

# **Essential Tools For Our Research**

# Cytogenomics

- ...involves the study of normal and abnormal chromosomes in health and disease.
- This includes examination of chromosome structure, relationships between chromosome structure and phenotype, and causes of chromosomal abnormalities.
- Essential tool for dx and rx of patients with malignancies and hematologic disorders.
- Reveals underlying genomic abnormalities in cancer.

# Spectral Karyotyping

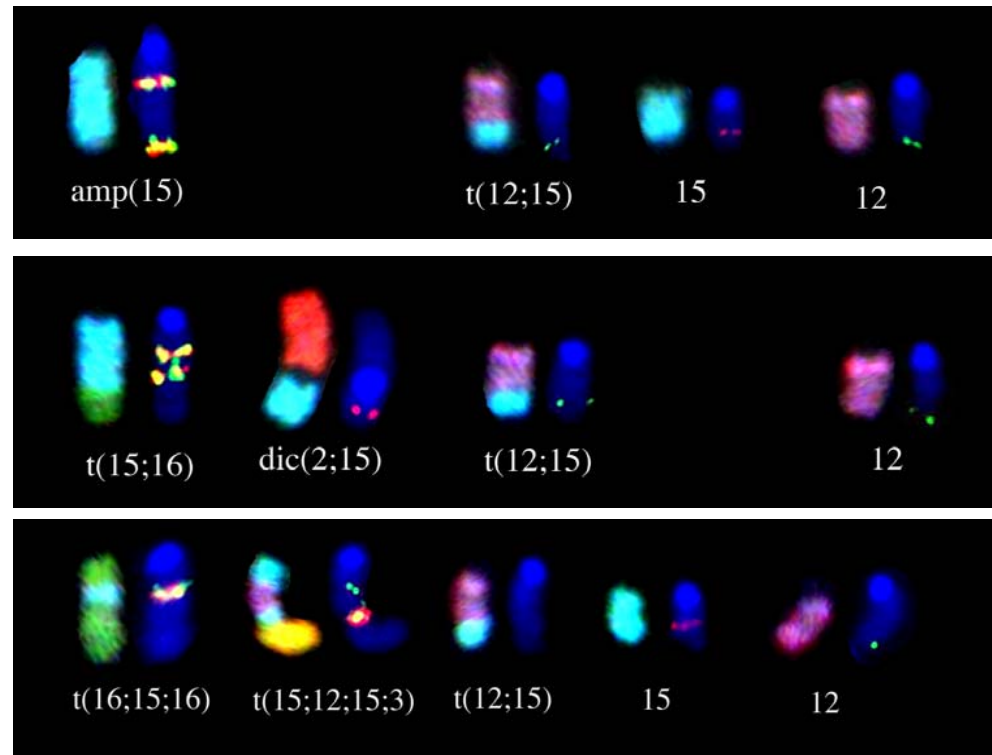
- ...is the visualization of all chromosome pairs - each chromosome “painted” in a different fluorescent color.
- Chromosomes in cancer cells frequently exhibit multiple aberrations: changes in chromosome # (ploidy), and structure including deletions, amplifications, and translocations.
- Identification of specific chromosome abnormalities is routinely used in dx and prognosis of cancer and in many genetic disorders.

Important research method to understand genetic mechanisms of cancer.

## Fluorescent In Situ Hybridization (FISH)

- ...uses fluorescently labeled DNA probes for specific regions of a chromosome permitting high resolution mapping of genomic abnormalities

# SKY and FISH in mouse models of leukemias



**This experiment reveals recurrent chromosomal translocations involving chromosomes 12 & 15 and the *IgH/c-Myc* loci respectively.**

Gladdy *et. al.*, 2003, *Cancer Cell*

# Gene Expression Microarray Analysis

- ...allows the simultaneous analysis of thousands of genes to compare their expression patterns between different cell types, developmental stages, disease states, or treatment conditions.
- Useful to compare the gene expression profiles of cancer vs. normal cells.
- Differential gene expression patterns aid in understanding of genetic alterations and their impact on gene expression in cancer and inherited genetic diseases.
- Generates hypotheses about underlying molecular mechanisms in cancer development, progression and response to therapy.

# The Process Involves....

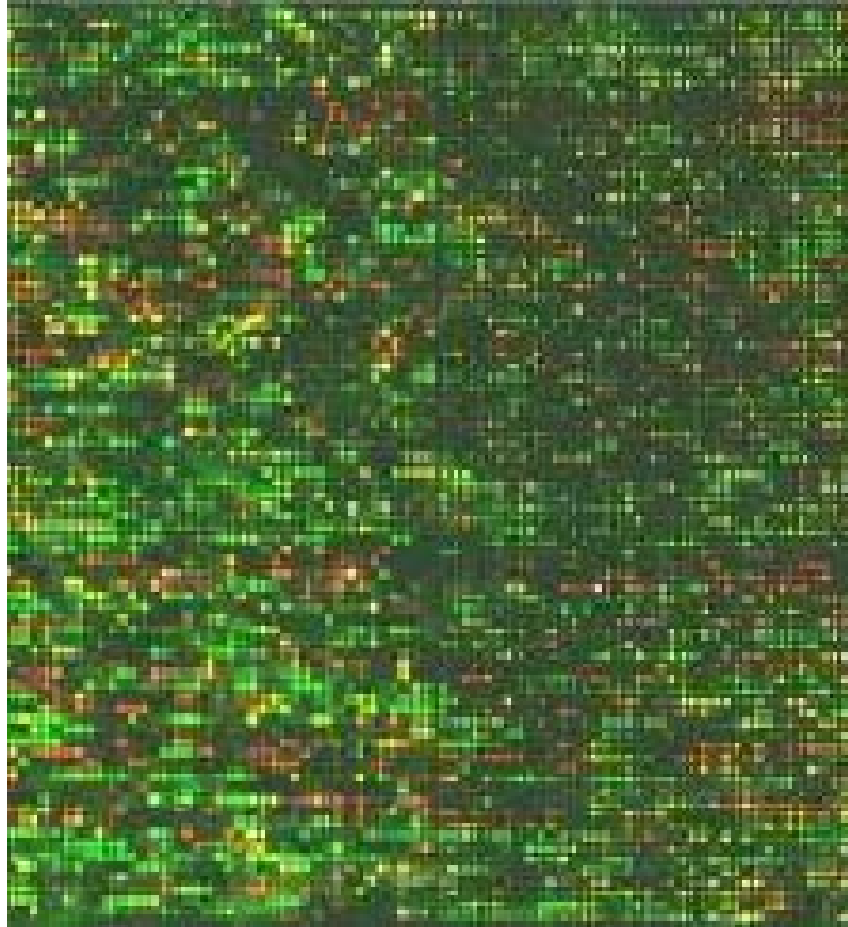
Isolate RNA  
from tissue  
of interest



microarray



Image array  
obtain signal  
intensity



Process the data



Perform statistical  
analysis

Flow cytometry

RT-PCR



Validate  
findings w/  
independent  
methods



## Our Experimental Success

- We've used gene expression arrays to compare expression profiles of normal precursor B-cells to immature B-cell leukemias.
- This provided us with a long list of potential candidate genes
- We validated key genes of interest by RT-PCR & flow cytometry

# Next Steps....

**Manipulate genes *in vivo***

**Identify diagnostic, prognostic and therapeutic targets**

**Does cancer develop, progress, and/or respond to treatment?**

# Flow Cytometry

- ...is the analysis of single cells in a fluid stream
- Normal lymphocytes, leukemias and lymphomas are heterogeneous populations.
- Subpopulations are identified by differential expression of cell-surface proteins that can be detected using specific antibodies.
- Flow cytometry allows the rapid detection, characterization, marker quantitation, and enumeration of individual cells within a heterogeneous population.
- Can also be used for the detection of intracellular proteins, cell cycle analysis, cell tracking, chromosome analysis, cell viability
- Specialized flow cytometers have the capacity to physically isolate homogeneous subpopulations of interest = cell sorting.

# How do Flow Cytometers Work?

- Several components make up a flow cytometer:
  - the fluidics system required for sample delivery
  - the optical system which performs the measurements
  - electronics used for signal detection, data processing and automation
  - the computer interface: controls the flow cytometer, collects, stores and displays the data

## Confocal Microscopy

- ...is the microscopic analysis of stained cells/tissues using a specialized microscope that enables the 3D reconstruction of images
- Detection reagents similar to those of flow cytometry, but confocal microscopy has certain advantages:
  - the ability to measure cell morphology even within tissues
  - the ability to preserve the relationships between cells within a tissue
  - the ability to determine the location of a target within a tissue (eg: nuclear *versus* cytoplasmic)
  - the ability to show co-localization of proteins of interest

# Bioinformatics

- ...is the use of computational tools for the interrogation of biological systems
- Used to identify several novel microsatellite markers to aid in the fine mapping of complex disease loci
- Used to annotate genes identified in flow cytometric and gene expression analyses to identify gene networks perturbed in disease states