Chapter 1

Overview

Chapter 1

Chapter Contents

This Chapter Contains:

- An overview of GeneChip® Expression Analysis.
- A summary of the procedures covered in the remainder of the manual.

Introduction and Objectives

Welcome to the *Affymetrix GeneChip® Expression Analysis Technical Manual*. This manual is a technical guide for using GeneChip expression analysis probe arrays. All protocols included in this manual have been used successfully by scientists at Affymetrix, or have been recommended by our collaborators during the development of particular products. The field of mRNA gene expression monitoring is rapidly evolving and periodic technical updates to this manual will reflect the newest protocols and information for using GeneChip probe arrays. This manual applies to all GeneChip 3' eukaryotic arrays in cartridge format and GeneChip prokaryotic arrays in cartridge format.

As an Affymetrix GeneChip user, your feedback is welcome. Please contact our technical support team with any input on how we can improve this resource.

Explanation of GeneChip® Probe Arrays

GeneChip probe arrays are manufactured using technology that combines photolithography and combinatorial chemistry.[1,2] Up to 1.3 million different oligonucleotide probes are synthesized on each array. Each oligonucleotide is located in a specific area on the array called a probe cell. Each probe cell contains hundreds of thousands to millions of copies of a given oligonucleotide.

Probe arrays are manufactured in a series of cycles. Initially, a glass substrate is coated with linkers containing photolabile protecting groups. Then, a mask is applied that exposes selected portions of the probe array to ultraviolet light. Illumination removes the photolabile protecting groups enabling selective nucleoside phosphoramidite addition only at the previously exposed sites. Next, a different mask is applied and the cycle of illumination and chemical coupling is performed again. By repeating this cycle, a specific set of oligonucleotide probes is synthesized with each probe type in a known location. The completed probe arrays are packaged into cartridges.

During the laboratory procedure described in this manual, biotin-labeled RNA or DNA fragments referred to as the "target" are hybridized to the probe array. The hybridized probe array is stained with streptavidin phycoerythrin conjugate and scanned by the GeneArray® Scanner or the GeneChip® Scanner 3000. The amount of

[1] *Sambrook, J., Fritsch, E.F., Maniatis, T. Molecular Cloning: A Laboratory Manual, v.1 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY p 21-52 (1989).*

[2] *See www.affymetrix.com for current GeneChip technology references.*

light emitted at 570 nm is proportional to the bound target at each location on the probe array.

GeneChip® Expression Analysis Overview

The following major steps outline GeneChip expression analysis:

1. Target Preparation
2. Target Hybridization
3. Fluidics Station Setup
4. Probe Array Washing and Staining
5. Probe Array Scan
6. Data Analysis

Due to the differences in the RNA species between eukaryotic and prokaryotic organisms, different target labeling protocols have been optimized. Chapters 2 through 6 provide detailed protocols for target preparation, hybridization, array washing, and staining for eukaryotic and prokaryotic arrays, respectively. Please refer to the sections in this manual for detailed protocols appropriate for your arrays.

STEP 1: TARGET PREPARATION

This manual describes procedures using GeneChip® reagent kits for preparing biotinylated target from purified eukaryotic and prokaryotic RNA samples suitable for hybridization to GeneChip expression probe arrays. For more information on these procedures, please contact Affymetrix Technical Support at 1-888-DNA-CHIP, +44 (0)1628 552550 in Europe, or +81-(0)3-5730-8200 in Japan.

For eukaryotic samples, using protocols referenced in Chapter 2, double-stranded cDNA is synthesized from total RNA or purified poly-A messenger RNA isolated from tissue or cells. An *in vitro* transcription (IVT) reaction is then done to produce biotin-labeled cRNA from the cDNA. The cRNA is fragmented before hybridization.

For prokaryotic samples, Chapter 4 describes a detailed protocol to isolate total RNA followed by reverse transcription with random hexamers to produce cDNA. After fragmentation by DNase I, the cDNA is end-labeled with biotin by terminal transferase.

STEP 2: TARGET HYBRIDIZATION

A hybridization cocktail is prepared, including the fragmented target, and probe array controls. It is then hybridized to the probe array during a 16-hour incubation. The hybridization process is described in the respective sections for the different probe array types. Refer to Chapter 3 for hybridization of eukaryotic samples, and Chapter 5 for prokaryotic samples.

STEP 3: FLUIDICS STATION SETUP

Specific experimental information is defined using Affymetrix® Microarray Suite or GeneChip Operating Software (GCOS) on a PC-compatible workstation. The probe array type, sample description, and comments are entered and saved with a unique experiment name. The fluidics station is then prepared for use by priming with the appropriate buffers. Refer to the *GeneChip® Expression Wash, Stain and Scan User Manual*, P/N 702731 for information on fluidics station setup for eukaryotic samples, and Chapter 6 for prokaryotic samples. For more information on the fluidics station, refer to the *GeneChip® Fluidics Station User's Guide*.

STEP 4: PROBE ARRAY WASHING AND STAINING

Immediately following hybridization, the probe array undergoes an automated washing and staining protocol on the fluidics station. The *GeneChip® Expression Wash, Stain and Scan User Manual*, P/N 702731 provides information for eukaryotic samples, and Chapter 6 provides information for prokaryotic samples.

STEP 5: PROBE ARRAY SCAN

Once the probe array has been hybridized, washed, and stained, it is scanned. Each workstation running Affymetrix Microarray Suite or GCOS can control one scanner. The software defines the probe cells and computes an intensity for each cell.

Each complete probe array image is stored in a separate data file identified by the experiment name and is saved with a data image file (.dat) extension.

Review the scanner user's manual for safety precautions and for more information on using the scanner.

STEP 6: DATA ANALYSIS

The .dat image is analyzed for probe intensities; results are reported in tabular and graphical formats. Information on data analysis is provided in the enclosed *GeneChip® Expression Analysis: Data Analysis Fundamentals* booklet (P/N 701190).

Precautions

1. FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. Avoid microbial contamination, which may cause erroneous results.

WARNING ▲

All biological specimens and materials with which they come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. This includes adherence to the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) for blood-derived and other samples governed by this act. Never pipet by mouth. Avoid specimen contact with skin and mucous membranes.

3. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic reagents.
4. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
5. Use powder-free gloves whenever possible to minimize introduction of powder particles into sample or probe array cartridges.

Terminology

Probes	The oligonucleotides on the surface of the probe arrays are called probes because they probe, or interrogate, the sample.
Target	The target is the labeled nucleic acid that is being interrogated. It is hybridized to the probes on the array.
Probe Cell	Specific areas on the probe array that contain oligonucleotides of a specific sequence.

Interfering Conditions

CAUTION

Wear powder-free gloves throughout procedure. Take steps to minimize the introduction of exogenous nucleases. Water used in the protocols below is molecular biology grade (nuclease free).

Proper storage and handling of reagents and samples is essential for robust performance.

All laboratory equipment used to prepare the target during this procedure should be calibrated and carefully maintained to ensure accuracy, as incorrect measurement of reagents may affect the outcome of the procedure.

Instruments

The *GeneChip Expression Analysis Technical Manual* is designed for use in a system consisting of a Fluidics Station, a Hybridization Oven 640, and a Scanner.

References

1. Sambrook, J., Fritsch, E.F., Maniatis, T. *Molecular Cloning: A Laboratory Manual, v.1* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY p 21-52 (1989).
2. See www.affymetrix.com for current GeneChip technology references.

Limitations

- The results of the assay are dependent upon the quality of the input RNA, subsequent proper handling of nucleic acids and other reagents.
- The results should be evaluated by a qualified individual.

IMPORTANT !

Do not store enzymes in a frost-free freezer.