Applied Biosystems 1700 Chemiluminescent Microarray Analyzer

Chemistry Guide

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

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Preface

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Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vi.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard

WARNING **CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Products 1

This chapter covers:

System Overview. 1-2
Reagents and Consumables 1-3

System Overview

Summary

The Applied Biosystems Expression Array System is a comprehensive gene expression profiling system that combines:

- Complete, annotated, fully curated human genome transcript data
- High-sensitivity chemiluminescence detection
- Advanced data management methods

System Products

The Applied Biosystems Expression Array System consists of the following products for profiling gene expression across entire genomes:

- Applied Biosystems 1700 Chemiluminescent Microarray Analyzer
- Applied Biosystems Chemiluminescent RT Labeling Kit
- Applied Biosystems Chemiluminescent RT-IVT Labeling Kit
- Applied Biosystems Chemiluminescence Detection Kit
- Applied Biosystems microarrays

Procedural Overview

Using the Applied Biosystems Expression Array System involves:

1. Using the RT labeling kit or the RT-IVT labeling kit to convert total RNA or mRNA sample to DIG-labeled cDNA targets or cRNA targets
2. Using the CL detection kit and microarrays to hybridize the targets to the probes on the microarray and to perfrom the chemiluminescent reaction
3. Using the 1700 instrument to detect and measure the chemiluminescent and fluorescent signals
4. Using data analysis software to profile gene expression

Results

The Applied Biosystems Expression Array System enables researchers to:

- Survey and measure gene expression over the entire human genome in a single experiment
- Determine differential gene expression over the entire human genome in various populations, tissues, developmental stages, disease states, or defined research conditions
- Isolate specific gene expression events
- Examine related annotations
- Select individual genes for validation of expression and quantitative transcript analysis

Features

The Applied Biosystems Expression Array System provides:

- Total genome analysis
- High sensitivity
- Gene expression ratios
- Reproducible results
- Extensive data quality checks
- Advanced microarray data management
- Celera genomic information
- Links to validated quantitative gene expression assays

Reagents and Consumables

Ordering Reagents and Consumables

Visit the Applied Biosystems Web site or contact your Applied Biosystems sales representative to order available products:

www.appliedbiosystems.com

Table 1-1 Reagents and consumables part numbers

Product	Applied Biosystems Part Number
Applied Biosystems Chemiluminescence Detection Kit	4342142
Applied Biosystems Chemiluminescent RT Labeling Kit	4340415
Applied Biosystems Chemiluminescent RT-IVT Labeling Kit	4340472
Applied Biosystems Human Genome Survey Microarrays (4)	4337467
Applied Biosystems Human Genome Survey Microarrays (12)	4337468

RT Labeling Kit

The Applied Biosystems Chemiluminescent RT Labeling Kit contains reagents and plastic consumables for producing single-stranded, digoxigenin (DIG)-labeled cDNA from mRNA samples and purifying the cDNA product.

RT-IVT Labeling Kit

The Applied Biosystems Chemiluminescent RT-IVT Labeling Kit contains reagents and plastic consumables for producing single-stranded, DIG-labeled cRNA from total RNA or mRNA samples and purifying the cRNA product.

Chemiluminescence Detection Kit

The Applied Biosystems Chemiluminescence Detection Kit contains reagents for hybridizing DIG-labeled cDNA or cRNA targets to probes on Applied Biosystems microarrays and preparing the microarray for chemiluminescence detection in the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer.

Assay Design and Theory 2

This chapter covers:

RT Labeling Processes 2-2
RT-IVT Labeling Processes 2-3
Microarray Design 2-5
Chemiluminescence Detection Processes 2-8

RT Labeling Processes

Flowchart

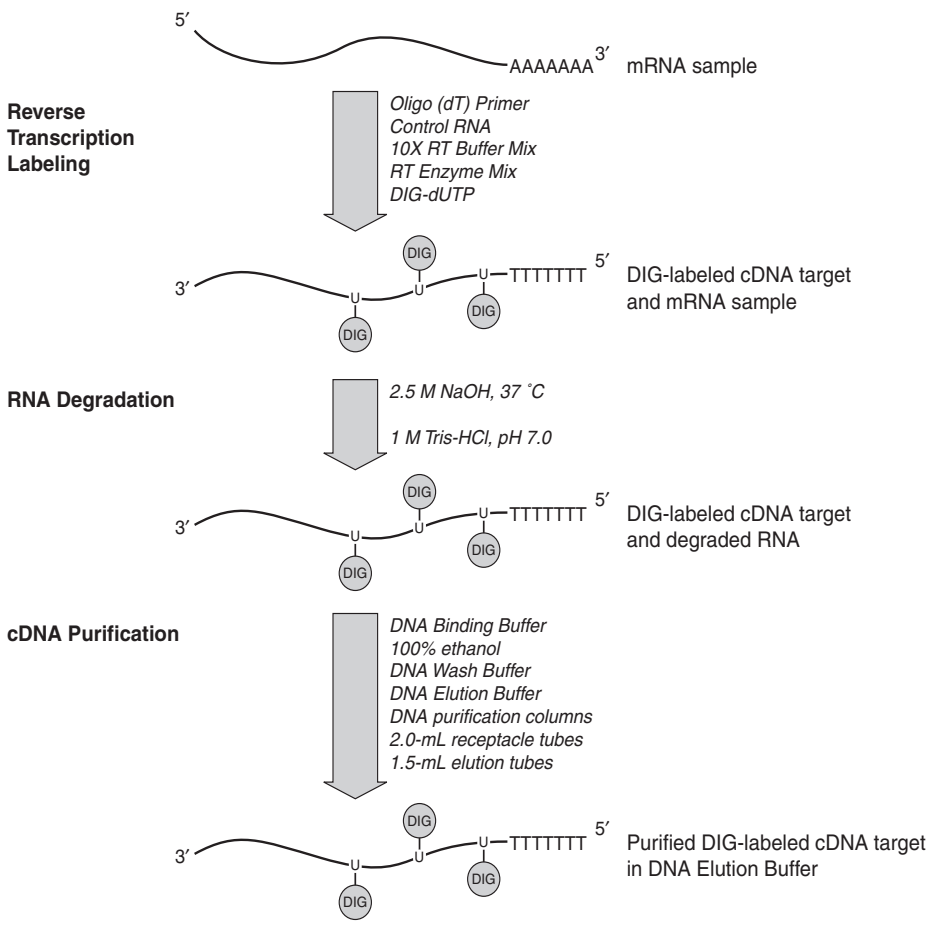

Figure 2-1 RT labeling flowchart

Reverse Transcription Labeling

The reverse transcriptase incorporates deoxynucleotides and digoxigenin-dUTP (DIG-dUTP) in the synthesis of single-stranded cDNA from sample RNA and RT Labeling Control RNA.

The reverse transcriptase used in this reaction is a modified version of Moloney murine leukemia virus (MMLV) reverse transcriptase. The modified reverse transcriptase has no RNase H activity. It also provides longer cDNA transcripts and higher yields than the wild type enzyme.

RNA Degradation

The template mRNA and the control RNA are hydrolyzed using sodium hydroxide and heat at 37 °C. The reaction is neutralized by the addition of 1 M Tris, pH 7.0.

cDNA Purification

cDNA purification washes away salts, buffers, unincorporated nucleotides, primers, and degraded RNA. The cDNA is eluted in a solution suitable for hybridization of the cDNA to 1700 microarrays.

RT-IVT Labeling Processes

Flowchart

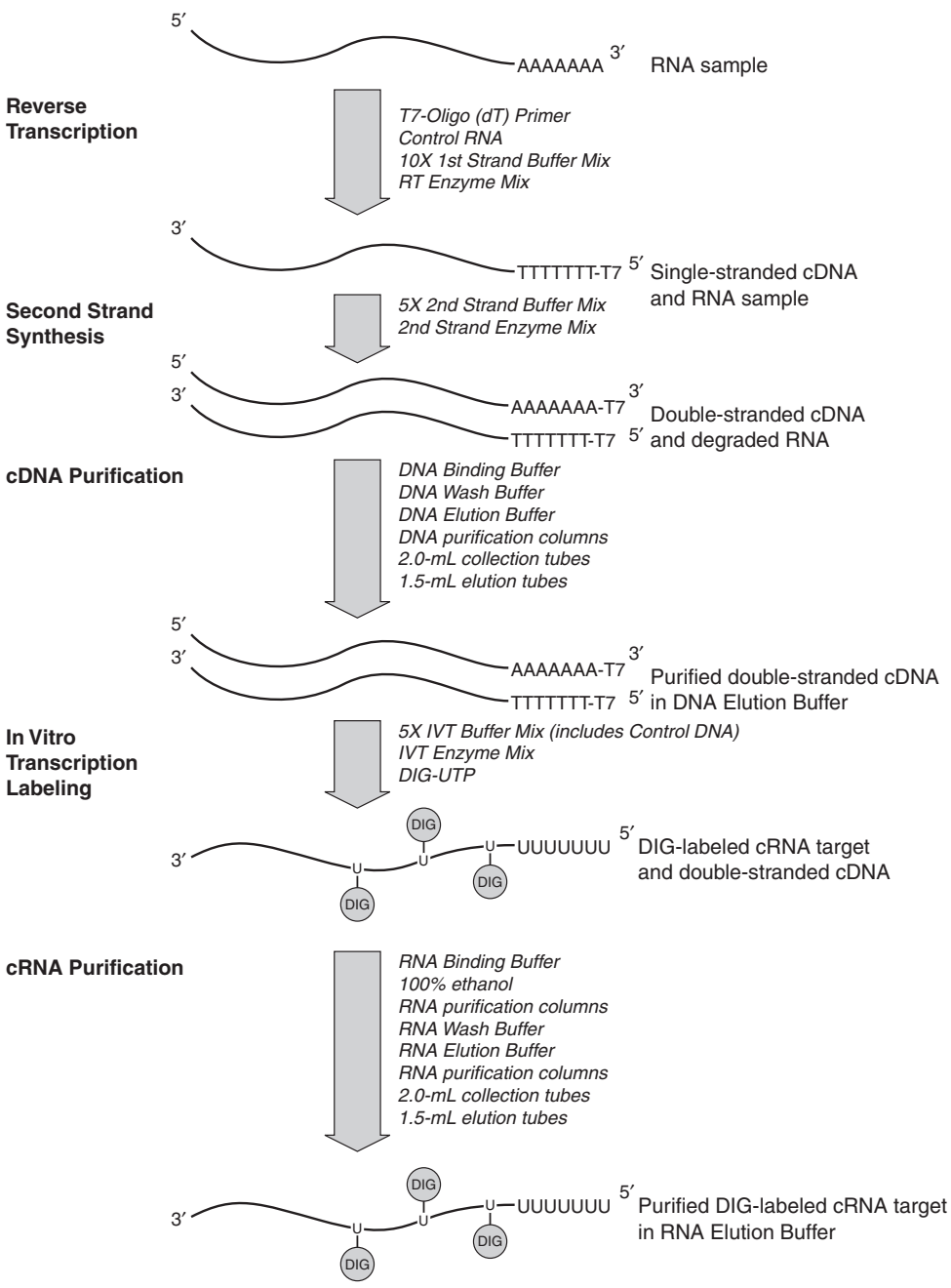

Figure 2-2 RT-IVT labeling flowchart

Reverse Transcription

The reverse transcriptase enzyme incorporates deoxynucleotides in the synthesis of single-stranded cDNA from sample RNA and RT Labeling Control RNA. Use of the T7-poly dT primer adds the T7 polymerase promoter to the 5′ end of the single-stranded cDNA transcript.

Note: The sequences of the RT Labeling Control RNA in the RT-IVT labeling kit are identical to those in the RT labeling kit. The concentration of RT Labeling Control RNA is lower in the RT-IVT labeling kit because of amplification during the IVT labeling step.

Second Strand Synthesis

RNase H degrades the RNA in DNA-RNA duplexes to provide priming sites for DNA polymerase to synthesize the second strand of cDNA.

cDNA Purification

cDNA purification washes away salts, buffers, and unincorporated nucleotides. The double-stranded (ds) cDNA is eluted in a solution suitable for the *in vitro* transcription (IVT) reaction.

IVT Labeling

T7 RNA polymerase incorporates ribonucleotides and DIG-UTP to synthesize copy RNA (cRNA) from ds cDNA containing the T7 promoter. This step results in 100-fold to 1000-fold amplification of targets.

cRNA Purification

cRNA purification washes away salts, unincorporated nucleotides, primers, and cDNA. The cRNA is eluted in a solution suitable for hybridization of the cRNA to 1700 microarrays.

Microarray Design

Layout

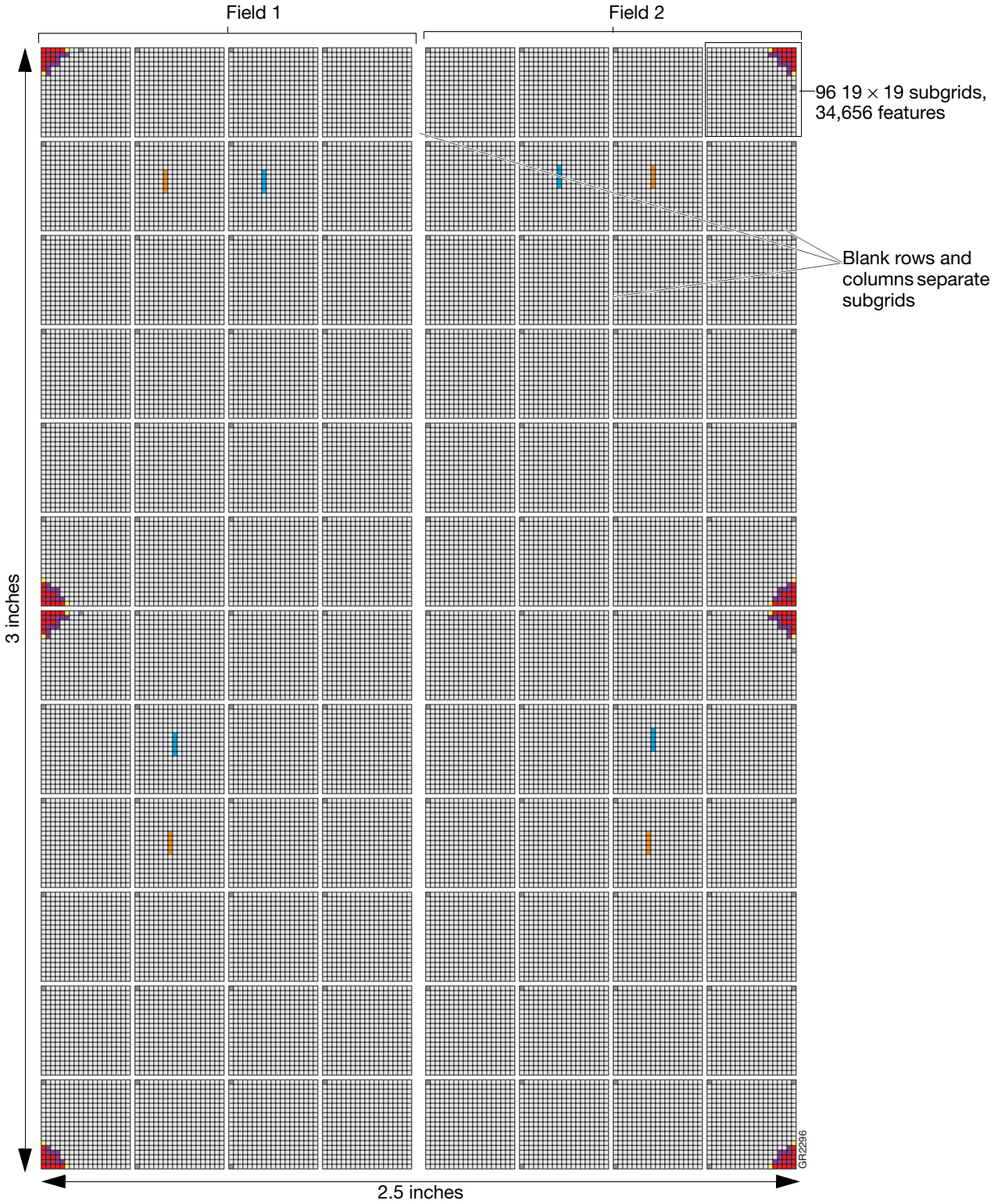

Figure 2-3 Microarray layout

Feature Sizes and Spacing

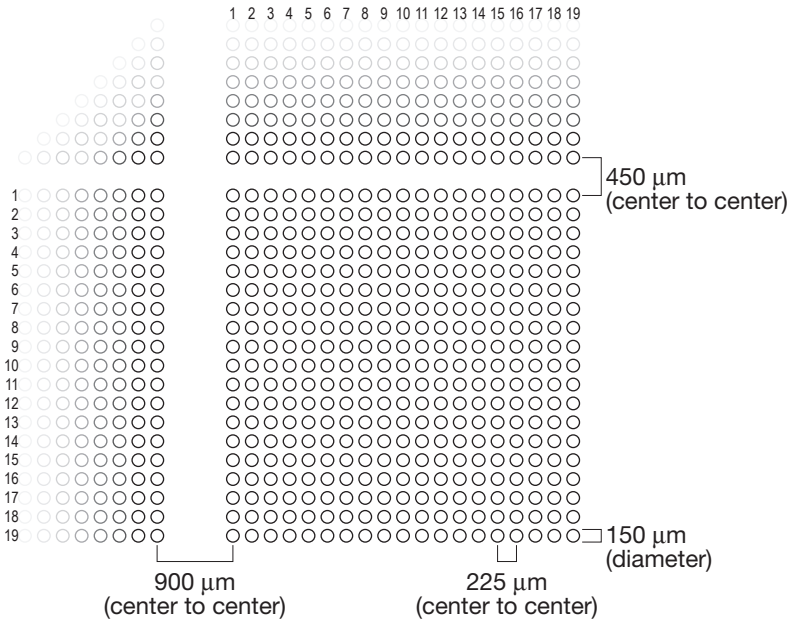

Figure 2-4 Feature sizes and spacing on the microarray

Sources

The probe sets used in Applied Biosystems microarrays are optimized by using high quality sequences from multiple sources:

- Celera expert-curated sequences
- Publicly available, validated mRNA sequences
- Wet chemistry-validated genes

Probe Design

Table 2-1 Probe design

Attribute	Probe Design	Design Result
Length	60 base pairs long	• High specificity • Optimal performance
Placement	Biased toward the 3′ end	High sensitivity because DIG-labeling process is more efficient at the 3′ end
Coverage	• Common regions between alternative transcripts • 3′ end defined as the most upstream polyadenylation site if possible	• Gene level coverage • Gene specificity
Number	One probe per gene	Genome-wide coverage
Overlap	Low probe overlap	Multiple independent measurements of mRNA levels

Probe Information

Information about each probe is available in the system database:

- 1500-bp sequence of the 3′ region for each gene represented by a probe
- Annotation and gene classification information from the Panther Protein Classification System.

Note: To obtain further annotated data for your selected gene targets through the Celera Discovery System™ online platform, contact an Applied Biosystems representative.

Chemiluminescence Detection Processes

Flowchart

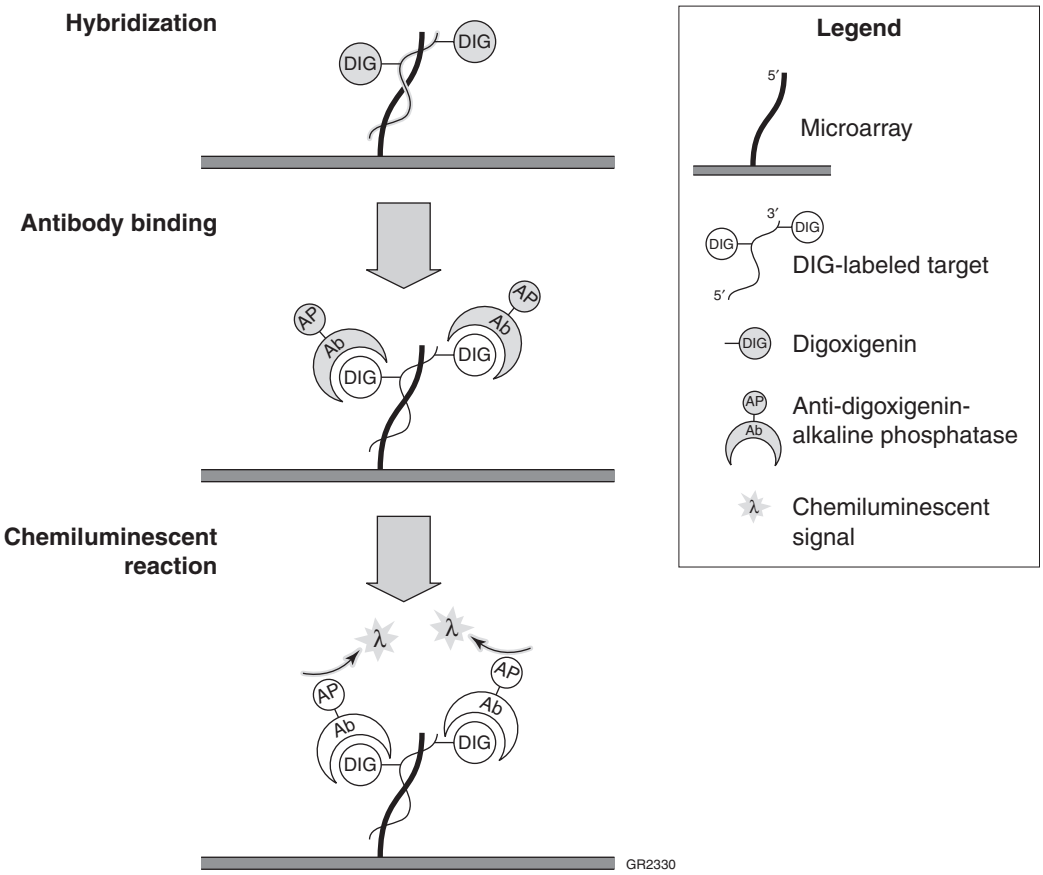

Figure 2-5 Chemiluminescence detection overview

Fragmenting cRNA Divalent cations catalyze 2′ hydroxyl cleavage of RNA. The cRNA fragmentation reduces secondary structure and improves hybridization kinetics.

Prehybridization Microarrays are incubated with blocking agents to prevent nonspecific hybridization.

Hybridization DIG-labeled targets hybridize to probes on the microarrays.

Washes Microarrays are taken out of the cartridges for the wash steps. The washes reduce nonspecific hybridization and prepare the microarray for antibody binding.

Antibody Binding

1. Blocking buffer is added to the microarray to prevent nonspecific antibody binding.
2. Anti-digoxigenin-alkaline phosphatase (AP) is added to bind to digoxigenin in:
   - cDNA or cRNA targets
   - RT controls and IVT controls
   - Chemiluminescent control probes on the microarray
3. Microarray rinses remove any unbound antibody and prepare the microarray for the chemiluminescence reaction.

Chemiluminescent Reaction

1. Enancing solution is added to the microarray to increase the quantum yield of the chemiluminescent reaction.
2. With the addition of substrate, the alkaline phosphatase in the anti-digoxigenin-AP conjugate dephosphorylates the substrate. This chemical reaction emits light.

Detection

1. The microarray is loaded onto the heated stage of the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer. The temperature of the stage is the optimal temperature for the chemiluminescent reaction.
2. The 1700 analyzer focuses on the microarray.
3. The 1700 analyzer obtains chemiluminescent and fluorescent images of the microarray.

Assay Workflow 3

This chapter covers:

Experimental Workflow. 3-2
RT Labeling and Detection 3-3
RT-IVT Labeling and Detection 3-4

Experimental Workflow

Gene Expression Assay Workflows

There are two workflows for performing gene expression assays using the Applied Biosystems Expression Array System:

- RT labeling and detection workflow
- RT-IVT labeling and detection workflow

Workflow Considerations

The workflows differ mainly in how labeled transcripts are produced. Use Table 3-1 to help you decide which workflow to use.

Table 3-1 Workflow considerations

Workflow	Sample Requirements	When to Use	Page
RT Labeling and Detection	2 to 5 µg mRNA	Abundant sample RNA	3-3
RT-IVT Labeling and Detection	1 to 10 µg total RNA or 0.05 to 2 µg mRNA	Limited sample RNA	3-4

RT Labeling and Detection

Products

- Applied Biosystems Chemiluminescent RT Labeling Kit
- Applied Biosystems Chemiluminescence Detection Kit
- Applied Biosystems microarrays

Documents

- *Applied Biosystems Chemiluminescent RT Labeling Kit Protocol* (PN 4339628)
- *Applied Biosystems Chemiluminescent RT Labeling Kit Quick Reference Card* (PN 4346876)
- *Applied Biosystems Chemiluminescence Detection Kit Protocol* (PN 4339627)
- *Applied Biosystems Chemiluminescence Detection Kit Quick Reference Card* (PN 4346875)
- *Applied Biosystems Chemiluminescent Microarray Analyzer Version 1.0 User Guide* (PN 4338852)

Procedural Overview

1. Obtain RNA samples.
2. Perform RT labeling kit procedures:
   a. Synthesize single-stranded, DIG-labeled cDNA from mRNA or total RNA samples.
   b. Degrade the RNA.
   c. Wash and purify the cDNA.
3. Perform CL detection kit procedures:
   a. Prepare the microarray for hybridization.
   b. Hybridize DIG-labeled cDNA targets to probes on an Applied Biosystems microarray.
   c. Bind anti-DIG antibody to DIG-labeled targets hybridized to the microarray.
   d. Perform the chemiluminescence reaction.
4. Load the microarray into the 1700 instrument.
5. Analyze the data.

RT-IVT Labeling and Detection

Products

- Applied Biosystems Chemiluminescent RT-IVT Labeling Kit
- Applied Biosystems Chemiluminescence Detection Kit
- Applied Biosystems microarrays

Documents

- *Applied Biosystems Chemiluminescent RT-IVT Labeling Kit Protocol* (PN 4339629)
- *Applied Biosystems Chemiluminescent RT-IVT Labeling Kit Quick Reference Card* (PN 4346877)
- *Applied Biosystems Chemiluminescence Detection Kit Protocol* (PN 4339627)
- *Applied Biosystems Chemiluminescence Detection Kit Quick Reference Card* (PN 4346875)
- *Applied Biosystems Chemiluminescent Microarray Analyzer Version 1.0 User Guide* (PN 4338852)

Procedural Overview

1. Obtain RNA samples.
2. Perform RT-IVT labeling kit procedures:
   a. Synthesize single-stranded cDNA from RNA samples.
   b. Convert single-stranded cDNA to double-stranded cDNA.
   c. Wash and purify the double-stranded cDNA.
   d. Synthesize single-stranded, DIG-labeled cRNA from the double-stranded cDNA.
   e. Wash and purify the cRNA.
3. Perform CL detection kit procedures:
   a. Fragment the cRNA.
   b. Prepare the microarray for hybridization.
   c. Hybridize DIG-labeled cRNA targets to probes on an Applied Biosystems microarray.
   d. Bind anti-DIG antibody to DIG-labeled targets hybridized to the microarray.
   e. Perform the chemiluminescence reaction.
4. Load the microarray into the 1700 instrument.
5. Analyze the data.

Assay Controls 4

This chapter covers:

Overview 4-2
Control Summaries 4-3
Blank Features 4-5
Control Ladders 4-6
Hybridization Controls 4-7
Internal Control Probe and Target 4-8
IVT Controls 4-9
Landmark Fiducials 4-10
Negative Controls 4-11
RT Controls 4-12
Spatial Normalization Controls 4-13
Quality Check Report 4-14

Overview

Purpose

The Applied Biosystems Expression Array System contains a suite of controls that can be used to check the quality of all aspects of an expression-profiling experiment.

About this Chapter

This chapter provides:

- Summaries of the system controls by products, workflow, and function (page 4-3)
- Descriptions and examples of each control (page 4-11 through page 4-14)
- Instructions for generating the quality check report (page 4-14)

Control Summaries

Products

Each chemistry product in the Applied Biosystems Expression Array System contains controls:

- Applied Biosystems Chemiluminescent RT Labeling Kit
- Applied Biosystems Chemiluminescent RT-IVT Labeling Kit
- Applied Biosystems Chemiluminescence Detection Kit
- Applied Biosystems microarrays

RT Labeling Kit Controls

RT controls (page 4-12)

RT-IVT Labeling Kit Controls

- RT controls (page 4-12)
- IVT controls (page 4-9)

CL Detection Kit Controls

- Hybridization controls (page 4-7)
- Internal control target (ICT) (page 4-8)

Microarray Controls

- Landmark fiducials (page 4-10)
- Control ladders (page 4-6)
- Spatial normalization controls (page 4-13)
- Negative controls (page 4-11)
- RT controls (page 4-12)
- IVT controls (page 4-9)
- Hybridization controls (page 4-7)
- Internal control probe (ICP) (page 4-8)

Functions

Table 4-1 summarizes the functions of the 1700 system controls.

Table 4-1 1700 system controls

Control Type	Features Contain ICP	Signal Requires Hybridization	Function	See Page
Blank features	No	No	Prevent cross-talk	4-5
Control ladder	No	No	Positive control for chemiluminescent detection chemistry acro+ss the dynamic range	4-6
Hybridization controls	Yes	Yes	Quality check for hybridization reaction	4-7
IVT labeling controls	Yes	Yes	Quality check for *in vitro* transcription in RT-IVT labeling kit	4-9
Landmark fiducials	No	No	System software uses to grid array during image analysis	4-10
Negative controls	Yes	Yes	Quality check for assay background	4-11
RT labeling controls	Yes	Yes	Quality check for reverse transcription in RT and RT-IVT labeling kits	4-12
Spatial calibration controls	Yes	Yes	Normalization of images across the array	4-13
Manufacturing QC controls	NA	NA	Used in manufacturing as a quality check	NA

Blank Features

Purpose The amount of signal produced at the blank features provides information about:

- The background signal for every region of the microarray
- Cross-talk from adjacent spots

Definition Blank features are located between the image zones and between the 19 × 19 subgrids on the microarray.

Function Nonspecific binding of DNA or RNA, antibodies, alkaline phosphatase enzyme, or other chemical or mechanical processes can result in background signal. Signals from the blank features indicate the level of nonspecific signal generated from the assay. The software calculates the amount of background chemiluminescent signals from the blank features and uses the correction to measure all other chemiluminescent signals on the microarray.

Control Ladders

Purpose The control ladders are used to:

- Perform quality checks of array spotting and chemistry
- Compare reproducibility of spotting across batches and many arrays.
- Perform quality checks of chemiluminescent detection chemistry
- Demonstrate sensitivity and dynamic range of chemiluminescent detection chemistry.

Definition There are two copies of the chemiluminescent fiducial ladder in each image zone.

Each chemiluminescent fiducial ladder consists of DIG-labeled probes spotted on the microarray in a five-fold dilution series: 625×, 125×, 25×, 5×, and 1×

Function Because the labeled probes are attached to the microarray, fiducial control ladders produce signals that are independent of labeling and hybridization. Signals from the fiducial control ladders indicate that the chemiluminescent reaction was successful. Signal intensity variability indicates differences in spotting and attachment efficiencies across various batches of manufactured arrays.

Hybridization Controls

Purpose

The hybridization controls can be used:

- To indicate successful hybridization
- To indicate hybridization stringency
- As a spatial normalization control

Definition

The hybridization controls consist of:

- Three DIG-labeled 60-mer oligo control targets supplied with the CL detection kit
  - HYB_Control_1_Ct
  - HYB_Control_2_Ct
  - HYB_Control_3_Ct
- Three unlabeled probes spotted on the microarray:
  - HYB_Control_1_Cp
  - HYB_Control_2_Cp
  - HYB_Control_3_Cp (see "Spatial Normalization Controls" on page 4-13)

Function

The DIG-labeled oligo targets are added to the microarray with the DIG-labeled cDNA or cRNA targets.

The three hybridization controls on the microarray are designed to hybridize to all the DIG-labeled oligo targets:

- Presence of signal indicates hybridization occurred.
- Signal strength indicates hybridization stringency.

See "Spatial Normalization Controls" on page 4-13 for more information about how HYB_Control_3_Cp functions as a spatial normalization control.

Internal Control Probe and Target

Purpose The internal control probe and target can be used to:

- Grid all features on the microarray
- Normalize the CL signal

Definition This control consists of:

- Internal control target (ICT) supplied with the CL detection kit: 24mer oligo labeled with LIZ dye
- Internal control probe (ICP) on the microarray: 24mer cospotted at every feature in the microarray that contains a 60mer gene expression probe

Function The ICT is added to the hybridization mixture. During the hybridization reaction, the ICTs hybridize to every ICP.

IVT Controls

Purpose When using the RT-IVT labeling kit, the IVT controls indicate if the *in vitro* transcription labeling reaction worked and how well the reaction worked. The IVT controls are not used when using the RT labeling kit.

Definition The IVT controls consist of:

- Three synthetic double-stranded cDNA with a T7 promoter and bacterial control gene sequences:
  - *bioB* — 1000-nt ds-cDNA with T7 promoter
  - *bioC* — 750-nt ds-cDNA with T7 promoter
  - *bioD* — 600-nt ds-cDNA with T7 promoter
- A total of 120 features on the microarray:
  - Fifteen probes on the microarray: five for each bacterial control gene, *BioB*, *BioC*, and *BioD*.
  - Each probe is spotted eight times: four times in each image zone.

Function The synthetic control cDNAs are added to the RNA sample when using the RT-IVT labeling kit. The control cDNA undergoes *in vitro* transcription in the presence of DIG to produce DIG-labeled cRNA.

The DIG-labeled control cRNAs hybridize to the probes on the microarray and, after a successful chemiluminescent reaction, generate signal.

Landmark Fiducials

Purpose The landmark fiducial controls are used by the system software to:

- Confirm that the placement of a subset of the features are in the correct position after manufacturing
- Determine the orientation of plates and of the array
- Define the edges and corners of the array
- Align the images

Definition The landmark fiducial controls consist of probes on the microarray labeled with DIG and LIZ dye.

Function After a successful chemiluminescent reaction, the landmark fiducial controls provide strong chemiluminescent and fluorescent signals independent of labeling and hybridization.

Negative Controls

Purpose The negative controls provide information about nonspecific background signals from all probes on the microarray.

Definition The negative controls consist of oligos designed to have low cross-reactivity with the genome being tested. The design strategy:

1. Applied Biosystems performed BLAST® (Basic Local Alignment Search Tool) searches with random sequences.
2. Sequences were ranked according to low predicted reactivity with the genome sequence of interest.
3. Sequences were refined further based on empirical data generated at Applied Biosystems.

Function Nonspecific binding to the negative controls results in signal.

Nonspecific binding can result from cross-hybridization, binding of alkaline phosphatase enzyme, or other chemical processes that lead to background chemiluminescent signal.

The software calculates the amount of background chemiluminescent signal and uses the correction to measure all other chemiluminescent signals on the microarray.

RT Controls

Purpose

The RT controls indicate:

- With the RT labeling kit, if the reverse transcription labeling reaction worked and how well the reaction worked.
- With the RT-IVT labeling kit, if the reverse transcription reaction worked and how well the reaction worked.

Definition

The RT controls consist of:

- Three synthetic mRNAs with bacterial control gene sequences:
  - *lys* — 1000-nt mRNA with poly(A) tail
  - *phe* — 1400-nt mRNA with poly(A) tail
  - *dap* — 1900-nt mRNA with poly(A) tail
- Features on the microarray:
  - Probes for each bacterial control gene: *DAP*, *LYS*, and *PHE*.
  - Each probe is spotted in each image zone.

Function

The synthetic mRNAs are added to the reverse transcription reaction with the RNA sample when using the RT labeling kit or the RT-IVT labeling kit:

- With the RT labeling kit, the control RNA undergoes reverse transcription in the presence of DIG-dUTP to produce DIG-labeled cDNA.
- With the RT-IVT labeling kit, the control RNA undergoes reverse transcription and second-strand synthesis into double-stranded cDNA. The control sequences then undergo *in vitro* transcription in the presence of DIG-dUTP to produce DIG-labeled cRNA.

The DIG-labeled control cDNAs (RT labeling kit) or DIG-labeled control cRNAs (RT-IVT labeling kit) hybridize to the probes on the microarray and, after a successful chemiluminescent reaction, generate signal.

Spatial Normalization Controls

Purpose

The software uses the spatial normalization control signal to remove any systematic spatial trends in feature ratios across the array. These spatial trends may be caused by nonuniform LED fluorescence illumination, nonuniform hybridization, or nonuniform focus of the microarray.

Definition

The spatial normalization controls consist of:

- HYB_Control_3_Cp, an unlabeled probe spotted on at least one corner of each 19 × 19 subgrid on the microarray, which should hybridize to the three DIG-labeled oligo control to produce chemiluminescent signal (see "Hybridization Controls" on page 4-7)
- Internal control probe, which should hybridize to the internal control target to produce fluorescent signal (see "Internal Control Probe and Target" on page 4-8)

Function

Spatial normalization controls are placed throughout the microarray and should produce the same chemiluminescent/fluorescent (CL/FL) signal ratios. After ICP normalization occurs (see "Internal Control Probe and Target" on page 4-8), the algorithm identifies spatial trends for CL/FL signal ratios and derives correction factors by interpolating between the spatial normalization control CL/FL ratios. The algorithm then removes the spatial trends from all feature ratios using local correction factors from the nearest spatial normalization control.

Quality Check Report

Overview

You can generate a quality check (QC) report using the system software to evaluate the performance of the system controls.

Performance Metrics

The performance metrics on the QC report include:

- Probe signal information
- Background signal
- Median signal-to-noise (S/N) values
- Number of genes detected above a standard threshold (S/N > 3)

Procedure

Generate a quality check report after you complete taking images of the microarray on the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer.

To generate a quality check report:

1. After performing the run, select the **QC** tab to display the quality check report.
2. Select **Results** to view the values for chemiluminescent signals and thresholds across the microarray:
   - Median Normalized Signal
   - Median Probe Signal
   - Median Normalized S/N
   - Median Probe S/N
   - Median Assay Background
   - Median Assay Background S/N
   - Median Background
   - Number of genes detected (S/N ≥ 3)
3. Select **Hybridization Controls** to view the values for chemiluminescent signals from the hybridization controls (see page 4-7).
4. Select **CL Control Ladder Table** to view the median CL signal, median S/N, and thresholds from the CL Control ladders (see page 4-6).
5. Select **CL Control Ladder Chart** to view a histogram showing the log(intensity) for the CL Control ladders.
6. Select **RT and RT-IVT Kit Controls** to view the values for chemiluminescent signals from the RT and IVT Controls (see page 4-9 and page 4-12):
   - Median CL signal
   - Median CL S/N

Troubleshooting A

This chapter covers:

Overview A-2
Troubleshooting Tables A-3

Overview

About This Appendix

This appendix contains troubleshooting tables for troubleshooting each step in the Applied Biosystems Expression Array System:

- Table A-1, “Troubleshooting RT labeling,” on page A-3
- Table A-2, “Troubleshooting RT-IVT labeling,” on page A-3

Procedural Overview

To troubleshoot system performance:

1. Generate a system quality check report (page 4-14).
2. Examine the system controls (Chapter 4, Assay Controls).
3. Use the troubleshooting tables (page A-3).

Troubleshooting Tables

Troubleshooting RT Labeling

If the level of chemiluminescent signals from the gene expression probes is low, check the level of chemiluminescent signals from the RT control probes (*lys, phe,* and *dap*). Use Table A-1 to identify possible causes of the problem and to determine recommended actions to resolve the problem.

Table A-1 Troubleshooting RT labeling

Level of Chemiluminescent Signals	Possible Cause	Action
Gene expression probes: low RT control probes: good	RNA sample does not meet the guidelines.	Extract more RNA from your samples and make sure that it meets the guidelines before proceeding.
Gene expression probes: low RT control probes: low	RNase activity in RT labeling reagents or chemiluminescence detection reagents.	1. Test reagents for RNase activity. 2. Discard all contaminated reagents. 3. Repeat the experiment using RNase-free products.
	Insufficient incorporation of DIG-dUTP.	Measure DIG incorporation.
	10X RT Buffer Mix contained precipitates.	Check the buffer mix for precipitates. If precipitates are present, make sure that you warm the buffer mix and vortex it well before using.
	Inactive enzyme failed to convert RNA to cDNA.	Make sure that you store the enzyme properly.
	DIG-UTP was used instead of DIG-dUTP.	Use the correct DIG product.

Troubleshooting RT-IVT Labeling

If the level of chemiluminescent signals from the gene expression probes is low, check the level of chemiluminescent signals from the RT control probes (*lys, phe,* and *dap*) and the RT-IVT control probes (*bioB*, *bioC*, and *bioD*). Use Table A-2 to identify possible causes and to determine recommended actions to resolve the problem.

Table A-2 Troubleshooting RT-IVT labeling

Level of Chemiluminescent Signals	Possible Cause	Action
Gene expression probes: low RT control probes: good IVT control probes: good	RNA sample does not meet the guidelines.	Extract more RNA from your samples and make sure that it meets the guidelines before proceeding.

Table A-2 Troubleshooting RT-IVT labeling *(continued)*

Level of Chemiluminescent Signals	Possible Cause	Action
Gene expression probes: low RT control probes: low IVT control probes: good	RNase activity in RT labeling reagents.	1. Test reagents for RNase activity. 2. Discard all contaminated reagents. 3. Repeat the experiment using RNase-free reagents.
	10× RT Buffer Mix contained precipitates.	Check the buffer mix for precipitates. If precipitates are present, make sure that you warm the buffer mix and vortex it well before using it.
	Inactive enzyme failed to convert RNA to cDNA.	Make sure that you store the enzyme properly.
Gene expression probes: low RT control probes: low IVT control probes: low	RNase activity in IVT labeling reagents or chemiluminescence detection reagents.	1. Test reagents for RNase activity. 2. Discard all contaminated reagents. 3. Repeat the experiment using RNase-free reagents.
	10× RT Buffer Mix or 5× IVT Buffer Mix contained precipitates.	Examine the buffer mixes for precipitates. If precipitates are present, make sure that you warm the buffer mix and vortex it well before using it.
	Inactive enzyme failed to convert cDNA to cRNA.	Make sure that you store the enzyme properly.
	Insufficient incorporation of DIG-UTP.	Measure DIG incorporation.
	DIG-dUTP was used instead of DIG-UTP.	Use the correct DIG product.

General RNA Procedures

B

This appendix covers:

RNase Detection B-2
RNase Decontamination of Plasticware B-3

RNase Detection

Overview

If you suspect that your reagents are contaminated with RNase, perform tests to detect RNase.

Methods

- TaqMan® RNase P Detection Reagents (PN 4316831)
- RNaseAlert® Lab Test Kit (PN 1964) from Ambion, Inc. (www.ambion.com, 2130 Woodward, Austin TX 78744-1832, telephone 1.800.888.8804, fax 512.651.0190)

If You Detect RNase Contamination

If you detect RNase contamination:

1. Decontaminate plasticware (see page B-3).
2. Discard RNase-contaminated solutions.
3. Replace the solutions.

RNase Decontamination of Plasticware

Overview

Applied Biosystems recommends that you decontaminate plasticware to remove and avoid RNase contamination. Removing and avoiding RNase contamination is especially important if you are using DIG-labeled cRNA targets. Below is one suggested procedure for decontaminating plasticware.

Materials Needed

- Detergent
- 95% ethanol
- 3% peroxide
- RNase-free water

Procedure

To decontaminate plasticware:

1.	Wash items with detergent.
2.	Rinse with 95% ethanol.  **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3.	Spray the items thoroughly with 3% peroxide and wait 10 minutes.
4.	Rinse items with RNase-free water.

Glossary

AP	Alkaline phosphatase.
cDNA	Copy DNA reverse transcribed from RNA.
cRNA	Copy RNA that is transcribed from cDNA.
C_V	Coefficient of variation.
DIG	Digoxigenin. Present in cDNA or cRNA targets (products of RT labeling kit and IVT labeling kit), RT controls, IVT controls, and chemiluminescent control probes on the microarray. Binds to anti-digoxigenin-alkaline phosphatase (AP) before the chemiluminescent reaction.
fiducial	Point of reference.
ICP	Internal control probe. 24mer cospotted at every feature in the microarray that contains a 60mer gene expression probe. Hybridizes to the ICT to produce fluorescent signal.
ICT	Internal control target. 24mer oligo labeled with LIZ dye that is supplied with the CL detection kit. Is added to the hybridization mixture and hybridizes to the ICP.
LIZ dye	Fluorescent dye.
probe	Sequence attached to the microarray.
target	Sequence added to the microarray during hybridization.

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