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Identification of protein/target molecule interactions using yeast surface-displayed cDNA libraries

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Abstract

We describe a novel expression cloning method based on screening yeast surface-displayed human cDNA libraries by direct affinity interaction to identify cellular proteins binding to a broad spectrum of target molecules. Being a eukaryote, yeast protein expression pathways are similar to those found in mammalian cells, and therefore mammalian protein fragments displayed on the yeast cell wall are more likely to be properly folded and functional than proteins displayed using prokaryotic systems. Yeast surface displayed human cDNA libraries have been successfully used to screen for proteins that bind to post-translationally modified phosphorylated peptides, small signaling molecule phosphatidylinositides, and monoclonal antibodies. In this article we describe protocols for using yeast surface-displayed cDNA libraries, coupled with fluorescence-activated cell sorting (FACS), to select protein fragments with affinity for various target molecules including post-translationally modified peptides, small signaling molecules, monoclonal phage antibodies, and monoclonal IgG molecules.

Keywords

Yeast surface cDNA display; expression cloning; phage antibody; antigen identification; small molecules; post-translationally modified ligands

1. Introduction

The external display of heterologous proteins or protein fragments incorporated into the *Saccharomyces cerevisiae* cell wall, termed yeast surface display, has been successfully utilized in various applications since the initial development of the technology by Boder and Wittrup (1). Yeast surface display technology has been most extensively applied to monoclonal antibody engineering, where it has been used to affinity mature human antibody fragments and map antibody-binding epitopes (2,3).

Recently, yeast surface-displayed human cDNA libraries have been constructed and used to screen for protein fragments with affinity for various types of molecules. In this novel expression cloning system, ligands of any chemical and molecule compositions can be used as “baits” to identify binding cellular proteins providing that the bait molecules can be labeled fluorescently or immobilized to a solid matrix. Yeast surface-displayed human cDNA libraries have been successfully used to screen for proteins that bind to post-translational modifications (phosphorylated peptides) (4), small molecules (phosphatidylinositides) (5), monoclonal antibodies (6), and serum autoantibodies (7). In

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this article we describe protocols for using yeast surface-displayed cDNA libraries, coupled with fluorescence-activated cell sorting (FACS), to select protein fragments with affinity for various soluble molecules.

2. Materials

2.1 Growth and induction of yeast surface-displayed human cDNA library

1. Yeast surface-displayed cDNA expression library (*see* Note 1)
2. 2x SR-CAA yeast growth media: 20 g raffinose, 14 g yeast nitrogen base w/o amino acids, 10 g bacto casamino acids, 5.4 g Na₂HPO₄, 7.4 g NaH₂PO₄, bring volume to 1 L with ddH₂O, filter sterilize.
3. 10x SD-CAA for making plates: 70 g yeast nitrogen base w/o amino acids, 50 g bacto casamino acids, 100 g dextrose, bring volume to 500 mL with ddH₂O, filter sterilize.
4. SD-CAA plates: 5.4 g Na₂HPO₂, 7.4 g NaH₂PO₄, 17 g agar, bring volume to 900 mL with ddH₂O and autoclave to sterilize, let cool until not too hot to touch and add 100ml 10x SD-CAA, pour plates (100mm and 150mm) and allow to cool at RT, store plates at 4°C until ready to use.
5. 20% Galactose: 100 g galactose, bring volume to 500 mL ddH₂O, filter sterilize.
6. Mouse anti-Xpress (Invitrogen)
7. Goat antimouse-phycoerythrin (PE) (Jackson ImmunoResearch)

2.2 Yeast surface-displayed cDNA library FACS sorting and analysis

1. PBS: 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na₂HPO₄, 0.24 g of KH₂PO₄, bring volume to 1 L with ddH₂O and adjust pH to 7.4, sterilize by autoclaving.
2. Biotinylated target phosphorylated peptide, biotinylated non-phosphorylated target peptide, and non-biotinylated, non-phosphorylated target peptide
3. Streptavidin-PE (SA-PE) (Invitrogen)
4. Streptavidin-647 (SA-647) (Invitrogen)
5. Biotinylated target phosphatidylinositides
6. Purified target phage antibody particles
7. Purified control phage (M13 helper phage)
8. EZ-Link Sulfo-NHS-LC-Biotin (Pierce)
9. 20% PEG/2.5 M NaCl: 100 g PEG-8000, 73 g NaCl, bring volume to 500 mL ddH₂O, filter sterilize.
10. Biotinylated anti-fd bacteriophage (Sigma)
11. Target IgG
12. Negative control IgG
13. Goat anti-human IgG-PE (Jackson ImmunoResearch)

¹The construction and application of yeast surface-displayed cDNA libraries has been previously described (4–6) and a detailed protocol is presented elsewhere in this volume.

2.4 Plasmid recovery from yeast and sequencing

1. Spin prep miniprep kit (Qiagen)
2. Acid-washed glass beads (Sigma)
3. Gap5 primer (5'-TTAAGCTTCTGCAGGCTAGTG -3')

3. Methods

The methods below are divided into six categories: 3.1) Growth and induction of the yeast surface-displayed cDNA expression libraries. 3.2) FACS-based selection of phosphopeptide-binding protein fragments. 3.3) FACS-based selection of phosphatidylinositide-binding protein fragments. 3.4) FACS-based selection of scFv phage antibody-binding protein fragments. 3.5) FACS-based selection of mAb IgG binding protein fragments. 3.6) Plasmid recovery from individual binding clones.

Although we provide specific examples using three different targets, these protocols can serve as a general template for designing methods to select protein fragments with affinity for any soluble molecule that can be fluorescently detected. However, each unique target molecule may require modifications or optimizations of the protocol. It is critical to do a full set of controls so that the selection progress can be monitored (*see* Note 2).

3.1 Induction of yeast surface-displayed human cDNA library

1. Pre-warm and dry four 150 mm SD-CAA plates in a 30°C incubator. Thaw 1 mL aliquot of yeast surface-displayed cDNA library at RT. To titer the library aliquot, dilute 2 µL of the thawed library into 10 mL sterile H₂O, mix well, and then evenly spread 5 µL of the dilution on a 150 mm SD-CAA plate. At the same time, plate the remaining library aliquot on the remaining three 150 mm SD-CAA plates (330 µL/plate). Incubate plates upside down at 30°C for 3 days.
2. Count colonies on the titer plate and multiply by 10⁶ to obtain the total colony forming units (CFU) for the aliquot. This number should be > 10x the size of the original library to allow adequate representation of the diversity. The recovered library can be kept for several weeks on the plates if necessary by sealing the plate edges with parafilm and storing at 4°C.
3. Recover library from plates by adding 5 mL 2x SR-CAA to each plate and scraping with a flame-sterilized spreader and then collect the resuspended cells by pipeting. Determine cell number by taking an OD₆₀₀ reading of a 1:50-diluted sample of the resuspended cells (1 OD₆₀₀ ≈ 2 × 10⁷ yeast cells/mL).
4. Using the resuspended library, start a 200 mL 0.5 OD₆₀₀ culture (approximately 2 × 10⁹ cells) in 2x SR-CAA. Grow at 30°C with shaking for 1 h. To induce surface expression of the library, add 22 mL sterile 20% galactose (final 2% galactose) and continue growing at 25°C with shaking for 16 h. The induced library can be stored for several weeks at 4°C.
5. Check induction of the library by FACS using the mouse anti-Xpress antibody. Spin down 100 µL of the induced library (10,000 rpm in microfuge) and wash twice with 1 mL PBS. Resuspend cells in 500 µL PBS and add 1 µL mouse anti-

²The controls should include incubations with secondary reagents only (previously used and used in the current round of selection) and incubations using all previous rounds so that the enrichment of target-specific binding clones can be observed from round to round. It is also important to alternate the secondary detection agent between rounds to minimize the chances of selecting clones with affinity for them. The use of proper controls and careful monitoring of the selection process is critical to maximize the chances for success and aid in troubleshooting.

Xpress antibody. Incubate at RT with rotation for 1 h. Wash three times with 1 mL PBS. Resuspend in 500 μ L PBS and add 1 μ L goat anti-mouse-PE. Incubate at RT with rotation for 30 min. Wash three times with PBS, resuspend in 500 μ L PBS, and place on ice.

6. Analyze cells by FACS. At least 40% of the population should be Xpress-positive (*see* Note 3). The induced library is now ready for FACS selection experiments.

3.2 FACS selection of phosphopeptide-binding protein fragments

1. Check the OD₆₀₀ of a 1:20 dilution of the induced library to determine cell density. For the first round of sorting, collect 2×10^8 yeast cells by centrifugation and wash them twice with PBS. For example, if the calculated OD₆₀₀ of the undiluted induced library is 5, 2 mL of culture will contain approximately 2×10^8 cells.
2. Resuspend the cells in 500 μ L PBS and add 10 μ M biotinylated phosphopeptide and the corresponding non-phosphorylated, non-biotinylated peptide at 40 μ M to compete away non phospho-specific binding. As a negative control for the sorting, set up an incubation with the same number of cells but without the peptides. Incubate for 4 h at 4 °C.
3. Wash cells twice with ice cold PBS and incubate with 500 μ L of 1:500 diluted SA-PE for 30 min at 4 °C (*see* Note 4). Wash cells three times with ice cold PBS and resuspend in 3 mL PBS. Keep cells on ice in the dark until sorting.
4. First analyze the negative control incubation by FACS (*see* Note 5). This will give you a zero baseline for adjusting the FACS parameters. It is advisable to be less stringent in the first round and the sort gate should be placed directly above the point where the vast majority of the negative control cells cut off in the PE channel (Fig 1). Use the “yield” or equivalent non-stringent sorting setting on your flow cytometer during the first round. The library should be comprehensively sorted for the first round of selection. Analyze at least 10^8 cells from the peptide selection incubation and sort PE-positive cells into an eppendorf tube containing 100 μ L PBS (*see* Note 6). Make a note of the total number of cells analyzed and the number sorted.
5. Plate the sorted cells on one or several large pre-dried SD-CAA plates by gently spreading. Incubate plates inverted at 30 °C until colonies form (3–4 days) (*see* Note 7).
6. Add 3 mL SR-CAA to plate(s) and recover cells by scraping with a sterile cell spreader. To prepare a freezer stock, add 250 μ L 50% glycerol to 500 μ L cells and store at –80 °C.

³There is always a negative population after induction when analyzed by FACS and the maximum induction will vary from experiment to experiment.

⁴We used a BD FACS Aria for sorting and a BD LSRII for analysis. The protocols are written generally and other FACS equipment can be used.

⁵We generally use SA-PE in the first round because it appears to give a cleaner background than SA-647 during sorting.

⁶At a sort rate of 50,000 events/sec, this should take about 35 min. The first round is sorted on “faith” - there should not be an obvious population of positive cells and there may not be any significant difference between the negative control and the selection incubation. It is critical to analyze enough cells to cover the full diversity of the library or else some binding clones may be lost immediately. Since the diversity is massively diminished after the first round selection, subsequent rounds take significantly less time to sort.

MACS bead selection (Miltenyi Biotec) could also be used in the first round, but we prefer to use FACS.

⁷We recommend that the sorted cells be directly plated without spinning them down. We have observed a poor rate of recovery when we attempt to centrifuge the cells prior to plating, even when great care is taken. We sometimes dry the SD-CAA plates overnight at RT prior to plating the sort output so that a larger volume of cells can be more quickly absorbed. The efficiency of recovery of the sort output (colonies recovered vs. events sorted) should be closely monitored, especially in the first round. Any clones lost at this step are lost for good.

7. To induce the first round output, inoculate a 10 mL culture at 0.5 OD₆₀₀ in SR-CAA + 2% galactose using the remaining cells from the round one output and grow at 25°C with shaking for 16 h.
8. Since the diversity is greatly reduced after the first round, it is not necessary to use as many cells during incubations in the subsequent rounds. Wash approximately 5 × 10⁷ cells (*see* Note 8) from the induced first round output and the starting library twice with PBS and set up control and selection incubations in the same manner as the first round and incubate for 4 h at 4 °C.
9. Wash cells twice with ice cold PBS and incubate with 500 µL of 1:500 diluted SA-647 for 20 min at 4 °C. It is important to use a different secondary detection reagent than was used in the first round (*see* Note 9). Set up a secondary only control with the first round output using SA-647. After incubations with the secondary reagents, wash cells three times with ice cold PBS and resuspend in 1 mL PBS. Keep cells on ice in the dark until sorting.
10. Analyze the selection incubation by FACS and compare it to the secondary only control to decide where to place the sort gate. Since the goal is to recover the maximum diversity of specific binding clones, we usually place the sort gate directly above the point where the negative control cells cut off in the 647 (aka APC) channel, in the same manner as during the first round sort. However, in the second and subsequent rounds, use the “purity” or equivalent more stringent sorting setting on your flow cytometer. If the selection is working, you are likely to observe increased binding in the selection incubation compared to the negative control. Analyze 10⁷ cells from the second round phosphorylated peptide selection incubation and sort 647-positive cells into an eppendorf tube containing 100 µL PBS. Again, make a note of the total number of cells analyzed and the number sorted and plate the sorted cells on one or several large SD-CAA plates. Incubate at 30 °C until colonies form.
11. Add 3 mL SR-CAA to plate(s) and recover cells by scraping. Prepare a frozen stock as previously described and store at −80 °C. Induce the second round output by inoculating a 10 mL culture at 0.5 OD₆₀₀ in SR-CAA + 2% galactose using the remaining cells and grow at 25°C with shaking for 16 h.
12. The third round is carried out identically to second round, except for returning to SA-PE for the secondary detection in the phosphorylated peptide selection incubation. It is absolutely critical to set up a SA-PE secondary only control and carefully analyze the results. It is also important to set up a control incubation with the biotinylated non-phosphorylated peptide control to determine if there is any non-phosphospecific binding. If the selection is working, there should be a significant population of binding clones (>5%) and very little background binding to the SA-PE negative control (<0.5%). If the specific binding population is >5% and the negative control background is comparably low (Fig 1), the output of this round of sorting is ready to be screened using individual clones (*see* Note 10). Sort the binders in the induced second round output and recover as previously described. If the intention is to screen individual clones from this sort output, some plates can be seeded at a lower density (500 cells/plate) based on the sorting data to facilitate the

⁸The cell number should be at least 20x more than the recovered output from the first round.

⁹Alternation between two detection reagents is usually sufficient to prevent the enrichment of secondary reagent binding clones. However, in the absence of real target binding clones (e.g. failed sorts), you will almost always end up with secondary binders if enough rounds of sorting are carried out.

¹⁰Sorting for too many rounds will reduce the output diversity and tend to favor high affinity or high expressing clones.

picking of individual colonies for screening. After the colonies have grown, make a glycerol freezer stock of the third round sort output as described previously.

13. Protocols for the screening of individual clones are similar to the sorting, but done on a smaller scale to save reagents and facilitate high throughput analysis (see Note 11). Inoculate 1 mL SR-CAA + 2% galactose cultures with a yeast colony from the SD-CAA sort output plates and grow at least 16 h with shaking at 30 °C.
14. Wash 200 µL of the cultures with PBS and pellet. Save the remaining culture at 4 °C so that binding clones can be identified and saved. Add 50 µL 10 µM phosphorylated biotinylated peptide or 50 µL non-phosphorylated biotinylated peptide (control) in PBS to the cell pellets, resuspend by pipeting or agitation, and incubate for 2 h at 25 °C or overnight at 4 °C.
15. Wash twice with ice cold PBS, add 50 µL 1:500 diluted SA-PE, and incubate at 25°C for 30 min. Wash twice with ice cold PBS, resuspend in 300 µL PBS, and keep on ice until FACS analysis.
16. Analyze by FACS. Phospho-specific binding clones will have binding in the phosphorylated peptide incubation, but not the non-phosphorylated control incubation (Fig 2). Clones that exhibit phospho-specific binding can be patched onto SD-CAA plates for temporary storage and frozen in SR-CAA + 15% glycerol for permanent storage at −80 °C. A protocol for plasmid recovery for sequencing of the clones is detailed in section 3.6 below.

3.3 FACS selection of phosphatidylinositide-binding protein fragments

1. The protocols for selection of phosphatidylinositide-binding protein fragments are similar to the protocols described above using the phosphorylated peptides. For the first round of selection, collect 2×10^8 cells from the induced library, wash twice with PBS, and incubate in 500 µL of PBS with 2 µM biotinylated phosphatidylinositides for 4 h at 4 °C.
2. Wash cells twice with PBS and incubate with 500 µL of 1:500 diluted SA-PE for 20 min at 4 °C. Wash cells twice with PBS and analyze, sort, and recover cells as described above.
3. Continue with subsequent rounds as described above, taking care to do appropriate negative controls and alternating secondary detection reagents between rounds. When the binding population is greater than 5% with very little background binding to the negative controls, individual colonies from the sort output from that round can be screened using 2 µM biotinylated phosphatidylinositides with a SA-PE secondary only control. Binding clones should be patched onto SD-CAA plates for further analysis and stored in glycerol freezer stocks.

3.4 FACS selection of phage antibody-binding protein fragments

1. The protocols for selection of phage antibody-binding protein fragments are similar to the protocols described above. We present a protocol for the biotinylation of phage antibody particles, but protocols and reagents exist that are suitable for the labeling of a wide variety of target molecules. To biotinylate the phage antibodies, make a fresh 10 mM biotinylation solution by adding 180 µL of ddH₂O to 1 mg of

¹¹The most efficient screening protocol will depend on the equipment available to each individual researcher. We describe a protocol based on single-tube FACS analysis, which is the format likely to be available to the most researchers. It is a simple matter to adapt the protocols to a 96 well or other more high throughput format if this equipment is available. The appropriate screening method will depend on the experimental goals of each project (i.e. strong binders vs. diversity).

EZ-Link Sulfo-NHS-LC-Biotin. Add 60 μL of this solution to 800 μL fd phage antibody in PBS (*see* Note 12). Incubate at RT for 15 min and then stop the reaction by adding 140 μL 1 *M* Tris.

2. Add 250 μL of 20% PEG/2.5 *M* NaCl solution and incubate on ice for at least 30 min to precipitate the labeled phage particles. Spin down precipitated phage at 16,000 g at 4 °C in a microcentrifuge for 20 min. Carefully remove as much of the PEG solution as possible from the phage pellet and resuspend in 1 mL PBS.
3. Precipitate the phage a second time using the same protocol, carefully remove the PEG solution, and resuspend in 1 mL PBS. Filter the labeled phage solution with a 0.45 μm syringe filter. The binding activity of the labeled phage should be confirmed using an appropriate assay.
4. The sorting process is similar to what has been previously described. In the first round of selection, collect 2×10^8 cells from the induced library, wash twice with PBS, and resuspend in 400 μL PBS. Add 50 μL labeled target phage and 250 μL unlabeled helper phage to compete away non-specific phage binding clones and incubate at RT for 1 h with rotation.
5. Wash cells twice with PBS and incubate with 500 μL of 1:500 diluted SA-PE for 20 min. Wash cells twice with PBS and analyze, sort, and recover binding clones as described above. In subsequent rounds be sure to do appropriate controls (e.g. control phage, secondary only, previous round outputs) and remember to alternate SA-647 with SA-PE or other detection reagents to minimize the selection of clones that bind the secondary detection reagent. When significant and specific binding (>5%) is observed in the sorted population, individual clones can be tested for phage binding by FACS.
6. It is possible to use a scaled down version of the above protocol with the biotinylated phage and appropriate secondary only controls. However, for screening many samples, we routinely use a biotinylated anti-fd bacteriophage antibody. Grow overnight cultures for screening as described above and wash 200 μL of the cultures with PBS and pellet. Add 200 μL of a 1:10 dilution of target phage and negative control helper phage in PBS to the cell pellets and incubate for 2 h at 25 °C or overnight at 4 °C.
7. Wash cells twice with PBS and incubate with 500 μL of 1:500 diluted anti-fd bacteriophage antibody for 1 h at RT.
8. Wash cells twice with PBS and incubate with 500 μL of 1:500 diluted SA-PE for 20 min. Wash cells twice with PBS and analyze by FACS.
9. Binding clones can be streaked on SD-CAA plates for temporary storage and should be stored permanently as glycerol stocks at -80°C as previously described.

3.5 FACS-based selection of mAb IgG binding protein fragments

1. To biotinylate the target and negative control IgGs, make a fresh 10 *mM* biotinylation solution by adding 180 μL of ddH₂O to 1 mg of EZ-Link Sulfo- NHS-LC-Biotin. Add 30 μL of this solution to 1 mL of 2 mg/mL IgG in PBS and incubate at RT for 30 min. Stop the reaction by adding 100 μL 1 *M* Tris.

¹²Phage particles must be in a solution that does not contain free primary amine groups that will interfere with the biotinylation reaction.

2. Remove excess biotin reagent by dialysis against PBS. The binding activity of the labeled IgG should be confirmed using an appropriate assay.
3. The sorting process is similar to what has been previously described. In the first round of selection, collect 2×10^8 cells from the induced library, wash twice with PBS, and resuspend in 400 μ L PBS. Add 10 μ L biotinylated target IgG and 100 μ L 1 mg/mL unlabeled negative control IgG to compete away non-specific IgG binding clones and incubate at RT for 1 h with rotation.
4. Wash cells twice with PBS and incubate with 500 μ L of 1:500 diluted SA-PE for 20 min. Wash cells twice with PBS and analyze, sort, and recover binding clones as described above. In subsequent rounds be sure to do appropriate controls (e.g. negative control IgG, secondary only, previous round outputs) and remember to alternate SA-647 with SA-PE or other detection reagents to minimize the selection of clones that bind the secondary detection reagent. When significant and specific binding (>5%) is observed in the sorted population, individual clones can be tested for target IgG binding by FACS.
5. To screen for binding clones in the sort output, grow overnight yeast cultures for screening as described previously and wash 200 μ L of the cultures with PBS and pellet. Add 200 μ L of a 1:100 dilution of target phage or negative control IgG in PBS to the cell pellets and incubate for 2 h at 25 °C or overnight at 4 °C.
6. Wash cells twice with PBS and incubate with 500 μ L of 1:500 diluted goat-antihuman-PE for 20 min. Wash cells twice with PBS and analyze by FACS.
7. Binding clones can be streaked on SD-CAA plates for temporary storage and should be stored permanently as glycerol stocks at -80 °C as previously described.

3.6 Plasmid recovery from individual binding clones

1. The method we describe is based on the Qiagen spin miniprep kit. The buffers used (P1, P2, N3, EB) are provided in the kit (*see* Note 13). Recover an approximately 50 μ L yeast cell pellet and wash twice with ddH₂O. The cells can be recovered from liquid cultures or by scraping from plates.
2. Resuspend the pellet in 400 μ L Qiagen buffer P1 and add approximately 200 μ L glass beads. Vortex at high speed for 3 min and remove 250 μ L of the cell slurry (leaving the glass beads behind) to a clean tube.
3. Add 250 μ L buffer P2, gently mix by inverting, and incubate at RT for 5 min.
4. Add 350 μ L buffer N3 (a cloudy precipitate will form) and spin at 16,000 g in a microcentrifuge for 15 min.
5. Apply supernatant to a Qiagen spin miniprep column and spin at 16,000 g for 1 min. Discard flow-through.
6. Add 750 μ L 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 and add 50 μ L elution buffer EB and spin at 16,000 g for 1 min to elute.
7. Transform the recovered plasmids into bacteria using any standard transformation protocol and plate on an LB-ampicillin plates. Prepare minipreps from the

¹³The protocol can also be scaled up to recover plasmids from a polyclonal population. These plasmids can then be sequenced as an alternative screening method. Plasmids of interest will have to be re-transformed into EBY100 and tested for target binding.

transformants using any standard method that is compatible with DNA sequencing. Use the Gap5 primer to sequence the plasmid's cDNA insert.

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Figure 1.

Selection of phosphopeptide binding clones from a yeast surface-displayed human cDNA library by FACS. Surface expression of the cDNA library was induced and the yeast cells were incubated with biotinylated, tyrosine-phosphorylated peptides derived from the major autophosphorylation sites of either EGFR (EGFRpY1173) or FAK (FAKpY397).

Enrichment of EGFRpY1173 and FAKpY397 binding clones is achieved through four rounds of FACS. The sort window is shown for the first round. In the fourth round, the upper left quadrant was sorted and the output was used for individual clone analysis. (This research was originally published in *Mol Cell Proteomics*. Bidlingmaier, S., and Liu, B. (2006) Construction and application of a yeast surface-displayed human cDNA library to identify post-translational modification-dependent protein-protein interactions. *Mol Cell Proteomics*. 5, 533–540. © the American Society for Biochemistry and Molecular Biology.)

Figure 2. Phosphorylation-dependent binding of selected clones. Induced yeast clones were tested for binding with 10 μ M of either phosphorylated (p) or non-phosphorylated (np) peptides. **A**, Cellular proteins (APS and PIK3R3) binding specifically to EGFRpY1173. **B**, Cellular proteins (APS, SH2B and Tensin) binding specifically to FAKpY397. (This research was originally published in *Mol Cell Proteomics*. Bidlingmaier, S., and Liu, B. (2006) Construction and application of a yeast surface- displayed human cDNA library to identify post-translational modification-dependent protein-protein interactions. *Mol Cell Proteomics*. 5, 533–540. © the American Society for Biochemistry and Molecular Biology.)