



## Using Microarrays to Study Leukemia Subtypes of ALL

### Introduction

- Some individuals diagnosed with ALL do not respond to the usual ALL treatment.
- Cells from non-responders are identical to cells from responders by the histo and immuno-chemical tests used to diagnose ALL. Thus there is no easy way to tell which patients will respond to the treatment for ALL and which won't.
- Most non-responders contain a mutation in a gene called MLL. This mutation is often a translocation, and can be difficult to detect. Thus it does not provide an easy test for this subtype of ALL.

In this activity you will use microarray technology to answer the following questions:

- Is there more than one definable category of ALL? Is ALL more than one distinct disease?
- If yes, how would you identify the two diseases?

This activity (and the 2 following activities) are based on work done by Dr. Eric Lander's team at the Whitehead Institute/MIT Center for Genome Research and is published in the following reference:

Armstrong, SA, Staunton, LS, Pieters, R, den Boer, ML, Minden, MD, Sallan, SE, Lander, ES, Golub, TR, and SJ Korsmeyer. 2002. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nature Genetics* (30)41-47  
Dr. Landers outlines much of this work in lecture 3 of the 2002 HHMI Holiday Lectures "Scanning Life's Matrix."

### ALL Subtypes

In this activity, you will explore whether gene expression patterns can be used to identify different subtypes of ALL. You will begin this portion of the activity by looking at microarray results from 12 different patients.

Methods:

#### The microarray

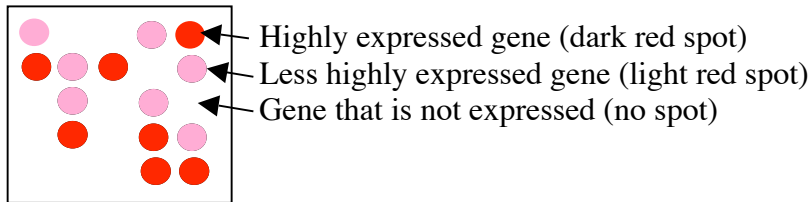
The researchers used a commercially available human gene chip with 30,000 human genes.

#### The samples

Messenger RNA was isolated from white blood cells from patients with ALL. Individually, a labeled cDNA probe was made from each mRNA sample. As was the case for the previous activity, only one color label was used. If a gene was expressed in a given sample, mRNA for that gene will be present, and a cDNA will be made. Further, the amount of cDNA produced for a given gene reflects the level of expression of that

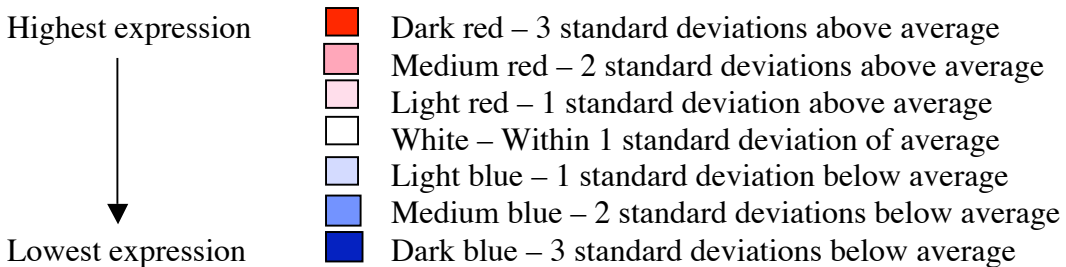
gene. The “probe” mixture that is used to anneal to the microarray will be made up of cDNAs complementary to all different mRNAs in the patient sample.

Each cDNA sample is then annealed to a microarray – one probe mixture – representing one patient – per microarray. Following annealing, a laser is used to measure how much cDNA had annealed to each spot. The amount of light measured for each gene correlates with how highly expressed each gene is in white blood cells from the patient tested.



How the strips will be working with were generated

In this activity, you will not be looking at raw microarray data. Rather, you will be looking at a computer-generated representation of increased or decreased gene expression. To generate the colored data you will be working with, the level of expression for each gene was averaged for all patients. Genes where there was a difference in expression were analyzed further. For each patient, the level of expression for genes of interest was compared to average and color-coded. In the strips you receive, the color and shade of the box in the strip represents the level of expression of the gene in the patient compared to the average expression for all 12 patients.



Note: You have been given a subset of genes (12) for which the expression differs among patients with ALL. If you were to look at all 30,000 genes, you would find that most had no difference in expression.

Note: Although you are working with two different colored boxes, these do not represent two different color labels used in two different samples as they did in the yeast microarray activity. Rather they represent whether a gene is expressed at a higher than average (red) or lower than average (blue) level.

The activity

In this activity you will be given sample microarray analyses from a variety of patients for a series of different genes. Your goal will be to decide whether you can find a pattern that distinguishes two different types of ALL, and if yes, what genes are differentially

expressed between the two leukemia types. Basically you are doing what a computer would do!

You will be given strips representing 12 different patients. 6 of the patients have ALL that is responding to treatment, while the other 6 are not responding to treatment. For the 12 patients you will see the gene expression profile generated by microarray. Patient #1 has the identity of the genes included next to the markers. For the other patients, the genes are in the same order.

Your goal is to separate the 12 patients into two groups of 6 patients each that can be distinguished by their gene expression profiles.

Hints:

- 1 - No complex calculations are necessary. Try to look for an overall pattern.
- 2 - Give more weight to darker colored boxes than lighter colored ones.

### Questions

1. Which patients are found in each of your two groups (assume patient 1 is in group 1)?

Group 1

Group 2

2. Next, in general which genes are

Overexpressed in group 1 and underexpressed in group 2?

Underexpressed in group 1 and overexpressed in group 2?

3. Describe one patient who you were able to place in group 1 or group 2 but who didn't exactly fit the typical gene expression profile you describe in your answer 2 above.

### Two subtypes of ALL

You have identified two distinct subtypes of ALL using microarrays and gene expression profiling. Remember that if you had looked at all 30,000 genes, most would not differ between the two types. Following identification of two distinct subtypes of ALL the authors gave names to the different cancers.

ALL (ALL1 in the DVD) – acute lymphocytic leukemia. This name refers to the patients who responded well to typical ALL treatment. Patients 1 and 2 have ALL.

MLL (ALL2 in the DVD) – mixed lineage leukemia. This name refers to patients who did not respond well to typical ALL treatment. The name comes from the fact that these patients usually have a mutation in the MLL gene. Additionally, WBCs from these patients tend to express low levels of myeloid surface markers as well as lymphocyte surface markers.

Note: The researchers noted that there are some similarities between MLL and AML. They thus compared gene expression profiles for patients with MLL, AML, and ALL. All three diseases have unique gene expression profiles.