invitrogen™

ProtoArray® Yeast Proteome Microarray nc v1.1

For detecting protein-protein interactions using a yeast proteome microarray

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User Manual

Table of Contents

Table of Contents iii
Kit Contents and Storage v
Accessory Products vi

Introduction 1

Overview 1
ProtoArray® Yeast Proteome Microarray 4
Experimental Overview 8

Methods 10

Preparing the Protein Probe 10
Guidelines for Probing the ProtoArray® Yeast Proteome Microarray 12
Probing Procedure 16
Scanning Arrays Using a Fluorescent Scanner 22
Data Acquisition and Analysis 24
Expected Results 28
Troubleshooting 30

Appendix 33

Technical Support 33
Purchaser Notification 34
References 36

Kit Contents and Storage

Shipping and Storage

The ProtoArray® Yeast Proteome Microarray nc (nitrocellulose) v1.1 is shipped on blue ice. Upon receipt, store the microarray at -20°C.

The microarray is stable for 12 months when stored properly.

Contents

Each ProtoArray® Yeast Proteome Microarray Box contains a mailer with 1 Yeast Proteome microarray.

Store the microarray at -20°C.

For more details on array specifications, see page 4.

Product Qualification

The ProtoArray® Yeast Proteome Microarray nc is visually examined for obvious defects. The quality of the printing process is verified by probing several arrays from each lot with an anti-GST (Glutathione-S-Transferase) antibody. The scanned image of the array must show a uniform spotting pattern.

The ProtoArray® Yeast Proteome Microarray nc is probed with the Array Control Protein (yeast calmodulin kinase with a BioEase™-V5-tag at the N-terminus) and interactions are detected using fluorescent detection with Anti-V5-Alexa Fluor® 647 or Streptavidin-Alexa Fluor® 647 conjugate as described in this manual. For details on the controls printed on the array, see page 6.

After probing and detection, the following results must be observed:

- Expected protein interactions with calmodulin and anti-biotin antibody using anti-V5 or streptavidin detection
- V5 Control Protein and Biotinylated Antibody produces signals at the expected locations using anti-V5 or streptavidin detection, respectively
- Alexa Fluor® 647 antibody signals are observed at the expected locations
- BSA and GST spots do not produce any signals when probed with the detection reagents

Accessory Products

Additional Products

The table below lists additional products available separately from Invitrogen. For more information about these products, visit www.invitrogen.com or contact Technical Support (page 33).

Product	Quantity	Catalog no.
ProtoArray® Protein-Protein Interaction Application Kit *for epitope tagged proteins*	1 kit	PA011
ProtoArray® Protein-Protein Interaction Application Kit *for biotinylated proteins*	1 kit	PA012
ProtoArray® Mini-Biotinylation Kit	1 kit	AL-01
ProtoArray® Protein-Protein Interaction Buffers Module	1 kit	PA014
ProtoArray® Human Protein Microarray nc v4.0	1 array	PAH052401
ProtoArray® Control Protein Microarray nc v4.0	1 array	PA1007
ProtoArray® Yeast Proteome Microarray PPI Complete Kit v1.1 *for epitope tagged proteins*	1 kit	PA0121013
ProtoArray® Yeast Proteome Microarray PPI Complete Kit v1.1 *for epitope tagged proteins*	1 kit	PA0121011
ProtoArray® Human Protein Microarray mg v4.0	1 array	PAH05406
ProtoArray® Yeast Proteome Microarray mg v1.1	1 array	PA012106
ProtoArray® Control Protein Microarray mg v4.0	1 array	PA1002
ProtoArray® Human Protein Microarray KSI Complete Kit v4.0	1 kit	PAH0524065
ProtoArray® Yeast Proteome Microarray KSI Complete Kit v1.1	1 kit	PA0121065
ProtoArray® Kinase Substrate Identification Application Kit	1 kit	PA015
Streptavidin-Alexa Fluor® 647 Conjugate (2 mg/ml)	0.5 ml	S-32357
Alexa Fluor® 647 Protein Labeling Kit	1 kit	A-20173
Alexa Fluor® 647 Goat Anti-Mouse IgG (H+L)	0.5 ml	A-21236
Anti-V5 Antibody	50 µl	R960-25
Anti-V5-HRP Antibody	50 µl	R961-25
Anti-V5-AP Antibody	50 µl	R962-25
HybriSlip™ Cover Slip	Set of 500	H-18202
Phosphate Buffered Saline (PBS), 1X	500 ml	10010-023
ProQuest™ Two-Hybrid System with Gateway® Technology	1 kit	PQ10001-01
ProQuest™ Two-Hybrid System	1 kit	PQ10002-01

Vectors

A variety of vectors is available for expression and purification of your protein of interest with different tags at the N- or C-terminus. For more information about these products, refer to our website (www.invitrogen.com) or call Technical Support (page 33).

The recommended tag for use with ProtoArray® Yeast Proteome Microarray is the V5 epitope tag.

Introduction

Overview

Introduction

The ProtoArray® Yeast Proteome Microarray nc (nitrocellulose) v1.1 allows rapid and efficient detection of protein-protein interactions on a proteome scale using a protein probe containing a suitable tag. The ProtoArray® is a proteome microarray containing >4000 purified yeast proteins from *Saccharomyces cerevisiae* printed in duplicate on a nitrocellulose-coated glass slide. See the next page for a system overview.

Since basic biological processes and protein interactions are well conserved between organisms, the ProtoArray® Yeast Proteome Microarray can be used to study protein-protein interactions in higher eukaryotes.

ProtoArray® Yeast Proteome Microarray

The ProtoArray® Yeast Proteome Microarray nc v1.1 is a high-density protein microarray containing >4000 purified yeast proteins from *Saccharomyces cerevisiae*. The ProtoArray® technology is based on the protein microarray technology developed by Zhu *et al*, 2001 to detect molecular interactions with proteins.

Each *S. cerevisiae* open reading frame (ORF) is expressed as an N-terminal GST (Glutathione-S-Transferase)-6xHis-fusion protein, purified, and printed in duplicate on a nitrocellulose-coated glass slide. Using a labeled protein probe and a suitable detection system, you can screen against >4000 *S. cerevisiae* ORF's within a day to elucidate protein interactions.

The use of nitrocellulose as a surface to print the arrays ensures maximum protein function since the nitrocellulose surface is known to be compatible with a variety of protein functions (Espejo *et al.*, 2002; Kukar *et al.*, 2002; Michaud *et al.*, 2003). The nitrocellulose coating is thin and does not interfere with scanning of the array using Alexa Fluor® 647 or Cy5™ dyes.

For specifications and more details on the ProtoArray® Yeast Proteome Microarray, see page 4.

Note: The subarray layout and controls are changed in ProtoArray® Yeast Proteome Microarray nc v1.1 as compared to the previously available ProtoArray® Yeast Proteome Microarray nc v1.0.

Applications

The ProtoArray® Yeast Proteome Microarray nc v1.1 allows you to:

- Detect novel protein-protein interactions
- Validate previously observed protein-protein interactions
- Confirm positive interactions using the identified interacting protein on the array as a probe in reciprocal experiments (page 6)
- Study protein-protein interactions in higher eukaryotes
- Test various experimental conditions for your protein-protein interactions

Continued on next page

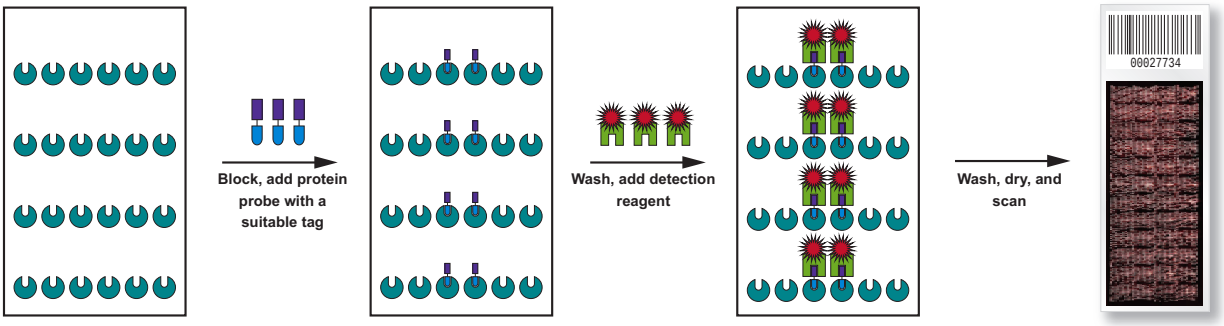
System Overview

To use the ProtoArray® Yeast Proteome Microarray nc v1.1 , you need to:

- Express your protein of interest as a fusion containing a suitable tag such as the V5 epitope tag at the N-or C-terminus and purify the fusion protein using a method of choice (page 10), **OR** *in vitro* biotinylate your protein of interest (page 10).
- Probe the ProtoArray® Yeast Proteome Microarray with the protein probe containing a suitable tag.
- Detect the protein-protein interaction using a labeled antibody specific for the tag or streptavidin conjugated to a fluorescent dye such as Alexa Fluor® 647.

The ProtoArray® detection protocol includes blocking the array, probing the array with your protein probe, washing to minimize non-specific interactions, detecting interactions using a labeled antibody or streptavidin, washing to remove unbound detection reagent, drying, scanning the array to view results, acquire the array image, and analyze results (see figure below).

For a detailed experimental workflow, see page 9.



Important

Since most of the yeast proteins printed on the microarray contain a GST (Glutathione-S-Transferase) fusion tag and some proteins also contain polyhistidine (6x) tag, **do not** use an anti-GST antibody or anti-polyhistidine antibody for detecting interactions on a ProtoArray® Yeast Protein Microarray nc. We strongly recommend that you probe the ProtoArray® Human Protein Microarray nc with only your detection reagent to detect signals resulting due to interactions between the detection reagent and proteins printed on the array.

Continued on next page

Advantages

Using the ProtoArray® Yeast Proteome Microarray to detect protein-protein interactions offers the following advantages:

- Provides a simple, rapid, sensitive, and efficient method to identify protein interactions within a day
- Allows screening of your protein of interest against >4000 yeast proteins from *S. cerevisiae*
- Suitable as a model to investigate interactions in higher eukaryotic systems
- Built-in controls printed on each array to control for background and detection
- Arrays compatible with most commercially available fluorescent microarray scanners

ProtoArray® Central Web Portal

The ProtoArray® Central Web Portal provides a web-based user interface to access ProtoArray® specific information including various applications, resources, and online tools. The portal is also used to retrieve ProtoArray® Lot Specific information (page 24) which is required for analyzing the array data and identifying statistically significant interactions.

Go to www.invitrogen.com/protoarray to visit the portal.

ProtoArray® Prospector

The ProtoArray® Prospector software quickly analyzes the microarray data acquired from the image acquisition software and easily identifies significant hits, saving you time and effort. In addition, the software has features that allow you to modify the analysis method and compare data obtained from different microarrays.

The ProtoArray® Prospector software and manual are available free-of-charge to ProtoArray® users, and are accessible online at the ProtoArray® Central Portal. To download the ProtoArray® Prospector software and manual, go to www.invitrogen.com/protoarray, and click on the Online Tools tab.

Purpose of the Manual

This manual provides the following information:

- An overview of the ProtoArray® Yeast Proteome Microarray
- Guidelines for preparing the protein probe and probing the microarray with your protein probe
- Scanning the microarray
- Guidelines for data analysis
- Troubleshooting.

ProtoArray® Yeast Proteome Microarray

Introduction

The ProtoArray® Yeast Proteome Microarray nc v1.1 is a high-density protein microarray containing the majority of proteins from *S. cerevisiae* for protein interaction screening. Each *S. cerevisiae* open reading frame (ORF) is expressed as a N-terminus GST-6xHis fusion protein, purified, and printed in duplicate on a nitrocellulose-coated glass slide.

Details on the ProtoArray® Yeast Proteome Microarray are described in this section.

Yeast Microarray Specifications

The specifications for the ProtoArray® Yeast Proteome Microarray nc v1.1 are listed below.

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with nitrocellulose membrane
Membrane Size:	20 mm x 60 mm
Membrane Properties:	Thickness: 15-20 µm; Pore Size: 0.2 µm

Each microarray has a barcode for tracking samples. The barcode is also used to retrieve array specific information from the ProtoArray® Central Web Portal (page 3).

Array Specifications

The array specifications for the ProtoArray® Yeast Proteome Microarray nc v1.1 are listed below.

The proteins on the microarray are printed in 48 subarrays and are equally spaced in vertical and horizontal directions.

For details on the subarray layout, and yeast protein and control spots on the ProtoArray® Yeast Protein Microarray nc, go to the ProtoArray® Central Portal at www.invitrogen.com/protoarray.

Note: The subarray layout and controls are changed in ProtoArray® Yeast Proteome Microarray nc v1.1 as compared to the previously available ProtoArray® Yeast Proteome Microarray nc v1.0.

Total Subarrays:	48 (4 columns x 12 rows)
Subarray Size:	4400 µm x 4400 µm
Subarray Dimensions:	**16 rows x 20 columns**
Median Spot Diameter:	~150 µm
Spot Center to Center Spacing:	220 µm
Distance Between Subarrays:	100 µm
Replicates per Sample:	2
Total Yeast Proteins on v1.1 Array:	>4000*

*Refer to ProtoArray® Central Portal for exact number of yeast proteins printed on the microarray.

Continued on next page

ProtoArray® Yeast Proteome Microarray, Continued

Preparing Yeast Proteins

The yeast proteome collection is derived from the *S. cerevisiae* clone collection of 5800 yeast ORFs (Zhu *et al.*, 2001). Each *S. cerevisiae* open reading frame (ORF) is expressed as a N-terminus-GST-6xHis fusion protein in the yeast expression vector pEG-KG (Mitchell *et al.*, 1993). The identity of each clone was verified using 5'-end sequencing and the expression of GST-tagged fusion protein by each clone was confirmed with western immunodetection using an anti-GST antibody. Once the identity of each clone was confirmed, the proteins from each clone were expressed and purified using high-throughput procedures. For a list of yeast proteins printed on the array and their description, download the Protein Information File from www.invitrogen.com/protoarray as described on page 25.

Briefly, yeast stocks were grown in growth media, protein expression was induced with galactose, and cell lysates prepared. The proteins were purified using glutathione affinity chromatography, eluted, and purified proteins were used for spotting the proteome microarray.

Printing Yeast ProtoArray®

The purified yeast proteins are printed on nitrocellulose-coated slides in a dust-free, and temperature and humidity controlled environment to maintain consistent quality of microarrays. The arrays are printed using an automated process on an arrayer that is extensively calibrated and tested for printing ProtoArray® Yeast Proteome Microarrays.

Maintaining Stringent Quality Control

The ProtoArray® Yeast Proteome Microarrays are produced using rigorous production and quality control procedures with an integrated data management system to ensure consistent results with every array and maximize inter-and intra-lot reproducibility.

Pre-Printing Quality Control

Prior to production, the arrayer and supporting components are tested and adjusted to production specifications. The quality and performance of pins is critical and all pins are extensively tested and calibrated. To maintain protein stability and function, arrays are printed at 6ºC under controlled environmental conditions.

Post-Printing Quality Control

After production each microarray is visually inspected for obvious defects that could interfere with the experimental results. To control for the quality of the printing process, several microarrays from each lot are probed with an anti-GST antibody. Since the proteins contain a GST fusion tag, probing the microarray with an anti-GST antibody allows identification of irregular spot morphology or missing spots. The arrays are functionally qualified by probing control proteins to detect the appropriate protein-protein interactions.

Detailed product qualification is on page v.

Continued on next page

ProtoArray® Yeast Proteome Microarray, Continued

Detecting Reciprocal Interactions

The ProtoArray® Yeast Proteome Microarray nc is ideal for detecting reciprocal protein-protein interactions since proteins are purified under native conditions and the microarrays are manufactured under highly controlled conditions to ensure maximum protein function.

Once you have identified a positive interaction using the ProtoArray® Yeast Proteome Microarray, use the identified interacting protein from the array as a probe for probing another microarray.

For example, perform an initial probing with calmodulin as a probe with a ProtoArray® Yeast Proteome Microarray to detect the interacting protein, calmodulin kinase. Then perform the reciprocal interaction with another microarray using calmodulin kinase as the probe to detect the interacting protein, calmodulin. The ability to observe reciprocal interactions indicates that the proteins maintain a proper folded state on the array.

Control Proteins

Various proteins and controls are printed on each ProtoArray® Yeast Proteome Microarray to verify background and detection.

The table below lists the controls printed on each ProtoArray® Yeast Proteome Microarray.

Protein	Function
Control Spots required for PPI Data Analysis	
Alexa Fluor® Antibody (Rabbit anti-mouse IgG Antibody labeled with Alexa Fluor® 647, Alexa Fluor® 555, and Alexa Fluor® 488)	Serves as a positive control for fluorescence scanning and for orientation of the microarray image.
Bovine Serum Albumin (BSA)	A negative control for non-specific protein interactions.
Biotinylated Anti-mouse Antibody	A positive control for interaction with streptavidin-labeled detection reagent.
Anti-biotin Antibody	Detects biotinylated probes.
V5 Control Protein (biotinylated, V5-tagged control protein)	A positive control for detection with the Anti-V5-Alexa Fluor® 647 Antibody.
Human IgG Protein Gradient	A positive control for the immune response serum profiling application. Interacts with Alexa Fluor® 647 goat anti-human IgG.
Anti-Human IgG Antibody Gradient (goat anti-human IgG)	A positive control for the immune response serum profiling application. Interacts with serum IgG antibodies which are then bound by Alexa Fluor® 647 goat anti-human IgG.

*The BioEase™ fusion tag directs *in vivo* biotinylation of the protein.

Continued on next page

ProtoArray® Yeast Proteome Microarray, Continued

Control Proteins, continued

Protein	Function
Yeast calmodulin (Cmd1p)	A positive control for protein-protein interaction application and interacts with the Array Control Protein.
GST Protein Gradient	Serves as a negative control and signals are used by ProtoArray® Prospector software for background and statistical significance calculations.
Control Spots NOT required for PPI Data Analysis	
Fiduciary Kinases	Kinases autophosphorylate and produce fiduciary (marker) signals which are used for orientation of the microarray image; also serves as a positive control for the radiolabel and assay conditions.
Control Kinase Substrate	A substrate for the Control Kinase used to verify assay conditions. The Control Kinase phosphorylates the Control Kinase Substrate.
CAMK2A (Calcium/calmodulin-dependent protein kinase II alpha)	A human protein kinase that is used as a positive control for the small molecule profiling application.

Experimental Overview

Experimental Outline

The experimental outline for probing ProtoArray® Yeast Proteome Microarray nc with your protein probe tagged with a suitable tag is shown below. See next page for the experimental workflow.

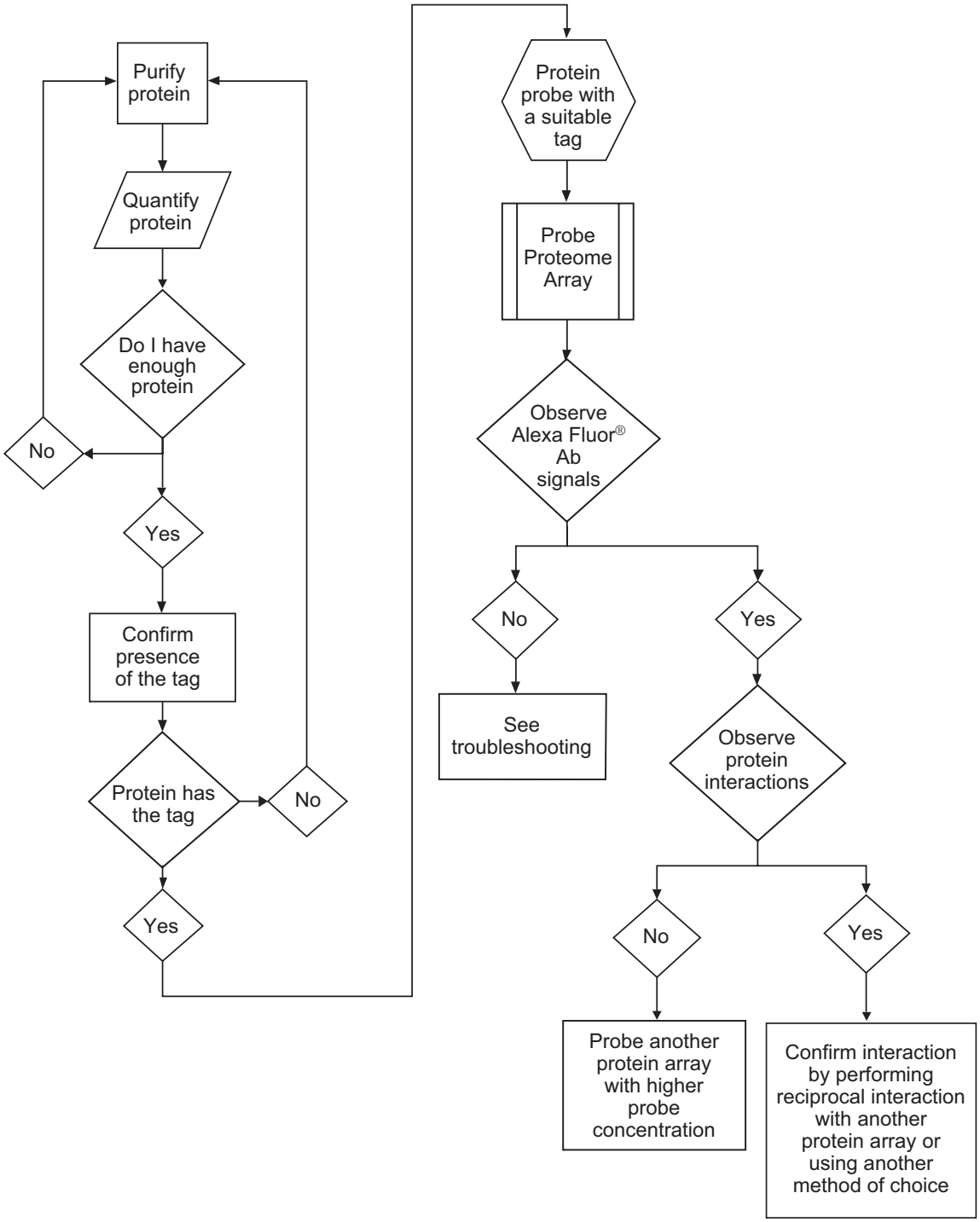
Step	Action	Page no.
1	Express your protein of interest as a fusion protein in an expression vector containing the desired tag at the N-or C-terminus of the protein and purify the protein. **OR** *In vitro* biotinylate your protein of interest using a method of choice.	10
2	Probe the yeast microarray with the tagged protein probe and perform detection using a suitable detection system. **Optional:** If you are a first time user of the ProtoArray® Yeast Proteome Microarray, perform a control probing using a ProtoArray® Control Microarray to verify probing and detection protocols.	12 13
3	Dry the microarray.	21
4	Scan the microarray using a suitable microarray scanner and save an image of the array.	22
5	Download the protein array lot specific information (mainly the .GAL file) from ProtoArray® Central Portal to acquire and analyze the data for identifying significant protein-protein interactions.	24

Continued on next page

Experimental Overview, Continued

Experimental Workflow

The experimental workflow for probing ProtoArray® Yeast Proteome Microarray with your protein probe tagged with a suitable tag is shown below.



Methods

Preparing the Protein Probe

Introduction

Before using the ProtoArray® Yeast Proteome Microarray nc, you will need your purified protein of interest to probe the microarray.

The protein of interest must contain a suitable tag at the N- or C-terminus of the protein (see below). You may purify proteins using a method of choice. You can use proteins purified from *E. coli*, yeast cells, or higher eukaryotes to probe the ProtoArray® Yeast Proteome Microarray.

The amount of protein and quality of protein required for probing are described in this section.

Protein Tags

The protein of interest can be tagged using an epitope tag or a biotin label.

Using an epitope tag at the N-or C-terminus of the probe allows the use of the recombinant fusion protein directly as a probe without any further modification wherein the tag is used as the marker for detection of interactions. The recommended epitope tag is *V5-epitope tag* at the N-or C-terminus of the protein to obtain the best results. Epitope tags such as FLAG, *myc*, or HA can also be used for probing the microarray using an appropriate labeled antibody.

The extremely high affinity of the biotin-streptavidin interaction makes biotin-protein conjugation an attractive method for probe labeling. Small amounts of the protein can be efficiently *in vitro* biotinylated using biotin in a simple procedure. The biotinylated protein probe is detected using a streptavidin detection system.

Generating Tagged Protein Probe

Epitope Tag

To generate your protein probe with an epitope tag, you need to express your protein of interest as a fusion protein in an expression vector containing the desired epitope tag at the N-or C-terminus of the protein.

A variety of vectors with different tags at the N- or C-terminus are available from Invitrogen for expression of your protein of. For more information about these products, refer to our website (www.invitrogen.com) or call Technical Support (page 33). The recommended epitope tag for use with ProtoArray® Yeast Proteome Microarray is the V5 epitope tag.

Biotin Tag

You may use any method of choice to *in vitro* biotinylate your protein of interest. We recommend using the ProtoArray® Mini-Biotinylation Kit available from Invitrogen (page vi) for efficient *in vitro* biotinylation of your protein of interest. The kit includes reagents and buffers for *in vitro* biotinylation, removal of free biotin, and assessing Biotinylation using western detection.

Continued on next page

Preparing the Protein Probe, Continued

Protein Amount and Quality

- **Purify the protein using native conditions.**
- Proteins should be >90% pure as determined by Coomassie® staining.
- Check the presence of the tag using western detection or ELISA. **Note:** To ensure that the tag is accessible under native conditions used for probing microarrays, perform ELISA of your protein probe with the tag.
- Check the functionality of the protein probe using a method of choice.
- Make sure the protein probe is soluble and active in buffers used for probing the microarray.
- The recommended protein concentration range for probing each yeast protein microarray is 100 nM-10 μM (for biotinylated proteins) and 10 nM-1 μM for V5-tagged proteins.

If you are using *in vitro* biotinylated proteins for probing:

- Resuspend the purified protein probe in a buffer (≤50 mM) that does not contain any primary amines such as ammonium ions, Tris, glutathione, imidazole, or glycine. If the buffer contains primary amines, sufficiently dialyze the protein probe against 50 mM HEPES buffer, pH 7.4 containing 100 mM NaCl, or PBS.
- You need to know the approximate molecular weight of your protein and the protein must be >15 kDa.
- For proteins purified using metal chelating column chromatography (ProBond™ resin or Ni-NTA resin), perform dialysis against 2 changes of PBS to significantly lower the imidazole concentration.
- Low concentrations (<0.1%) of sodium azide or thimerosal in the protein solution have no effect on the biotinylation reaction.

Guidelines for Probing the ProtoArray® Yeast Proteome Microarray

Introduction

To perform probing you need to use an appropriate detection system (see below). Various options are available for performing the probing procedure (see next page for details). An experimental workflow for probing is shown on page 14.

Important

- **The ProtoArray® Yeast Proteome Microarray can only be used once. Do not re-use the array or re-probe the same array with another probe.**
- The ProtoArray® Yeast Proteome Microarray is not compatible for use with Alexa Fluor® 555 or Cy3™ dyes. Use of these fluorescent dyes results in high background on the array as the nitrocellulose surface has high intrinsic fluorescence at the wavelength used to visualize Alexa Fluor® 555 or Cy3™ dyes. Always use Alexa Fluor® 647 or Cy5™ dyes for detection with nitrocellulose-coated ProtoArray® Microarrays (see below).

Detection Methods

Detection of protein-protein interactions on the ProtoArray® Yeast Proteome Microarray can be performed using fluorescence, chemiluminescence, or radioactivity.

The high sensitivity, low background, signal stability, and commercial availability of fluorescence microarray scanners makes fluorescence detection the preferred and recommended method for detecting protein-protein interactions on the microarray.

Based on the tag or label on your protein probe, use the appropriate detection method to identify protein interactions.

Epitope Tag

To detect the epitope tag on your protein probe, use a labeled antibody specific for the tag. The antibody can be directly labeled with a fluorescent dye such as Alexa Fluor® 647 or labeled with a secondary antibody conjugated to a fluorescent dye such as Alexa Fluor® 647.

Note: Be sure to check that direct labeling of the antibody does not affect the antibody activity.

Biotin Label

To detect the biotin label on your protein probe, use streptavidin conjugated to a suitable label such as a fluorescent dye (Alexa Fluor® 647) providing signal amplification and increased sensitivity.

Alexa Fluor® Detection

The Alexa Fluor® detection system available from Invitrogen (page vi) is the recommended fluorescent detection method. Alexa Fluor® 647 fluorophore is brighter and more stable than other commercially available dyes such as Cy5™ Dyes and is more sensitive for detecting interactions on protein arrays. We have demonstrated that detection with Alexa Fluor® 647 produces approximately 2-fold higher signal/background ratios than Cy5™ detection.

Continued on next page

Guidelines for Probing the ProtoArray® Yeast Proteome Microarray, Continued

Probing Options

The recommended protein probe concentration range for probing the array is 100 nM-10 µM (for biotinylated proteins) and 10 nM-1 µM (for V5-tagged proteins).

Various options are available for probing the yeast microarray with the protein probe of interest using application kits (contain pre-made reagents including buffers and detection reagents), pre-made buffers only, or your own buffers and detection reagents as described below. Review the information below, before proceeding with the probing procedure.

Probing Array with…	…Then Choose	… And Use Protocol
Biotinylated probe using application kit	ProtoArray® Protein-Protein Interaction Application Kit for *in vitro* biotinylated proteins (page vi)	Supplied in the manual shipped with the application kit.
V5-tagged protein probe using application kit	ProtoArray® Protein-Protein Interaction Application Kit for epitope tagged proteins (page vi)	Supplied in the manual shipped with the application kit.
Protein Probe using pre-made buffers only	ProtoArray® Protein-Protein Interaction Buffers Module (page vi)	On page 16.
Protein Probe using your own buffers and detection reagents	Your own buffers and reagents	On page 16.

Additional probing options include:

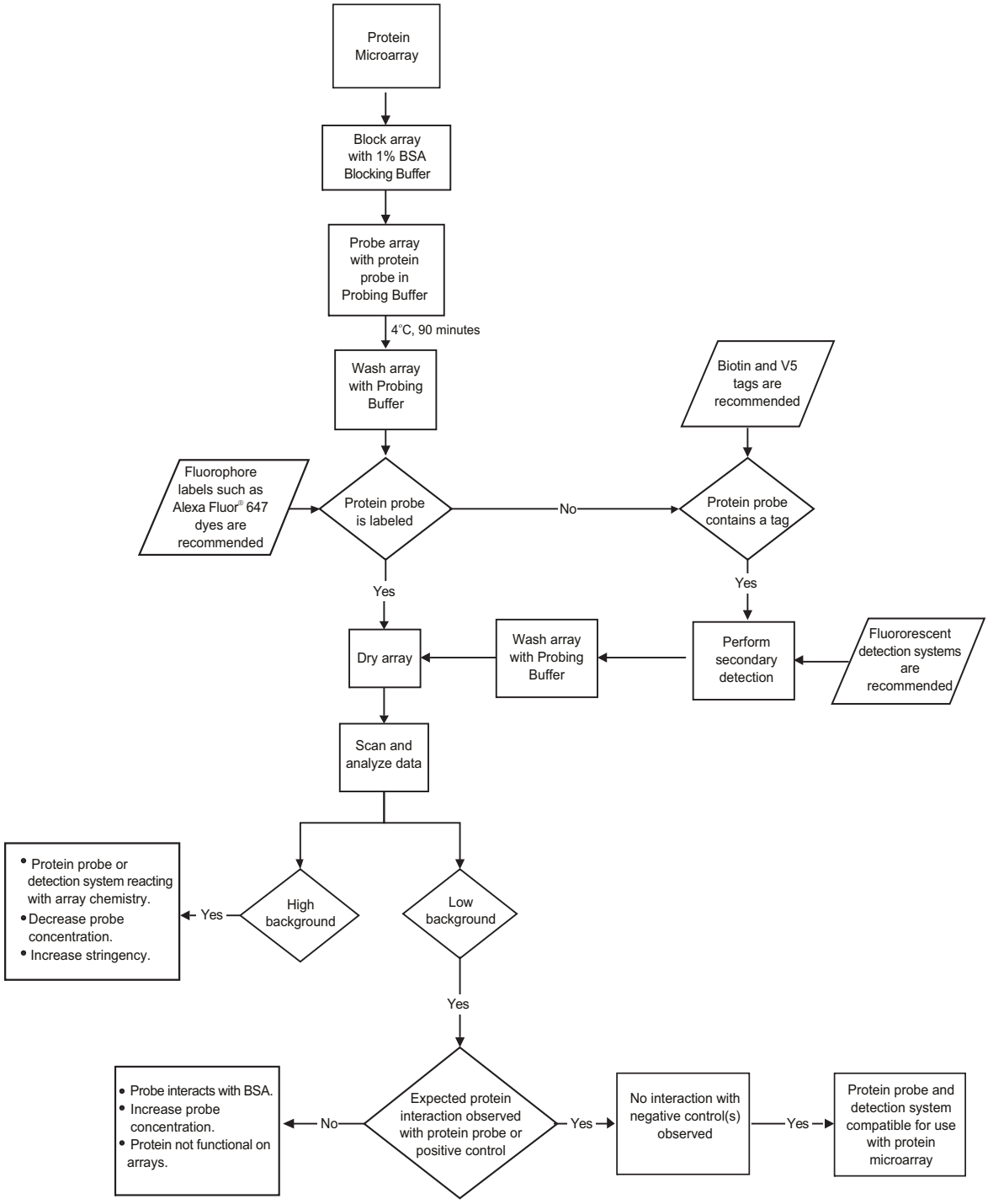
- If you are first time user of the ProtoArray® Yeast Proteome Microarray, we recommend that you also probe a ProtoArray® Control Protein Microarray available from Invitrogen (page vi) prior to probing the yeast microarray. The Control Protein Microarray contains various controls and protein interactors printed on the array, and is supplied with an Array Control Protein to allow you to validate probing and detection protocols.
- You can probe two arrays simultaneously, probing one protein array with your protein probe and the second protein array with no protein probe (negative control). The negative control allows you to determine signals specific to your probe.
- You can probe one array with an initial probe concentration. If the initial signal is strong with low background, confirm the initial results with a second array using the same experimental conditions. If the initial results indicate weak signal and unacceptable signal-to-noise ratio, probe a second array with a different probe concentration as described in the table below:

Probe first array….	And….	Then Probe Second Array….
With 10 nM probe	Weak signal	With 1-10 µM probe
With 10 µM probe	High background	With 10-100 nM probe

Guidelines for Probing the ProtoArray® Yeast Proteome Microarray, Continued

Experimental Workflow

The experimental workflow for probing the ProtoArray® Yeast Proteome Microarray nc is shown below.



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Guidelines for Probing the ProtoArray® Yeast Proteome Microarray, Continued

Important Guidelines

Since proteins are sensitive to various environmental factors, each array is produced in an environment-controlled facility to ensure protein integrity and maintain consistency.

To obtain the best results and avoid any damage to the array or array proteins, always handle the ProtoArray® Microarray with care using the following guidelines:

- Always wear clean gloves while handling the microarray
- **Do not** touch the surface of the array to avoid any damage to the array surface resulting in uneven or high background
- Maintain the array and reagents at 2-8°C during the experiment
- To prevent condensation on the array that may reduce protein activity or alter spot morphology, remove array from the mailer and immerse the array immediately in blocking solution when performing an experiment
- Perform array experiments at a clean location to avoid dust or contamination and filter solutions if needed (particles invisible to eyes can produce high background signals and cause irregular spot morphology)
- Avoid drying of the array during the experiment and ensure the array is completely covered with the appropriate reagent during all steps of the protocol
- Always dry the array prior to scanning and scan the array on the same day at the end of the experiment
- Do not dry the array using compressed air or commercial aerosol sprays
- Avoid exposing the array to light after probing with a fluorescent detection reagent

Probing Procedure

Introduction

After purifying the protein probe and verifying the presence of the tag or label on the protein, probe the ProtoArray® Yeast Protein Microarray nc using your protein probe.

Instructions are included in this section to probe the ProtoArray® Yeast Proteome Microarray using the ProtoArray® PPI Buffers Module available from Invitrogen (page vi) or your own buffers.

If you are preparing your own buffers, see page 18 for buffer recipes.

ProtoArray® PPI Buffers Module

The ProtoArray® PPI Buffers Module available from Invitrogen (page vi) includes qualified reagents for blocking and washing the ProtoArray® Yeast Proteome Microarray. The pre-made buffers provide consistent results and eliminate the time required to prepare reagents.

The module also includes HybriSlip™ cover slips that hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. Array Chambers are also included in the module for washing the microarray.

ProtoArray® Application Kit

The ProtoArray® Protein-Protein Interaction Application Kit includes ProtoArray® PPI Buffer Modules A and B and the appropriate Alexa Fluor® detection reagent. The use of the application kit provides consistent results and eliminates the time required to prepare reagents.

The ProtoArray® PPI Buffer Modules A and B include qualified reagents for blocking, washing, and probing during the microarray probing procedure (see above).

To use the ProtoArray® Yeast Protein Microarray nc with the ProtoArray® Application Kit, refer to the manual supplied with the application kit.

Experimental Outline

1. Block the ProtoArray® Yeast Proteome Microarray.
2. Probe with your tagged protein probe.
3. Perform detection using an appropriate detection system.
4. Dry the array for scanning.

Continued on next page

Probing Procedure Using the ProtoArray® PPI Buffers Module, Continued

Using Your Own Buffers

If you are preparing your own buffers, follow the guidelines listed below for buffer preparation to obtain the best results with microarrays. The buffer recipes are listed on the next page.

- Always use protease-free BSA for preparing buffers
- Perform blocking using BSA (gelatin or casein blocking is not recommended)
- Always use ultra pure water to prepare reagents and buffers
- You may use non-ionic detergents and reducing agents during probing to minimize non-specific interactions
- If the protein interaction requires certain co-factors, be sure to include the co-factors in the probing buffer during probing

Array Chamber

The microarray is placed in the Array Chamber during the blocking and washing steps. The Array Chamber should be able to hold ~25 ml reagent. Be sure the Array Chamber is made of non-protein binding material.

Cover slips

HybriSlip™ cover slips are available from Invitrogen (page vi) and hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. If you are using any other cover slip, be sure the cover slip is able to completely cover the printed area (20 mm x 60 mm) of the glass slide and the cover slip is made of non-protein binding material. Untreated glass cover slips are not recommended.

Materials Needed

- ProtoArray® PPI Buffers Module (page vi)
- Protein probe containing the suitable tag in Probing Buffer (next page)
- Appropriate -Alexa Fluor® 647 conjugate or equivalent (page vi); keep on ice in dark until immediately before use
- Antibody against the epitope tag for epitope tagged protein probe
- Sterile 50 ml conical tube
- Ice bucket
- Deionized water
- Array Chambers for holding the arrays (included in the ProtoArray® PPI Buffer Modules)
- HybriSlips™ or equivalent cover slips (included with the ProtoArray® PPI Buffer Modules)
- *Optional* microarray slide holder and centrifuge equipped with a plate holder

Continued on next page

Probing Procedure, Continued

- Prepare the PBST Blocking Buffer and Probing Buffer **fresh** prior to use. The recipes below provide sufficient buffers to probe one microarray. To probe more than one microarray, scale up the amount of reagents accordingly.
- Use the recipes described below to prepare your own buffers. Recommended buffers are listed below for blocking and washing the arrays. You can perform array probing using the recommended buffers and then based on your initial results optimize the buffer formulation.

Preparing PBST Blocking Buffer

1X PBS
1% BSA
0.1% Tween 20

1. Use reagents provided in the ProtoArray® Buffers Module to prepare 30 ml PBST Blocking Buffer as follows:

Blocking Buffer (10X)	3 ml
30% BSA	1 ml
Deionized water	to 30 ml

2. Mix well (do not vortex) and store on ice until use.

Immediately return the remaining 10X Blocking Buffer to 4°C and 30% BSA to -20°C.

Preparing Probing Buffer

1X PBS
5 mM $MgCl_2$
0.5 mM DTT
0.05% Triton X-100
5% Glycerol
1% BSA

1. Use reagents provided in the ProtoArray® Buffers Module to prepare 180 ml Probing Buffer as follows:

Probe Buffer (5X)	36 ml
1 M DTT	90 µl
1 M $MgCl_2$	0.9 ml
30% BSA	6 ml
Deionized water	to 180 ml

2. Mix well (do not vortex) and store on ice until use.

Immediately return the remaining 5X Probe Buffer and 1 M $MgCl_2$ to 4°C, and 1 M DTT and 30% BSA to -20°C.

Preparing the Probe

You need 120 µl of your protein probe containing a suitable tag. Dilute the probe to the recommended starting concentration (page 13) in Probing Buffer. Mix well (do not vortex) and store on ice until use.

Continued on next page

Probing Procedure, Continued

Before Starting

- Before starting the probing procedure, make sure you have all items on hand especially buffers (previous page), probe in Probing Buffer (previous page), Array Chambers (included in the ProtoArray® PPI Buffers Module), and HybriSlips™ (included in the ProtoArray® PPI Buffers Module).
- Make sure the buffers are cold. Store buffers on ice until use. Place the Array Chamber on ice to chill the chamber until use.
- Review **Important Guidelines** on page 15 prior to starting the probing procedure.

Important

Due to the large variety of protein probes and detection systems that can be used for probing the yeast microarray, it is not possible to have a single probing protocol that is suitable for all proteins and detection systems. Use the probing procedure from this section as a starting protocol and based on your initial results, empirically determine the probing protocol by optimizing the probe concentration, buffer formulation, incubation time, or detection reagents.

Optimization of probing protocol can be easily and rapidly achieved using multiple yeast microarrays.

Blocking Step

Instructions for blocking the microarray are described below:

1. Remove the mailer containing the ProtoArray® Yeast Protein Microarray nc from storage at -20°C and immediately place the mailer at 4°C.
2. Allow the mailer containing the array to equilibrate at 4°C for at least 15 minutes prior to performing the blocking step.
3. Perform blocking in the mailer. Ensure that the microarray is placed properly in the mailer with the printed (white) side facing up. Add 30 ml PBST Blocking Buffer (page 18) to the mailer containing the array.

   Note: You can block 2 arrays simultaneously in the mailer using 30 ml PBST Blocking Buffer.
4. Incubate for 1 hour at 4°C with gentle shaking (~50 rpm).
5. Decant the PBST Blocking Buffer. Drain excess buffer by inverting the mailer on paper towels for a few seconds. Remove the array from the mailer. Tap one edge of the array gently on a laboratory wipe for a few seconds to drain any buffer **without allowing the array to dry**. Place the array on a clean, flat surface with the printed side of the array facing up.
6. Proceed immediately to **Probing the Array**, next page.

Continued on next page

Probing Procedure, Continued

Probing the Array

1. Pipette 120 µl of the protein probe prepared in Probing Buffer (page 18) on top of the array without touching the array surface. The liquid quickly spreads over the nitrocellulose membrane.
2. Carefully lift the HybriSlip™ cover slip (supplied with the ProtoArray® PPI Buffers Module) from the support liner with forceps and lay the clear side of HybriSlip™ cover slip on the array to cover the membrane area without trapping any air-bubbles. The HybriSlip™ is designed to exactly cover the membrane area. Gently adjust the HybriSlip™ to remove any air-bubbles.
3. Insert the array with HybriSlip™ into a separate 50 ml conical tube with the printed side of the array facing up. Cap the conical tube.
4. Place the conical tube on a flat surface such that the printed side of the array is facing up and the tube is as level as possible. If needed, you can tape the conical tube on the flat surface to avoid any accidental disturbances. Incubate the array in the tube for 1.5 h at 2-8°C **without shaking**.
5. Remove the array from the conical tube and insert the array diagonally (see Note below) into the Array Chamber kept on ice.

   Note: The microarray with HybriSlip™ does not fit on the rails of the chamber. You need to insert the microarray diagonally into the chamber.
6. Using a sterile pipette, add 25 ml Probing Buffer (page 18) to the chamber wall while keeping the chamber on ice. **Avoid pipetting buffer directly onto the array surface.** The addition of buffer usually separates the HybriSlip™ from the array.
7. Carefully remove the HybriSlip™ with forceps without touching the array surface with forceps. Discard the HybriSlip™. The array can now be repositioned on the chamber rails, if desired.
8. Incubate the array in Probing Buffer for ~1 minute on ice. Decant the Probing Buffer. Invert chamber on paper towels for a few seconds to drain excess buffer.
9. Add 25 ml Probing Buffer to the chamber and incubate the array in Probing Buffer for ~1 minute on ice. Decant the buffer. Repeat this wash step once.
10. Prepare the appropriate dilution of the labeled antibody solution or labeled streptavidin solution with 25 ml Probing Buffer.
11. After the 1 minute incubation with Probing Buffer, decant the buffer. Invert chamber on paper towels for a few seconds to drain excess buffer. Add 25 ml labeled antibody or streptavidin solution from Step 10 to the chamber.
12. Incubate the chamber for 30 minutes on ice in dark (if needed). Decant the solution. Invert the chamber on paper towels to drain excess buffer.
13. Slowly add 25 ml Probing Buffer onto the chamber wall while keeping the chamber on ice. Avoid pipetting buffer directly onto the array surface.
14. Incubate the array in Probing Buffer for ~1 minute on ice. Decant the buffer. Drain excess buffer by inverting chamber on paper towels for a few seconds.
15. Repeat Steps 13-14 twice, using 25 ml Probing Buffer each time.
16. Proceed to **Drying the Array**, next page.

Continued on next page

Probing Procedure, Continued

Drying the Array

1. Remove the array from the chamber at the end of the probing procedure. Tap one edge of the array gently on a laboratory wipe for a few seconds to drain any buffer.
2. Place the array in a slide holder (or a sterile 50 ml conical tube, if you do not have a slide holder) in a vertical orientation. Ensure the array is properly placed and is secure in the holder to prevent any damage to the array during centrifugation.
3. Centrifuge the array in the slide holder or 50 ml conical tube at 800 x g for 3-5 minutes in a centrifuge (equipped with a plate rotor, if you are using the slide holder) at room temperature.
4. Place the array in a slide box and keep the box with the lid open in **dark** for 30-60 minutes at room temperature for drying the array.
5. Scan the array using a fluorescent microarray scanner (page 23 for details) after the array is completely dry with no translucent areas.

Data Analysis

After scanning and saving an image of the array, analyze results to identify positive interactors. For more details, see page 24.

1. To acquire data from the scanned image, use the barcode on the array to download the .GAL file from ProtoArray® Central as described on page 24.
2. Use the .GAL file and a suitable microarray data acquisition software to acquire pixel intensity values for all features on the control array.
3. Analyze data using the guidelines on page 24 to determine significant signals.

Cleaning the Chamber

At the end of probing experiments, clean the Array Chambers properly and rinse with sterile water before re-using the chambers.

Scanning Arrays Using a Fluorescent Scanner

Introduction

Once you have probed the ProtoArray® with your protein, scan the microarray using a suitable microarray scanner. Instructions are included in this section to scan the microarray using a fluorescent microarray scanner.

Non-Fluorescent Scanners

If you have used a non-fluorescent detection system such as chemiluminescence or radioactivity, you need to use an imaging system with a CCD camera such as the Alphaimager™ Imaging System (for chemiluminescence detection) or a phosphoimager scanner such as PerkinElmer Cyclone phosphor imaging system (for detecting radioactivity).

If you are not using a fluorescent detection system, use the manufacturer's recommendations to scan the microarray.

Materials Needed

You need a suitable scanner to scan the ProtoArray® Yeast Proteome Microarray. To acquire ProtoArray® data from the image, you need appropriate microarray data acquisition software.

The recommended microarray data acquisition software for analysis is GenePix® Pro (Molecular Devices Corporation) or ScanArray® Software (PerkinElmer, Inc.).

The scanner specifications are listed on the next page.

Experimental Outline

1. Insert array into the fluorescent microarray scanner.
2. Adjust scanner settings.
3. Preview the microarray and adjust settings, if needed.
4. Scan the microarray.
5. Align grid over spots and use image analysis software to align features.
6. Export and analyze results.

Scanner Specifications

The fluorescence microarray scanner specifications required to image the ProtoArray® Yeast Proteome Microarray are listed in the table below.

A list of recommended fluorescent microarray scanners is available from ProtoArray® Central at www.invitrogen.com/protoarray.

Array Compatibility	**Size**	Standard 1" x 3" or 25 mm x 75 mm microscope slides
	Thickness	1 mm
Detection	**Light and Detector Orientation**	Facing array
	Scanned Area	22 mm x 73 mm
	Focus	Auto focus or adjustable ($\pm$ 200 µm)
	Excitation	Depends on the fluorophore used for detection
	Detection limit	0.1 fluor/µm^2
	Resolution	$\leq$10 µm
	Dynamic Range	>3 orders of magnitude
	Output	16-bit TIFF

Continued on next page

Scanning Arrays Using a Fluorescent Scanner, Continued

Note

Unlike DNA microarrays, scan the ProtoArray® Yeast Proteome Microarray using only one color.

Scanning Procedure

A brief procedure for scanning the ProtoArray® Yeast Proteome Microarray with a fluorescent microarray scanner is described below.

For details on using a specific scanner or non-fluorescent scanner, refer to the manual supplied with the scanner.

The scanning time for each array is ~7-8 minutes.

1. Start the appropriate array acquisition and analysis software on the computer connected to the fluorescence microarray scanner.
2. Open the microarray enclosure on the scanner.
3. Place the ProtoArray® Yeast Proteome Microarray in the holder such that the nitrocellulose-coated side of the array faces the laser source and barcode on the array is closest to the outside of the instrument.
4. Close the microarray enclosure on the scanner.
5. Set the following settings to image the microarray:
   - Wavelength: Choose the appropriate wavelength based on the fluorophore used for detection
   - PMT Gain: 600
   - Laser Power: 100%
   - Pixel Size: 10 μm
   - Lines to Average: 1.0
   - Focus Position: 0 μm
6. Perform a preview to quickly scan the microarray. Adjust the PMT Gain, if needed. **Note:** The image should have very few saturated spots (white).
7. Select the area of the array to scan in detail (include the barcode in the area for record) and then scan the array to provide a high-resolution image.
8. After acquiring the image, save the image to a suitable location as 'multi-image TIFF file'. Be sure the barcode is included in the name of the image.
9. Open the microarray enclosure and remove the microarray from the holder.
10. Proceed to downloading lot-specific information available on the ProtoArray® Central Portal, next page.

Data Acquisition and Analysis

Introduction

After scanning and saving an image of the array, download the protein array lot specific information (including the .GAL file) from the ProtoArray® Central Portal. Use the lot specific information to acquire and analyze the data to identify protein-protein interactions.

Note: To familiarize yourself with the array and subarray layout, you may also download a file showing the subarray layout from ProtoArray® Central. To access the file, go to www.invitrogen.com/protoarray and click on Online Tools.

Important

While downloading the lot specific information files, ensure that you are downloading files that are associated with your specific barcode on the array. Since lot specific information files are updated frequently based on recently available sequence or protein information, make sure that you download the latest version of the lot specific information files.

GAL File

The .GAL (GenePix Array List) files describe the location and identity of all spots on the protein microarray and are used with the microarray data acquisition software to generate files that contain pixel intensity information for all features on the array.

The .GAL files are available for downloading from the ProtoArray® Lot Specific Information available on ProtoArray® Central, see below.

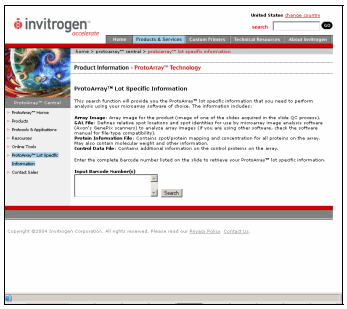
Note: The .GAL files are text files that contain the data in a format specified by GenePix® Pro Microarray data acquisition software. If you are using any other microarray data acquisition software, you can use data from the .GAL files to generate files that are compatible with your microarray data acquisition software.

ProtoArray® Central

The ProtoArray® Central provides a web-based user interface to retrieve ProtoArray® Lot Specific information. This information (.GAL file) is required for acquiring the array data.

If the scanner computer is connected to the internet, click on the link below to connect to the portal. If the scanner computer is not connected to the internet, download the array-specific information to portable media as described below and then download the information onto the scanner computer.

1. Connect to the portal at www.invitrogen.com/protoarray and then click on the Online Tools tab.
2. A ProtoArray® Lot Specific Information page is displayed.
3. Enter the array barcode in the Input Barcode Number box. Click on the Search button.



Continued on next page

ProtoArray® Central, continued

4. For each input barcode, the following files are displayed:

   .GAL file (LotNumber.gal):

   This file is essential for data acquisition by the software and defines spot locations and identities of all protein spots on the array. The file also includes the "equivalent solution protein concentration" in nM for use during data analysis.

   Protein Information File (LotNumber_info.txt):

   This file contains a listing and description of human proteins on the array.

   Protein Sequence File (LotNumber_seq.txt):

   This tab-delimited text file lists the GenBank® accession number, Ultimate™ ORF Clone ID number (if available), FASTA header, and amino acid sequence of the ORF for each array protein.

   Control Data File (LotNumber_control.txt):

   This file contains a description of control spots on the array.

   Protein Application File (LotNumber_application.PAI):

   ProtoArray® Prospector uses the Protein Application Files for data analysis. Different PAI files are designed for different applications. For example, ProtoArray® Prospector uses the file HA10756 PPI.PAI to analyze data from PPI experiments performed on array from lot HA10756.

   Slide Information File (LotNumber_slide.txt):

   This file contains a listing of all barcodes associated with a specific lot of arrays.

5. Download the files listed above for yeast array-specific information from a specific lot. Use these files to interpret your results with the ProtoArray® Yeast Microarray as described below.

   Note: The file size for some files such as the Protein Sequence File may be> 1 MB.

6. Start the microarray data acquisition software on the computer and open the saved image (.tiff) from Step 8, page 23.

7. To acquire data from ProtoArray® experiments,

   - For GenePix® Pro Software, download the .GAL files from ProtoArray® Central for protein arrays which defines the array grid required by the microarray data acquisition software.
   - For other microarray data acquisition software, use data from the .GAL files from ProtoArray® Central for protein arrays to generate files that are compatible with your microarray data acquisition software to define the array grid.

   Scroll through the image to ensure that the grid is in proper location for each subarray. Adjust the subarray grid, if needed.

8. After the grid is properly adjusted and all of the features are aligned, save/export the results as a .GPR (GenePix® Results) file for data analysis using ProtoArray® Prospector (next page). The results contain the pixel intensity information for each spot/feature on the array and information on additional parameters depending on the type of software used for data acquisition.

Alternatively, save/export the results with an .xls extension or rename the .tab or .gpr file using the .xls extension for data analysis using Microsoft® Excel.

Continued on next page

Data Acquisition and Analysis, Continued

Analyzing Data

After data acquisition, analyze the data to identify protein interactions.

Visual identification of interactions can be performed after initial identification of significant interactions is done using the data analysis guidelines listed below.

We recommend using the ProtoArray® Prospector software available from Invitrogen for data analysis. This software allows rapid data analysis without the need to perform any manual calculations. For more information, see below.

Performing the data analysis by importing the data file into Microsoft® Excel or an equivalent spreadsheet program to identify potential interactions is not recommended. This approach requires a certain degree of expertise with statistics and Excel or another spreadsheet program.

Data Analysis Using ProtoArray® Prospector

The ProtoArray® Prospector software quickly analyzes the data acquired from the image acquisition software and easily identifies statistically significant interactors, saving you time and effort. In addition, the software has features that allow you to modify the analysis method and compare data obtained from different slides.

The ProtoArray® Prospector software and manual are available free-of-charge to ProtoArray® users. To download the ProtoArray® Prospector software or manual, go to www.invitrogen.com/protoarray, and click on the Online Tools tab. Install the **Basic** version of ProtoArray® Prospector for data analysis.

The ProtoArray® Prospector software currently accepts the output files (.GPR) generated by the GenePix® Pro microarray data acquisition software, and analyzes the data using specified algorithms to generate a list of human proteins showing significant interactions with the protein probe. If .GPR files are not available, consult the ProtoArray® Prospector manual for guidelines to format a results file that is compatible for import into ProtoArray® Prospector.

Analyzing ProtoArray® Prospector Results

After data analysis, ProtoArray® Prospector presents a summary of the analyzed data in a table format (see ProtoArray® Prospector manual for details).

The proteins that score as positive in the experiment are proteins that satisfy the basic program options. Review the information on page 28, **Expected Results**, to help with data interpretation.

Based on the Z-score and available protein sequence information, we recommend validating the interactions as described on the next page.

Continued on next page

The Next Step

After identifying a positive interaction on the ProtoArray® Yeast Microarray, you may validate the protein interaction using the ProtoArray® Technology or other methods.

Using the ProtoArray® Technology, validate the protein-protein interactions by performing experiments with additional arrays to ensure:

Reproducibility

Probe protein arrays using a similar or a different probe concentration to observe similar interactions.

Specificity

Probe protein arrays with different proteins containing the tag to identify interactions specific to your protein probe of interest and also identify any non-specific interactions.

Reciprocal Interactions

Determine reciprocal interactions as described on page 6 using a purified protein probe.

Other methods for validating protein-protein interactions include:

- Yeast Two-Hybrid Systems (page vi)
- Co-immunoprecipitation
- Gel-shift assay

Expected Results

Introduction

The controls printed on the ProtoArray® Yeast Proteome Microarray are useful in verifying the probing, detection, and scanning protocols as described below.

Based on the initial results, you may need to optimize the probing and detection protocol by optimizing the probe concentration, buffer formulation, incubation time, or detection reagents.

Note: To identify protein-protein interaction specific to your protein probe, we recommend probing a second array with another protein probe that enables you to determine probe-specific interactions and identify any non-specific interactions.

Control	Description	Function	Verification
Alexa Fluor® Ab	A rabbit anti-mouse IgG antibody labeled with Alexa Fluor® dye is printed on the microarray	The fluorescent antibody signals indicate proper scanning procedure and are used as reference spots to orient the microarray and help assign spot identities.	Scanning procedure using a fluorescent microarray scanner.
Anti-biotin Antibody	A mouse anti-biotin antibody is printed on the microarray.	The biotinylated protein binds to the anti-biotin antibody on the array. Signals at this spot indicate that the probe is biotinylated. A weak or low signal at the Anti-biotin spots indicates poor biotinylation. If free biotin is not removed after biotinylation, the free biotin binds non-specifically to the microarray increasing the background and decreasing the signal with the Anti-biotin spot.	Proper *in vitro* biotinylation of proteins and check background levels.
		A secondary anti-mouse labeled antibody binds to Alexa Fluor® Ab to produce signals and can be used as reference spots.	Proper probing and detection reagents.
Biotinylated Antibody	A goat anti-mouse IgG labeled with biotin is printed on the microarray	The streptavidin-conjugate binds to the biotinylated antibody.	Proper probing procedure.

Continued on next page

Expected Results, Continued

Control	Description	Function	Verification
V5 Control Protein	A protein with a BioEase™ (biotin)-V5-tag on the N-terminus is printed on the microarray	An anti-V5-antibody or streptavidin conjugate binds to the V5 Control protein. The signals indicate that the antibody is functional and probing is performed properly. The signal is also used to check the background.	Detection reagents and probing
Calmodulin	Yeast calmodulin is printed on the array.	The Array Control Protein (calmodulin kinase with N-terminus BioEase™-V5 tag) supplied with the Control Protein Microarray binds to the calmodulin on the array.	Probing procedure
GST Protein Gradient	A GST protein gradient is printed on the array	Detects GST-tagged protein probe or non-specific binding to GST and serves as a negative control	Negative Control

Troubleshooting

Introduction

The table below provides some solutions to possible problems you might encounter when using the ProtoArray® Yeast Proteome Microarray.

Review the expected results section (page 28) to verify the probing, detection, and scanning procedures are performed correctly.

Problem	Cause	Solution
Protein Probe		
No signal after western detection using an antibody against the protein	Poor or incomplete transfer	Monitor the transfer of pre-stained protein standard bands to determine the transfer efficiency.
	Insufficient exposure time	Increase the exposure time.
	Epitope tag not present or cleaved	Confirm the presence of the tag by sequence analysis and ensure the tag is cloned in frame. Perform all purification steps at 4°C and use protease inhibitors to prevent proteolytic cleavage of the tag.
Poor or no biotinylation for your protein probe	Incorrect buffers used or the biotinylation reaction is not performed correctly	Make sure the protein is in a buffer that does not contain any primary amines such as ammonium ions, Tris, glutathione, imidazole, or glycine.
		Make sure that the biotinylation reaction was performed correctly using the specified molar ratios and at pH ~8.0. Check that the calculations and serial dilutions are performed correctly.
	Protein has low lysine content or lysine residues are not readily available for biotinylation	Perform the biotinylation reaction at a higher molar ratio. You may express your protein as fusion to a tag that contains lysine.
Additional biotinylated bands observed	Protein impurities present that undergo biotinylation and may cause high background during probing	Purify protein to remove impurities and perform biotinylation to ensure the absence of additional biotinylated bands.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Protein Array Results		
Weak or no signal with protein probe	Epitope tag not present or not accessible	Confirm the presence of the tag by western detection. Ensure the tag is accessible under native conditions by performing an ELISA.
	Poor biotinylation of protein probe	See previous page for details.
	Low probe concentration	Perform probing with higher probe concentration or increase the incubation time.
	Incorrect probing procedure	Follow the recommended protocol for probing on page 16. Be sure all incubations are performed at 4°C. Prepare the PBST Blocking Buffer and Probing Buffer **fresh** as described on page 18.
		Do not allow the array to dry during the probing procedure.
		Avoid prolonged exposure of detection reagents labeled with fluorescent dye to light.
	Incorrect scanning or imaging	Scan the array at suitable wavelength for the detection system used and place the array in the slide holder such that the proteins on the array are facing the laser source.
	Decrease stringency	Decrease the number of washes. Perform probing and washing in the absence or lower concentration of detergent or salts.
High background	Improper blocking	Prepare the PBST Blocking Buffer **fresh** as described on page 18.
	Improper washing	To obtain the best results, perform the recommended washing steps. Prepare the Probing Buffer **fresh** as described on page 18.
	Used Alexa Fluor® 555 or Cy3™ dyes for detection	Always use Alexa Fluor® 647 or Cy5™ dyes for detection as the nitrocellulose surface has high intrinsic fluorescence at the wavelength used to visualize Alexa Fluor® 555 or Cy3™ dyes.
	Array dried during probing	Do not allow the array to dry during probing.
	Array not dried properly before scanning	Dry the array as described on page 21 before scanning.
	High probe concentration	Decrease the probe concentration or decrease the incubation time.
	Antibody cross-reactivity	Probe a protein array using only the antibody without the protein probe to detect cross-reactivity with the Ab only.

Continued on next page

Problem	Cause	Solution
Uneven background	Uneven blocking or washing	During the blocking or washing steps, ensure the array is completely immersed in blocking solution or Probing Buffer and use at least 25 ml buffer in the Array Chamber to cover the array completely with buffer.
	Improper washing	To obtain the best results, perform the recommended washing steps. Prepare the Probing Buffer **fresh** as described on page 18.
	Portions of array have dried	Do not allow the array to dry during probing.
	Improper array handling	Always wear gloves and avoid touching the surface of the array with gloved hands or forceps. Take care while inserting the array into the Array Chamber to avoid scratching the array surface.
	Protein probe not applied properly	Apply the probe solution and HybriSlip™ or equivalent cover slip to the array as described in the manual. To avoid drying of the membrane, make sure the cover slip covers the membrane area of the array and adjust the cover slip, if needed.
	Probe or detection reagents contain precipitates	Centrifuge the probe or detection reagents to remove precipitates prior to probing the array.

Appendix

Technical Support

Web Resources

Visit the Invitrogen Web site at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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MSDS

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

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