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Identification of Novel Protein–Ligand Interactions by Exon Microarray Analysis of Yeast Surface Displayed cDNA Library Selection Outputs

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Abstract

Yeast surface display is widely utilized to screen large libraries for proteins or protein fragments with specific binding properties. We have previously constructed and utilized yeast surface displayed human cDNA libraries to identify protein fragments that bind to various target ligands. Conventional approaches employ monoclonal screening and sequencing of polyclonal outputs that have been enriched for binding to a target molecule by several rounds of affinity-based selection. Frequently, a small number of clones will dominate the selection output, making it difficult to comprehensively identify potentially important interactions due to low representation in the selection output. We have developed a novel method to address this problem. By analyzing selection outputs using high-density human exon microarrays, the full potential of selection output diversity can be revealed in one experiment. FACS-based selection using yeast surface displayed human cDNA libraries combined with exon microarray analysis of the selection outputs is a powerful way of rapidly identifying protein fragments with affinity for any soluble ligand that can be fluorescently detected, including small biological molecules and drugs. In this report we present protocols for exon microarray-based analysis of yeast surface display human cDNA library selection outputs.

Keywords

Yeast surface display; cDNA library; Exon microarray; Phosphatidylinositide; Novel nuclear phosphatidylinositide-binding proteins

1 Introduction

Identifying proteins that interact with small bioactive molecules such as phosphatidylinositides is an important, but often challenging and rate-limiting step in understanding cellular signaling pathways. Using yeast surface display techniques, heterologous protein fragments can be efficiently displayed on the surface of the *Saccharomyces cerevisiae* yeast cell as C-terminal fusions to the yeast a-agglutinin subunit, Aga2p [1] and this technology has been extensively utilized in protein engineering applications such as antibody affinity maturation and epitope mapping [2–5]. We have previously described the construction of large (2×10^7) yeast surface-displayed human protein fragment libraries [6–8]. When coupled with fluorescence-activated cell sorting

(FACS), yeast surface-displayed human protein fragment libraries can theoretically be used to identify protein fragments with affinity for any soluble molecule that can be fluorescently detected. These libraries have been used to identify human protein fragments with affinity for tyrosine-phosphorylated peptides [6], tumor-targeting antibodies [9], and phosphatidylinositides (PtdIns) [7].

Often a small number of clones become dominant during selection, making it likely that rare binding clones in the selection output will be missed by monoclonal screening. To capture the full diversity of yeast surface display selection outputs, we have developed methods using high-density human exon microarrays (*see* Fig. 1 for method outline). We applied these methods to PtdIns-binding enriched polyclonal yeast selection outputs that had been previously analyzed by standard monoclonal screening and sequencing. In addition to the nine PtdIns-binding protein fragments previously identified by monoclonal screening and sequencing [7], we identified an additional 17 novel PtdIns-binding protein fragments [10] (Table 1). Our results suggest that affinity-based selections with yeast human cDNA display libraries coupled with comprehensive analysis of the outputs using DNA microarrays is a rapid and efficient method for identifying protein interactions. In this report we describe protocols for the analysis of yeast surface display human cDNA library selection outputs using human exon microarrays.

2 Materials

2.1 Plasmid Recovery

1. Polyclonal yeast surface display selection outputs (*see* Note 1).
2. 10× SD-CAA for making plates: 70 g yeast nitrogen base w/o amino acids, 50 g Bacto casamino acids, 100 g dextrose; bring the volume to 500 mL with ddH₂O and filter-sterilize.
3. SD-CAA plates: 5.4 g Na₂HPO₂, 7.4 g NaH₂PO₄, 17 g agar. Bring the volume to 900 mL with ddH₂O, autoclave to sterilize, let the agar cool until it is cool enough to touch, add 100 mL 10× SD-CAA, mix and pour into plates (100 or 150 mm) and allow to cool at RT, and store the plates at 4 °C until ready to use.
4. Acid-washed glass beads (Sigma).
5. Spin miniprep kit (Qiagen).
6. 10G Supreme electrocompetent cells (Lucigen).
7. Transformation recovery medium (supplied with 10G Supreme cells).
8. 1 mm Electroporation cuvettes (Molecular BioProducts).
9. Electroporators (Eppendorf 2510, Bio-Rad Gene Pulser II).

¹We have previously described protocols for the construction of yeast surface-displayed human cDNA libraries [4, 8] and their application to identify human protein fragments with affinity for tyrosine-phosphorylated peptides [2], tumor-targeting antibodies [5], and phosphatidylinositides [3]. As a starting point for exon microarray analysis we use polyclonal yeast surface displayed human cDNA selection outputs enriched for clones that bind to the desired target ligand. We enrich for target binding by FACS until the fraction of binding clones in the polyclonal outputs is greater than 90 %.

10. 2× YT: 16 g tryptone, 10 g yeast extract, 5 g NaCl, bring volume to 1 L with ddH₂O, adjust pH to 7.0, and sterilize by autoclaving.
11. 1,000× ampicillin: 1 g ampicillin, add ddH₂O to 10 mL, sterilize by filtration and aliquot, and store aliquots at −20 °C.
12. 150 mm 2× YT ampicillin plates: 16 g tryptone, 10 g yeast extract, 5 g NaCl, 17 g agar, bring volume to 1 L with ddH₂O, adjust pH to 7.0, sterilize by autoclaving, let cool until not too hot to touch and add 1 mL 1,000× ampicillin.
13. Maxiprep kit (Qiagen).

2.2 Preparation of Biotinylated cRNA Probes

1. Plasmids recovered from polyclonal selection output (described in Subheading 3.1).
2. *Xho*I restriction enzyme.
3. *Not*I restriction enzyme.
4. 10× React 3.
5. Qiagen spin prep kit.
6. Biotin-16-UTP.
7. Ambion MAXIscript T7 kit (Ambion).
8. RNeasy Mini Kit (Qiagen).

2.3 Exon Microarray Data Analysis

1. Affymetrix GeneChip® Human Exon 1.0 ST Array (Affymetrix) (*see* Note 2).
2. Affymetrix Expression Console software (Affymetrix) (*see* Note 3).
3. Integrated Genome Browser software [7] (*see* Note 4).
4. Excel (Microsoft).

2.4 Recovery of Putative Binding Clones

1. cDNA-specific primer sets designed using the exon microarray data (*see* Note 5).
2. pYD1-specific primers: pYD1 Forward 5′-AGTAACGTTTGTCTAGTAATTGC-3′, pYD1 Reverse 5′-GTCGATTTTGTACATCTACAC-3′ (*see* Note 6).
3. 10× PCR buffer.

²The GeneChip® Human Exon 1.0 ST Array has 1.4 million total probe sets (approximately four probes per exon and roughly 40 probes per gene). The probes tiled on the array are designed in the antisense orientation, requiring sense-strand labeled targets to be hybridized to the array. For more information about the GeneChip® Human Exon 1.0 ST Array consult the Affymetrix website.

³Expression Console Software is used to analyze microarray data and is freely available on the Affymetrix website.

⁴The Integrated Genome Browser is an application that allows the graphic visualization of exon microarray-generated data in the context of an annotated human genome.

⁵After estimating the 5′ and 3′ boundaries of the cDNA candidates, internal primers should be designed to amplify the 5′ and 3′ cDNA/vector junctions. We find it convenient for downstream gel isolation to design the primers to produce an approximately 500 bp product.

⁶If a yeast display vector other than pYD1 is utilized then different primers will need to be designed.

4. dNTPs.
5. Taq polymerase.
6. Primers for sequencing: Gap5 5'-TTAAGCTTCTGCAGGCTAGTG-3', Gap3 5'-GTTAGGGATAGGCTTACCTTC-3' (see Note 6).
7. Primer sets based on the 5' and 3' boundaries of the cDNA inserts with added EcoRI overhangs for cloning into pYD1 (see Note 7).
8. *EcoRI* restriction enzyme and 10× *EcoRI* buffer.
9. pYD1 or suitable yeast display vector.
10. T4 DNA ligase and 10× ligation buffer.
11. Chemically competent *E. coli* cells.
12. Heat block at 42 °C.
13. 1,000× Ampicillin: 1 g ampicillin, add ddH₂O to 10 mL, sterilize by filtration and aliquot, and store aliquots at −20 °C.
14. 100 mm 2× YT ampicillin plates: 16 g tryptone, 10 g yeast extract, 5 g NaCl, 17 g agar, bring volume to 1 L with ddH₂O, adjust pH to 7.0, sterilize by autoclaving, let cool until not too hot to touch and add 1 mL 1,000× ampicillin and pour.
15. Spin miniprep kit (Qiagen).
16. *S. cerevisiae* strain EBY100 (MATa GAL1-*AGA1*::URA3 *ura3-52 trp1 leu2 1 his3 200 pep4::HIS3 prb1 1.6R can1 GAL*).
17. YPD: 10 g of yeast extract, 20 g of bacteriological peptone, 20 g dextrose, bring volume to 1 L with ddH₂O, and filter-sterilize.
18. 50 % PEG-3350: 250 g PEG-3350, bring volume to 500 mL with ddH₂O, and filter-sterilize.
19. 1 M lithium acetate: 33 g lithium acetate, bring volume to 500 mL with ddH₂O, and filter-sterilize.
20. 10× SD-CAA for making plates: 70 g yeast nitrogen base without amino acids, 50 g Bacto casamino acids, 100 g dextrose, bring volume to 500 mL with ddH₂O, and filter-sterilize.
21. SD-CAA plates: 5.4 g Na₂HPO₂, 7.4 g NaH₂PO₄, 17 g agar, bring volume to 900 mL with ddH₂O and autoclave, let cool until not too hot to touch and add 100 mL 10× SD-CAA, pour plates of desired size (100 or 150 mm) and allow to cool at RT, and store plates at 4 °C until ready to use (see Note 8).

⁷Design primers to amplify the candidate cDNAs based on the sequencing data. Take care to ensure that the cDNA coding regions will be in frame with Aga2 after cloning into the display vector.

⁸Antibiotics (ampicillin and/or tetracycline) can be added to the plates if contamination is an issue.

2.5 FACS

1. 2× SR-CAA yeast growth media: 20 g raffinose, 14 g yeast nitrogen base, 10 g Bacto casamino acids, 5.4 g Na₂HPO₄, 7.4 g NaH₂PO₄, bring volume to 1 L with ddH₂O, and filter-sterilize.
2. 20 % Galactose: 100 g galactose; bring the volume to 500 mL with ddH₂O and filter-sterilize.
3. PBS: 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na₂HPO₄, 0.24 g of KH₂PO₄, bring the volume to 1 L with ddH₂O, adjust pH to 7.4, and sterilize by autoclaving or filtration.
4. Biotinylated or fluorescently labeled target molecules (*see* Note 9).
5. Mouse anti-Xpress (Invitrogen).
6. Goat anti-mouse-647 (Jackson ImmunoResearch).
7. Streptavidin-PE (SA-PE) (Invitrogen).

3 Methods

Protocols detailing the construction and use of yeast surface-displayed human protein fragment libraries for FACS-based enrichment of protein fragments with affinity for various target ligands have been described previously [8, 11]. Here we describe protocols for the comprehensive analysis of enriched polyclonal yeast surface display selection outputs using exon microarrays. It is assumed that a core facility or commercial service will be performing the chip hybridization and data collection steps and therefore protocols for these steps are not described. Additionally, since the Affymetrix Expression Console software used to process the raw chip data is frequently updated, detailed protocols for the software-based data processing will not be described. Instead, a brief outline of the data analysis we performed will be presented. Affymetrix provides detailed instruction manuals and video tutorials for the current version of the Expression Console software package on their website. The core facility or commercial service provider may also offer data analysis services. The methods below are divided into five categories: Plasmid Recovery From Polyclonal Yeast Display Selection Outputs (Subheading 3.1); Preparation of Biotinylated cRNA probes (Subheading 3.2); Exon Microarray Data Analysis (Subheading 3.3); Recovery of putative binding clones (Subheading 3.4); Testing candidate binding clones by FACS (Subheading 3.5).

3.1 Plasmid Recovery from Poly clonal Yeast Display Selection Outputs

1. Prewarm and dry one 150 mm SD-CAA plate for each selection output to be analyzed in a 30 °C incubator. Thaw freezer stocks of the selection outputs at RT and evenly spread 100 µL of each selection output on a 150 mm SD-CAA plate. Incubate the plates upside down at 30 °C for 1–2 days (*see* Note 10).

⁹It is best to use the same labeled target reagents that were utilized during the selection process.

¹⁰The yeast should form an almost continuous layer of colonies. If the number of colonies seems too low to cover the expected diversity, more volume can be plated.

2. Add 5 mL of 2× SR-CAA to each plate, resuspend the cells by scraping with a flame-sterilized spreader, and then collect the resuspended cells by pipetting. Recover an approximately 200 µL yeast cell pellet by centrifugation and wash twice with ddH₂O.
3. Resuspend the pellet in 400 µL Qiagen buffer P1 (*see* Note 11) and add approximately 400 µL glass beads (the beads should be about 50 % of the total volume). Vortex at high speed for 10 min and remove 250 µL of the cell slurry (leaving the glass beads behind) to a clean tube.
4. Add 250 µL buffer P2, gently mix by inverting, and incubate at RT for 5 min.
5. Add 350 µL buffer N3 (a cloudy precipitate will form) and spin at 16,000 × *g* in a microcentrifuge for 15 min.
6. Apply supernatant to a Qiagen spin miniprep column and spin at 16,000 × *g* for 1 min. Discard flow-through.
7. Add 750 µL buffer PE and spin at 16,000 × *g* for 1 min. Discard flow-through and spin at 16,000 × *g* for 2 min to remove residual buffer PE. Replace collection tube with a clean Eppendorf tube, add 50 µL ddH₂O, and spin at 16,000 × *g* for 1 min to elute.
8. Prewarm transformation recovery medium at 37 °C. Place electroporation cuvettes on ice. Thaw 10G supreme cells completely on ice (10–20 min) and aliquot 25 µL of cells for each output to be recovered to prechilled 1.5 mL Eppendorf tubes on ice. Add 4 µL of each of the polyclonal output yeast plasmid preps to the tubes with cells and stir gently without pipetting up and down.
9. Transfer 25 µL of the cell/ligation mixture to the chilled electroporation cuvettes, and electroporate using the following conditions (*see* Note 12).

10 µF

600 Ω

1,800 V

Immediately add approximately 1 mL of the prewarmed recovery medium to the cuvette, resuspend the cells, and transfer to 17 mm culture tubes.
10. Incubate tubes at 37 °C with shaking at 250 rpm for 1 h. Dry 2× Y T-ampicillin plates at 37 °C while transformations are recovering.
11. Plate 1/10,000 of each transformation on a 2× YT-ampicillin plate for titrating, and plate the remaining transformation cultures on three more plates at approximately 350 mL/plate. Incubate the plates overnight at 37 °C (*see* Note 13).

¹¹The method we describe is based on the Qiagen spin miniprep kit. The buffers used (P1, P2, N3, and EB) are provided in the kit.

¹²We use an Eppendorf 2510 electroporator. Other electroporators should work similarly using the same settings. The time constant should be 3.5–4.5 ms.

¹³There should be at least 100 colonies on the 1/10,000 titer plate. If the transformation efficiency is low, it will need to be repeated.

12. Add 3 mL 2× YT + ampicillin to each of the plates, resuspend cells with a flame-sterilized spreader, and collect by pipetting.
13. Prepare bacterial freezer stocks of the library by mixing 0.5 mL 50 % glycerol and 0.5 mL of the resuspended transformants in a cryotube and store at −80 °C.
14. Prepare several minipreps or a Qiagen maxiprep using the remaining resuspended transformants according to manufacturer's protocols. Determine the concentration of the library DNA prep using a spectrophotometer.

3.2 Preparation of Biotinylated cRNA Probes

1. Set up the following digestions:
 - 4 µL 10× React 3
 - 10 µg recovered selection output plasmid
 - 2 µL *NotI*
 - Bring volume to 40 µL with ddH₂O
 - 4 µL 10× React 3
 - 10 µg recovered selection output plasmid
 - 2 µL *XhoI*
 - Bring volume to 40 µL with ddH₂O
 - Incubate at 37 °C for 3 h (see Note 14).
2. Run the digestions on a 0.75 % agarose gel, cut out linearized bands with a clean razor blade, and isolate DNA using Qiagen gel isolation kit following manufacturer's protocols. Elute in 50 µL EB buffer, combine the gel purified *NotI* and *XhoI* digestions, and measure the concentration with a spectrometer.
3. Thaw the frozen reagents from Ambion MAXIscript T7 kit. Vortex the 10× Transcription Buffer and ribonucleotide solutions until they are completely in solution. Once they are thawed, store the ribonucleotides on ice, but keep the 10× Transcription Buffer at room temp. Assemble transcription reaction at room temperature, adding the 10× Transcription Buffer after the water and template DNA are already in the tube:

Nuclease-free water to	20 µL
DNA template	1 µg
10× transcription buffer	2 µL
10 mM ATP	1 µL
10 mM CTP	1 µL

¹⁴We use two different enzymes to linearize the plasmid to minimize the chances of cutting in the cDNA insert.

10 mM GTP	1 μ L
10 mM UTP	0.6 μ L
10 mM biotin-16-UTP	0.4 μ L
T7 enzyme mix	2 μ L

4. Incubate for 1 h at 37 °C
5. Add 1 μ L TURBO DNase, mix well, and incubate at 37 °C for 15 min
6. Purify the biotinylated cRNA probe from the in vitro transcription reaction using the RNeasy Mini Kit according to manufacturer's instructions and quantitate using spectrophotometer (*see* Note 15).

3.3 Exon Microarray Data Analysis

Since it is assumed that that probe hybridization and data collection will be performed by a core facility or commercial service, detailed protocols for the exon chip hybridization and data collection will not be presented here. Briefly, the biotinylated cRNA probes were fragmented by sonication and 5 μ g of each was hybridized to an Affymetrix GeneChip® Human Exon 1.0 ST Array and scanned with an Affymetrix GeneChip Scanner 3000 (Affymetrix) according to manufacturer's instructions at a core facility.

Although it is beyond the scope of this review to provide a detailed protocol for the software-based data analysis, a brief outline of the strategy we used to analyze the raw data with Affymetrix Expression Console and Integrated Genome Browser software [12] and generate a list of binding candidates for validation is presented below. Please refer to the Affymetrix website for detailed manuals and tutorials that describe use of current software versions.

1. Process .cel files using Expression Console software.
2. Generate list of ranked probeset intensities using Expression Console.
3. Calculate gene expression level using Expression Console and transfer annotated results to excel.
4. Visualize .chp files using Integrated Genome Browser software and examine high scoring genes and top probeset intensities. Look for genes with multiple continuous high-scoring probesets within the exons (*see* Fig. 2 for examples) (*see* Note 16).
5. Make a list of candidate binders and estimate the boundaries of the cDNA for each candidate based on the probeset intensity values (*see* Note 17).

¹⁵We hybridized 5 μ g of each biotinylated cRNA probe to the exon array chip. If less than this amount is recovered after purification, the in vitro transcription reaction will need to be repeated.

¹⁶There will be some background from the common vector sequences in the in vitro transcribed probes. If data from several selection outputs is simultaneously visualized in the Integrated Genome Browser, it is easy to identify probesets with a high nonspecific background signal.

¹⁷The sequences of the high signal intensity probes within a candidate gene can be recovered from within the Integrated Genome Browser.

3.4 Recovery of Putative Binding Clones

1. Design a forward and reverse primer located near the middle of each predicted candidate gene cDNA (see Note 18).
2. Set up standard PCR reactions using candidate cDNA-specific and vector-specific (pYD1 Forward and pYD1 Reverse) primers and 1 ng of the appropriate selection output plasmid prep as template.
3. Run PCR reactions on 1.5 % agarose gel, cut out bands with a clean razor blade, and isolate DNA using Qiagen gel isolation kit following manufacturer's protocols. Elute in 50 μ L EB buffer or ddH₂O.
4. Sequence purified PCR products using the appropriate primer (Gap5 for PCRs done with pYD1 Forward or Gap3 for PCRs done with pYD1 Reverse).
5. Analyze sequence data to define the precise 5' and 3' cDNA insert boundaries and determine if the cDNA encoded protein is in-frame with AGA2 in the pYD1 yeast display vector.
6. Design new primer sets based on the newly determined 5' and 3' boundaries of the cDNA inserts, adding EcoRI overhangs to facilitate cloning (see Note 7).
7. PCR amplify the cDNA inserts using the cDNA-specific primer sets and gel purify.
8. Digest pYD1 and the candidate cDNA PCR products with *EcoRI*, purify, ligate, transform, miniprep colonies using standard molecular biology procedures, and sequence to confirm using the Gap5 sequencing primer.
9. Inoculate a 5 mL YPD culture with EBY100 and grow overnight with shaking at 30 °C and 200 rpm.
10. Determine concentration of the overnight yeast culture by measuring the OD₆₀₀ of a 1:20 dilution using a spectrophotometer ($1 \text{ OD}_{600} = 2 \times 10^7/\text{mL}$).
11. Use the overnight culture to inoculate a culture in YPD (the inoculation volume should be at least 1 mL/transformation) at 0.5 OD₆₀₀ and incubate at 30 °C, 200 rpm for 4–5 h (at least two cell divisions).
12. For each transformation, pellet 1 mL of the EBY100 culture by centrifugation at $3,000 \times g$ for 3 min, wash with 1 mL sterile ddH₂O, and remove ddH₂O.
13. To each tube of cells, add the following in this order:
 - 240 μ L 50 % PEG-3350
 - 36 μ L 1.0 M lithium acetate
 - 5 μ g plasmid (candidate cDNAs cloned into pYD1).
 - Bring volume to 360 μ L with sterile ddH₂O.

¹⁸We designed the primers to produce products about 500 bp in size for efficient PCR and gel-based purification.

14. Vortex transformation mix vigorously and incubate tubes at 42 °C in a water bath for 40 min.
15. Spin transformation tubes at top speed for 30 s and remove transformation mix. Resuspend pellet in 100 µL sterile ddH₂O and plate on prewarmed SD-CAA plates. Incubate the plates inverted at 30 °C for 3–4 days until colonies grow. Clones can be stored in SD-CAA + 20 % glycerol at –80 °C.

3.5 Testing Candidate Binding Clones by FACS

1. Inoculate 2 mL 2× SRG-CAA (2× SR-CAA + 2 % galactose) cultures with yeast colonies from candidate cDNA transformations and grow for at least 16 h with shaking at 30 °C.
2. Pellet 200 µL of the overnight cultures using a tabletop microfuge and wash twice with 1 mL PBS.
3. Incubate yeast for 1 h at room temperature with 100 µL of an appropriate concentration of biotinylated or fluorescently labeled target molecule (*see* Note 9) and mouse anti-Xpress antibody (1/1,000 dilution of stock solution) in PBS (*see* Note 19).
4. Wash yeast twice with 1 mL PBS and incubate with 100 µL 1/1,000 SA-PE and 1/1,000 Alexa fluor 647-conjugated anti-mouse secondary antibody in PBS for 30 min.
5. Wash twice with 1 mL PBS and analyze the yeast by FACS.

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¹⁹There is an Xpress epitope upstream of the cDNA inserts in the pYD1 yeast display vector allowing the efficiency of surface display to be monitored using anti-Xpress antibody.

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Fig. 1. Diagram of procedure for enrichment of clones displaying PtdIns-binding protein fragments and comprehensive exon microarray analysis of polyclonal selection output. A yeast library displaying human protein fragments was incubated with labeled PtdIns and binding yeast clones were enriched through several rounds of FACS. Polyclonal plasmid containing cDNAs encoding PtdIns-binding protein fragments was isolated from the polyclonal selection output and used as a template for in vitro transcription to generate biotin-labeled RNA. The labeled RNA was hybridized to the exon microarray and data was collected and

analyzed. (This research was originally published in Mol Cell Proteomics [10] © the American Society for Biochemistry and Molecular Biology)

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Fig. 2. Visualization of probeset intensity data collected from an Affymetrix GeneChip® Human Exon 1.0 ST Array hybridized with cRNA probes prepared from polyclonal yeast cDNA display selection outputs enriched for binding to PtdIns [6]. The gene exon structure is shown in *green*. Intensity values for exon-associated probesets are represented by *red bars* below the gene exon structure. The exon structure and probeset intensity data is visualized using Integrated Genome Browser software. *Grey bars* represent the total protein with *boxes* indicating the location of known functional domains (PH, pleckstrin homology domain; PTB, phosphotyrosine-binding domain). The *blue bars* below represent the protein fragments encoded by the cDNA inserts determined by sequencing. Since the gene exon structures are interrupted by non-protein coding intronic sequences, they do not precisely align with the proteins depicted above. (This research was originally published in Mol Cell Proteomics [10] © the American Society for Biochemistry and Molecular Biology)

Table 1

FACS-validated phosphatidylinositide-binding protein fragments identified by exon microarray analysis of yeast surface displayed cDNA library selection outputs. Expressed regions and total aa lengths are shown

Protein	Expressed aa	Total aa	PtdIns-binding domain (aa)
APOH	94–345	345	
ARNO	226–399	399	PH (261–386)
ATN1 ^a	1–229	1,190	
CRABP1 ^a	1–137	137	
CRIP1A ^a	1–140	164	
DAB2	2–221	771	PTB (36–176)
FAM71B ^a	267–546	605	
GAB2	1–125	676	PH (9–116)
HMGN2 ^a	6–90	90	
HOXA5 ^a	206–270	270	
HOXB6 ^a	136–224	224	
HOXC6 ^a	150–235	235	
HOXD4 ^a	144–255	255	
NUCKS1 ^a	54–243	243	
OSBP2	5–290	915	PH (185–272)
PDK1	232–429	429	PH (330–394)
PNPLA7 ^a	1,013–1,274	1,317	
POLS ^a	412–542	542	
PSD	739–1,024	1,024	PH (758–874)
PTPN5 ^a	1–60	565	
RNPS1 ^a	1–230	305	
SBF1	1,638–1,893	1,893	PH (1,790–1,890)
SFRS4 ^a	409–494	494	
SPTBN2	2,101–2,390	2,390	PH (2,221–2,304)
WDR60 ^a	60–260	1,066	
WNK1 ^a	924–1,156	2,382	

aa amino acids, C2C2 domain, PH pleckstrin homology domain, PTB phospho-tyrosine binding domain

^aPtdIns-binding protein fragments identified by human exon microarray analysis that were missed by monoclonal screening