Lecture 15: Functional analysis—proteome

Protein expression pattern
Immunohistochemistry
Western blot
Mass spectrometry
ICATs

Protein-protein Interaction
Co-Immunoprecipitation
Yeast two hybrid screen
Protein chip

Read 444-451, Fig. 12.13-16, 12.7, 12.18
Western blot
(a) Asymmetric neuroblast stem cell divisions

(b) Asymmetric distribution of Prospero protein
Fig. 12.13

- Protein
- Peptides
- MS base peak
- Tandem mass spectrum
- Q1, Q2, Q3 (collision cell)
- Computer sequence database searching

Theoretical vs. Acquired:
- Peptide identification and protein identification
- m/z range: 400 to 1200
Quantifying changes in protein concentration in different cell or tissue states

• Isotope analysis using isotope-coded affinity tags (ICATs)
  – Analyze protein expression in two different cellular states.
  – Three components of ICATs:
    • A biotin tag
    • A linker
    • A chemical group that reacts with cysteine amino acids
ICAT

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(a) ICAT reagents:
Heavy reagent: d8-ICAT (X=deuterium)
Light reagent: d0-ICAT (X=hydrogen)

(b) Mixture 1
ICAT-labeled cysteines
Optional fractionation
Combine and proteolyze
Affinity separation on avidin column
Quantitation and protein identification

Membrane
Nuclear
Cytosolic

Fig. 12.14
Protein arrays can identify protein interactions and potential for chemical modification.

A protein array of different types of protein kinase

Fig. 12.7
Identifying protein-protein interactions

Affinity capture/mass spectrometry

- Antibody ‘pulls down’ a protein from complex mixture.
- Identified in the max spectrometer

Fig. 12.15
Co-immunoprecipitation

Protein G

Sepharose bead
Identifying protein-protein interactions
Yeast two-hybrid interactions

DBD indicates the Gal4 DNA-binding domain.
B (Bait) indicates the protein of interest.
AD is the activator domain of Gal4.
ORF is the open reading frame of proteins tested against bait.

DBD expression vector put in one set of yeast cells.
AD/ORF put in another set.
DBD/B and AD/ORF cells fused.

If B and ORF proteins interact, Gal4 turns on expression of HIS.
HIS expressing strains selected.

Strains not expressing HIS die.

Fig. 12-16
Yeast Two Hybrid (Y2H) Assay

to test interaction between two proteins

![Diagram showing the Y2H assay process](image)
LUG/SEU represses AGAMOUS

AG second intron
Yeast Two-Hybrid Assay for Interaction Between LUG and SEU

LUFS
LUFS+Q-rich
Q-rich+WD
LUFS+Q-rich+WD
LUFS+Q-rich

Positive Control

LUG-GAL4-DB constructs vs. SEU-GAL4-AD

LUFS
LUFS Q-rich (89-184, 449-470)
LUFS Q-rich (89-184, 449-470) 7 WD
Q-rich (89-184, 449-470) 7 WD