Direct Targets Identification of a Bioactive Compound using Chemical Biology

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ABSTRACT

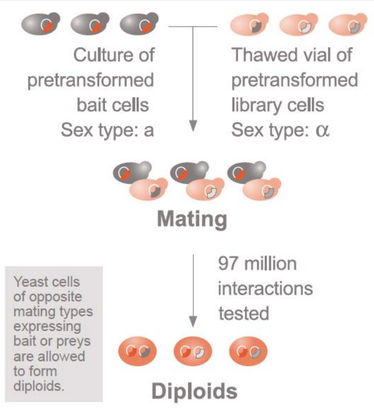
Identifying protein partners of a small bioactive molecule is of great interest in many aspects of life sciences and specifically in the drug discovery and development process cycle. It is a support to (i) decipher the mechanism of action after for example a “High Content” screening, (ii) study “off-target” effects, (iii) adjust therapeutic indications and clinical regimens of a drug and (iv) consider drug repositioning.

ULTImate YChemH is a chemical biology tool for direct targets identification. This method is based on the well-established yeast two-hybrid (Y2H) technology. The small molecule of interest is used as a bait to screen highly complex protein domain libraries. The small molecule - protein interactions are detected thanks to the reconstitution of an active transcription factor from DNA Binding Domain (DBD) and Activation Domain (AD) moieties. When an interaction takes place, the bait derivative bridges the gap between the DBD and the AD. This enables the expression of the reporter gene and subsequent yeast growth on a selective medium. Positive clones are then analyzed by sequencing to identify the protein partners.

One example with a hit coming from a “black box” screen highlights how this technology can be easily applied for target identification.

Hybrigenics’ ULTImate Y2H™ HERITAGE

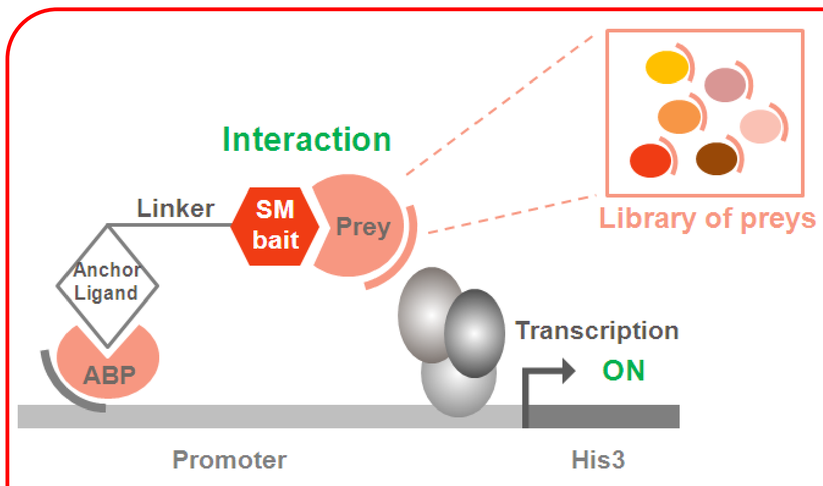
- **Random-primed cDNA library screening approach** (domain-based, no full-length matrix pair-wise testing of ORFs)
- **Highly complex libraries to ensure representation of all mRNAs** (≥ 10 million fragments in yeast of 800 bp on average)
- **Exhaustive and reproducible screening :** cell-to-cell mating enabling the routine testing of about 100 million interactions per screen (10-fold library coverage)
- **Delineation of a minimal interacting domain (SID)** from prey fragments identified
- **Computation of a confidence score** (PBS) for each interaction



WHAT FOR ?

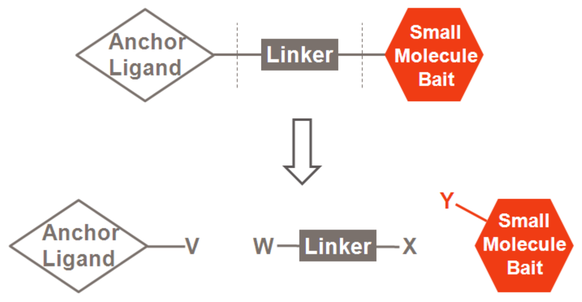
- ✓Identification of “hit” targets and deciphering mechanisms of action following a high content screening
- ✓Study of “off-target” effect(s) of an active compound
- ✓Safety evaluation of chemicals (Toxico Proteomics)
- ✓Drug repositioning support in new therapeutic areas

ULTImate YChemH PRINCIPLE

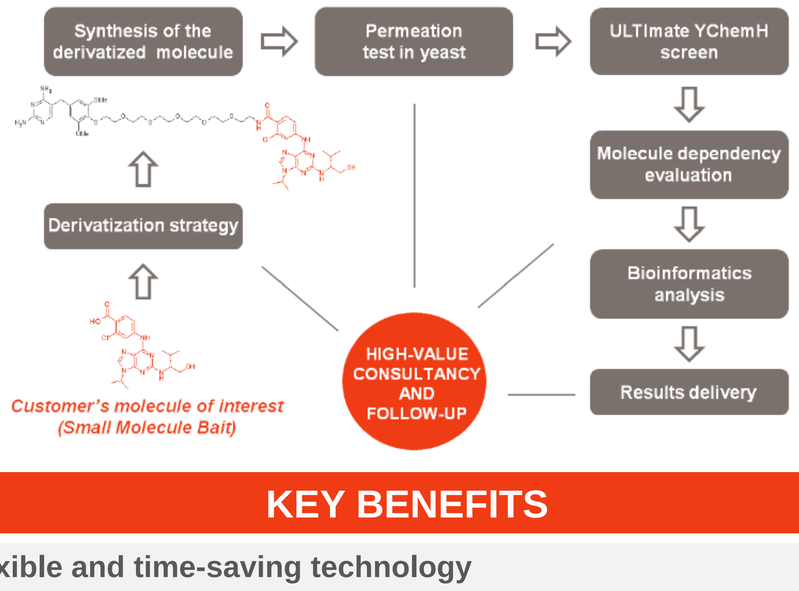


SPECIFIC TOOLS DEVELOPED FOR ULTImate YChemH

- ➢**2 Anchor systems**
  - Dehydrofolate reductase (DHFR) / Trimethoprim (TMP)
  - SNAP-Tag / Benzylguanine (BG) derivative (1)
- ➢**Yeast strain permeability optimized**
- ➢**Derivatization of the Bioactive Molecule**
  - Several attachment points considered
  - Building blocks readily available
  - Coupling reactions optimized



WORKFLOW

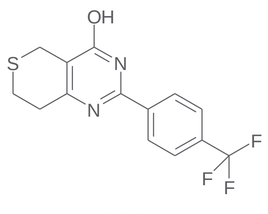


KEY BENEFITS

- ➢**Flexible and time-saving technology**
- ➢**Gain in productivity** with additional resources
- ➢**Benefit from our expertise** in both chemistry and biology
- ➢**Understand** how your bioactive molecule works
- ➢Select **the most promising molecule** & develop it **the optimal way**

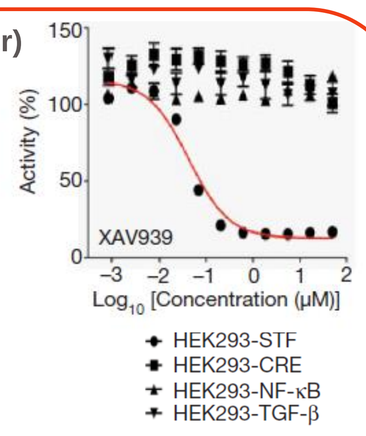
TARGET IDENTIFICATION OF A HIT FROM A BLACK BOX SCREEN (HCS)

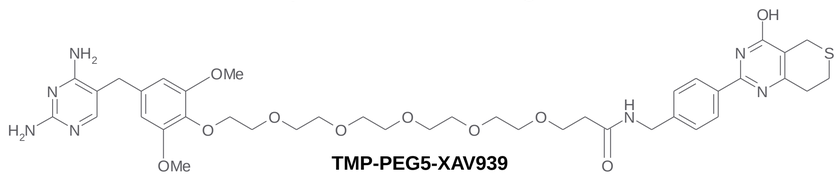
Black box screen on Wnt pathway (transcriptional reporter)



XAV939

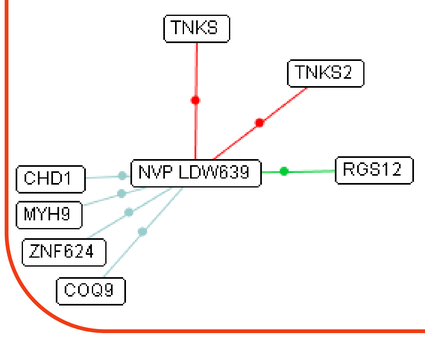
- •XAV939 was identified as a small molecule inhibitor of the Wnt/β-cathenin patway from a HTS using a Wnt-responsive luciferase reporter assay in HEK293 cells (2).
- •To identify cellular efficacy targets of XAV939, Huang et al. used a three-channel iTRAQ quantitative chemical proteomic approach: immobilization of bioactive analogue of XAV939, and competition experiments.
- •Our Ultimate YChemH approach allowed within only one screen experiment to identify TNKS1 and TNKS2 as partners with a high confidence score





Library of cDNA Preys	Human placenta
Anchor Binding Protein	DHFR
Protocol	mating
CID concentration	20µM
# of interactions tested	132 10^6
# of clones	346

2 Protein partners (TNKS1 and TNKS2) identified with a PBS A



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1) C. Chidley, *et al.* H. Haruki, M. Gronlund Pedersen, E. Muller, K. Johnsson, A yeast-based screen reveals that sulfazalazine inhibits tetrahydrobiopterin biosynthesis, *Nat. Chem. Biology*, **2011**, *7*, 375-383

2) S-M. Huang, *et al.* , Tankirase inhibition stabilizes axin and antagonizes Wnt signalling, *Nature*, **2009**, *461*, 614-620.