The protein interactions expert
Dear colleague,

Welcome to our new catalog of services.

Whether you are involved in basic, pharmaceutical, cosmetic or agro-biotech research, we know that deciphering protein interactions is key for your projects.

We now bring you a full range of protein interaction services, with a vast array of applications: from understanding the mechanisms of cell physiology to identifying new intervention targets and uncovering the mode of action of drugs.

With over 7,000 projects performed on 20 species, and more than 1,000 academic and industrial customers worldwide, Hybrigenics Services enjoy an unparalleled commercial and scientific recognition.

We look forward to sharing this expertise with you to advance your research.

Yours sincerely,

Etienne Formstecher,
Deputy General Manager, Hybrigenics Services.

Visit our website

www.hybrigenics-services.com
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Assay development
High-throughput screening
Hit-to-lead services
EXPERTISE
With more than 15 years of experience, Hybrigenics Services is the leading provider of services to study protein interactions.
Our expertise extends to all kind of protein interaction techniques, from a high-quality yeast two-hybrid platform to time-resolved FRET and Surface Plasmon Resonance.
Protein interactions with DNA, short RNA and small molecules can also be investigated using the same protein domain libraries and ISO 9001-2000 quality-certified platform.
All projects benefit from our unique bioinformatics capabilities and scientific support for time-saving analysis and publication-grade presentation of the results.

DEDICATION
We are proud to serve academic and industrial researchers from all life science areas.
With an ever-growing list of more than 60 libraries available, prepared from various organisms, tissues or cell types, we strive to meet the exact needs of our customers, from plant research to neurobiology, host-pathogen interactions or cancer biology.
Over 200 publications in top ranking journals based on the results we delivered are our best track record.

OUR RANGES OF SERVICES
Our comprehensive ranges allow you to:
> DISCOVER the protein partners of any protein, DNA, short RNA or small molecule
> VALIDATE interactions in vitro and in cells, map critical domains and amino acids
> INHIBIT interactions with small molecules or natural products
"I was extremely pleased with a recent yeast two-hybrid screen that was conducted for our laboratory by Hybrigenics. Their technology is phenomenal and yielded impressive results that are sure to give us fascinating projects for years to come. Every member of the staff that I contacted was very professional, knowledgeable and friendly on all occasions. I certainly recommend the Hybrigenics services."

Clifford Toleman,
St. Jude Children’s Research Hospital, USA
ULTImate Y2H™ is an optimized version of the yeast two-hybrid (Y2H) screening technique that tackles most of the false positives and false negatives issues arising from the original protocol developed by Stanley Fields in 1989. Unlike classical Y2H sequential transformation protocols, ULTImate Y2H™ is derived from a unique and patented cell-to-cell mating process that allows to test 83 million interactions on average per screen (2011 average number).

HIGHLY COMPLEX LIBRARIES SCREENED TO SATURATION

• Choose from over 60 cDNA and genomic libraries or ask for a custom library
• Benefit from random-primed cDNA libraries of 10 million primary clones in yeast
• All libraries screened to saturation by covering on average 10-fold their complexity

THE CONFIDENCE SCORE

Hybrigenics assigns to each protein interaction a statistical confidence score, the Predicted Biological Score (PBS™). Interacting proteins are ranked according to technical parameters such as the number of independent prey fragments and information derived from all screens performed at Hybrigenics on the same organism.

The PBS™ is computed as an e-value and thresholds are attributed to define categories from high confidence (A) to lower confidence (D) interactions, with E and F as distinct categories flagging highly connected prey domains and technical false positives, respectively.

DELIVERABLES

The results of each screen are displayed in three different files:
• A results summary with prey identity and Predicted Biological Score (PBS™)
• An Excel spreadsheet containing raw data with prey clone sequences
• A ‘DomSight’ file providing a graphical comparison of the smallest interacting domains (SID™) with the functional domains present in the preys
**Molecules Tested**
- Full-length proteins or fragments, peptides
- Cytoplasmic or extracellular proteins
- Loops and tails of membrane proteins

**What For?**
To discover novel protein partners of your favorite protein

**Key Benefits**
- Fast and exhaustive screening of highly complex libraries thanks to Hybrigenics patented cell-to-cell mating protocol
- Detection of even weak interactions and interactions from the rarest transcripts
- Full sophisticated bioinformatics analysis of the results including confidence scores
- Up to 380 positive clones analyzed (5’ and 3’ sequences)
- An additional screening included if the initial results provide less than 20 positive clones
- Scientific assistance for the best outcome of your project

**References**
- And since then over 200 publications based on Hybrigenics technology in top-ranking journals

**Screening Services for Specific Protein Baits**
We offer **ULTiMate Y2H+1**, a modified version of **ULTiMate Y2H™** that allows the expression in yeast of any co-factor required for the proper folding or post-translational modification of the bait.

Should you wish to identify the interacting partners of an integral transmembrane (TM) protein, you can take advantage of **MBMate Y2H**. Based on the split-ubiquitin system from Dualsystems Biotech AG®, this unique screening service is the only solution to screen full TM baits.

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**Example of deliverable: Extract of the ‘DomSight’ file**

**Your Bait**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
<th>Percent Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMAD5</td>
<td></td>
<td>267/465</td>
</tr>
</tbody>
</table>

**Prey 1**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td></td>
<td>225/314</td>
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</tbody>
</table>

**Prey 2**

<table>
<thead>
<tr>
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<th>Percent Complete</th>
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</thead>
<tbody>
<tr>
<td>PP1N2</td>
<td></td>
<td>98/336</td>
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</tbody>
</table>

**Prey 3**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
<th>Percent Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRM1</td>
<td></td>
<td>93/364</td>
</tr>
</tbody>
</table>

**Prey 4**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
<th>Percent Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMAD/RIA</td>
<td></td>
<td>172/329</td>
</tr>
</tbody>
</table>

**Legend**

- **Bait Fragment**
- **SID Fragment**
- **Known Domain (INTERPRO, PFAM, SMART...)**
- **Transmembrane Domain**
- **Cgo/Dco (cylindrical) Domain**
- **Signal Peptide**

**SID: Selected Interaction Domain** - It is the amino acid sequence shared by all prey fragments matching the same reference protein. SIDs have been found in numerous cases to correspond to a known structural or functional domain.
ULTImate YChemH (YeastChemHybrid) is a chemical proteomics technique which allows to identify protein partners of a given bioactive small molecule. It is adapted from Hybrigenics ULTImate Y2H™ technology and takes advantage of its key benefits.

**WHAT FOR?**
To identify the protein partners of a small molecule:
- Identify “hit” targets and decipher mechanisms of action following a high-content screening
- Study the “off-target” effects of an active compound
- Support drug repositioning in new therapeutic areas
- Evaluate safety of chemicals (toxico-proteomics)

**KEY BENEFITS**
- Exhaustive screening of highly complex protein fragment libraries
- Multi-anchor and multi-linker approach to optimize the accessibility of the molecule of interest
- Detection of even weak “small molecule-protein” interactions
- Full sophisticated bioinformatics analysis of the results including confidence scores
- Flexible and time-saving technology

**REFERENCES**

**EXAMPLE OF A BAIT DERIVATIVE**

**HOW DOES IT WORK?**
ULTImate YChemH is based on the Y2H principle that consists in the detection of protein interactions thanks to the reconstitution of an active transcription factor from DNA Binding Domain and Activation Domain moieties. For ULTImate YChemH, three hybrid molecules are used:
- A hybrid protein containing a DNA Binding Domain (DBD) fused to a small molecule Anchor Binding Domain (ABD)
- A hybrid protein containing a transcriptional Activation Domain (AD) fused to a ‘prey’ protein fragment from the library
- A bait derivative molecule consisting of three components, an “anchor” with an affinity for the ABD, the small molecule of interest (“bait”) and a “linker” which covalently links these together

When a “small molecule-prey protein” interaction takes place, the bivalent hybrid molecule bridges the gap between the DBD and the AD enabling the expression of the reporter gene in yeast. The DNA of the positive clones is then sequenced and analyzed to identify the protein partners.

For more details about the Y2H technology, please refer to the ULTImate Y2H™ pages (p.8-9).

**DELIVERABLES**
Deliverables are similar to those for ULTImate Y2H™ (p.8-9) and include the list of protein partners, the interacting domains and confidence scores.
ULTimate RNA Y3H (RNA yeast three-hybrid) derives from the ULTImate Y2H™ technology with a RNA molecule as a bait. It allows to screen highly complex domain-based libraries for protein partners of any short RNA.

**HOW DOES IT WORK?**

ULTimate RNA Y3H is based on the Y2H principle that consists of the detection of ‘bait-prey’ interactions thanks to the reconstitution of a full transcription factor from DNA Binding Domain and Activation Domain moieties. In the RNA Y3H approach, three hybrid molecules are used:

- A hybrid protein containing a DNA Binding Domain (DBD) fused to a RNA Binding Domain (RBD)
- A hybrid protein containing a transcriptional Activation Domain (AD) fused to a ‘prey’ protein fragment from the library
- A hybrid RNA molecule consisting of two RNA fragments, the RNA anchor interacting with RBD and the RNA bait

Upon interaction of the RNA bait with the prey, the gap between the DBD and the AD is bridged, enabling the transcription of the reporter gene in the yeast cells. The DNA of the positive clones is then sequenced and analyzed to identify the protein partners.

For more details about the Y2H technology, please refer to the ULTImate Y2H™ pages (p.8-9).

**DELIVERABLES**

Deliverables and information on the protein partners identified are similar to those for ULTImate Y2H™ (p.8-9).

**MOLECULES TESTED**

- Short RNA

**WHAT FOR?**

To identify and characterize RNA-protein interactions

**KEY BENEFITS**

- Fast and exhaustive screening thanks to Hybrigenics patented cell-to-cell mating protocol
- Detection of even weak interactions and interactions with proteins whose transcripts are rare
- Full sophisticated bioinformatics analysis of the results including confidence scores
- Up to 380 positive clones analyzed (5' and 3' sequences)
- Scientific assistance for the best outcome of your project

**REFERENCES**


**ASSOCIATED SERVICE**

- Domain libraries for ULTImate screening (p.13)
ULTImate Y1H

MOLECULES TESTED
• DNA sequences (e.g. promoter regions)

WHAT FOR?
To identify and characterize DNA-protein interactions

KEY BENEFITS
• Fast and exhaustive screening thanks to Hybrigenics patented cell-to-cell mating protocol
• Detection of even weak interactions and interactions with proteins whose transcripts are rare
• Full sophisticated bioinformatics analysis of the results including confidence scores
• Up to 380 positive clones analyzed (5’ and 3’ sequences)
• Scientific assistance for the best outcome of your project

REFERENCES
• Li, J.J. and Herskowitz, I. Use of a one-hybrid system to identity and clone ORC6, a gene encoding the 50kd subunit of the yeast origin recognition complex. Science 262, 1870-1874 (1993)

ASSOCIATED SERVICE
• Domain libraries for ULTImate screening (p.13)

ULTImate Y1H (yeast one-hybrid) derives from the ULTImate Y2H™ technology with a DNA sequence as a bait. The technique tests the interaction between this DNA bait and a library of protein fragments.

HOW DOES IT WORK?
To conduct an ULTImate Y1H assay, a DNA sequence of interest, the ‘DNA bait’, is first cloned upstream of a reporter gene to create a ‘DNA bait - reporter’ construct. The ‘DNA bait’ attached to the reporter is integrated into the genome of a dedicated yeast strain by site-specific recombination. A cell-to-cell mating is then performed between the ‘DNA bait - reporter’ yeast strain and a yeast strain pre-transformed with a high complexity domain library to identify protein partners of the ‘DNA bait’. For more details about the Y2H™ technology, please refer to the ULTImate Y2H™ pages (p.8-9).

DELIVERABLES
Deliverables and information on the protein partners identified are similar to those for ULTImate Y2H™ (p.8-9).
Over 60 libraries are ready to screen. Custom libraries that meet your need can be constructed on demand.

**LIST OF AVAILABLE LIBRARIES**

**HUMAN**
- **Tissues**
  - Brain adult; Brain fetal
  - Breast
  - Colon
  - Heart adult/fetal
  - Liver
  - Lung
  - Muscle skeletal adult/fetal
  - Pancreas
  - Placenta
  - Reconstituted skin (EpiSkin)
  - Retina
  - Testis
- **Primary cells**
  - Fibroblasts
  - Leukocytes + activated mononuclear cells
  - Melanocytes
  - Thymocytes (CD4+ and CD8+)
- **Cell lines**
  - B cell lymphoma
  - Bone marrow endothelial cells
  - Breast tumor epithelial cells
  - Brown adipocytes
  - Colon tumor epithelial cells
  - HeLa cells
  - Lung tumor cells
  - Pre-adipocytes
  - T-cells
- **RODENT**
  - **Mouse**
    - Brain adult; Brain embryo; Neurospheres adult
    - Embryonic stem cells
    - Inner ear
    - Kidney
    - Pancreatic beta-cells
    - Spleen
    - Rat
    - Hippocampus
    - Rear brain

**MODEL ORGANISMS**
- *Caenorhabditis elegans*: Mixed stages; Embryo
- *Dicyostelium discoideum*
- *Drosophila melanogaster*: Adult head; Embryo; Larvae brain; Ovary; Third instar larvae
- *Medaka (Oryzias latipes)*: Embryo
- *Zebrafish (Danio rerio)*: Embryo

**FISH**
- Rainbow trout (*Onchorhynchus mykiss*): Embryonic male gonades
- *Arabidopsis thaliana*: Seedlings; Flowers
- *Nicotiana benthamiana*: Mixed Tissues
- *Nicotiana tabacum*: Leaves
- *Rice (Oryza sativa)*: Leaves and Roots
- *Tomato (Solanum lycopersicum)*: Fruits; Meristems, Leaf Primordia, Stems and Floral Buds
- *Wheat (Triticum aestivum)*: Heads, Leaves and Roots

**GREEN ALGAE**
- *Chlamydomonas reinhardtii*

**YEAST**
- *Saccharomyces cerevisiae (Genomic)*
- *Schizosaccharomyces pombe (cDNA)*

**BACTERIA (GENOMIC DNA)**
- *Helicobacter pylori*
- *Escherichia coli*
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

**PARASITES AND HOSTS**
- *Trypanosoma brucei* (Genomic)
- *Aedes albopictus* (cDNA)

**STRUCTURALLY CONSTRAINED PEPTIDES**
- Cysteine-rich
- Proline-rich

Please check our website for an updated list and more details about our libraries.
PIMRider® is Hybrigenics functional proteomics software dedicated to the exploration of Protein Interaction Maps (PIM®). As a visualization interface fed by ULTimate Y2H™ screens results and data available from the literature, PIMRider® is a powerful tool to understand biological functions and pathways.

**PROTEIN VIEWER™ - BROWSE THE LIST OF INTERACTING PROTEINS AND RELATED ANNOTATIONS**
- Browse the list of interacting proteins with their confidence score (PBS™, Predicted Biological Score)
- Access interactions from public interaction databases (e.g. Intact, Biogrid, Mint, etc.)
- Review functional and structural annotations: description, aliases, function, localization, links to bibliographic and external databases, protein sequence and genomic information

**PIM VIEWER™ - RIDE THE INTERACTIONS**
- Explore your PIM®: different graph layouts, path search functions
- Display multiple PIM® for comparative proteomics
- Filter interactions by technology, confidence score, library or subcellular localization
- Save, load and print graphs
**DISCOVER / PIMRider®**

**WHAT FOR?**
- To explore and analyze your protein interaction data in a user-friendly way
- To combine your interaction data with other biological information (e.g. functional annotations, protein interactions from the literature, genetics, expression profile)

**KEY BENEFITS**
- Straightforward analysis and navigation inside complex networks
- Immediate overview of biological connections involving your favorite proteins
- Direct access to protein annotations and publications
- Comparison of interacting and functional domains

You can view a flash presentation of the PIMRider® and browse our published data sets at: [http://pimr.hybrigenics.com](http://pimr.hybrigenics.com)

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**INTERACTION VIEWER™ - REVIEW THE DETAILS OF Y2H EXPERIMENTAL DATA**

- Access each interacting clone and its associated experimental sequence
- Delineate the minimal interacting domain (SID™, Selected Interacting Domain) on each partner

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**DOMAIN VIEWER™ - DISPLAY AND COMPARE INTERACTING, STRUCTURAL AND FUNCTIONAL DOMAINS**
I used HYBRIGENICS 1-by-1 Y2H service to test specific protein-protein interactions. The result was very satisfying. HYBRIGENICS staff was very friendly and helpful, from the design of the protein fragments to the interpretation of the results. It is a pleasant experience to work with them and I would highly recommend HYBRIGENICS to colleagues.

Dr. Paul Wu, Cambridge University, United Kingdom.
**Molecules Tested**
- Full-length wild-type or mutant proteins
- Protein fragments or peptides
- Cytoplasmic or extracellular proteins
- Loops and tails of membrane proteins

**What For?**
- To test the interaction between two candidate proteins
- To compare the binding of wild type and mutant proteins
- To map the exact interacting domain on a protein

**Key Benefits**
- Simple, fast and robust pairwise interaction test
- Quantification of the strength of the interactions
- Deeper characterization of your interaction and the domains involved
- Publication-grade illustrations including all positive and negative controls
- Scientific assistance to select the domains to be tested

**References**

**1-by-1 Y2H & Interaction Domain Mapping Principle**

**How Does It Work?**

1-by-1 Y2H and Interaction Domain Mapping take advantage of our Ultimate Y2H™ Yeast Two-Hybrid technology (see p.8-9).

The two proteins or protein fragments to be tested are cloned in frame with LexA or Gal4 DNA binding domain and Gal4 activation domain. In yeast, the interaction between the two fragments leads to the reconstitution of a functional transcription factor and activates the transcription of HIS3 and LacZ reporter genes. HIS3 allows yeast cells to grow on a medium lacking histidine, whereas LacZ offers quantitative measurement of the interaction’s strength thanks to colorimetric or luminescent β-galactosidase substrates.

To delineate the interaction domain on each protein, multiple fragments are cloned by gap repair in yeast and tested for their interaction with the protein partner.

**Deliverables**
- Full report with electronic pictures of solid growth assays
- Graphs for the quantitative LacZ assays
- Detailed experimental methods

**Associated Services**
- Loss of Affinity Mutants screening (LAM) to identify amino acids which are critical for the interaction (p.19)
- 1-by-1 with HRTF (p.20) and 1-by-1 with BIACORE (p.21)
A Loss of Affinity Mutants (LAM) screening yields point mutants of a protein which have lost their ability to bind their protein partner. LAM takes advantage of Hybrigenics ULTImate Y2H™ technique to functionally screen the mutants in yeast.

**HOW DOES IT WORK?**

A mutagenesis is conducted by PCR and experimental conditions are adapted to each target gene to get a single mutation per clone on average.

The mutant library is then screened against the interacting partner by yeast two-hybrid using the LacZ reporter gene. White or light blue yeast clones in which no or a weak interaction occurs are selected. 192 clones are fully sequenced to delineate their mutation. Their phenotypes are confirmed in a secondary, semi-quantitative LacZ assay. Up to 25 point mutants are then selected by the customer, further characterized in a quantitative colorimetric interaction assay and delivered.

**DELIVERABLES**

Full report with:

- Pictures of the solid LacZ assay
- Nucleic and protenic sequences of 192 mutant clones
- Quantitative colorimetric LacZ results for 25 selected mutants, ready for publication
- Delivery of up to 25 mutants as DNA minipreparations
- Detailed experimental methods

**MOLECULES TESTED**

- Full-length proteins or fragments, peptides
- Cytoplasmic or extracellular proteins
- Loops and tails of membrane proteins

**WHAT FOR?**

- To identify critical amino acids required for your favorite interaction
- To correlate loss of interaction with loss of function in cells

**KEY BENEFITS**

- Powerful point mutant screening method based on ULTImate Y2H™ yeast two-hybrid
- Deeper understanding of your interaction and the amino acids involved
- Generation of invaluable tools for functional studies in cells or in vitro
- Easily launched after an ULTImate Y2H screen or a 1-by-1 Y2H assay

**REFERENCES**


**ASSOCIATED SERVICES**

- 1-by-1 Y2H to test the identified mutants or other protein interactions (p.18)
- High-throughput screening for small molecules inhibiting the interaction (p.25)
1-BY-1 WITH HTRF

MOLECULES TESTED
- Full-length proteins or fragments, peptides
- Cytoplasmic or extracellular proteins
- Loops and tails of membrane proteins

WHAT FOR?
- To test the direct biochemical interaction of two candidate proteins (in vitro)
- To compare the binding of wild type and mutant proteins

KEY BENEFITS
- Highly sensitive homogeneous assay – no washing steps required
- Miniaturized 384-well format requiring only minute amount of proteins
- For each protein pair, simultaneous evaluation of multiple tags and FRET configurations to achieve proper protein folding and fluorescence transfer

Further to an ULTImate Y2H™ screen:
- Confirmation of your ULTImate Y2H™ interaction in an in vitro biochemical system
- Full compatibility of Y2H™ and HRTF* plasmids for fast and efficient subcloning

REFERENCES

1-by-1 with HTRF* is a powerful and highly sensitive interaction assay based on TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer).

**HOW DOES IT WORK?**

The two proteins or fragments tested are fused to different tags and produced in bacteria. Antibodies coupled to a fluorescence donor (Europium cryptate; EuK) or acceptor (XL665, d2), recognize the tags. If the two candidate proteins interact, energy is transferred from the donor to the acceptor upon excitation at 337 nm, and an emission at 665 nm is detected.

Because Europium cryptate has a much longer half-life than standard fluorescence donors, the specific FRET signal can be monitored several dozens of µs after excitation, once the fluorescence background has collapsed. This accounts for 1-by-1 with HTRF* unprecedented sensitivity and robustness.

The assay has been miniaturized in a 384-well homogeneous format, requiring only minute amounts of each candidate protein and no washing steps. As a consequence, multiple tags and TR-FRET configurations can be investigated simultaneously for each protein pair, to achieve proper recombinant protein folding and fluorescence transfer.

Interaction assays set up in this miniaturized format can subsequently be screened against libraries of small molecules to identify bioactive compounds disrupting or modulating the protein interaction (see p.25).

**DELCIVERABLES**

Full report with:
- Detailed experimental procedures
- SDS-PAGE gel pictures
- Raw fluorescence data and publication-grade graphs

**ASSOCIATED SERVICE**

- High-throughput screening for small molecules inhibiting the interaction (p.25)

* Homogeneous Time-Resolved Fluorescence (HTRF®) is a registered trademark of Cisbio International.
1-by-1 with BIACORE® relies on the versatile label-free BIACORE® technique to detect and characterize protein interactions. Based on the Surface Plasmon Resonance (SPR) phenomenon, 1-by-1 with BIACORE® allows to monitor the interactions in real time: association rate ($k_{on}$), dissociation rate ($k_{off}$) and affinity constant of the interaction can be measured.

**HOW DOES IT WORK?**

SPR occurs when polarized light is reflected under certain conditions on the surface of a thin metallic chip. The reflection angle is modified upon binding of biomolecules to the chip. One protein (the ligand) is immobilized on the chip and the putative partner in solution (the analyte) is passed over the surface. Real-time measurements give direct access to the association and dissociation kinetics of the interaction, as well as the affinity.

Other biomolecules such as glycans or small molecules can be tested as ligand or analyte for their ability to form or disrupt interactions.

**DELIVERABLES**

Full report with:
- Detailed description of the protocols
- SDS-PAGE pictures of the purified recombinant proteins
- Association and dissociation data as sensorgrams, $k_{on}$, $k_{off}$ and affinity ($K_d$).

**REFERENCES**

Hybrigenics’ contribution to our HIV target identification and drug discovery programs was absolutely decisive. They identified an interaction between a key HIV enzyme and an obligatory host protein. Then, they set up an in vitro assay for the high throughput screening of this interaction against diverse small molecule libraries. This culminated in the identification of lead compounds that modulate the interaction and inhibit HIV-1 replication in cells. Hybrigenics’ staff is very competent and dedicated. I would highly recommend their outstanding services.

Richard Benarous, Mutabilis, France
ASSAY DEVELOPMENT
FOR SMALL MOLECULE SCREENING

ASSAY TYPES
Protein interaction and enzymatic assays using:
- Homogeneous Time-Resolved Fluorescence (HTRF®)
- Fluorescence Polarization (FP)
- ‘Permeable’ yeast two-hybrid (Y2H)

WHAT FOR?
To set up an assay for the high-throughput screening of small molecule libraries

KEY BENEFITS
- Thorough expertise in protein-protein interactions and enzyme activities
- Flexible offer adapted to your requirements
- High-value scientific assistance thanks to a dedicated team made of a biologist, a chemist and an assay development engineer

REFERENCES

ASSOCIATED SERVICES
- High-Throughput Screening of small molecule libraries (p.25)
- HTS follow-up and Hit-to-lead services (p.26)

DELIVERABLES
- Full assay development report including operating procedures
- Technical support for assay transfer

HOW DOES IT WORK?
The dissociation of a fluorophore-labeled peptide from a protein caused by a small molecule leads to an increase in peptide rotation, resulting in a decrease in polarization upon excitation with polarized light.

HOW DOES IT WORK?
Antibodies bearing fluorescence donor (EuK) or acceptor (XL) recognize the tags on each protein tested. The interaction between protein 1 and protein 2 is detected by energy transfer (excitation at 337nm, emission at 665nm) and suppressed upon inhibition by a small molecule.

HOW DOES IT WORK?
Upon disruption of the interaction between protein 1 and protein 2 by a small molecule, specific reporter genes are no longer transcribed in yeast. To increase compound efficacy, several transporters responsible for drug efflux are down-regulated in Hybrigenics’ ‘permeable’ yeast two-hybrid strains.

* Homogeneous Time-Resolved Fluorescence (HTRF®) is a registered trademark of Cisbio International.
The High-Throughput Screening (HTS) of small molecule libraries is a key method to discover new drug candidates and pharmacological tools. Miniaturized HTS allows to conduct thousands of tests in parallel to identify modulators of protein interactions or enzymatic activity.

Compound libraries available at Hybrigenics include:
- The Prestwick Chemical Library® (FDA approved drugs)
- Hybrigenics highly diverse library of 100,000 drug-like molecules, built from multiple providers.
- A ready-to-screen representative subset of 10,000 molecules
- A selection of 2,000 pure natural compounds
- A selection of 2,000 natural product mimetics
- A Protein-Protein Interaction (PPI) focussed library

**DELIVERABLES**
- Full report detailing the results at each screening step
- Chemical database displaying biological activity and structures for all screened compounds in standard formats
- Detailed raw data

**HTS process**
- Automation of the assay
- Primary screening
- Cherry-picking and confirmation assay
- Analytical validation
- Results delivery

- Statistical quality assessment using Z’ and Z factors
- Selection of primary hits
- List of confirmed hits
- List of validated hits

**WHAT FOR?**
To discover small molecules modulating a protein interaction or an enzymatic activity

**LIBRARIES AVAILABLE**
- Hybrigenics highly diverse libraries of small molecules and collection of natural compounds
- Your own compound library

**KEY BENEFITS**
- State-of-the-art facilities and equipment
- Expert result analysis combining signal and chemical structures
- Flagging of the frequent hitters
- Analytical validation of the hits

**REFERENCES**

**ASSOCIATED SERVICES**
- Assay development for small molecule screening (p.24)
- HTS follow-up and Hit-to-Lead services (p.26)
HIT-TO-LEAD SERVICES

WHAT FOR?
- To further characterize and improve hits from a high-throughput screening
- To test compounds selected by rational drug design, virtual screening or other approaches
- To support your drug discovery program

KEY BENEFITS
- Integrated and flexible solutions to improve primary hits
- Time-saving and cost-effective services
- Expert support from dedicated medicinal chemists and chemo-informaticians

ASSOCIATED SERVICES
- Assay development for small molecule screening (p.24)
- High-Throughput Screening of small molecule libraries (p.25)

Whether you want to characterize and improve hits identified in a high-throughput screening or compounds selected by other techniques, Hybrigenics can assist you with a range of services to search and order commercially available analogs, determine IC50, assess specificity on secondary assays and analyze structure-activity relationships. You can also benefit from our staff’s experience in drug discovery to support your medicinal chemistry program and generate leads from promising hit series.

ANALOGING
Analogs of hits are designed or selected from providers by a medicinal chemist according to different substitution patterns, isosteric replacements or following a structure-activity relationship analysis. They can be further tested on the assay used for the screening or developed for their evaluation.

COMPOND EVALUATION
To further characterize hits from a high-throughput screening or their analogs, IC50 can be determined from dose-response experiments in the same test or in secondary assays. The results will fuel the structure-activity relationship analysis used to rank them.

SECONDARY ASSAY DEVELOPMENT AND SCREENING
Hits and their analogs can be further evaluated in secondary assays involving related proteins, domains or interactions to investigate specificity. Dedicated cell-based assays to confirm compound efficacy and mechanism of action can also be developed using specific cellular models with increased or reduced expression of the target proteins.

STRUCTURE-ACTIVITY RELATIONSHIP (SAR) ANALYSIS
Chemical structures of the hits and their analogs are quantitatively correlated with biological activity to guide the improvement of the series. Other relevant parameters and predictors of “drug-likeness” are taken into account to select compounds for further development.

DELIVERABLES
- Full report with criteria for analog selection
- Detailed experimental procedures for secondary assays
- Chemical database displaying biological activity and structures for all screened compounds in standard formats
At Hybrigenics Services, we know that working in close collaboration with our customers is the best way to share our respective knowledge and to accelerate research.

Each project is followed by a dedicated team composed of a sales engineer and a protein interactions expert, with input from our chemistry and bioinformatics specialists when needed.

We will share our experience and expertise with you for the best design and outcome of your project.

Do you prefer face-to-face discussions? We would be happy to visit you or to welcome you at Hybrigenics!

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Contact us with details on your project, and we will get back to you with a quotation.

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