National Cancer Institute

OFFICE OF CANCER CLINICAL PROTEOMICS RESEARCH

Bioengineering for Medical Diagnostics, Therapeutics, and Imaging:

1-2 Diagnostic Biomarkers, Technology, and Regulatory Considerations

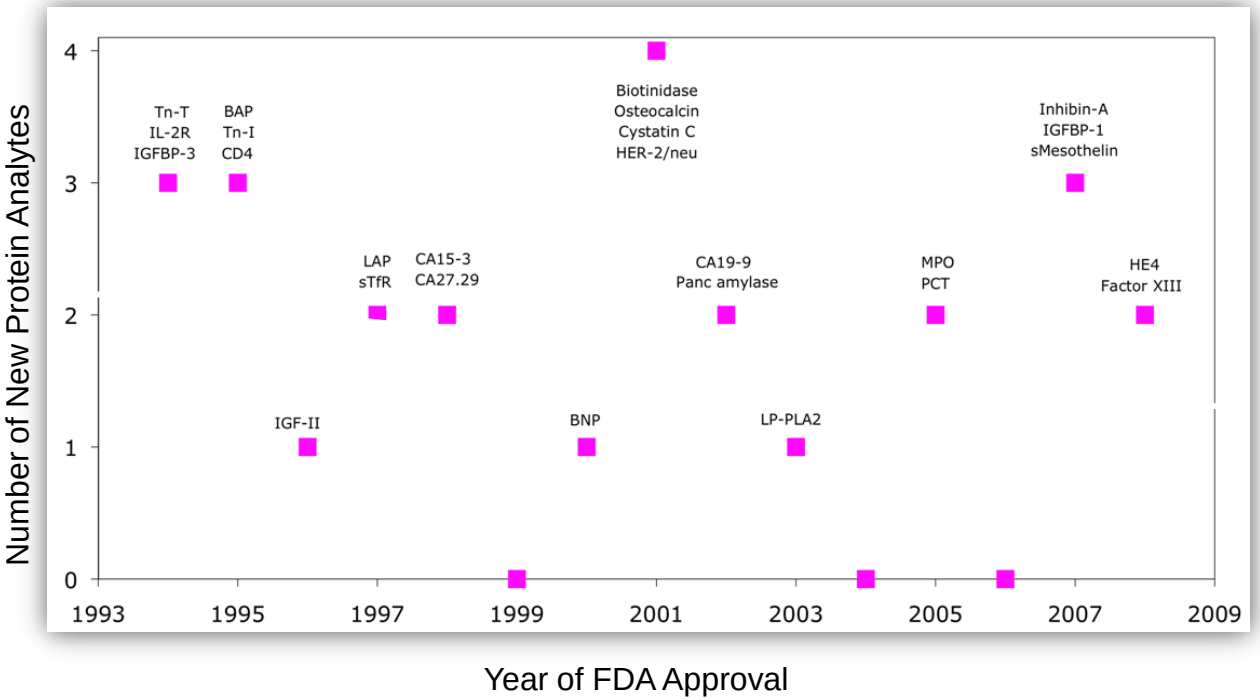
Session Chair: Christopher Kinsinger

February 5, 2013

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Where Clinical Proteomics Is Today

Few biomarker candidates translating into clinical utility

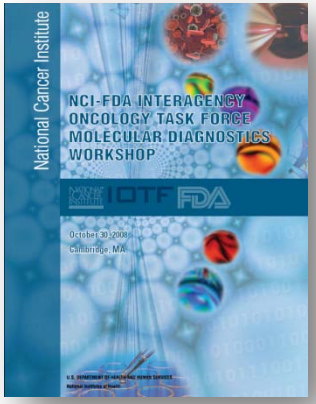


- 109 FDA-approved protein biomarkers in total
- 96 LDTs with protein biomarkers

Source: Anderson, N. L. Clin. Chem. 2010, 56, 1.

Coordination with FDA

- NCI-FDA Interagency Oncology Task Force (Molecular Diagnostics Subcommittee)
- Workshop: Identify analytical validation needs for proteomic technologies (e.g., mass spectrometry and affinity arrays) in the context of intended use.
- Outputs: Summary Document and Mock 510(k) Pre-Applications to serve as guidance to the proteomics community
  - Multiplex MRM assay
  - Multiplex affinity-based assay



Regnier et al. *Clin Chem.* 56:2 165, **2010**

Pathway to FDA clearance

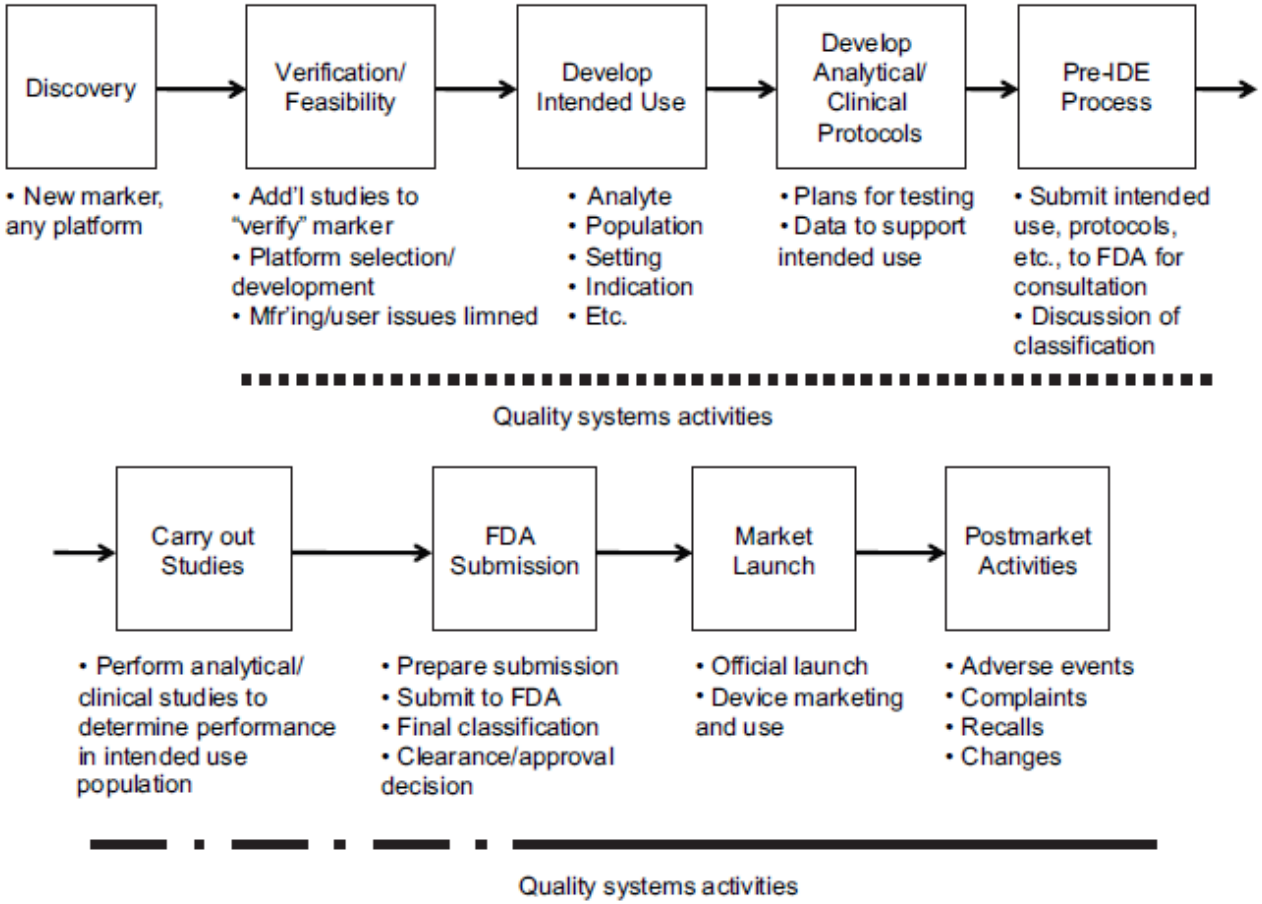


Fig. 1. Schematic description of the process that may lead from a biomarker discovery to an FDA-cleared or approved diagnostic test.

Nine steps (shown in 2 rows) can generally occur in succession, and activities governed by quality systems as defined by the FDA [FDA *(4)*] in some cases may start right after the first step.

High-Quality Affinity Reagents
(Ab Characterization Program)

National Cancer Institute at Frederick
NCI-Frederick

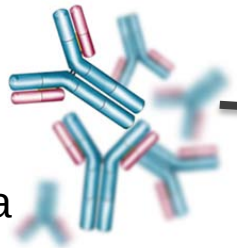
CENTER FOR CANCER RESEARCH

Isotype	Western	ELISA	SPR	SPR-Pairing	Immuno-MS	IHC	NCI-60 Protein Array

Industry

and/or

Academia



NATIONAL CANCER INSTITUTE

AC Labs

NAPPA	IHC-HPA

ASU BIODESIGN INSTITUTE ARIZONA STATE UNIVERSITY

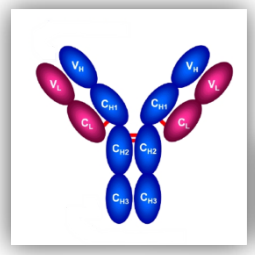
HIP

KTH VETENSKAP OCH KONST

C O M M U N I T Y A C C E S S

http://antibodies.cancer.gov

Data



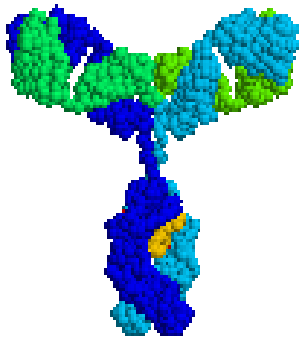
Reagents

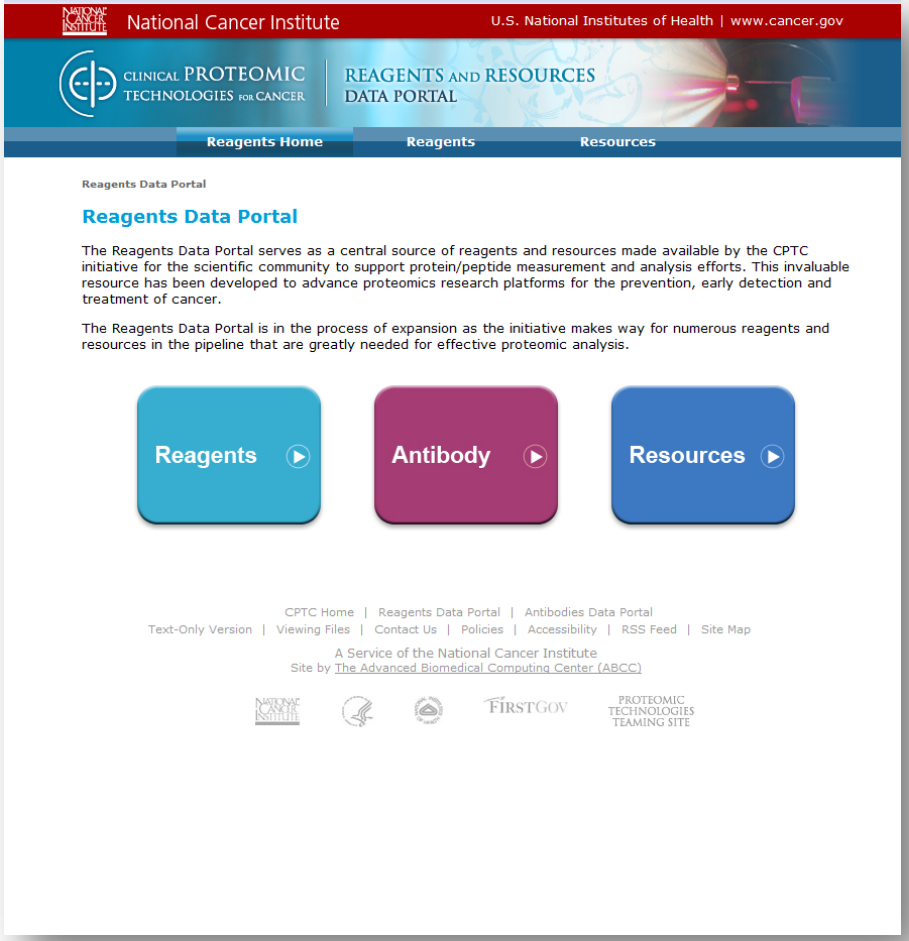
(mAb and Hybridoma)

DSHB

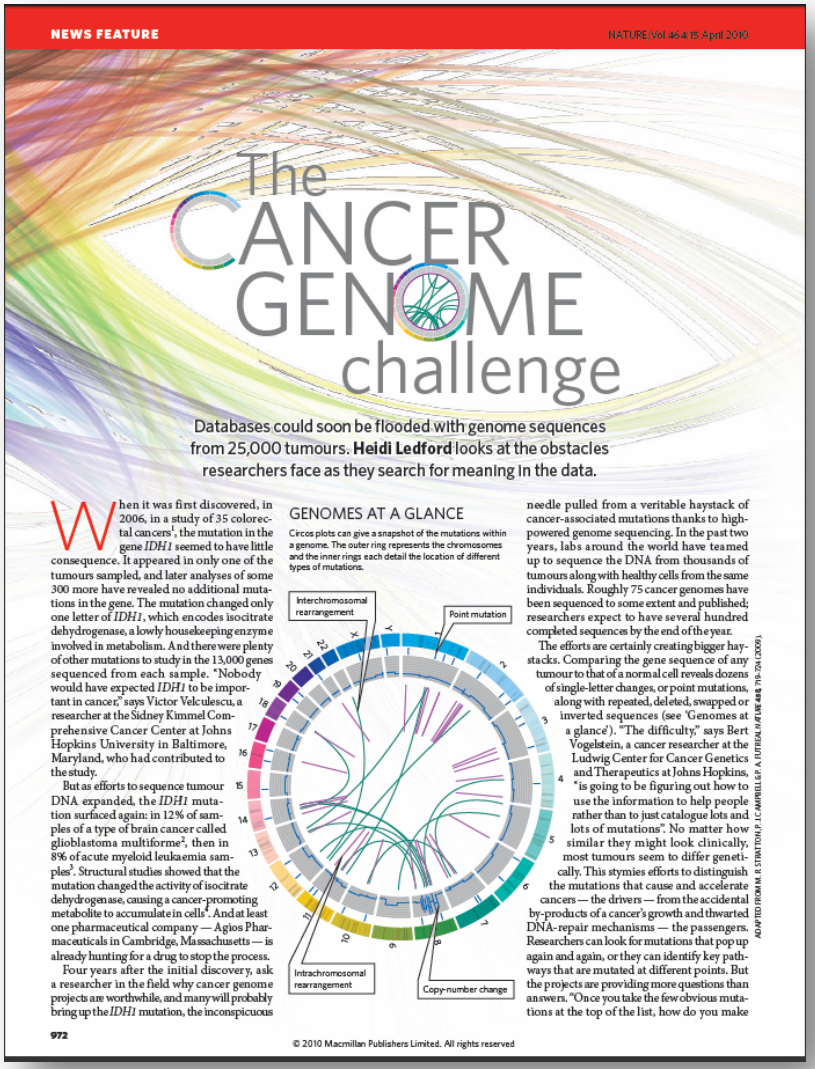
Reagents Currently Available

- **Antigens**
  - > 80 purified soluble proteins (> 10 mg)
  - Available at http://antigens.anl.gov
- **Antibodies**
  - 220 monoclonal Abs to 80 targets
  - Available at http://antibodies.cancer.gov





Understanding the cancer genome: what role for proteomics?



NEWS FEATURE

NATURE|Vol 464|15 April 2010

The CANCER GENOME challenge

Databases could soon be flooded with genome sequences from 25,000 tumours. **Heidi Ledford** looks at the obstacles researchers face as they search for meaning in the data.

When it was first discovered, in 2006, in a study of 35 colorectal cancers[1], the mutation in the gene *IDH1* seemed to have little consequence. It appeared in only one of the tumours sampled, and later analyses of some 300 more have revealed no additional mutations in the gene. The mutation changed only one letter of *IDH1*, which encodes isocitrate dehydrogenase, a lowly housekeeping enzyme involved in metabolism. And there were plenty of other mutations to study in the 13,000 genes sequenced from each sample. "Nobody would have expected *IDH1* to be important in cancer," says Victor Velculescu, a researcher at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University in Baltimore, Maryland, who had contributed to the study.

But as efforts to sequence tumour DNA expanded, the *IDH1* mutation surfaced again: in 12% of samples of a type of brain cancer called glioblastoma multiforme[2], then in 8% of acute myeloid leukaemia samples[3]. Structural studies showed that the mutation changed the activity of isocitrate dehydrogenase, causing a cancer-promoting metabolite to accumulate in cells[4]. And at least one pharmaceutical company — Agios Pharmaceuticals in Cambridge, Massachusetts — is already hunting for a drug to stop the process.

Four years after the initial discovery, ask a researcher in the field why cancer genome projects are worthwhile, and many will probably bring up the *IDH1* mutation, the inconspicuous needle pulled from a veritable haystack of cancer-associated mutations thanks to high-powered genome sequencing. In the past two years, labs around the world have teamed up to sequence the DNA from thousands of tumours along with healthy cells from the same individuals. Roughly 75 cancer genomes have been sequenced to some extent and published; researchers expect to have several hundred completed sequences by the end of the year.

The efforts are certainly creating bigger haystacks. Comparing the gene sequence of any tumour to that of a normal cell reveals dozens of single-letter changes, or point mutations, along with repeated, deleted, swapped or inverted sequences (see 'Genomes at a glance'). "The difficulty," says Bert Vogelstein, a cancer researcher at the Ludwig Center for Cancer Genetics and Therapeutics at Johns Hopkins, "is going to be figuring out how to use the information to help people rather than to just catalogue lots and lots of mutations". No matter how similar they might look clinically, most tumours seem to differ genetically. This stymies efforts to distinguish the mutations that cause and accelerate cancers — the drivers — from the accidental by-products of a cancer's growth and thwarted DNA-repair mechanisms — the passengers. Researchers can look for mutations that pop up again and again, or they can identify key pathways that are mutated at different points. But the projects are providing more questions than answers. "Once you take the few obvious mutations at the top of the list, how do you make

GENOMES AT A GLANCE

Circos plots can give a snapshot of the mutations within a genome. The outer ring represents the chromosomes and the inner rings each detail the location of different types of mutations.

ADAPTED FROM M. R. STRATTON, P. J. CAMPBELL & P. A. FUTREAL, NATURE 458, 719–724 (2009).

- TCGA is generating huge datasets on genomic characteristics of human cancers
  - 20 types of cancer; 1000 tumors/cancer type
- Biggest challenge is to translate genomic variation to function and cancer phenotypes

***Nature* April 15, 2010**

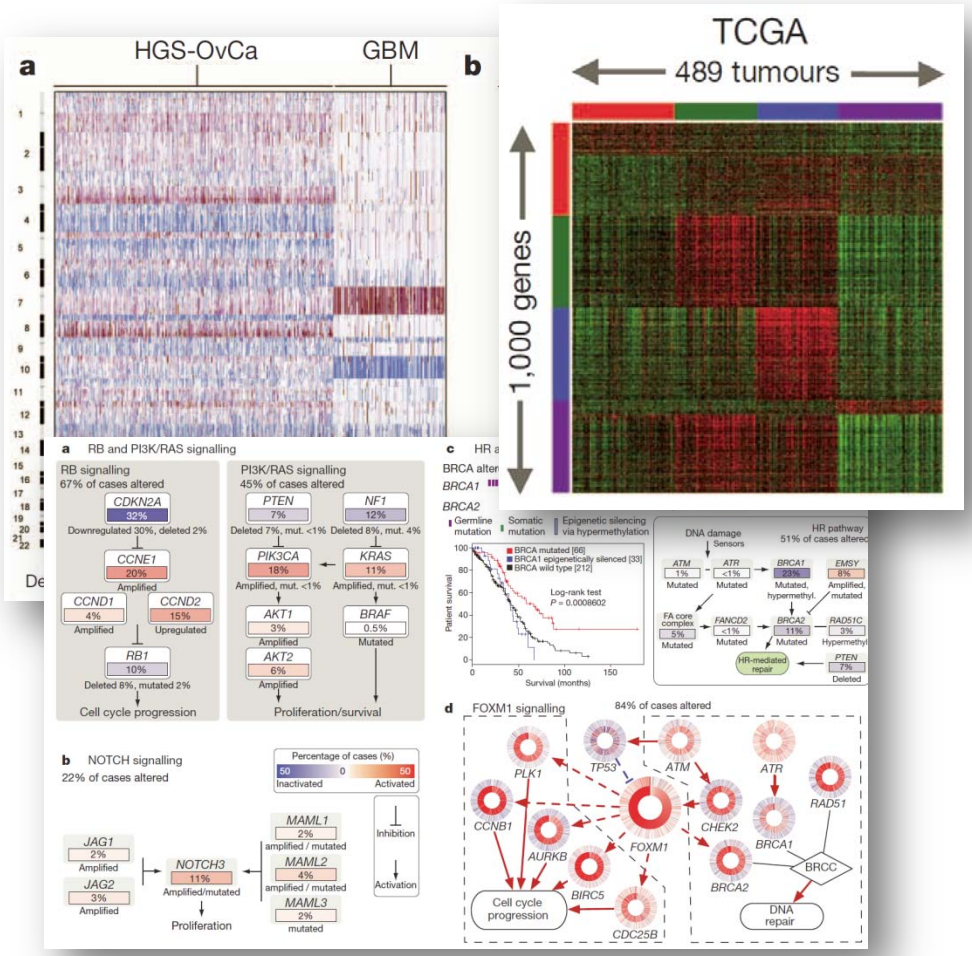
NCI-CPTAC: Proteomics can help answer questions about the molecular mechanisms of cancer

Biological mechanisms:

- Are genomic aberrations detectable at protein level?
- What is their effect on protein function?
- Which events are drivers? Which are passengers?

Clinical applications:

- Can proteomic information provide a better molecular taxonomy of cancer?
- Can genotypic information guide protein marker development?



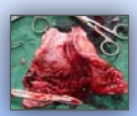
Source: The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature, 30 June 2011, Vol. 474, p. 609.

Map proteome/PTMs to each patient's genome; develop assays for pathways and candidate biomarkers

Inputs

TCGA retrospective tumor collections

Prospective tumor collections



Verification sample collections

Analyses

Analyze >100 tumors/cancer
- breast, ovarian, colon
- Proteome
- Phosphoproteome
- Other PTMs

Targeted protein / PTM
- Cancer pathways
- Protein isoforms

Targeted Verification of "biology driven" biomarker candidates

Outputs

- Molecular signatures
- Genome-proteome relationships
- Genome-signaling relationships
- Protein targets
- Assays for newly discovered proteins

- Protein, PTM verification
- Assays, reagents, SOPs

Cancer Biology Arm Clinical Arm

The Centers: Broad/FHCRC; PNNL; Vanderbilt; Wash U.; Johns Hopkins

TCGA biospecimen collection and QC focused on DNA and RNA, not proteins/PTMs

- Primary, *adult* tumors (except for melanoma and triplets)
- Malignant (no *in situ* cases)
- No neo-adjuvant or chemotherapy prior to sample collection
- Pathology review: > 60% tumor cellularity and < 20% necrosis
- Sufficient sample to yield 15 micrograms of DNA and RNA
- Snap frozen in OCT
  - Ligation time not recorded
  - Up to 60min from excision to freezing
- Matched germline DNA: blood, saliva or skin
  - few/no “normal” tissue collected

Post excision delay-to-freezing time could have profound effects on posttranslational modifications

- Time between ligation, excision and freezing for the TCGA samples (post-excision delay, PDT) varied from many minutes to >1 hour
- Effects of ischemia and physical tissue trauma on PTM's not well studied
- Activated kinases and phosphatases can act in seconds-minutes
  - Alterations in phosphosignaling in cancer well established
- Prior studies have shown that the phosphorylation site stoichiometry can change significantly post tumor excision
  - Duration from ligation of blood flow to excision highly variable and often not taken into account (shortest time evaluated ca. 15 min.)
  - few p-sites evaluated (RPPA)

Study goal: evaluate changes in proteome and phosphoproteome (<1 min and longer) induced by PDT using quantitative LC-MS/MS

Study Design

Samples: two xenografted human breast cancer tumors (basal-like; luminal-like) and five patient-derived ovarian cancer tumors

Collection: excision prior to ligation; immediate LN2

Timepoints: “0” (≤60s from excision to freezing); 5 minutes; 30 minutes and 60 minutes

Tumor processing: Covaris “Cryoprep” freeze-fracturing to prepare identical powdered tumor (WHIM or OC) to each analysis site

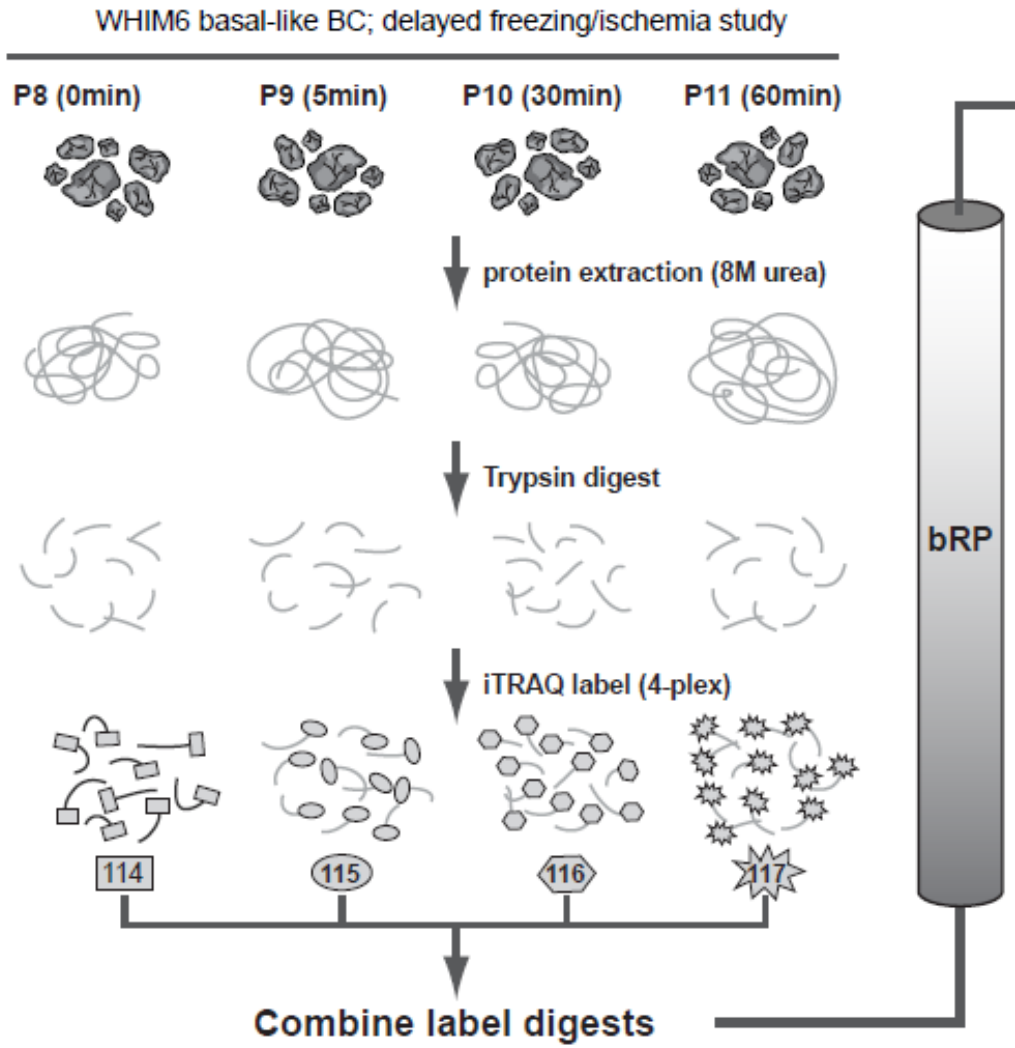
Proteomic Data Generation: high performance instruments capable of robust iTRAQ mass-tag generation

Quantification method: 4-plex iTRAQ labeling

Proteomic Data Analysis: Groups used software they are experienced with for identifying and quantifying peptides and PTMs with iTRAQ labeling

- Use common agreed upon DB to search
- Searched data through two common analysis pipes

Broad and PNNL: An integrated workflow for global proteomic and phosphoproteomic analysis



98% iTRAQ labeling efficiency; 1 - 1.5mg total protein per label

Cold ischemia times up to 1 hour cause no change in proteome but up to 17% change in phosphoproteome

		Basal Breast Cancer Xeno					Luminal Breast Cancer Xeno				
		quantified	Reg-ulated	≥2fold at 60min	up at 60min	down at 60min	quantified	Reg-ulated	≥2fold at 60min	up at 60min	down at 60min
Proteins	Avg. per replicate	12,279					9,637				
	in ≥2 replicates	11,586	0	0	0	0	9,175	0	1	0	0
pSTY-sites	Avg. per replicate	27,883					28,851				
	fully localized sites	15,942					19,412				
	in ≥2 replicates	24,607	1,129	996	791	338	26,686	4,623	1,766	3599	1,024
			4.6% *	4.0%				17% *	6.6%		
phosphorylated proteins	in ≥2 replicates	7,072	778				6,920	2,285			
kinase pSTY-sites	in ≥2 replicates	1,350	77				1,466	252			
pTyr-sites	in ≥2 replicates	442	27				441	62			

* Moderated F-test, p=0.01

Summary

- http://antibodies.cancer.gov
- Regnier et al. *Clin Chem.* 56:2 165, **2010**