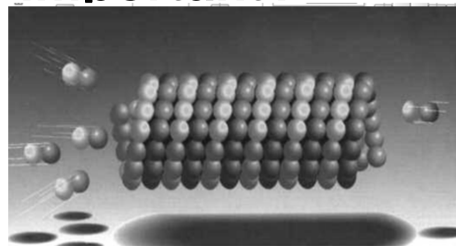
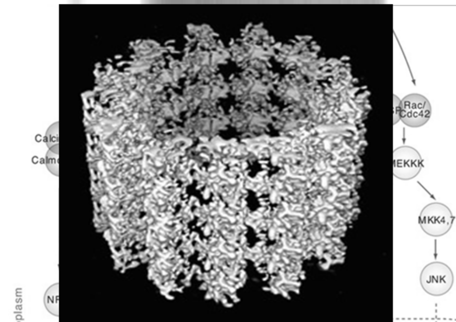
Protein-Protein interactions

Reducing the complexity

Why are protein-protein interactions important?

- Identify proteins in complexes.
- Identify proteins that are in a metabolic or signaling pathway.
- Identify members of a non-enzymatic structures.





Protein complexes

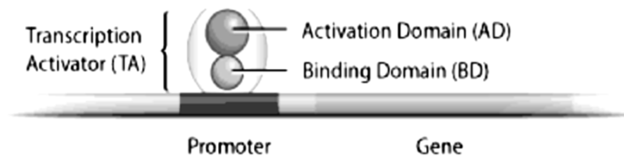
- Protein complexes can be made up of 2-50 identical or different proteins (subunits).
- Some proteins in complexes may not have any apparent activity.
- One protein subunit could have two activities, one not related to the complex function.

Methods of studying protein-protein interactions.

- Yeast two hybrid
- Pull down or co-purification
  - (co-immunoprecipitation)
- Fluorescence Resonance Energy Transfer (FRET)

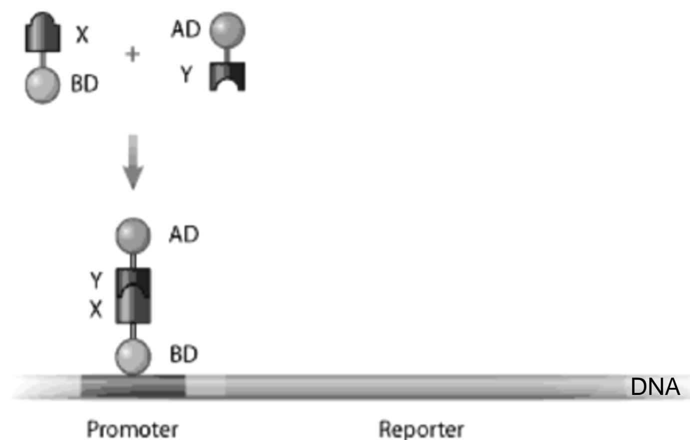
Yeast Two Hybrid Analysis

- Need a way to identify if two proteins are near each other.
- Many transcription promoters are composed of two domains which can be separated.
  - the binding domain
  - the activator domain



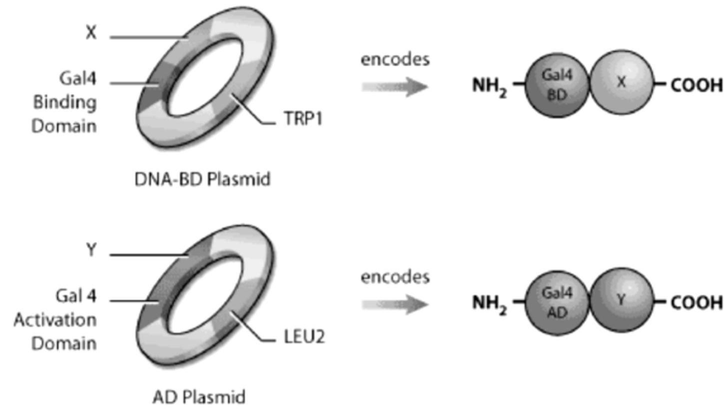
•http://www.scq.ubc.ca/the-yeast-two-hybrid-assay-an-exercise-in-experimental-eloquence/

How Does it Work ?

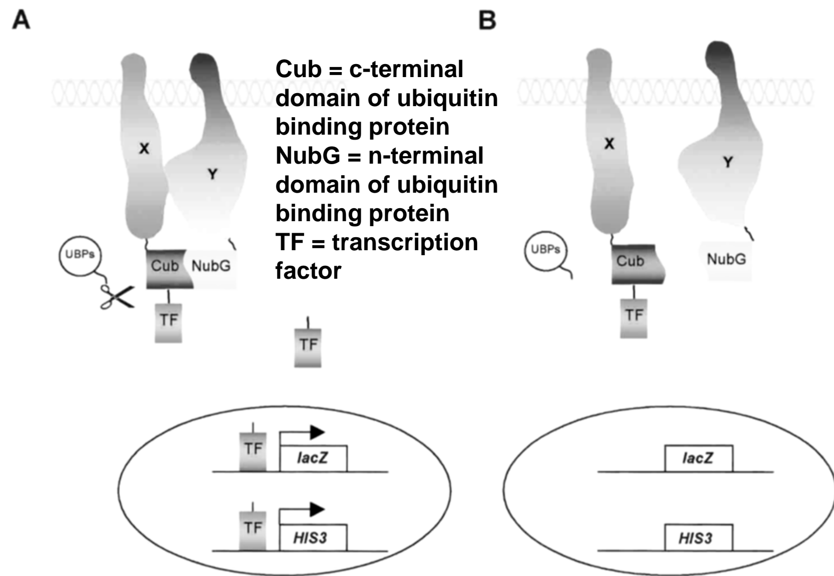


How Does it Work?

The process is usually performed with a single bait and it is probed with a genomic library of all the other proteins



Cytosolic and Membrane Proteins



Cub = c-terminal domain of ubiquitin binding protein
NubG = n-terminal domain of ubiquitin binding protein
TF = transcription factor

***Genome Res.* 2003 13: 1744-1753**

Genomic scale screening

- Use yeast mating types and put bait in one mating type and prey in the other.
- Mixing methods
  - Mix one prey or bait with the whole genome with the other plasmid.
  - Mix batches of genomic bait and prey molecules.
- Sequence plasmids from surviving colonies.

Problems

- Many false positive and negatives.
- Fusion structure may affect protein interactions.
- Proteins may not express well in yeast.
- Transient interactions may be missed.
- Rate of synthesis and degradation might be rapid.
- Difficulty with multiple subunit proteins.

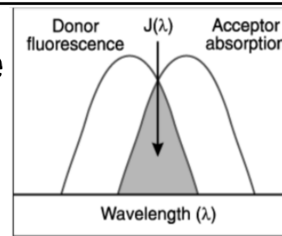
Co-purification/ Immuno-trapping

- Based on the principle that interacting proteins will stay together during purification.
- Tag protein with antibody or affinity tag
- Bind tag to column or plate.
- Remove bound proteins and separate.
- Sequence by LC/MS/MS.

Affinity tags/columns

- glutathione S-transferase
  - Binds to glutathione coupled column
  - Removed with glutathione
- hexyl histidine
  - Binds to Ni+ chelating column
  - Removed by addition of imidazole
- calmodulin binding protein
  - Binds to calmodulin coupled column
- protein A
  - Binds to antibody column
  - Removed by denaturation
- maltose binding protein
  - Binds to Maltose coupled column
  - Removed with maltose
- FLAG
  - Binds to FLAG antibody column
  - Removed by denaturation
- Strep II tag
  - Binds to Avidin coupled column
  - Can be removed with desthiobiotin

Fluorescence Resonance Energy Transfer



- **When a fluorescent molecule is excited it will emit light as fluorescence.**
- **If a second fluorescent molecule is present that absorbs in the wavelength range of the emission of the first fluorescent molecule it can absorb the light and emit the energy at a second wavelength.**
- **Alternatively a second molecule can absorb the fluorescence and not emit the energy as light. This is called quenching.**

FRET

- **Processes is highly distance dependent, drops off by r^{-6}.**
  - **Efficiency of transfer = $1 - \phi_{D+A}/\phi_D$**
  - **Efficiency of transfer = $r_0^6 / (r_0^6 + r^6)$**
  - **Quantum Yield = ϕ_D**
  - **r_0 represents the distance for 50% efficiency**
- **The bait protein(s) is (are) tagged with one fluorescent molecule.**
- **The prey protein(s) is (are) tagged with a complementary fluorescent molecule or quencher.**
- **Look for change in fluorescence wavelength or quenching. Should occur only when two proteins are very close together (connected).**

Donor Acceptor Pairs Used in FRET

Donor	Acceptor	Ro (Å)
• Fluorescein	Tetramethylrhodamine	55
• IAEDANS	Fluorescein	46
• EDANS	DABCYL	33
• Fluorescein	QSY-7 dye	61
• BFP	GFP	35
• CFP	YFP	50

Ro is the distance at which the efficiency of energy transfer is 50%