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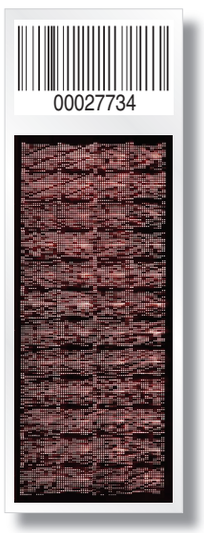
Revolutionize the way you study protein-protein interactions



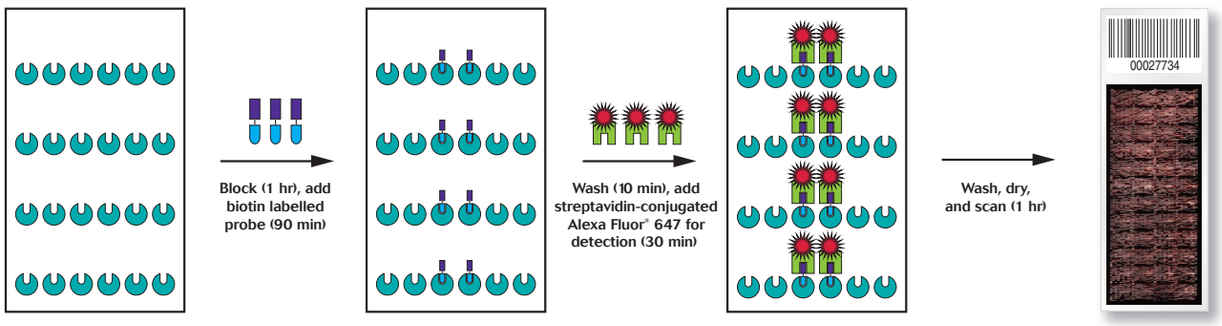
The Yeast ProtoArray™ PPI (Protein-Protein Interactions) microarray is a high-density protein microarray for the elucidation of protein-protein interactions on a proteome scale. In a single experiment you can rapidly and efficiently assay your protein of interest against the proteome of *S. cerevisiae*. With a ProtoArray™ PPI microarray you can:

- Quickly identify novel protein interactions in a single day instead of several weeks using alternate systems such as yeast two-hybrid
- Easily assay protein-protein interactions under different conditions to elucidate the dynamics of the interaction

Simple procedure, powerful results

The Yeast ProtoArray™ PPI microarray contains 4088 *S. cerevisiae* open reading frames (ORFs) expressed as 5´-Glutathione S-Transferase (GST) fusions, purified and spotted in duplicate on a nitrocellulose-coated 1 inch x 3 inch glass slide. Using the Yeast ProtoArray™ PPI microarray is a simple procedure (Figure 1). All of the yeast proteins are immobilized on the slide, allowing you to readily assay the proteome in one simple experiment. Other protein-protein interaction detection methodologies, such as the yeast two-hybrid assay, require labour-intensive steps and can take several weeks to perform. With the Yeast ProtoArray™ PPI microarray, you can screen your labelled protein of interest against the *S. cerevisiae* proteome in as little as 4 hours and elucidate protein-protein interactions against over 4000 proteins. Since it is estimated that 50% of yeast proteins have a human homolog*, the Yeast ProtoArray™ PPI is a powerful model for investigating interactions in higher eukaryotic systems.

Figure 1 — Using the Yeast ProtoArray™ PPI microarray to detect protein interactions is simple



* Based on BLASTP analysis of yeast protein sequences versus human RefSeq protein sequences, with matches of ≤ e^{-10} over greater than 100 amino acids.

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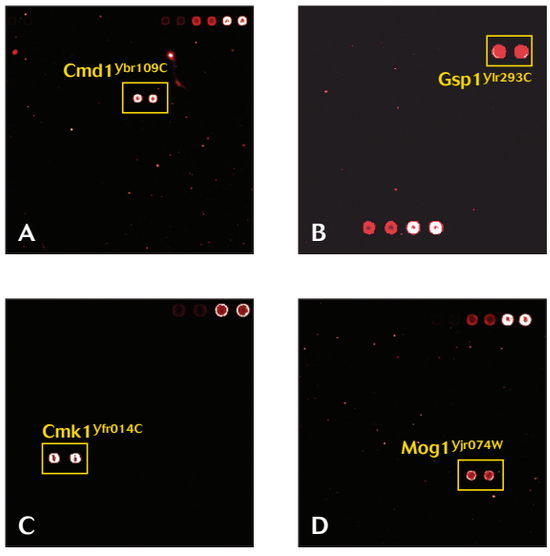
Easily detect and validate interactions

The Yeast ProtoArray™ PPI microarray allows you to detect novel interactions as well as to validate previously detected protein-protein interactions. In addition, once positive interactions are identified, you can easily confirm these interactions by performing the 'reciprocal' experiments using the identified interacting protein as the probe. To demonstrate, two well-characterized proteins, calmodulin and Gsp1p, were screened against the Yeast ProtoArray™ PPI microarray. For both Calmodulin and Gsp1p, known and novel interactions were detected (Figure 2). Panel A shows the known interaction of calmodulin with calmodulin kinase 1, a calmodulin-dependent protein kinase involved in signal transduction. Panel B shows the known interaction of Gsp1p with Mog1p, a nuclear protein involved in, and required for, nuclear protein import. To rapidly validate these interactions, 'reciprocal' experiments were performed using calmodulin kinase and Mog1p as probes. In both cases the expected reciprocal interactions were observed; calmodulin kinase was confirmed to bind calmodulin (Figure 2C) and Mog1p bound to Gsp1p (Figure 2D). Additional interactions were also observed for both Calmodulin and Gsp1p, including many novel interactions (Table 1).

Figure 2 — Identification of protein-protein interactions using the Yeast ProtoArray™ PPI microarray



Calmodulin and Gsp1p were biotin labeled using the *in vitro* biotinylation kit and probed against Yeast ProtoArray™ PPI microarrays. Interactions were detected using Alexa Fluor® 647-conjugated streptavidin and scanned using an Axon GenePix® 4000B scanner.

A positive interaction from each screening was identified. Calmodulin kinase demonstrated a positive interaction with calmodulin (A), and Mog1p demonstrated a positive interaction with Gsp1p (B). These were then used to perform the 'reciprocal' experiment (C and D, respectively). (Note: Unlabelled spots are reference spots.)

Table 1 — Proteins identified as interacting with Calmodulin or Gsp1p

Probe	Known interacting proteins*	Novel interacting proteins
Calmodulin	YFR014C (Cmk1p)	YBR169C (HSP70 family member)
	YML057W (Cmp2)	YGL105W (Arc1p)
	YOL016C (Cmk2p)	YLR022C (uncharacterized ORF)
		YPL106C (HSP70 family member)
Gsp1p	YJR074W (Mog1p)	YJL184W (ORF, unknown function)
		YIL108W (hypothetical ORF)

*Previously identified as interactors in the GRID database: http://biodata.mshri.on.ca/yeast_grid/servlet/SearchPage

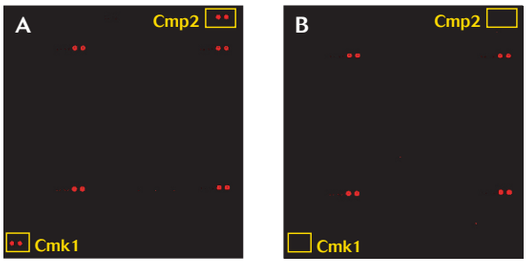
Investigate interaction dynamics

Identifying a protein-protein interaction is often only the beginning of your research. The next step is to more precisely understand the biochemistry of the interaction under investigation. You can do this by testing the affinity of your identified interaction under a range of experimental conditions. With the Yeast ProtoArray™ PPI microarray you can easily and rapidly assay your protein-protein interactions using:

- Different protein modification states such as phosphorylation or glycosylation
- Different protein concentrations
- Alternate reaction conditions such as ionic strength, pH, or temperature
- Presence of cofactors such as divalent cations

As an example of this, the Yeast ProtoArray™ PPI microarray was probed with calmodulin in the presence of either Ca^{++} or EGTA. The calmodulin:calmodulin kinase interaction was abolished in the presence of EGTA consistent with the known calcium dependence of this interaction (Figure 3). Using a Yeast ProtoArray™ PPI microarray you can easily assay alternate reaction conditions for your protein-protein interactions.

Figure 3 — Effect of probing conditions on calmodulin binding interactions

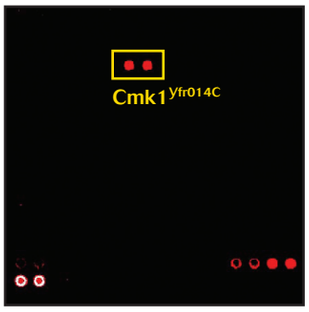


Yeast ProtoArray™ PPI microarrays were probed with biotinylated calmodulin in the presence of Ca^{++} (A) or EGTA (B). Locations of calmodulin kinase (Cmk1) and a calcineurin homolog (Cmp2) are indicated. Binding of calmodulin to both of these proteins is clearly abolished in the presence of EGTA. (Note: Additional spots are Alexa Fluor® 647 fluorescent reference spots.)

Model system for analysis of human proteins

Because many basic biological processes are well conserved between organisms, yeast has long served as a useful model for studying protein biology in other eukaryotes. In fact, approximately 50% of yeast proteins have human homologs, including proteins such as Ras, histone deacetylases, cell division control proteins, protein kinases and calmodulin. Not surprisingly, protein interactions are often conserved across eukaryotes as well. To demonstrate the value of using the Yeast ProtoArray™ PPI microarray as a model for protein interactions in higher eukaryotes, the calmodulin:calmodulin kinase interaction detected in the previous experiment (Figure 2) was repeated using human calmodulin. Screening with human calmodulin also detected a positive interaction with calmodulin kinase (Figure 4). This demonstrates how the Yeast ProtoArray™ PPI microarray can be used to investigate interactions not only in yeast, but as a model for interactions in higher eukaryotic systems.

Figure 4 — Using Yeast ProtoArray™ PPI microarray as a model for human protein interactions



A Yeast ProtoArray™ PPI microarray was screened using biotin-labelled human calmodulin. The interaction was detected using Alexa Fluor® 647-conjugated streptavidin. The positive interaction with calmodulin kinase (Cmk1p) was detected. Unlabelled spots are reference spots.

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Functional proteins for reliable results

Ensuring maximum function

Maintaining the arrayed proteins in a state capable of functional interactions is an important prerequisite to their use in interaction assays. While obviously impossible to guarantee this for every protein, several steps have been taken to ensure maximum protein function. These steps include precisely controlled purification conditions and methods, as well as specialized handling and arraying. In addition, the surface on the glass slide chosen to produce the Yeast ProtoArray™ PPI microarray is made of nitrocellulose, a surface known to be compatible with a wide variety of protein functions (1-4). To specifically test the nitrocellulose surface for compatibility with yeast protein-protein interactions, eight unique yeast protein interaction pairs were identified from the literature. These proteins were expressed, purified, and their interactions validated through non-denaturing polyacrylamide gel electrophoresis (Table 2). In all but one case (87.5%), the interactions observed in solution were also observed with the Yeast ProtoArray™ PPI microarray.

Proven interactions

The ability to observe 'reciprocal' interactions, as previously described (page 2), serves as another test of protein function. For instance, Figure 2A shows calmodulin (probe) interacting with calmodulin kinase. The ability to observe calmodulin kinase (probe) binding to calmodulin (Figure 2C), however, is dependent upon calmodulin maintaining a proper folded state on the array. The same logic applies to the reciprocal interactions between Gsp1p and Mog1p (Figures 2B and 2D). In fact, in a set of 9 proteins tested, 8 (~90%) showed the expected reciprocal interactions (Table 3, page 5). This high level of protein function combined with the high number of proteins available on the array allows you to get robust results in your protein interaction studies.

Table 2 — Demonstrating protein function on the Yeast ProtoArray™ PPI microarray

Yeast protein pairs*		Interaction assay	
		Gel Shift	Yeast ProtoArray™ PPI Screen
YOR117W	YOR259C	Yes	Yes
YLR423C	YDR022C	Yes	Yes
YMR146C	YDR429C	Yes	Yes
YPR181C	YHR098C	Yes	Yes
YDL160C	YCR077C	Yes	Yes
YOR185C	YJR074W	Yes	Yes
YBR109C	YFR014C	Yes	Yes
YLR291C	YGR083C	Yes	No

Eight interacting protein pairs were identified from the literature. These proteins were expressed, purified and assayed for protein-protein interactions by non-denaturing polyacrylamide gel electrophoresis as well as by screening on a Yeast ProtoArray™ PPI microarray. For all protein pairs except one (87.5%) the protein-protein interaction was detected by both assay methods.

* Yeast proteins in the first column were immobilized on Yeast ProtoArray™ PPI microarray. Proteins in the second column were used as probes in the Yeast ProtoArray™ PPI microarray screening.

Functional proteins for reliable results, continued

Table 3 — Reciprocal interactions tested on yeast arrays

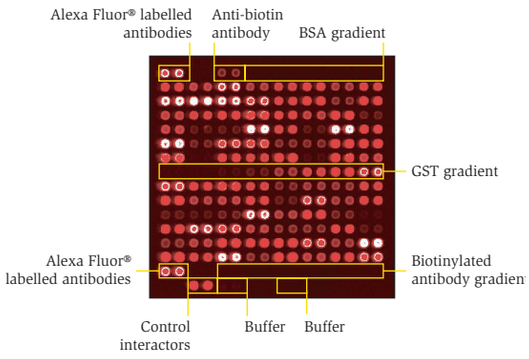
Yeast protein pairs		Reciprocal interaction
YKL166C	YIL033C	Yes
YOR117W	YOR259C	Yes
YPL203W	YIL033C	Yes
YLR423C	YDR022C	No
YMR146C	YDR429C	Yes
YPR181C	YHR098C	Yes
YDL160C	YCR077C	Yes
YOR185C	YJR074W	Yes
YBR109C	YFR014C	Yes

Nine yeast protein pairs were screened for protein-protein interactions. First one protein from each protein pair was chosen to be used as a probe to screen a Yeast ProtoArray™ PPI microarray. To perform the 'reciprocal' experiment, the second protein of the protein pair was then used as the probe. In all but one case (88%) using either protein from the pair as the probe yielded a positive protein-protein interaction.

Built-in controls make your work easier

To facilitate analysis of your results, controls are built into each Yeast ProtoArray™ PPI microarray. As an internal control for detected interactions, each yeast protein is printed in duplicate on the array. In addition, several controls for background, labelling, and detection are also printed on each slide (Figure 5, Table 4). These controls provide additional information, ensuring you will be confident of your results.

Figure 5 — Control spots on a Yeast ProtoArray™ microarray.



A Yeast ProtoArray™ PPI micoarray was probed with an anti-GST antibody. Control spots are identified in the figure, and their utility is decribed in Table 4. Other spots are from yeast proteins. Note that all spots are printed in duplicate.

Table 4 — Control spots for confident results

Protein	Analysis value
Buffer only	Detects non-specific interaction with buffer
BSA gradient	Negative control for protein interactions
Alexa Fluor® labelled antibody	Internal reference for determining protein location on the microarray
Anti-biotin antibody	Detection of biotin labelled probe
Biotinylated antibody	Detection of streptavidin-conjugated Alexa Fluor® 647
GST protein gradient	Used for protein quantitation, detects non-specific binding to GST
Control interactors	Positive control for interactions using calmodulin or calmodulin kinase

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Quality content the first step to success

The limiting factor in most protein microarray efforts is the ability to produce quality proteins. The Yeast ProtoArray™ PPI microarray starts with superior content derived from the Yeast collection created in Mike Snyder's lab at Yale University (5). This collection consists of full-length yeast open reading frames cloned as 5′ GST fusions in a yeast expression vector. By incorporating the GST tags on the proteins they can be readily purified under native conditions in a high-throughput manner, increasing the likelihood of functional protein. To further validate the collection before inclusion in the Yeast ProtoArray™ PPI microarray, each clone was:

- 5′ end sequenced to confirm the identity of the construct
- Analyzed by western blot to confirm size of the fusion protein

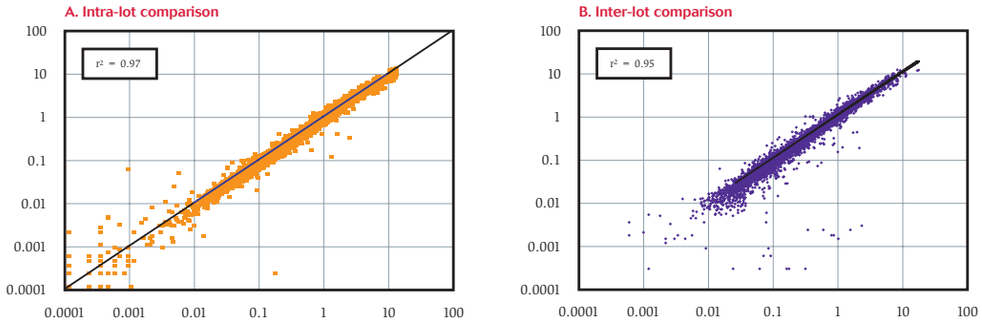
This means that the proteins on each Yeast ProtoArray™ PPI microarray are likely to be full length and your protein interaction screens will yield valuable results.

Rigorous production and quality control standards ensure reproducible results

Reproducibility between experiments is vital to the success of your research. The Yeast ProtoArray™ PPI microarray is produced with an integrated data management system, as well as rigorous production and quality control procedures, to ensure your results in every experiment. Prior to production, instrumentation and pins are calibrated and extensively quality controlled. To maintain protein stability and function, slides are printed at +6ºC in controlled environmental conditions. After production, each slide is visually inspected for obvious imperfections that could interfere with your experimental results. To control for the quality of the printing process, representative slides from each lot are probed using an anti-GST antibody. Since all the yeast proteins contain a GST tag, this allows ready identification of irregular spot morphology and missing spots. In addition, an assay is performed to detect the protein-protein interaction between calmodulin and calmodulin kinase. This assay ensures the utility of the microarray for your experiments.

Knowing how much protein is in each spot is useful for the analysis of your results. To calculate the amount of protein deposited in each spot, a dilution series of purified GST protein is run on each slide (Figure 5, page 5). The signal generated by the GST protein dilution series is used to generate a standard curve that can then be used to calculate the amount of protein in each spot. For your convenience this information is readily available for download from our website at **www.invitrogen.com/protoarray** under the 'online resources' tab. These quality control experiments, combined with rigorous process control and automated systems, mean you can count on maximum inter- and intra-lot reproducibility (Figure 6). You'll get consistent quality and reproducibility in every experiment you perform.

Figure 6 — Inter- and intra-lot reproducibility of the Yeast ProtoArray™ PPI microarray



Easy use and high sensitivity with Alexa Fluor® dye-mediated detection

Critical to the outcome of your experiments is the method used for detecting protein-protein interactions. You can start with as little as 150 µg of purified protein and in a few steps get right to screening for protein interactions. Simply:

1. Use the *in vitro* biotinylation kit to biotin label your probe protein at three different concentrations
2. Use the included biotinylation assessment kit to assay which of the experimental concentrations yield the best biotinylation for probing
3. Probe the Yeast ProtoArray™ PPI with your biotin-labelled probe
4. Follow with detection using Alexa Fluor® 647 conjugated streptavidin
5. Allow the microarray to dry and read it in any compatible microarray scanner

That's it. Using Alexa Fluor® 647 dye-mediated detection means you will get high sensitivity, low background, and stable signal.

Save money by using standard detection equipment

Once you've completed your experiment using the Yeast ProtoArray™ PPI microarray, you can read it in almost any microarray scanner (Table 5) and use standard microarray analysis software for data capture. For the analysis of your results, you'll need protein location and quantity of protein in each spot. To make your analysis as easy as possible, visit **www.invitrogen.com/protoarray** to download a file that includes this information and can be readily imported into your microarray analysis software of choice. We even provide guidelines for data analysis to ensure your success. Since you can use existing equipment and software already in your lab or facility, there are no large capital expenditures or learning curves required to use the ProtoArray™ Technology.

Table 5 — Reading your results

Compatible	Axon GenePix® 4000A
	Axon GenePix® 4000B
	Axon GenePix® Professional 4200A
	PerkinElmer ScanArray™ Lite
	Tecan LS Series Laser Scanner
	PerkinElmer ScanArray™ Express
	PerkinElmer ScanArray™ Express HT
Should be Compatible	AlphaInnotech AlphaArray™
	AppliedPrecision® arrayWoRx®ᵉ 4-color Biochip Reader
	Telechem SpotLight®
Not Compatible	Affymetrix GeneChip® Scanner 3000
	Agilent DNA Microarray scanner

These scanners have all been tested to be compatible with the Yeast ProtoArray™ PPI microarray. Other scanners may be compatible. For a complete list of compatible and incompatible scanners please visit our website at **www.invitrogen.com/protoarray**.

Complete kit ensures your success

To ensure your success, each Yeast ProtoArray™ PPI kit comes complete with:

- Two Yeast ProtoArray™ PPI microarrays to allow you to perform two screening assays under differing conditions
- Two control slides to assay for background and non-specific binding of your probe protein on the array
- *In vitro* biotinylation kit for convenient biotin labeling of your probe protein
- Biotinylation assessment kit
- Alexa Fluor® 647 detection reagents for high sensitivity and low background detection
- Buffers for probing and washing so you don't have to spend time making buffers

Revolutionize your protein-protein interaction studies

Revolutionize the way you study protein-protein interactions. With the Yeast ProtoArray™ PPI Kit you can screen your protein of interest against the proteome of yeast and get results in as little as four hours. Never have you been able to do so much in so little time. Visit **www.invitrogen.com/protoarray** to order today.

Product	Quantity	Cat. no.
Yeast ProtoArray™ PPI Kit *for protein-protein interactions with in vitro biotin-labelling*	1 kit	PA0121011
ProtoArray™ Biotinylation Kit	1 kit	AL01

For Custom ProtoArray™ Services email: **custom.services@invitrogen.com**

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European headquarters:
Invitrogen Ltd • Inchinnan Business Park • 3 Fountain Drive • Paisley PA4 9RF, UK • Tel: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com

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