

Yeast 2 Hybrid Screens & Genome-wide study of PDZ interactions

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What is the Yeast Two Hybrid (Y2H) system?

A way to find protein-protein interactions

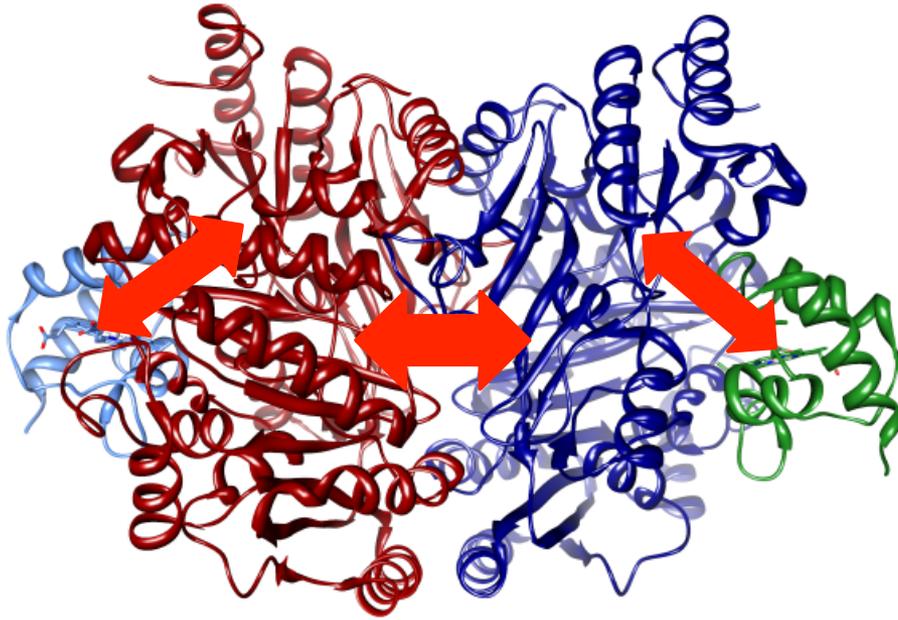
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A way to find protein-protein interactions

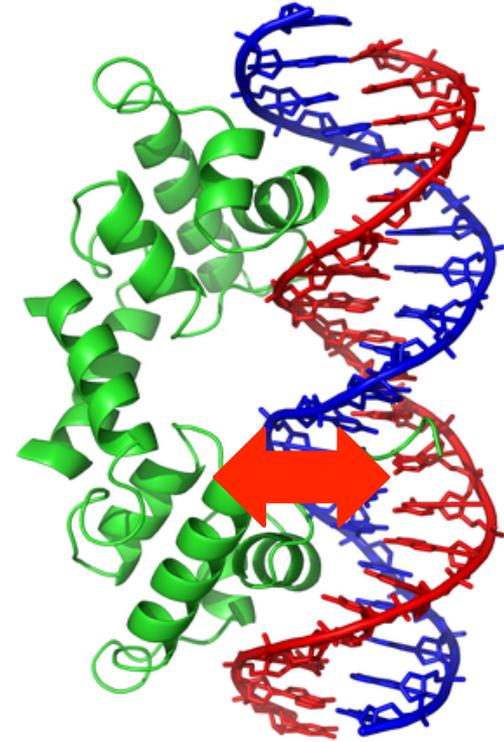


Tells you how your protein might function in the cell

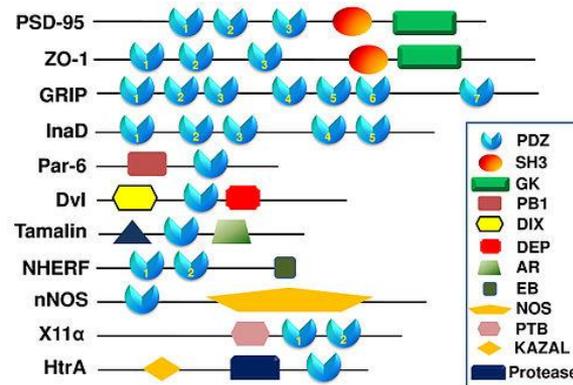
Why is Y2H important?



Protein - Protein interactions

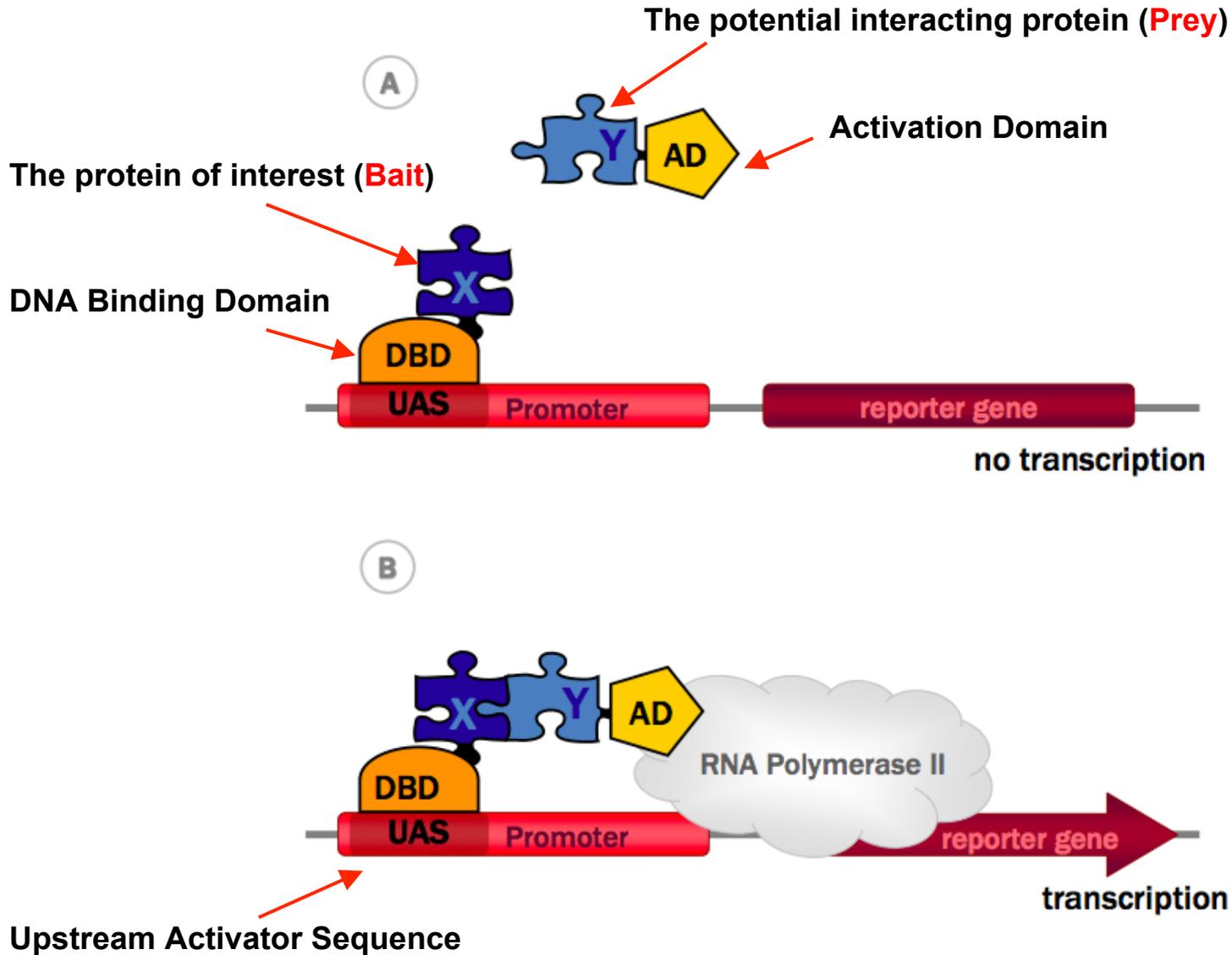


Protein - DNA interactions



Characterization of protein domains

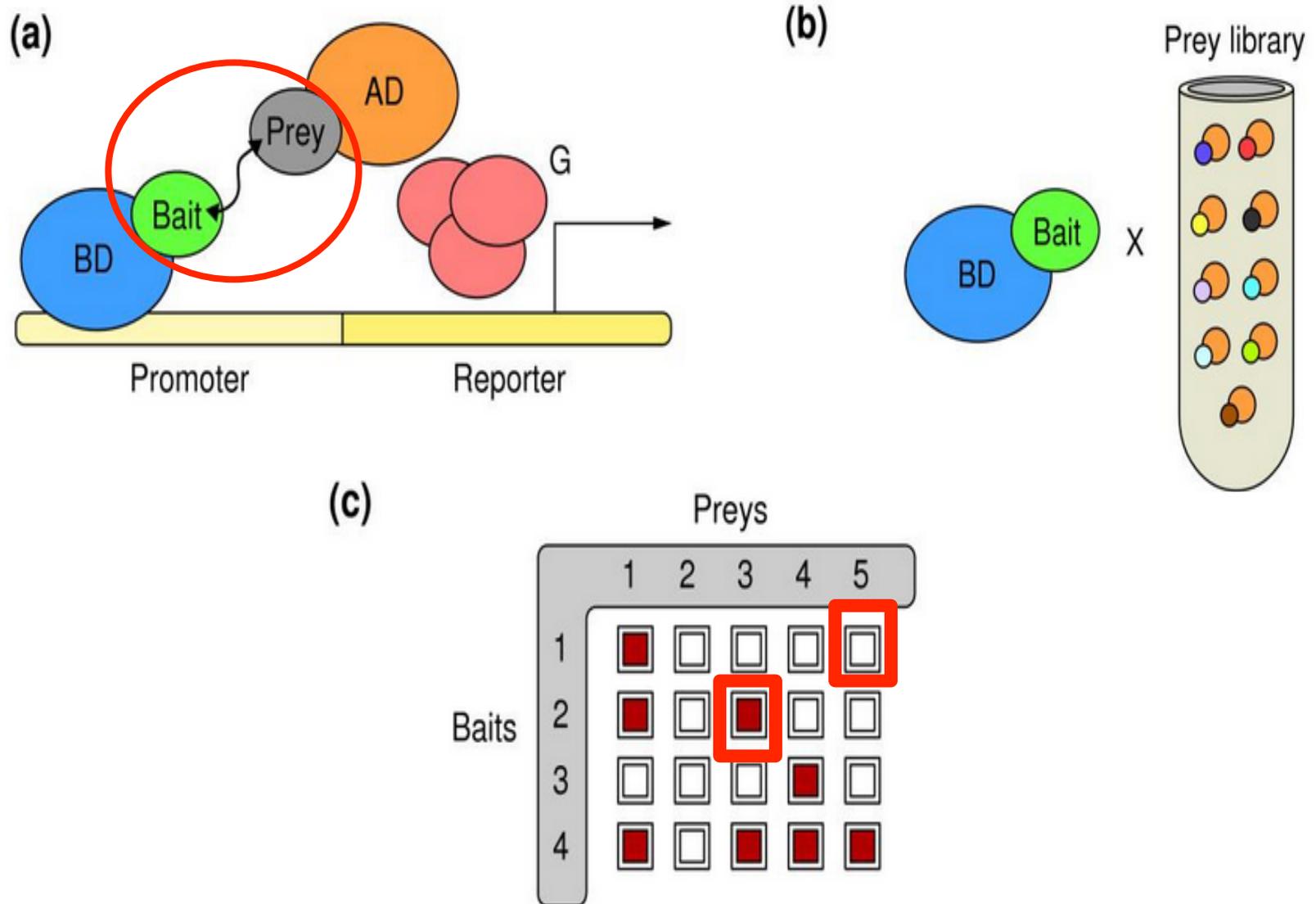
How does Y2H work?



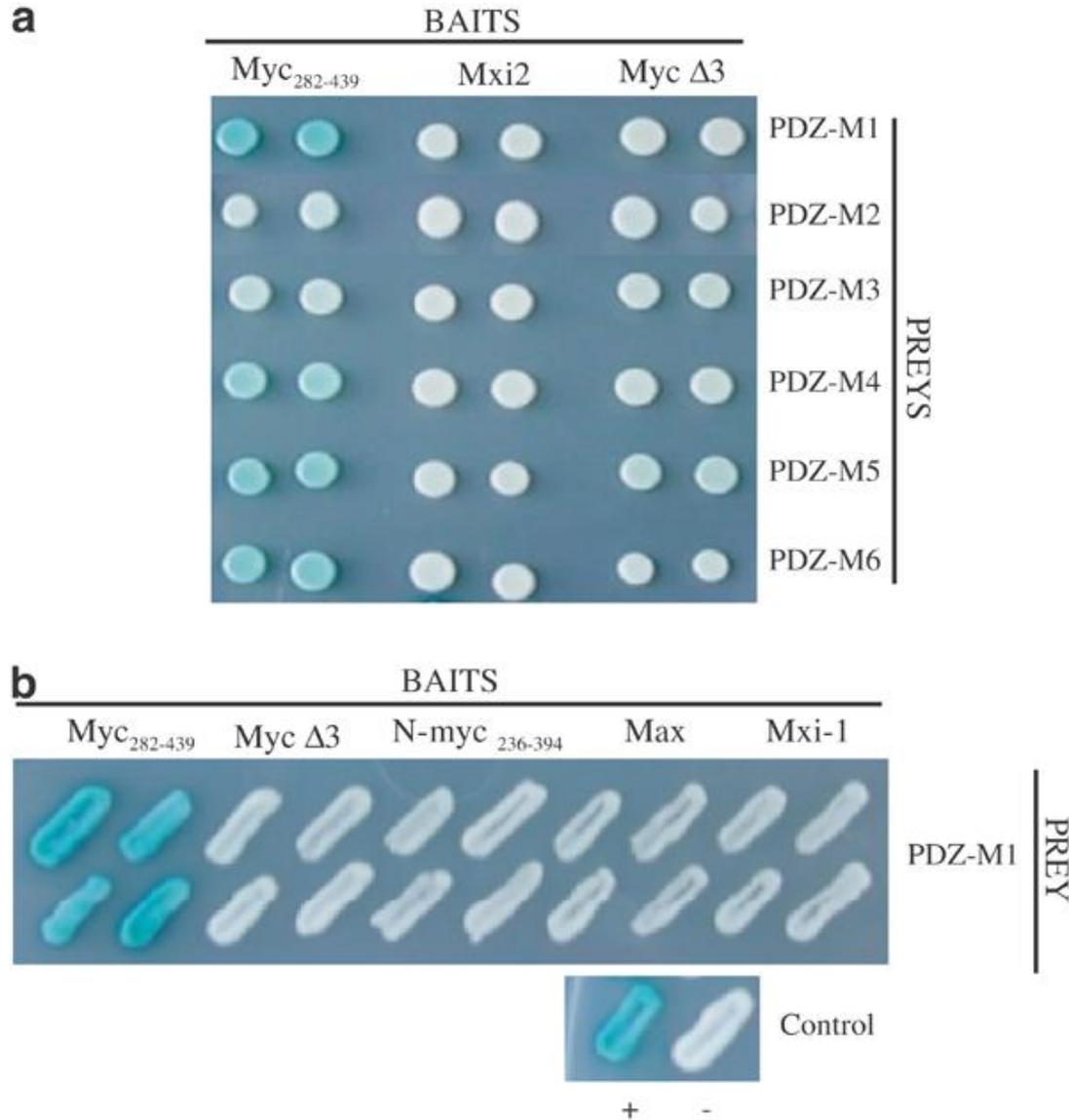
How does Y2H work?

<https://www.youtube.com/watch?v=bW6wFXKc-mw>

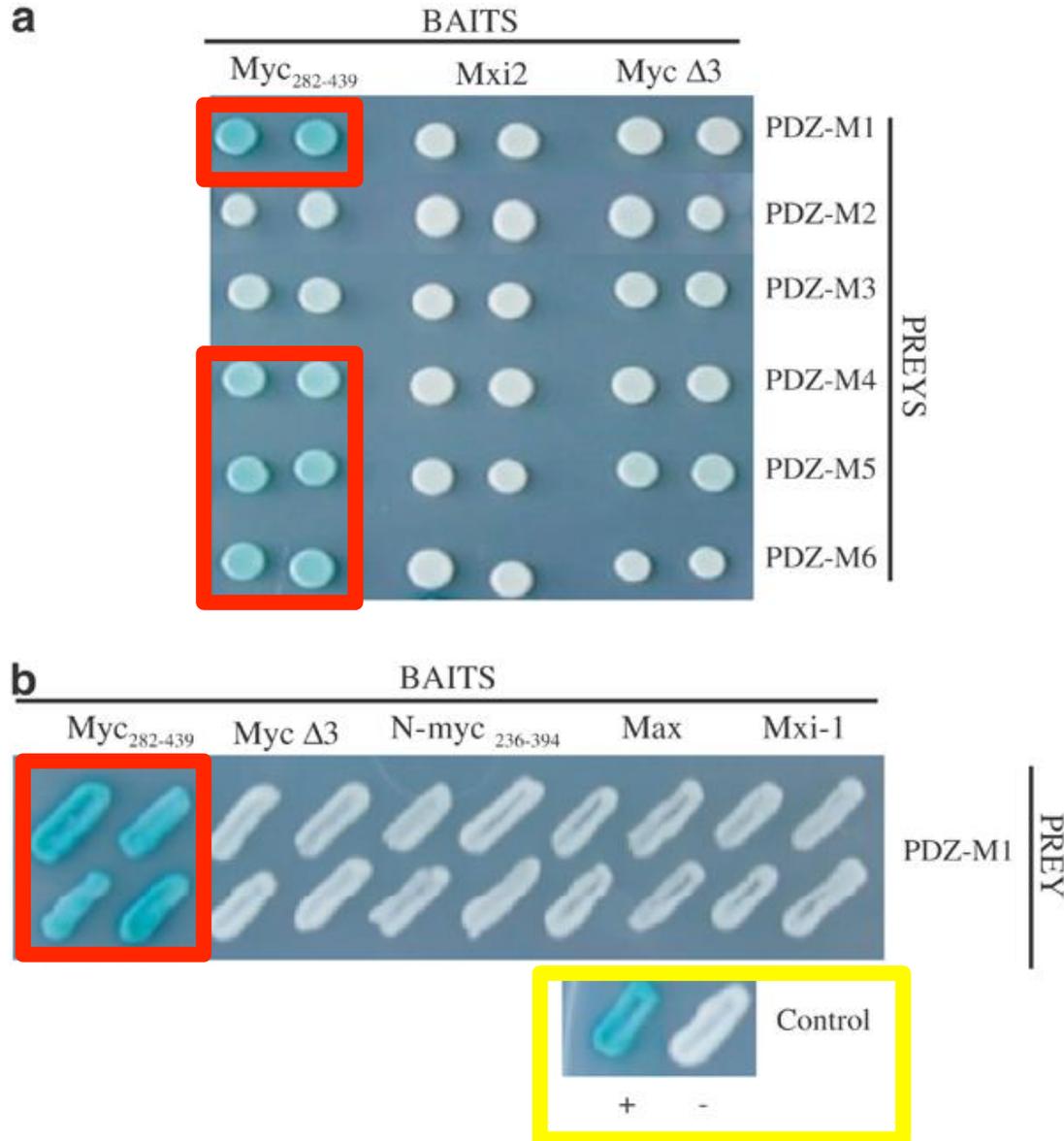
How does a Y2H screen work?



What does the Y2H screen look like?

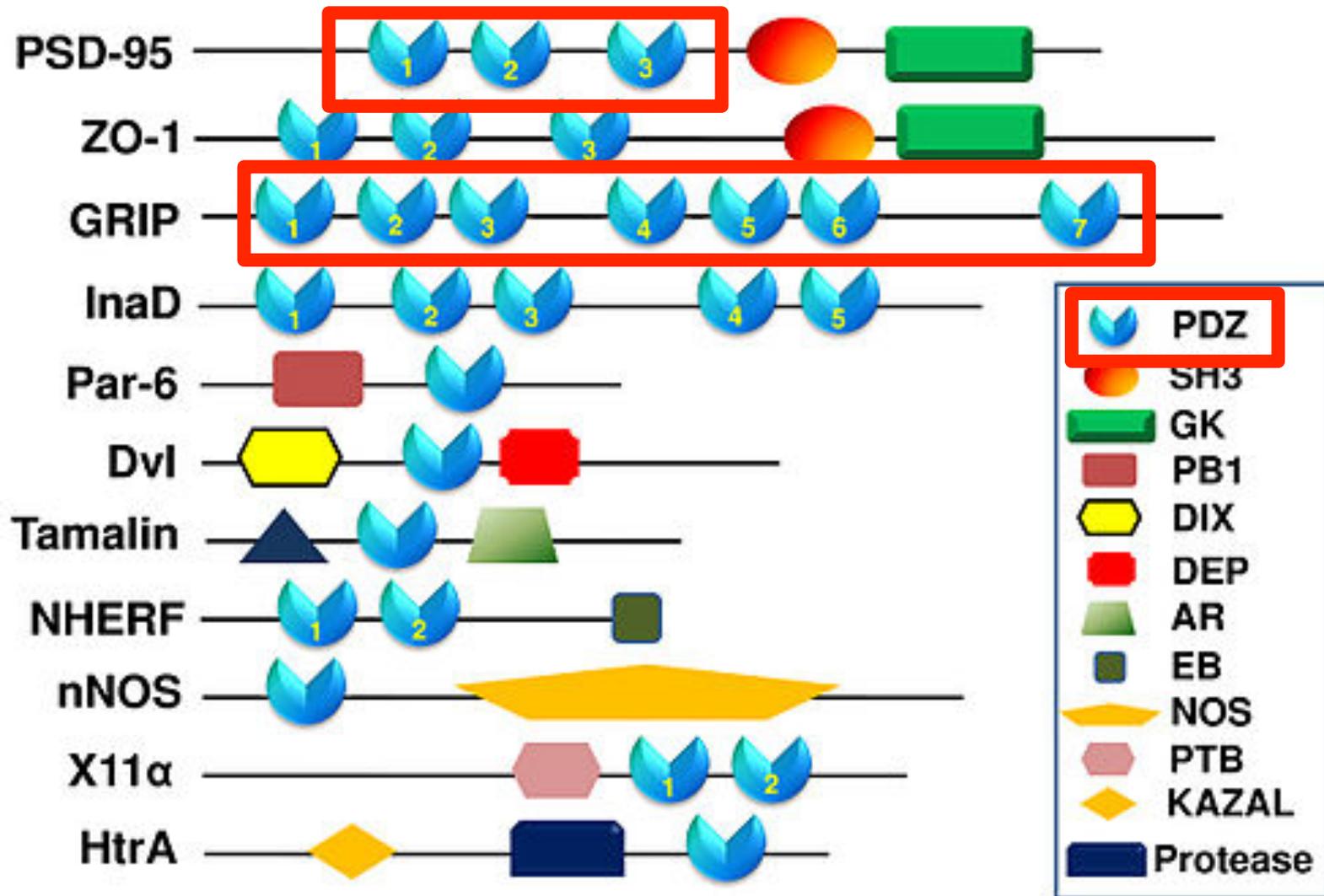


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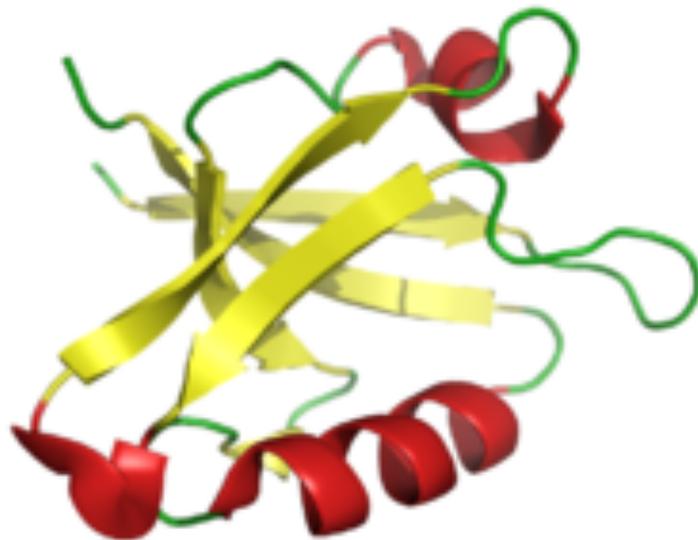
What are protein domains?

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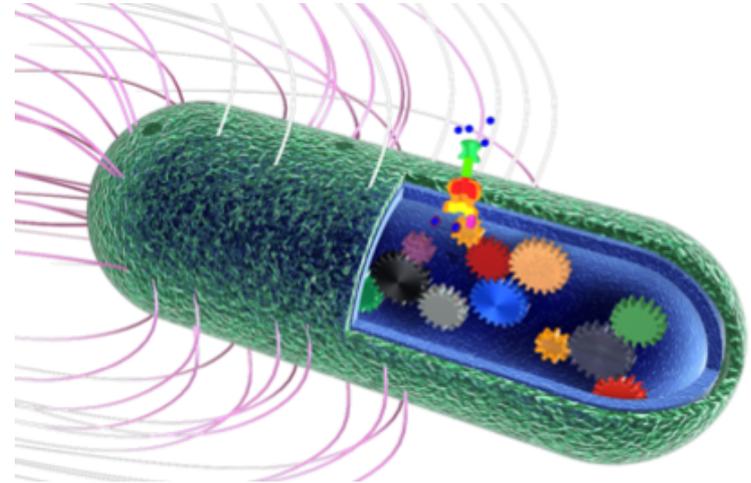
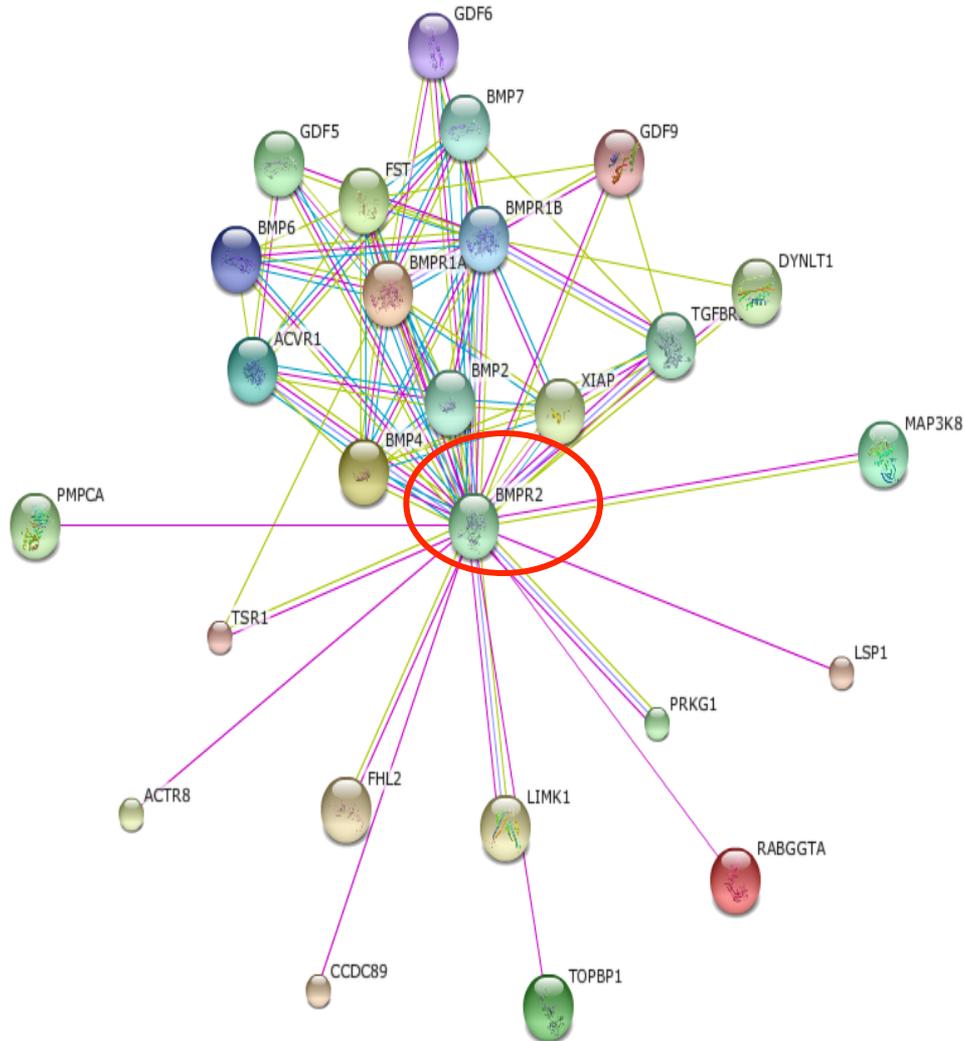


Why is domain characterization important?

- Structural and functional protein information



What information can we determine using Y2H?



What are the pros & cons of the Y2H system?



Pros

- Inexpensive
- Large output
- Fast results



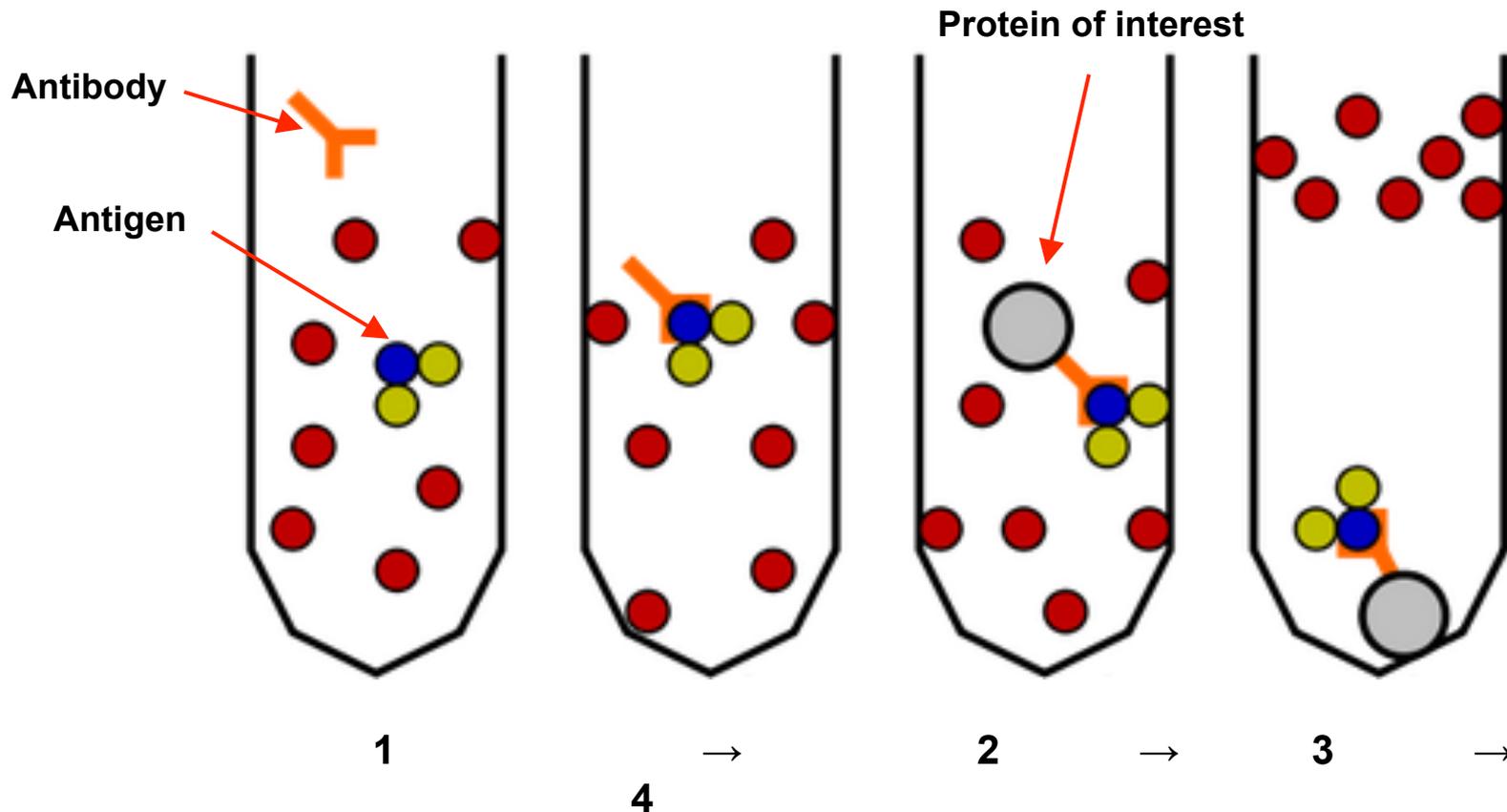
Cons

- No indicated Interaction mechanism
- Time consuming
- High error rates

How do we verify the Y2H results?

Co-IP (Protein complex immunoprecipitation)

- Antigen - Antibody - Protein of Interest
- Analyzing or confirming the interactions



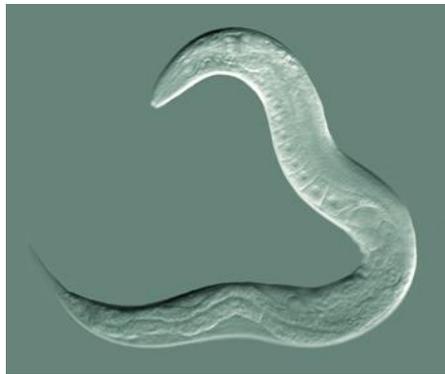
Questions?



**A genome-wide study of PDZ-
domain interactions in *C. elegans*
reveals a high frequency of non-
canonical binding**

**Nicolas Lenfant, Jolanta Polanowska, Sophie Bamps,
Shizue Omi, Jean-Paul Borg, Jerome Reboul**

What is the purpose of this study?



http://en.wikipedia.org/wiki/Caenorhabditis_elegans

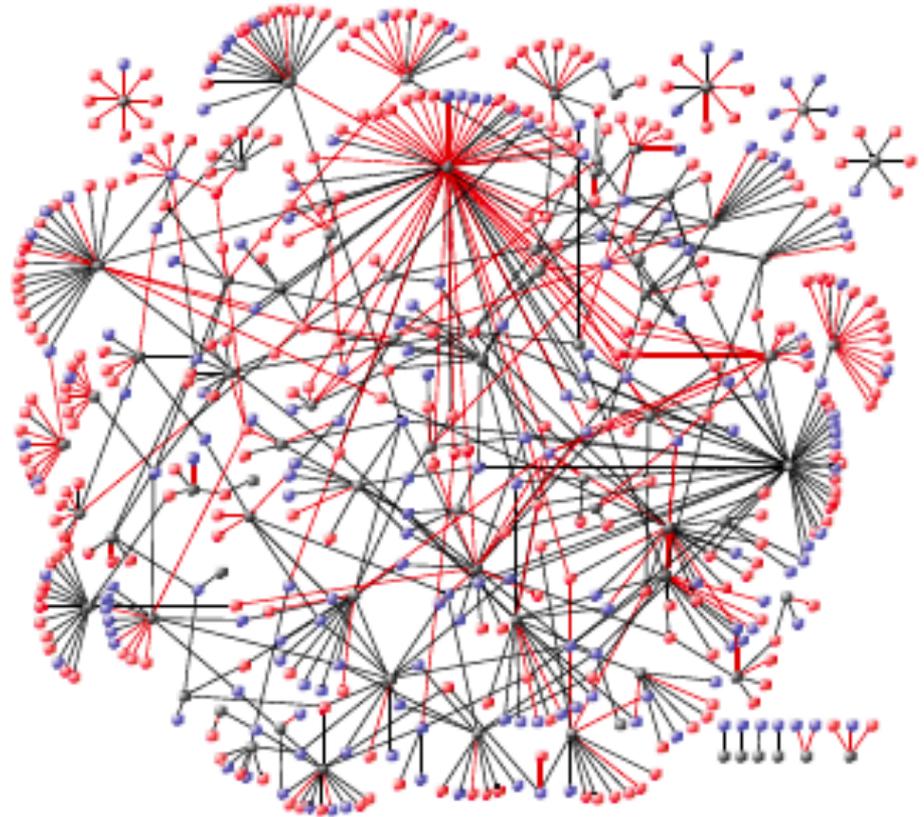
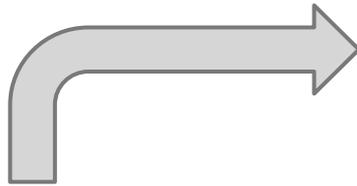
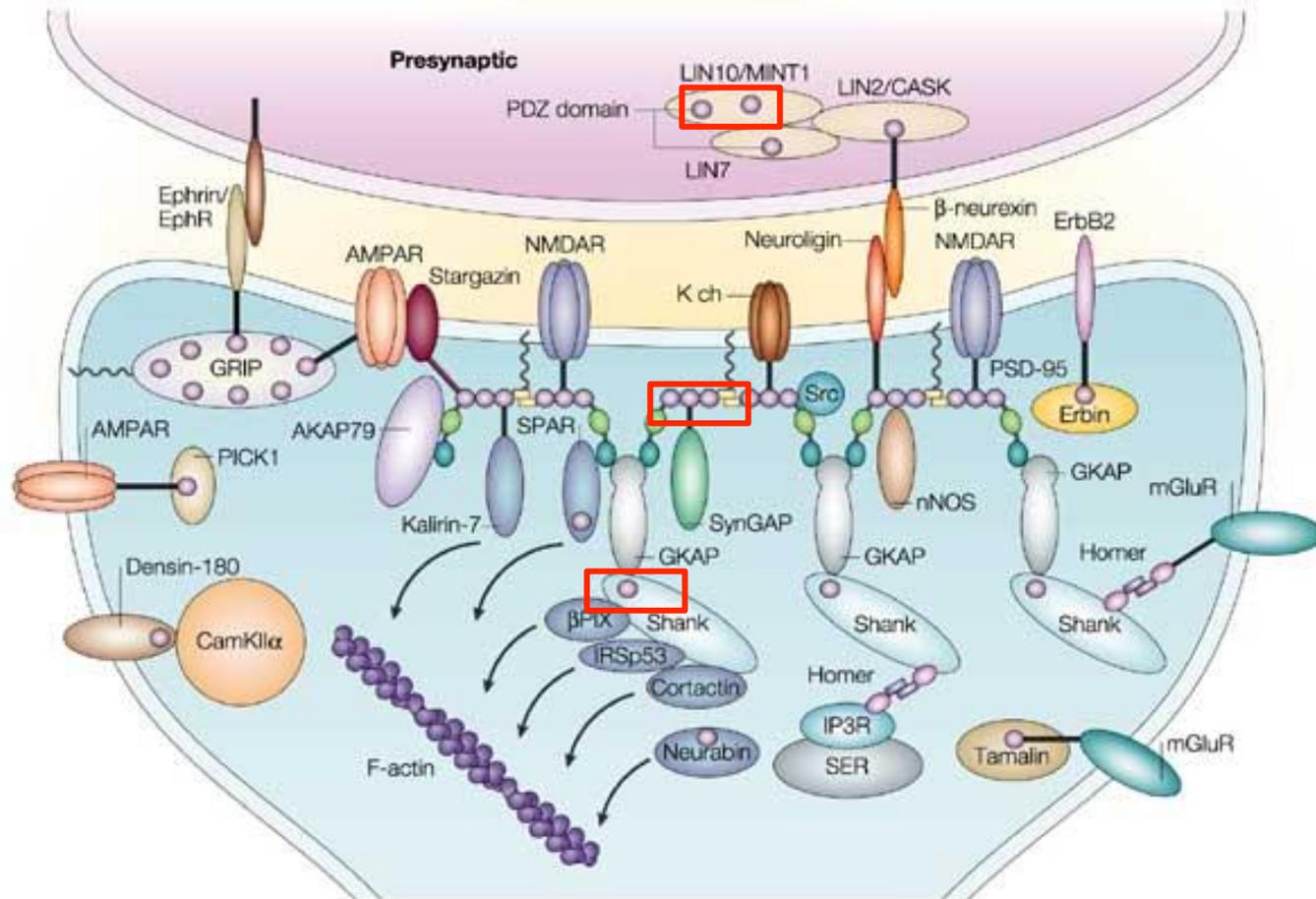


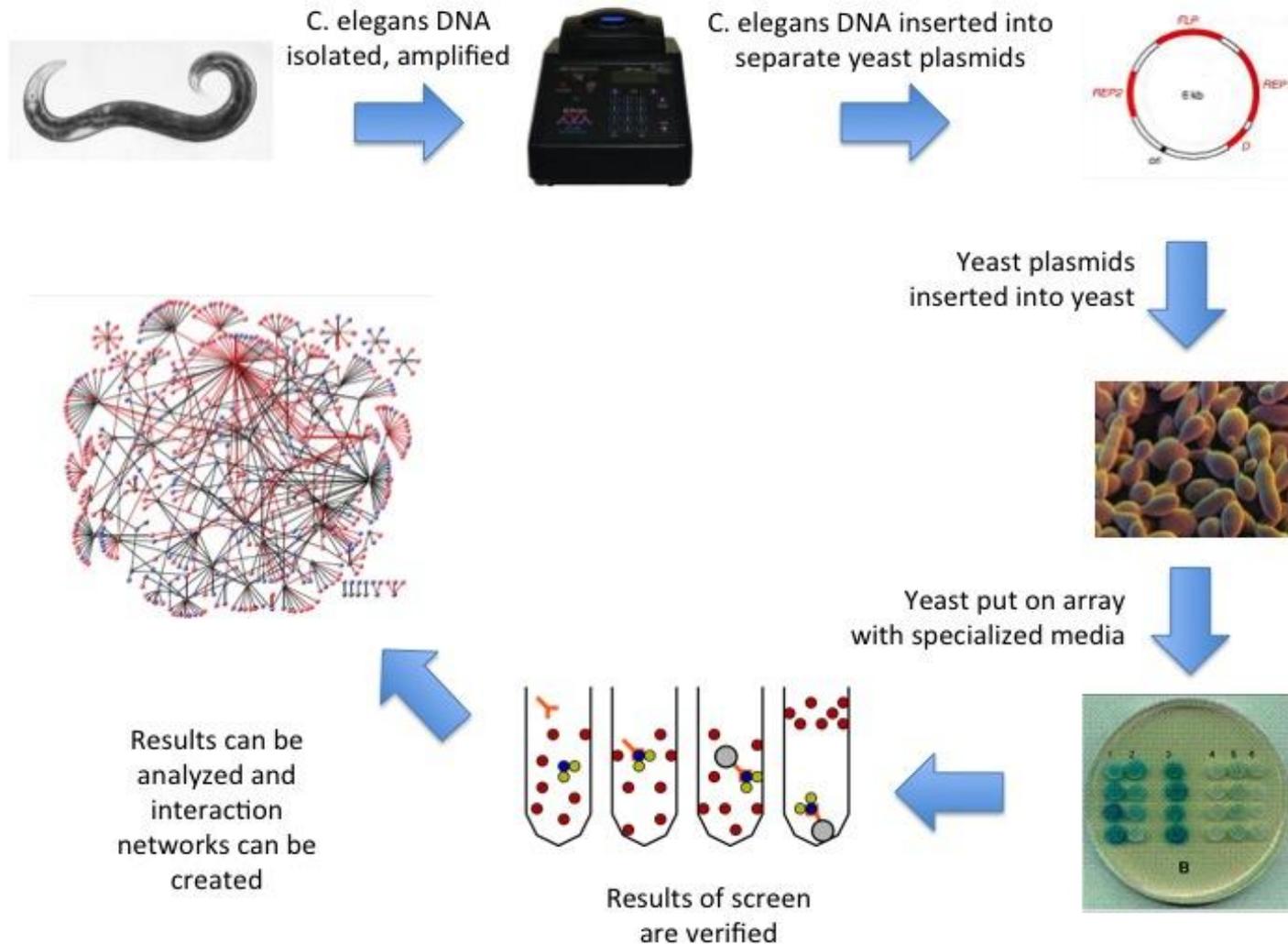
Figure 1. "A genome-wide study of PDZ-domain interactions in *C. elegans* reveals a high frequency of non-canonical binding." Lenfant et al.

Genome-wide study for interactions with PDZ-domain-containing proteins using Y2H screens

What is the PDZ domain?



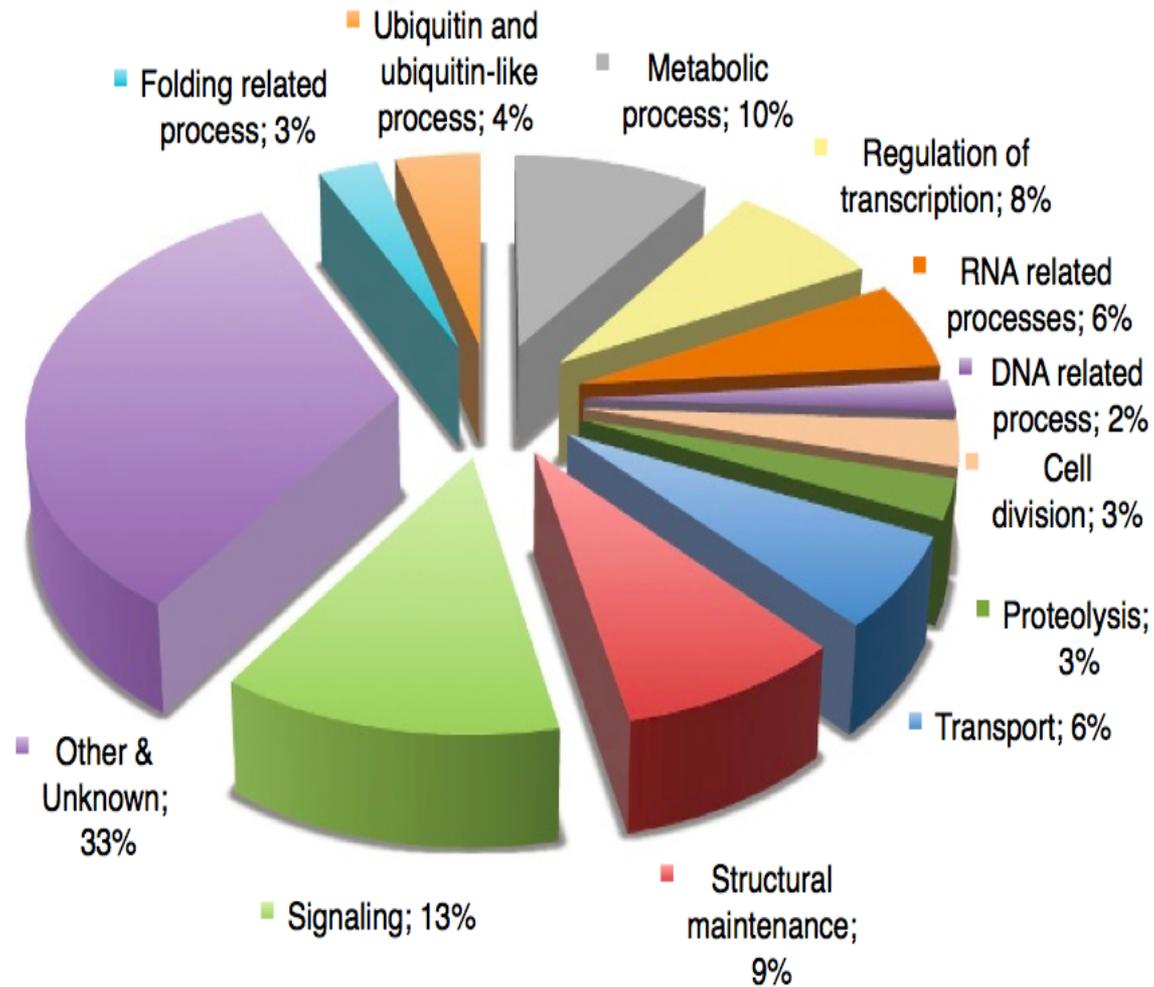
How did they perform this Y2H screen in *C. elegans*?



Images, in order: 1. <http://fangyenlab.seas.upenn.edu> 2. <http://www.medwow.com/med/pcr/mj-research/ptc-200/41156.model-spec> 3. http://mol-biol4masters.masters.grkraj.org/html/Genetic_Engineering2A-Molecular_Tools-General_Vectors.htm

4. <http://www.kurzweilai.net/how-to-synthesize-a-new-kind-of-yeast-cell-or-person> 5. <http://www.nature.com/protocolexchange/protocols/314> 6. <http://www.piercenet.com/method/co-immunoprecipitation-co-ip> 7. Figure 1. "A genome-wide study of PDZ-domain interactions in *C. elegans* reveals a high frequency of non-canonical binding." Lenfant et al.

Figure 2: How did they sort their Y2H data?



Using Gene Ontology: Biological Process

How could their data presentation be improved?

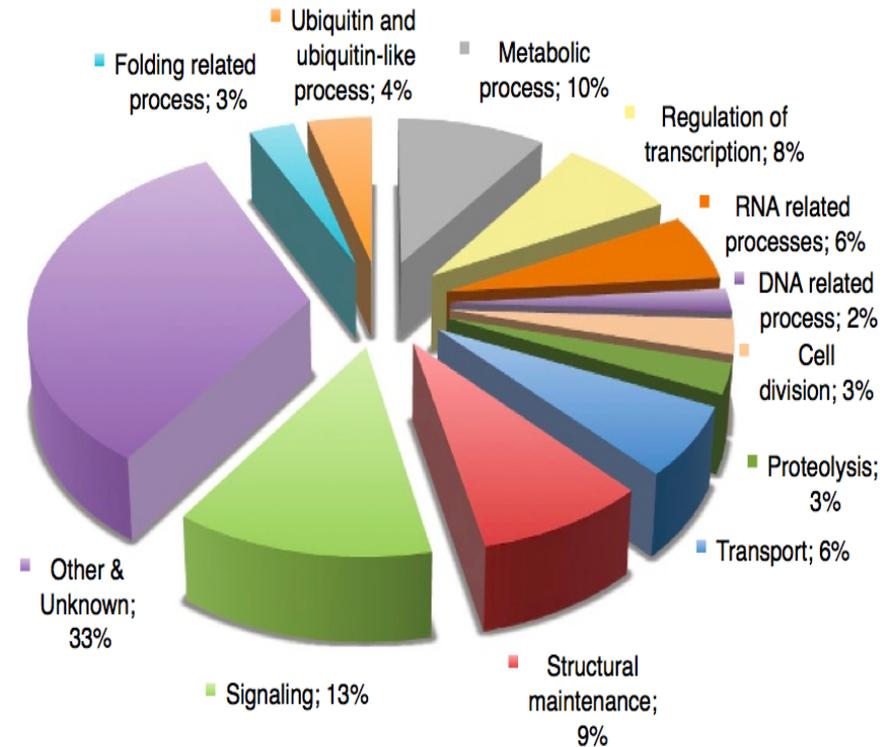
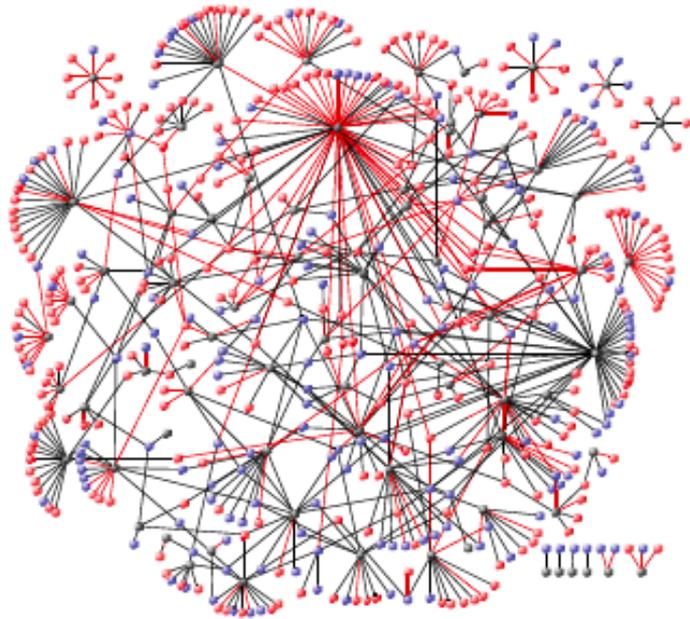
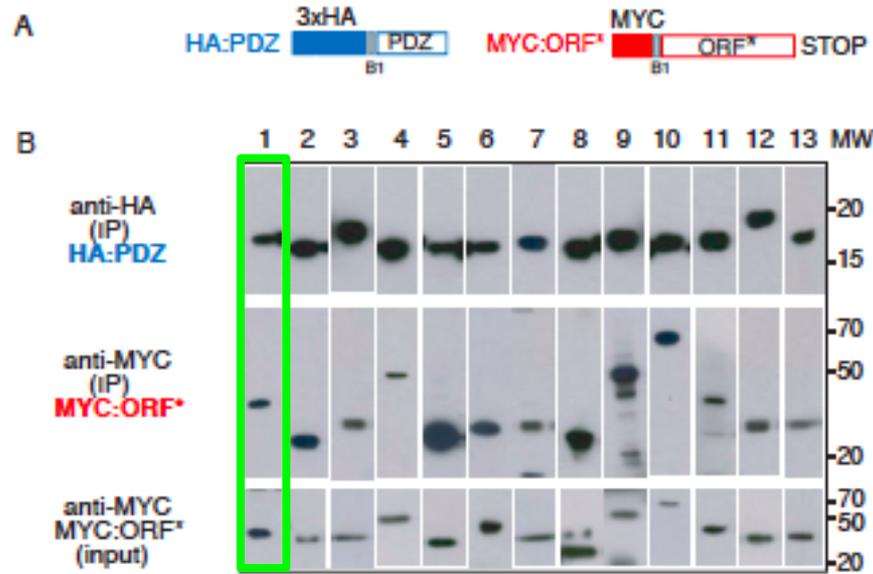


Figure 3: How did they verify their Y2H protein-protein interactions?

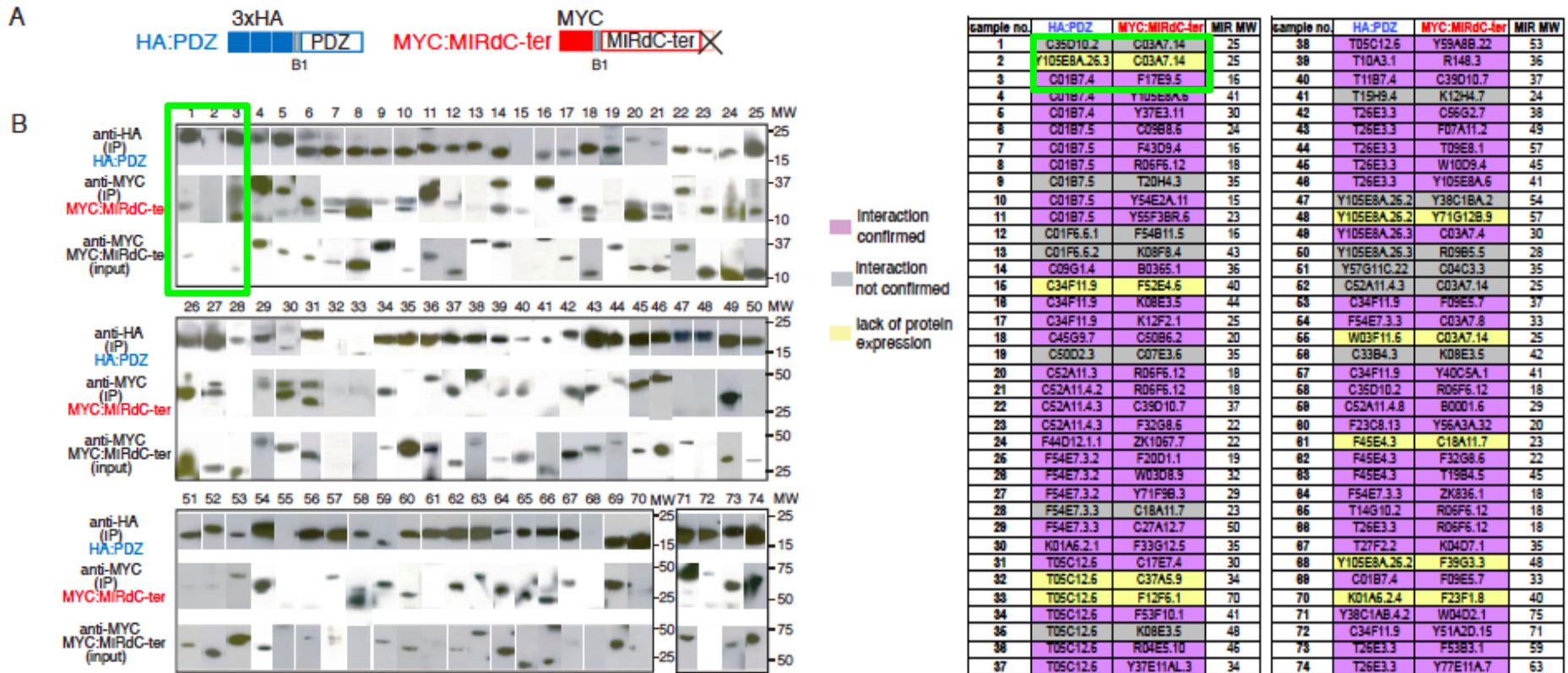


sample no.	HA-PDZ	MYC-ORF*	ORF MW
1	C01B7.4	Y105E8A.6	41
2	C01B7.5	Y55F3BR.6	20
3	C09H6.2.2	Y48E1B.12	28
4	C25F6.2.3	C34C6.6	55
5	C34F11.9	Y40C5A.1	29
6	K01A6.2.2	F54D10.7	30
7	K01A6.2.3	Y48E1B.12	28
8	T05C12.6	F53F10.1	28
9	T28E3.3	F53B3.1	60
10	T28E3.3	Y77E11A.7	69
11	T28E3.3	Y105E8A.6	41
12	W03F11.6	Y48E1B.12	28
13	Y54G11A.10	Y48E1B.12	28

Interaction not confirmed
 Interaction confirmed
 lack of protein expression

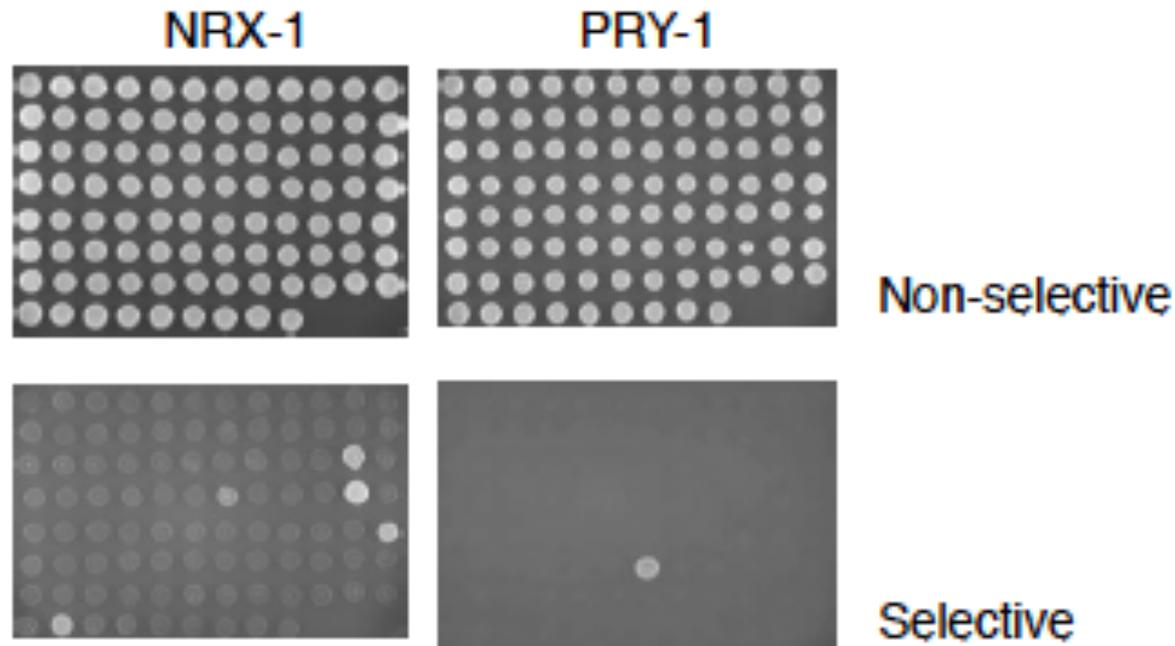
Using co-IP

Figure 4: How did they verify protein-protein interactions at non-C-terminus sites?



51% of interacting proteins did not possess a C-terminal consensus motif

Figure 5: How did they array all of the *C. elegans* PDZ-domain-containing proteins for this study?



674 total PDZ domain interactions

469 interacting proteins

78 active PDZ domains out of 93 (6 auto-activators)

Conclusions

High degree of promiscuity between PDZ domains and their protein binding partners

Fraction of binding that takes place at C-terminus is smaller than originally thought

Questions?



http://www.clipartof.com/gallery/clipart/c_elegans.html

Thank you!