Breast Cancer Biomarker Research in Washington State

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What is a Biomarker?

- A detectable change in biological system that conveys information about disease state, susceptibility, or exposure

- Surrogate biomarkers
  - Statistically correlated with a disease state
  - Provides adequate sensitivity and selectivity
    - Sensitivity - low false negative rate
    - Selectivity - low false positive rate
  - Mechanistic relationship to disease process may be unknown

- Therapeutic biomarkers
  - Mechanistically involved in the disease process
  - Provides potential therapeutic target

The Life Cycle of a Biomarker

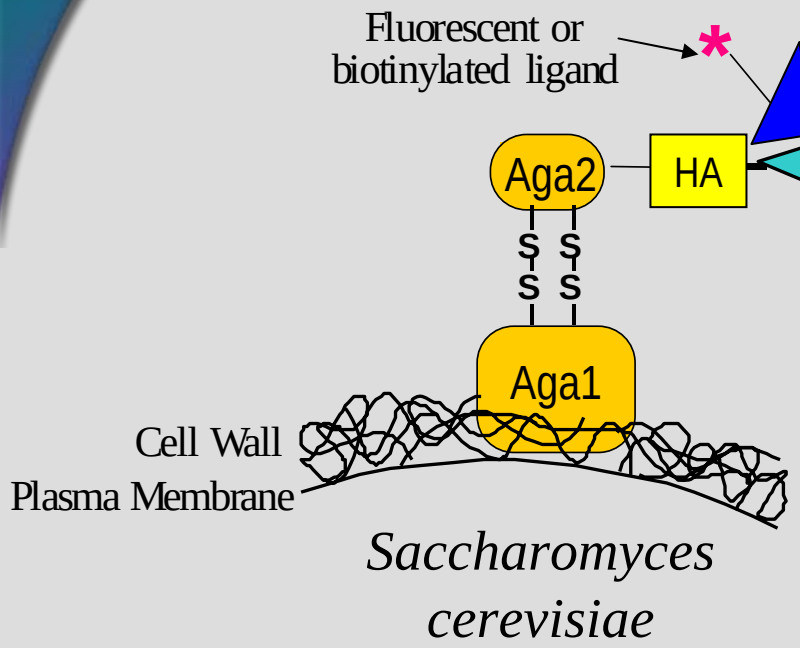
- Discovery
  - What important molecules have changed in the diseased cell?
    - DNA mutations or SNPs
    - mRNA expression
    - Protein abundance
    - Protein post-translational modifications
    - metabolites
  - Can these altered proteins or nucleic acids be detected in blood?

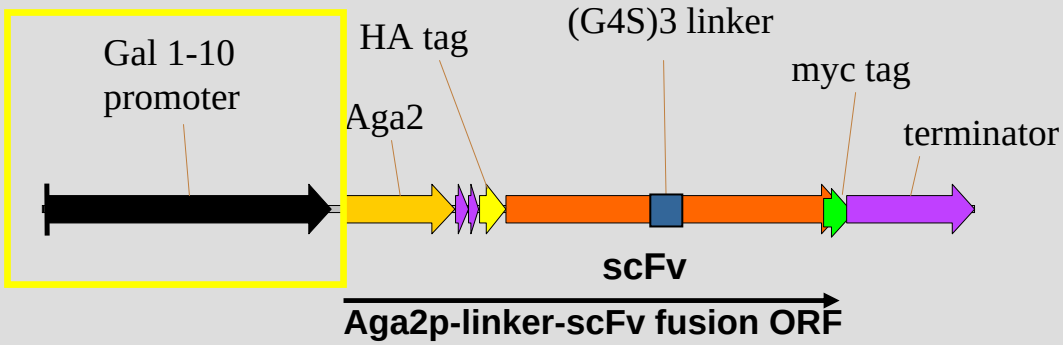
- Clinical Validation
  - How robust and reproducible is the observed change?
  - Pre-clinical trials with limited samples
  - Large scale clinical trials with lots of samples
  - Does the biomarker have predictive value
  - Is it better than current tests?

PNNL and HUPO Plasma Proteomics Project

- HUPO Plasma Proteomics Project started with ‘competition’ between 47 laboratories using distinct platforms
- HUPO reference samples consisted of serum and citrated plasma from African American, Asian American, and Caucasian American blood donors
  - Each lab sent separate aliquots from pooled samples representing each population group
- PNNL analysis used immunoglobulin depletion and AMT approach including FTICR-MS
  - Complete analysis for eight samples in triplicate completed in four days
  - Stringent filters used for peptide identification
- Results (Dec 2003 filters / June 2004 filters - only tryptic peptides allowed):
  - High Confidence Peptide-protein pairs (not unique) 2811 / 2298
  - High Confidence Uniquely Identified Proteins: 1366 / 842
  - Dynamic range covered: 5000 fold - down to ng/ml cytokines

Yeast Surface Display

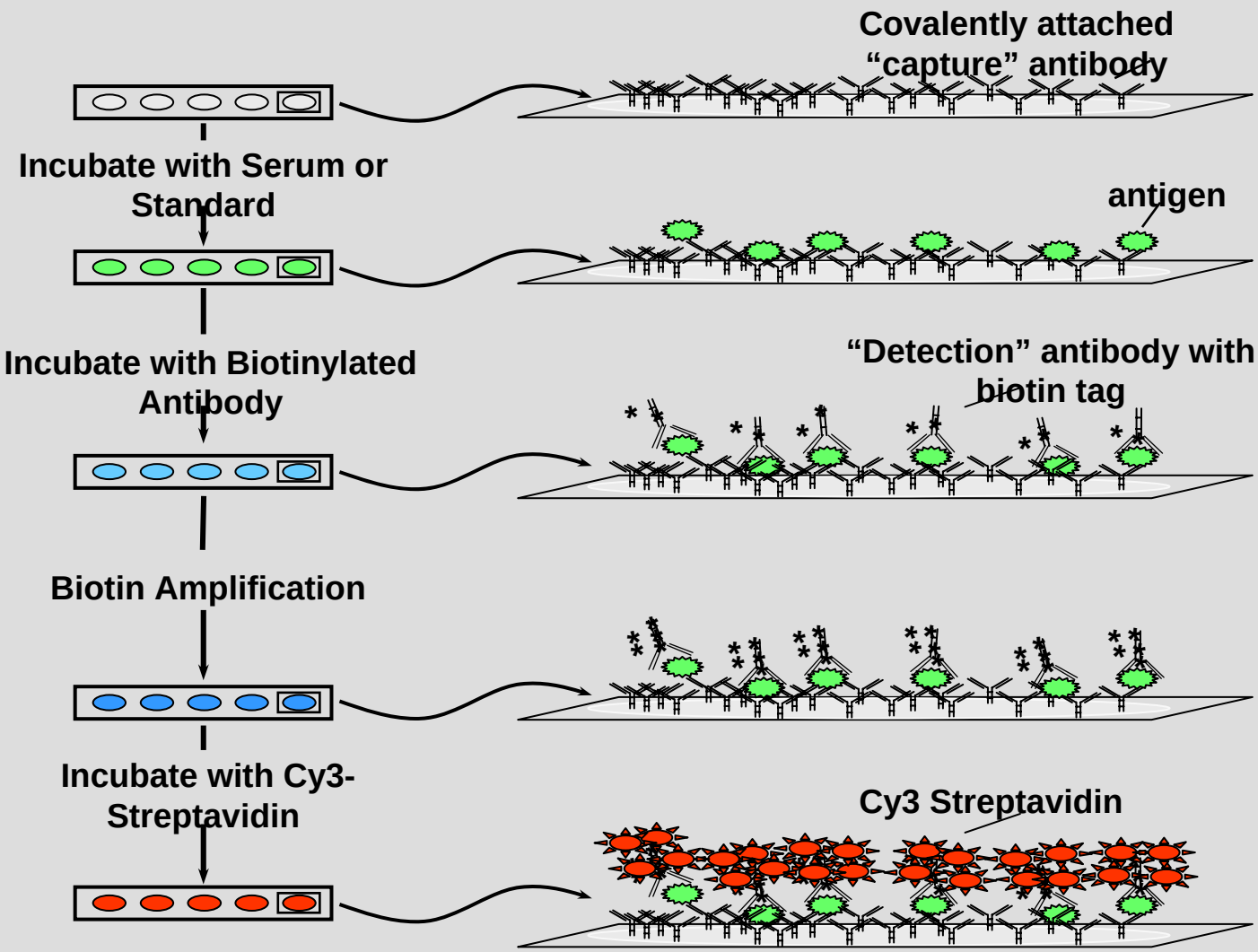




Tight, inducible regulation

Boder & Wittrup, Nature Biotech, Vol. 15 (1997)
Boder, Midelfort & Wittrup, PNAS, Vol 97 (2000)

Creation of a Protein Microarray



NCI Award to Accelerate Cancer Biomarker Discovery

- **Goal:** ***To accelerate the rate at which early biomarkers for cancer are detected through the use of mouse models of cancer***

- **Objectives:**
  - Evaluate proteomic techniques for tissue collection, sample preparation and measurement
  - Developing more effective proteomic technologies for detecting and identifying proteins and protein fragments in serum
  - Creating resources, such as serum and plasma specimen repositories, antibodies and peptide reagents, to serve as common community resources
  - Establishing a publicly available data bases, including bioinformatics support, for analyzing proteomics

- **Fred Hutchinson Cancer Research Center Funding:**
  - Sam Hanash, auto-antibodies
  - Martin MacIntosh, protein biomarkers
    - PNNL funded directly under award to FHCRC
    - Dick Smith group responsible for mass spectrometry

Entertainment Industry Fund Breast Cancer Biomarker Study

- Goal: To identify very early plasma biomarkers for breast cancer
- Multi-institutional consortium headed by Lee Hartwell, Fred Hutchinson Cancer Research Center
- PNNL Role:
  - Global proteomic analysis of matched nipple aspirate fluid, tumor, and plasma samples for detection of differentially expressed proteins
- Funding from Entertainment Industry Fund

NCI Protein Array Research Program

- Goal: To produce a protein array of 100 biomarkers useful for the early detection of cancer
- PNNL Role:
  - Development of recombinant scFv antibodies as detection reagents
  - Co-development of protein array format with NCI-Frederick
- Phased Implementation
  - Demonstration Project
    - Production of 50 purified antigens and selection of 50 scFv
    - Demonstrate utility of recombinant affinity reagents
  - Full Implementation
    - Development of assay format
    - Distribution of prototype assays for definition of QC and QA
    - Testing by Early Detection Research Network investigators and NCI intramural staff
- Funding by Interagency Agreement