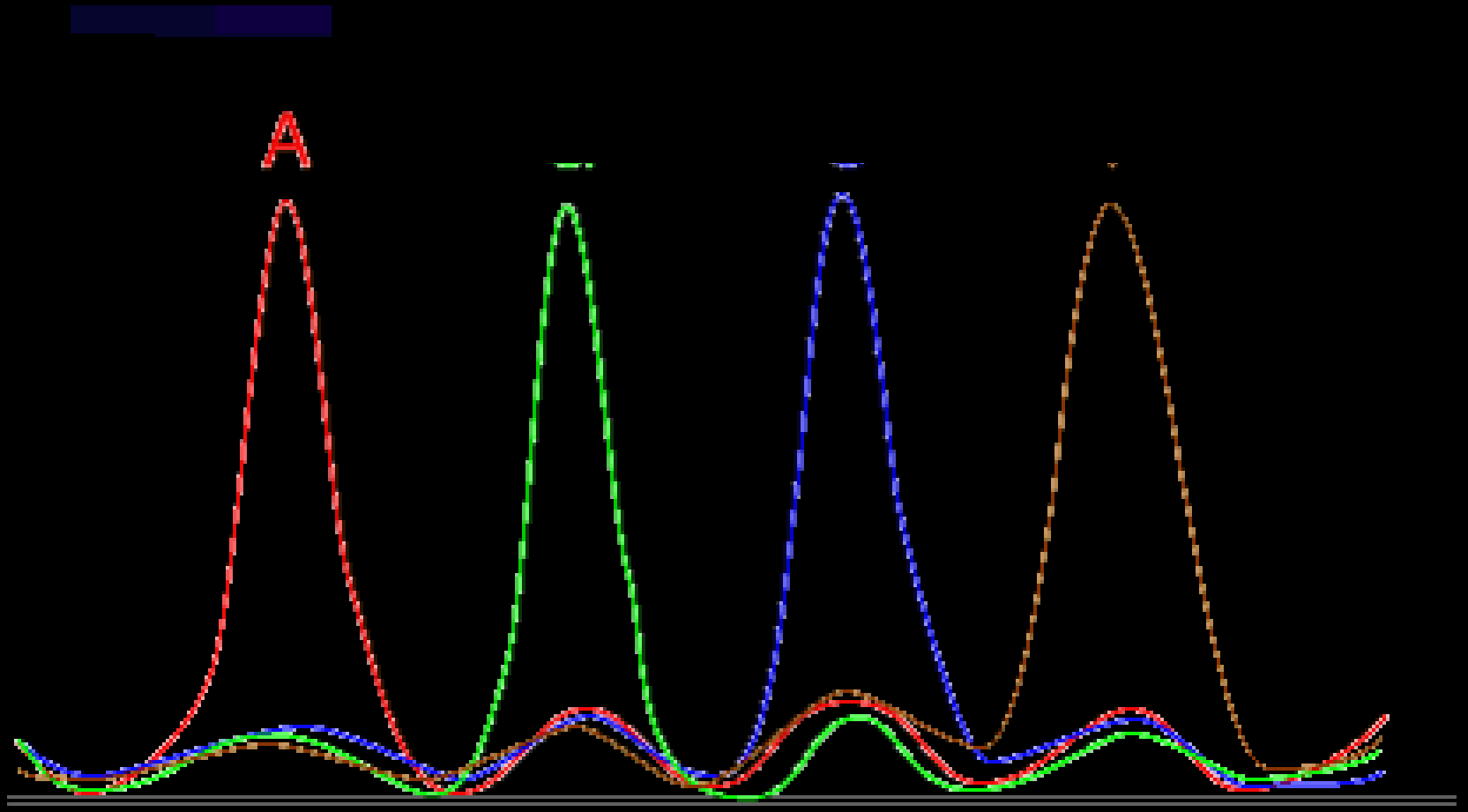
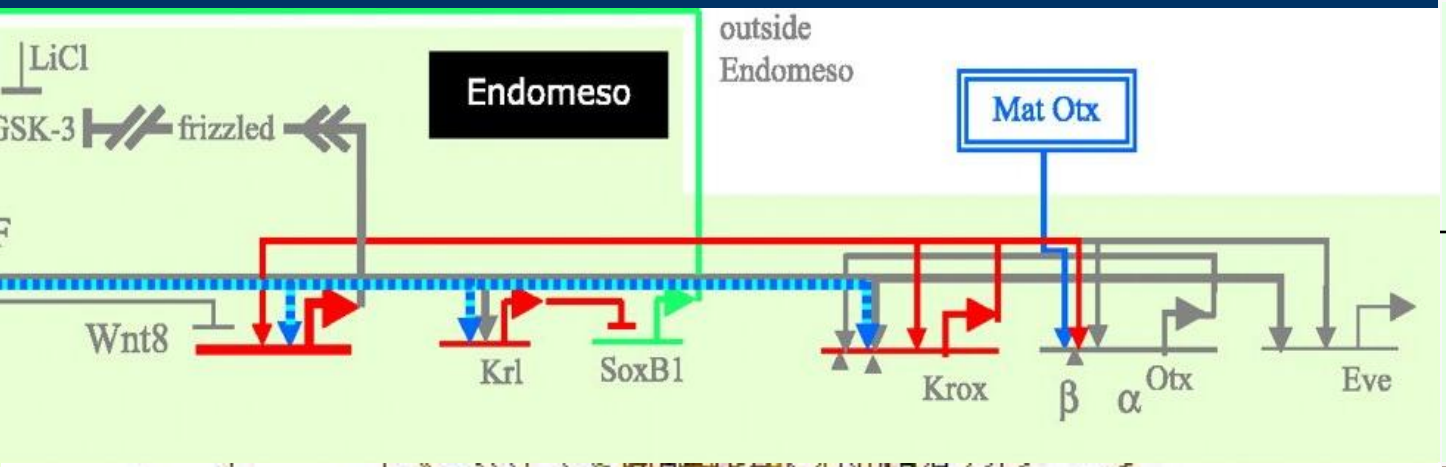
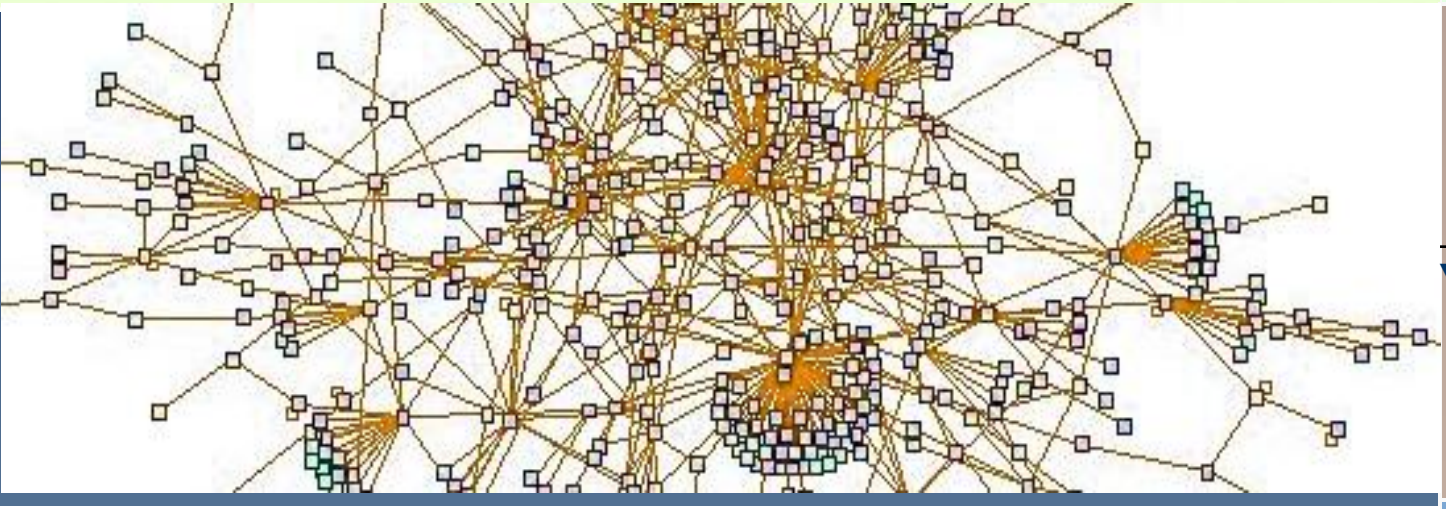
BENG 183

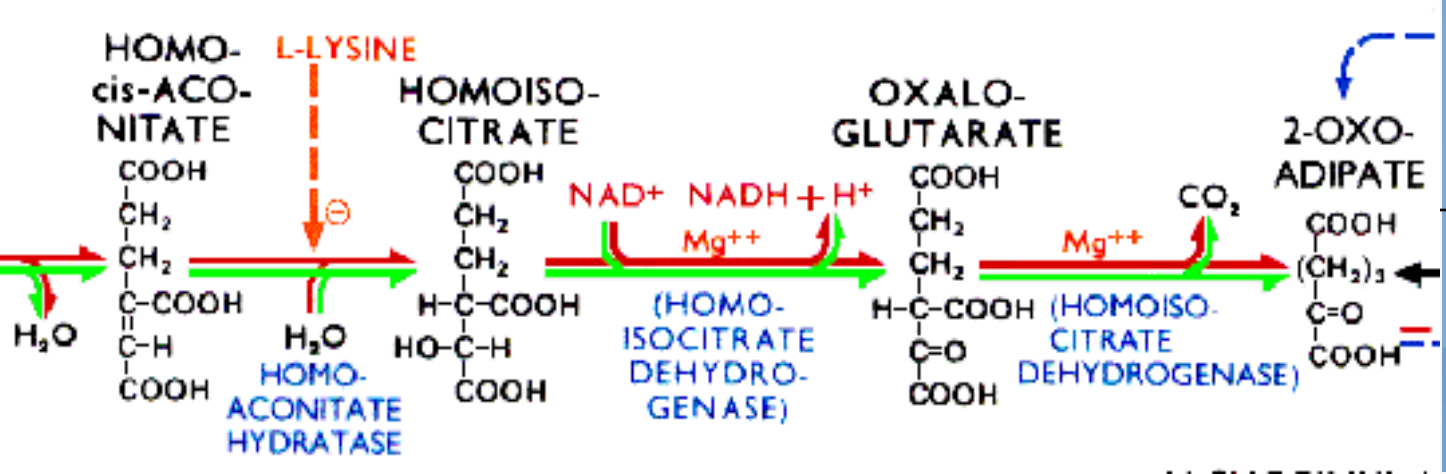
Trey Ideker

Protein interactions









Protein→DNA interactions

▲ Chromatin IP

▼ DNA microarray

Gene levels (on/off)

Protein—protein interactions

▲ Protein coIP

▼ Mass spectrometry

Protein levels (present/absent)

Biochemical reactions

▲ Not yet!!!

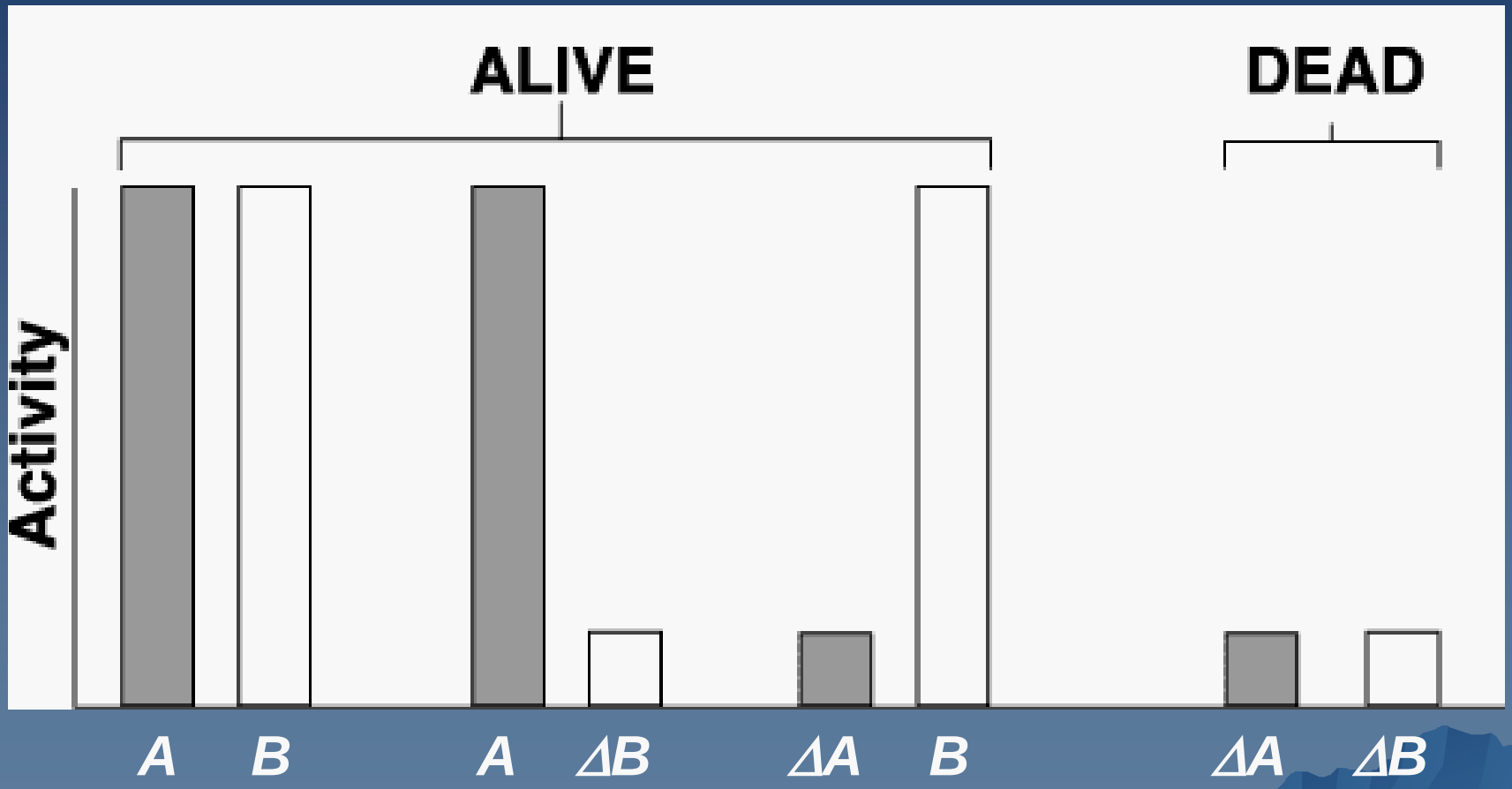
Metabolic flux ▼ measurements

Biochemical levels

Classical methods for identifying protein-protein interactions

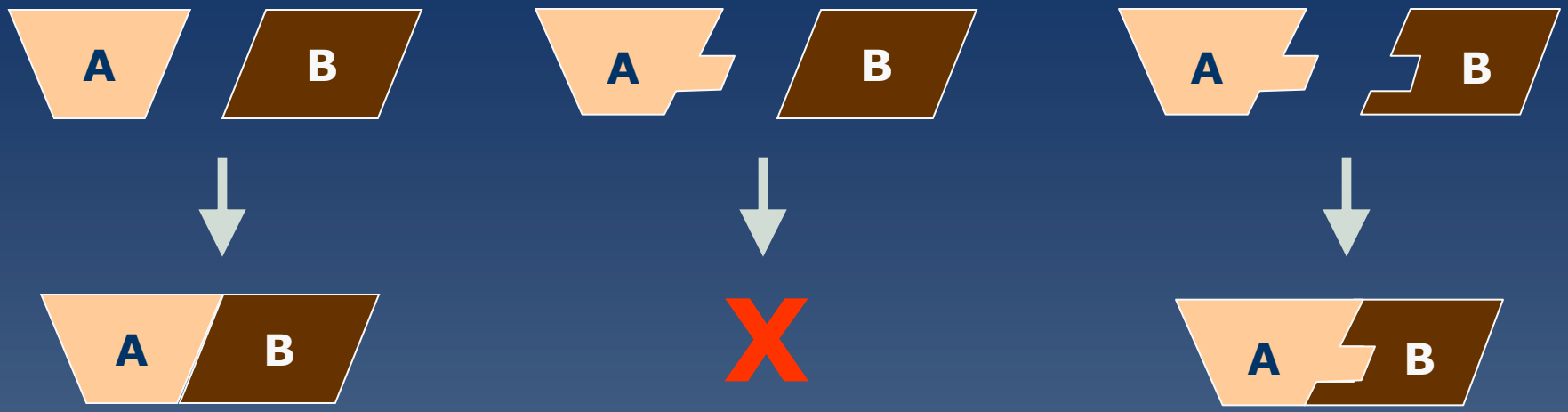
- Co-immunoprecipitation / affinity chromatography / crosslinking
- Suppressor and synthetic lethal mutations
- Fluorescence energy transfer (FRET)
- Dominant negatives– overexpression of the mutant form X of a protein causes loss of function despite the presence of wt proteins. One explanation is that X forms a multimer that sequesters wt proteins.

Most recorded genetic interactions are *synthetic lethal* relationships

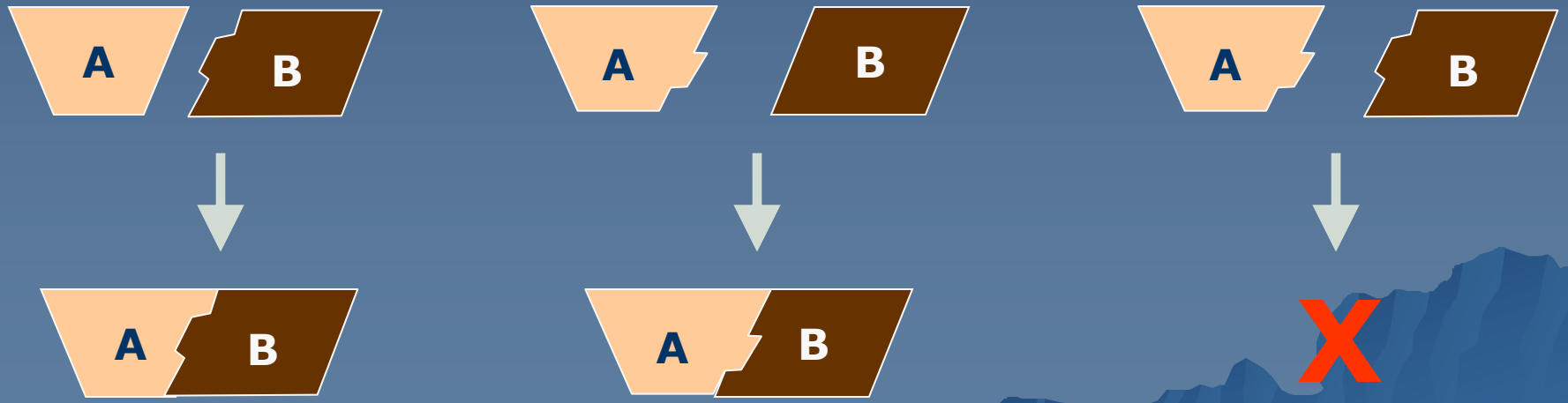


Adapted from Hartman, Garvik, and Hartwell, *Science* 2001

Suppressor protein interaction

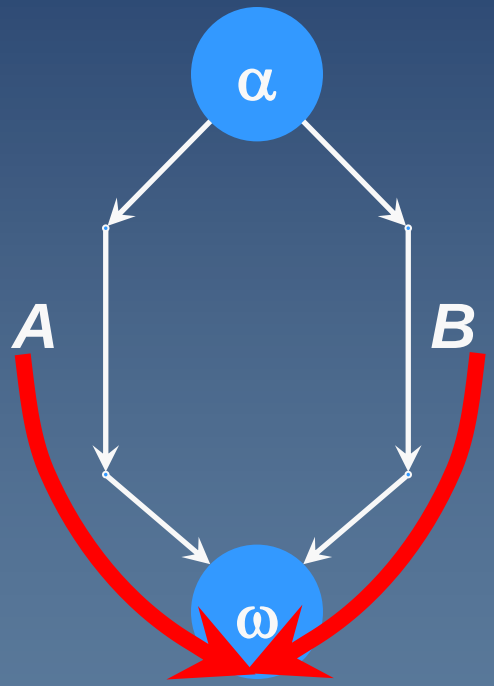


Synthetic-lethal protein interaction



Interpretation of genetic interactions (Guarente *T.I.G.* 1990)

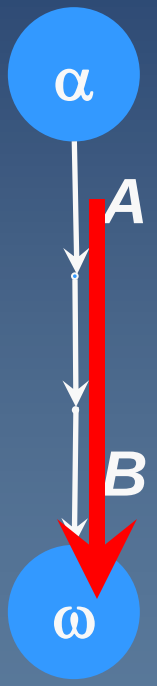
Parallel Effects (Redundant or Additive)



Single A or B mutations typically *abolish* their biochemical activities

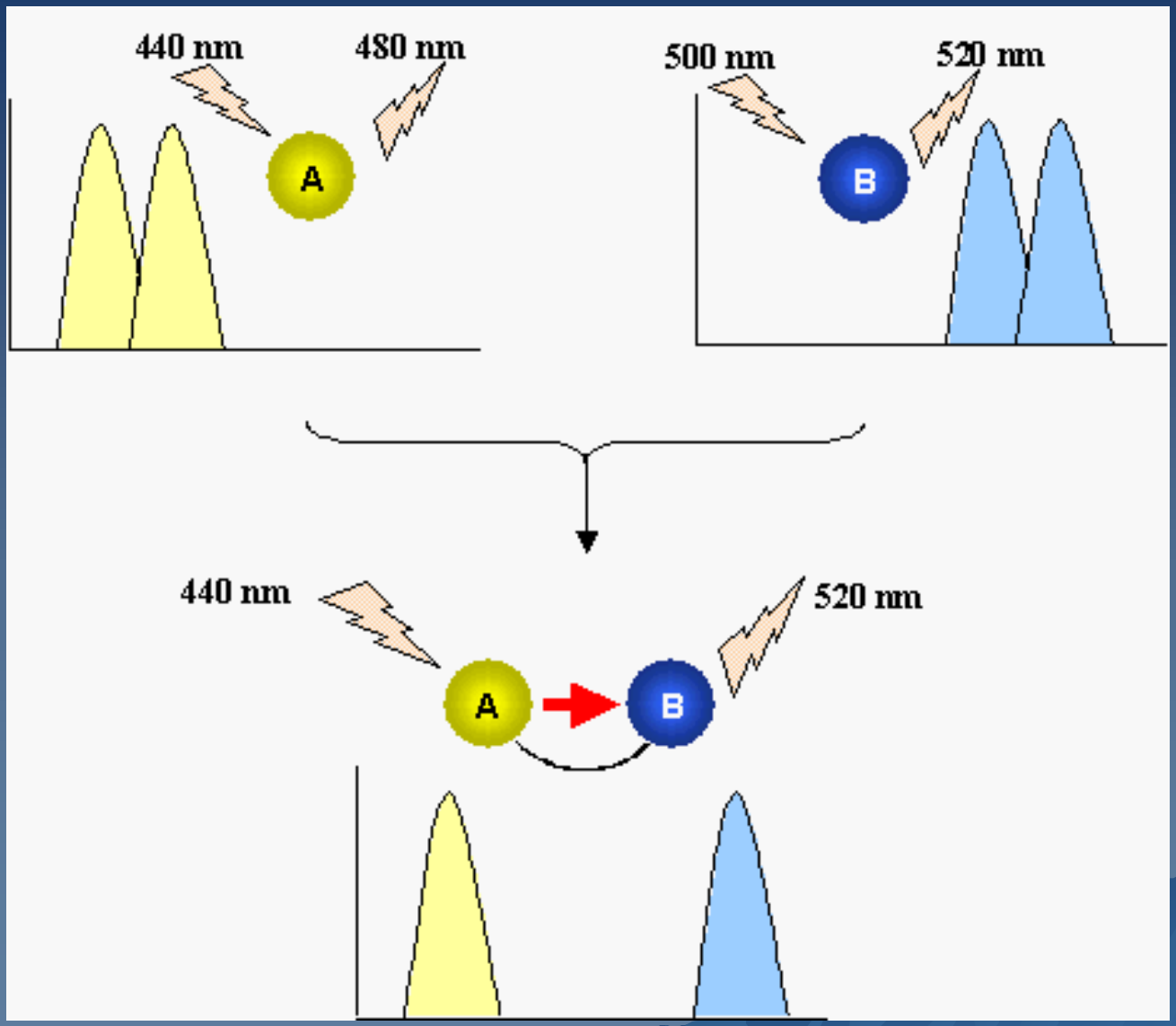
GOAL: Identify downstream physical pathways

Sequential Effects (Additive)

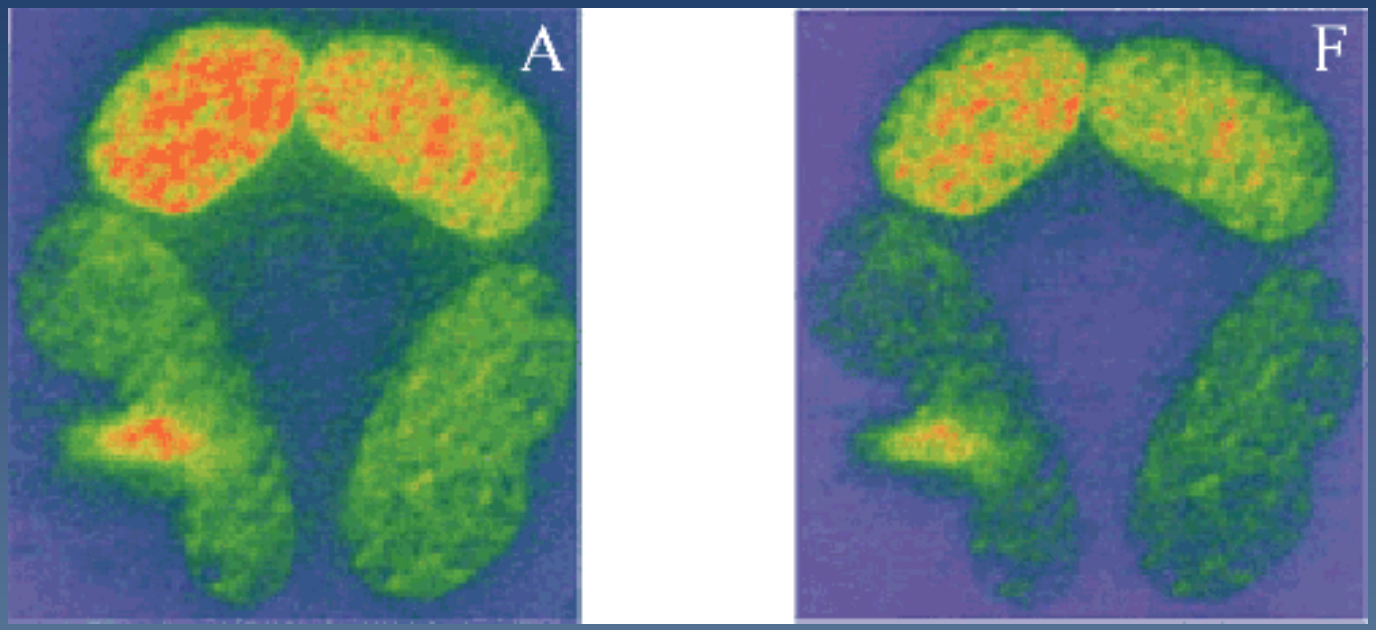


Single A or B mutations typically *reduce* their biochemical activities

Fluorescence energy transfer (FRET)



FRET (continued)



HeLa cells (immortalized cervical tumor from Henrietta Lacks)

Transfected with both PIT1-GFP and PIT1-BFP

PIT1-GFP excited with laser

Red indicates FRET & argues for the dimerization of PIT1

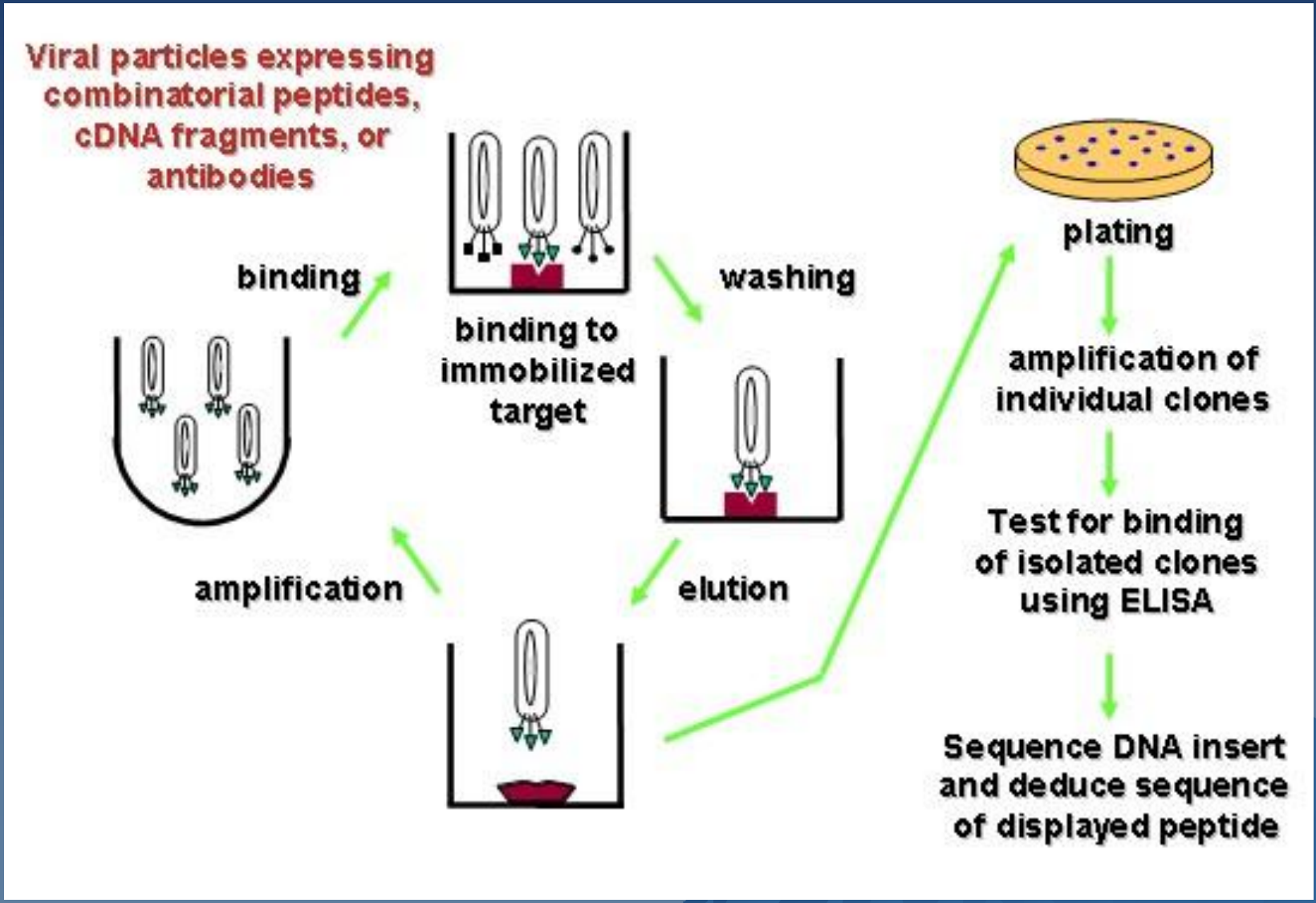
High-throughput methods for measuring interactions

- Phage display
- 2-hybrid
- co-immunoprecipitation
- Protein arrays
- chIP-on-chip
- systematic genetic analysis

Phage display

1) Expression of fusion proteins so that a foreign peptide sequence is displayed on a bacteriophage surface
2) Libraries of phage are produced by infected *E. coli* and screened to identify peptides that interact with a probe (e.g. an antibody)
3) Screening is an iterative affinity purification process, called *panning*, in which only the interacting (bound) phage are retained and used to reinfect *E. coli* in step (2) above.
4) After several rounds of panning, the remaining tightly bound phage are isolated and the inserts sequenced to identify the interacting peptides.
5) Screening and panning includes intrinsic amplification at each step and can be carried out in high-throughput array format.

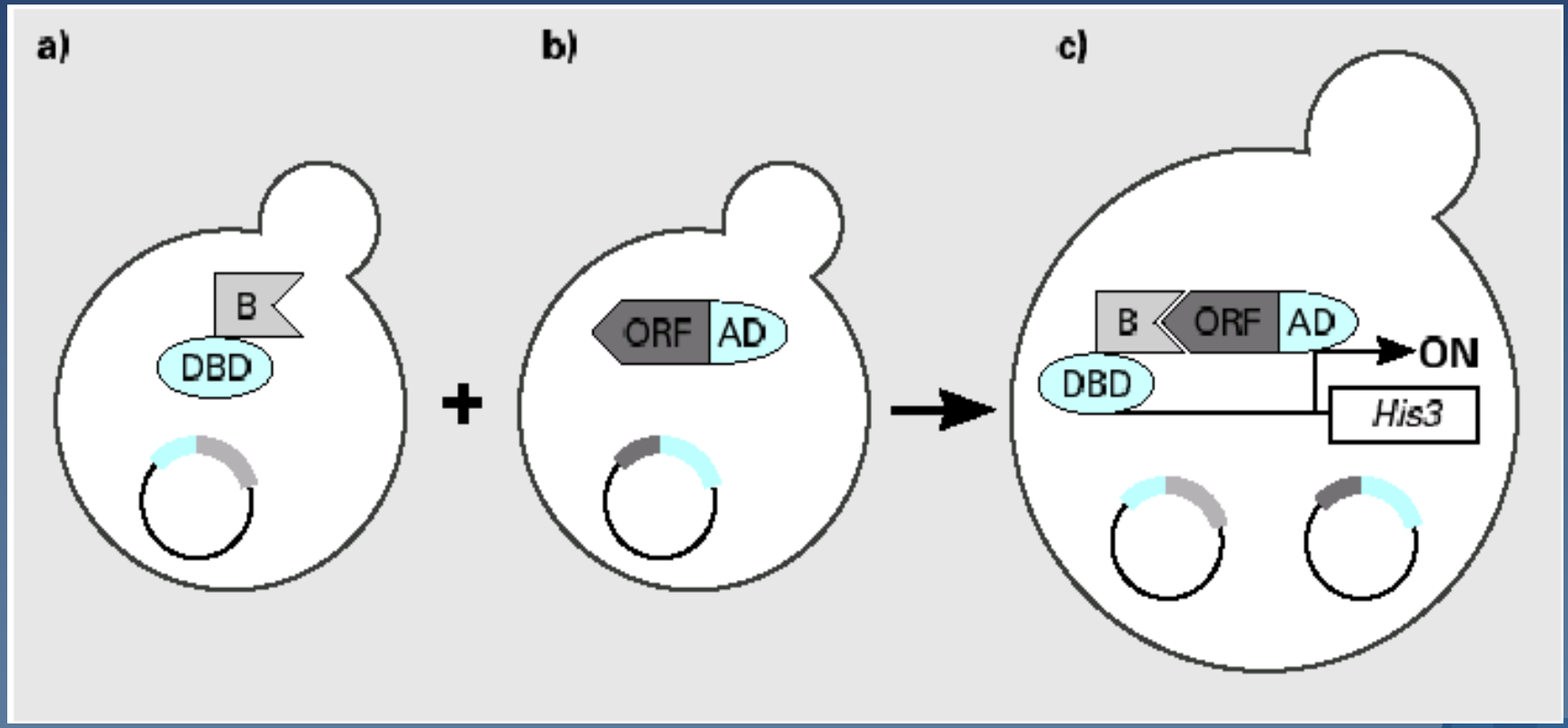
Phage display (continued)



Yeast Two Hybrid (Y2H) Method

- One problem with phage display and other *in vitro* technologies is that the measured binding may not actually occur.
- Y2H assays interactions *in vivo*.
- Uses property that transcription factors generally have separable *transcriptional activation* (AD) and *DNA binding* (DBD) domains.
- A functional transcription factor can be created if a separately expressed AD can be made to interact with a DBD.
- A protein 'bait' B is fused to a DBD and screened against a library of protein 'preys', each fused to a AD.

Yeast two-hybrid method

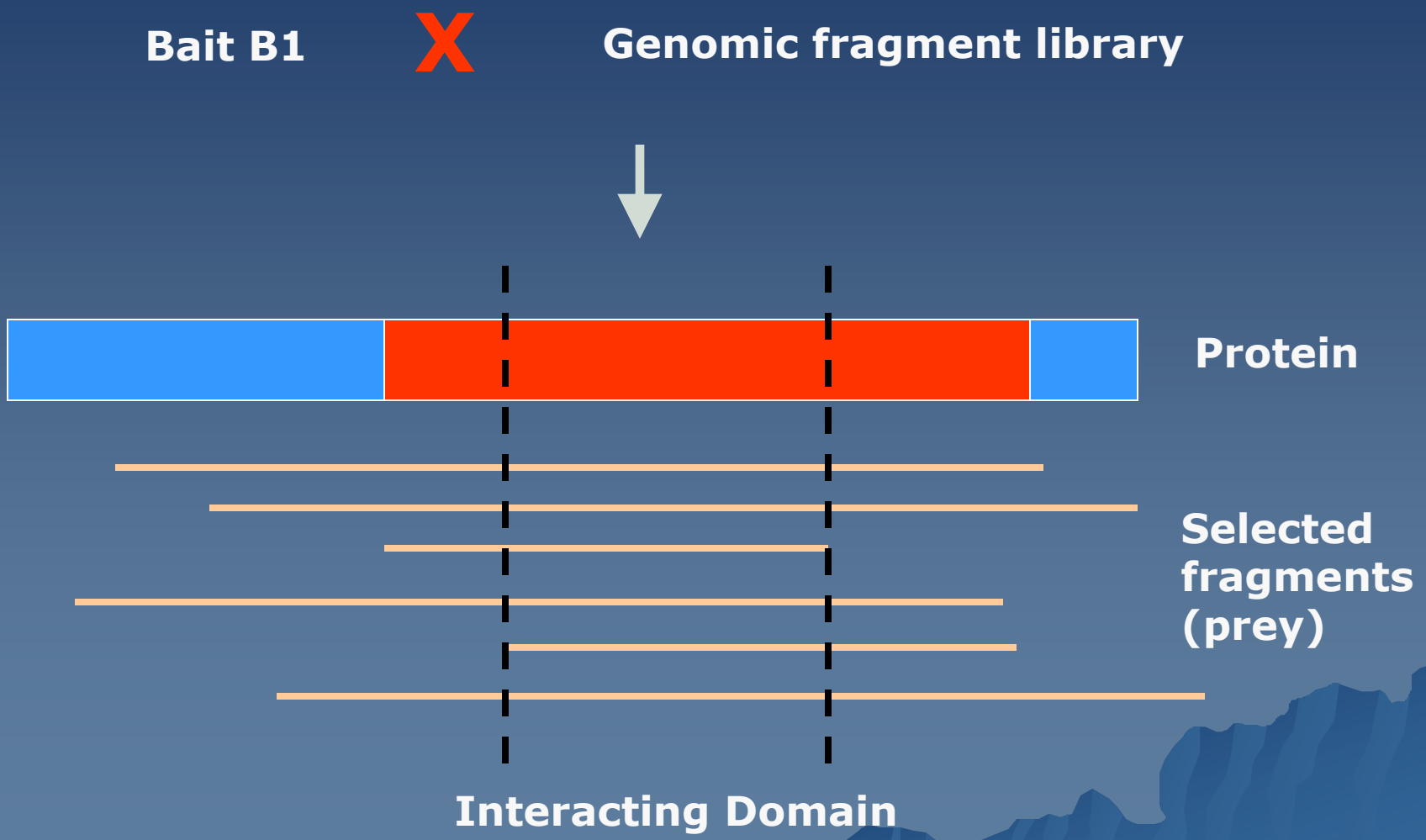


Fields and Song

Y2H matrix approach

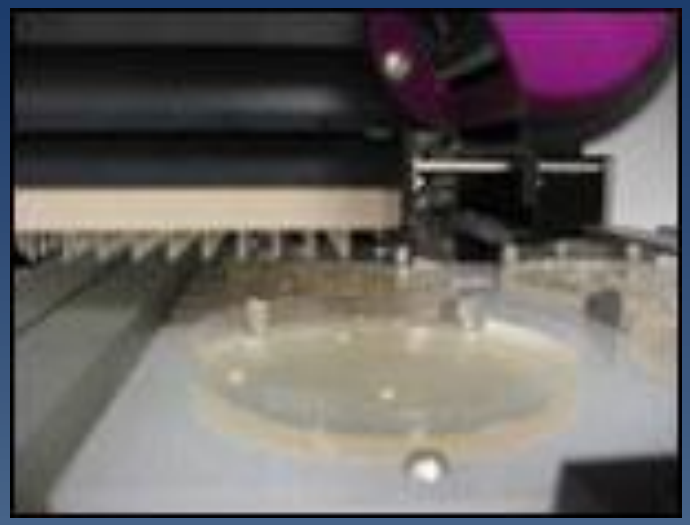
Prey / Bait	P1	P2	P3	P4	P5
B1	■				
B2	■		■		
B3					
B4	■	■	■	■	■
B5	■				■

Y2H random library approach



Prolexys high-throughput Y2H

Automated plating and mating



Robot picking a yeast colony

PCR and sequencing

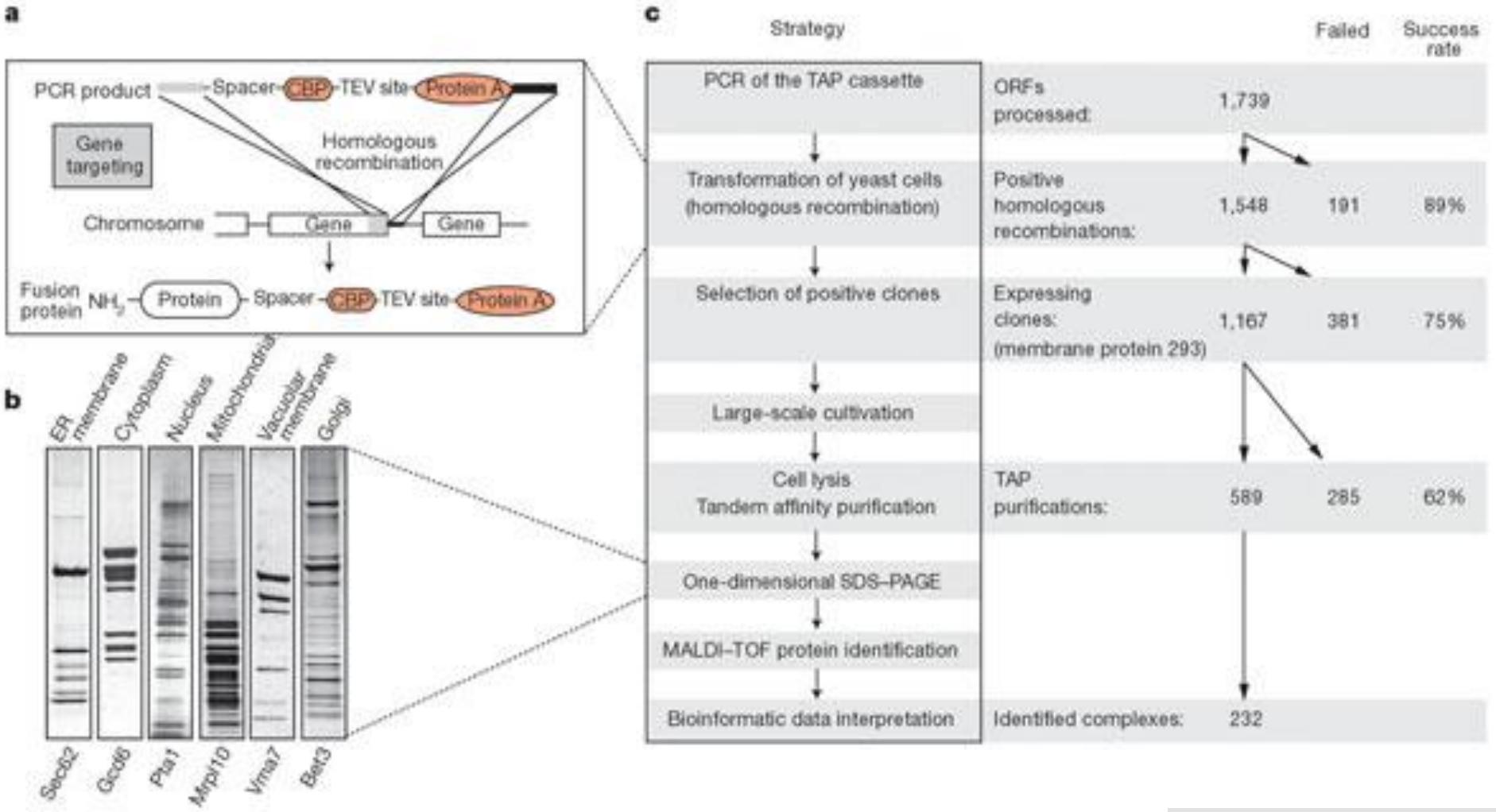
Issues with Y2H

- False positive interactions due to:
  - Autoactivation
  - 'Sticky' prey
  - Genuine but biologically irrelevant interactions
- False negative interactions
  - Similar studies often reveal different sets of interacting proteins
  - Matrix method appears to suffer from higher degree of false negatives.
  - The random library method perhaps does better because each protein is represented by a series of overlapping peptide fragments.

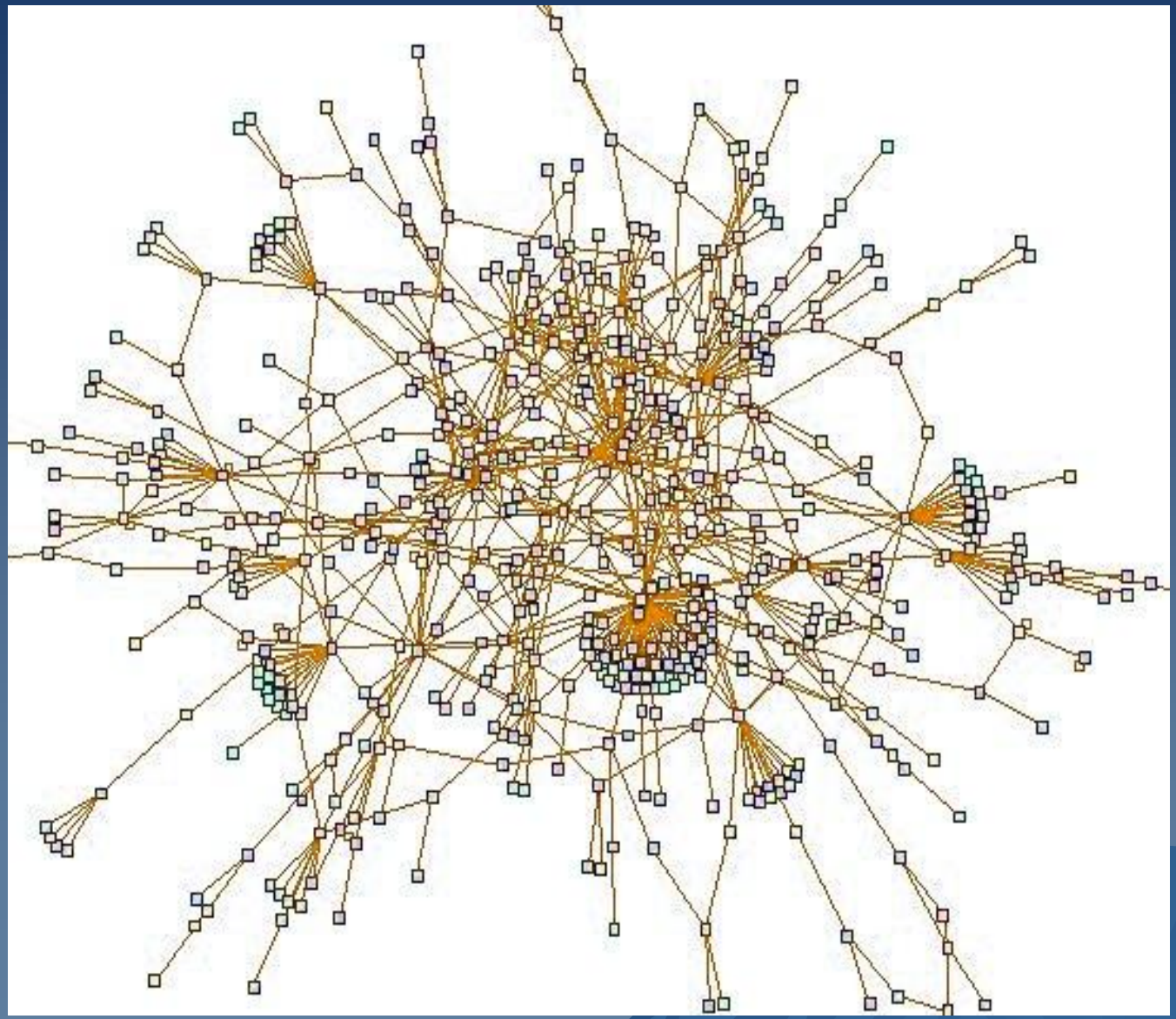
Protein interactions by protein immuno-precipitation followed by mass spectrometry

- We previously discussed mass spec. as a method for identifying proteins and protein abundances, but it can also be applied to protein interactions
- Start with affinity purification of a single epitope-tagged protein
- This enriched sample typically has a low enough complexity to be fractionated on a standard polyacrylamide gel.
- Individual bands can be excised from the gel and identified with mass spectrometry.

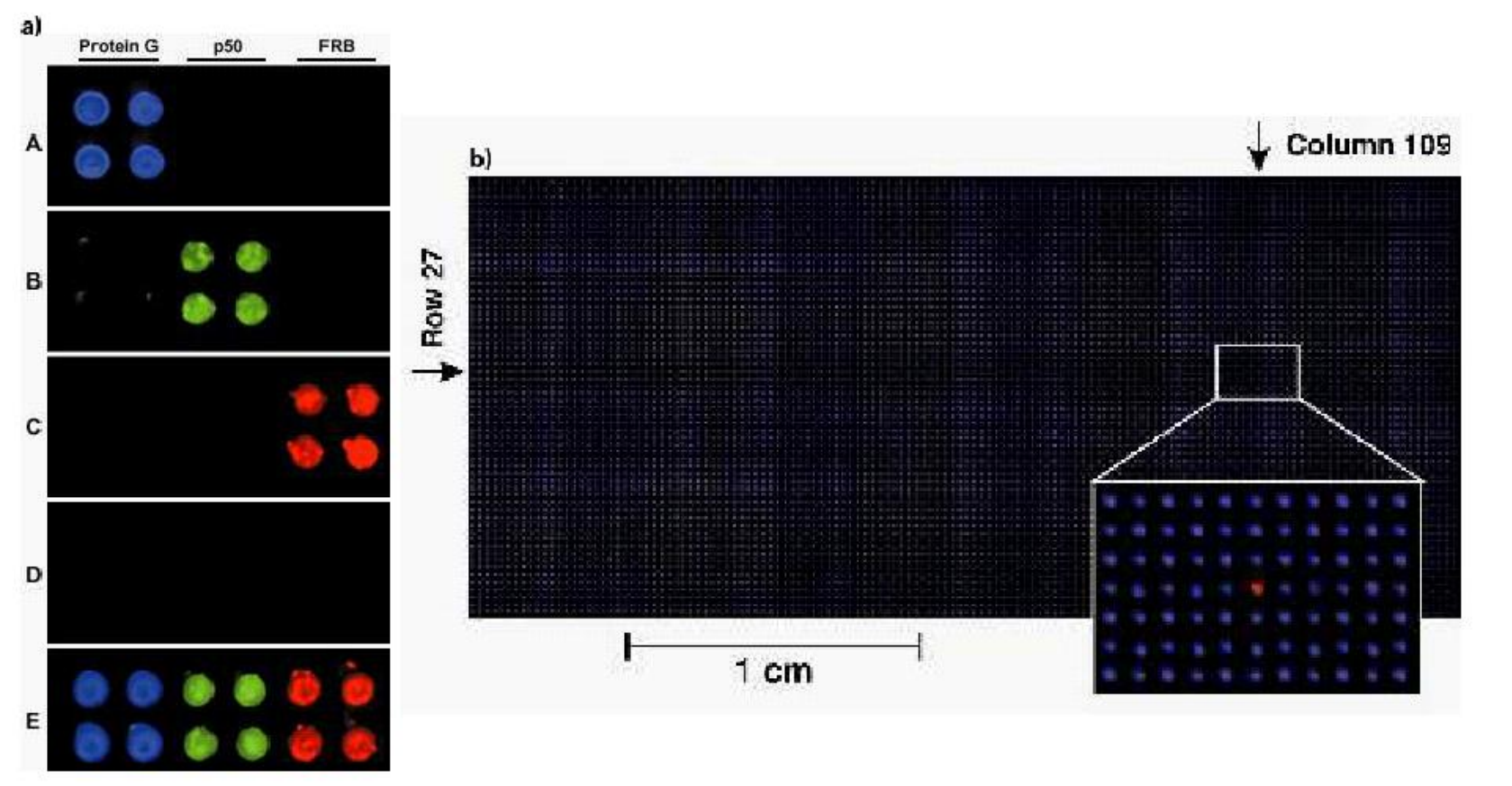
Protein interactions by protein immuno-precipitation followed by mass spectrometry



Helicobacter pylori Y2H network

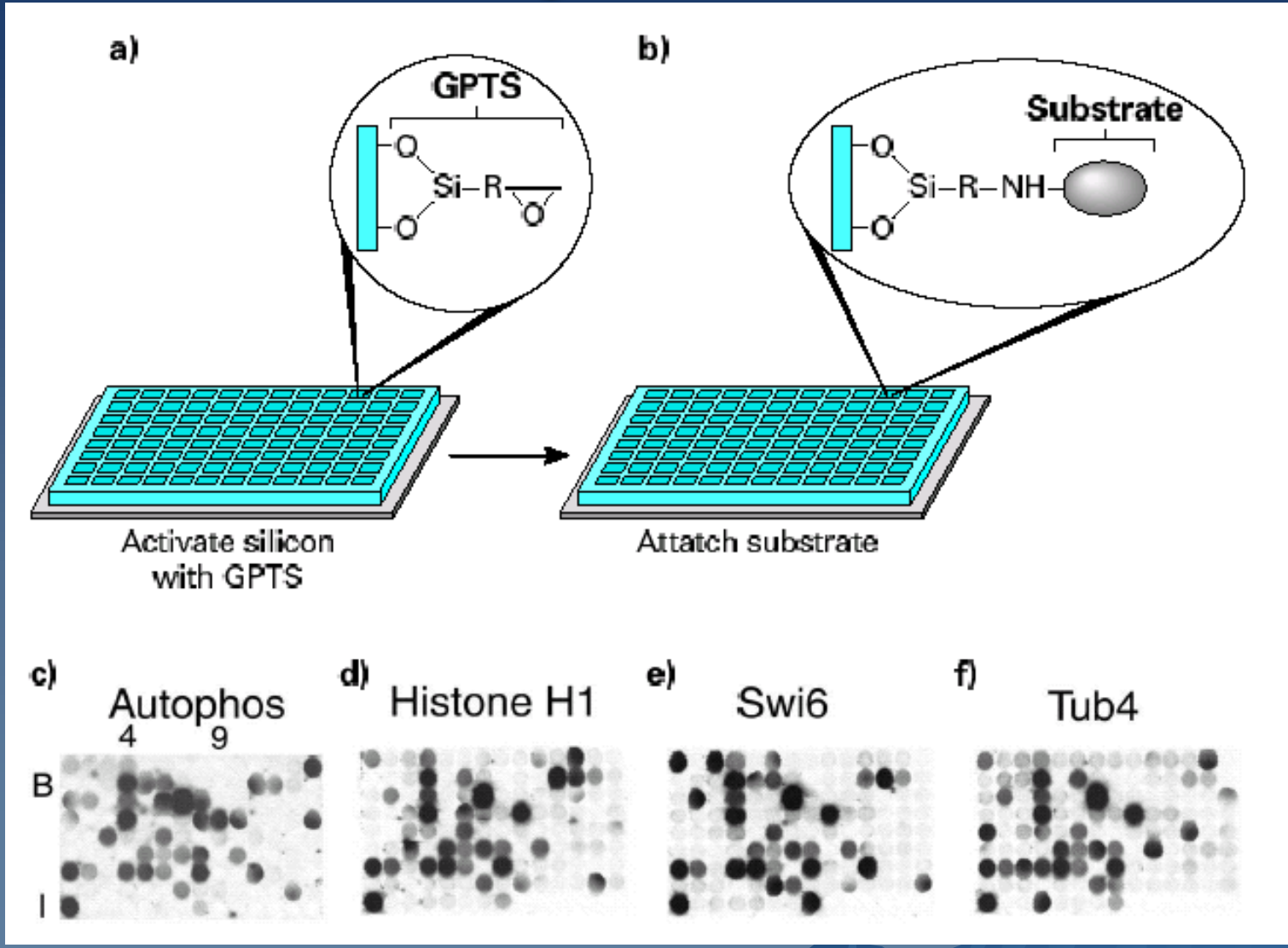


Detection of protein interactions with antibody arrays



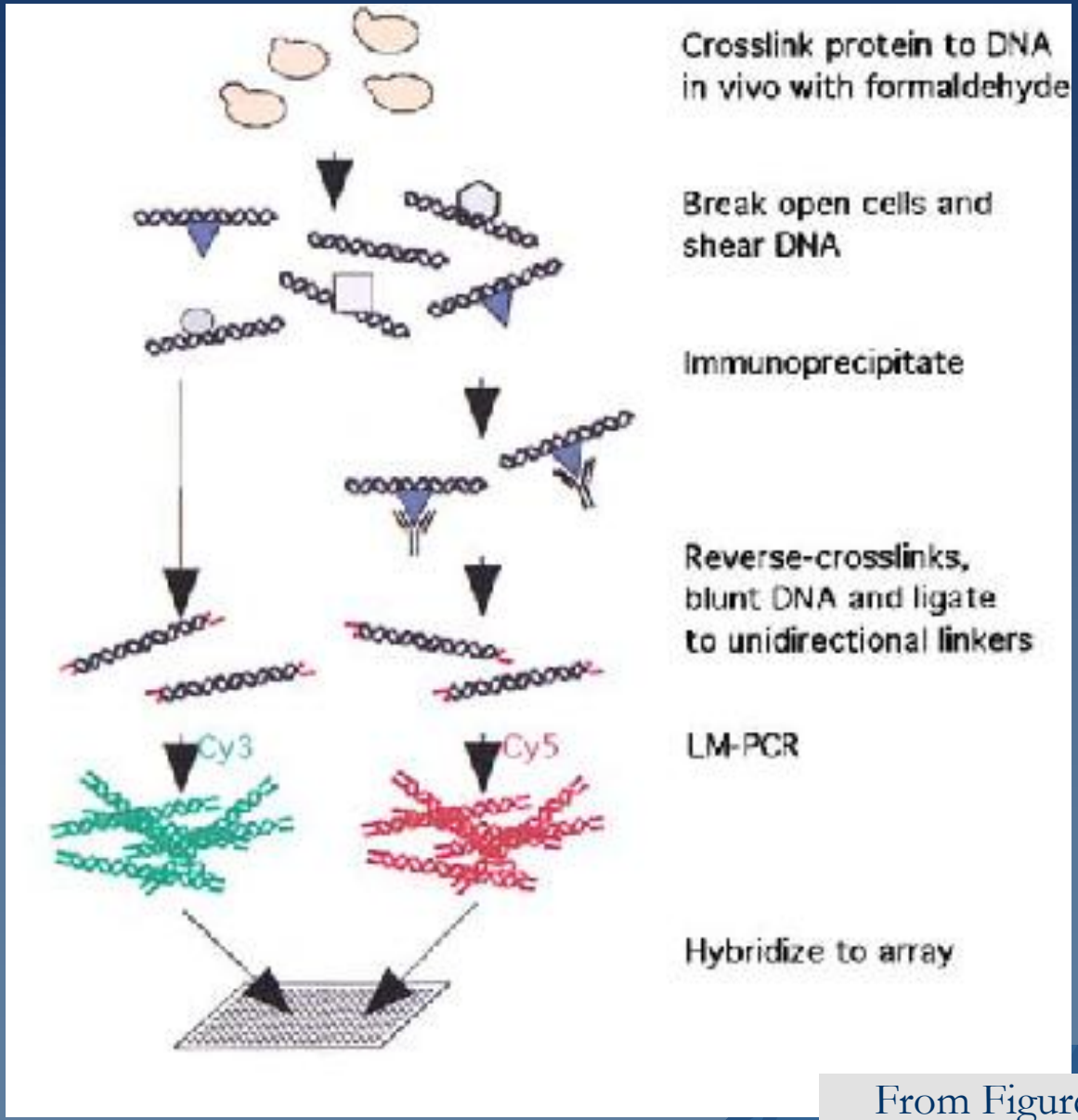
McBeath and Schreiber

Kinase-target interactions



Mike Snyder and colleagues

ChIP-chip measurement of protein→DNA interactions

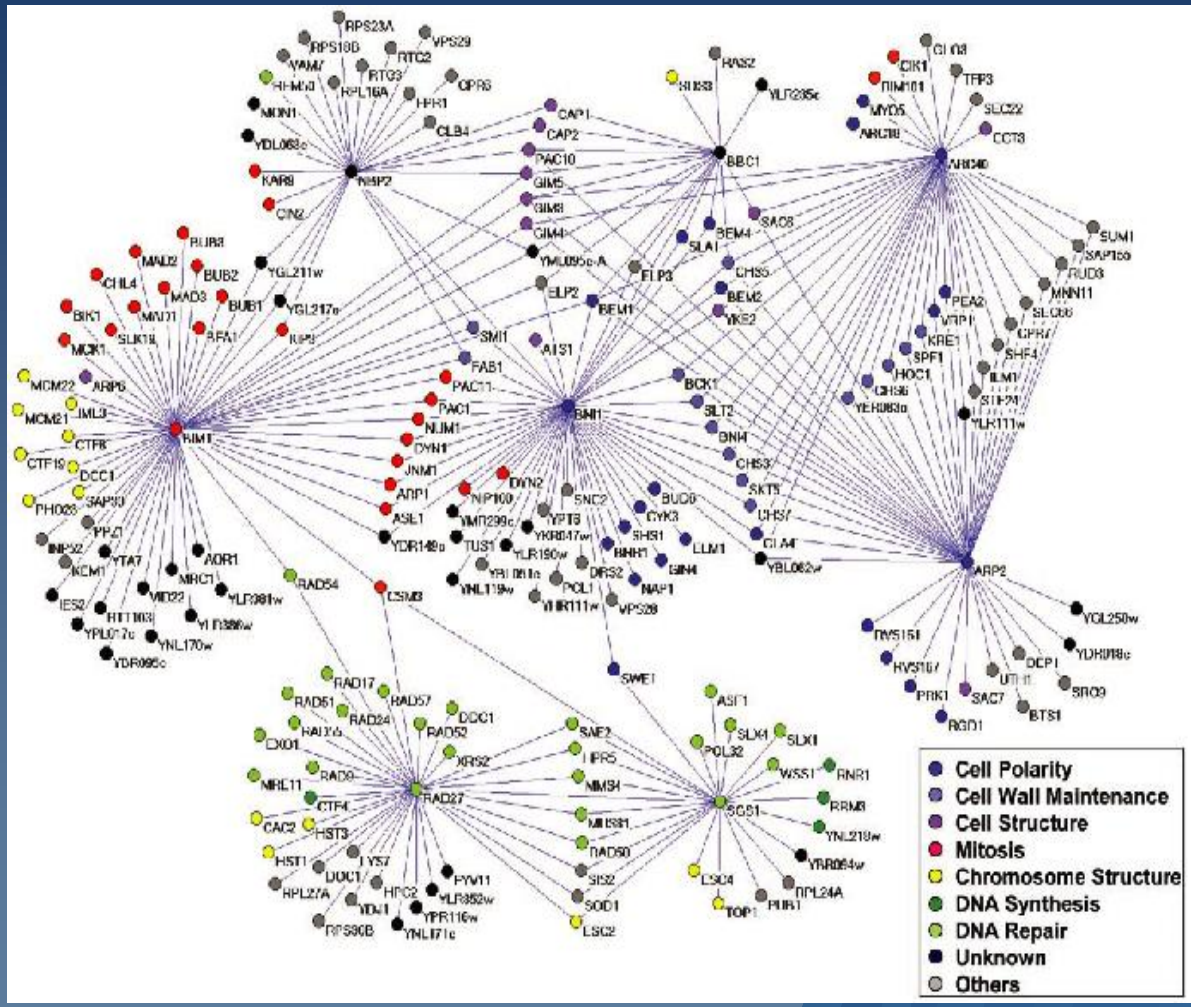


From Figure 1 of Simon et al. *Cell* 2001

Genetic interactions: synthetic lethals and suppressors

Genetic Interactions:

- Widespread method used by geneticists to discover pathways in yeast, fly, and worm
- Implications for drug targeting and drug development for human disease
- Thousands are now reported in literature and systematic studies
- As with other types, the number of known genetic interactions is *exponentially increasing…*



Adapted from Tong *et al.*, *Science* 2001

SUMMARY: High-throughput methods for measuring interactions

- Phage display
- 2-hybrid
- co-immunoprecipitation
- Protein arrays
- chIP-on-chip
- systematic genetic analysis