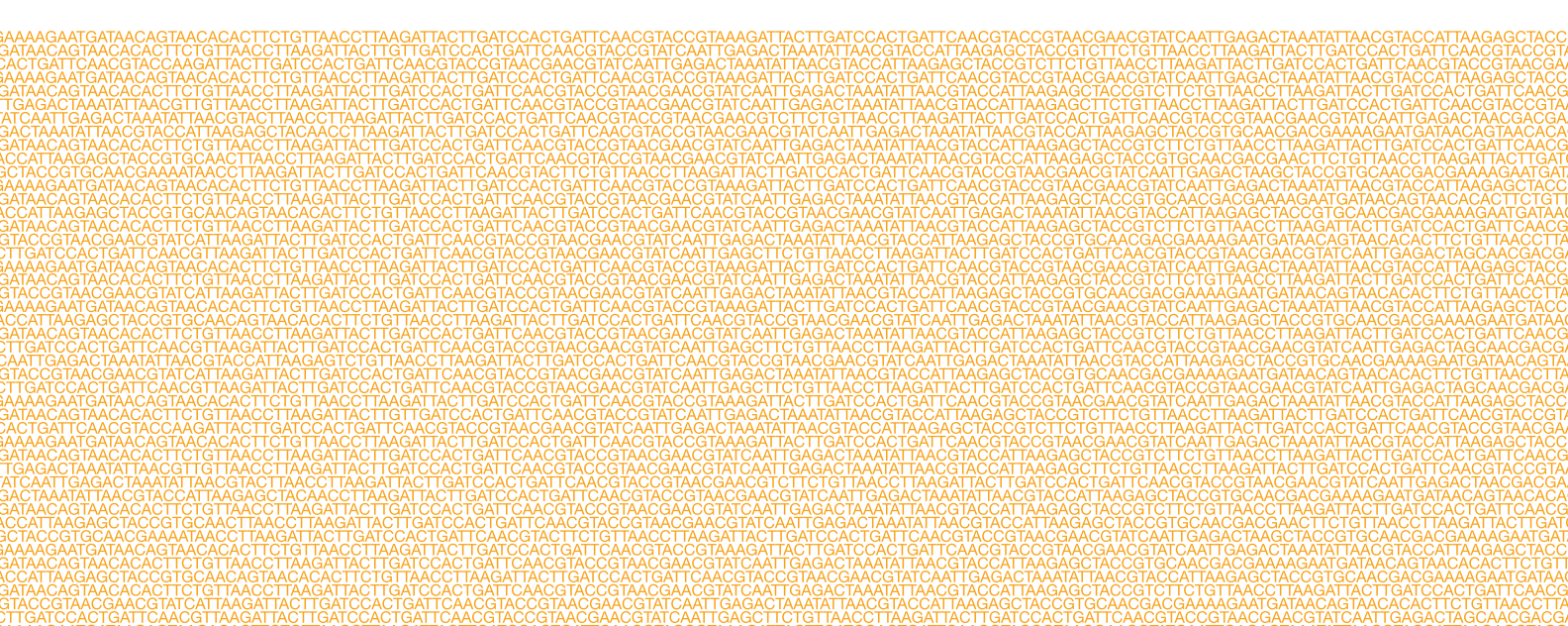
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

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

Product Documentation

You can obtain PDFs of additional product documentation from the Illumina website. Go to http://www.illumina.com/support and select a product. To download documentation, you will be asked to log in to MyIllumina. After you log in, you can view or save the PDF. To register for a MyIllumina account, please visit https://my.illumina.com/Account/Register.



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