invitrogen™

ProtoArray® Yeast Proteome Microarray mg

For identifying protein kinase substrates using a yeast proteome microarray

Catalog no. PA012106

Version B

18 October 2006
25-0837

User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

The ProtoArray® Yeast Proteome Microarray mg (modified glass) is shipped on blue ice. Upon receipt, store the microarray at -20°C. The **expiration date** is printed on the package for each array. Use the array before the expiration date for best results.

Contents

Each ProtoArray® Yeast Proteome Microarray box contains a mailer with one yeast proteome microarray.

Store the microarray at -20°C.

For best results, use the microarray before the specified expiration date.

For details on array specifications, see pages 4.

Product Qualification

The ProtoArray® Yeast Proteome Microarray is visually examined for obvious defects.

The ProtoArray® Yeast Proteome Microarray is probed with the Control Kinase in the presence of radiolabeled ATP as described in this manual. For details on the controls printed on the array, see page 6.

After scanning and analysis, the following results must be observed:

- Fiduciary Kinase signals are observed at the expected locations
- Signals observed at the Control Kinase substrate location
- GST (Glutathione-S-Transferase) spots do not produce any significant signals

Accessory Products

Additional Products

The table below lists additional products available separately from Invitrogen. For more information about these products, refer to our website (www.invitrogen.com) or call Technical Support (page 29).

Product	Quantity	Catalog no.
ProtoArray® Human Protein Microarray mg v4.0	1 array	PAH052406
ProtoArray® Control Protein Microarray mg v4.0	1 array	PA1002
ProtoArray® Control Protein Microarray nc v4.0	1 array	PA1007
ProtoArray® Human Protein Microarray nc v4.0	1 array	PAH052401
ProtoArray® Yeast Proteome Microarray nc v1.1	1 array	PA012101
ProtoArray® Human Protein Microarray KSI Complete Kit v4.0 *for kinase substrate identification*	1 kit	PAH0524065
ProtoArray® Yeast Proteome Microarray KSI Complete Kit v1.1 *for kinase substrate identification*	1 kit	PA0121065
ProtoArray® Protein-Protein Interaction Buffer Modules	1 kit	PA014
ProtoArray® Human Protein Microarray PPI Complete Kit v4.0 *for biotinylated proteins*	1 kit	PAH0524011
ProtoArray® Yeast Proteome Microarray PPI Complete Kit v1.1 *for biotinylated proteins*	1 kit	PA0121011
ProtoArray® Protein-Protein Interaction Application Kit *for biotinylated proteins*	1 kit	PA011
ProtoArray® Human Protein Microarray PPI Complete Kit v4.0 *for epitope-tagged proteins*	1 kit	PAH0524013
ProtoArray® Yeast Proteome Microarray PPI Complete Kit v1.1 *for epitope-tagged proteins*	1 kit	PA0121013
ProtoArray® Mini-Biotinylation Kit	1 kit	AL-01
ProtoArray® Kinase Substrate Identification Application Kit	1 kit	PA015
ProtoArray® Immune Response Biomarker Profiling Application Kit	1 kit	PA016
NuPAGE® Novex® 4-12% Bis-Tris Gel (1.0 mm, 10-well)	1 box	NP0321BOX
NuPAGE® MOPS SDS Running Buffer (20X)	500 ml	NP0001
NuPAGE® MES SDS Running Buffer (20X)	500 ml	NP0002
NuPAGE® Transfer Buffer (20X)	125 ml	NP0006
NuPAGE® Antioxidant	15 ml	NP0005
NuPAGE® Sample Reducing Agent (10X)	250 µl	NP0004
NuPAGE® LDS Sample Buffer (4X)	10 ml	NP0007
Phosphate Buffered Saline (PBS), 1X	500 ml	10010-023

Kinase

A variety of purified kinases is available from Invitrogen for use with ProtoArray® Microarrays. For more information about these products, refer to our website at www.invitrogen.com or contact Technical Support (page 29).

Introduction

Overview

Introduction

The ProtoArray® Yeast Proteome Microarray mg for Kinase Substrate Identification (KSI) allows rapid and efficient identification of potential kinase substrates using a protein kinase of interest. The ProtoArray® Yeast Proteome Microarray contains >4000 purified proteins from *Saccharomyces cerevisiae*, printed in duplicate on a modified glass (mg) slide. See the next page for an overview of the system.

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

ProtoArray® Yeast Proteome Microarray

The ProtoArray® Yeast Proteome Microarray mg (modified glass) 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. The ProtoArray® Technology has recently been shown to be a powerful method to rapidly identify substrates for protein kinases (Mah *et al.*, 2005; Ptacek *et al.*, 2005).

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 modified glass slide. Using a protein kinase of interest in the presence of radioalabeled ATP, you can screen against >4000 *S. cerevisiae* ORF's within 2 days to identify potential kinase substrates.

The modified glass array is a thin nitrocellulose coated slide from GenTel® BioSciences, Inc., and is specifically selected for superior performance of the array in the KSI application. Thin-film nitrocellulose slides are manufactured by GenTel® BioSciences, Inc. using a proprietary surface chemistry owned by Decision Biomarkers, Inc.

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

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

ProtoArray® Microarray KSI Applications

The ProtoArray® Yeast Proteome Microarray mg for KSI allows you to:

- Identify potentially biologically relevant protein kinase substrates
- Validate previously observed signals for KSI applications
- Test various experimental conditions for the kinase

Continued on next page

System Overview

To use the ProtoArray® Yeast Proteome Microarray mg for KSI, you will:

- Purify your kinase of interest using a method of choice or purchase a purified protein kinase from Invitrogen (page vi).
- Probe the ProtoArray® Yeast Proteome Microarray mg with your kinase of interest in the presence of labeled [γ-^{33}P]ATP to identify potential substrates for your specific kinase.

The ProtoArray® KSI protocol includes instructions to block the array, probe the array with your kinase in the presence of radiolabeled [γ-^{33}P]ATP, wash to minimize non-specific binding, dry the array, expose the array to phosphorscreen or X-ray film, and acquire the array image to view results and analyze data. For a detailed experimental workflow, see page 9.

Advantages

Using the ProtoArray® Yeast Proteome Microarray mg for KSI offers the following advantages:

- Provides a simple, rapid, and sensitive method to identify kinase substrates
- Allows screening of your kinase of interest against thousands of full-length yeast proteins in an easy-to-use format
- Built-in controls are printed on each array to control for background, detection, and analysis
- Simple signal detection using autoradiography or phosphorimaging

ProtoArray® Central Application Portal

The ProtoArray® Central Application Portal at www.invitrogen.com/protoarray provides a web-based user interface to access ProtoArray® specific information including online tools, applications, and other resources. You also use the portal to retrieve ProtoArray® Lot Specific information (see page 22), which is required for analysis of the array data and identification of statistically significant hits (potential substrates).

ProtoArray® Prospector Software

The ProtoArray® Prospector software version 4.0 (includes Imager and Analyzer) quickly analyzes the microarray data 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 or manual, go to www.invitrogen.com/protoarray, and click on the Online Tools tab.

Continued on next page

Expected Results

ProtoArray® Yeast Protein Microarrays are designed for kinase substrate identification. After performing the KSI assay and identifying potential kinase substrates, we recommend that you validate the observed substrate phosphorylation using another method such as *in vitro* solution assay.

Using ProtoArray® Yeast Protein Microarrays, we have typically observed a true positive rate of ~80% for serine-threonine protein kinases.

A true positive signal is defined as a phosphorylation signal observed on a protein microarray that is validated as a substrate using an *in vitro* solution assay.

The kinase substrate identification assay depends on various factors such as the buffer composition, kinase activity/concentration, assay conditions, ATP concentration, protein sequence, and the amount of protein on the array.

It is possible that some proteins reported in literature as substrates for the kinase may not be identified as kinase substrates on the array. When comparing the kinase substrate data obtained from ProtoArray® experiments to kinase annotated substrates as reported in the literature, it is important to review the experimental conditions used for identifying a protein as a substrate for the kinase. In many cases, several proteins annotated in the literature as kinase substrates have been identified using *in vivo* based approaches, which are usually not conclusive. Sometimes the identified signals on the array may be due to the interaction of an array protein with radiolabeled ATP or autophosphorylated protein kinase, thereby causing false positive results. To minimize the number of false positive signals arising due to non-specific interaction and to decrease the number of signals not arising from protein kinase phosphorylation of array proteins, wash the kinase-treated microarray with denaturing SDS as described in the assay protocol.

Purpose of the Manual

This manual provides the following information:

- An overview of the ProtoArray® Yeast Proteome Microarray mg for KSI
- Instructions to probe the ProtoArray® Microarray with your protein kinase
- Guidelines to perform data analysis
- Troubleshooting

ProtoArray® Yeast Proteome Microarray

Introduction

The ProtoArray® Yeast Proteome Microarray mg (modified glass) is a high-density protein microarray containing the majority of proteins from *S. cerevisiae* for kinase substrate identification. Each *S. cerevisiae* open reading frame (ORF) is expressed as an N-terminus GST-6xHis fusion protein, purified, and printed in duplicate on a modified glass slide.

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

Yeast Proteome Microarray Specifications

The specifications for the ProtoArray® Yeast Proteome Microarray mg are listed below.

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with a proprietary polymer.

The modified glass array is a thin nitrocellulose coated slide from GenTel® BioSciences, Inc., and is specifically selected for superior performance of the array in the KSI application. Thin-film nitrocellulose slides are manufactured by GenTel® BioSciences, Inc. using a proprietary surface chemistry owned by Decision Biomarkers, Inc.

Each microarray has a barcode for tracking samples. The barcode is also used to retrieve array specific information from the ProtoArray® Central Application Portal (page 22).

Array Specifications

The array specifications for the ProtoArray® Yeast Proteome Microarray mg for KSI 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 Proteome Microarray mg, go to the ProtoArray® Central Application Portal at www.invitrogen.com/protoarray.

Note: The subarray layout and controls are different 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 using a modified version of the yeast expression vector pEG-KG (Mitchell *et al.*, 1993). The identity of each clone was verified using 5'-end DNA sequencing and the expression of expected 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 that are designed to maximize protein integrity, function, and activity.

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 printing the proteome microarray.

Printing the Yeast ProtoArray®

The purified yeast proteins are printed on modified glass slides in a dust-free, temperature, and humidity controlled environment to maintain consistent quality of the microarrays. The arrays are printed using an automated process on an arrayer that is extensively calibrated and tested for printing ProtoArray® 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. To maintain protein stability and function, arrays are printed at 6°C 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 microarrays with an anti-GST antibody allows identification of irregular spot morphology or missing spots. The arrays are functionally qualified by probing with radiolabeled ATP in the absence and presence of the Control Kinase to confirm phosphorylation of the Control Kinase substrate and activity of Fiduciary Kinases.

For detailed product qualification, see page v.

Continued on next page

ProtoArray® Yeast Proteome Microarray, Continued

Controls Printed on Each ProtoArray® Microarray

Various proteins and other controls are printed on each ProtoArray® Yeast Proteome Microarray mg to allow you to verify reagents, background, and detection conditions used during probing.

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

Note: Some of the controls printed on the arrays are not required for analysis using the KSI protocol.

Protein	Function
Control Spots required for KSI 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.
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 KSI 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.
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.
Yeast calmodulin (Cmd1p)	A positive control for protein-protein interaction application and interacts with the Array Control Protein.
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.

Working with Radioactive Material

Introduction

This section provides general guidelines and safety tips for working with radioactive material. For more information and specific requirements, contact the safety department of your institution.

Use extreme caution when working with radioactive material. Follow all federal and state regulations regarding radiation safety. For general guidelines when working with radioactive material, see below.

General Guidelines

Follow these general guidelines when working with radioactive material.

- Do not work with radioactive materials until you have been properly trained.
- Wear protective clothing, vinyl or latex gloves, and eyewear, and use a radiation monitor.
- Work in areas with equipment and instruments that are designated for radioactive use.
- Plan ahead to ensure that all the necessary equipment and reagents are available and to minimize exposure to radioactive materials.
- Monitor work area continuously for radiation contamination.
- Dispose of radioactive waste properly.
- After you have completed your experiments, monitor all work areas, equipment, and yourself for radiation contamination.
- Follow all the radiation safety rules and guidelines mandated by your institution.

Important

Any material in contact with a radioactive isotope must be disposed of properly. This includes any reagents that are discarded during the probing procedure (*e.g.* washes). Contact your safety department for regulations regarding radioactive waste disposal.

Experimental Overview

Experimental Steps

The recommended experimental steps are outlined below. Detailed experimental workflow is shown on the next page.

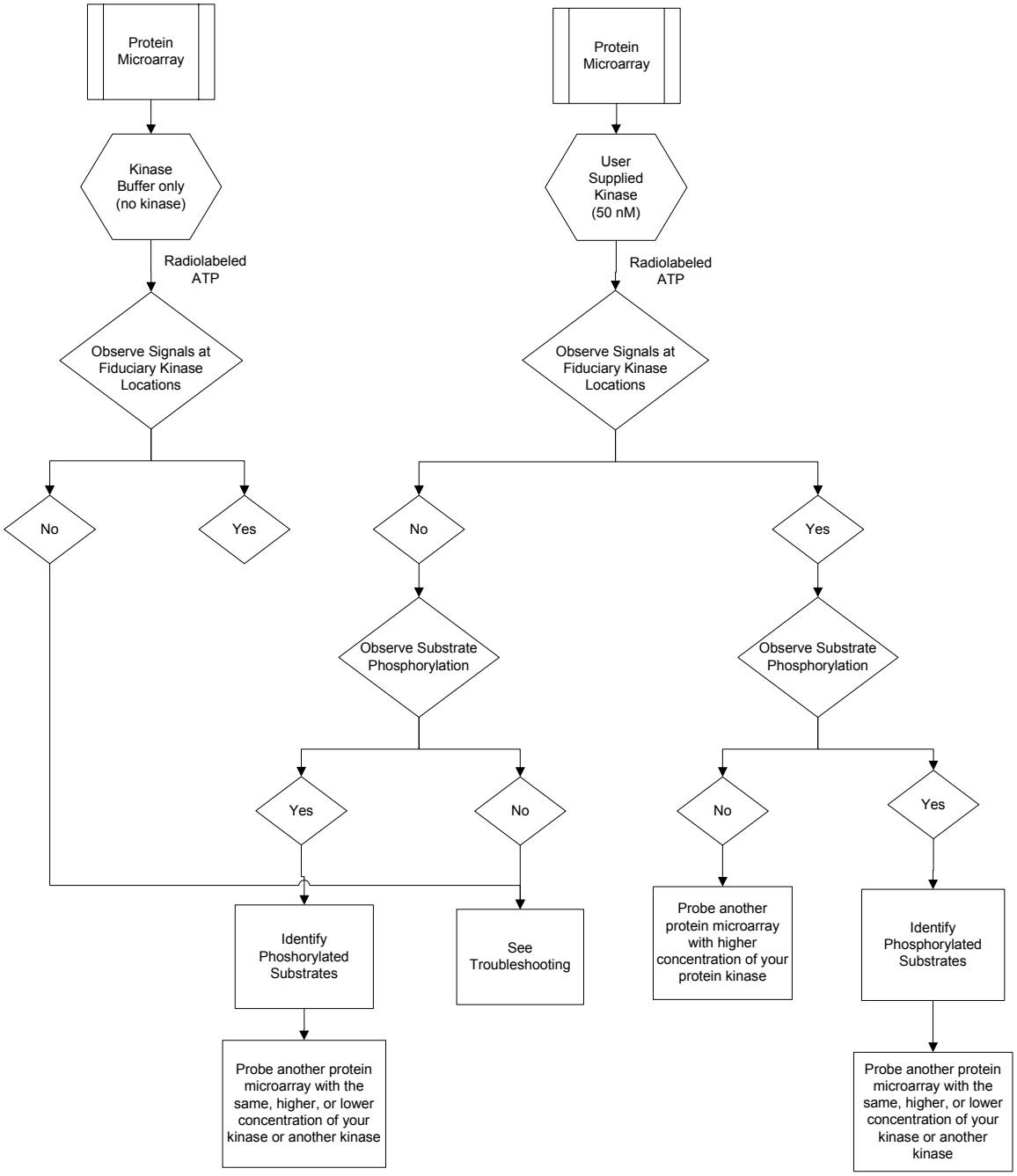
Step	Action	Page no.
1	Purify your protein kinase of interest using a method of choice or purchase the protein kinase of interest from Invitrogen.	10
2	Probe the yeast microarray with the protein kinase of interest in the presence of radiolabeled ATP. **Optional:** If you are a first time user of the ProtoArray® Yeast Proteome Microarray mg, perform a control probing using a ProtoArray® Control Microarray mg to verify the assay protocol.	11
3	Dry the microarray.	19
4	Expose the microarray to X-ray film or phosphorscreen for 18-24 hours.	19
5	Scan the developed X-ray film or phosphorscreen and save an image of the array.	20
6	Download the protein array lot specific information (mainly the .GAL file) from ProtoArray® Central Portal to acquire and analyze the data using ProtoArray® Prospector to identify protein kinase substrates.	22

Continued on next page

Experimental Overview, Continued

Experimental Workflow

The experimental workflow for probing ProtoArray® Yeast Proteome Microarray mg with your protein kinase of interest is shown below.



Methods

Preparing the Protein Kinase

Introduction

Before using the ProtoArray® Yeast Proteome Microarray mg for KSI, you need to purchase or purify the protein kinase of interest to probe the microarray.

You may purify the protein kinase using any method of choice. You can use proteins purified from *E. coli*, yeast cells, or higher eukaryotes to probe the ProtoArray® Microarray.

A large variety of highly purified protein kinases are available from Invitrogen. For details, visit www.invitrogen.com or contact Technical Support (page 29).

The amount of protein and quality of protein required for probing are described below.

Protein Amount and Quality

- **Purify the protein kinase under native conditions.**
- Proteins should be > 90% pure as determined by Coomassie® staining.
- Check the activity of the protein kinase after purification using a method of choice.
- Dilute the kinase for use during probing in the Kinase Buffer (see recipe on page 15).
- Make sure the protein kinase is soluble and active in buffers used for probing the microarray (see recipe on page 16).
- You need at least 120 µl of your purified protein kinase at a recommended initial protein concentration of **50 nM** to probe each ProtoArray® Microarray.

Probing the ProtoArray® Yeast Proteome Microarray

Introduction

Instructions are included in this section for probing the ProtoArray® Yeast Proteome Microarray mg using your protein kinase. Follow the guidelines provided in this section.

To perform the probing, you will use radiolabeled ATP. Various options are available for performing the probing procedure (see below). An experimental workflow for probing is shown on page 9.

Important

Each ProtoArray® Yeast Proteome Microarray can only be used once. Do not re-use the array or reprobe the same array with another kinase.

Probing Options

The recommended protein kinase concentration for probing the Yeast Proteome Microarray is 50 nM.

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

Probing Array with…	…Then Choose	… And Use Protocol
Protein kinase using application kit	ProtoArray® Kinase Substrate Identification Application Kit (page vi)	Supplied in the manual shipped with the application kit.
Protein kinase using your own buffers	Your own buffers and reagents	On page 17.

Additional probing options include:

- If you are a first time user of the ProtoArray® Yeast Proteome Microarray, we recommend that you also probe a ProtoArray® Control Protein Microarray mg available from Invitrogen (page vi) prior to probing the yeast microarray. The Control Microarray contains various controls and a protein kinase substrate printed on the array to allow you to validate the assay conditions and scanning protocols.
- We **strongly recommend** probing **two** ProtoArray® Yeast Proteome Microarrays simultaneously as described on the next page. You need to purchase additional ProtoArray® Yeast Proteome Microarrays (page vi).
    - Probe the first array using your kinase (supplied by the user) at 50 nM in the presence of radiolabeled [γ-^{33}P]ATP to identify potential substrates
    - Probe the second array using only buffer and no kinase (negative control) in the presence of radiolabeled [γ-^{33}P]ATP to determine which signals are specific to your kinase

Continued on next page

Probing the ProtoArray® Yeast Proteome Microarray, Continued

Recommended Workflow

The recommended workflow for probing the ProtoArray® Yeast Proteome Microarray mg is described below. If you are using this workflow, you need to purchase an additional ProtoArray® Yeast Proteome Microarray mg (page vi).

The recommended protein kinase concentration for probing each array is 50 nM.

1. Probe **two** ProtoArray® Yeast Proteome Microarrays simultaneously as follows:
   - Probe the first array using your kinase (supplied by the user) at 50 nM in the presence of radiolabeled [γ-^{33}P]ATP to identify potential substrates
   - Probe the second array using only buffer and no kinase (negative control) in the presence of radiolabeled [γ-^{33}P]ATP to determine which signals are specific to your kinase
2. After the probing procedure, expose arrays to X-ray film or a phosphor screen for 18-24 hours. Acquire the array image to produce a 16-bit TIFF file. The array image can be acquired by scanning the phosphorscreen using a phosphorimager or develop the X-ray film and scan the X-ray film using a scanner.
3. Process the microarray images, and acquire and analyze data using ProtoArray® Prospector (recommended).

Application Kit

The ProtoArray® Kinase Substrate Identification Application Kit contains qualified pre-made reagents including buffers and Control Kinase. The use of application kit provides consistent results and eliminates the time required to prepare reagents.

The ProtoArray® KSI Buffer Modules A and B are supplied in the application kit and include qualified reagents for blocking, washing, and probing during the microarray probing procedure.

Continued on next page

Probing the ProtoArray® Yeast Proteome Microarray, Continued

Important Guidelines

Since proteins are sensitive to various environmental factors, each array is produced in an environmentally 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® Microarrays with care using the following guidelines:

- Always wear clean gloves while handling microarrays
- **Do not** touch the surface of the array to avoid any damage to the array surface resulting in uneven or high background
- To prevent condensation on the array that may reduce protein activity or alter spot morphology, allow the mailer containing the array to equilibrate at 4°C for at least 15 minutes prior to removing the array from the mailer and immerse the array immediately in blocking solution equilibrated at 4°C
- **Do not** use [γ-^{32}P]ATP for the assay, use [γ-^{33}P]ATP as the use of [γ-^{33}P]ATP supports increased signal resolution during data acquisition while [γ-^{32}P]ATP can be used for the assay but data quantitation with [γ-^{32}P]ATP is not supported
- 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 exposing to X-ray film or phosphor screen
- Do not dry the array using compressed air or commercial aerosol sprays

Materials Needed

- ProtoArray® Yeast Proteome Microarray mg

  Note: You need to purchase an additional ProtoArray® Yeast Proteome Microarray mg if you are using the recommended workflow for probing the array.
- [γ-^{33}P]ATP (3000 Ci/mmol, 10 μCi/μl)
- ProtoArray® Kinase Substrate Identification Application Kit (page vi) or see page 15 for buffer recipes if you are preparing your own buffers
- Protein Kinase supplied by the user in Kinase Buffer (page 16)
- Incubator set to 30°C
- Sterile 50 ml conical tubes
- Cover slips (VWR catalog no. 48404-454)
- Ice bucket
- Deionized water
- Shaker
- X-ray film or phosphorscreen (provide at least 50 μm resolution) and instrumentation to acquire the image (provide at least 50 μm resolution)
- X-ray film cassette
- Clear plastic wrap
- *Optional*: Microarray slide holder and centrifuge equipped with a plate holder

Continued on next page

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 kinase assay requires certain co-factors, be sure to include the co-factors in the kinase buffer during probing

Cover slips

You will need cover slips that are able to completely cover the printed area (20 mm x 60 mm) of the glass slide and hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. We **recommend** using glass cover slips (VWR catalog no. 48404-454).

- To perform the washing and probing steps, we recommend using a sterile 50-ml conical tube or a sterile petri dish.
- Array Chamber included with the ProtoArray® PPI Kits or other hybridization chambers may not be suitable for use as you will need a container that seals tightly to prevent any leakage of radioactive material during the washing steps.
- **Do not** use any cold ATP for the kinase probing steps. If your kinase is stored in a buffer containing ATP, make sure the final concentration of cold ATP is less than 1 nM during the kinase probing step.
- Avoid adding more than 10% (v/v) of your protein kinase sample to 120 µl of Kinase Buffer. Addition of more than 10% of your kinase to the Kinase Buffer can decrease the assay performance.

Continued on next page

Preparing Blocking Buffer

Prepare the Blocking Buffer **fresh** using the reagents supplied in the ProtoArray® Kinase Substrate Identification Application Kit. The recipe below provides sufficient buffer to probe two microarrays.

If you are preparing your own buffer, use the recipe below.

Blocking Buffer

1X PBS
1% BSA

1. Use reagents provided in the application kit to prepare 30 ml Blocking Buffer as follows:

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

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

After preparing Blocking Buffer, immediately return the remaining 30% BSA to -20°C.

Preparing Kinase Buffer

The Kinase Buffer and 1 M DTT are included in the ProtoArray® Kinase Substrate Identification Application Kit. If you are preparing your own Kinase Buffer, use the recipe below and filter the buffer using a 0.45 µm filter to remove any particulate material.

Kinase Buffer

100 mM MOPS, pH 7.2
1% Nonidet P40 (NP 40)
100 mM NaCl
10 mg/ml BSA
5 mM $MgCl_2$
5 mM $MnCl_2$

You will need 120 µl Kinase Buffer with 1 mM DTT for probing one microarray.

1. Use reagents provided in the application kit to prepare 500 µl Kinase Buffer with 1 mM DTT as follows:

Kinase Buffer	500 µl
1 M DTT	0.5 µl

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

After preparing the Kinase Buffer with DTT, immediately return the remaining Kinase Buffer and 1 M DTT to -20°C.

Continued on next page

Preparing 0.5% SDS

You need 200 ml 0.5% SDS for washing two microarrays.

Prepare 0.5% SDS **fresh** from 10% SDS included with the application kit as follows:

10% SDS	10 ml
Ultrapure water	190 ml
Total Volume	200 ml

Mix well and store at room temperature until use.

Calculating the Protein Molar Concentration

You need to calculate the molar concentration of your protein kinase.

Use the protein concentration and molecular weight of your protein kinase for the calculation using the formula listed below.

Protein Concentration (µM) = [Protein concentration in mg/ml] x [1/(protein molecular weight in grams x 10^{-6})]

Example:

For a kinase (50,000 Da) at a protein concentration of 0.5 mg/ml, the µM protein concentration is:

µM = [0.5 mg/ml] x [1/(50,000 x 10^{-6})]
µM = 10

Preparing the Kinase

You need 120 µl Kinase Buffer with 1 mM DTT containing your kinase to probe **one** ProtoArray® Yeast Proteome Microarray mg.

Note: Prepare dilutions of your kinase in the Kinase Buffer.

Component	**User Kinase**
Kinase	50 nM
Kinase Buffer with 1 mM DTT	to 120 µl

Mix well (do not vortex) and store on ice until use. Immediately return the remaining kinase to -80°C.

Before Starting

- Before starting the probing procedure, make sure you have all items on hand especially buffers (previous page), kinase in Kinase Buffer (above), and cover slips.
- Make sure the kinase in Kinase Buffer and Kinase Buffer are cold and stored on ice until use. Place a 50-ml conical tube on ice to chill the tube until use.
- Do not store the 0.5% SDS solution on ice. Store the 0.5% SDS solution at room temperature.
- Review **Important Guidelines** on page 13 and **Working with Radioactive Material** on page 7, prior to starting the probing procedure.

Continued on next page

Probing the ProtoArray® Yeast Proteome Microarray, Continued

Blocking Step

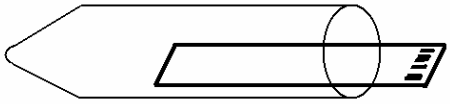
Instructions for blocking two Yeast Microarrays are described below.

Note: To enable data analysis and identify signals specific to your kinase, we recommend probing two Yeast Microarrays as outlined in the **Recommended Workflow** (page 12).

1. Remove two mailers containing ProtoArray® Yeast Proteome Microarrays mg from storage at -20°C and immediately place the mailers at 4°C (be sure to use the microarray **before** the expiration date printed on the box).
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 the microarray is placed in the mailer with the printed (barcode) side facing up. You can block 2 arrays simultaneously in the mailer using 30 ml Blocking Buffer. Add 30 ml Blocking Buffer (page 16) to the mailer containing the ProtoArray® Yeast Proteome Microarray mg. Incubate for 2-3 hours at 4°C with gentle shaking (~50 rpm).
4. Decant the 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**.
5. Proceed immediately to **Probing the Array**, below.

Probing the Array

1. Place each Yeast Microarray horizontally in a separate sterile 50-ml conical tube with the printed side (barcode) of the array facing up and about 1/3 of the array (barcode side) protruding to the outside of the conical tube as shown in the figure below.



2. To 120 µl kinase mixture (page 16), add 1 µl [γ-^{33}P]ATP (3000Ci/mmol, 10 µCi/µl) to obtain a final [γ-^{33}P]ATP concentration of 33 nM for each array.

   Note: Once the ATP is added to the kinase, use the kinase-ATP mixture **immediately** for probing the array. **Do not** store the prepared kinase-ATP mixture on ice for more than 2 minutes prior to use on the array.
3. Pipet Kinase Buffer with the radiolabel and kinase on top of the array without touching the array surface
   - First Yeast Microarray: add 120 µl Kinase Buffer containing 50 nM **your kinase** and 33 nM [γ -^{33}P]ATP (Step 2)
   - Second Yeast Microarray: add 120 µl Kinase Buffer containing 33 nM [γ-^{33}P]ATP (Step 2) but **no kinase**
4. Carefully remove a cover slip from the package with forceps and lay the cover slip on the array to cover the array without trapping any air-bubbles. Align the cover slip flush with the top edge of the array to ensure the printed area of the array is completely covered. Gently adjust the cover slip to remove any air bubbles.
5. Gently slide each array with a cover slip into the conical tube with the printed side (barcode) of the array facing up. Cap the conical tube.

Continued on next page

Probing the ProtoArray® Yeast Proteome Microarray, Continued

Probing the Array, Continued

Protocol continued from the previous page.

6. Place each conical tube horizontally on a flat surface in an incubator set to 30°C 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.
7. Incubate the array in the tube for 1 hour at 30°C **without shaking**.
8. Remove the tubes from the incubator. Using a sterile pipette, add 40 ml 0.5% SDS (page 16) to the sides of the tube. **Avoid pipetting SDS directly onto the array surface.**
9. Incubate the array in SDS for 1 minute at room temperature. Gently move the array in the tube to dislodge the cover slip. Do not remove the cover slip with forceps if the cover slip is not dislodged from the array.
10. Using forceps, carefully remove the dislodged cover slip without touching the array surface. Discard the cover slip appropriately as radioactive waste.
11. Cap the conical tubes and incubate arrays in 0.5% SDS for 15 minutes at room temperature.
12. Decant the 0.5% SDS. Be sure to dispose radioactive waste properly.
13. Slowly add 40 ml 0.5% SDS to tubes, cap the tubes, and incubate for 15 minutes at room temperature.
14. Decant the 0.5% SDS. Be sure to dispose radioactive waste properly.
15. Add 40 ml ultrapure water to the tubes and incubate the arrays in the tube for 15 minutes at room temperature.
16. Decant the water. Be sure to dispose radioactive waste properly.
17. Add 40 ml ultrapure water to the tubes and incubate the arrays in the tube for 15 minutes at room temperature.
18. Decant the water. Be sure to dispose radioactive waste properly.
19. Proceed to **Drying Arrays**, next page.

Continued on next page

Probing the ProtoArray® Yeast Proteome Microarray, Continued

Drying Arrays

1. Remove arrays from the tubes 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 each 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. Make sure that the array is completely dry; there should be no translucent areas.
4. Place the array in an X-ray film cassette. Cover the array with a clear plastic wrap. You can check the radioactivity on the array using a Geiger counter.
5. Overlay the array with an X-ray film or a phosphorscreen (at least 50 μm resolution). Be sure the phosphorscreen was erased prior to exposure.
6. Expose the arrays for 18-24 hours.
7. Perform microarray image data acquisition and data analysis as described below.

Imaging and Data Analysis

Analyze the image and data to identify potential substrates as below. For details, see page 22.

1. Acquire an image (.tiff) from the X-ray film or phosphorscreen (next page).
2. Use barcode on the array to download the .GAL file from ProtoArray® Central as described on page 22.
3. Use the .GAL file and ProtoArray® Prospector to acquire pixel intensity values for all features on the control array and analyze data (page 24) to determine significant signals.

   Note: The expected results obtained after probing a Yeast Microarray are described on page 26. For troubleshooting, see page 27.

Scanning and Image Analysis

Introduction

Once you have exposed the ProtoArray® to X-ray film or phosphorscreen, scan the film or phosphorscreen to acquire a TIFF image that is required for microarray data analysis.

To make the image compatible with the microarray data acquisition software, process the image using ProtoArray® Prospector (recommended) or Adobe® Photoshop® image analysis software as described on the next page.

Materials Needed

Scanning the X-ray film

You need a standard desktop film scanner that provide at least 50 µm resolution (>600 dpi) to scan the X-ray film after developing the film to produce a 16-bit TIFF files.

Scanning the Phosphorscreen

You need a phosphorimager that provides at least 50 µm resolution to acquire the microarray image from the phosphorscreen to produce a 16-bit TIFF file.

The following phosphorimagers have been tested with the ProtoArray® Microarrays:

- Cyclone® Storage Phosphor System (PerkinElmer, Inc.)
- Typhoon™ Imager (Amersham Biosciences)

Data acquisition software

To acquire ProtoArray® data from the image, you need ProtoArray® Prospector 4.0 or higher (page 22). Microarray data acquisition software such as GenePix® Pro (Molecular Devices Corporation) or ScanArray® Software (PerkinElmer, Inc.) are also suitable for data acquisition.

Experimental Outline

1. Develop the X-ray film or process the phosphorscreen according to the manufacturer's recommendations.
2. Scan the X-ray film on a standard scanner or scan the phosphor screen on a phosphorimager to generate a 16-bit TIFF image file.
3. Process the image using ProtoArray® Prospector.
4. Save the adjusted microarray image.

Scanning Guidelines

After exposing the X-ray film or phosphor screen to the ProtoArray® Microarray, scan the film or phosphorscreen to obtain a 16-bit TIFF image file that is required for microarray data analysis. Brief scanning guidelines are described below. For details, refer to the manufacturer's recommendations on using the scanner or phosphorimager.

1. Remove the X-ray film or phosphorscreen from the cassette. Keep the array covered in clear plastic wrap in the dark for use later if a longer exposure time is needed.
2. Develop the X-ray film.
3. Scan the X-ray film using a standard scanner or scan the phosphorscreen using a phosphorimager to obtain a 16-bit TIFF file. Include the barcode in the area for maintaining a record and scan the array to provide a high-resolution image (~50 µm).
4. Save the image file to a suitable location.

Continued on next page

Scanning and Image Analysis, Continued

Image Processing Using ProtoArray® Prospector Imager

ProtoArray® Prospector software version 4.0 (includes Imager and Analyzer) is available from Invitrogen at www.invitrogen.com/protoarray, and then click on the Online Tools tab. The ProtoArray® Prospector Imager allows image processing for data analysis. Install the **Complete** version of ProtoArray® Prospector installation package to install ProtoArray® Prospector Imager.

1. Start ProtoArray® Prospector Imager on the computer.
2. Open the microarray image (.tiff) acquired in Step 4, previous page.
3. Perform the following adjustments to the image (refer to ProtoArray® Prospector Imager manual for detailed instructions)
   - Invert the data (convert the image from white background with black spots to black background with white spots which is required for analysis).
   - Rotate the image such that the array image is vertical and the barcode is located at the bottom
   - Crop a fixed rectangular area (600 x 1800 pixel, if scanned at 600 dpi) from each image (.tiff) file corresponding to the array. If the spots are not aligned vertically, rotate the crop rectangle by holding the Ctrl key and rotating the selection angle with the mouse.

     First rotate and align the rectangle against the Fiduciary Kinase spots, release the Ctrl key and move the rectangle to cover the whole array area. Crop the image using the Crop button. If needed, adjust the image contrast/brightness in Imager for better visualization, which will not affect the final saved image.

     Note: If the image is scanned at a different dpi, set the fixed rectangular area accordingly. For example, if the image is scanned at 300 dpi, set the fixed rectangular area to 300 x 900 pixel to cover the 1″ x 3″ array area.
4. Save the cropped and resized image (.tiff) file with a new name to a suitable location. Be sure the barcode is included in the name of the image.
5. Download lot-specific information from ProtoArray® Central, see next page.

Note: Follow instructions in the Prospector manual to download lot-specific information and analyze data.

Image Processing Using Adobe® Photoshop®

1. Start Adobe® Photoshop® on the computer.
2. Open the microarray image (.tiff) acquired in Step 4, previous page.
3. Perform the following adjustments to the image:
   - Crop a fixed rectangular area (1″ x 3″) from each image (.tiff) file corresponding to the array. If the spots are not aligned vertically, rotate the image to correctly align the spots.
   - Invert the data (convert the image from white background with black spots to black background with white spots).
   - Resize the image file to 2550 x 7650 pixels (constrained proportions).

     Important: Do not adjust the image quality (such as contrast or level) which can compress the dynamic range of the data set and affect data analysis.
4. Save the cropped and resized image (.tiff) file with a new name to a suitable location. Be sure the barcode is included in the name of the image.
5. Download lot-specific information from ProtoArray® Central, see next page.

Data Acquisition and Analysis

Introduction

Download the protein array lot specific information (mainly the .GAL file) from ProtoArray® Central Portal. Use the lot-specific information to acquire and analyze the data to identify potential kinase substrates as described in this section.

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 Human, Yeast, and Control Microarrays and are used with the microarray data acquisition software to generate files that contain pixel intensity information for feature/spot and non-features of the slide.

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.

Materials Needed

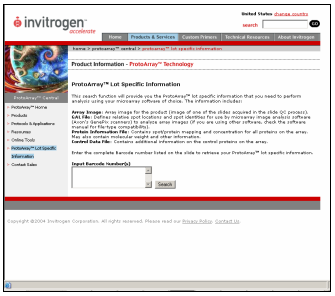
To acquire ProtoArray® data from the image, you need ProtoArray® Prospector 4.0 or higher (page 22). Microarray data acquisition software such as GenePix® Pro (Molecular Devices Corporation) or ScanArray® Software (PerkinElmer, Inc.) are also suitable for data acquisition.

ProtoArray® Central

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

If the scanner computer is connected to the Internet, then 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. Go to www.invitrogen.com/protoarray and then click on the Online Tools tab.
2. Click on the link to ProtoArray® Lot Specific Information.
3. Enter the array barcode in the Input Barcode Number(s) box. Click on Search.



Continued on next page

ProtoArray® Central, continued

4. For each input barcode, the following files are available for downloading:

.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 the human proteins on the microarray.
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 KSI.PAI to analyze data from KSI 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 larger than 1 MB.

6. Start the ProtoArray® Prospector Imager, GenePix® Pro Software, or equivalent microarray data acquisition software on the computer.

7. Open the saved image (16-bit TIFF file) from Step 4, page 21.

   Note: If the image is not saved as a 16-bit TIFF file, GenePix® Pro software is unable to open the file (image).

Continued on next page

ProtoArray® Central, continued

8. Acquire data from ProtoArray® experiments as follows:
   - For ProtoArray® Prospector Imager, download the .GAL files from ProtoArray® Central, which defines the array grid required by the microarray data acquisition software. Load the .GAL file into Imager using the Array List button. Make adjustments to the blocks as described in the Imager manual. Use spots corresponding to the Fiduciary Kinase as reference spots to orient the microarray image. Scroll through the image to ensure that the grid is in the proper location for each subarray. Adjust the subarray grid manually, if needed. After the grid is adjusted properly and all features are aligned, save the Project and analyze the results. Imager automatically opens the Analyzer component of ProtoArray® Prospector for data analysis, and allows you to select the KSI application and specify the experimental conditions. Analyzer then performs the data analysis and shows a summary of results (see ProtoArray® Prospector manual for details).
   - For GenePix® Pro Software, download the .GAL files from ProtoArray® Central, which defines the array grid required by the microarray data acquisition software. Analyze the data and save/export the results as a .GPR (GenePix® Results) file for data analysis using ProtoArray® Prospector (see 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.
   - For other microarray data acquisition software, use data from the .GAL files from ProtoArray® Central to generate files that are compatible with your microarray data acquisition software to define the microarray grid.

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.

Analyzing Data

After data acquisition, analyze the data to identify potential kinase substrates. Once significant signals are identified, we recommend confirming these signals using visual identification.

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 substrates is not recommended. This approach requires a certain degree of expertise with statistics and Excel or another spreadsheet program.

Continued on next page

Data Acquisition and Analysis, Continued

Data Analysis Using ProtoArray® Prospector

The ProtoArray® Prospector Analyzer software quickly analyzes the data acquired from the ProtoArray® Prospector Imager or image acquisition software and easily identifies statistically significant hits (potential substrates), saving you time and effort. The Analyzer software is designed to analyze data and identify potential substrates with a low false positive rate as compared to performing manual calculations using a spreadsheet program. 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 for FREE to ProtoArray® users. To download the ProtoArray® Prospector software and manual, go to www.invitrogen.com/protoarray, and click on the Online Tools tab. Install the **Complete** version of ProtoArray® Prospector installation package to install ProtoArray® Prospector Imager and Analyzer.

The ProtoArray® Prospector software also 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 as potential substrates with the protein kinase.

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.

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.

Based on the Z-score and available protein sequence information, we recommend reproducing the results using ProtoArray® Technology or other methods as described on the next page.

The Next Step

After identifying potential kinase substrates on the ProtoArray® Yeast Microarray, you may reproduce the result using:

The ProtoArray® Technology with additional arrays to ensure:

- **Reproducibility:** Probe the yeast array using a similar or a different kinase concentration to address reproducibility.
- **Specificity:** Probe a yeast array with different kinase to identify substrates specific to your protein kinase of interest.

OR

A solution assay as described briefly below:

To verify substrate phosphorylation in solution, perform solution assays in the presence of radiolabeled ATP using the purified protein kinase and potential kinase substrate using the probing conditions described in this manual. Be sure to include appropriate positive and negative control reactions. Analyze the results using SDS-PAGE and autoradiography.

A true positive signal identified on the array should also produce positive results using the solution assay while a false positive signal identified on the array should not produce any positive results using the solution assay.

Expected Results

Introduction

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

Note: To identify kinase substrates specific to your protein kinase, we recommend probing a second array simultaneously with buffer only that enables you to determine kinase-specific signals.

Control	Description	Function	Verification
Fiduciary Kinases	Fiduciary Kinases are printed on the microarray	The Fiduciary Kinases are autophosphorylated during the labeling reaction. The signals are used as reference spots to orient the microarray image and help assign spot identities	Proper probing and scanning procedures
Control Kinase Substrate	The Control Kinase substrate is printed on the microarray.	The Control Kinase (included in the application and complete kits) phosphorylates the Control Kinase substrate producing a signal.	Proper probing and scanning procedures
GST Protein Gradient	A GST protein gradient is printed on the array	Detects non-specific binding to GST and serves as a negative control. The signals are also used for background calculation by ProtoArray® Prospector software	Negative Control

Troubleshooting

Introduction

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

Problem	Cause	Solution
Weak or no signal with your protein kinase	Kinase of interest is not active or is inactivated by the assay buffer	Check the activity of the kinase after purification using a method of choice. Ensure the kinase is active under the conditions used for probing. Avoid repeated freezing-thawing of your kinase.
	Low specific activity of the kinase	Perform probing with higher kinase concentration, higher kinase specific activity, or increase the incubation time. Avoid repeated freezing-thawing of your kinase.
	Incorrect scanning or imaging	For X-ray film, develop the film and acquire the image using a standard scanner. For phosphorscreen, acquire the image using a phosphorimager. Follow the manufacturer's recommendations on using the scanner or phosphorimager to scan the array correctly. Be sure to use a scanner or phosphorimager that provides at least 50 µm resolution and generates 16-bit TIFF image files.
	Incorrect assay conditions	Perform incubation of the array at 30°C during the probing procedure. Use the Kinase Buffer included with the application kit for best results.
	Poor incorporation of radiolabel	Use fresh [γ-^{33}P]ATP. Be sure to check the array using a Geiger counter to verify that the radioactive signal is obtained after the probing procedure.
	Kinase-ATP mixture not added immediately to the array	After preparing the kinase-ATP mixture, immediately add the mixture to the array. Do not store the prepared kinase-ATP mixture on ice for more than 2 minutes prior to use on the array.
	Kinase specific substrates are not present on the array	Use another kinase.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
High background	Improper blocking	Prepare the Blocking Buffer **fresh** as described on page 16.
	Improper washing	For the best results, perform the recommended washing steps using 0.5% SDS and water as outlined in the protocol.
	Array dried during probing or washing	Do not allow the array to dry during probing or washing procedure. Ensure the cover slip completely covers the printed area of the array. During the incubation step at 30°C, make sure the 50-ml conical tube is capped to minimize drying. During all wash steps, ensure the array is completely covered in buffers.
	Array not dried properly before scanning	Dry the array as described on page 19 before scanning.
	High kinase concentration	Decrease the kinase concentration/specific activity or decrease the incubation time.
Uneven background	Uneven blocking or washing	During the blocking or washing steps, ensure the array is completely immersed in buffers and use at least 40 ml buffer in the 50-ml conical tube to cover the array completely with buffer.
	Improper washing	To obtain the best results, perform the recommended washing steps. Prepare the 0.5% SDS solution **fresh** as described on page 16.
	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 tube to avoid scratching the array surface.
	Radiolabeled ATP or buffer contains precipitates	Centrifuge the [γ-^{33}P]ATP or buffer to remove precipitates prior to probing the array.
Poor spot resolution	Incorrect scanner or phosphorimager used	Be sure the scanner or phosphorimager is capable of providing at least 50 μm resolution.
	Improper handling of arrays	Be sure to allow the mailers with arrays to equilibrate at 4°C for at least 15 minutes prior to use.
	Improper covering of arrays	Properly cover the array with a clear plastic wrap without any creases.
Signals from duplicate spots are merged	--	It is normal for signals from duplicate spots to merge sometimes. The merging of spots does not affect data analysis.

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).

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
Invitrogen Japan
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS

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

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Support Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

Purchaser Notification

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Invitrogen Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com

Limited Use Label License No. 125: GST

This product is the subject of WIPO patent WO8809372 and foreign equivalents to be used for scientific investigation and research and for no other purpose whatsoever. Licenses for commercial use of the above mentioned patents must be negotiated directly with Amrad Corporation, 576 Swan Street, Richmond, Victoria Australia 3121, Telephone: 61 3 9208 4000.

Continued on next page

Purchaser Notification, Continued

Limited Use Label License No. 221: Microarrays of Biological Samples

This product may be covered by one or more of U.S Patent numbers 5,807,522, and 6,110,426 licensed exclusively to Incyte Corporation. The purchase of this product conveys to the buyer (whether employed in academia, government, not-for-profit entity or a for-profit entity) the limited, non-exclusive, non-transferable right (without the right to resell, repackage, or further sublicense) under these patents to use this product for research and development purposes. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of this product does not include or carry any right or license to use, develop, or otherwise exploit this product commercially. This product is sold pursuant to authorization from Incyte Corporation and Incyte Corporation reserves all other rights under these patents. For information on purchasing a license for purposes other than research and development, please contact Incyte Corporation, Corporate Licensing, Route 141 and Henry Clay Boulevard, Wilmington, DE 19880. Phone 302-498-6825, Fax 302-498-2707.

Limited Use Label License No. 295: Polypeptides Expressed in Yeast

This product is the subject of one or more of U.S. Patent Nos. 5,618,676, 5,854,018, 5,856,123, 5,919,651 and foreign equivalents. Rights to use this product are limited to research use only. Rights are available from Washington Research Foundation to practice under the above-referenced patents for any use by contacting Washington Research Foundation, 2815 Eastlake Avenue East, Suite 300, Seattle, Washington 98102. Tel: 206-336-5600. Fax: 206-336-5615.

References

Mah, A. S., Elia, A. E., Devgan, G., Ptacek, J., Schutkowski, M., Snyder, M., Yaffe, M. B., and Deshaies, R. J. (2005) Substrate Specificity Analysis of Protein Kinase Complex Dbf2-Mob1 by Peptide Library and Proteome Array Screening. BMC Biochem *6*, 22-33

Mitchell, D., Marshall, T., and Deschenes, R. (1993) Vectors for the Inducible Overexpression of Glutathione S-Transferase Fusion Proteins in Yeast. Yeast *9*, 715-722

Ptacek, J., Devgan, G., Michaud, G., Zhu, H., Zhu, X., Fasolo, J., Guo, H., Jona, G., Breitkreutz, A., Sopko, R., McCartney, R., Schmidt, M., Rachidi, N., Lee, S. J., Mah, A., Meng, L., Stark, M., Stern, D., De Virgilio, C., Tyers, M., Andrews, B., Gerstein, M., Schweitzer, B., Predki, P., and Snyder, M. (2005) Global Analysis of Protein Phosphorylation in Yeast. Nature *438*, 679-684

Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., Mitchell, T., Miller, P., Dean, R. A., Gerstein, M., and Snyder, M. (2001) Global Analysis of Protein Activities Using Proteome Chips. Science *293*, 2101-2105

invitrogen™

Corporate Headquarters
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech.service@invitrogen.com

For country-specific contact information visit our web site at **www.invitrogen.com**