

Lab Techniques for Systems Biology

- A virtual tour of :
- a. the brain of a biologist
 - b. the Pilot Proteomics Facility

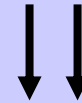
Lecture 7

Review

• Gene (DNA) → mRNA → protein



Two red arrows branch from the word 'protein' to the words 'structural' and 'catalytic (enzymes)'.



• Pathways (complexes)

a. energy metabolism

C-cpd + O₂



CO₂ + H₂O

ATP
electrons
concentration
gradient

b. signal transduction (how cells talk)

• Computers/Bioinformatics

• Biology Methods

- What do we want?

- Understand cell's response to changes in environment—involves observation of changes in all relevant gene products (proteins)

- How are we going to get there?

- Use all scientific tools at hand, including complete sequenced genomes

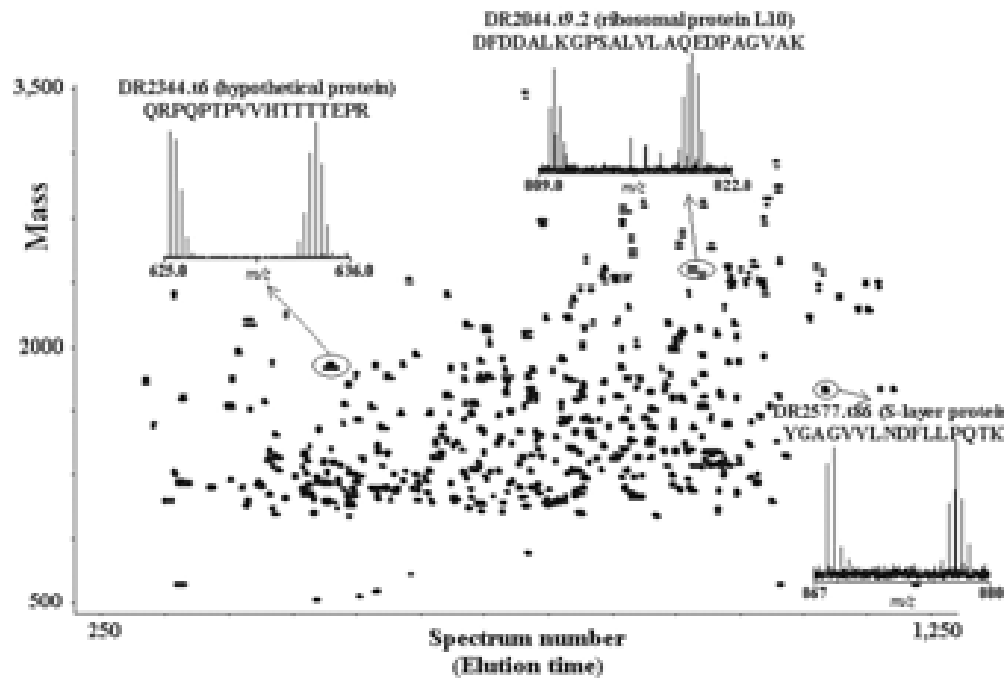
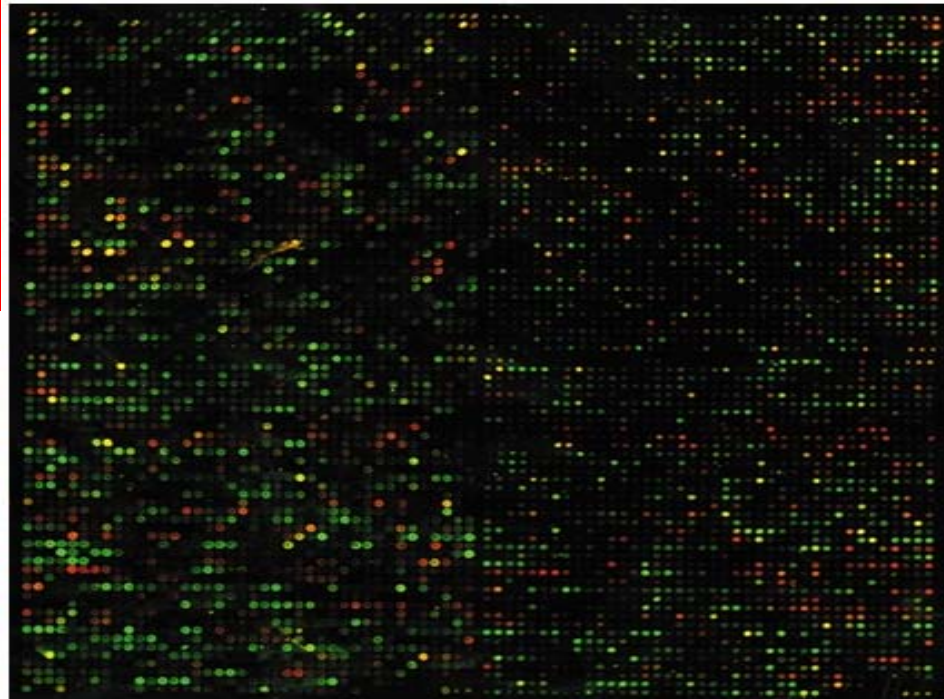
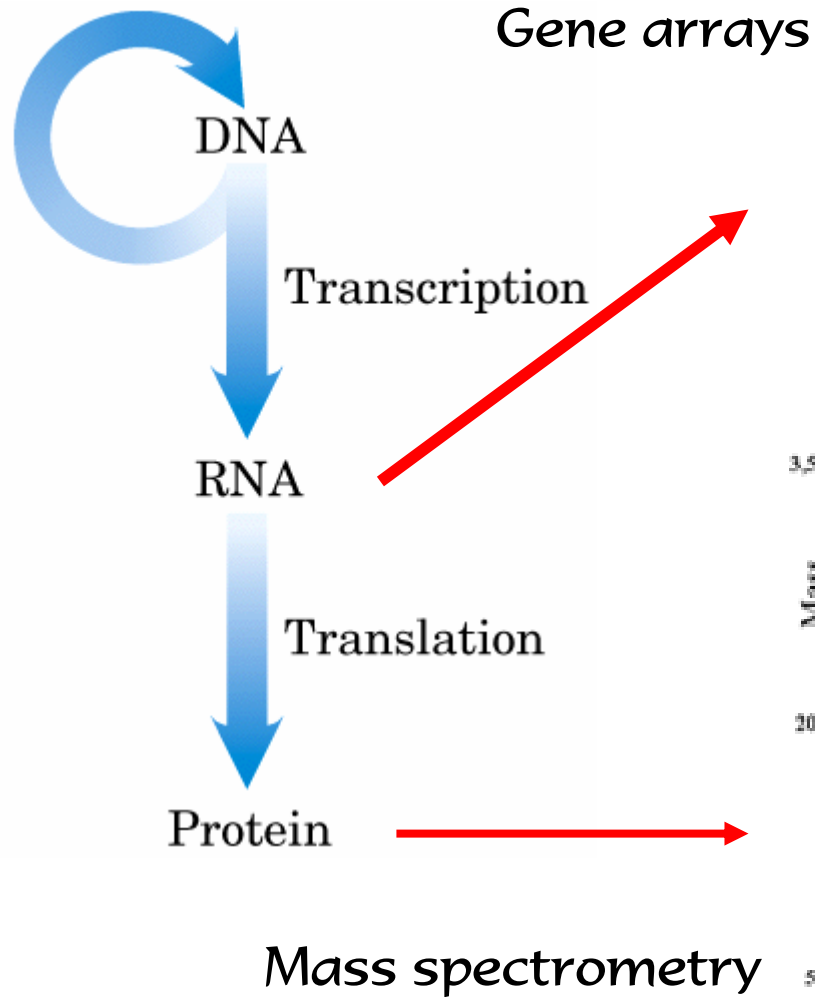
Scientific Method

- Make an observation (**discovery phase**)
 - Focus on an interesting aspect
 - (Ignore other aspects)
- Make a (testable) hypothesis
- Design/perform experiment (test the hypothesis)
- Make a conclusion
- Refine thinking
 - Make new hypothesis, etc.

Hypotheses in Systems Biology

- **Systems Biology focuses on many aspects of an observation**
 - Hypothesis example:
 - cell stimulus $X \rightarrow$ stress proteins are up-regulated
 - » Experimental results will enforce a focus on one or a few gene products
 - » Hypothesis: Stress protein A is the initial stimulus within the cell.

Discovery



Discovery Phase

capabilities for multiplex profiling of the cell

- Stimulus → cell response?
 - Change in cell morphology/cell fate (proliferation/cell death/differentiation)
 - Change in metabolites (metabolomics)
 - Change in protein functions
 - New genes transcribed (gene arrays)
 - New proteins translated (proteomics)
 - Post-translational modifications (proteomics)
 - Protein Translocation (imaging)

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Have Gene, Will Clone

using cell's machinery to make protein

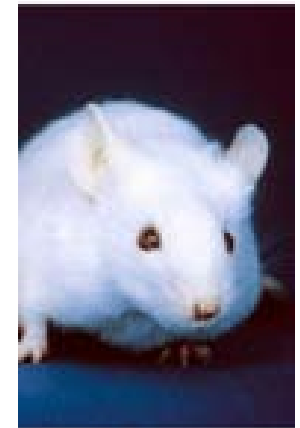
Wildtype or mutant gene

Protein
expression
& purification



(E. coli)

Cell culture
Tissue specific



Transgenic

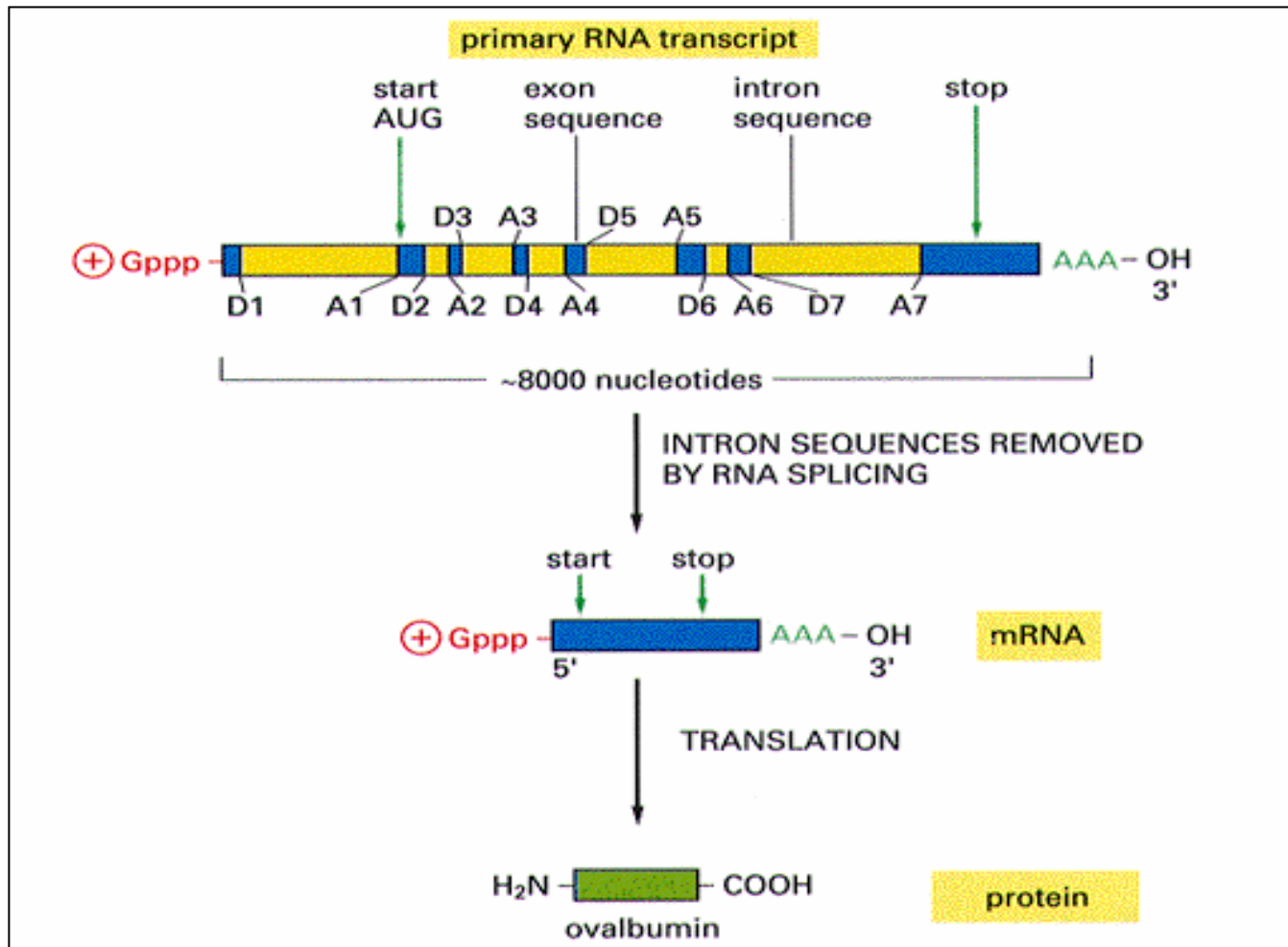
Cloning Strategy

- Step 1. Select a gene from a genome.
- Step 2. Make lots of that gene.
- Step 3. Insert the gene into a vector.
- Step 4. Insert the vector into a cell.
- Step 5. The cell makes lots of the protein.
- Step 6. Purify the protein.
- Step 7. Perform quality control.

Step 1. Select a gene from a genome.

- Sequenced genome of interest
 - human genome
 - mouse genome
 - *Shewanella* genome
- GGCAAAGCCACATAATGATGCCGGCGCGGATATCTGGTGATACA
ATGCCTATGAATGCGTACTAGGTCAGATTTGGACTCTCACG-
- *unrelenting sequence!!*
- need to know expressed genes

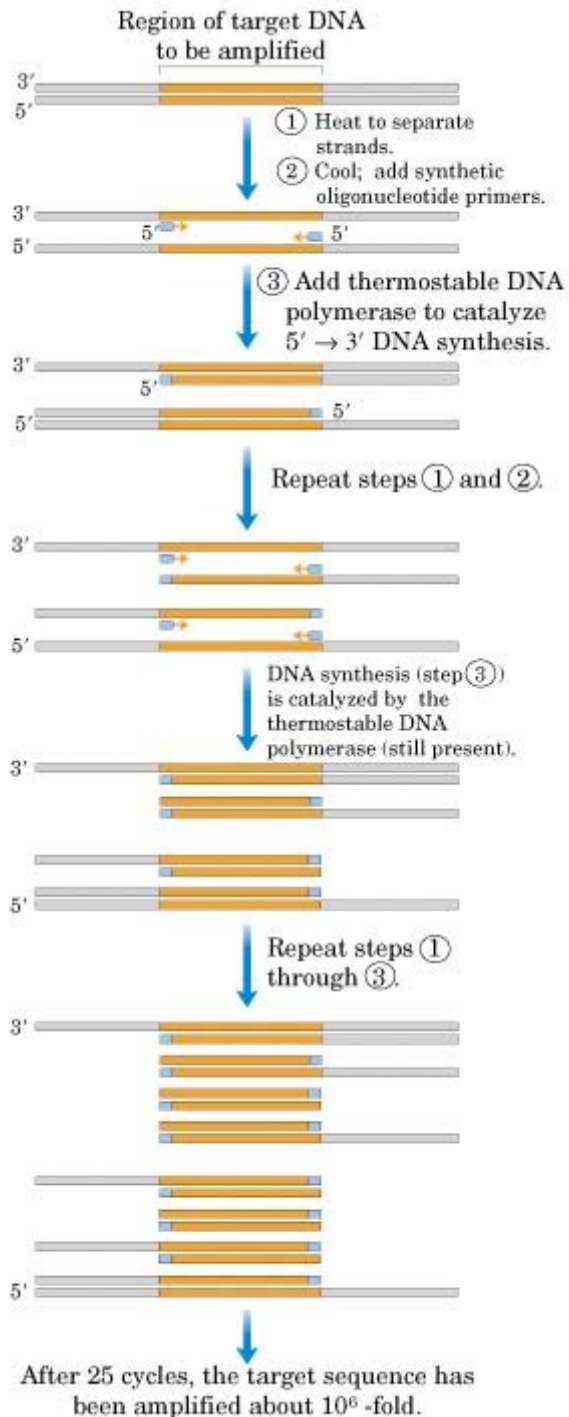
Annotated Sequence → ORFs (open reading frames) *Ready-to-Read*



Strategy to Select a Protein of Interest

- Select from an annotated genome
- Translate ORFs to protein sequences
- Look in databases (BLAST) for protein identity
 - Protein analogs in other species aid ID
 - (~ ½ human ORFs = unknowns)
 - NIH Structural Genome Consortia

» Your Pick!



Step 2. Make lots of that gene.

Polymerase Chain Reaction (PCR)

Uses heat-stable DNA polymerase (TaqI from hot springs bacteria)

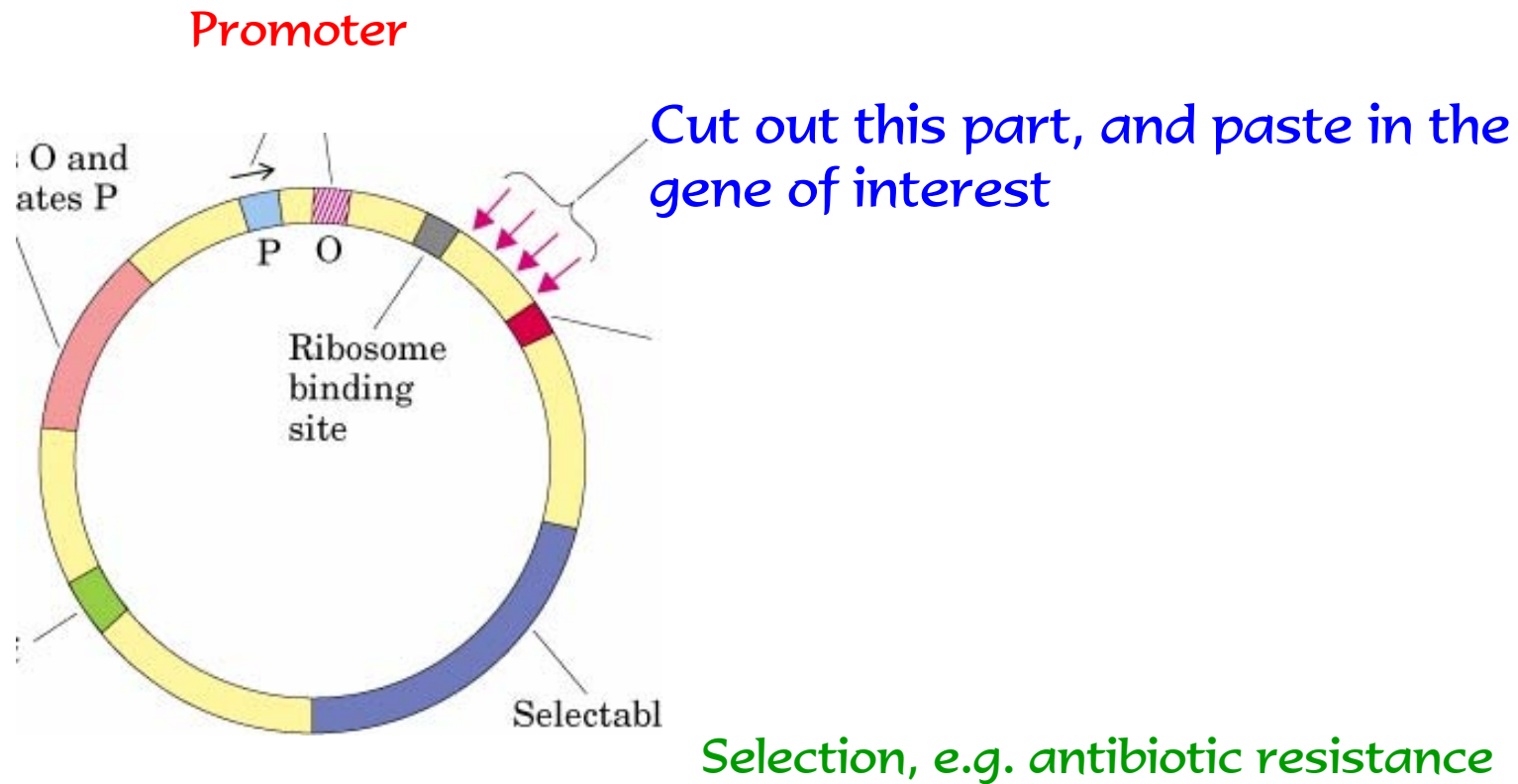
Able to amplify one DNA molecule (40,000 yrs old)

e.g., woolly mammoths

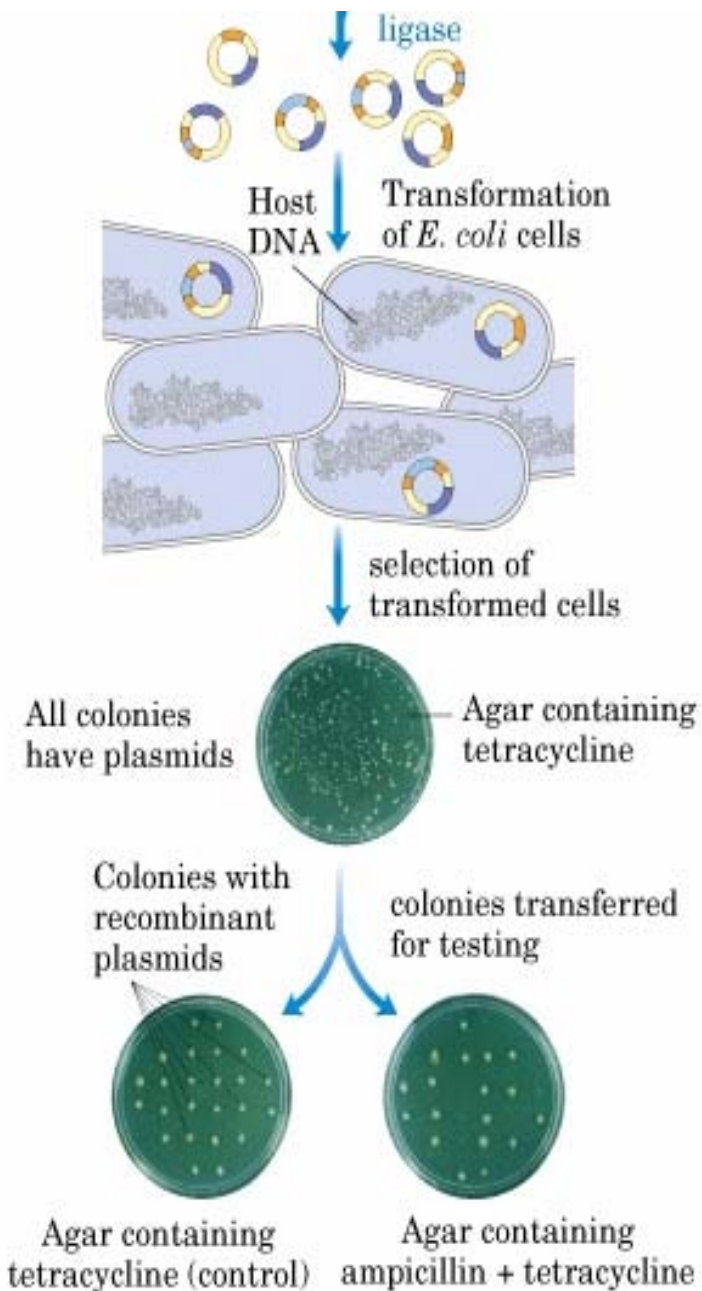
Jurassic Park?

forensics

Step 3. Insert the gene into a vector.



Step 4. Insert the vector into a cell.



1. E. Coli (disabled) + vector
2. Perturb cell membrane → DNA entry (**small % success**)
3. Select transformed cells

GM bacteria

Step 5. The cell makes LOTS of the protein (with a little help).

1. Grow cells in a batch culture.
2. Use a promoter to turn on protein expression.

Step 6. Purify the protein.

Break cells



Isolate expressed protein from other cell proteins

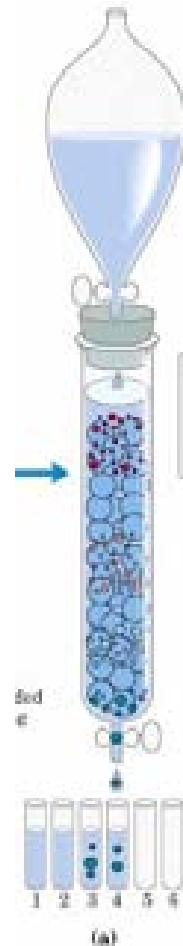
Separate by:

Size

Charge

Ligand binding

Engineered tags



Step 7. Perform quality control.

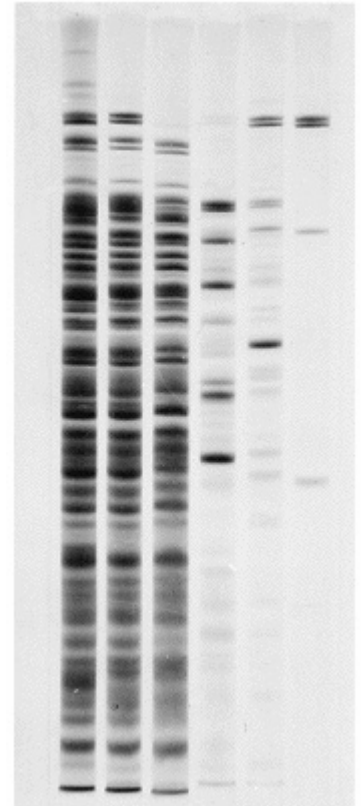
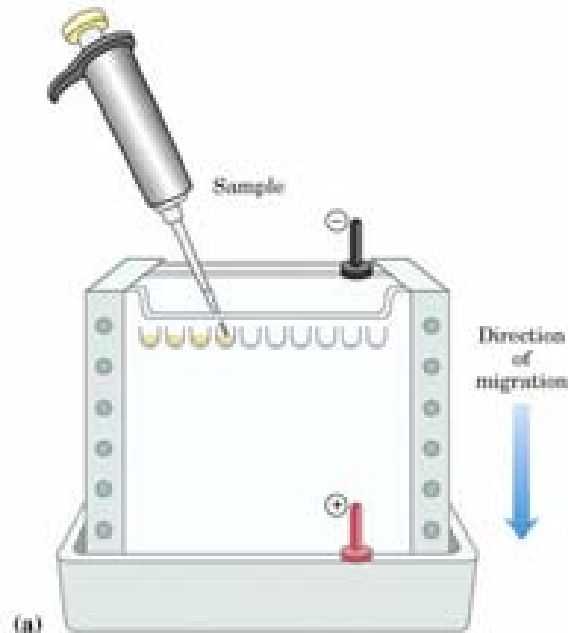
Electrophoresis

Separate molecules by size/charge in an electric field through a matrix of:

- a. polyacrylamide (proteins)
SDS-PAGE or native
- b. agarose (DNA)

Visualize bands

Coomassie blue (wool dye)



Tour of the Pilot Proteomics Facility

Stimulus → Proteome Response?

Proteomic profile provides a process

1st Question: Which proteins change function, and how?

Are new proteins translated? Which ones, how much?

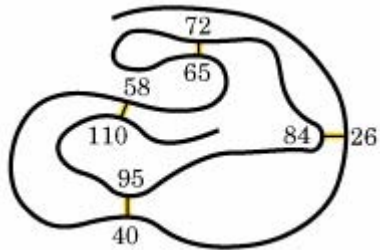
Are new or old proteins modified? Which proteins/ how much?
(phosphorylation, oxid mod., ubiquitinated, etc.)

Do cellular locations of proteins change? (Use imaging?)
Which proteins/ how much?

Does structure/binding change? (function of structural proteins)

Do amounts of small molecule regulators change?
(metabolomics)

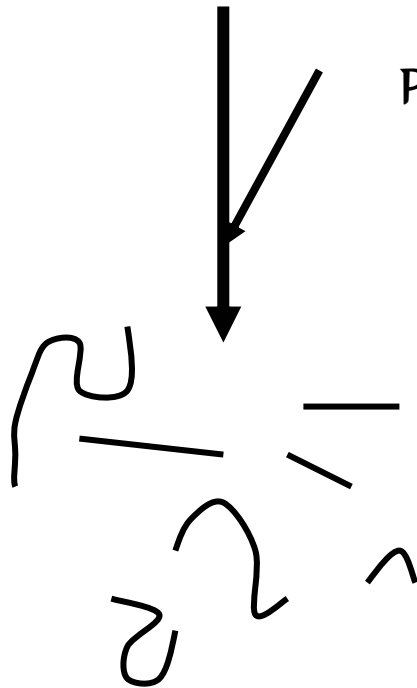
New Protein Translation?



Protein: known
sequence
= known mass

Mass spectrometer
measures the mass of
protein fragments

Protease



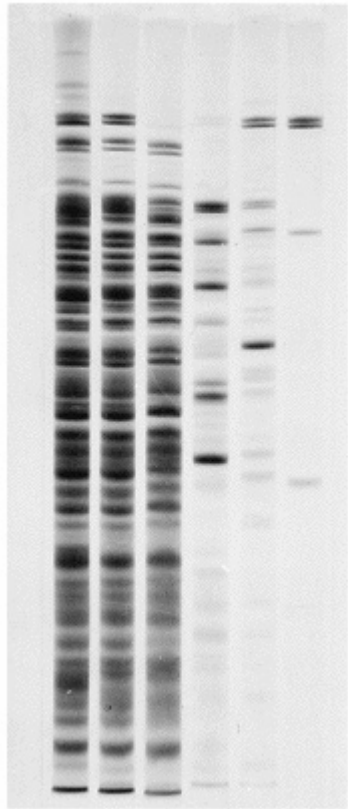
Protein
fragments
of known mass

ID protein from
database by the mass
of one or more of its
fragments

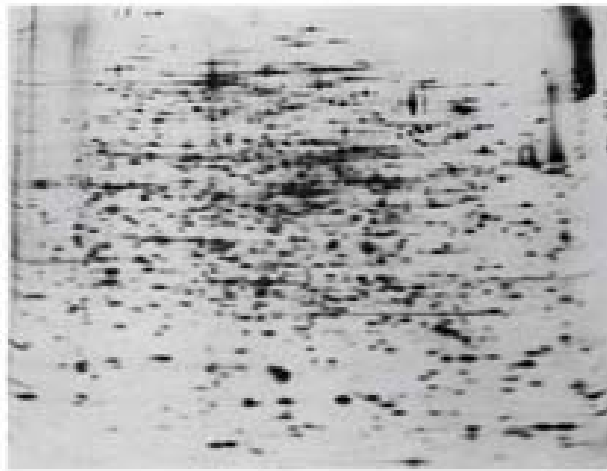
11.4 Tesla FT-ICR Mass Spectrometer



How Much of Any New Protein?

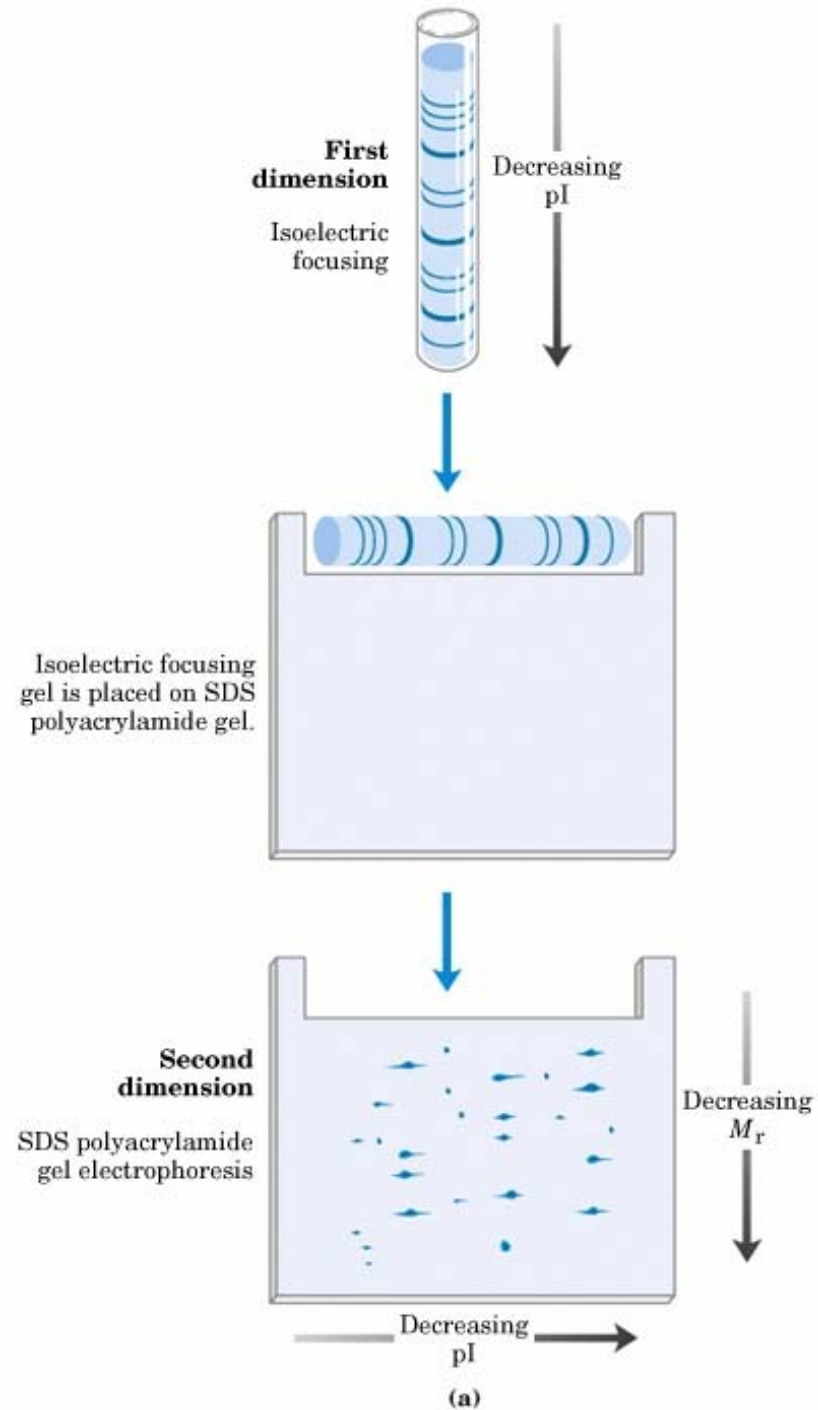


1-D



2-D

electrophoresis



Post-Translational Modifications?

Build new mass spectrometer databases for modified proteins??

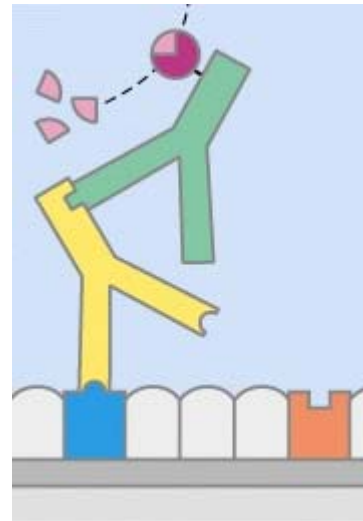
Many proteins have multiple modifications

Or, enrich modified proteins for MS

Antibody-on-a-bead purification:

Anti-phosphoprotein

Anti-oxi-protein



Proteomic Methods

- Cloning
- Gene arrays
- Separation and enrichment strategies
- Electrophoresis (1-D, 2-D)
- Surface plasmon resonance
- Mass spectrometry
- FT-IR
- Fluorescence