Pronetbio

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PronetBio

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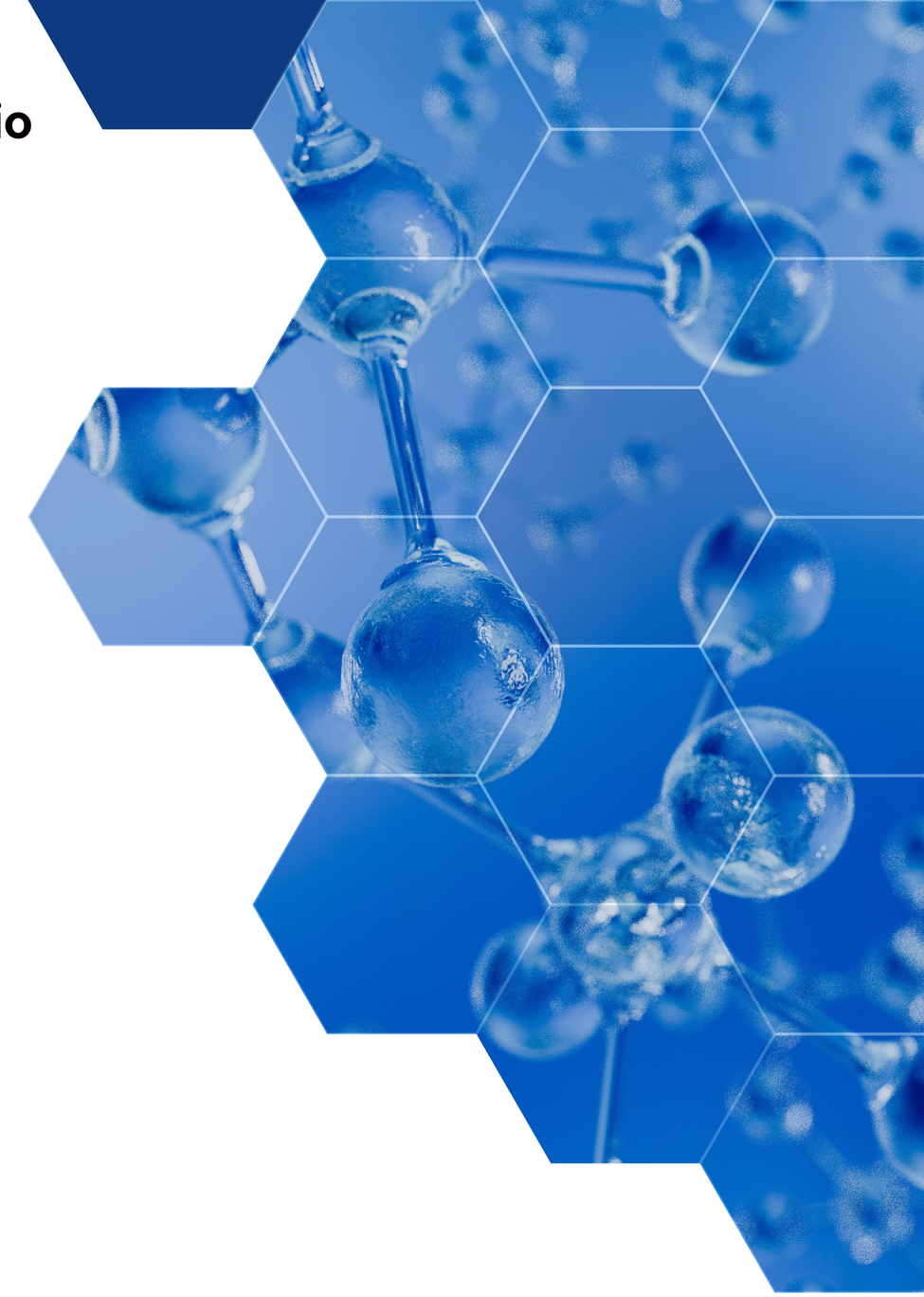

Yeast Technology Service Solutions

Customer-centric and committed to scientific research

Company Profile

ProNet Biotech Co., Ltd. (established in 2019) is located in Jiangsu Life Science and Technology Innovation Park, Qixia District, Nanjing City.

Relying on independent innovation technology platforms such as "high-throughput yeast interaction ", "yeast secretion expression", and "yeast surface display" etc., we provide full chain and scenario application solution for yeast. Our vision is that to lead global biosynthesis towards a high-speed development era, and accelerate the transformation of yeast technology from scientific research to industrial application. We have established the "Institute of Biosynthesis Process Technology" with Nanjing Agricultural University in 2022.

Our R&D break the monopoly and greatly surpass from international yeast technology. We modify yeast strains and optimize and transformation process to solve the application challenges of yeast technology in different scenarios, and enter the billions of yuan biosynthetic market.

We committed to becoming an international leading provider for multi scene technology services in yeast, focus on the fields of Molecular pairing, Antibody discovery, Protein synthesis, and Enzyme engineering, for promoting life science and biotechnology and contributing the new human manufacturing industry.

Current Partners

Catalog

Yeast Technology Platform

The yeast technology platform encompasses a range of techniques and tools that utilize yeast as a versatile model organism for various applications in biology and iotechnology.

Model Organism: Yeast, specifically S. cerevisiae, is a single-celled eukaryotic organism that shares many fundamental cellular processes with higher eukaryotes, including humans.

Genetic Manipulation: Yeast can be easily manipulated genetically, allowing researchers to introduce specific mutations, over-express or delete genes, and study their effects on cellular functions.

Functional Genomics: Large-scale gene deletion collections and mutant libraries are available, allowing systematic analysis of gene function on a genome-wide scale.

Protein Expression: Yeast expression systems offer advantages in terms of protein folding, post-translational modifications, and scalability.

Biotechnology and Industrial Applications: Used in the production of biofuels, pharmaceuticals, industrial enzymes, and food ingredients. Such as yeast surface display and protein engineering techniques, are utilized for directed evolution and protein engineering to optimize enzyme activity or generate novel functionalities.

Synthetic Biology: Yeast serves as a foundational organism for synthetic biology, enabling the design and construction of artificial gene circuits and pathways.

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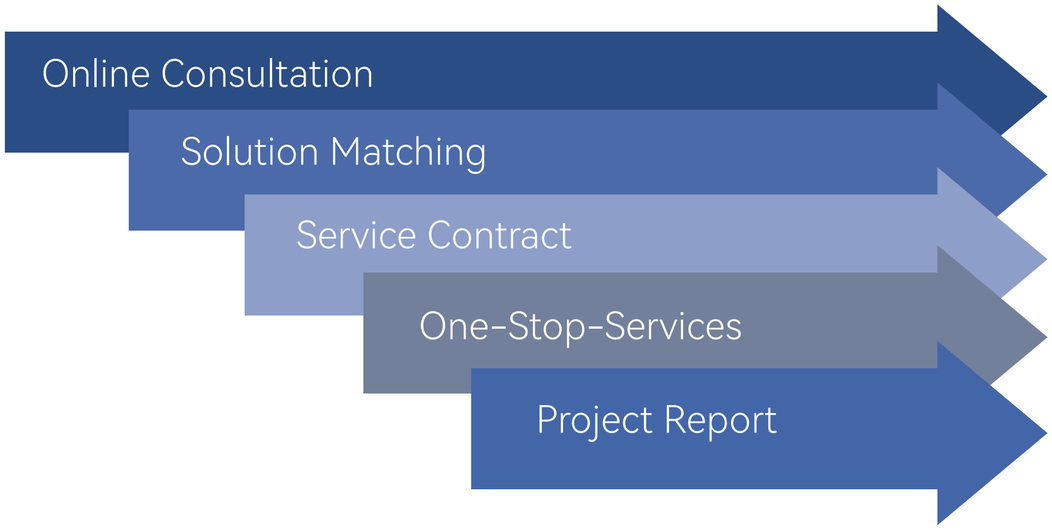
Pronetbio Conventional Yeast Platform

ProNet has a world-leading, complete and constantly updated yeast center technology platform. We provide project services such as yeast one-hybrid, yeast two-hybrid and yeast three-hybrid experiments.

Our Next-generation sequencing, Genome Scanning Analysis and ProS-3D Structural Analysis take scientists' study to the higher level and provide more research directions for downstream experiments.

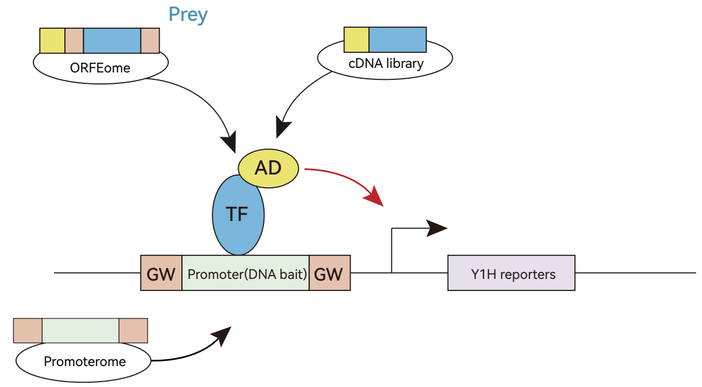
Services Workflow



Gene-centered Y1H (Nuclear System)

Technology

Yeast one-hybrid system is a technique developed from yeast two-hybrid to study DNA-protein interactions, and is widely used to study the regulation of gene expression in eukaryotic cells, such as identifying DNA binding sites to discover potential binding protein genes, analyzing DNA binding structural domain information, etc.



(A Gene-Centered C. elegans Protein-DNA Interaction Network: Cell)

Application

- dentify transcription factors that directly bind to the promoter region of a specific gene, reveal on the regulatory mechanisms involved in gene expression. Identification and characterization of cis-regulatory elements within the pro-
- Identification and characterization of cis-regulatory elements within the promoter region of a gene, such as enhancers or repressors.

  Construction of gene regulatory networks.
- Functional analysis of specific regulatory elements within the promoter region of a gene by mutating or deleting these elements and assessing their impact on protein-DNA interactions.

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Service Features

1. 100% guaranteed to screen out TF (Next-generation sequencing)
2. Ensure at least 5 TF sequences (No Frameshift Mutation)
3. Recommended Whole Sequencing Library

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
1. Gene synthesis (Codon Optimization) /Vector Construction	15 d	Third Party: Price Based on gene length. Price: 0.25 EUR/bp, usually ≥ 200 EUR; Vector Construction 300 EUR/ One Vector	
2. Self-activation of bait plasmid	10 d		
3. Co-transfer Bait plasmid and library plasmid 4. Selective medium for yeast screening 5. Sanger sequence of screening results (typically test 96 clones)	15 d	4,000 EUR	1. Vector Construction result; 2. Temporary Data Report (Self-activation, Rotation results)
6. Verify by Rotation 7. Temporary Data Report	5 d		
8. Next-generation sequencing 9. Complete Project Data Report	10 d	1,000 EUR	1. All Positive clone sequencing results (Based on Positive clone Numbers, including Sanger sequence results). 2. At least 5 TF sequences (No Frameshift mutation, based on NGS results). 3. Project Report and All Data documents (Electronic Version).
Total	55 d	5,500 EUR (Including Gene synthesis/ Vector Construction Price)	

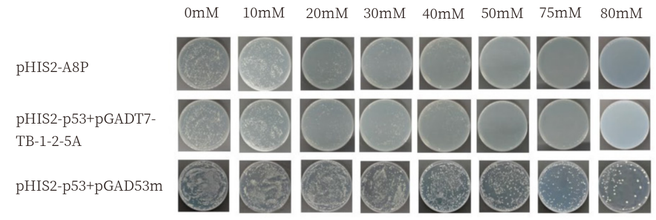
Delivery Standards

1. All Positive clone sequencing results (Based on Positive clone Numbers)
2. ≥ 5 TF sequences (No Frameshift mutation, based on NGS results)
3. Project Report and All Data documents (Electronic Version)

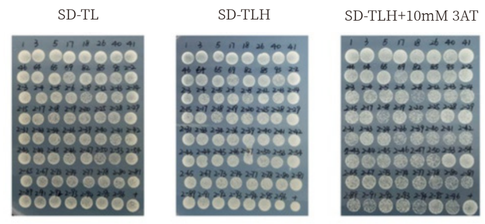
Notice

1. 'Positive clones' refers to the minimum requirement of positive clones that have been screened from the Media Triple Dropouts (TDO)
2. If the delivered Positive clone sequences number is “0”, Party A shall refund 100% of the project fees which the full payment has been received. Notice that Party A will not refund the Gene synthesis and Vector Construction payment.
3. If 1≤ the delivered TF sequences number ＜5, Party A shall repeat the screening experiment up to three rounds and finally delivers the actual TF sequences (No Frameshift mutation).
4. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Case diagram



(Self-activation test results of bait plasmid)



（Rotation verification results）

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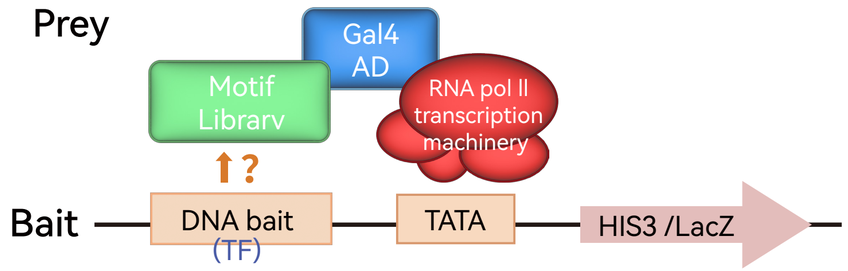
TF-Centered Y1H (Nuclear System)

Technology

There are two complementary approaches used to detect the interactions between a transcription factor (TF) and DNA, i.e., the TF-centered or protein-DNA approach, and the gene-centered or DNA-protein approach.
We provide a TF-centered method based on the Y1H system to identify the motifs recognized by a defined TF, termed TF-centered Y1H. In this system, a random short DNA sequence insertion library is generated as the prey DNA sequences to interact with a defined TF as the bait. TF-centered Y1H could identify quickly the motifs bound by a defined TF, representing a reliable and efficient approach with the advantages of Y1H. Therefore, this TF-centered Y1H may have a wide application in protein-DNA interaction studies.



(Transcription Factor-Centered Yeast One-Hybrid Assay. 2018)

Application

- It can be combined with ChIP-seq to reveal the regulatory role of elements.
- In the absence of ChIP-seq results, the elements identified by TF-centered Y1H can be scanned for promoter elements of these genes based on downstream target genes obtained by qRT-PCR or RNA-seq.
- TF target identification: Used to identify the target genes regulated by a specific TF. By screening a library of DNA fragments containing potential regulatory elements, the interacting DNA sequences can be identified and associated with specific target genes.

Service Features

- 1. Motif Synthetic Library (authorized), No Species Limited
  3*4^7 = 49,512 fragments (7bp per sequence), No Frameshift Mutation
- 2. Select 5 motifs for Genome Scanning Analysis
- 3. Replace the Chromatin Immunoprecipitation (ChIP) Assay

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
1. Gene synthesis (Codon Optimization) /vector Construction	15 d	Third Party: Price Based on gene length. Price: 0.25 EUR/bp, usually ≥ 200 EUR; Vector Construction 300 EUR/ One Vector	1. Vector Construction result; 2. Temporary Data Report (Self-activation, Rotation results)
2. Self-activation of bait plasmid	10 d	4,000 EUR	
3. Co-transfer Bait plasmid and library plasmid 4. Selective medium for yeast screening 5. Sanger sequence of screening results	20 d		
6. Verify by Rotation 7. Temporary Data Report	5 d		
8. Genome Scanning Analysis 9. Complete Project Data Report	5 d	1,000 EUR	1. All Positive clone Sanger sequencing results (Based on Positive clone Numbers) 2. Select 5 motifs for Genome Scanning Analysis; 3. Project Report and All Data documents (Electronic Version).
Total	55 d	5,500 EUR (Including Gene synthesis/ Vector Construction Price)	

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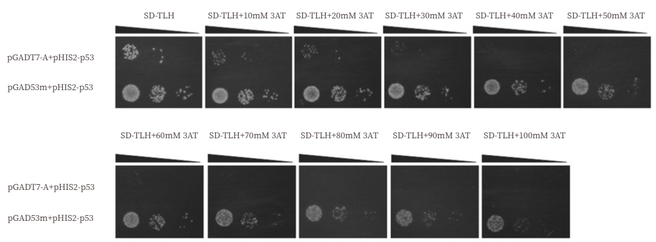
Delivery Standards

1. All Positive clone Sanger sequencing results (Based on Positive clone Numbers);
2. Select 5 motifs for Genome Scanning Analysis;
3. Project Report and All Data documents (Electronic Version);

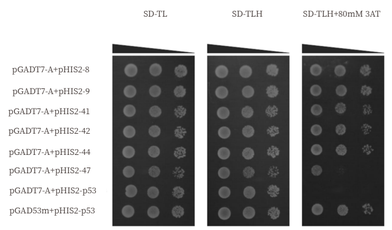
Notice

1. 'Positive clones' refers to the minimum requirement of positive clones that have been screened from the Media Triple Dropouts (TDO)
2. If the delivered Positive clone sequences number is “0”, Party A shall refund 100% of the project fees which the full payment has been received. Notice that Party A will not refund the Gene synthesis and Vector Construction payment.
3. If 1≤ the delivered motifs number ＜5, Party A shall repeat the screening experiment up to three rounds and finally delivers the actual motifs sequences.
4. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Case diagram



（Self-activation test results）



（Rotation verification results）

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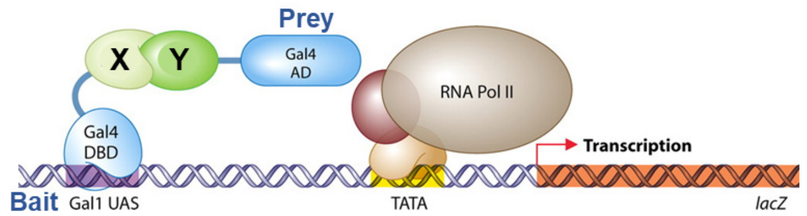
Two-Hybrid (Nuclear System)

Technology

The yeast two-hybrid system is a research method for identifying and detecting protein interactions in living cells, which is performed in the eukaryotic model organism yeast. The protein of interest X, is fused to the DNA binding domain (DBD), a construct called bait. The potential interacting protein Y is fused to the activation domain (AD) and is called prey. The bait, i.e. the DBD-X fusion protein, binds the upstream activator sequence (UAS) of the promoter. The interaction of bait with prey, i.e. the AD-Y fusion protein, recruits the AD and thus reconstitutes a functional transcription factor, leading to further recruitment of RNA polymerase II and subsequent transcription of a reporter gene.



（Diversity in Genetic In Vivo Methods for Protein-Protein Interaction Studies from the Yeast Two-Hybrid System to the Mammalian Split-Luciferase System 2012）

Application

- Protein-protein interaction mapping.
- Validation of protein-protein interactions.
- Used to identify and validate therapeutic targets.
- Screening for inhibitors or ligand.
- Analysis of protein complexes and signaling pathways.

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Service Features

- 1. Based on NGS, delivery more positive clone sequences
- 2. Ensure at least 8 positive clone sequences (No Frameshift Mutation)
- 3. ProS-3D Structural Analysis
- 4. Recommended Whole Sequencing Library

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
1. Gene synthesis (Codon Optimization) /Vector Construction	15 d	Third Party: Price Based on gene length. Price: 0.25 EUR/bp, usually ≥ 200 EUR; Vector Construction 300 EUR/ One Vector	1. Vector Construction result; 2. Temporary Data Report (Self-activation, Rotation results);
2. Self-activation of bait plasmid	10 d	4,000 EUR	
3. Co-transfer Bait plasmid and library plasmid 4. Selective medium for yeast screening 5. Sanger sequence of screening results (typically test 96 clones)	15 d		
6. Verify by Rotation 7. Temporary Data Report	5 d		
8. Next-generation sequencing 9. Complete Project Data Report	10 d	1,000 EUR	1. All Positive clone sequencing results (Based on Positive clone Numbers). 2. ≥ 8 Positive clone sequences (No Frameshift Mutation, Sanger sequence results); 3. Project Report and All Data documents (Electronic Version);
Total	55 d	5,500 EUR (Including Gene synthesis/ Vector Construction Price)	

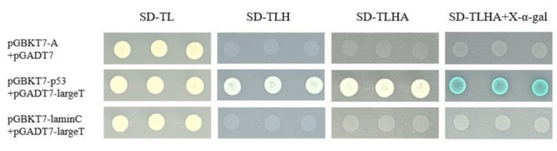
Delivery Standards

1. All Positive clone sequencing results (Based on Positive clone Numbers);
2. ≥ 8 Positive clone sequences (No Frameshift Mutation, Sanger sequence results);
3. Project Report and All Data documents (Electronic Version);

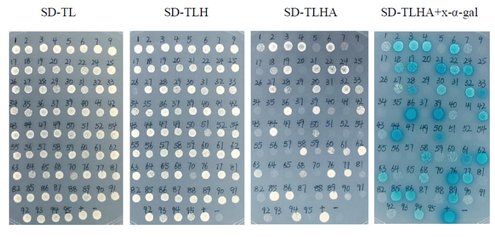
Notice

1. 'Positive clones' refers to the minimum requirement of positive clones that have been screened from the Media Triple Dropouts (TDO)
2. If the delivered Positive clone sequences number is “0”, Party A shall refund 100% of the project fees which the full payment has been received. Notice that Party A will not refund the Gene synthesis and Vector Construction payment.
3. If 1≤ the delivered Positive clone sequences number＜8, Party A shall repeat the screening experiment up to three rounds and finally delivers the actual Positive clone sequences.
4. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Case diagram



（Self-activation test results）



(Rotation verification results)

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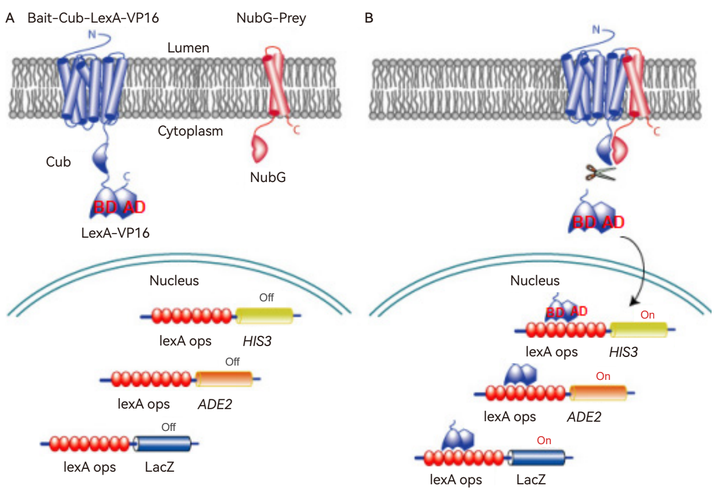
Two-Hybrid (Membrane System)

Technology

DUAL membrane system designed to identify interactions involving integral membrane proteins or membrane-associated proteins is derived from Dualsystems Biotech AG.
The membrane-based yeast two-hybrid (Y2H) system utilizes a split-ubiquitin approach and is specifically designed to investigate interactions involving transmembrane prteins or proteins localized to the cell membrane. The principle of the membrane-based Y2H system remains similar to the conventional Y2H assay, but with modifications to accommodate the membrane environment.



(Utilizing the Split-Ubiquitin Membrane Yeast Two-Hybrid System to Identify Protein-Protein Interactions of Integral Membrane Proteins. 2005)

Application

- Studying transmembrane protein interactions.
- Mapping membrane protein interaction networks.
- Validation of protein-protein interactions at the cell membrane.
- Functional analysis of membrane protein domains.

Service Features

- 1. Research Proteins located on cell membrane, or other organelles membrane, or membrane-associated receptors.
- 2. Based on NGS, delivery more positive clone sequences.
- 3. Ensure at least 8 positive clone sequences (No Frameshift Mutation).
- 4. ProS-3D Structural Analysis.
- 5.Recommended Whole Sequencing Library.

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
Analysis of transmembrane structures	0 d	Vector Selection 0 EUR	
1. Gene synthesis (Codon Optimization) /Vector Construction	15 d	Third Party: Price Based on gene length. Price: 0.25 EUR/bp, usually ≥ 200 EUR; Vector Construction 300 EUR/ One Vector	
2. Self-activation of bait plasmid	10 d	5,000 EUR	1. Vector Construction result; 2. Temporary Data Report (Self-activation, Rotation results);
3. Co-transfer Bait plasmid and library plasmid 4. Selective medium for yeast screening 5. Sanger sequence of screening results (typically test 96 clones)	15 d		
6. Verify by Rotation 7. Temporary Data Report	5 d		
8. Next-generation sequencing 9. Complete Project Data Report	10 d	1,000 EUR	1. All Positive clone sequencing results (Based on Positive clone Numbers). 2. ≥ 8 Positive clone sequences (No Frameshift Mutation, Sanger sequence results); 3. Project Report and All Data documents (Electronic Version);
Total	55 d	6,500 EUR (Including Gene synthesis/ Vector Construction Price)	

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Delivery Standards

1. All Positive clone sequencing results (Based on Positive clone Numbers);
2. ≥ 8 Positive clone sequences (No Frameshift Mutation, Sanger sequence results);
3. Project Report and All Data documents (Electronic Version);

Notice

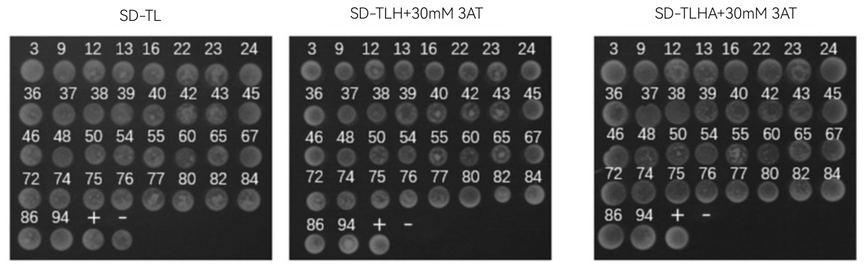
1. 'Positive clones' refers to the minimum requirement of positive clones that have been screened from the Media Triple Dropouts (TDO);
2. If the delivered Positive clone sequences number is “0”, Party A shall refund 100% of the project fees which the full payment has been received. Notice that Party A will not refund the Gene synthesis and Vector Construction payment.
3. If 1≤ the delivered Positive clone sequences number＜8, Party A shall repeat the screening experiment up to three rounds and finally delivers the actual Positive clone sequences.
4. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Bait Vector selection

N-terminal	C-terminal	Vector
Intracellular	Extracellular	pBT3-N
Extracellular with signal peptide	Intracellular	pBT3-SUC
Extracellular without signal peptide	Intracellular	pBT3-STE
Intracellular	Intracellular	pBT3-N or pBT3-STE

Case diagram

（Self-activation test results）



（Rotation verification results）

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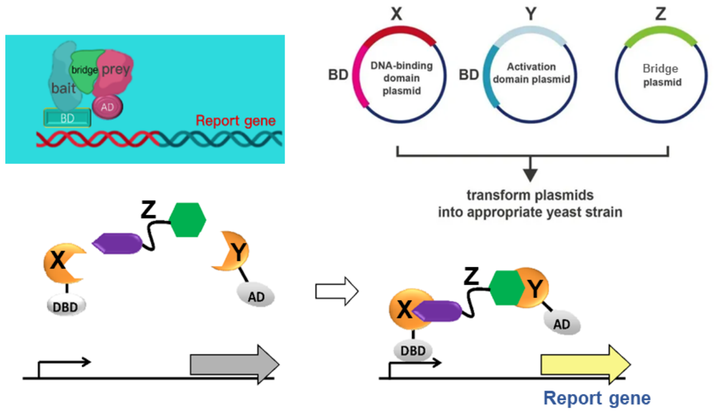
Three-Hybrid (Nuclear System)

Technology

The yeast three-hybrid system is an extension of the two-hybrid system to study more complex macromolecular interactions including three components, providing a new method for the study of protein-protein-protein, protein-RNA-protein, and protein-small molecule-protein interactions. Protein interactions can be affected by the presence of third protein. e.g., Proteins X and Y do not interact in isolation, but Protein Z connects X and Y as a “bridge” by associating X and Y at two surfaces.



（A Modular Approach to Triazole-Containing Chemical Inducers of Dimerisation for Yeast Three-Hybrid Screening 2013）

Application

- Ternary complexes Interactions.
- Ligand-Receptor Interactions.
- Signaling Pathways exploration.
- Protein Function and Localization.
- Drug discovery and the development of therapeutic interventions.

Service Features

- 1. Further research basis on yeast two-hybrid.
- 2. Identify a third protein is an activator or inhibitor.
- 3. Saving Time and Cost-effective.

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
1. Gene synthesis (Codon Optimization) /Vector Construction (Include Yeast pBridge vector)	15 d	Third Party: Price Based on gene length. Price: 0.25 EUR/bp, Usually ≥ 400 EUR; Vector Construction 600 EUR / Two Vectors;	1. Vector Construction result; 2. Temporary Data Report (Self-activation, Rotation results);
2. Self-activation of bait plasmid	10 d		
3. Co-transfer Bait plasmid and library plasmid 4. Selective medium for yeast screening 5. Sanger sequence of screening results (typically test 96 clones)	30 d	6,000 EUR	1. All Positive clone sequencing results (Based on Positive clone Numbers). 2. ≥ 15 Positive clone sequences (Sanger sequence results) 3. Project Report and All Data documents (Electronic Version);
6. Verify by rotation 7. Complete Project Data Report	10 d		
Total	65 d	7,000 EUR (Including Gene synthesis/ Vector Construction Price)	

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Delivery Standards:

1. All Positive clone sequencing results (Based on Positive clone Numbers);
2. ≥ 15 Positive clone sequences (Sanger sequence results);
3. Project Report and All Data documents (Electronic Version);

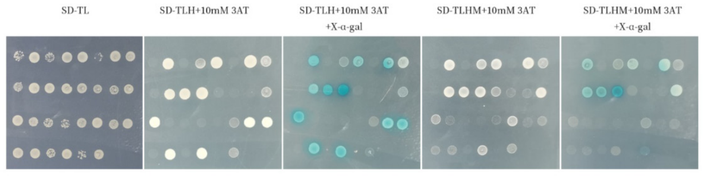
Notice

1. 'Positive clones' refers to the minimum requirement of positive clones that have been screened from the Media Triple Dropouts (TDO)
2. If the delivered Positive clone sequences number is “0”, Party A shall refund 100% of the project fees which the full payment has been received. Notice that Party A will not refund the Gene synthesis and Vector Construction payment.
3. If 1≤ the delivered Positive clone sequences number＜15, Party A shall repeat the screening experiment up to three rounds and finally delivers the actual Positive clone sequences.
4. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Case diagram

▲ *Self-activation test results*

▲ *Screening for minimum 3AT self-activation concentration.*



▲ *Rotation verification results*

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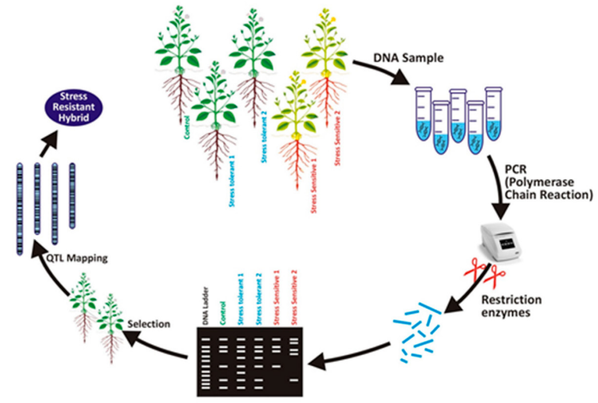
Abiotic-Stress Resistance Gene Screening

Technology

Saccharomyces cerevisiae belongs to eukaryote, which is closer to the expression system of plants and animals. This expression system includes the following processes: glycosylation, disulfide bond formation, and Post-translational modification of protein folding. Therefore, the protein encoded by the plant gene screened out by the yeast system can function normally, and false positive probability of multiple resistance genes is greatly reduced. The resistance-related genes were screened from the whole genome through the resistance gradient experiment, and the screening results were analyzed by bioinformatics to classify the resistance genes.



（*Molecular Markers Improve Abiotic Stress Tolerance in Crops: A Review 2020*）

Application

- ▲ Crop Improvement and Molecular Breeding.
- ▲ Understanding Stress Response Mechanisms.
- ▲ Functional Annotation of Genomes.
- ▲ Biotechnological Applications promote the development of stress-tolerant crops, biofuel production, and environmental remediation.

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Service Features

- Recommended Species .
- Screen single or multiple genes.
- Combined with next generation sequencing.

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
Yeast working solution preparation 1. Yeast working solution preparation. 2. PCR quality control for working solution.	10 d	1,000 EUR	1. Vector Construction result; 2. Temporary Data Report (Rotation results);
Stress condition optimization 3. Five different concentration gradient mediums for condition optimization.	10 d	1,000 EUR	
Yeast Screening 4. yeast suspension coating on 20 plates (diameter: 15cm).	10 d	3,500 EUR	
Positive clone identification 5. PCR identification of yeast colonies 6. Sanger sequence of screening results(typically test 96 clones)	5 d		
Verify the Screening results 6. Verify by Rotation 7. Temporary Data Report	10 d		
NGS 8. Next-generation sequencing 9. Complete Project Data Report	15 d	1,000 EUR	1. All Positive clone sequencing results (Based on Positive clone Numbers). 2. ≥ 15 Yeast Positive clone sequences (Sanger sequence results); 3. Project Report and All Data documents (Electronic Version);
Total	60 d	6,500 EUR	

Delivery Standards

1. All Positive clone sequencing results (Based on Positive clone Numbers);
2. ≥ 15 Yeast Positive clone sequences (Sanger sequence results);
3. Project Report and All Data documents (Electronic Version);

Notice

1. If 1≤ the delivered Yeast Positive clone sequences number＜15, Party A shall repeat the experiment up to three rounds and finally delivers the actual Yeast Positive clone sequences.
2. If the delivered Yeast Positive clone sequences number is ”0”, Party A shall refund 100% of the project fees which the full payment has been received.
3. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Case diagram

(Stress condition optimization results)

(Rotation verification results)

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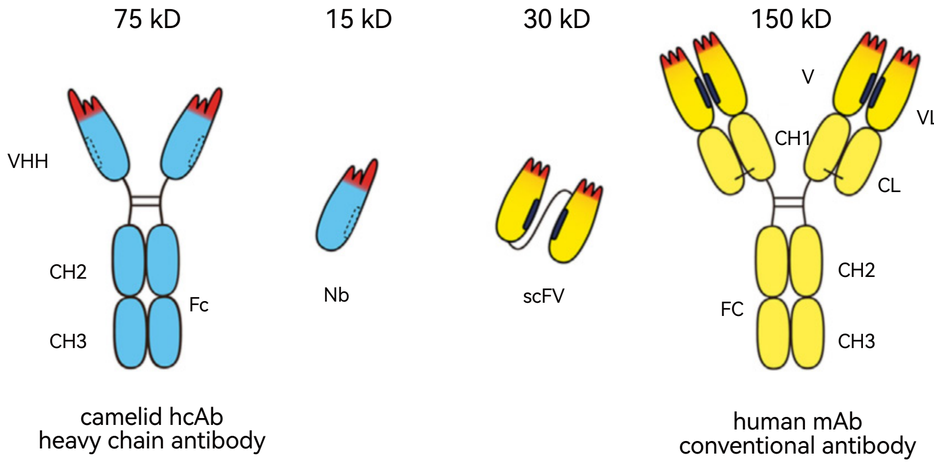
ProNet Nanobody Platform

Nanobody is the variable domain of heavy-chain-only antibody (HcAbs), which is 4 nm in length, 2.5 nm in width, and only 15 kD in molecular weight, due to the lack of the light chains (L) and heavy chain constant domain (CH) compared with conventional monoclonal antibodies (mAbs).

Nanobodies have unique properties such as small size, excellent solubility, superior stability, quick clearance from blood, and deep tissue penetration. As a result, nanobodies have become a promising tool for the diagnosis and therapy of diseases.

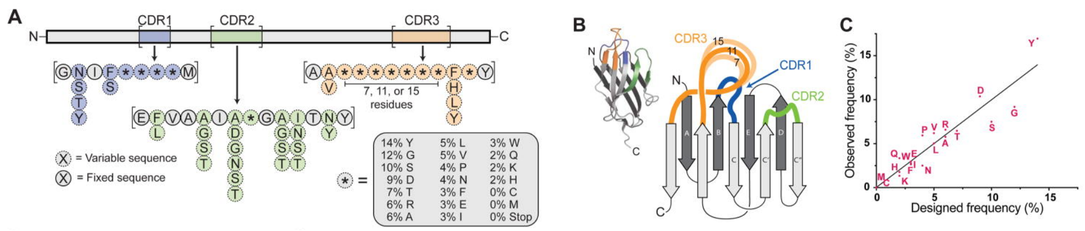


(Nanobodies and Nanobody-Based Human Heavy Chain Antibodies As Antitumor Therapeutics. 2017)

Nanobody Screening (Nuclear System)

Technology

The antigen-binding capacity of nanobodies is similar to conventional antibodies for three reasons. First, the complementarity-determining region 3 (CDR3) of nanobodies is similar or even longer than that of human VH domain (variable domain of heavy immunoglobulin chain). Second, nanobodies can form finger-like structures to recognize cavities or hidden epitopes that are not available to mAbs. Third, nanobodies exhibit excellent stability, hydrophilicity, and water solubility that help maintain their binding affinity across different conditions. The CDR3 corresponds to the unique region of the antibody molecule that is encoded by a DNA element newly generated during B-cell development. Genetic recombination results in the fusion of a D-element with flanking V- and J-elements. During recombination further genetic diversity is generated by addition and/or deletion of nucleotides at the junctions. Thereby, the CDR3 loop provides the major contribution to antibody diversity and specificity. There is a much lower contribution to diversity by the CDR1 and CDR2 loops, since these loops are germline encoded by a limited number of different V-elements.



Application

- Research and diagnostics: used as research tools to study protein structure and function, cellular processes, and intracellular localization.
- Imaging and molecular biology: conjugated with fluorescent dyes, radionuclides, or other imaging agents to visualize specific targets in cells or tissues.
- Industrial applications: such as enzyme stabilization, biosensors, and affinit chromatography.

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Service Features

- Synthetic Library, No Species Limited
  10^8 = 100 million fragments, and No Frameshift Mutation.
- Ensure at least 5 CDR3 positive clone sequences.
- Comparable accuracy but Lower Cost.

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
1. Gene synthesis (Codon Optimization) /Vector Construction	15 d	Third Party: Price Based on gene length. Price: 0.25 EUR/bp, usually ≥ 200 EUR; Vector Construction 300 EUR/ One Vector	1. Vector Construction result; 2. Temporary Data Report (Self-activation, Rotation results);
2. Self-activation of bait plasmid	10 d	6,000 EUR	
3. Co-transfer Bait plasmid and VHH library plasmid 4. Selective medium for yeast screening 5. Sanger sequence of screening results (typically test 96 clones)	20 d		
6. Verify by rotation 7. Complete Project Data Report	5 d		1. All Positive clone sequencing results (Based on Positive clone Numbers); 2. ≥5 CDR3 positive clone sequences (Sanger sequence results); 3. Project Report and All Data documents (Electronic Version);
Total	50 d	6,500 EUR	

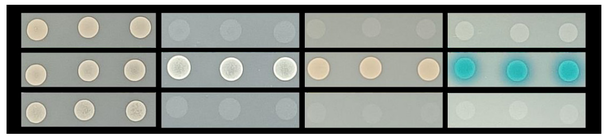
Delivery Standards

1. All Positive clone sequencing results (Based on Positive clone Numbers);
2. ≥ 5 CDR3 positive clone sequences;
3. Project Report and All Data documents (Electronic Version);

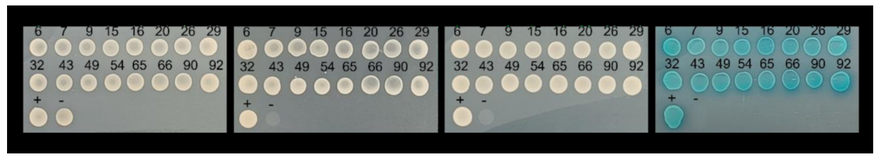
Notice

1. 'Positive clones' refers to the minimum requirement of positive clones that have been screened from the Media Triple Dropouts (TDO)
2. If the delivered Positive clone sequences number is “0”, Party A shall refund 100% of the project fees which the full payment has been received. Notice that Party A will not refund the Gene synthesis and Vector Construction payment.
3. If 1≤ the delivered CDR3 positive clone sequences number＜5, Party A shall repeat the screening experiment up to three rounds and finally delivers the actual CDR3 positive clone sequences.
4. Delivery time: Calculated from receiving the Purchase Order, Bait Gene Sequence and signed contract.

Case diagram



(Self-activation test results)



(Rotation verification results)

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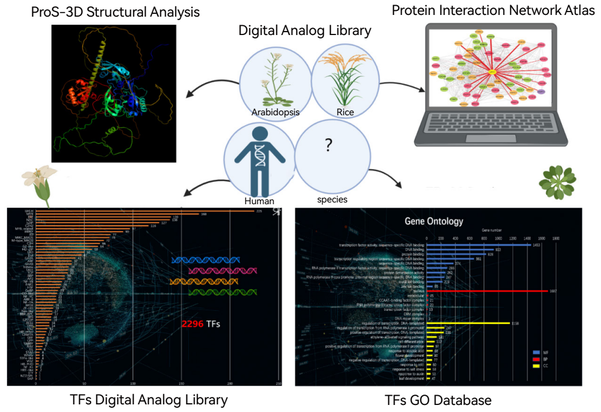
Pronetbio AI Protein Interaction Platform

Technology

Digital screening library is a high-throughput method using computer programs to simulate yeast hybridization library screening experiments, which can more quickly, efficiently and comprehensively discover genes that interact with specific proteins.
We also provide Point-to-Point verification experiment service to increate Credibility and Accuracy.



TFs Digital Analog Library　　TFs GO Database

Citations:

▲ *MEGADOCK 4.0:*
an ultra-high-performance protein-protein docking software for heterogeneous supercomputers, Bioinformatics, 30(22): 3281-3283, 2014.

▲ *Protein-Protein Docking on Hardware Accelerators:*
Comparison of GPU and MIC Architectures, BMC Systems Biology, 9(Suppl 1): S6, 2015.

▲ *Addressing recent docking challenges:*
A hybrid strategy to integrate template-based and free protein-protein docking. Proteins 2017;85:497-512.

Application

▲ Protein-protein docking is an important computational tool for predicting protein-protein interactions.
▲ Incorporate the biological information into traditional ab initio of protein-protein docking.
▲ Double validation or replacement of yeast hybrid assays.

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AI Protein Interaction Screening

Service Features

- ProNet's program algorithm.
- ProS-3D Structural Analysis.
- Customized Library.
- Double verification for Yeast hybridization Assay
- Binding Domain Analysis of 5 candidate genes (free of charge)

Delivery Time & Cost

Service content	Working days	Price (EUR) One gene	Delivery
Customer provide Amino Acid sequence or full length CDS sequence	2 d	2,500 EUR	1.Bait Gene of ProS-3D Structural Confidence Analysis Table； 2. Prey Digital Library of ProS-3D Structural Confidence Analysis Table （Customer provides gene sequence for positive control）； 3. Candidate interaction genes table with Confidence Analysis; PDB
Bait Gene of ProS-3D Structural Confidence Analysis	5 d		
Run the library screening program: High-throughput simulate library screening experiment	5 d		
Total	12 d	2,500 EUR	
Binding Domain Analysis of 5 candidate interaction genes.	*NA*	*Free of charge*	*PDB file*

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Service Advantages

Reliable Confidence Analysis:

Significantly narrow down DNA or Protein Interaction scope to avoid unnecessary work.

Exceptional Result Reliability:

AI screening results have been validated that cover experimental operations re-

Protein Interaction Core Site Analysis:

High-definition 3D protein structure analysis diagrams meet the format require-

Extremely Fast Screening:

Getting results within 12 working days, instead of 3-4 months.

No Experimental Operations and Reagent Consumables:

AI fully automated service eliminates tedious experimental operations and expensive reagent consumables.

Extremely Competitive Costs:

Saving 65% cost, compared with traditional hybridization experiment.

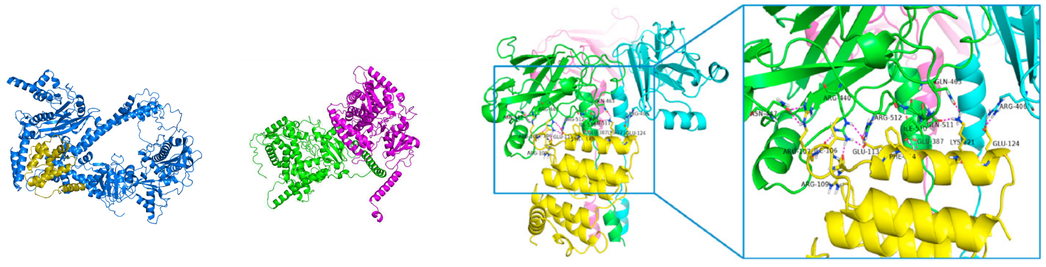
Applicable Species Coverage

Diverse Species:

Covers Plant, Animal, Fungal, Archaeal, Bacterial and Protist Kingdoms.

Specialized for Unique Species:

Particularly appreciable for symbiote and other challenging species which has difficulty in RNA extraction, full-length cDNA obtainment, and cDNA library construction.



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Diverse Species:

Covers Plant, Animal, Fungal, Archaeal, Bacterial and Protist Kingdoms.

Specialized for Unique Species:

Particularly appreciable for symbiote and other challenging species which has difficulty in RNA extraction, ful-length cDNA obtainment, and cDNA library construction.

★ **We also accept customized AI CDNA libraries**

Model Organisms' Complete Genes Digital Library

Species	Latin Name	Whole Genes Number
Arabidopsis	Arabidopsis thaliana	27434
Soybeans	Glycine max	55799
Rice	Oryza sativa	43649
Corn	Zea mays	39299
Human	Homo sapiens	23391
Drosophila melanogaster	Drosophila melanogaster	13458
Mice	Mus musculus	21615
Rat	Rattus norvegicus	21270
Zebrafish	Danio rerio	24664
Caenorhabditis elegans	Caenorhabditis elegans	19694
Saccharomyces cerevisiae	Saccharomyces cerevisiae	6039
Fission yeast	Schizosaccharomyces pombe	5128
Candida albicans	Candida albicans	5974
Methanocaldococcus annaschii	Methanocaldococcus jannaschii	1773
E. coli	Escherichia coli	4363

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Common Plant Tanscription Factors Digital Library

Species	Latin Name	TF Number
Soybeans	glycine max	6150
Barley	Barley	2620
Tomato	Solanum lycopersicum	1845
Potato	Solanum tuberosum	2405
Populus trichocarpa	Populus trichocarpa	4224
Upland cotton	Gossypium hirsutum	5022
Cassava	Manihot esculenta	2676
Apple	Malus domestica	3119
Wheat	Triticum aestivum	3606
Com	Zea mays	3308
Rapeseed plant	Brassica napus	5985
Melon	Cucumis melo	1537
Strawberry	Fragaria X ananassa	1247

Other Pathogens' Complete Genes Digital Library

Latin Name	Whole Genes Number	Latin Name	Whole Genes Number
Ajellomyces capsulatus	9199	Nocardia brasiliensis	8372
Brugia malayi	8743	Onchocerca volvulus	12047
Campylobacter jejuni	1620	Paracoccidioides lutzii	8794
Cladophialophora carrionit	11170	Plasmodium falciparum	5187
Dracunculus medinensis	10834	Pseudomonas aeruginosa	5556
Enterococcus faecium	2823	Salmonella typhimurium	4526
Fonsecaea pedrosoi	12509	Schistosoma mansoni	13865
Haemophilus influenzae	1662	Shigella dysenteriae	3893
Helicobacter pylori	1538	Sporothrix schenckii	8652
Klebsiella pneumoniae	5727	Staphylococcus aureus	2888
Leishmania infantum	7924	Streptococcus pneumoniae	2030
Madurella mycetomatis	9561	Strongyloides stercoralis	12613
Mycobacterium leprae	1602	Trichuris trichiura	9564
Mycobacterium tuberculosis	3988	Trypanosoma brucei	8491
Mycobacterium ulcerans	9033	rypanosoma cruzi	19036
Neisseria gonorrhoeae	2106	Wuchereria bancrofti	12721

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Notice

Yeast two-hybrid is only a preliminary screening method and needs to be combined with other experimental methods and bioinformatics analysis to further confirm the protein interactions.

According to the statistics from some articles of Journals as "Nature", "Molecular & Cellular Proteomics", and "Journal of Proteome Research", the success rate of interaction results obtained by yeast two-hybrid, verified by co-immunoprecipitation is approximately between 40% and 60%.

The reliability of AI results is 50% of yeast two-hybrid.
(It means that AI Library Screen result covers Yeast-Hybrid Assay result)

Currently, all 20 customer cases have a 100% success rate in obtaining interaction genes (verified by co-immunoprecipitation or Yeast-Hybrid Assay).

If there is none of the suggested proteins finally interacts, we will refund all customers' payment.

Citations

1. *Addressing recent docking challenges: A hybrid strategy to integrate template-based and free protein-protein docking. Proteins 2017;85:497-512.*
2. *MEGADOCK 4.0: an ultra-high-performance protein-protein docking software for heterogeneous supercomputers, Bioinformatics, 30(22): 3281-3283, 2014.*
3. *Protein-Protein Docking on Hardware Accelerators: Comparison of GPU and MIC Architectures, BMC Systems Biology, 9(Suppl 1): S6, 2015.*
4. *Highly accurate protein structure prediction with AlphaFold. Nature, 2021*
5. *AlphaFold-Multimer predicts cross-kingdom interactions at the plant-pathogen interface. Nature Communications | (2023) 14:6040*
6. *HNSPPI: a hybrid computational model combing network and sequence information for predicting protein–protein interaction. Briefings in Bioinformatics, 2023, 1–14*

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