



CONTENTS

About us

- > Edito: Life after a Yeast Two-Hybrid Screen
- > 1by1 Y2H
- > 1by1 HTRF

News

- Events
- Interview

NEWS

Hybrigenics has created a **new PBS F category** that represents proven (tested in our labs) Y2H technical artifacts. Technical artifacts were one of the three categories of highly connected proteins previously contained among PBS E interactions. This new PBS adds even more value to the in-depth analysis of your preys.

We have also decided to correlate the threshold for considering an interaction as highly connected (PBS E) to the intensity of library screening. In particular, libraries from human, Drosophila, mouse and Arabidopsis have been screened intensively and we have **increased their threshold for high connectivity to 10**.



EXPERTISE #3

hybrigenics services | Newsletter June 2008

LIFE AFTER A YEAST TWO-HYBRID SCREEN

When I started my Ph.D. in 1995, I was trained to perform a Y2H screen the “hard way” (by sequential transformation): transform the bait in yeast, test it for autoactivation, then transform the library in the same yeast cells, pick, grow and lyse the positive clones obtained on selective medium, transform the yeast extracts in a not-so-easy-bacteria-to-work-with, spread, select and grow the prey plasmid containing bacteria, mini prep and sequence.

Thanks to ULTImate Y2H™, this cumbersome work is now performed with the most reliable screening tools to date, and prey sequences are analyzed with the most sophisticated bioinformatics. In 86 days on average, bait cloning included!

Now that the screening is done, what's next? We offer a series of pairwise testing tools in yeast called 1-by-1 Y2H. These tools will allow you to illustrate your interactions for publication and to quantify and compare the binding between your wild-type bait, isoforms or point mutants and your preys. We also propose 1-by-1 HTRF® (Homogeneous Time-Resolved FRET) as our preferred choice to confirm your interactions with another detection system.

Because we have been dealing with Y2H screens for 15 years, we know what researchers need afterwards !

Luc Selig, Ph.D.
Director, Sales and Marketing

1-by-1 Y2H: ILLUSTRATE, TEST AND COMPARE INTERACTIONS

PUBLICATION-READY INTERACTIONS

To illustrate your screening data, we start with preys extracted from yeast and sequence checked. They are retransformed individually with your bait, co-transformants are grown, and drops at various serial dilutions are deposited by a robot on solid plates containing selective media. The interactions are tested at various 3-amino triazole (3-AT) concentrations to semi-quantify the interaction strength.

As always, several controls are included : growth control in a non-selective medium, autoactivation of the bait and of the preys, positive and negative interaction controls. As deliverables, we provide you with solid growth assay pictures together with the Mat&Meths.

EVENTS

We will be present at the **ASPB Plant Biology 2008** meeting, June 26th – July 1st in Merida, Mexico. Please **visit us at booth 412!**

To improve our level of service to the plant community, we have constructed a new ***Arabidopsis thaliana* flower library**. This library contains 10 million independent and primary random-primed cDNA clones in yeast, and is ready for cell-to-cell mating. The first screen results of the *A. thaliana* flower library will be presented at the ASPB meeting.

With the increasing interest of biofuels, we are in the process of constructing an *A. thaliana* silique (seed) library. More plant libraries are to come: rice, *Nicotiana benthamiana*... do not hesitate to enquire about your favorite library.



INTERVIEW

Vincent Collura, Bioinformatics Support, Hybrigenics Services

"Hybrigenics has spent 10 years developing sophisticated tools for the automatic analysis of ULTImate Y2H screen data. The analyses have now reached a high level of complexity and maturity. My daily mission is to answer specific questions from customers who would like to know what lies behind our algorithms for PBS computation and prey identification in particular. I appreciate customers' feedback very much."



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TEST THE INTERACTION BETWEEN CANDIDATE PROTEINS

Should the suspected mechanism of action (MOA) of a protein involve its interaction with another candidate protein, the same protocol can be applied to the proteins that are suspected to interact together.

In the case of an interaction that has been found using biochemical interaction assays (GST, IP...), 1-by-1 Y2H is a straightforward method to confirm the previously observed interactions.

COMPARE THE INTERACTION STRENGTH BETWEEN WT and MUTANTS

Cell biologists have been using mutants extensively to study the functions of their favorite protein in cells, and when a mutant of function is isolated the next question is: "how different is its MOA compared to the wild-type protein?" One way to address this question is to look at the differences in the binding of a wt protein and a mutant (or isoform) with the preys that came out of the screening with the bait. Correlating the loss of function with the loss of an interaction is surely a big step towards the understanding of the bait's MOA.

In addition to, or separately from, the solid growth assay that is depicted in the above paragraphs, we can perform a lacZ colorimetric test to quantify the binding between a bait and its relatives, and selected preys. A graphical output of the colorimetric tests, including positive and negative controls, will be provided to you together with the Mat&Meths.

1-by-1 HTRF: CONFIRM YOUR Y2H INTERACTIONS *in vitro*

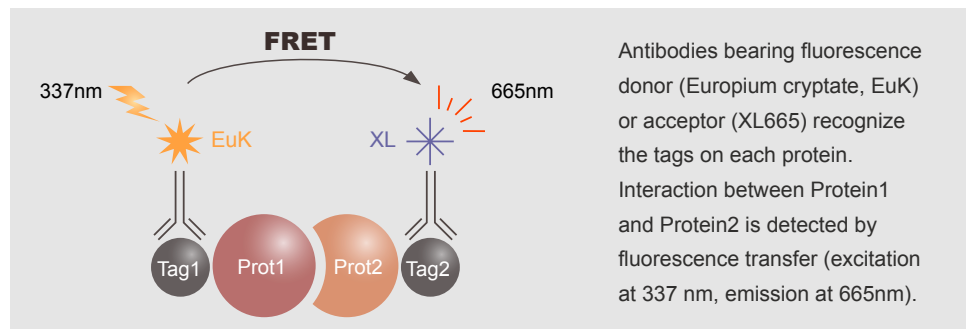
2 TECHNOLOGIES BETTER THAN 1

Frequently after ULTImate Y2H we are asked if we can "confirm" the interaction using 1-by-1 Y2H between the bait and a few promising preys. Indeed, when the now old-fashioned sequential transformation of the library was in use, it was common to extract the prey clones and retransform them with the bait to check for the pertinence of the interaction. We do not recommend performing any of these steps except for PBS D interactions which may contain background noise.

Because our cell-to-cell mating protocol to screen libraries is much more reliable than sequential transformation, each time we have re tested an interaction with a PBS A, B or C coming from an ULTImate Y2H screen with 1-by-1 Y2H, the interaction came positive again. That's why we do not usually propose 1-by-1 Y2H to "confirm" an interaction found with ULTImate Y2H. Besides, peer-reviewed journals will ask you to test the interaction with another detection system, the reason being that different technologies show different false positives and false negatives.

WE HAVE CHOSEN HTRF

Among GST pulldowns, Co-IP, TAP-TAG and other biochemical technologies, we like Homogeneous Time-Resolved Fluorescence (HTRF) better. HTRF is based on Time-Resolved FRET (Fluorescence Resonance Energy Transfer), a combination of FRET chemistry and the use of fluorophores with long emission half-lives. The use of a radiometric measurement enables correction for quenching and sample interferences.



WHAT WE OFFER

Your two proteins or protein domains are produced in bacteria as fusions to different tags, and labeled either directly or indirectly with anti-tag antibodies coupled to a fluorescence donor and acceptor.

Our multi-tag approach and the use of the two donor-acceptor orientations for each protein pair allow investigating systematically a large panel of combinations, thus increasing the chance of proper protein folding and fluorescence transfer.

Because the assay is homogeneous and requires only low nanomolar concentrations of proteins, multiple interactions can be evaluated simultaneously in a 384-well format.

This makes 1-by-1 HTRF the perfect complement to confirm ULTImate Y2H interactions. Our Y2H and HTRF plasmids are fully compatible for direct and efficient subcloning.

Don't hesitate, contact us to confirm your interactions!

**PROTEIN INTERACTIONS,
WHO ELSE CAN DO IT?**