Yeast Two Hybrid Assay as a Host Protein- Effector Interaction Detection Tool

Catherine Ma
Lesser Lab, Infectious Disease MGH/HMS, Cambridge, MA

Introduction

The Lesser lab is interested in how different pathogenic bacteria manipulate host cell processes and interact with host proteins during an infection. One way to study these infections is to find interactions between the bacterial protein (the effector) and host proteins. Specifically, the lab is interested in pathogens that cause gastrointestinal diseases such as *Shigella, Salmonella, Yersinia* and enteropathogenic *E. coli.* Using Yeast-Two-Hybrid Assay, an existing protein-protein interaction detection assay, we discovered novel interactions and investigated these interactions through further experimentation. However, the Y2H assay has several limitations; the lab is testing an alternative method to detect protein-protein interaction- the Protein-Interaction-Platform (PIP) Assay.

Activity (Methods)

My primary role was to make the plasmids containing the DNA that codes for the protein of interest through a series of Gateway reactions and to use Y2H assay to screen for potential host protein-effector interactions. I used the following methods:

- PCR/DNA purification
- Bacteria and yeast transformation chemical/electrical
- Mini prep
- Spectrophotometry
- Yeast fluorescence microscopy

Additionally, a high throughput colony inoculating robot is used to facilitate yeast mating and growth.

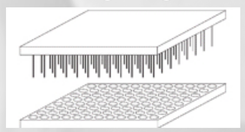

Figure1, 96x Pinning Tool for inoculating colonies

Figure 2. BM3-BC Colony Processing Robot
Automated colony picking between selective media

Outcomes (Results)

Experiment 1

Low copy number plasmid (1-3 copies per cell)

No interactions were found.

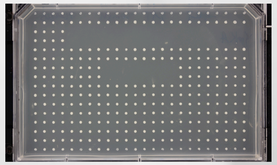

Figure 3. –HLT (30mM 3AT)
YpkA x host proteins
Negative interactions (low copy number plasmid)

Experiment 2

Modifications-

High copy number plasmid- 2 micron (40-60 copies per cell)

Addition of catalytically dead effector proteins (mutation in catalytic site)

35 interactions were found, some were unexpected.

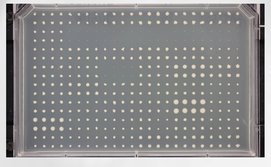

Figure 5. –HLT (30mM 3AT)
SifA x host proteins (2μ)

Table 1. Host protein master plate. Proteins are are fused to activation domain (AD). 53 effector proteins and mutants fused to binding domain (BD) were screened for interaction against the host protein master plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Rac1	Rac1	RhoG	RhoG	Cdc42	Cdc42	RhoA	RhoA	Rab1	Rab1	Arf1	Arf1
B	Arf6	Arf6										
C	**MAP2K2**	**MAP2K2**	**MAP2K3**	**MAP2K3**	**MAP2K6**	**MAP2K6**	**MAP2K7**	**MAP2K7**	**MAPK1**	**MAPK1**	**MAPK11**	**MAPK11**
D	**MAPK3**	**MAPK3**	**MAPK8**	**MAPK8**			MAP3K7IP1	MAP3K7IP1	CASP1	CASP1	CASP4	CASP4
E	CASP5	CASP5	CASP8	CASP8	CASP9	CASP9	FADD	FADD	IKBKB	IKBKB	IKBKG	IKBKG
F	MYD88	MYD88	MALT1	MALT1	NFKBIA	NFKBIA	NOD1	NOD1	NR2C2	NR2C2	PYCARD	PYCARD
G	RELA	RELA	RIPK2	RIPK2	TNIP1	TNIP1	TRADD	TRADD	TRAF6	TRAF6	UBE2N	UBE2N
H	UBE2V1	UBE2V1	TRAF3	TRAF3	TRAF2	TRAF2	TAB1	TAB1	TAB3	TAB3	IKKalpha	IKKalpha

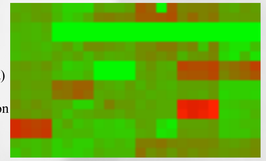

Figure 6. Heat map
SifA x host proteins (2μ)
Red indicate strong growth, hence interaction

Limitations with using the Y2H Assay:

- Use growth as read out
- Able to detect ~30% of known host protein- effector interactions.
- Fails when an effector is toxic to yeast.
- Protein must be able to get into nucleus for Gal4 transcription.
- Protein must have a stable conformation.

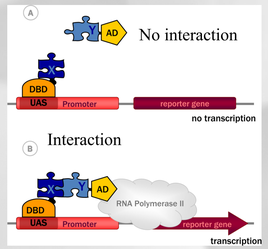

Figure 4. The Y2H Assay using Gal4 promoter

The PIP Assay

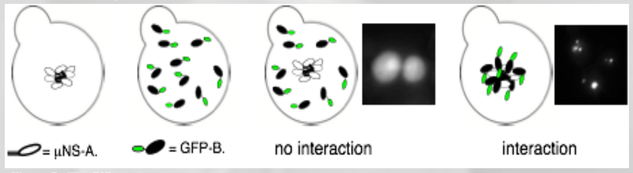

Figure 7. The PIP assay

- Fluorescence: GFP is fused to a protein and uNS is fused to another protein. Interaction between two proteins creates a foci that can be visualized using fluorescence microscopy.
- Interaction is not limited to just the nucleus- can happen anywhere in the cell
- May be able to capture more transient interactions.

Reflection (Conclusions)

The most valuable lesson I learned on this co-op is how to deal with failure. Failure is associated with such negativity that I'd rather never identify with. Yet, in a professional setting, failure is unavoidable, even for the most experience individuals. Learning to keep modifying the experiment via trial and error and using controls to find the source of the problem is a skill that will be beneficial in future work place and in everyday life.

Additionally, I learned to design experiments and write my own protocol using skills I have learned from biochemistry and genetics classes.

Science never stops. There's always a next project that builds on our current findings to advance our knowledge. For example, further experiments are needed to verify the accuracy and validity of the PIP Assay and we need to find ways to justify the new interactions through other protein interaction assays.

Acknowledgments

This co-op experience is made awesome through the Lesser Lab:

Cammie Lesser MD PhD., Analise Reeves PhD., Nadja Ernst, Xiangyu Mou PhD., Neha Godbole and Kristen Fang