



Identifying Ribosomal Protein Interacting Partners

By Akhilesh Kesavan

Identifying Interacting partners of Ribosomal Proteins

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Saccharomyces Genome
Resequencing Project

Contains sequence data of
38 unique strains
of *S.cerevisiae*

Ribosomal proteins despite being largely
conserved show a large variation in growth
in deletion strains across different
environments

Is it possible RP genes are
involved in pathways other than
translation?

Yes

Identify naturally occurring RP gene
alleles across the 38 strains of
S.cerevisiae listed in the SGRP database

81 allelic variants in 52 RP genes

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Thank you for attending my talk

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Create clones of RP gene alleles using
Gateway Cloning

Use generated clones as baits in an Y2H
system against a library of prey proteins
composing of all the genes in *S.
cerevisiae*

Yes

Is there a difference in the
interaction partners between the
alleles of the RP?

The difference in interaction partners
indicates the involvement of the RPs in
pathways other than translation

Introduction



Ribosomes are the cellular machinery that translate mRNA to synthesise proteins



Made of 2 subunits; A small subunit with about 32 different ribosomal proteins(RPS) and a large subunit with about 43 different ribosomal proteins(RPL)



These proteins are primarily involved in translation



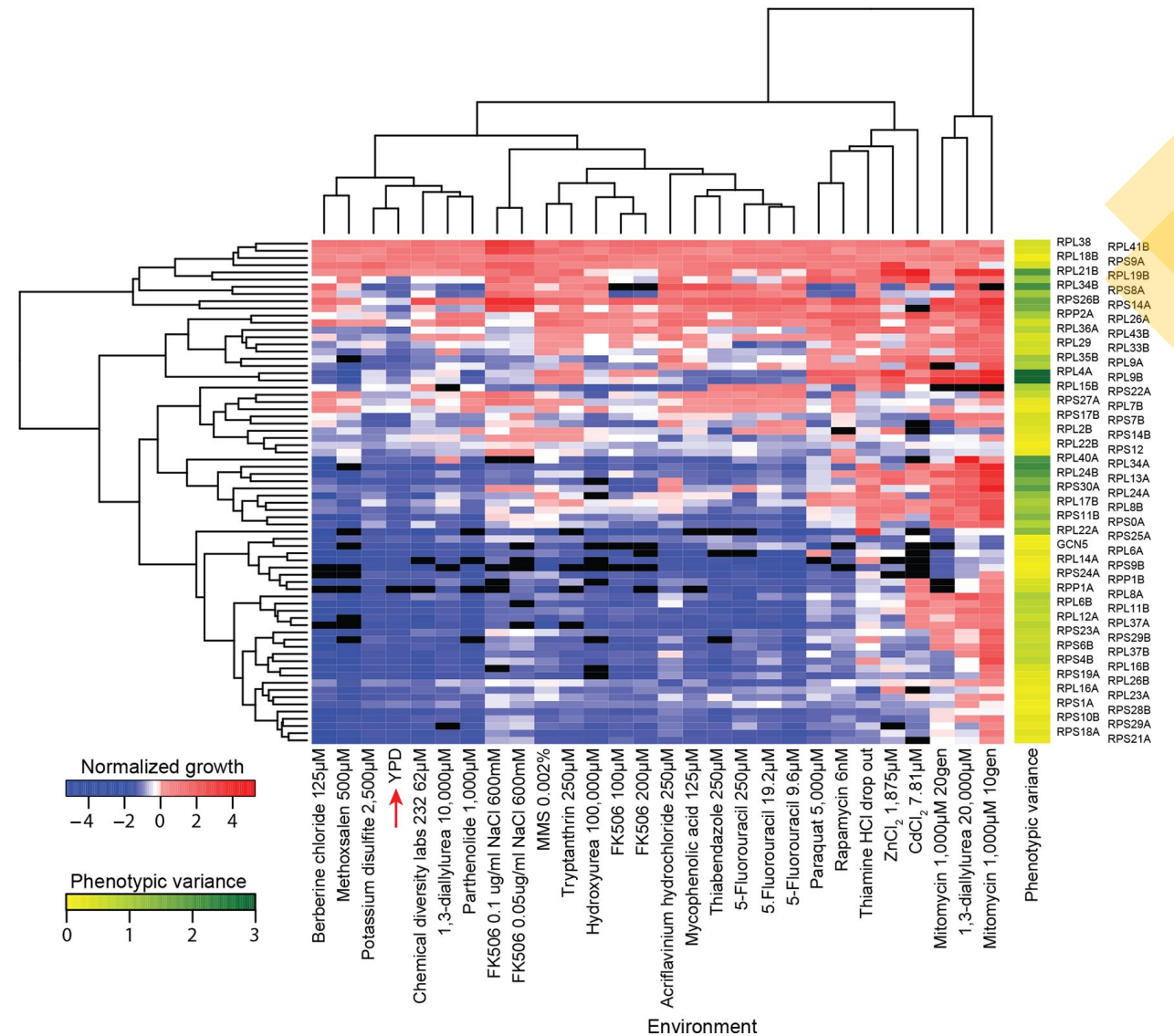
However recent studies suggest that they may have interactions in other pathways



The aim of the study is to identify the interacting partners of the certain RPs that have polymorphisms across different strains

Phenotypic Variability

- 68 single deletions in 26 environments
- RPs show higher phenotypic variance



Identification of RP Variants

Obtain sequence from SGD

The screenshot displays the SGD (Saccharomyces Genome Database) website. The top navigation bar includes links for Analyze, Sequence, Function, Literature, and Community. A search bar is located on the right. The main content area is divided into several sections: "About SGD" (providing an overview of the database), "Meetings" (listing upcoming events like TAGC 2020), "New & Noteworthy" (highlighting recent research), and "Tweets by @yeastgenome". Below these, the "RPL4A / YBR031W Sequence" page is shown, featuring a sidebar with navigation links (Summary, Sequence, Protein, Gene Ontology, Phenotype, Interactions, Regulation, Expression, Literature) and a main content area with details about the gene, its aliases, protein product, feature type, description, and paralog. A "Reference Strain: S288C" section is also present, showing the gene's location on Chromosome II and a table of coordinates. The bottom section displays the "Sequence - S288C" with a "Genomic DNA" dropdown and a list of nucleotide sequences.

SGD Saccharomyces GENOME DATABASE

Analyze Sequence Function Literature Community

Search: actin, kinase, glucose

About SGD

The Saccharomyces Genome Database (SGD) provides comprehensive integrated biological information for the budding yeast *Saccharomyces cerevisiae* along with search and analysis tools to explore these data, enabling the discovery of functional relationships between sequence and gene products in fungi and higher organisms.

Meetings

The Allied Genetics Conference - TAGC 2020
April 22 to April 26, 2020 - Metro Washington, DC

Fungal Pathogen Genomics
May 11 to May 16, 2020 - Houston, TX

New & Noteworthy

Apply Now for the 2020 Yeast Genetics and Genomics Course - March 03, 2020
For over 50 years, the legendary Yeast Genetics & Genomics course has been taught each summer at Cold Spring Harbor Laboratory, (OK, the name didn't include "Genomics" in the beginning...) The

Tweets by @yeastgenome

RPL4A / YBR031W Sequence

Aliases: rp2², L2A², L4A², YL2², L4³, uL4^{1,7}

Protein Product: ribosomal 60S subunit protein L4A

Feature Type: ORF, Verified

Description: Ribosomal 60S subunit protein L4A; N-terminally acetylated; homologous to mammalian ribosomal protein L4 and bacterial L4; RPL4A has a paralog, RPL4B, that arose from the whole genome duplication^{1,2,3,4,5}

Paralog: RPL4B⁴

Reference Strain: S288C

RPL4A Location: Chromosome II 300166..301254

Sequence - S288C

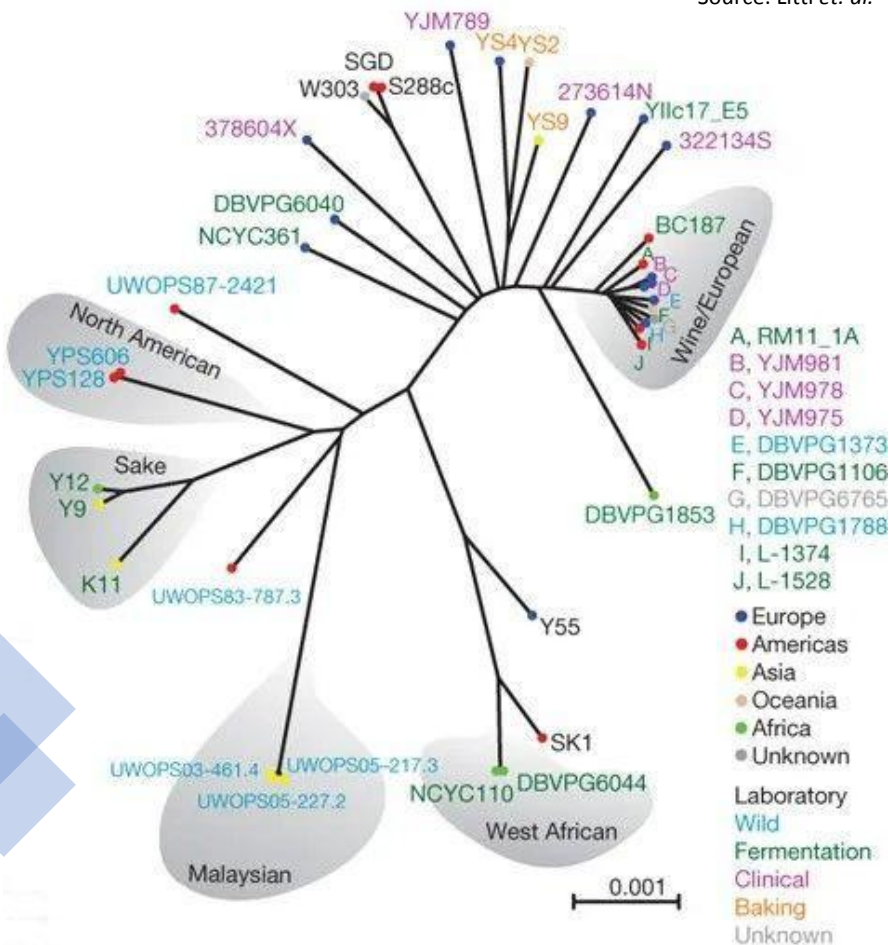
Genomic DNA

```
1 ATGTCCCTTC CAGAGTTAC TGTCACTTC TTGACTGTTG AAGCTACTGC CAATGCTTGG
61 CCAITTCGAG CTGTCTCTCT CGTCTCTATC GTTCCAGACA TTGTCCAGAC TGTTTTCAAC
121 TCTGTGACGA AGAACAAGAG ACAAGCTTAC GCTGTTTCTG AAAAGGCTGG TCACCAAAAC
181 TCGCTGGAAT CTTGGGGTAC CGTGTGTGCC GTCTCTGTTA TTCCAAAGAT TGTGTGTGTG
241 GTTACCGTTA GATCGGTGCA AGTGTCTCTC GTTAACTGTT GTCTGTGTGG TGTATGTTT
301 GTTCAACTTA AGACTGTGAG AAGTGTGAGC GTTAAAGTTA ACCACAAGCA AAGAGCTTAC
361 GCACTGTCTT CTGTATTGTC TGTACTGTCT GTTGTCTCTT TGTGTGTGGC CAGAGTCTAC
421 AAGTGTGAAA AAGTGTGAAA AAGTGTGAAA GTTGTGTCTA CTGACTGTGA ATTATGTGAA
481 AAGTGTGAAA AAGTGTGTTC TGTGTGTGAG GTTGTGTGTT GTACTGTGAA CTGTGTGAG
541 GTTGTGAGTT CCAAGAAATT GAGAGCTGTT AAGTGTGAGT ACAGAGACAG AAGATGAGT
601 CAAAGAGAGG GTTCAATGAT TGTCTACGTC GAAGACACAG GTATGTGCAA GGCTTGAGA
661 AAGTGTGAGG GTTGTGAAAC TGGCAAGCTT GCTTCTTTGA ACTTGTGTGA ATTGTGTCCA
721 GTTGTGTGAT TGTGTGAGAT GTTATCTTGG ACAGAGCTGT CTTTGACGAA GTTGTGACAA
781 GTTGTGTGAT CCAAGAACTT TGTGTGTCTC AAGTGTGAGT ACATTTGTGC ATCCATATGC
841 ATCTGACTCT CTGATGTGAC CAGATATATC AACTGTGCTG AAGTGTGAGT TGTGTGAG
```


Identification of RP Variants

Obtain sequence from SGD

Source: Litti *et. al.*



SGRP
Saccharomyces Genome Resequencing Project

Home Download Data **Blast Litti 2009** Search Bergström 2014

SGRP Blast Server

Enter the sequence to Blast in fasta format in the text box below. [Example](#)

```

121 TCTGAGAA AGACAGAG AGACGTTAC GTGTTTCG AAGAGGCTG TACACACCC
181 TCCGCTGAT CTTGGGATAC CGTGGTGGC GTGCTGGTA TTCCAGAGT TGGTGGTGT
241 GGTACCGTA GATCGGTCA AGTGGCTTC GTTACAGAT GTCTGGTGG TGGTATGTT
301 GTCCCACTA AGACCTGGG AAGTGGGAA GTTAAGTTA ACCACACGA AAGCGTTAC
361 GCCACTGCT CTGCTATTG TGTACTGCT GTTGCTCTT TGGTCTGGC CAGAGGTCAC
421 AGAGTCGAA AGATTCCGA AATCCCATG GTTGCTTCA CTGACTTGA ATCTATTCR
481 AAGACCAAG AAGCTGTTC TGTCTTGA GCTGTGGTG CTGACTTGA CTGTTTGA
541 GTTGTAGT CAGAGGAT CAGAGGAT AAGGTAGT ACAGAGAG AAGAGGAT
601 CAGAGAGAG GTCCATTAG TGTCTAGCT GAAGACACG GTATGTCR GSCCTTGAA
661 AAGCTTCAG GTGTTGAAC TGCCACGTT GCTTCTTGA ACTTGTGCA ATTGGTCCA
721 GGTGCTCAC TGGGTAGAT CGTTATCTG ACCGAGCTG CTTCACCAA GTTGACCAA
781 GTCTGGGTT CCGAAGCGT TGTCTCTCC AAGTGGGCT ACACCTTGC ATCCCATATC
841 ATCTGAGT CTGATGTC CAGATATTC AACTCTTGC AATCTATC TGTCTATCA
901 CAGCTGGCC AAGCTACTA AAGCGTACT CAGCTTTGA AAGAGACCC ATTGAAGAC
961 AAGCAAGCT TGTGTAGAT GAACCTTAC GCCAAGGCT TTGCTGCTA AAGCTAGGT
1021 TCCAGAGAG CTGAAAGAC TGGTACCA CAGCTGCTG TTTTCAACG AACTTTGAA
1081 CAGATTA
    
```

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Linked Table Tab Delimited Select a display

Search

www.moseslab.cib.utoronto.ca/sgrp/blast_original/

Query	Subject	% Identity	Aligned Length	Mismatch	Gaps	Query Start	Query End	Subject Start	Subject End	E-value	Score
Request	S288c.chr02	100.00	1089	0	0	1	1089	300152	301240	0.0	2159
Request	REF.chr02	100.00	1089	0	0	1	1089	300166	301254	0.0	2159
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Identification of RP Variants

- Align protein sequences to identify non-synonymous variants

Gene	Strain 1	Variant1	Strain 2	Variant2
RPL04A	YS4	168S (502T)	S288c	168A (502G)
RPL04A	RM11_1A	256A (766G)	S288c	256T (766A)



So what can do we do?



SELECT RPS WITH HIGH VARIANCE, GENERATE
ALLELE SPECIFIC POPULATIONS AND STUDY
GROWTH IN DIFFERENT ENVIRONMENTS



PROVIDES IN DEPTH ANALYSIS FOR SPECIFIC **RP**
ALLELE



NOT VIABLE FOR LARGE SCALE ANALYSIS OF
ALL VARIANTS

Now what?



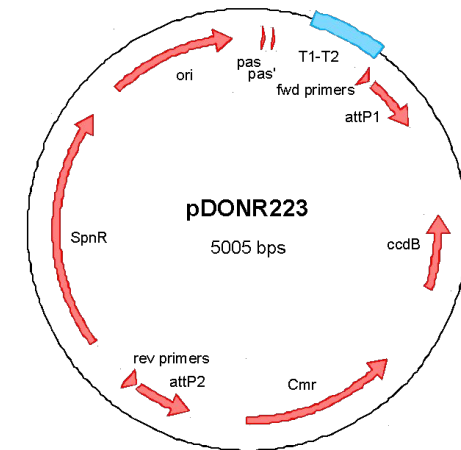
PCR amplify the RP of interest



Clone into plasmid of interest

pDONR223

- AttB sequence + Specific Sequence
- Forward: GGGGACAAC TTTGTACAAAAAAGTTGGCACC
ATGTCCCGTCCACAAGTTAC
- Reverse: GGGGACAAC TTTGTACAAGAAAGTTGGCAA
TTAATCGTGTTTCAAAGTTTCGGT



Gateway Cloning

- Inspired by lambda phage's mechanism of inserting its genetic material into host by homologous recombination
- BP reaction mediated by integration host factor and integrase
- LR reaction mediated by integrase, integration host factor and excisionase

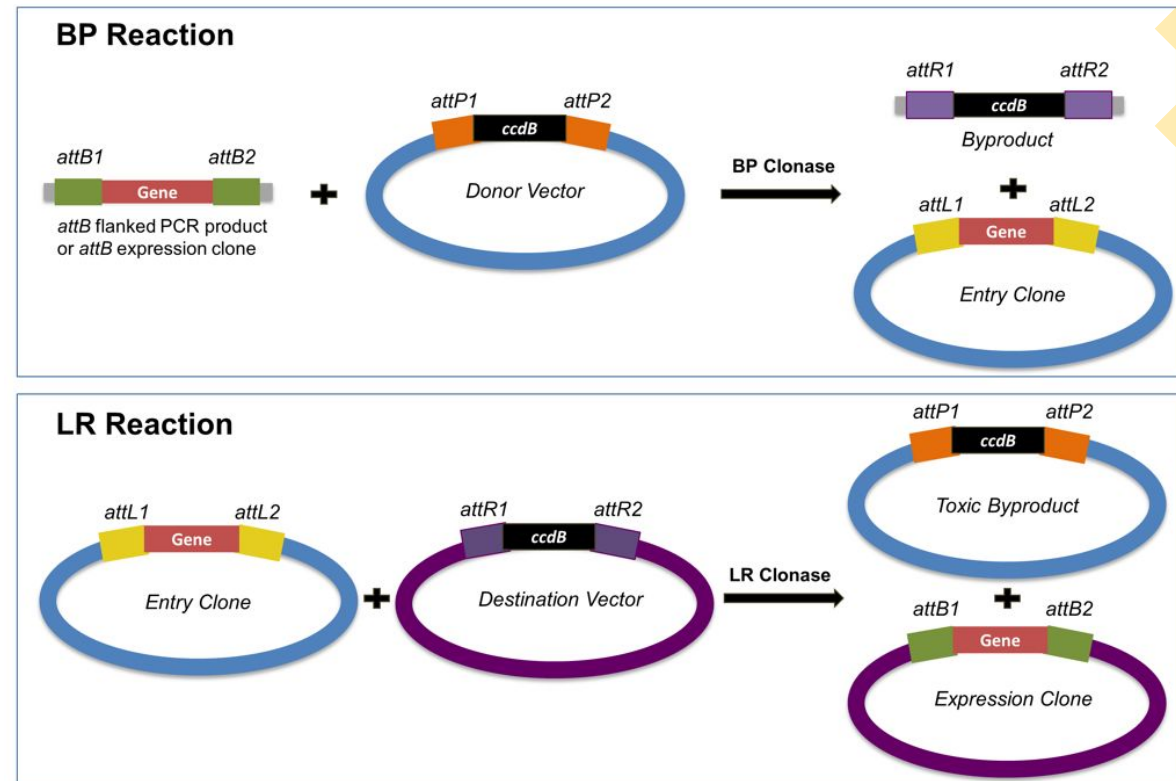
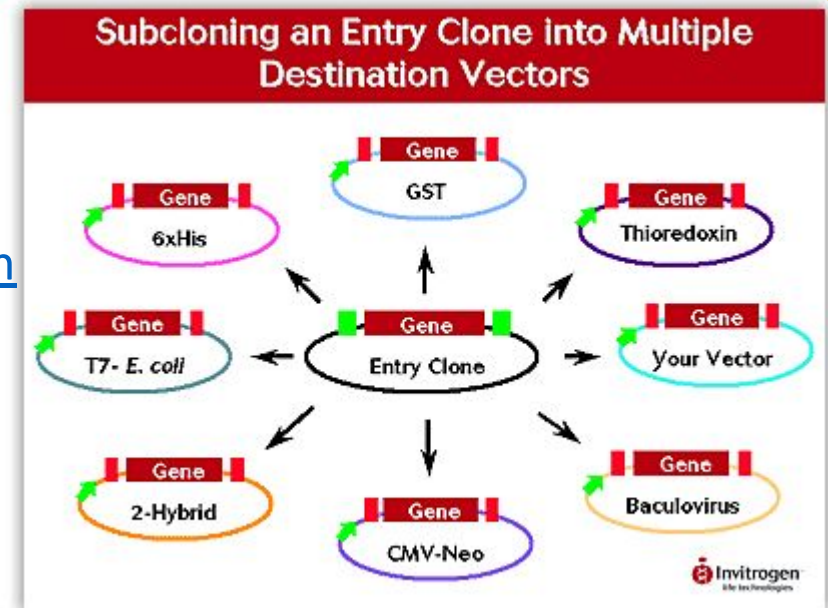


Figure 2: The Gateway system adopts phage integration into the BP and LR reactions. The BP reaction creates an *attL*-flanked entry clone. The LR reaction creates an expression clone with all of the components necessary for gene expression.

Advantages

- Fast reactions—1 hour room-temperature cloning reactions
- Accurate results—cloning reactions achieve >95% efficiency to deliver the clone you need
- Versatile technology—easily shuttle DNA material/ insert from vector to vector
- Source:
<https://www.thermofisher.com/in/en/home/life-science/cloning/gateway-cloning/protocols.html>



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What Next?

- Naive GAL4 (transcription factor) contains a DNA binding domain and an activating domain
- The GAL4 protein is modified as following:
 - Binding domain fused to protein X (Bait)
 - Activating domain fused to protein Y (Prey)
- Only when the bait and prey interact, transcription machinery is recruited and the cell shows β -Galactosidase activity

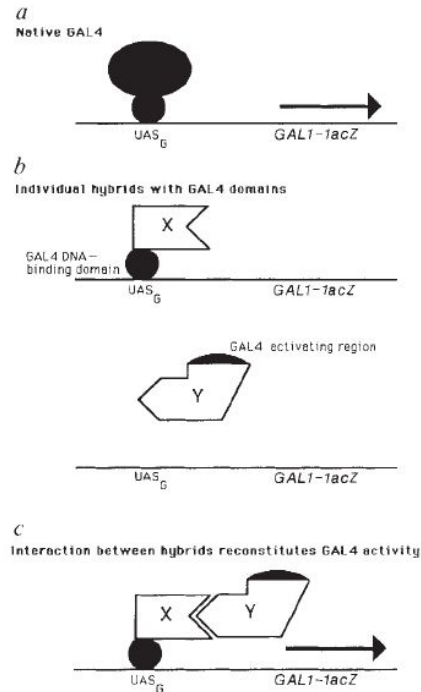
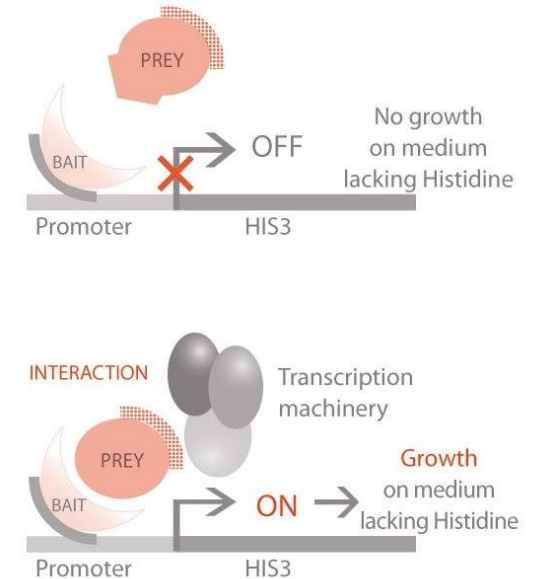


FIG. 1 Model of transcriptional activation by reconstitution of GAL4 activity. *a*, The native GAL4 protein contains both DNA-binding and activating regions and induces *GAL1-lacZ* transcription. *b*, Hybrids containing either the DNA-binding domain (upper) or activating region (lower) are incapable of inducing transcription. *c*, A protein-protein interaction between proteins X and Y brings the GAL4 domains into close proximity and results in transcriptional activity.

Source: Fields, S. & Song, O., 1989. A novel genetic system to detect protein-protein interactions. *Nature*, 340(6230), pp.245–246



LexA or Gal4 DNA Binding Domain

Gal4 Activation Domain

The interaction of 2 proteins reconstitutes an active transcription factor and enables yeast growth

BAIT = your protein of interest

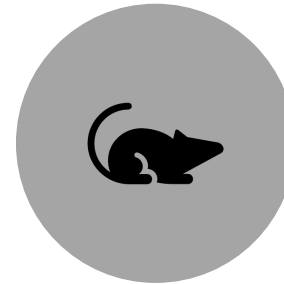
PREY = protein partner of the bait

Source:
<https://www.hybrigenics-services.com/contents/resources/yeast-two-hybrid-principle>

Why Y2H?



Flexible and less time consuming compared to biochemical methods



In-vivo technique using a eukaryotic model system giving more accurate results compared to in-vitro or bacterial systems



No need of high quality purified proteins and antibodies



Capable of screening large scale libraries more quickly and efficiently