ECM 2025 Conference
The 3rd International Electronic Conference on Microbiology
01–03 April 2025 | Online
MDPI
HYBRIGENICS SERVICES

ULTImate Y2H: A Powerful Platform to decipher Host-Pathogen Protein Interactions and Biological Pathways

Susanne REICHINNEK1, Chloe Antoine1, Petra Tafelmeyer2 and Jean-Christophe Rain1
1Hybrigenics Services, Evry-Courcouronnes 91000, France, 2Hybrigenics Corporation, Boston MA 02109, USA

INTRODUCTION & AIM

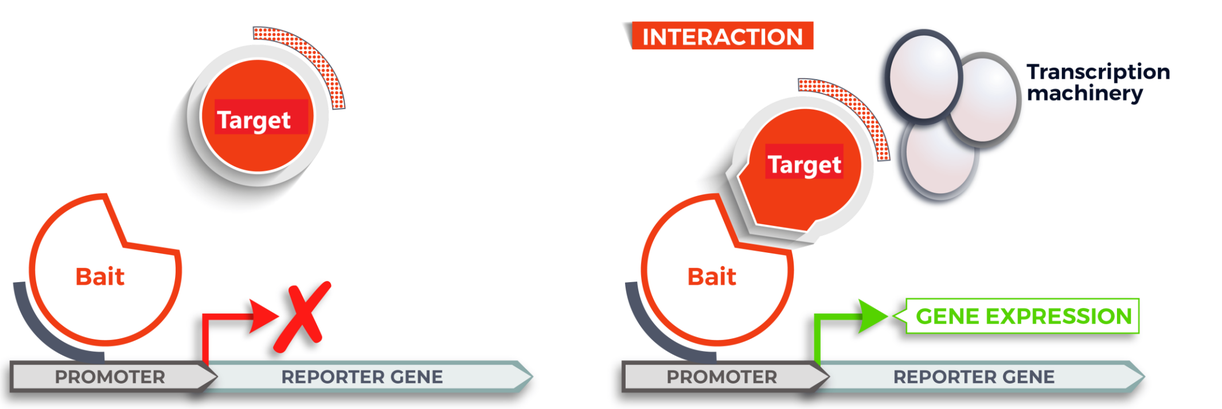
The understanding of the molecular mechanisms by which pathogens like viruses, bacteria, fungi, and parasites operate, particularly in relation to their host interactions, is essential to effectively develop therapeutic lead candidates. Protein–protein interactions serve as fundamental components of cellular protein complexes and pathways and play a crucial role in determining protein functionality. The yeast two-hybrid (Y2H) screening method remains a valuable tool for analyzing the interactomes of various model organisms and pathogens, as well as for investigating host–pathogen interactions.

In order to achieve reproducible and comprehensive Y2H outcomes, we developed a patented mating protocol that allows 83 million interactions to be tested, on average, per screen. Additionally, we used a domain-based strategy to create highly intricate, random-primed cDNA/genomics libraries with several million independent clones in yeast derived from several bacteria and viruses, such as Caulobacter crescentus, Escherichia coli, and Streptococcus pneumoniae, as well as human tissues, rodents, flies, and plants. The use of protein domain libraries allows for the isolation of multiple independent fragments for each interactant, facilitating the immediate identification of minimally interacting domains and the calculation of confidence scores.

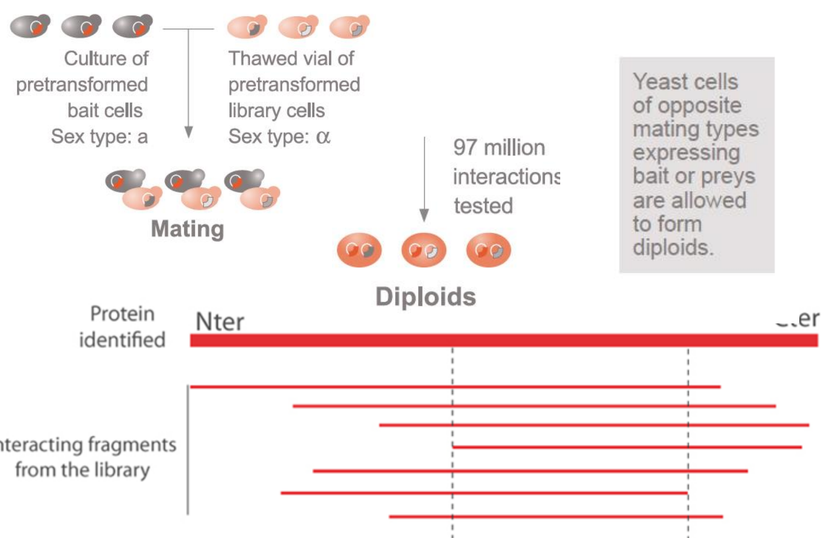
We used this methodology to investigate biological pathways in Staphylococcus aureus.

METHOD

ULTImate Y2H™, our exhaustive Yeast Two-Hybrid screening

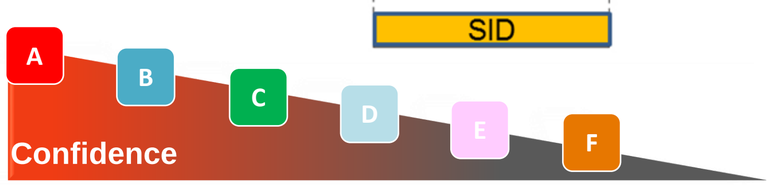


Exhaustive and reproducible screening
80 million interactions tested on average, 8-fold library coverage



Delineation of a minimal interacting domain (SID)

Computation of a confidence score (PBS) for each interaction



Starting material: *Staphylococcus aureus COL* genomic DNA
Library construction: - nebulization of DNA (average fragment size 1000 +/- 500 bp)
- blunt ending of fragments with a cocktail of mung bean nuclease, T4 and Klenow polymerase
- ligation of adapters containing SfiI restriction sites
- cloning into pP6 library vector derived from the original pGADGH1[4] and transformation in *E. coli* (>10 million independent clones)
- transformation in yeast (2 million independent colonies were collected, pooled and stored at -80°C as equivalent aliquot fractions of the same library)

Other bacterial libraries available for interaction screening: *Helicobacter pylori , Escherichia coli , Streptococcus pneumoniae , Streptococcus pyogenes, Caulobacter crescentus, Enterococcus faecalis, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Clostridium difficile*

CONTACT US : Hybrigenics Services SAS
services@hybrigenics.com www.hybrigenics.services.com

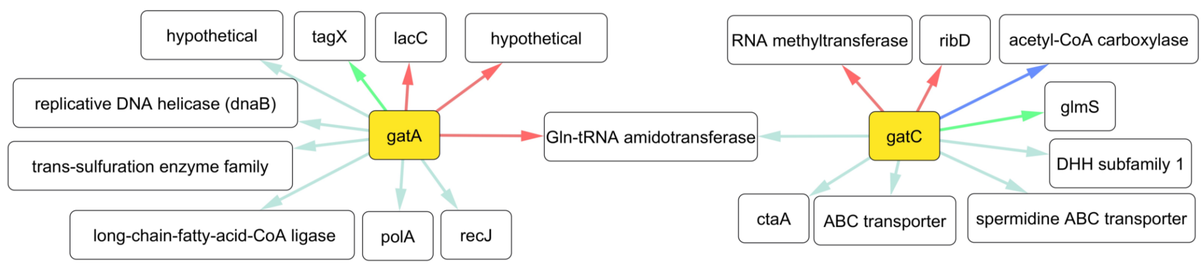
RESULTS & DISCUSSION

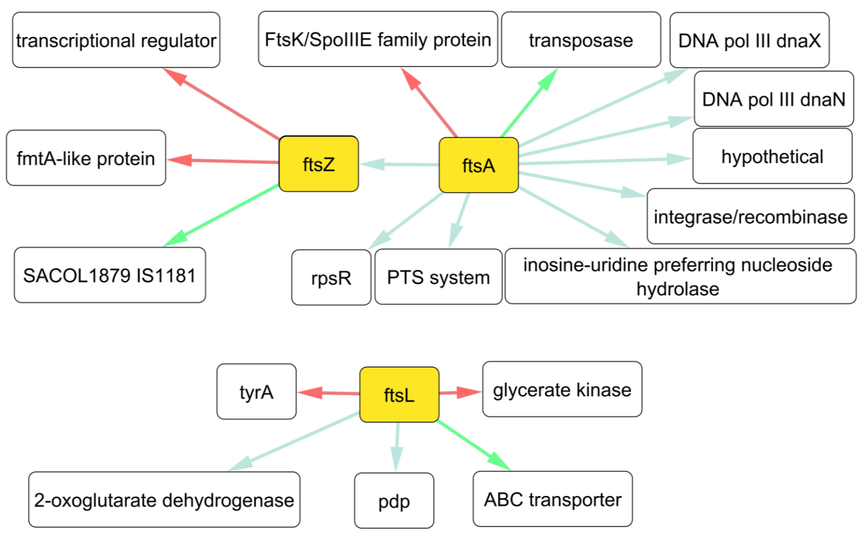
A total of 26 essential proteins from S. aureus were screened against our highly complex genomic library of the same organism.

- 30 screens were performed with 25 different bait proteins
- 167 CDS are connected by 191 PPI
- on average, every bait protein has 9.6 PPI

Bait protein	Full name	GO
argC2	accessory gene regulator protein C	ATP binding
dnaA	chromosomal replication initiation protein	regulation of DNA replication
era	GTPase Era	ribosome biogenesis
frr	ribosome recycling factor	translation
ftsA	Cell division protein ftsA	cell cycle
ftsL	Cell division protein FtsL	cell cycle
ftsZ	cell division protein FtsZ	cell cycle
gatA	Glutamyl-tRNA(Gln) amidotransferase subunit A	translation; ATP binding
gatB	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B	translation; ATP binding
gatC	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	regulation of translational fidelity
glyS	Glycyl-tRNA synthetase	glycyl-tRNA aminoacylation
mecA	Penicillin-binding protein 2'	peptidoglycan-based cell wall biogenesis; response to antibiotic
msrA	Peptide methionine sulfoxide reductase MsrA 2	oxidation-reduction process; protein modification process
murAA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	cell cycle
murD	UDP-N-acetylmuramoylalanine--D-glutamate ligase	cell cycle
pbp1	Penicillin-binding protein 1	peptidoglycan-based cell wall biogenesis; penicillin binding
pbp2	Penicillin-binding protein 2	Cell wall biogenesis/degradation; Peptidoglycan synthesis
pbp4	Penicillin-binding protein 4	proteolysis
rpoA	DNA-directed RNA polymerase subunit alpha	DNA binding; DNA-directed RNA polymerase activity; protein dimerization activity
SACOL1779	Transglycosylase domain protein	Cell wall biogenesis/degradation; Peptidoglycan synthesis
SACOL1933	Uncharacterized protein SACOL1933	hydrolase activity, acting on glycosyl bonds
SACOL2560	Hydroxymethylglutaryl-CoA reductase, degradative	coenzyme A metabolic process; oxidation-reduction process
vraR	Response regulator protein vraR	regulation of transcription, DNA-dependent
yycF	Transcriptional regulatory protein walR	Transcription; Transcription regulation; Two-component regulatory system
yycG	Sensor protein kinase walK	Two-component regulatory system

Among the identified proteins are critical genes associated with cell wall biosynthesis, cell division, and antibiotic resistance to allow for an understanding of the underlying molecular pathways and the development of therapeutic targeting and antimicrobial resistance research.





- **Strategy:** pathogen proteins used as baits to screen host cDNA libraries for interacting partners
- More than 130 cDNA libraries from human, rodents, plants and model organisms currently available for Y2H screening

The resulting protein interaction map identified 167 CDSs that are connected by 191 protein–protein interactions (PPIs), and, on average, every bait protein has 9.6 PPIs and can therefore can serve as a foundational resource for identifying potential new targets for antimicrobial drug development and future treatments.

CONCLUSION

- Identification of direct targets, unbiased, robust & exhaustive screens (~ 8-fold library coverage)
- Interactions (= putative partner) are screened individually in a different yeast colony, high sensitivity transcriptional readout
- Simplified & fastened analysis of the results (integrated bioinformatics analysis), screening capacity adaptable to projects specificities

REFERENCES

Exploiting bacterial effector proteins to uncover evolutionarily conserved antiviral host machinery
Aaron Embry, Nina S Baggett, David B Heisler, Addison White, Maarten F de Jong, Benjamin L Kocsis, Diana R Tomchick, Neal M Alto, Don B Gammon
(2024) PLoS Pathog
A bacterial virulence factor interacts with the splicing factor RBM5 and stimulates formation of nuclear RBM5 granules
Renaud Pourpre, Goran Lakisic, Emma Desgranges, Pascale Cossart, Alessandro Pagliuso & Hélène Bierne
(2022) Nature, Scientific Reports volume 12, Article number: 21961