tion necessary to analyze the ELISA data. We believe that this straightforward approach may be used for measuring other cell surface cytokines or antigens found on cells. The only limitation that we can envision is that this technique should be done with either homogeneous or cloned cells.

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M. Ly, M.C.N. Irwin, M.R. Irwin, H.T. Wepsic and M.R. Jadus
Veterans Affairs Medical Center
Long Beach, CA, USA

Techniques for Colony DNA Hybridization and Protein Immunoassays for a Broad Spectrum of Yeast Genera

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Both filter techniques presented here are based on the new principle of disruption of yeast cells. This appears to be the limiting step of all current techniques, where yeast cell walls must be degraded by action of enzymes specific for particular yeast species. Degradation of cell walls is efficient only in exponentially growing cells, and there are some yeasts for which such enzymes are not available. In our approach, we used autolysis for disruption of yeast cells followed by any standard hybridization (Table 1) or immunodetection (Table 2) procedure. The advantages of our methods are as follows: *(i)* it is possible to induce the autolysis of cells of a broad spectrum of yeast genera by the same procedure (7) without addition of any lytic enzymes; *(ii)* yeast clones can be autolyzed on membranes in any phase of their growth; *(iii)* a great number of yeast clones can be analyzed in parallel; and *(iv)* both methods are cheap and simple.

We demonstrate our filter techniques on the detection of specific DNA fragments in five different yeast genera (*Saccharomyces, Schizosaccharomyces, Kluyveromyces, Endomycopsis* and *Schwanniomyces*) and on immunodetection of human MN-protein (10) intracellularly expressed in *Saccharomyces cerevisiae*.

We applied the described protocol for the detection of the autonomously replicating plasmid pJDBαCHE, both in *S. cerevisiae* GRF18/pJDBαCHE (4) (Figure 1, lane 1) and in *Schizosaccharomyces pombe* LP36/pJDBαCHE (5) (Figure 1, lane 4), and for the detection of the integrative plasmid pUCL (pUC18 with the LEU2 gene inserted in the *Pst*I site) in *S. cerevisiae* GRF18/pUCL (Figure 1, lane 3). The plasmid pHC624 (1), digested with *Eco*RI and labeled by using the random prime extension technique with [α-^{32}P]-dCTP, was used as the specific probe against bacterial sequences (ori, Amp^{R}) present in both tested plasmids. The parental strains, *S. cerevisiae* GRF18 (α, his3-II, his3-I5, leu2-3, leu2-II2) and *S. pombe* LP36 (h^{-s}, leu1-32), were used as the negative controls (Figure 1, lanes 2 and 5). The presence of specific DNA sequences in three more different yeast genera was analyzed to prove the universality of the technique: cytoplasmic linear double-stranded (ds)DNA plasmids pGKL1 and pGKL2 (2) were detected in *Kluyveromyces lactis* IFO1267 using the agarose-gel-purified [α-^{32}P]dCTP labeled pGKL DNA as the probe (Figure 1, lane 6). The genes for glucoamylase (GLU1) and α-amylase (Soa) were detected in *Endomycopsis fibuligera* CCY42-3-2 (Figure 1, lane 8) and *Schwanniomyces occidentalis* CCY47-1-1 (Figure 1, lane 10) genomes, respectively. Agarose purified and radioactively labeled DNA fragments, containing either GLU1 from plasmid pSfGlu1 (9) or Soa from plasmid pRS306-Soa (3), were used for hybridization. The strain, *S. cerevisiae* N247 (a, leu2, ura3) (Figure 1, lanes 7, 9 and 11), in which pGKL, GLU1 and Soa sequences were absent, was used as the negative control.

Intracellular production of human MN protein in *S. cerevisiae* GRF18 from an inducible GAL7 promoter placed on plasmid pWYGLMN (11)

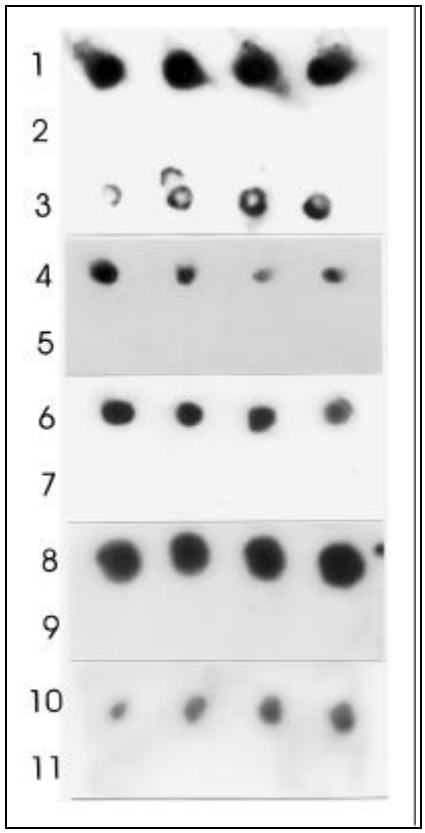

Figure 1. Detection of specific DNA sequences in yeast. Lane 1, *S. cerevisiae* GRF18/pJDBαCHE; lane 2, *S. cerevisiae* GRF18; lane 3, *S. cerevisiae* GRF18/pUCL; lane 4, *S. pombe* LP36/pJDBαCHE; lane 5, *S. pombe* LP36; lane 6, *K. lactis* IFO1267; lanes 7, 9 and 11, *S. cerevisiae* N247; lane 8, *E. fibuligera* CCY42-3-2; and lane 10, *S. occidentalis* CCY47-1-1.

Table 1. Protocol for Colony DNA Hybridization

1. The yeast clones were grown on nitrocellulose (or nylon) membrane placed on SD-CA (0.67% Bacto-yeast nitrogen base, 2% glucose, 2% Bacto-agar, 0.5% Casaminoacids) agar plate for 2–4 days (dependent on the strain) or, alternatively, cell suspensions were spotted on a membrane.
2. Membranes with colonies were dried at 45°C (1–2 h), laid on Whatman paper (Clifton, NJ, USA) soaked with toluene in a glass petri dish, incubated 5 min and dried lying on Whatman paper in a fume hood at room temperature for 15 min.
3. Dried membranes were incubated at 37°C overnight on Whatman paper soaked with TESP (20 mM Tris-HCl, pH 8.0, 50 mM EDTA-NaOH, pH 8.0, 2% sodium dodecyl sulfate [SDS], 0.05% pronase E) in a glass petri dish. During this step, the cells became disrupted by autolysis.
4. Total yeast DNA was denatured and neutralized by soaking the membrane gradually with: *(i)* 0.1 M NaOH (twice for 5 min); *(ii)* 1 M Tris-HCl, pH 7.5 (twice for 5 min); *(iii)* 2× SSC (300 mM NaCl, 30 mM sodium citrate) (twice for 2 min) and then fixed by ultraviolet (UV)-cross-linking or baking the membrane in a vacuum oven (8). At this stage, filters were ready for the standard hybridization procedure (8).

Table 2. Protocol for Immunodetection of Specific Proteins

1. As described in Table 1 (Step 1).
2. As described in Table 1 (Step 2).
3. To autolyze yeast clones, dried membranes were laid on Whatman paper soaked with TEDS (20 mM Tris-HCl, pH 8.0, 100 mM EDTA-NaOH, pH 8.0, 2% SDS) in a glass petri dish and incubated at 37°C overnight.
4. Membranes were dried at room temperature and then used for standard immunodetection (8).

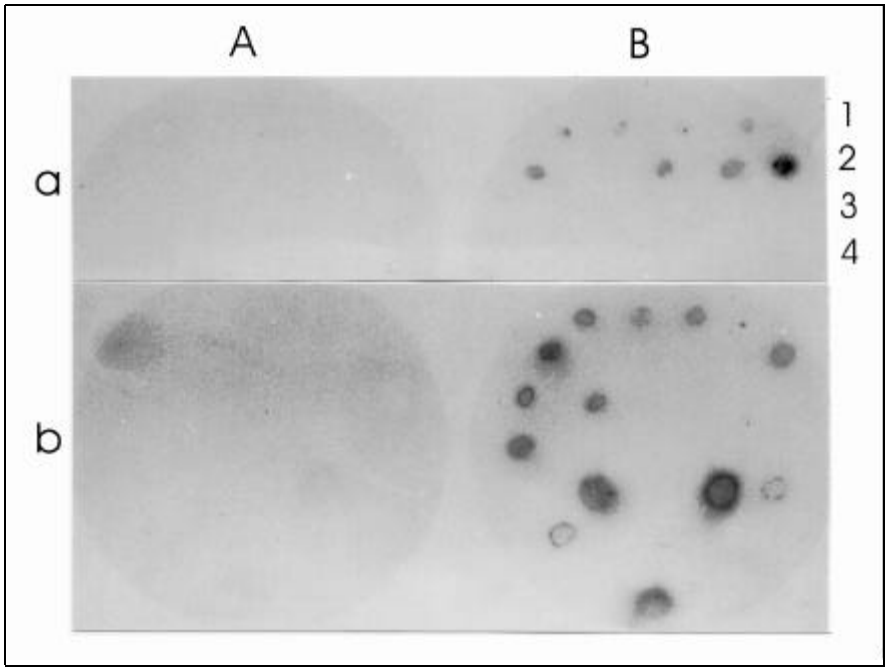

Figure 2. Immunodetection of human MN protein produced in *S. cerevisiae.* (a) Lanes 1 and 2, *S. cerevisiae* GRF18/pWYGLMN; and lanes 3 and 4, *S. cerevisiae* GRF18. (b) Individual subclones of *S. cerevisiae* GRF18/pWYGLMN after several passages. (A) SD-GL-his; (B) SD-GAL-his (induction of GAL7 promoter).

was detected using the ^{125}I-labeled monoclonal antibody M75 (6) (Figure 2). The individual clones of *S. cerevisiae* GRF18/pWYGLMN were grown on SD-GAL-his (0.67% Bacto-yeast nitrogen base, 2% galactose, 50 mg/mL histidine) (Figure 2B) or on SD-GL-his (0.67% Bacto-yeast nitrogen base, 2% glucose, 2% Bacto-agar, 50 mg/mL histidine) (Figure 2A). The parental strain, *S. cerevisiae* GRF18, was used as the negative control (Figure 2a, lanes 3 and 4). The MN protein production was found only in clones of *S. cerevisiae* GRF18/pWYGLMN growing on galactose medium where the expression from the GAL7 promoter was induced (Figure 2a, lanes 1 and 2). The method helped us to identify non-producing subclones, which lost the MN gene after several passages (Figure 2b).

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We thank Dr. J. Forstová for critical reading of the manuscript. This work was partially supported by Grant No. 20107 from Charles University, Prague, Czech Republic. *Address correspondence to Zdena Palková, Department of Genetics and Microbiology, Charles University, Viničná 5, 12844 Prague 2, Czech Republic. Internet: zdenap@prfdec.natur.cuni.cz*

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Zdena Palková, Martin Pospíšek and Blanka Zikánová
Charles University
Prague, Czech Republic

Immunopurification of Polyclonal Antibodies to Recombinant Proteins of the Same Gene Family

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Polyclonal antibodies raised against bacterially expressed fusion proteins commonly cross-react with undesired proteins, such as: *(i)* bacterial antigens that are co-injected with the fusion protein or may be present in animal sera due to previous bacterial infections; *(ii)* proteins (β-galactosidase, MS2 RNA polymerase, glutathione S-transferase [GST]) in fusion with the protein of interest that are useful for enhancement of the immune response and/or purification schemes; and *(iii)* related proteins of the same gene family. These undesirable "contaminating" antibodies are likely to interfere with several immunological assays. In techniques such as the enzyme-linked immunosorbent assay (ELISA), immunofluorescence and immunohistological staining, which do not involve protein fractionation, it is difficult to distinguish between specific and nonspecific recognition. In Western blots and immunoprecipitation, cross-reacting antibodies may reveal undesirable bands with molecular weights close to those of the proteins of interest. High background and nonspecific results may lead to deceiving conclusions that can be avoided by the use of immunopurified antibodies.

Conventional methods to remove undesirable antibodies are based on adsorption to nitrocellulose filters, Sepharose beads or insoluble bacterial or cellular extracts (5). The most commonly used technique to clear anti-fusion-protein and anti-related protein members antibodies also involves nonspecific antigen binding to Sepharose beads (3). These methods are time-consuming and expensive, in addition to resulting in low yields and contaminated antisera. Recently, a new method was described (2) to prepare bacterial extracts for antibody purification, based on adsorption of anti-bacterial antibodies. This technique is important for the adequate screening of bacterial expression libraries. Here we describe a modification of this technique, which results in

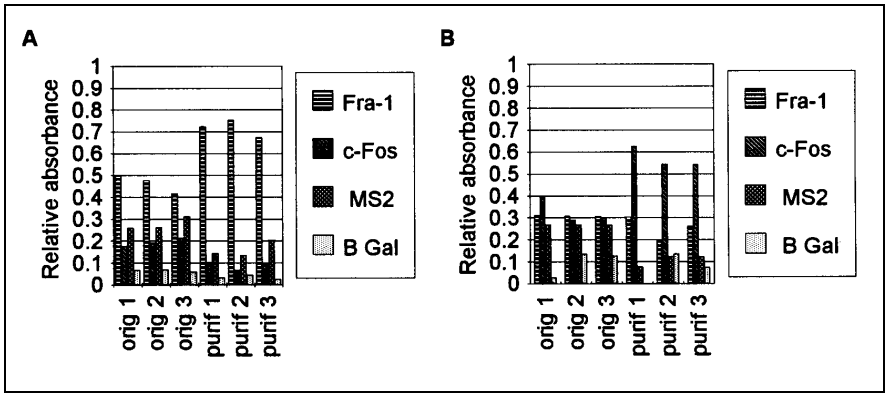

Figure 1. ELISA for original (orig) and purified (purif) anti-Fra-1 (panel A) and anti-c-Fos (panel B) antisera. K537 *E. coli* bacteria transformed with pEXc-*fos* (coding for MS2-polymerase c-Fos protein) or pEX*fra*-1 (coding for MS2-polymerase Fra-1) were used to generate bacterial extracts for purification of anti-Fra-1 and anti-c-Fos antisera, produced against recombinant bacterial proteins in fusion with MS2-polymerase (panels A and B, respectively). ELISA plates were coated with a semi-purified protein solution (10 μg/μL) and incubated overnight at room temperature. The following proteins used were: MS2-polymerase-cFos (c-Fos); MS2-polymerase-Fra-1 (Fra-1); MS2-polymerase (MS2) and β-galactosidase (β-gal). Plates were washed and incubated for 30 min in blocking solution (0.5% BSA and 0.05% Tween® 20 in PBS). Original (orig) or purified (purif) rabbit antisera against Fra-1 protein (panel A) or c-Fos (panel B), diluted 1:4000 in blocking solution, were added in triplicates and incubated for 2 h at room temperature. Antisera were removed, plates were washed in PBS and incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase diluted 1:25 000 (Sigma Chemical, St. Louis, MO, USA). Antibody concentration was determined by measurement of absorbance at 495 nm upon reaction with 0.04% *o*-phenylenediamine (OPD) and 0.04% (vol/vol) hydrogen peroxide in 0.1 M phosphate-citrate solution (pH 5.0), and the reaction was interrupted with 1 vol of 2.5 M HCl. Chart shows relative recognition of each protein by original or purified antisera in three experiments (1, 2, 3). The reactivity of the pre-immune sera in relation to the antisera against the protein used as antigen is 0.15 for c-Fos and 0.02 for Fra-1.