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NEWS

Paris, December 1st, 2008 – Hybrigenics receives EU funding for protein interaction assay development and chemical screening

A consortium of 16 academic labs and biotech companies, coordinated by Pr. Tom Huizinga (Leiden University Medical Center, NL), will receive a 5-year grant in the European '7th Framework program' scheme for a project tackling chronic inflammatory diseases.

As a partner, Hybrigenics will develop a high throughput protein interaction assay and screen its chemical libraries to identify small-molecule inhibitors of a therapeutically relevant interaction.

A hit-to-lead optimisation program will then be conducted in close collaboration with Dr. Ponchel (Leeds Institute of Molecular Medicine, UK) and Pr. Aspinall (Translational Medicine, Cranfield University, UK).

Happy New Year 2009

EXPERTISE #4

hybrigenics services | Newsletter January 2009

ACCEPT ONLY EXHAUSTIVITY

Exhaustivity : 'Treating all parts or aspects without omission'

Exhaustive : 'Performed comprehensively and completely'

Making sure that we have not missed anything is what we all need when screening. What are the components of exhaustivity? What are the benefits for the users?

Find out in this Newsletter that exhaustivity not only permits the discovery of many more interactions than what Y2H reagent kits allow but is also the necessary basis to identify false positives. One thing is to miss interactions, another is to work 3 years on a fake one!

Do you intend to use highly-complex randomly generated fragments and a powerful screening technology?

Exhaustivity is what you should always look for.

Luc Selig, Ph.D

Director, Sales and Marketing

WHY IS ULTImate Y2H™ THE ONLY EXHAUSTIVE SCREENING TECHNOLOGY

PREFER RANDOM PROTEIN DOMAINS

Sources of missed interactions include incorrect folding and expression, inappropriate subcellular localization and toxicity of the proteins.

To circumvent these limitations, we use a domain-based strategy to construct random-primed (RP) cDNA libraries.

Several random fragments of each mRNA transcript are generated, increasing the probability to test properly folded and localized, correctly expressed and not toxic protein domains.

Randomness does not prevent high selectivity and high specificity (overlapping fragments containing the interaction domain). Indeed, from 97 million interactions that are tested per screen, 114 prey fragments are analyzed on average (selectivity of 1 in 1 million).



To further demonstrate the **absolute benefit of RP fragments over oligo-dT-primed cDNA libraries** (biased towards full-length proteins and C-terminal fragments), we have screened a dozen of baits with RP and oligo-dT libraries (same starting RNAs, same final complexity) and have identified more families of proteins with the RP libraries.

When dealing with protein-protein interactions, one must think that the functional entity at stake is a protein domain, not a protein as a whole.

HIGHLY-COMPLEX LIBRARIES: A MUST HAVE

If the prey protein that interacts with your bait is not represented in the library you want to screen, it is the most obvious reason to miss it. For the human genome, as many as 25,000 genes are predicted. Alternative gene splicing events are predicted for 5% to 75% of the genes, significantly increasing the diversity of transcripts in a cell.

Typically, at least 10E6 independent full-length cDNA clones are required to ensure that low-abundance transcripts are represented in the library.

RP-generated libraries must be even more complex to represent their higher diversity. All random-primed cDNA libraries we offer for screening contain a minimum of **10 million independent and primary fragments** in ready-to-be-mated yeast cells. Rare transcripts will not be present in cDNA libraries from higher eukaryotes with lower complexities.

ALL PROTEINS MUST BE TESTED FOR INTERACTION

To ensure exhaustive screening, libraries must be screened to saturation. Statistically, there is a 98% probability that all fragments are tested at least once when the **number of tests is 10-fold greater than the complexity of the library**.

In other words, when Hybrigenics tests 97 million interactions per screen on average, it means that **98% of each individual fragment are tested at least once!**

This extremely high number of interactions tests can only be obtained with the optimized and patented cell-to-cell mating procedure of ULTimate Y2H. Sequential transformation or lousy mating will omit many interactions.

THE BENEFITS OF EXHAUSTIVITY

DON'T MISS ANY INTERACTIONS

Exhaustive screening is a combination of using highly complex libraries containing randomly generated fragments, screening these fragments to saturation, and – of course – analyzing all positive clones selected from the screen. This is the only way not to miss any interactions.

FOCUS ON REAL INTERACTIONS

A confidence score, the Predicted Biological Score (PBS, see Expertise #2), is attributed to each interaction identified in a screen. PBS can only be computed if screens are exhaustive, and allows the ranking of the interactions.

We have published (Rain et al., Nature 2001 and Formstecher et al., Genome Research 2005) that **high scores (A, B and C) correlate with the occurrence of the interactions in their cellular context**. Exhaustivity tells you which interactions are more likely to exist *in vivo!*

STOP WORKING ON FALSE RESULTS

We have performed more than 4,000 ULTimate Y2H screens. The prey proteins of a new screen are systematically compared to the ones present in our database and found in libraries from the same organism.

Prey proteins found many times in many other exhaustive screens are flagged for low specificity and technical false positives are further tested and confirmed in our labs.

Exhaustivity saves you a tremendous amount of time and money.

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

EVENTS

We were present at the **ASCB Cell Biology annual meeting December 13th – 17th in San Francisco, CA, USA.**

We took this opportunity to visit customers at UC Davis, Berkeley and Stanford. Our favorite duo (a protein interaction expert and a sales engineer) had also one-to-one meetings to discuss new projects on protein interactions. In 2009, we will be attending meetings in the following cities: Berlin, Denver, Lille, Vienna, Chicago. Scotland and Italy will also be part of our destinations.

Should you wish to be visited, **please contact us**



INTERVIEW

Laura Watremez, Yeast Production Lab, Hybrigenics Services

“Scientific research is a day to day engagement. My aim is to find the most pertinent answers to scientific needs. Knowing that a major discovery can come out of the ULTimate Y2H exhaustive screens I perform every day brings an additional satisfaction”.



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