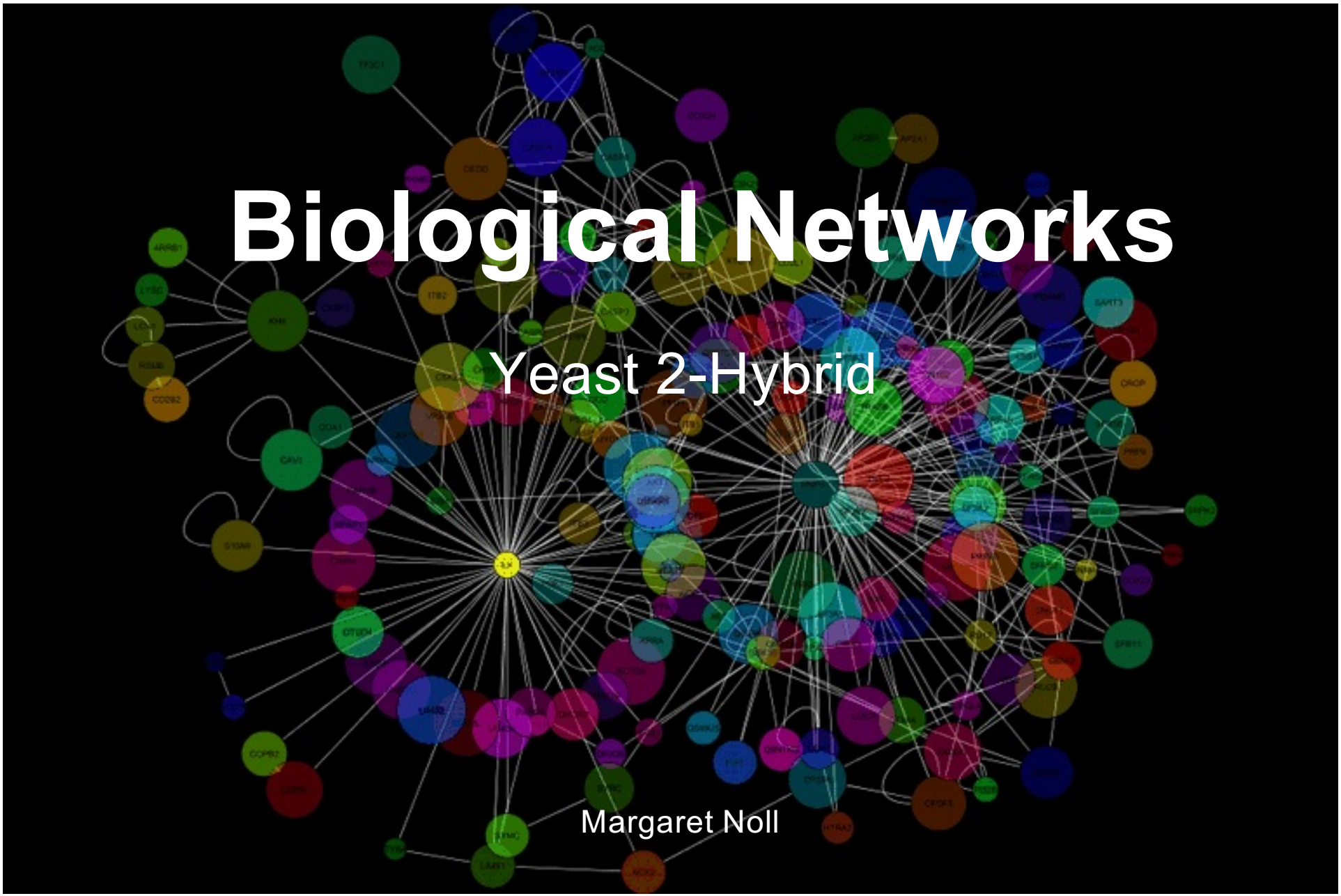


Biological Networks

Yeast 2-Hybrid

Margaret Noll

http://fiehnlab.ucdavis.edu/staff/kumar/metabolic_networks/networks_tools

Why build networks?

- Biological functions regulated by protein interactions
- Can serve in understanding molecular mechanisms
- Provide framework onto which to build more complex regulatory information

Three identification methods:

Yeast Two-Hybrid

Affinity Purification

Protein Chips

Yeast Two-Hybrid

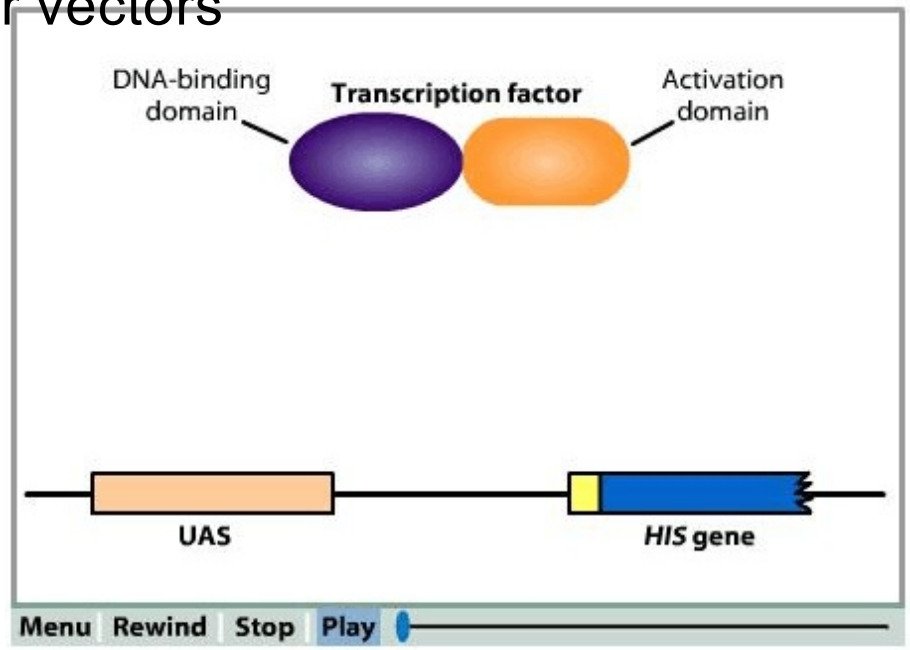
- Domain structure of transcription factors
- DNA Binding Domain (DMD)
- Activation Domain (AD)

http://eng.b-bridge.com/index.php?main_page=services

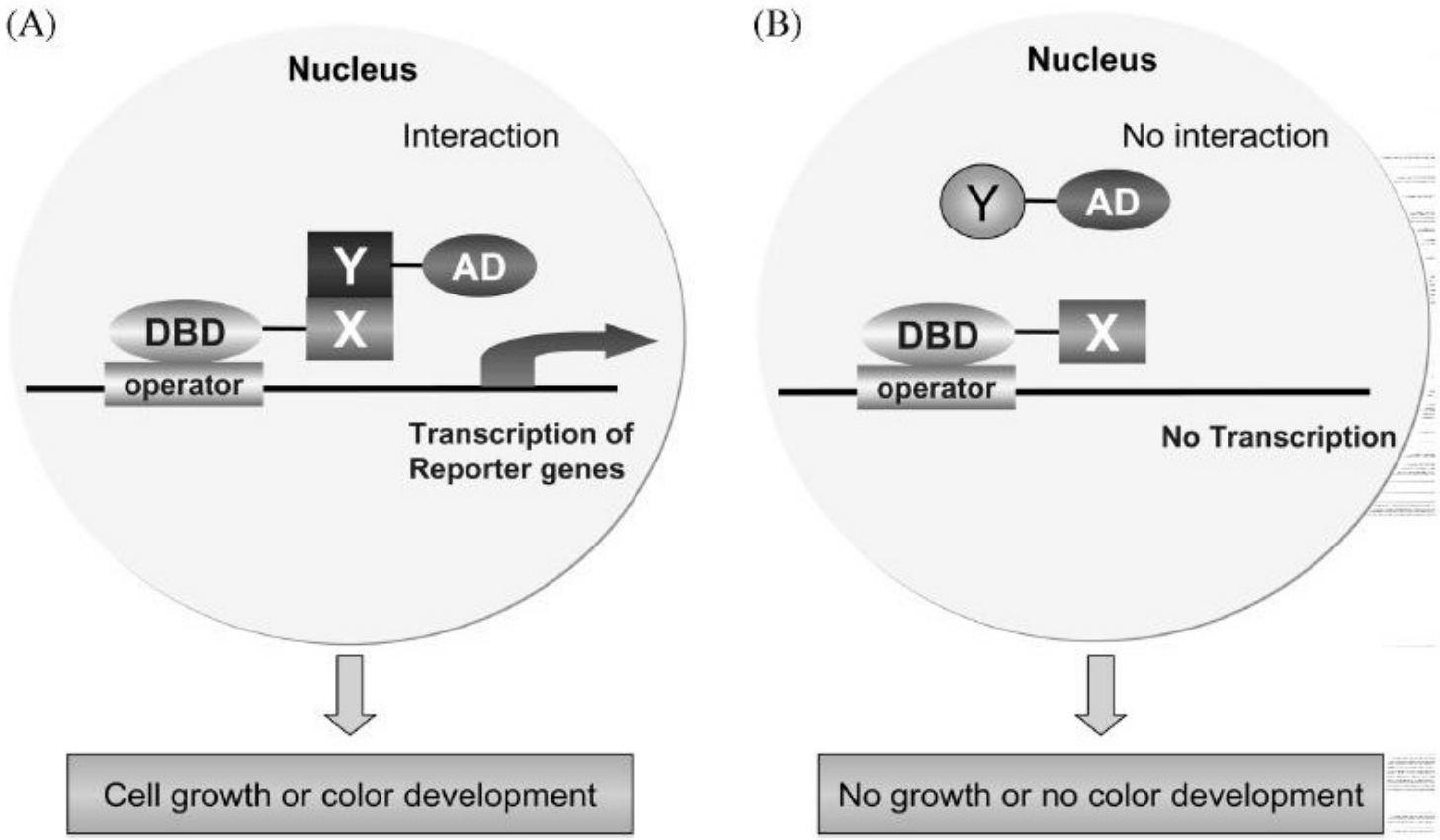
Yeast Two-Hybrid

Three Components:

- Bait vector
- Prey vector
- Reporter vectors



http://www.sumanasinc.com/webcontent/animations/content/yeasttwohybrid.html

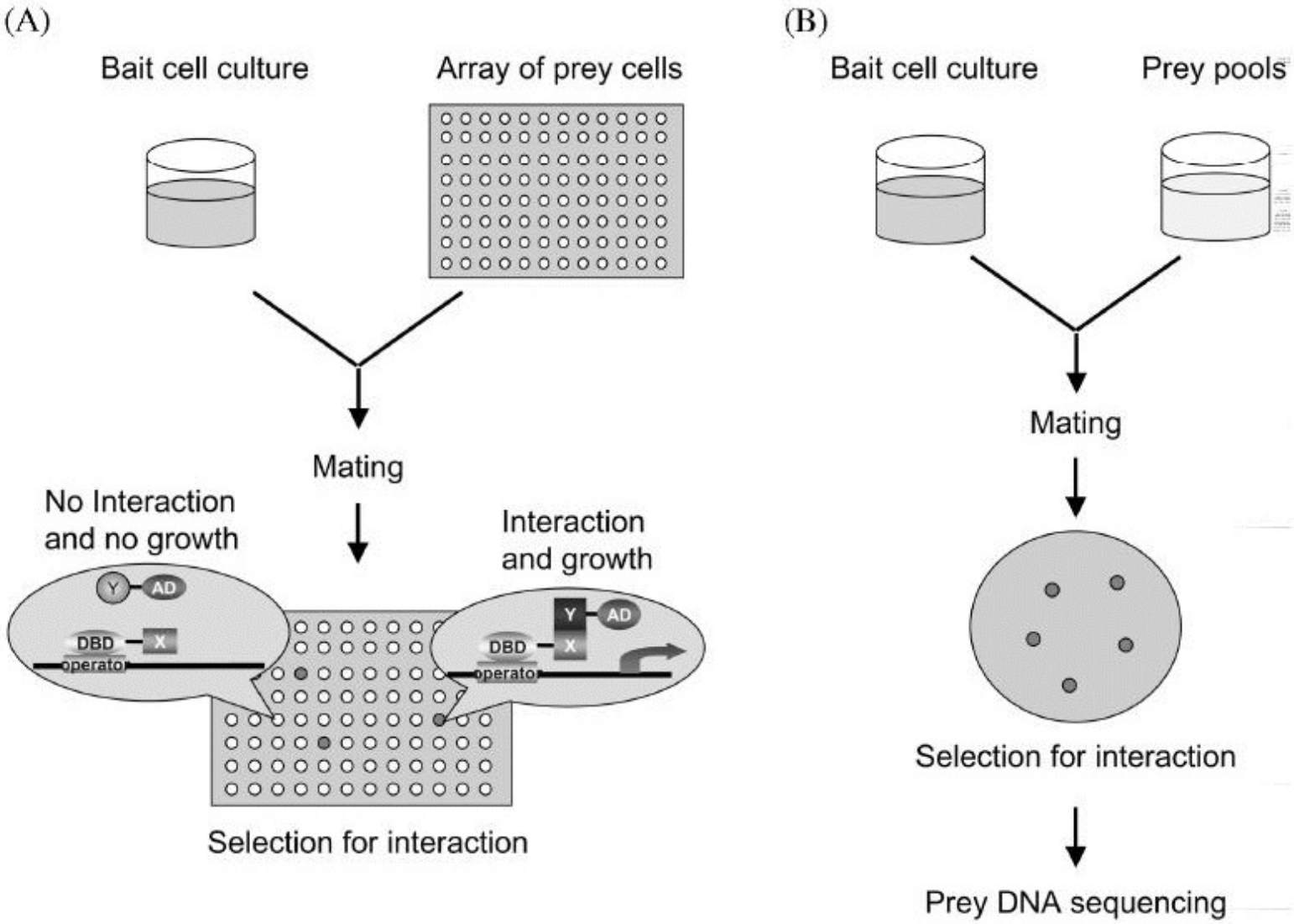

(A)
Nucleus
Interaction
Y
AD
DBD
X
operator
Transcription of
Reporter genes
Cell growth or color development
(B)
Nucleus
No interaction
Y
AD
DBD
X
operator
No Transcription
No growth or no color development

Cho *et al.*

Table 1. Comparison of high-throughput protein interaction analysis methods

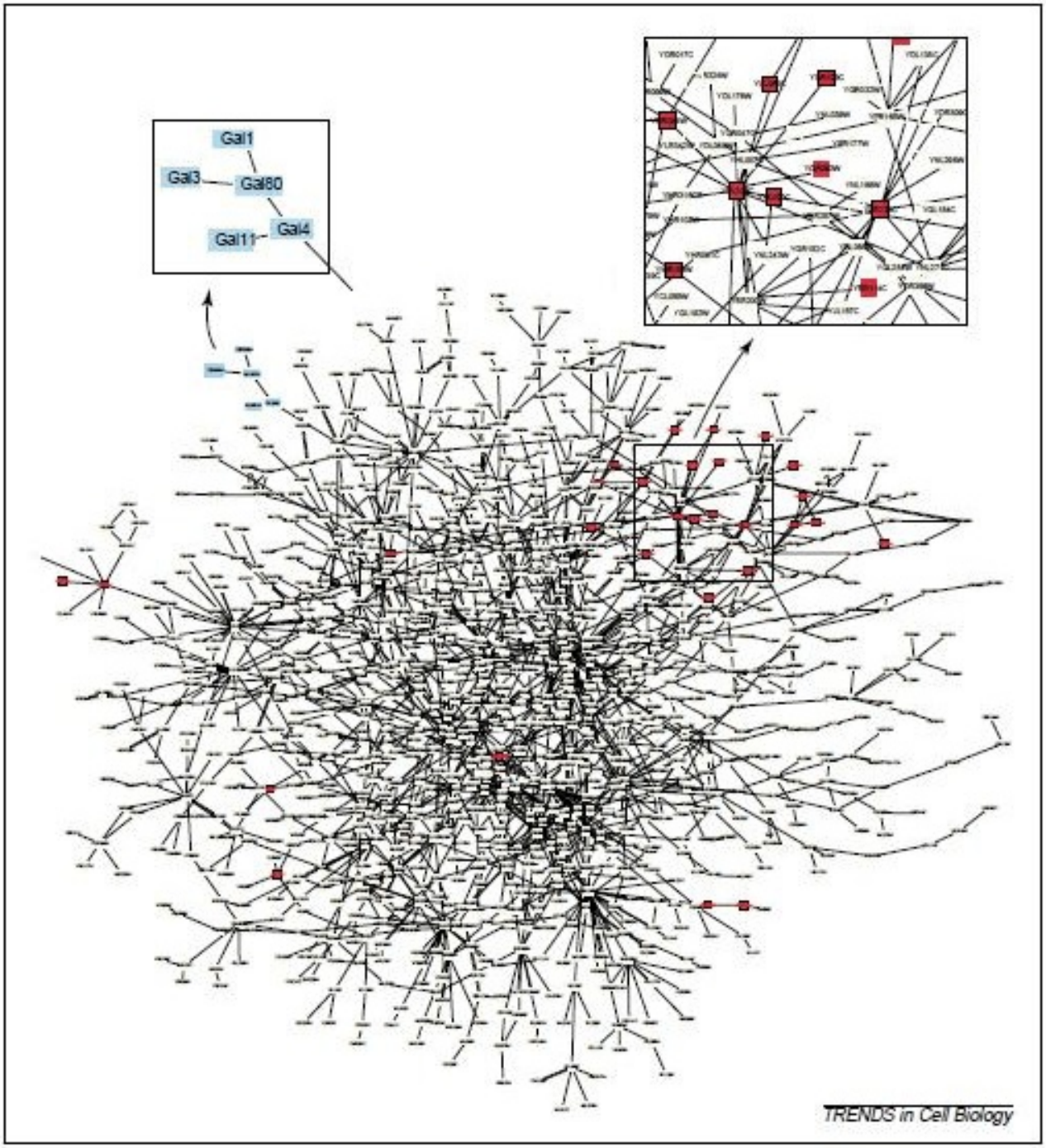
Method	Advantages	Disadvantages
Yeast Two Hybrid	Easy to obtain target genes (using cDNA library) Easy to identify genes (DNA sequencing) Highly sensitive to detect low abundant proteins Can detect weak and transient interactions Less expensive	Detects mostly direct protein interactions False-positives and false-negatives are often detected due to forced nuclear localization Difficult to detect interactions caused by protein modification

Cho *et al.*

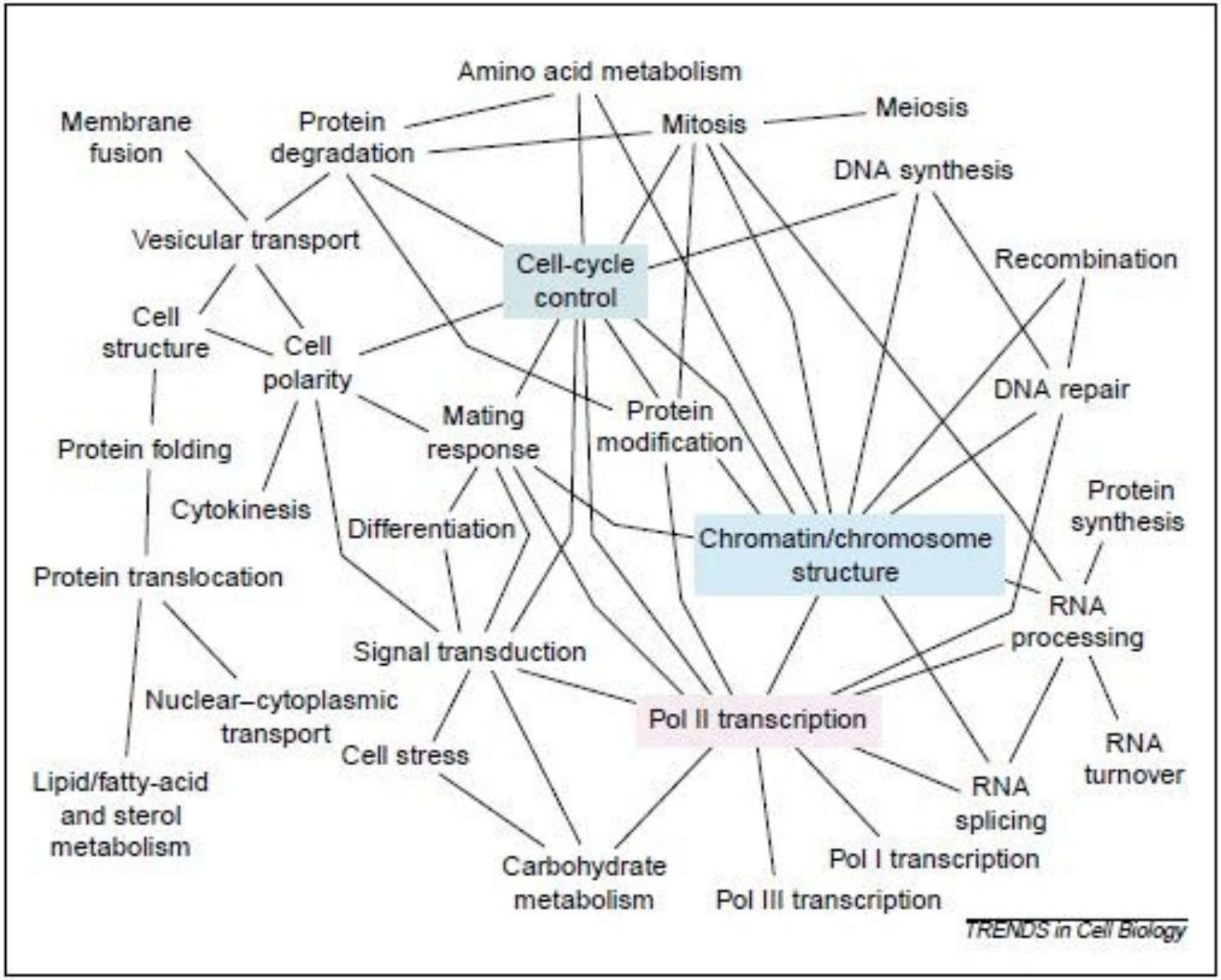

(A)
Bait cell culture
Array of prey cells
Mating
No Interaction
and no growth
Interaction
and growth
Y
AD
DBD
X
operator
Selection for interaction
(B)
Bait cell culture
Prey pools
Mating
Selection for interaction
Prey DNA sequencing

Cho *et al.*

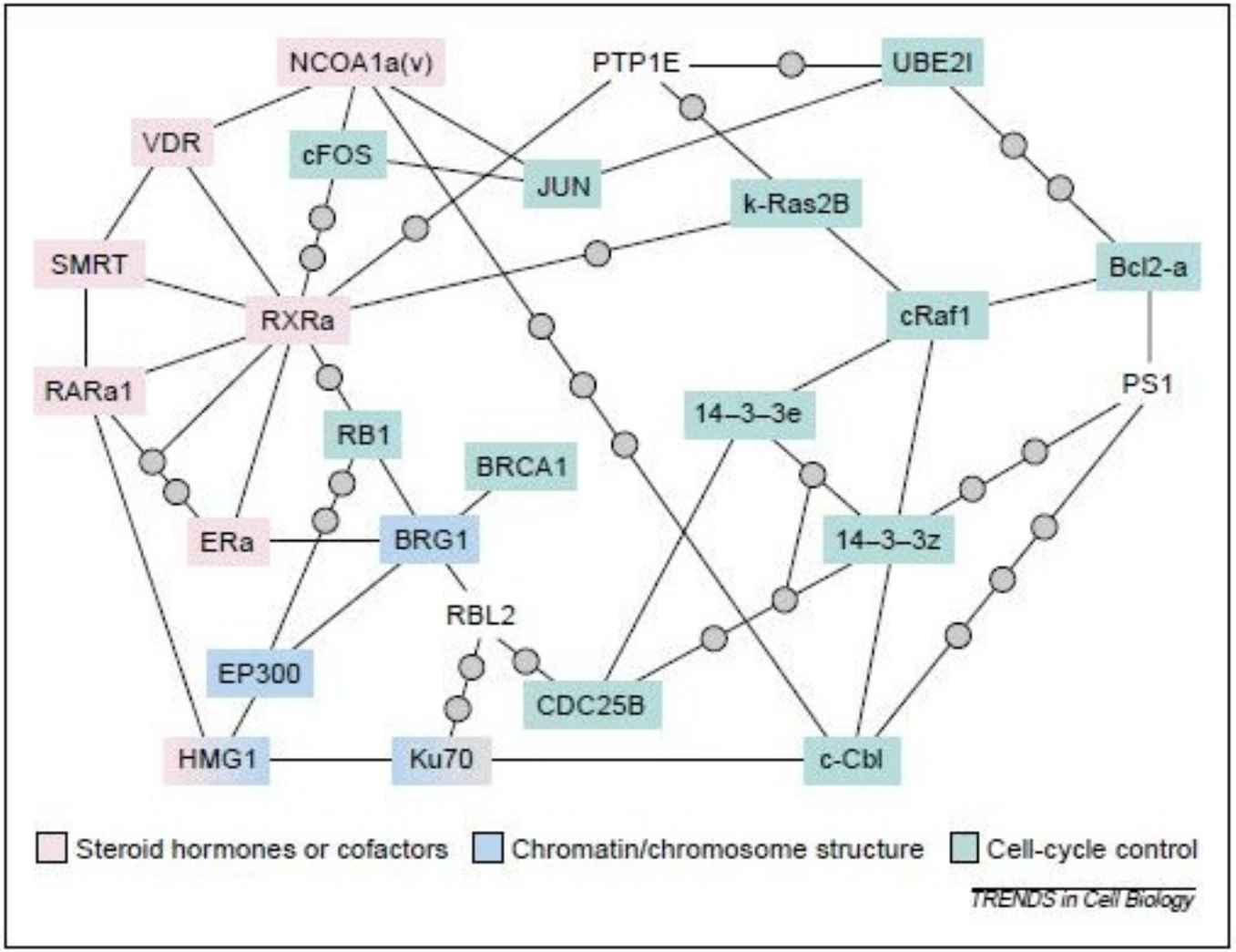
Protein Networks



Tucker *et al.*


Amino acid metabolism
Membrane fusion
Protein degradation
Mitosis
Meiosis
DNA synthesis
Vesicular transport
Cell-cycle control
Recombination
Cell structure
Cell polarity
DNA repair
Mating response
Protein modification
Protein folding
Cytokinesis
Differentiation
Chromatin/chromosome structure
Protein synthesis
Protein translocation
RNA processing
Signal transduction
Nuclear–cytoplasmic transport
Pol II transcription
Cell stress
RNA turnover
Lipid/fatty-acid and sterol metabolism
RNA splicing
Pol I transcription
Carbohydrate metabolism
Pol III transcription
TRENDS in Cell Biology

Tucker *et al.*


NCOA1a(v)
PTP1E
UBE2I
VDR
cFOS
JUN
k-Ras2B
SMRT
Bcl2-a
RXRa
cRaf1
RARa1
PS1
14–3–3e
RB1
BRCA1
ERa
BRG1
14–3–3z
RBL2
EP300
CDC25B
HMG1
Ku70
c-Cbl
Steroid hormones or cofactors
Chromatin/chromosome structure
Cell-cycle control
TRENDS in Cell Biology

Tucker *et al.*

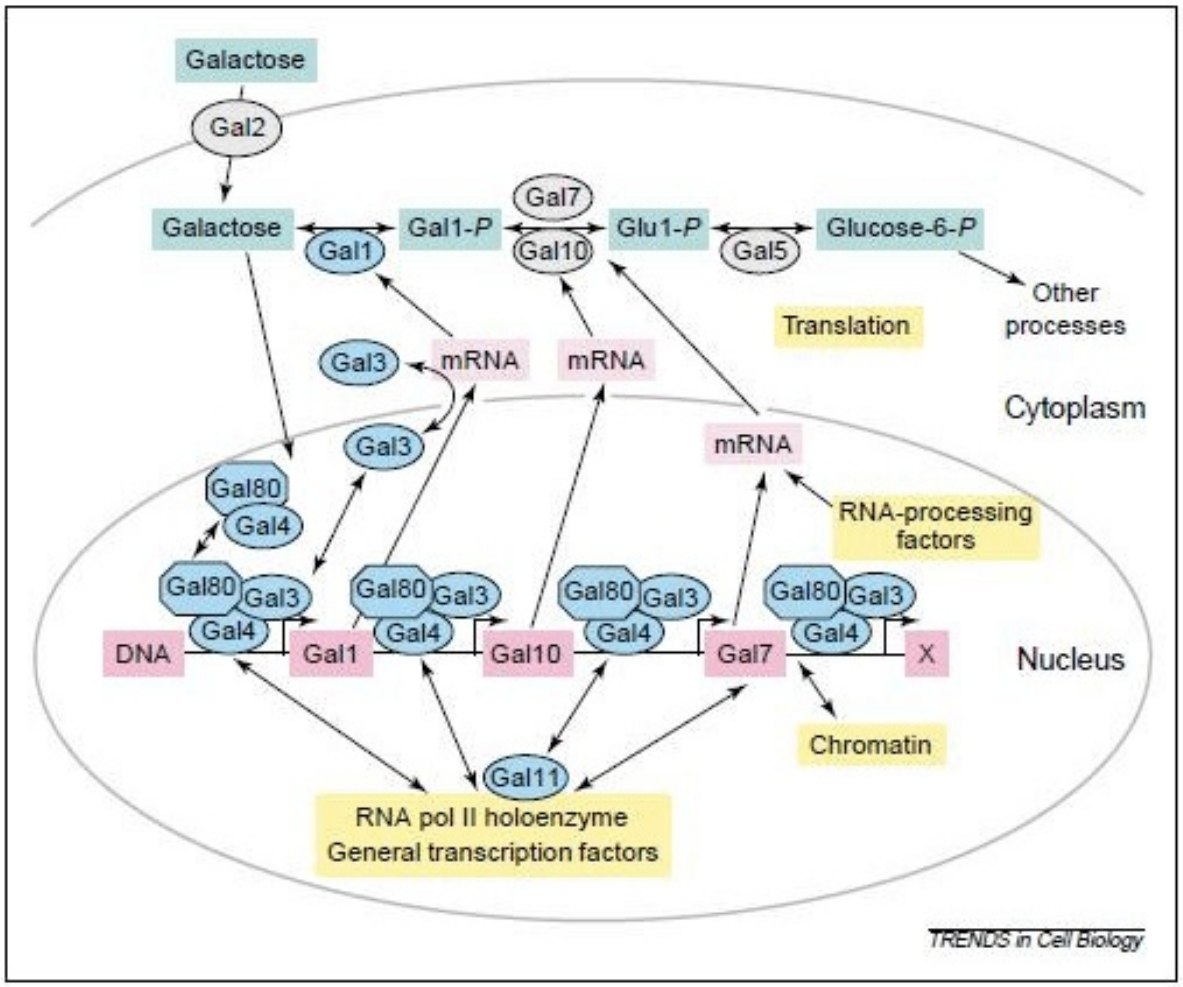
Main Goals of Protein Networks

To learn about individual proteins

Potetial partners

Potential functions

Potential interactive complexes


Galactose
Gal2
Galactose
Gal1
Gal1-P
Gal7
Gal10
Glu1-P
Gal5
Glucose-6-P
Other processes
Translation
Gal3
mRNA
mRNA
Cytoplasm
Gal3
mRNA
Gal80
Gal4
RNA-processing factors
Gal80
Gal3
Gal4
Gal80
Gal3
Gal4
Gal80
Gal3
Gal4
Gal80
Gal3
Gal4
DNA
Gal1
Gal10
Gal7
X
Nucleus
Chromatin
Gal11
RNA pol II holoenzyme
General transcription factors
TRENDS in Cell Biology

Tucker *et al.*

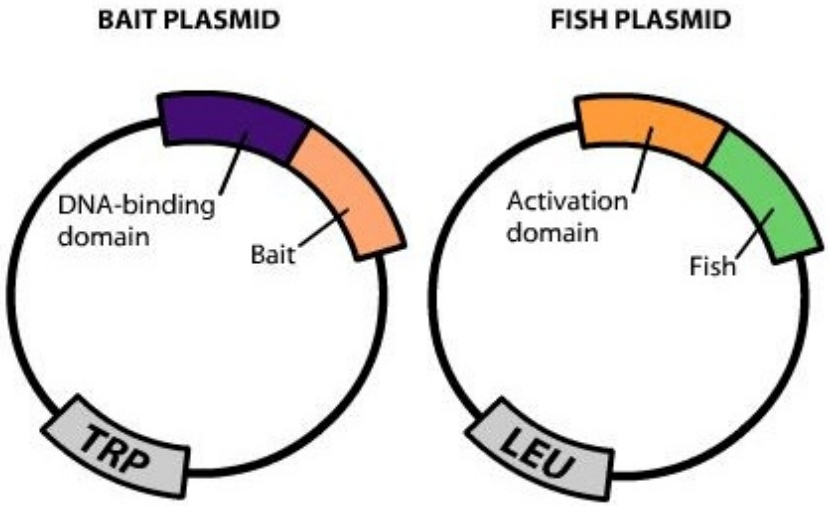
A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*

Uetz *et al.*

Two approaches used:

Protein array of activation-domain hybrids

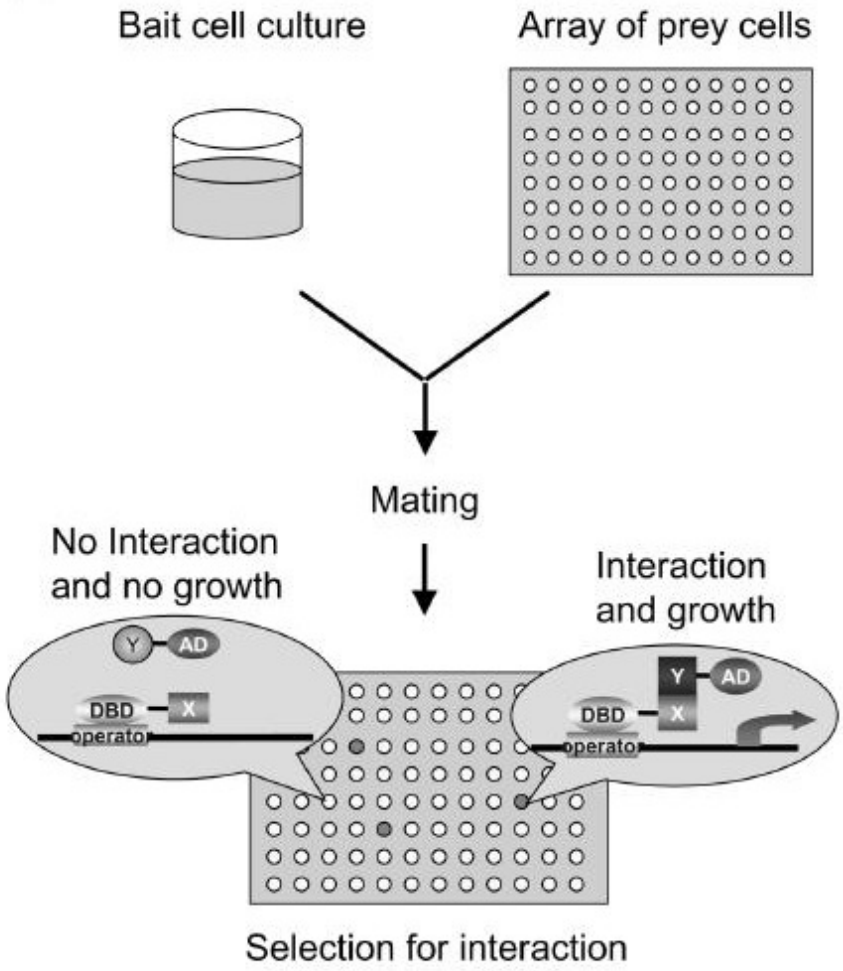
High-throughput screens of activation-domain library



http://www.sumanasinc.com/webcontent/animations/content/yeasttwohybrid.html

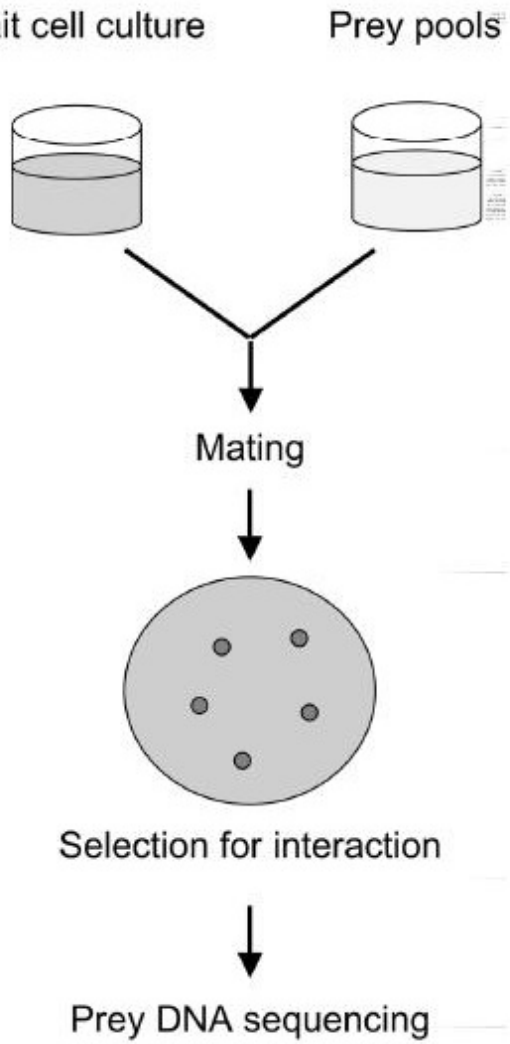
Library Screen

(A)

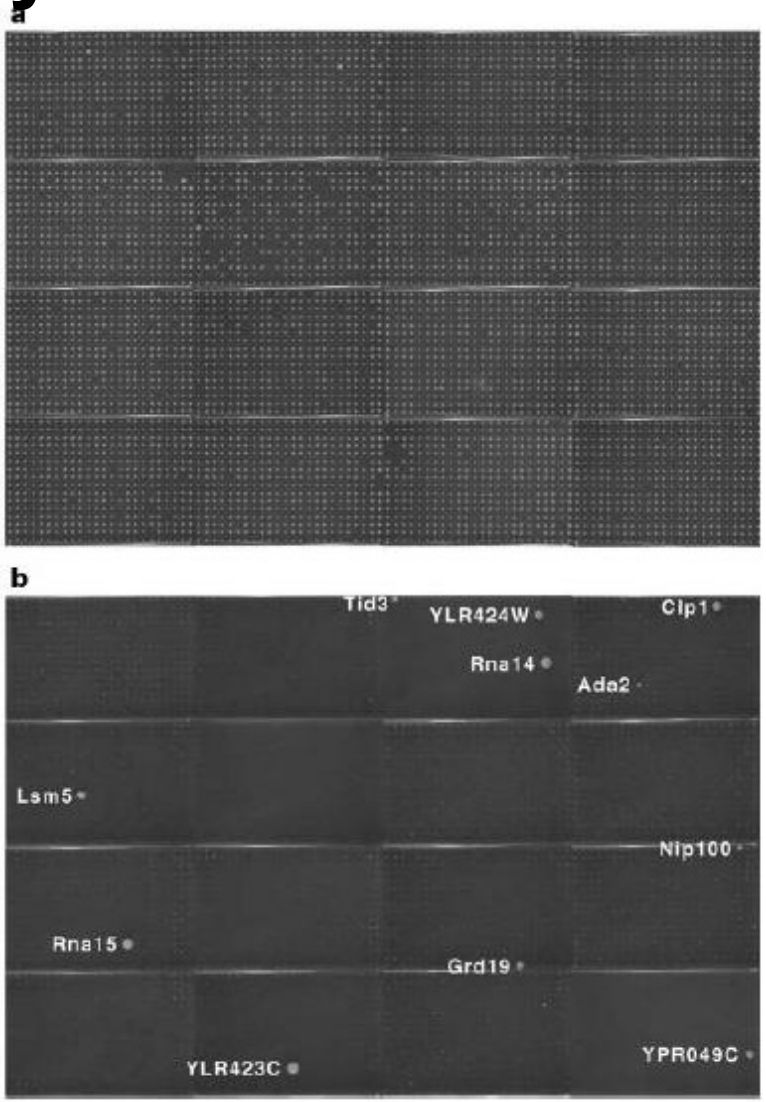


Protein Array

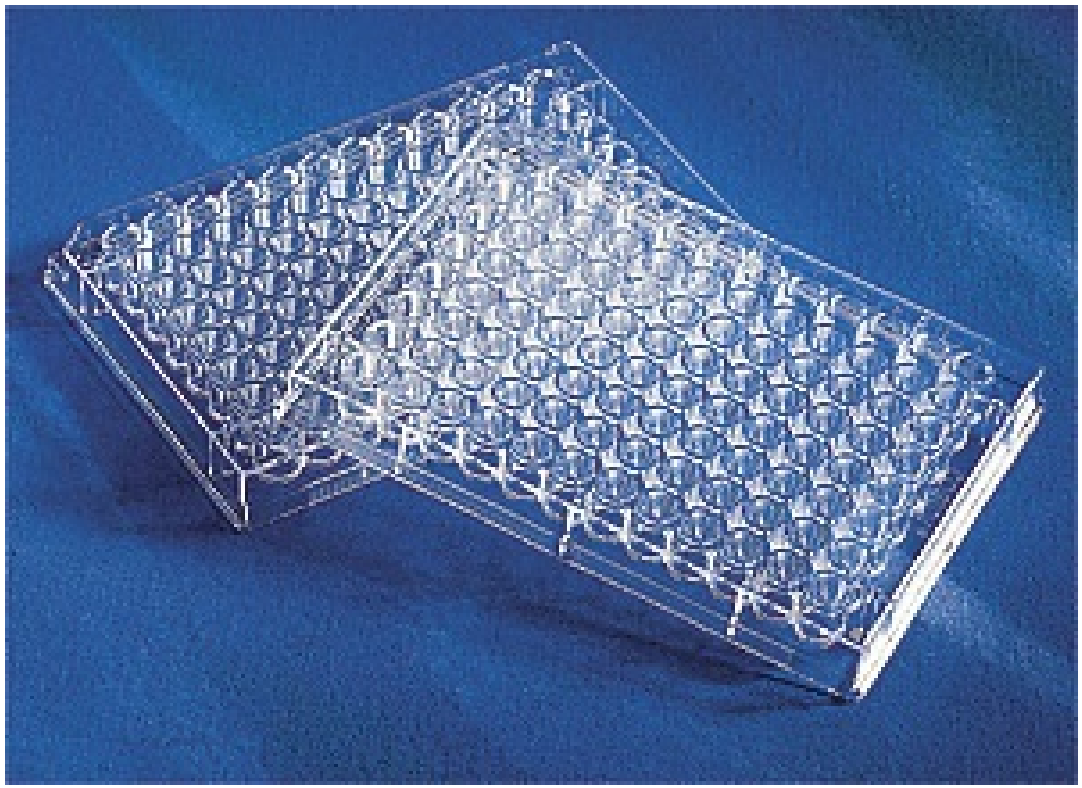
(B)



Protein Array



High-throughput screens



https://www.eandkscientific.com/product.php?productid=19309

Comparison

Table 1 Summary of experimental results

(a) Protein array

Description	Total
Yeast ORFs screened	192
Yeast ORF yielding reproducible interactions	87
Total discrete interacting protein pairs (reproduced in a second screen)	281

(b) High-throughput screens

Description	Total
Yeast ORF PCR products	6,144
Yeast ORFs cloned*	5,345
ORFs pooled to generate the activation domain library	5,341†
ORF activating transcription on their own	680
Yeast ORFs identified to have interactions‡	817
Total discrete interacting protein pairs	692
Interactions identified in independent experiments§	286
Interactions identified multiple times in a single experiment‖	186
Interactions identified only once	220

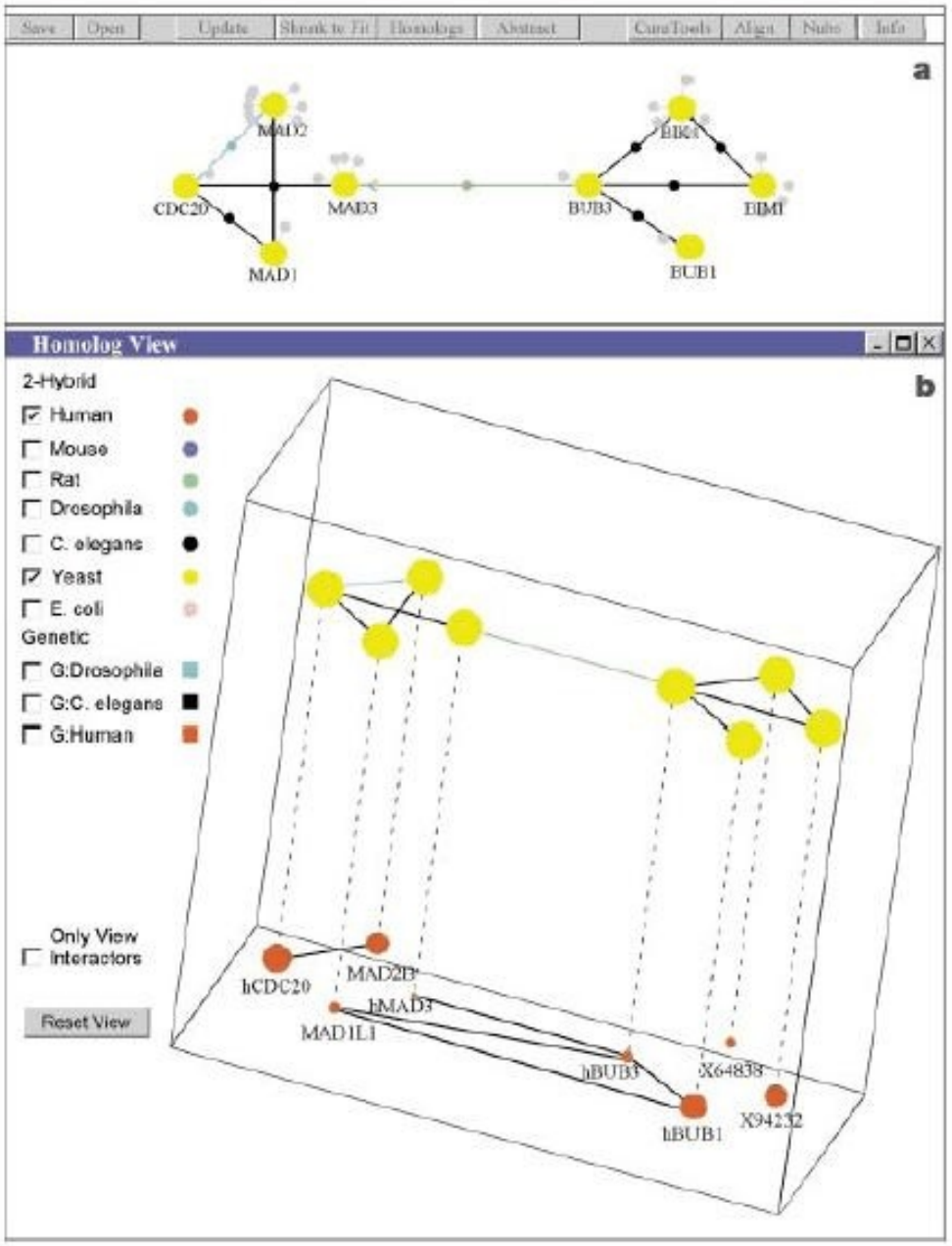
* Number of PCR products giving transformants in both plasmids (pOBD2 and pOAD).
† One yeast ORF activation domain construct was excluded from the pool owing to self-activation in a test screen (YJR009C—glyceraldehyde-3-phosphate dehydrogenase) and three yeast ORF activation domain constructs were excluded because they encoded proteins that could affect the selection process (YPL248C—Gal4, YML051W—Gal80 and YEL021W—Ura3).
‡ The total number of yeast ORFs found as interacting binding domain clones and/or interacting activation domain clones in the screens.
§ All screening experiments were performed in duplicate.
‖ Interactions that were identified several times in only one mating.

Results

BD fusion	AD fusion	Source
YMR093W (un)	YDR398W (un)	*
YMR102C (un)	YNL218W (ds)	*
YMR210W (un)	MRP8 (ps)	*
YMR269W (un)	GCN3 (ps)	*
YMR312W (un)	IKI1 (un)	*
YMR322C (un)	SNZ1 (cs)	*
YMR322C (un)	SNZ2 (cs)	*
YMR322C (un)	SNZ3 (cs)	*
YNK1 (nm)	YNK1 (nm)	*,∫
YNL201C (cm)	GDS1 (un)	*
YNL201C (cm)	STD1 (cm,cs,sm)	*
YNL201C (cm)	YPR115W (un)	*
YNL218W (ds)	MAD2 (cc,mi)	*
YNL218W (ds)	YNL218W (ds)	*
YNL311C (un)	MET14 (aa,cs,nm,om)	*
YNL311C (un)	YIL074C (un)	*
YNR004W (un)	YPL157W (un)	*
YNR025C (un)	SEC35 (vt)	*
YNR029C (un)	YJL064W (un)	*
YNR029C (un)	YJL065C (un)	*
YNR068C (un)	YNR069C (un)	*
YOL034W (un)	SPC24 (mi)	*,‡
YOL070C (un)	YNL078W (un)	*
YOL106W (un)	SMX3 (ch,ds,rp,rs)	*
YOL111C (un)	SGT2 (un)	*
YOR138C (un)	NPR2 (sm)	*
YOR138C (un)	YGR268C (un)	*
YOR215C (un)	YHR115C (un)	*
YNL056W (un)	SIW14 (cc)	*
YNL056W (un)	YNL099C (un)	*
YNL078W (un)	NAP1 (ch)	*
YNL086W (un)	YKL061W (un)	*
YNL091W (un)	GDS1 (un)	*
YNL091W (un)	YKL075C (un)	*
YNL091W (un)	YNL164C (un)	*
YNL091W (un)	YNL288W (un)	*
YNL091W (un)	YPL229W (un)	*
YOR220W (un)	CUP2 (cs,p2)	*
YOR264W (un)	YCR086W (me)	*
YOR264W (un)	YGR058W (un)	*
YOR353C (un)	NRK1 (wm,mi)	*
YOR353C (un)	SEC35 (vt)	*
YOR353C (un)	YOL082W (un)	*
YPL019C (ca)	VMA22 (ca,sm)	*
YPL110C (un)	YGR024C (un)	*
YPL151C (un)	PEP12 (mf,vt)	*
YPL192C (un)	YPL192C (un)	*
YPL222W (un)	UFD1 (pd)	*
YPL260W (un)	TID3 (mi)	*
YPR105C (un)	PEX14 (i,pt)	*
YPR105C (un)	SEC35 (vt)	*
YPR105C (un)	TIP20 (vt)	*
YPR105C (un)	VMA22 (ca,sm)	*
YPR105C (un)	YLR315W (un)	*
YPR105C (un)	YMR181C (un)	*
YPR105C (un)	YOR164C (un)	*
YNL094W (un)	SSN8 (p2)	*
YNL094W (un)	YAL049C (un)	*
YNL122C (un)	YKL061W (un)	*
YNL127W (un)	RHO4 (cp)	*
YNL127W (un)	TPD3 (cs,p3)	*
YNL157W (un)	ACE2 (p2)	†
YNL157W (un)	YJL075C (un)	†
YNL171C (un)	YCR106W (p2)	*
YNL201C (cm)	AAD6 (mr)	*
YPR105C (un)	YOR331C (un)	*
YPR152C (un)	YBR194W (un)	*
YPT1 (vt)	BOS1 (vt)	†
YPT1 (vt)	KTR3 (pm)	†
YPT1 (vt)	PEP12 (mf,vt)	†
YPT1 (vt)	YPL246C (un)	†
YPT31 (vt)	PEP12 (mf,vt)	†
YPT31 (vt)	YKR030W (un)	†
YPT31 (vt)	YNL146W (un)	†
YPT31 (vt)	YPL192C (un)	†
YPT53 (vt)	SIW14 (cc)	*
YRB1 (nc)	NAP1 (ch)	*
YSC84 (un)	GFD1 (un)	*
YSC84 (un)	SLA1 (cp,st)	*
YSC84 (un)	YGR268C (un)	*
YSC84 (un)	YLR243W (un)	*
YTH1 (rp)	FIP1 (rp)	*,‡
YTH1 (rp)	KTR3 (pm)	*
ZRC1 (cs,sm)	MRP8 (ps)	*

* High throughput screen
† Protein array
‡ Previously known from two-hybrid screens
∫ Previously known to interact or to occur in the same complex as shown by other methods
¶ Found in the other orientation, that is, inverted BD and AD fusions
'Cellular roles' are in parentheses; abbreviations are explained in Table 3.

Software


Save
Open
Update
Shrink to Fit
Homologs
Abstract
CuraTools
Align
Nulvs
Info
a
MAD2
CDC20
MAD3
MAD1
BUB3
BUB1
Homolog View
b
2-Hybrid
Human
Mouse
Rat
Drosophila
C. elegans
Yeast
E. coli
Genetic
G:Drosophila
G:C. elegans
G:Human
Only View Interactors
Reset View
hCDC20
MAD2B
hMAD3
MAD1L1
hBUB3
X64838
X94232
hBUB1

Conclusions

Data represents new potential interactions

Screens should be viewed as potential positives to motivate further experiments

Studies represent first comprehensive biological screens that use complete set of ORFs

Questions?